Application of PHYSIOLOGY in Wheat Breeding

M.P. Reynolds,
J.I. Ortiz-Monasterio,
and A. McNab,
Editors

CIMMYT®

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CIMMYT®

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PREFACE

We applaud this practical guide to the application of physiology in wheat breeding, which brings together in one volume the working knowledge of a broad range of experts in salinity, drought, cold, waterlogging, micronutrients, and other key topics.

The more understanding plant breeders have of the physiological processes that underlie plant performance, the more efficiently they can exploit relevant physiological mechanisms to improve crop performance. Wheat breeders have become increasingly able to use physiological traits directly as selection criteria, as their knowledge of physiological processes has expanded and as traits have been identified that can be used as selection criteria to achieve results more quickly and efficiently than selecting for yield performance alone.

Nonetheless, there are still major gaps in our understanding of how crops adapt to the environment, and this calls for further physiological research. Indeed, a more complete understanding of crop physiology will be a prerequisite to the effective application of new techniques such as genetic transformation, functional genomics, and marker-assisted selection in wheat breeding.

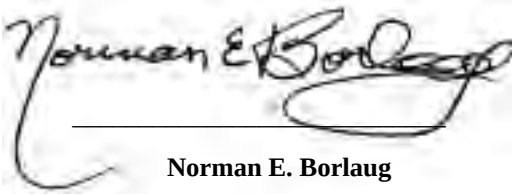
The improved varieties developed though wheat breeding are important catalysts for increasing crop performance at the farm level, where a range of biotic and abiotic stresses impinge on yields. However, for the maximum genetic yield potential of improved varieties to be fully expressed, scientists must also pay due attention to crop management practices. Without adequate soil fertility, appropriate planting methods, effective control of weeds and pests, and efficient water management, the full economic benefits of genetic improvement can never be realized.

Brief theoretical explanations are provided throughout this book, but the main focus is on practical procedures breeders can readily apply. Such topics as economic issues related to the role of physiology in wheat breeding and the search for genetic diversity that could contribute to increasing yield will help breeders take full advantage of existing methodologies and resources to do their work more efficiently. The chapter on the genetic basis of physiological traits brings out the point that though field testing is indispensable, proper combination with molecular data could lead to more efficient use of limited resources.

The collected wisdom contained in this book was generously contributed by the authors, and we thank them for sharing the fruits of their varied experience. Through this book, their expertise will be accessible to breeders everywhere, but especially in developing countries, where information on this newly emerging field is rarely available.

Sanjaya Rajaram

Director
CIMMYT Wheat Program



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General Considerations in Physiological Breeding

INTRODUCTION

Application of Physiology in Wheat Breeding

M.P. Reynolds, R.M. Trethowan, M. van Ginkel, and S. Rajaram[1]

How can disciplinary research in physiology complement wheat breeding? This introductory chapter is intended to provide broad guidelines to help breeding programs: 1) assess whether physiological criteria should be included in a breeding strategy; 2) evaluate specific physiological selection traits and determine their usefulness in breeding. The other chapters in this book provide more explicit information on how physiological approaches can be used in breeding work for a variety of environmental conditions.

Physiological criteria are commonly though not explicitly used in breeding programs. A good example is selection for reduced height, which improves lodging resistance, partitioning of total biomass to grain yield, and responsiveness to management. Another is differential sensitivity to photoperiod and vernalizing cold, which permit adaptation of varieties to a wide range of latitudes, as well as to winter- and spring-sown habitats. Despite a lack of detailed understanding of how photoperiod and vernalization sensitivity interact with each other and the environment, the relatively simple inheritance of photoperiod (*Ppd*) and vernalization (*Vrn*) sensitivity genes and their obvious phenotypic expression (i.e. earliness versus lateness) has permitted them to be modified in many breeding programs. The same is true for the height reduction (*Rht*) gene. In the future an increased understanding of the genetic basis of these traits may enable breeding programs to exploit them further.

Selection for reduced height and improved adaptation to environment has had a profound impact on modern plant breeding, and the improvement in yield potential of spring wheat since the Green Revolution has been shown to be associated with a number of other physiological factors (Reynolds et al., 1999). Nonetheless, most breeding programs do not put much emphasis on selecting physiological traits *per se* (Rajaram and van Ginkel, 1996). Exceptions would include: 1) the stay-green character, which has been selected for in relation to improved disease resistance and is associated with high chlorophyll content and photosynthetic rate in Veery wheats, for example Seri-82 (Fischer et al., 1998), and 2) more erect leaf angle, a common trait in many high yielding bread and durum wheat plant types that was introgressed into the CIMMYT germplasm pool in the early 1970s (Fischer, 1996).

A recent survey of plant breeders and physiologists addressed the question of how physiological approaches in plant breeding could have greater impact (Jackson et al., 1996). According to the survey, while the impacts of physiological research on breeding programs have been limited in the past, future impacts may arise through:

- Focusing physiological work on an appropriate range of germplasm (which will depend on the specific breeding objectives);
- Working with larger populations to enable extrapolation of findings to breeding methods;
- Identifying traits for use as indirect selection criteria, in addition to those already used in core breeding programs;
- Identifying traits for use as selection criteria in introgression programs;
- Conducting selection trials in more representative environments, and
- Developing tools that could be quickly and easily applied to large numbers of segregating lines.

In this and the following chapters, many of these suggestions are incorporated into a research framework for assessing the value of physiological selection traits in a breeding context.

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Assessing the Potential of Physiological Selection Criteria to Complement Breeding Strategies

The Art, Science, and Empiricism of Breeding

Breeding is frequently referred to as a blend of science and art, as well as an empirical process. The science refers to the routine application of established facts, such as the documented role of specific genes in conferring disease resistance or environmental adaptation. The art of breeding refers to the intuition gained by working with germplasm and the integration of that experience with established knowledge. In other words, intuition enables good breeding decisions to be made based on our incomplete knowledge of the biology and ecology of plants. Empiricism refers to the use of multiple crossing and selection strategies to achieve a single objective, sometimes loosely referred to as "the numbers game." Physiological understanding adds to the science, and as such complements the intuitive knowledge required to conduct good breeding. Use of physiological selection criteria can improve the probability of success by making empirical selection more efficient.

Theoretical Basis for Using Physiological Traits

Assuming significant genetic diversity for a trait is established, the question of how its use as a selection criterion would improve breeding efficiency can be addressed. Without experimentation, this cannot be predicted with any certainty, any more than a breeder can know in advance which of many crosses will produce the desired variety. An essential question is whether selection for a given physiological trait as part of an integrated breeding approach will achieve results more quickly and efficiently than selecting parents and/or progeny for performance alone.

Many traits may appear to be of potential benefit to yield. To assess which trait(s) should be prioritized, alternate hypotheses may be tested empirically, based on a conceptual model that incorporates current understanding of physiological and biochemical constraints to performance (Figure 1). For example, if the principal yield-limiting factor is water stress, physiological understanding would suggest that genotypes with deeper roots will have an advantage over others, assuming moisture is available deeper in the soil profile. However, selection for yield alone will not guarantee that good lines have the deepest roots, because drought tolerance may be conferred through genetic superiority of other mechanisms, such as osmotic adjustment, accumulation and remobilization of stem reserves, superior spike photosynthesis, heat tolerant metabolism, and good emergence and establishment under moisture stress (Figure 1).

Genetic advance in dry environments has been quite limited in most breeding programs. Slower progress in moisture stressed environments compared to irrigated environments is usually ascribed to the heterogeneity of selection nurseries under dry conditions, which renders performance-based selection unreliable. Selection for specific traits in more controlled environments is likely to be more effective. In addition, if more than one mechanism is involved in drought tolerance, deliberate selection with a view to combining synergistic traits is likely to achieve results sooner than adopting a strategy whereby parents and progeny are selected on the basis of performance alone (see chapter by Richards et al.).

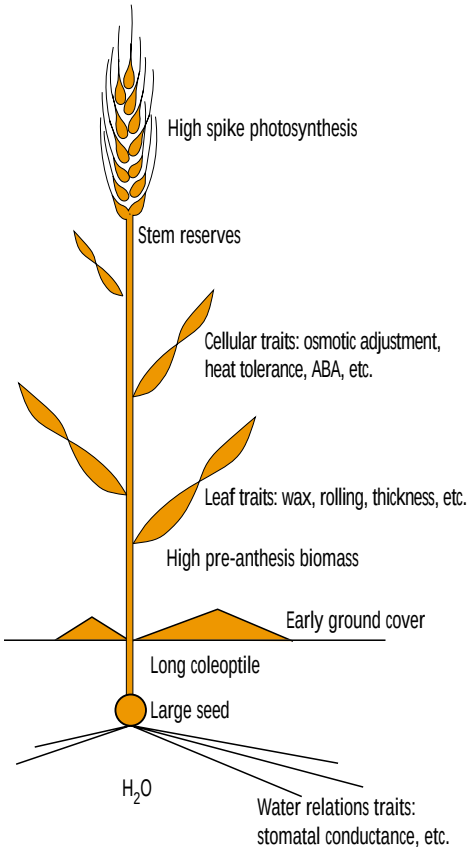

Figure 1. A conceptual model for drought tolerance in wheat. The theoretical ideotype has high expression of the following traits (not all of which would be useful in all drought environments): seed size & coleoptile length (improve early crop establishment), early ground cover & pre-anthesis biomass (reduce evaporation of soil moisture), stem reserves / remobilization & spike photosynthesis (help grainfilling during severe post-anthesis stress), stomatal conductance (indicative of roots which are able to extract soil water at depth), osmotic adjustment (maintains cell functions at low water potential), accumulation of abscisic acid (pre-adapts cells to stress), heat tolerance (heat stress may be caused by low leaf transpiration rates under drought), leaf anatomical traits e.g. waxiness, pubescence, rolling, thickness (reduce risk of photo-inhibition), high tiller survival and stay-green (easily observed integrative traits indicative of good drought tolerance).

Establishing the Genetic Bases of Physiological Traits

Once the value of a physiological trait has been established, it may be useful to determine its genetic basis, such as the number and location of genes involved in its expression. (Genetic studies, including the identification of molecular markers, can be conducted on the same kinds of populations developed to establish genetic gains associated with selection of phenotypic traits.) In theory, such an investment would enable fingerprinting for stress tolerance, or other desirable traits, on fixed lines in any breeding program worldwide. This information would allow strategic crossing programs to improve the likelihood of pyramiding drought tolerance traits, without necessarily having to measure phenotypic expression in the parents or progeny. If a trait is genetically complex and its expression a function of epistatic and other interactions among genes, then genetic markers would need to be identified in several different genetic backgrounds to gain comprehensive information about which *loci* may be involved.

Although identifying adaptive physiological mechanisms and their genetic markers may be time-consuming and costly, once the initial investment is made, the information is permanently available. The information can be used at different stages of the breeding process, depending on the resources available.

In a relatively low investment scenario, information on important physiological traits can be collected on potential parental lines. For example, it might be worth screening an entire crossing block or a subset of commonly used parents to produce a catalogue of useful physiological traits or their genetic markers. The information can be used strategically in designing crosses, thereby increasing the likelihood of transgressive segregation events that bring together desirable traits. In a scenario where more resources are available to screen for physiological traits, the same selection criteria could be applied to segregating generations, in yield trials, or any intermediate stage, depending on where genetic gains from selection are optimal.

The rest of this chapter will describe generalized procedures for evaluating physiological criteria within a breeding program, and how they might be applied.

Standard Procedures for Incorporating Physiological Criteria into a Breeding Strategy

The procedure for incorporating physiological criteria into a breeding program has two phases, each of which consists of a number of experimental steps (see boxes).

Phase 1. Identifying Traits Associated with Performance

Define the target wheat-growing environment

Before designing a research program, it is crucial to define the physical and agricultural characteristics of the target environment (Table 1), for a number of reasons. First, this information enables selection traits to be chosen which are most relevant to the environmental factors limiting performance. For example, when aiming to improve performance under drought, there is no point in selecting for deep rooting capacity if target environments are characterized by soil profiles that lack additional water at depth. Similarly, it would not be beneficial to select for early ground cover with a view to conserving soil moisture if farmers practice residue retention or soil mulching.

Second, an accurate description of the target environment is important to facilitate experimental design by permitting identification of appropriate research sites (based on temperature profile, latitude, soil type, etc.) as well as choice of experimental treatments (i.e. sowing dates, crop management, etc.). By matching the conditions of the experimental environment with those of the target environment as far as possible, results are more likely to be representative of the target environment as a whole. In many instances it is advisable to replicate trials across a number of locations within the target environment. It may not be possible to mimic the target environment precisely at any experimental site, and for strategic reasons these sites may be managed differently. Nonetheless, decisions of this type should be based on as complete a knowledge base as possible of the target environment (see chapter by Hobbs and Sayre).

Identify physiological traits/ selection criteria

When considering the incorporation of physiological criteria into a breeding strategy, previously published work on traits and methodologies can be

Steps for Incorporating Physiological Criteria into a Breeding Strategy

Phase 1: Identifying Traits Associated with Performance

- Define the target wheat-growing environment
- Identify physiological traits/selection criteria
- Choose genotypes appropriate for evaluating trait expression
- Design the experimental environment
- Develop protocols to optimize trait expression
- Measure trait expression and its association with performance

Table 1. Key parameters defining target and experimental environments.

Parameter	Units
Climate	
Maximum daily temperature	ºC (monthly mean)
Minimum daily temperature	ºC (monthly mean)
Sunhours or incident radiation	h/day or joules/m^2/d
Annual rainfall	mm/month
Relative humidity	(% max/min)
Crop environment	
Average yield	t/ha
Preceding crop(s)	e.g. summer crop species
Biotic stresses	Typical % yield loss
Soil	
Soil type	e.g. clay/loam/sand
Soil pH	pH
Physical properties	e.g. compaction zones
Organic matter	%
Rooting depth	approx in cm
Management	
Typical fertilizer rates	NPK etc. kg/ha
Typical irrigation schedule	Frequency/mm applied (if available)
Disease, pest, and weed control	Frequency

consulted. Much of this literature has been reviewed—for example, by Blum (1988) for abiotic stresses, by Evans (1993) for yield potential, and by Loss and Siddique (1994) for wheat under drought. In addition, many traits and references will be included in subsequent chapters related to yield potential, heat stress, drought stress, etc. To be of potential use in breeding, a trait must meet two broad criteria 1) evidence of significant genetic variability for the trait must exist, and 2) selection for the trait must be economically advantageous based on relative costs and benefits (see chapter by Brennan and Morris).

In choosing traits it may be helpful to think in terms of their level of integration at the whole plant level. Traits can be classified into two broad categories: simple traits associated with a particular morpho-physiological attribute, such as leaf waxiness, and integrative traits produced by the net effect of a number of simpler traits—for example, canopy temperature. Being a function of several simpler traits, integrative traits are potentially powerful selection criteria for evaluating breeding progeny; however, the heritability (measured as the ratio of genetic variance σ^2_g to phenotypic variance σ^2_p) of such traits is generally lower than that of simple traits. Clearly the heritability of a target trait will influence the ease with which it can be measured and employed in a breeding program.

Choose genotypes appropriate for evaluating trait expression

The initial choice of germplasm is critical since conclusions will hinge on it being representative of current breeding objectives. Crossing block materials and advanced breeding lines are good sources. Germplasm collections may provide useful sources of genetic diversity, especially if the accessions originate from environments where the yield constraints are similar to those in the target environment. Ideally germplasm should have a number of common characteristics that will facilitate experimental work and interpretation of the results (Table 2). Differences in height, maturity, adaptation, disease susceptibility, etc., are all factors potentially confounding to the trait under study, and variation may increase experimental error. It is unlikely that all material will meet all of the criteria; however, research findings will be greatly enhanced if most of them are met.

If significant genetic diversity for the trait cannot be found in agronomically acceptable backgrounds, it may be necessary to introgress the trait into commonly used parental lines before trait evaluation can begin. Since this requires considerable resources, there has to be good reason to believe that the trait is economically advantageous, based on, for example, studies in related species or preliminary studies on lines in which modest genetic diversity for the trait indicated a strong association with performance.

An important additional point to check before proceeding is the current status of germplasm coming out of ongoing selection programs with respect to the trait of interest. If that material already shows high expression for the trait of interest with little significant genetic

Table 2. Desirable characteristics for germplasm used in physiological breeding experiments.

- Generally adapted to the target environment
- Acceptable range of maturity
- Acceptable agronomic type
- Resistant to prevalent diseases and pests
- Broad genetic background
- Not contrasting in height class†
- Similar response to photoperiod and vernalization†

† Unless characteristics are those under investigation.

diversity, then the methodologies being used are effective in making genetic gains for the trait. Developing physiological selection methodologies is only worthwhile if they can achieve greater efficiency than existing approaches. Otherwise, the only role of physiological measurements may be to identify new and better genetic sources of the trait.

The number of lines studied must be sufficient to ensure a range of genetic diversity for the trait of interest, preferably in several diverse but locally adapted genetic backgrounds. It may be sensible to start with a large number of lines for preliminary observations. This could run into a hundred or so, depending on the complexity of the trait; the precise number will depend on the likelihood of finding genetic diversity for the trait of interest. Once diversity has been established from preliminary observations in a controlled test cycle, numbers can be reduced (e.g. 20-50) to include the best germplasm, encompassing the full range of genetic diversity, for more detailed observations in subsequent cycles.

Design the experimental environment

Trials should be managed optimally because factors such as disease pressure and irregular irrigation can introduce errors into the expression of physiological traits. Besides choosing an appropriate test site that is representative of the target environment, a number of management factors need to be considered (Table 3). Some factors, such as sowing dates that mimic temperature and photoperiod regimes, should be as representative as possible of the target environment to avoid expression of traits not relevant to the experimental objectives. Others, such as land preparation and pest control, should be managed optimally to reduce experimental error. Seed quality is another factor which should be managed optimally to ensure good stand establishment. In addition, seed should come from the same growing environment. Differing seed sources may constitute a serious confounding factor, for example when studying responses to micro-elements known to be stored to varying degrees in the seed, depending on local growing conditions (see chapter by Ascher-Ellis et al.). More details on management of trials is given in the chapter by Hobbs and Sayre.

Develop protocols to optimize trait expression

The efficiency of physiological trait selection will be related to how well a trait is expressed and measured. Therefore, for any trait, experimentation must take place to establish how and when measurements should be made to maximize genetic resolution for its expression. Three groups of factors may interact with the expression of a trait: 1) macro-environment, i.e. temperature, radiation, irrigation status, nutritional status, and soil type; 2) micro-environment, i.e. small daily fluctuations in temperature and radiation, etc., as well as small environmental differences among plots or between plants caused, for example, by soil heterogeneity, weeds, pests, etc.; and 3) physiological factors, i.e., age of plant or its organs, diurnal rhythms of plants, and small amounts of genetic diversity that may exist within so-called fixed lines.

Table 3. Management factors in trait evaluation work.

Factors that should be representative of target environment:
• Crop rotation
• Sowing date (to mimic target photoperiod and temperature regime)
• Planting method
• Seed rate
• Fertilizer regime
• Irrigation regime
Factors that should be managed optimally:
• Seed quality and source
• Land preparation
• Pest and disease control
• Weed control
• Appropriate statistical design

For example, leaf chlorophyll is relatively simple in its expression in that it is not affected by diurnal changes. However, its expression may vary under different nutritional regimes. Chlorophyll is also a function of leaf age, and some standardization in measurement will be necessary to take this into account. One might choose to measure chlorophyll in the flag-leaf at flowering or in the youngest fully expanded leaf at regular intervals after sowing. On the other hand, traits like leaf conductance or canopy temperature depression (CTD) are strongly affected by temperature and relative humidity (i.e. vapor pressure deficit) and have a diurnal function. Studies in CIMMYT (Amani et al., 1996) have shown that CTD is best expressed on warm, sunny, cloudless afternoons, in well-watered plots. The trait is also affected by phenology and, while pre-heading readings are usually higher, readings made during grainfilling are best associated with yield potential.

Avoiding confounding factors and use of experimental design. While it is impossible to completely avoid confounding factors in field experiments, much can be done to minimize their effect through planning, good crop management, and appropriate experimental design. For example, it has already been mentioned that germplasm should be chosen to avoid excessive contrasts in maturity date and height (Table 2). In addition, if test sites are carefully chosen and managed

optimally, plot-to-plot variability can be reduced. For traits affected by weather conditions, investigators may need to be flexible in the timing of data measurement, collecting readings only under relatively favorable conditions.

Fortunately, appropriate statistical procedures can help attenuate some of these problems. The use of replication in combination with appropriate blocking structures is very powerful and will help control the effects of heterogeneity inherent in all field experimental sites. Covariance analysis can help reduce the confounding effect of plant age and phenology, provided the trait and maturity class are not genetically linked. Multiple sampling will reduce errors associated with measurement (human and instrument error). These strategies, in combination with advanced data analysis procedures (i.e., spatial analysis), will reduce error associated with environmental variability.

Genotype by environment interaction. The factors that can affect the expression of a trait (i.e., macro-environment, micro-environment, and physiological factors) may show interaction with genotype, accounting for what is collectively called genotype by environment interaction (G×E). Some traits demonstrate little G×E; that is to say that genotypes ranked based on these traits will largely maintain this rank across different environments, regardless of the absolute expression of the trait. These traits are highly heritable, as environment has little influence on their expression. Therefore, selection for these traits will be effective across locations and years. In general, the greater the genetic complexity of a trait, the greater is the probability of obtaining significant G×E.

Measure trait expression and its association with performance

Once experimental protocols have been refined, data must be collected in at least two or three environments (these may be different representative sites and/or years), and assessed for 1) significant and consistent expression of the trait of interest, and 2) association of the trait with performance among genotypes. For the latter, correlation between the trait and performance should be tested using the mean values for both, averaged across replications within environments. (Correlations should not be interpreted from individual replication data, since these may be highly confounded by environmental differences among replications; ideally genetic correlations should be calculated.)

Any interpretation of data from unrelated fixed lines is speculative, since the association between traits and performance may be confounded by other genetic factors, such as differences in phenology and plant type. For this reason, assuming the above criteria are met, a second phase of experimentation is needed to demonstrate a definitive genetic linkage between the trait and performance in more closely related materials such as homozygous sister lines. Genetic gains resulting from selection and measured as improved performance can then be estimated.

Phase 2. Estimating Heritability of Traits and Response to Selection

Make experimental crosses with parents contrasting in trait

The initial objective of producing experimental germplasm is the generation of homozygous sister lines, i.e., recombinant inbred lines (RILs), which may be F_4 (or later) generation derived so as to be reasonably homozygous and homogenous.

Experimental germplasm can, in theory, be derived from any cross. In practice the parents should be sufficiently well adapted to the target environment to permit field experimentation, and show genetic diversity for the selection trait under investigation; preferably, they should also meet the additional criteria listed in Table 2. Lines on which initial studies were made are a good source, since they will have been well characterized in Phase 1. Two or three crosses would probably be a minimum since traits of interest may interact within different genetic backgrounds.

Develop randomly-derived homozygous sister lines

To demonstrate a genetic linkage between traits in homozygous lines, ideally there should have been no selection pressure applied during their development, thereby ensuring that all lines are randomly derived. One way to achieve this is through using the single seed descent (SSD) method. However, SSD is a resource-intensive way of producing germplasm. The cheapest alternative is to simply advance each generation in bulk without deliberate selection. However, the disadvantage of bulking is that natural selection and

Steps for Incorporating Physiological Criteria into a Breeding Strategy

Phase 2: Estimating Heritability of Traits and Response to Selection

- Make experimental crosses with parents contrasting in trait
- Develop randomly-derived homozygous sister lines
- Test genetic links between quantitative traits and performance
- Estimate heritability and genetic gains from selection
- Apply physiological traits to complement breeding

genotypic competitivity (e.g., tillering ability, height) will influence gene frequencies. A reasonable compromise is to grow bulks at low density starting with F_2 seed, such that individual plants can be recognized. To minimize the effects of interplant competition, the bulk is maintained by harvesting a single spike from each plant, rather than the whole plant.

An alternative to the above is to produce doubled haploid populations. This strategy allows the researcher to produce a large number of totally unselected and genetically fixed lines in a relatively short space of time; however, the technique is quite costly and resource dependent (Snape, 1989).

Relevance of "unselected" germplasm to breeding objectives. There are a number of practical problems associated with working on "unselected" materials. The most obvious relates to relevance to a breeding program, in which unsuitable materials are normally discarded as early as possible. Conclusions based on studies with unselected material may thus not be relevant to a practical breeding program.

A second problem relates to potentially confounding factors inherent in unselected material with respect to measuring physiological traits. For example, expression of many physiological traits may be seriously confounded by phenotypic variability in maturity date or height. Unless the choice of experimental germplasm has avoided all potentially confounding factors (Tables 2 and 4), segregating lines are likely to show considerable variation for such characteristics.

As a compromise between maintaining maximum genetic diversity on the one hand and, on the other, obtaining results that are relevant to breeding objectives and still experimentally valid, negative selection can be practiced on experimental germplasm to remove totally unsuitable material. While the precise nature of such characteristics will vary with the target environment, some common ones are listed (Table 4), bearing in mind that lines with undesirable characteristics should never be removed if they are in any way related or linked to the trait under study. Undesirable traits are the same ones that would normally be discarded during the breeding process.

Test genetic links between quantitative traits and performance

The relevant generation at which to look for a genetic link between a trait and performance will depend on how complex the trait is. Due to the nature of genetic segregation, the number of heterozygous *loci* in a genotype is approximately halved after each generation of self-fertilization. Thus, based on probability, any given genotype will be approximately 50% homozygous at all *loci* in the F_2, 75% in the F_3, 87.5% in the F_4, etc., such that the probability of being genetically stable increases with each subsequent generation. As a consequence, traits controlled by few genes are more likely to become fixed in earlier generations than more complex ones. In addition, the more genes involved in the expression of a trait, the greater the number of different genotypes possible.

Table 4. Undesirable agronomic characteristics that may confound results in experimental breeding.

- Extremes of height
- Unsuitable phenological development
- Strong lodging tendency
- Very poor tillering ability
- Shriveled kernels
- Very low yield potential
- Chlorotic and necrotic symptoms
- Susceptibility to diseases that are widespread and difficult to control

Since in practice it is difficult to establish the number of genes involved for any but the most simple of traits, we must assume that a trait is relatively complex or relatively simple based on segregation ratios for qualitative (or Mendelian) traits or, in the case of quantitatively inherited characters, the degree of G×E observed in the trait's expression. The heritability of a trait in early generations will also indicate its genetic complexity. For simply inherited characters such as the presence or absence of awns, the heritability of expression is 100%; however, the expression of quantitatively inherited characters such as grain yield will be greatly influence by G×E.

Based on the above considerations, one might harvest a number of (relatively) randomly derived lines anywhere from F_4 to F_8 by taking the seed of individual plants in the requisite generation and multiplying it for yield testing. The procedure is then the same as described in Phase 1, when looking for the association between a trait and performance in fixed lines. The genetic link will be validated when tested in an adequate number of environments.

Evaluating physiological traits independently of biotic stress. A major confounding factor in evaluating physiological traits is disease incidence. Generally speaking diseases should be controlled chemically, if not genetically, so as not to confound conclusions. This is reasonable considering the very different nature of the genetic mechanisms involved. For example, when comparing resistance to biotic versus abiotic stresses, it is clear that germplasm with improved abiotic stress tolerance will maintain its superior performance indefinitely under a defined physical environment. Resistance to

pests and diseases, on the other hand, may break down very rapidly. Hence, within the context of experimental germplasm at least, it makes no sense to restrict genetic variability for a physiological trait based on disease resistance criteria that may become obsolete in time.

One exception to the above would be particularly intractable disease problems that are of major economic importance in the target area, where maintenance breeding is costly. In that case, not only would it be necessary for germplasm to be derived from at least one resistant parent, but susceptible progeny would need to be eliminated systematically. Although it may be decided to control diseases in experimental work, disease expression should be encouraged in ongoing breeding work so that susceptible lines can be eliminated before they are evaluated for physiological criteria.

Estimate heritability and genetic gains from selection

If a trait is highly heritable, it is more efficient from a breeding point of view to select lines as early as possible in the breeding process. If shuttle breeding is being applied, the environment most suitable for expressing a specific trait may coincide with a particular generation or generations. In either case, it is necessary to establish the heritability of the trait at that generation, so that genetic gains can be evaluated using the formula

$$R = ih^2\sigma_p \qquad (1)$$

where i = intensity of selection or the proportion of the population included in the selected group and σ_p is the phenotypic standard deviation.

In this case, realized heritability (estimated as σ^2_g/σ^2_p) is perhaps the most relevant parameter to calculate. This is done by measuring trait expression in a population of lines or bulks from a cross and, using an arbitrary selection intensity, dividing the population into high and low groups. These are advanced one generation, and then the trait is measured again. The smaller the difference between the high and low groups in subsequent generations, the lower the heritability. This can be represented by the following equation:

$$h_r = (F_{n+1}\ \text{high} - F_{n+1}\ \text{low}) / (F_n\ \text{high} - F_n\ \text{low}) \qquad (2)$$

Where F_n high is the mean trait value of the plants selected for high expression in the n^{th} generation; F_n low is the mean trait value of plants selected for low expression in the n^{th} generation; F_{n+1} high is the mean value in F_{n+1} of the same high lines from F_n; and F_{n+1} low is the mean value in F_{n+1} of the same low lines in F_n (Falconer, 1990).

Apply physiological traits to complement breeding

When a trait shows a strong association with performance in unrelated fixed lines (Phase 1) as well as in homozygous sister lines (Phase 2), it is probably worth applying selection pressure for the trait in preliminary trials (PTs). However, the nature of the association should also be examined, i.e., the distribution of the values of the trait in relation to performance of lines. For example, when the distribution is linear at first but flattens off at higher yields (Figure 2), selection for the trait may only be effective in eliminating the poorest material.

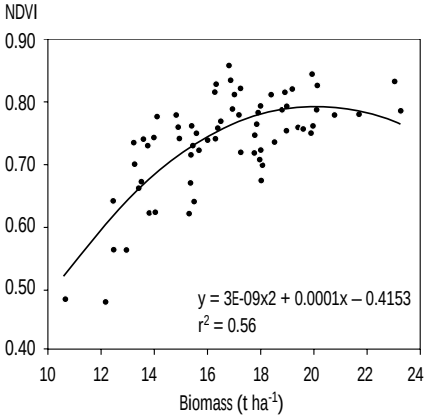

Figure 2. Relationship between spectral reflectance index (NDVI, normalized difference vegetation index) measured during grainfilling and biomass of irrigated spring wheat advanced lines, Obregon, northwestern Mexico, 1996-97.
Source: Reynolds et al. (1999).

When a trait shows high heritability and good association with performance in sister lines, it may lend itself to early generation selection (EGS), instead of or in addition to selection in PTs (Table 5). Selection pressure might be applied in F_3 plants or $F_{3:4}$ plots etc., depending on the sensitivity of trait expression to planting method. Even for a trait that is relatively weakly associated with performance, but highly heritable, early generation selection may be a useful tool for eliminating the poorest material (Table 5). Assuming that the data point convincingly to the use of a physiological trait in breeding, it must be selected for within the overall framework of the breeding program. Disease resistance, agronomic type, and industrial quality are among economically important traits that are essential if a new variety is to succeed. A theoretical scheme for incorporating physiological traits into a conventional breeding program is presented below (Table 6).

Table 5. Criteria for applying physiological traits in a breeding program.

Trait heritability	Association of trait with performance	
	Strong	Weak
Low	Selection in PYTs	No application
High	Early and/or late generation selection	Negative selection in early generations

Table 6. Theoretical scheme for incorporating some physiological selection criteria into a conventional breeding program showing different alternatives for when traits could be measured, depending on resources available.

	Breeding generation when selection should be conducted			
	All generations	F3	F4-F6	PYTs / Advanced lines
Simple traits				
Disease	visual			
Height	visual			
Maturity	visual			
Canopy type		visual	†	
Complex traits				
Yield			visual	yield plots
Industrial quality			grain	grain
Lodging		small plots	small plots	yield plots
Canopy temperature depression			small plots	yield plots
Stomatal conductance		plants	small plots	yield plots
Leaf chlorophyll		plants	small plots	†
Spectral reflectance			small plots	yield plots

† Selection in early/intermediate generations is probably sufficient, as GxE is low.

In summary, the advantages of EGS of physiological traits over later generation selection are 1) resources may be saved by eliminating physiologically inferior material from the program, and 2) the likelihood of discarding favorable genetic diversity is decreased. The potential disadvantages are 1) without close interdisciplinary collaboration, time may be wasted by measuring traits on agronomically unsuitable material, or even promoting it, and 2) in early generations large numbers of plants must be tested, and some currently available physiological tools are either too expensive or cannot be applied quickly enough.

Conclusions

This chapter attempts to provide basic guidelines for evaluating and applying physiological selection traits in breeding work. If adopted, physiological criteria represent a refinement of a breeding program and in no way replace traditional methods of selection. Nonetheless, breeding efforts aimed at meeting ongoing challenges, such as breaking yield barriers and improving performance under abiotic stresses, are more likely to achieve success if physiological understanding is used to complement traditional approaches.

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CHAPTER 1

Directions for Physiological Research in Breeding: Issues from a Breeding Perspective

P.A. Jackson[1]

Plant breeding has traditionally applied a trial-and-error approach in which large numbers of crosses are made from many sources of parental germplasm. Progenies are evaluated for characters of direct economic interest (e.g., grain yield and grain quality) in target environments. Good performing parental germplasm, crosses, and progenies are selected for further use or testing. In many programs "breakthroughs" in improvement are made simply by finding superior sources of parental germplasm among the numerous sources tested. This conceptually simple approach has been highly successful in many crop species and numerous breeding programs.

The approach has often succeeded in the absence of in-depth knowledge about the physiological basis for superior performance. In some crops such knowledge has been obtained by doing retrospective analyses of prior genetic gains. Breeders have not applied this knowledge to a significant extent as a guide to further improvements, but instead have taken any avenue of improvement that happens to arise from direct selection for yield and economic performance.

Given the success of such approaches to date, to what extent can plant breeding programs benefit from physiological research? A recent survey of plant breeders and physiologists (reported in Jackson et al., 1996) indicated a general view among both groups that in the future physiological research would have an increasing role in plant breeding programs. However, the same survey also indicated that many respondents felt outputs from physiological research to date had not developed into practical improvements to the extent they had expected or thought was desirable.

There has been considerable discussion in the literature about the potential role of physiological research in plant breeding. Much of this discussion has been from a physiological perspective—i.e., examining the potential merits of different plant traits for improving yield under different environmental conditions. The aim of this chapter is to take a breeding perspective of ways in which physiological understanding may be applied in traditional breeding approaches. The assumption is that plant breeding programs will continue to rely heavily on large scale evaluation of parental and progeny populations. Physiological understanding could enhance and refine this approach.

Applying Physiological Understanding in Breeding

Illustrated in Figure 1 are the ways in which physiological understanding may be applied in a breeding program; this provides the basis for the subsequent discussion. The figure also shows possible ways to enhance this process, all of them based on physiological research or understanding.

Understanding yield-limiting factors

Understanding the biological factors limiting the performance of genotypes across target environments is essential for improving breeding programs through physiological research. Examples of such factors are moisture stress during different phenological periods (Fischer, 1979; Woodruffe and Tonks, 1983), soil fertility constraints (Carver and Ownby, 1995), production of sink capacity (grain number) and subsequent partitioning of dry matter to grain (Gallagher et al., 1975), canopy light interception during reproductive growth (Lawn and Byth, 1974), and presence of a plant disease. The defining feature of a limiting factor in this context is that improving genetic response to that specific factor would result in higher yields. In all physiological research targeting crop improvement, knowledge of what these limiting factors are for a particular crop species x target environmental domain combination is

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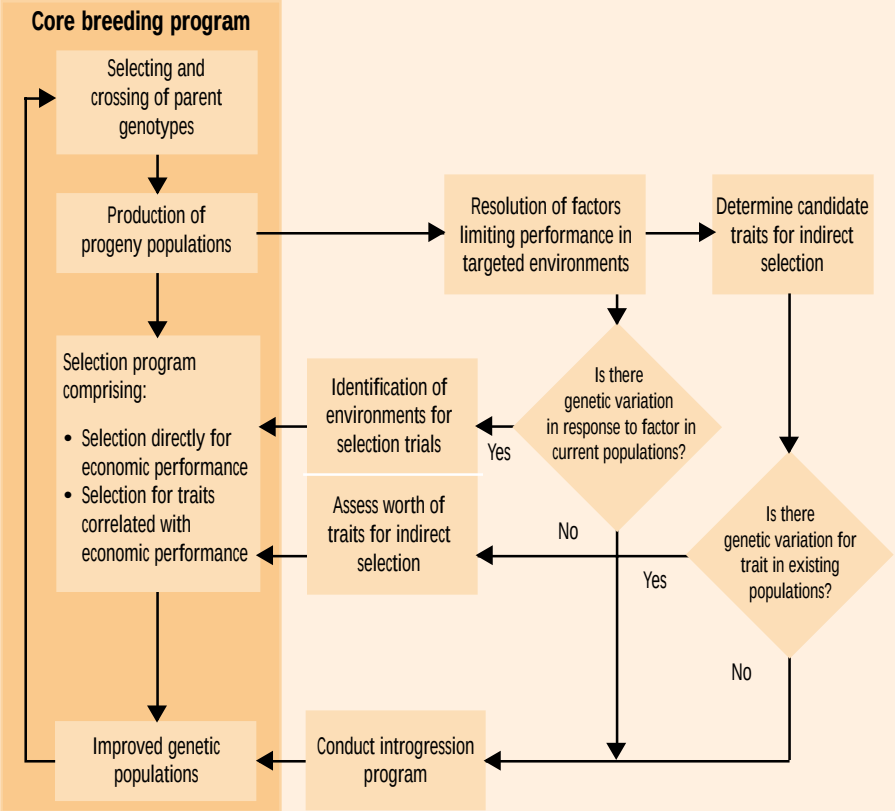

Figure 1. Key steps in a generalized breeding program (on the left) and the potential role of physiological research or understanding.

either taken from previous experience or research, or established at the outset as an objective of the research.

Knowledge of the important limits to better performance may lead to more effective approaches to crop improvement, which will be discussed later. An example of the application of such knowledge is the use of disease screening trials in breeding programs. In such cases, the breeder has previously recognized that the disease in question is an important limiting factor in at least some target environments. Genotypes will be deliberately evaluated in the presence of that disease at some stage of the selection process, and an appropriate weighting given to the results. Furthermore, if necessary, sources of parental germplasm that exhibit favorable responses to the disease may also be sought outside the breeding program. Thus breeding is more focused and effective than when such a limiting factor is not known.

While a breeder may have some knowledge of the major constraints to higher yields in his or her target environments, often it may be superficial or deficient. Physiological research can help to fill the gap. Different approaches have been used, including focused agronomic trials in which factors suspected of limiting performance are manipulated to verify and quantify their effect (Nix, 1980). At a slightly more sophisticated level, simple but very informative conceptual models of yield accumulation processes (e.g., Fischer, 1979) may be developed. Finally, crop growth models to quantitatively assess constraints have been advocated (e.g., Muchow and Carberry, 1993) and may have particular value where highly variable seasonal conditions exist. However, it should be noted that development of crop growth models is expensive and, in many cases, may not be necessary to gain an adequate level of knowledge.

Identification of the environmental or physiological constraints to higher yield does not automatically lead to more effective breeding and selection approaches. However, it is a starting point for developing the approaches discussed below.

Choosing environments for selection trials

Most resources in large plant breeding programs are devoted to conducting selection trials for progeny lines. The aim is to select superior lines that will be more precisely evaluated in subsequent trials, and eventually to release worthwhile lines. Selection trials are usually conducted in environments considered representative of target production areas.

Limiting factors are significant enough to be the object of selection trials if: 1) they are economically important, i.e., they have a widespread or large impact on yield in the target region, and 2) there is genetic variation for response to them, i.e., they allow discriminating among the genetic materials being evaluated. Enhanced knowledge of these factors will help the breeder choose sites for selection trials and decide how to manage them. The breeder evaluates genetic materials so as to be able to observe genotypic response to the limiting factors. Based on these observations, he can select genotypes with the desired combination of favorable responses.

If the occurrence or intensity of the limiting factors varies across the target region, this will lead to genotype x environment (G×E) interactions in multi-environment trials. Large G×E interactions may result in smaller realized gains from selection if the

factors causing the interactions are not adequately sampled at a particular stage of selection. If there is adequate sampling of such factors in selection trials, selection should produce good results.

A stratified random sampling of environments will usually be conducted across the target region if limiting factors are not known or poorly defined. The stratification may be based on, for example, geographic location, soil type, management regimes (e.g., irrigation versus rainfed), or other factors that might possibly affect the relative response of genotypes. This approach will be effective to some extent; however, sampling of some factors may be deficient with such a hit-and-miss approach, and the relative weighting to apply to results from different trials during selection will be unknown.

In this situation physiological research clearly has a role to play by helping to identify the significant constraints to higher yields and determine the level of genetic variation for response to those constraints in particular populations. Using the approaches outlined in the previous section, physiological research on one or a few genotypes may help identify economically important constraints (determined as previously described). However, comparing responses of an adequate sample of genotypes representative of those being evaluated is necessary to determine the extent to which the various factors elicit genetic variation. This may sometimes be done in conjunction with selection trials already established in the breeding program (e.g., Jackson et al., 1994). Improved knowledge of the factors involved in generating variation among materials being tested will nearly always facilitate more focused and efficient selection strategies.

One way this information may be used in breeding programs is via the use of managed environments for selection trials. In Queensland, Australia, wheat (Cooper et al., 1995) and barley (Jackson et al., 1994) lines were found to exhibit large variation in grain number and grain yield under favorable growing conditions (influenced by water and nitrogen availability) at around anthesis. The responsiveness of different lines to good conditions at around anthesis accounted for a large proportion of G×E interactions exhibited by breeding populations in a sample of production environments. It was therefore suggested that wheat and barley lines should be evaluated in one or more high input (water and N) environments in the early stages of selection (Jackson et al., 1994). This would allow effective discrimination among lines for adaptation across the target environments.

Cooper et al. (1995) tested the value of using a small number of high input environments for selection trials in wheat. They showed that mean yields of wheat lines across three such environments had a high genetic correlation with mean yields across a larger number (16) of random production environments. They concluded that high input managed environments could be used at the preliminary yield evaluation stage to facilitate efficient and effective discrimination among unselected lines. This approach should provide more reliable selection data, at a lower cost, than using a larger number of random production environments for selection trials.

Using physiological traits as indirect selection criteria

Identification of yield limiting factors may suggest physiological traits that breeders could use as indirect selection criteria. Although this topic has received considerable attention in physiological research and literature, successful application in breeding programs appears rare. Possible reasons for this lack of success and ways to enhance application in breeding programs are discussed below.

Once identified, physiological traits affecting response to important limiting factors may be used in two ways: 1) as indirect selection criteria in progeny populations in core breeding programs, and 2) to define objectives for introgression activities (Figure 1).

Genetic response for yield using another trait as an indirect selection criterion may be predicted from the following formula (Falconer and Mackay, 1996):

$$CR = i\, h_x \cdot r_g \cdot \sigma_{gy}, \qquad (1)$$

where CR is the correlated response for yield, from selection based on character x; i is the standardized selection differential (related to the proportion of genetic population selected); h_x is the square root of the heritability for character x; r_g is the genetic correlation between character y and character x; and σ_{gy} is the genetic standard deviation for character y.

The genetic correlation is the correlation between genetic effects for yield and the trait used for selection, and may be estimated from analyses of covariance. Many physiological studies reported in the literature have described only phenotypic correlations (i.e., correlations based on means), which may be seriously biased (either upward or downward) by error effects or correlated environmental effects. Genetic correlations are more relevant than phenotypic correlations for examining the value of traits to be used as selection criteria.

Equation 1 suggests that using a trait as an indirect selection criterion will be effective only under rare circumstances. First, the heritability (i.e., the ratio of

variation due to genetic variance compared with error, G×E interaction) of the trait has to be high. Second, the genetic correlation between the trait and yield (or other character of primary interest) also has to be high. A further consideration is the cost of measuring the trait. When this cost is high (assuming fixed budgets), smaller numbers of genotypes may be screened, reducing the intensity of selection and the component *i* in equation 1. Despite the fundamental importance of these parameters in determining the value of traits for use as selection criteria, they are often ignored in discussions of the subject in the literature.

For a trait to be relevant to breeding programs, it is important that its heritability and genetic correlations be estimated in genetic populations representative of those being evaluated. This is because such parameters will differ among different genetic populations (Falconer and Mackay, 1996), and misleading or irrelevant information could be obtained if atypical populations are used. For example, the use of highly diverse genotypes would produce estimates of genetic variance, heritability, and, probably, genetic correlations that are greater than those of more homogeneous genetic populations in advanced breeding trials. Similarly, these genetic parameters in highly selected varieties or lines in advanced breeding trials would probably be very different from those in less selected early generation materials.

It is also important that an adequate sample of the representative genetic population(s) be used in estimating genetic parameters. Breeders usually consider a minimum of 30-40 genotypes to be an adequate sample for estimating variance components and other statistical data. Methods for determining standard errors of estimated genetic statistics have been developed (Falconer and Mackay, 1996) and are helpful in assessing whether sampling and experimental design are adequate for precise estimation.

In practice, there are few cases where indirect selection alone will be more effective than direct selection for yield, particularly if labor-efficient and low-cost methods have been developed for conducting large scale yield trials, as is usually the case. However, sometimes it is possible to identify traits having all the desired features—high heritability, high genetic correlation with characters of economic interest, low cost of measurement. Greater gains can be made by using such traits together with yield as a selection index than by using yield alone. The use of selection indices has been reviewed elsewhere (Baker, 1986).

Well documented examples of the successful application of indirect selection have been reported by Fischer et al. (1989) and Bolanos and Edmeades (1993a; 1993b). The latter selected maize populations using a selection index based on grain yield across environments where water was managed and other traits such as relative leaf elongation under stress, anthesis to silking interval, and leaf death score. Fischer et al. (1989) compared recurrent selection using a selection index to selection for grain yield *per se*. Gains in grain yield under severe moisture stress conditions were greater when using the selection index than based on yield *per se* (Table 1). These authors suggest that traits other than yield were more useful as selection criteria because they had greater heritability than yield under severe moisture stress. This may be because such traits were less influenced by competition effects in small plots and by soil variability, which is sometimes a problem in trials under severe moisture deficits.

By extension, the identification of individual traits for use as selection criteria is selection toward an ideotype in which the ideotype predicts what the characteristics of a genotype ideal for a target environmental domain should be. Perhaps the major limitation of such an approach is that it does not, by itself, account for the level of genetic variation in genetic populations, nor for genetic correlations among traits. In some cases, there may be little genetic variation for traits viewed as desirable and, therefore, selection based on such traits will be ineffective and wasteful.

Table 1. Performance of maize populations after recurrent selection using different criteria.

	Grain yield at soil moisture deficit level			Character†		
Population‡	Mild	Medium	Severe	ASI	RLE	LDS
Original	5240	2780	1520	6.5	75	4.2
Yield-mild	6170	2580	1330	6.3	69	4.0
Yield-severe	5420	3160	1680	4.7	77	3.8
Index	5950	3670	2300	3.7	81	3.1
SE	496	399	297	0.6	4	0.3

† ASI: anthesis to silking interval (days); ALE: relative leaf extension (%); LDS: leaf death score.

‡ Original: the population before any selection; yield-mild: the population selected based on grain yield under limited moisture stress; yield-severe: the population selected based on grain yield under severe moisture stress; index: the population selected based on a selection index.

Source: Adapted from Table 3 in Fischer et al. (1989).

Negative genetic correlations may exist between many physiological traits and other useful characters (e.g., Misken and Rasmusson, 1970); this may result in low or zero genetic correlations with economically important characters. For example, many yield components are negatively correlated (Adams, 1967), so that gains from selecting for one component will inevitably result in a decrease in other important components. Genetic linkage or pleiotropy may cause negative genetic correlations and will either reduce the rate of progress made in introgression (in the case of linkage) or reduce the value of the trait being introgressed (with pleiotropy). Rasmusson (1991) found pleiotropy and trait compensation were major factors limiting progress in an extensive barley breeding program applying an ideotype approach.

Thus selection for traits that could be useful as selection criteria simply on the basis of physiological understanding may result in small or no gains for characters of direct interest, such as yield. Further, this approach may not identify traits having a positive genetic correlation with yield and in which gains via breeding may be easiest to achieve. If yield itself (or other characters of economic interest) is used as the key selection criterion, physiological traits influencing yield, and for which genetic variation exists, will automatically be changed.

In summary, when considering the use of physiological traits as indirect selection criteria, expected results should be compared with predicted gains using yield itself. The search for, and assessment of, traits that may be useful as selection criteria should be based on estimation of their heritability, their measurement cost, and their genetic (not phenotypic) correlation with yield. Using traits in association with yield as a combined selection index should also be considered.

Using physiological understanding to define objectives of introgression programs

The aim of the main steps in a core breeding program (Figure 1) is normally the direct development of new cultivars, and the selection of parents and progeny is usually based on overall estimates of economic performance. However, in a more strategic phase of genetic improvement, the breeder may select parent germplasm on the basis of a specific trait such as disease resistance or tolerance to some abiotic stress. The aim here is to introgress—i.e., introduce—a specific trait into locally adapted breeding stocks (Simmonds, 1993). Successful introgression of germplasm from diverse outside sources has often resulted in quantum gains in improvement. Examples include N.E. Borlaug's use of dwarf wheat germplasm as parental material to reduce lodging, and the use of *Saccharum spontaneum* in sugarcane to improve ratooning and stress tolerance in noble varieties (Berding and Roach, 1987).

Donor germplasm may be identified from any source outside the locally adapted materials being selected. For example, it may include improved materials from other breeding programs which, though not locally adapted, may have desirable characters; materials from germplasm collections of the same species; or materials from related species. Physiological understanding may be useful or even necessary for choosing donor material to be used in introgression. Its role here would be to define specific traits or responses of particular value for which little genetic variation exists in core breeding populations (see chapter by Skovmand et al.).

It should be emphasized that introgression is sometimes a difficult, long-term process and often unsuccessful; it therefore requires careful consideration and planning. The donor germplasm of the trait being introduced will nearly always be inferior from an overall agronomic point of view. For this reason, several generations of backcrossing toward locally adapted material and selection between each generation of crossing are required to combine adequate expression of the trait in a suitable agronomic background.

In the future, genetic engineering approaches will increasingly be used to provide 'new' genes, efficiently incorporate them into adapted germplasm, and control their expression. The aim here is in many ways similar to that of introgression based on more conventional breeding approaches. However, the new approaches are potentially more powerful in that they make a wider range of genes accessible for improving plant traits. This research will require an important and complementary effort by plant physiologists and biochemists to define or suggest specific genetic manipulations needed to overcome constraints to better productivity or improved quality in target environments.

Conclusions

This chapter outlines three ways in which knowledge developed from physiological research may be used to assist plant breeding programs:

- to improve sampling of environments for selection trials;
- to identify traits that may be used as indirect selection criteria, most commonly in an index combined with direct measurements of economic performance;
- to assist in determining the objectives of introgression programs and, increasingly in the future, of genetic engineering approaches.

Physiological research needs to be more closely integrated with active breeding programs for its application to yield practical outcomes. If this occurs, physiological research will be conducted on relevant genetic populations and, more importantly, outputs and suggestions from physiological research can be rapidly evaluated, compared, and redeveloped in the context of existing breeding approaches. A plant breeding perspective of the research and potential opportunities for its application should be determined using simple quantitative genetics models (see equation 1). Both the physiologist and the breeder need to continually address hard questions about how physiological research will improve existing breeding approaches. This should help maintain a focus on producing practical breeding outcomes.

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CHAPTER 2

Searching Genetic Resources for Physiological Traits with Potential for Increasing Yield

B. Skovmand,[1] M.P. Reynolds,[1] and I.H. Delacy[2]

World demand for wheat is growing at approximately 2% per year (Rosegrant et al., 1995), while genetic gains in yield potential of irrigated wheat stand at less than 1% (Sayre et al., 1997). Thus global demand for wheat is growing at about twice the current rate of gain in genetic yield potential, with progress in rainfed environments being even lower. Meeting these demands by continuing to expand agricultural production into remaining natural ecosystems is environmentally unacceptable, and the economic costs of increasing yields through the intensification of agronomic infrastructure are high. Hence a cost-effective and environmentally sound means of meeting global demand for grain is through the genetic improvement of wheat.

Increases in wheat yield potential to date have resulted mostly from manipulation of a few major genes, such as those affecting height reduction (*Rht*), adaptation to photoperiod (*Ppd)* and growth habit (*Vrn).* Future gains in yield potential, especially under stressed conditions, will almost certainly require exploitation of the largely untapped sources of genetic diversity housed in collections of wheat landraces and wild relatives. Though these sources of genetic diversity have been exploited to improve disease resistance in wheat (e.g. Villareal et al., 1995), little use has been made of them for physiological improvement. Nonetheless, many traits reportedly have potential to enhance yield, and high expression of these can be found in germplasm collections. Seed multiplication nurseries can be used for characterizing and evaluating germplasm collections for non-disease and non-destructive traits (DeLacy et al., 2000). Since seed regeneration activities are carried out anyway, they can be an economic way of collecting data. Recent work (Hede et al., 1999; DeLacy et al., 2000) has indicated that agronomic traits (including those with low heritability) measured on small, seed-increase hillplots or miniplots can be used for such purposes.

Genetic Resources

The genetic resources available to plant physiologists and breeders are found in several Triticeae genepools recognized by Von Botmer et al. (1992) and described as concentric circles (Figure 1). The concept of the genepool was first proposed in 1971 by Harlan and deWet (Harlan, 1992), who suggested a circular way of demonstrating the relationships among genepools. The primary genepool consists of a given biological species including, in the case of a crop species, its cultivated, wild, and weedy forms. Gene transfer within species of the primary genepool is not

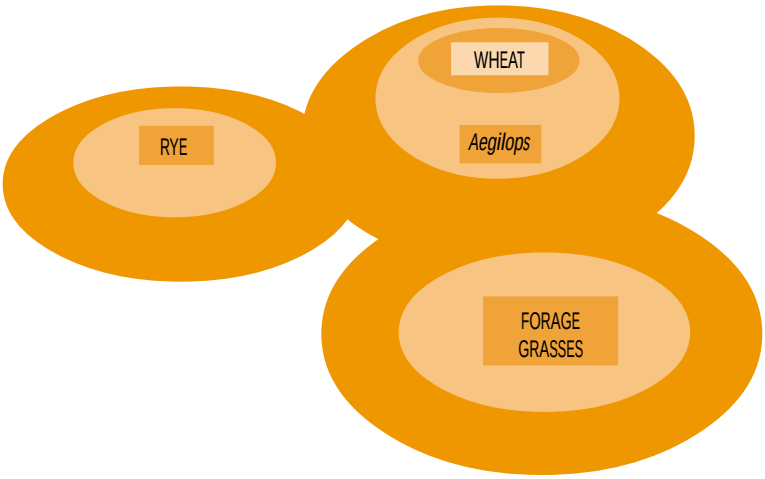

Figure 1. Schematic diagram of the concentric circles illustrating genepools of the Triticeae.
Source: Adapted from Botmer et al. (1992).

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difficult. Table 1 lists the diploid, tetraploid, and hexaploid species in cultivated wheat's primary genepool, listing their common names and indicating the genomes of each. The secondary genepool comprises the cenospecies, i.e., a group of related species with which gene transfer is possible but difficult. Most species in wheat's secondary genepool, along with their synonyms and genomic constitution, are given in Table 2. Wheat's tertiary genepool is composed of related genera of annual and perennial grasses from which gene transfer can only be achieved through the use of special techniques. The genera and species in the tertiary genepool, described by Dewey in 1984, are too numerous to be listed here.

Genetic resources of cultivated plant species were categorized by Frankel (1977) and the Food and Agriculture Organization of the United Nations (FAO) Commission on Plant Genetic Resources (FAO, 1983); however, this categorization is not followed by all centers involved in genetic resource conservation and utilization. The categories are:

- modern cultivars in current use
- obsolete cultivars, often the elite cultivars of the past, many of which are found in the pedigrees of modern cultivars
- landraces
- wild relatives of crop species
- genetic and cytogenetic stocks
- breeding lines

Recently the International Plant Genetic Resources Institute (IPGRI) and FAO jointly developed a list of multi-crop passport descriptors to provide coding schemes consistent across crops. These descriptors should be compatible with future IPGRI crop descriptors and with those used by the FAO World Information and Early Warning System (WIEWS) on Plant Genetic Resources (PGR) (Hazekamp et al., 1997). The descriptors are: 1) unknown; 2) wild; 3) weedy; 4) traditional cultivar/landrace; 5) breeding/research material; 6) advanced cultivar; and 7) other. Because they are more generic (so as to fit multi-crop classification), these descriptors are also less useful in single-crop classification systems.

The classification system used in CIMMYT's wheat collection is based on the categories outlined by Frankel and the FAO Commission on PGR (Skovmand et al., 1992). Recently, however, a list including 21 categories was defined in the GRIP project (Skovmand et al., 2000) to describe the biological status of materials in CIMMYT's collection and other genetic resources. When such specific categories are applied to collections, utilization efficiency is enhanced, making it easier for users to know exactly what they are working with.

Table 1. Summary of taxa in the primary and secondary genepools of cultivated wheat.

I. Sect. **Monococcon** Dumont; Ploidy level: diploid; genome type (female x male parent): AA **('A')**

1. ***Triticum monococcum*** L.
   a. ssp. *monococcum*; cultivated form; einkorn or small spelt wheat
   b. ssp. *aegilopoides* (Link) Thell.; wild form; synonym: *T. boeoticum*
2. ***Triticum urartu*** Tumanian ex Gandilyan; wild form

II. Sect. **Dicoccoidea** Flaksb.; Ploidy level: tetraploid; genome type (female [B] x male [A] parent): BBAA **('BA')**

3. ***Triticum turgidum*** L.; cultivated and wild forms
   a. ssp. *turgidu*; rivet, cone, or pollard wheat
   b. ssp. *carthlicum* (Nevski) A. Loeve & D. Loeve; Persian wheat, Persian black wheat
   c. ssp. *dicoccon* (Schrank) Thell.; emmer wheat
   d. ssp. *durum* (Desf.) Husn.; macaroni wheat, hard wheat, or durum wheat
   e. ssp. *paleocolchicum* (Menabde) A. Loeve & D. Loeve
   f. ssp. *polonicum* (L.) Thell.; Polish wheat
   g. ssp. *turanicum* (Jakubz.) A. Loeve & D. Loeve; Khorassan wheat
   h. ssp. *dicoccoides* (Koern. ex Asch. & Graebn.) Thell.; wild emmer wheat
4. ***Triticum timopheevii*** (Zhuk.) Zhuk.
   a. ssp. *timopheevii*; cultivated and wild forms
   b. ssp. *armeniacum* (Jakubz.) van Slageren; wild form

III. Sect. **Triticum;** Ploidy level: hexaploid; genome type (female [BA] x male [D] parent): BBAADD **('BAD')**

5. ***Triticum aestivum*** L.
   a. ssp. *aestivum*; bread wheat
   b. ssp. *compactum* (Host) Mackey; club, dwarf, cluster, or hedgehog wheat
   c. ssp. *macha* (Dekapr. & Menabde) Mackey
   d. ssp. *spelta* (l.) Thell.; large spelt, spelt or dinkel wheat
   e. ssp. *sphaerococcum* (Percival) Mackey; Indian dwarf wheat or shot wheat
6. ***Triticum zhukovskyi*** Menabde & Ericz.; Ploidy level: hexaploid; genome type (female [GA] x male [A] parent: GGAAAA **('GAA')**

Source: van Slageren (1994).

The concentric circles proposed by Harlan and deWet (Harlan 1992) to describe the different genepools have been very useful, and the concept has provided a rational basis for comparing taxonomies. However, it gives the impression that separations among the pools are clear-cut, with distinct divisions between one pool and another, though Harlan (1992) did state that the lines of demarcation may be fuzzy. Furthermore, the circles do not reflect the relative difficulty of utilizing the genepools, nor the cost of utilizing genetic resources within a genepool or within a species.

A schematic diagram of the effort needed to transfer traits from genetic resources to farmers' fields is given in Figure 2. Within the primary genepool, the utilization cost increases as the genetic distance increases. Within a species there are also levels of genetic resources (from current high yielding cultivars to landraces) that may determine the cost of using those resources.

As one moves away from the primary genepool, the effort required to utilize genetic resources in the secondary and tertiary genepools increases geometrically. It is difficult to release a commercially acceptable cultivar that does not have previously released cultivars in its pedigree (Rajaram, pers. comm.) because crosses with species in the secondary and tertiary genepools tend to disunite favorable gene complexes, which affects performance. Technology extends the genepools and reduces costs, as, for example, embryo rescue has done in the recent past and genetic engineering promises to do in the future. Also, species in the secondary genepool, such as *Aegilops tauschii*, can now be used as readily as species in the primary genepool through the production of hexaploid synthetic wheats using embryo rescue followed by chromosome doubling using colchicine (Mujeeb-Kazi, 1995).

Table 2. Species of *Aegilops*, their genomic formula and synonyms (when available) when *Aegilops* and *Amblyopyrum* are placed within *Triticum emend.*

	Species of *Aegilops*	Genome	Species of *Triticum*
1	*Aegilops bicornis* (Forssk.) Jaub. & Spach	S^b	*Triticum bicorne* Forssk.
2	*Aegilops biunciales* Vis.	UM	*Triticum macrochaetum* (Shuttlew. & A. Huet ex Duval-Jouve) K. Richt.
3	*Aegilops caudata* L.	C	*Triticum dichasians* Bowden
4	*Aegilops columnaris* Zhuk.	UM	*Triticum* – none
5	*Aegilops comosa* Sm. in Sibth. & Sm.	M	*Triticum comosum* (Sm. in Sibth. & Sm.) K. Richt.
6	*Aegilops crassa* Boiss.	DM	
		DDM	*Triticum crassum* (Boiss.) Aitch. & Hemsl.
7	*Aegilops cylindrica* Host	**DC**	*Triticum cylindricum* (Host) Ces., Pass. & Gibelli
8	*Aegilops geniculata* Roth (*Ae. ovata*)	MU	*Triticum* – none
9	*Aegilops juvenalis* (Thell.) Eig	DMU	*Triticum juvenale* Thell.
10	*Aegilops kotschyi* Boiss.	SU	*Triticum kotschyi* (Boiss.) Bowden
11	*Aegilops longissima* Schweinf. & Muschl.	S^l	*Triticum longissimum* (Schweinf. & Muschl.) Bowden
12	*Aegilops neglecta* Req. ex Bertol.	UM	
		UMN	*Triticum neclectum* (Req. ex Bertol.) Greuter *Triticum recta* (Zhuk.) Chennav.
13	*Aegilops peregrina* (Hack. in J. Fraser) Maire & Weiller	SU	*Triticum peregrinum* Hack. in J. Fraser
14	*Aegilops searsii* Feldman & Kislev ex Hammer	S^s	*Triticum* – none
15	*Aegilops sharonensis* Eig	S^l	*Triticum longissimum* (Schweinf. & Muschl.) Bowden spp. Sharonense (Eig) Chennav.
16	*Aegilops speltoides* Tausch	S	*Triticum speltoides* (Tausch) Gren. ex K.Richt.
17	*Aegilops tauschii* Coss.	D	*Triticum aegilops* P.Beauv. ex Roem. Ex Schult.
18	*Aegilops triuncialis* L.	UC	
		CU	*Triticum triunciale* (L.) Rasp. (var. *triunciale*) (*T. triunciale* spp. Persicum)
19	*Aegilops umbellulata* Zhuk.	U	*Triticum umbellulatum* (Zhuk.) Bowden
20	*Aegilops uniaristata* Vis.	N	*Triticum uniaristatum* (Vis.) K.Richt.
21	*Aegilops vavilovii* (Zhuk.) Chennav.	DMS	*Triticum syriacum* Bowden
22	*Aegilops ventricosa* Tausch	DN	*Triticum ventricosum* (Tausch) Ces. Pass. & Gibelli
	Species of *Amblyopyrum*		
1	*Amblyopyrum muticum* (Boiss.) Eig	**T**	*Triticum tripsacoides* (Jaub. & Spach) Bowden

Source: van Slageren (1994).

Global wheat genetic resources and their availability

About 640,000 accessions of *Triticum* spp., *Aegilops* spp., and X *Triticosecale* (a man-made crop, a cross between wheat and rye) can be found in collections around the world (Table 3). The degree of duplication in these collections is difficult to ascertain without some type of global wheat genetic resources database. Given this situation, the level of priority that should be placed on collecting more materials is uncertain, except where

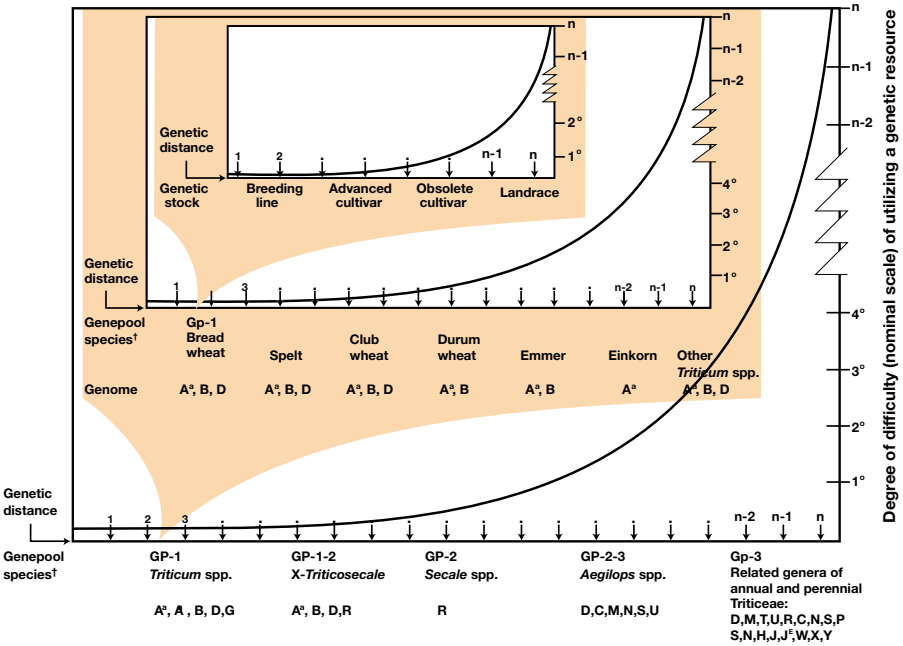

Figure 2. Schematic diagram of the effort required to transfer adaptive traits from genepools of wheat to farmers' fields.

† Not in strict phylogenetic order.

there is a real threat of genetic erosion to native species. Accessions in collections around the world may or may not be properly preserved, and some may not even be catalogued. It may thus be more cost effective to place such collections in secure storage than to collect more materials in the field. Most major wheat-producing countries have *ex-situ* collections, and genetic resources can be obtained from these collections by writing to the curator.

Table 3. Number of accessions available in collections around the world.

Type of wheat	Number of accessions
Hexaploid	266,589
Tetraploid	78,726
Diploid	11,314
Unspecified *Triticum*	252,530
Aegilops spp.	17,748
Triticale	23,659
Total	640,603

Source: Information collated from IBPGR (1990).

Conservation can be either *in situ* or *ex situ*, but most wheat genetic resources have been conserved *ex situ*. Only in the last few years has *in-situ* conservation been seriously considered; the World Bank recently supported such an undertaking in Turkey. The exception is the natural habitats in Eastern Galilee, Israel, where the Ammiad wild wheat study was undertaken in the 1980s. Shands (1991) and Hawkes (1991) summarized a symposium where the findings in this *in-situ* field laboratory were discussed. *Ex-situ* conservation of Triticeae genetic resources is easy and cost effective (Pardey et al., 1998), since they are adapted to long-term storage conditions.

The key to accessing wheat genetic resources is the development of a database, or interconnected database systems, with the capacity to manage and integrate all wheat information, including passport, characterization, and evaluation data. In the early 1990s, the CIMMYT Wheat Program established just such a strategy for integrating and managing all data pertaining to germplasm regardless of where they were generated (Skovmand et al., 1998). The goal was to facilitate the unambiguous identification of wheat genetic resources and remove barriers to handling and accessing information. The resulting database, the International Wheat Information System (IWIS), seamlessly joins the conservation, utilization, and exchange of genetic materials. The system is fast, user-friendly, and available on an annually updated CD-ROM (Skovmand et al., 2000a).

IWIS has two major components: the Wheat Pedigree Management System, which assigns and maintains unique wheat identifiers and genealogies, and the Wheat Data Management System, which manages performance information and data on known genes. Another information tool, the Genetic Resource Information Package (GRIP), was developed using IWIS for data warehousing. One of the functions of GRIP attempts to collate passport information across genebanks to identify duplications and unique genetic resources (Table 4) (Skovmand et al., 2000b).

The Genetic Resources Information Network (GRIN) and the System-Wide Information Network for Genetic Resources (SINGER) are other publicly available databases on wheat and Triticeae genetic resources. GRIN contains information about the USDA Small Grains Collection stored in Aberdeen, Idaho, and can be reached on the Internet (http://www.ars-grin.gov/) . SINGER (http://singer.cgiar.org/), developed and made available under the leadership of System Wide Genetic Resources

Program (SGRP), gives access to all FAO/CGIAR Center accessions, including wheat and other cereals.

In the 1980s there was an increasing trend towards greater application of intellectual property protection (IPP), which contrasted with the pervading attitude during the 1960s and 1970s, where IPP within the context of international plant improvement was seen as an obstacle to progress. Since then, the view that strong IPP can help maintain technological leadership has gained respectability, especially in the United States (Siebeck, 1994). Several international initiatives have resulted, such as the strengthening of the UPOV Convention in 1991, which narrowed the breeder's privilege to use protected cultivars as breeding parents. However, according to Siebeck (1994), the most significant initiative was instigated as part of the Multilateral Trade Negotiating Round in the General Agreement on Tariffs and Trade that ended in 1993. At the insistence of industrialized nations, strengthening of IPP was included as a key negotiating point. Efforts in UPOV and GATT to widen IPP on inventions and breeding technologies were paralleled by efforts to regulate international access to genetic resources.

The "International Undertaking on Plant Genetic Resources," established by FAO in 1983, was an attempt to stop genetic erosion and protect plant genetic resources. The Undertaking initially subscribed to the rule of free germplasm exchange and recognized plant genetic resources as the "heritage of mankind." However, disagreements later arose over the issue of genetic resources ownership. The idea of compensation was introduced in 1989 and modified in 1991, when FAO adopted the common heritage principle but subordinated it to "the sovereignty of states."

Unlike the FAO Undertaking, which was voluntary, the Convention on Biological Diversity (CBD) of 1992 is an internationally ratified treaty among nations. The CBD officially recognizes sovereign control by individual nations over biological diversity and resources within their territories. The convention excludes material collected before 29 December 1993, when the CBD took effect, but any germplasm collected after that date in a country that has signed the CBD comes under the provisions of the Convention. A result of the discussions on the ownership of genetic resources was the signing of an agreement between the CGIAR and FAO that places the germplasm collections held in trust by the CGIAR system under the auspices of FAO.

Consequently, plant genetic resources may not be freely available to everyone in the future but likely to be made available under some type of intellectual property rights (IPR) agreement. For example, the accessions in the CIMMYT collection that come under the FAO/CIMMYT in-trust agreement are shared under a Materials Transfer Agreement that states the accessions can be utilized but not protected by IPR. However, products derived from research and breeding with such materials can be protected, since they are deemed to be different and belong to the scientist or breeder who developed them.

The Search for New Genetic Variation

A classic method of identifying new genetic variation is the recognition of potentially useful traits by experienced scientists and research staff in the course of routine maintenance activities, special studies, or as an offshoot of prebreeding and breeding exercises. This should not be underestimated, given that much of the useful novel variation deployed in cultivated crops today was recognized in this way.

Augmented use of seed multiplication nurseries

Seed multiplication nurseries can be used to characterize and evaluate germplasm collections for non-disease and non-destructive traits. Since routine seed regeneration activities have to be carried out anyway, they can be an inexpensive means of collecting data. Recent work has indicated that traditional agronomic traits (including those with low heritability) measured on small, seed-increase hillplots can be used for such purposes (Hede et al., 1999; DeLacy et al., 2000). Curators of

Table 4. Biological status classification used in GRIP II.

GRIP code	Status
BL	Breeding Line
CV	Cultivar
LV	Landrace
X	No data
AL	Addition Line
BL	Apomixis Line
BL	Breeding Population
BL	Cross
BL	Genetic Population
GS	Genetic Stock
ML	Multi Line
MTL	Mutation Line
NIL	Near Isogenic Line
RCMS	CMS Restorer
RF	Fertility Restorer, non specific
SL	Substitution Line
TL	Translocation Line
CMS	Cytoplasmic Male Sterile
GMS	Genetic Male Sterile
RG	Genetic Restorer
MS	Male Sterile, non specific

Source: Skovmand et al. (2000b).

germplasm banks have traditionally avoided these traits, which are useful for plant improvement programs. Descriptions of germplasm based on "useful" attributes are immediately advantageous to practical plant improvement programs because they indicate where useful variation may be found in the collection.

A description based on useful attributes also allows more directed search strategies than those derived from traditional characterization attributes or random DNA markers with high heritabilities. Provided that random markers adequately cover the genome, they give information on the amount of variation at and between sites, thus indicating whether adequate collection has been done. However, until adequate linkages to known functions have been established, they, like traditional characterization attributes, provide little information on the type of variation present.

When low(er) heritability data are used, means and variances change in different seasons, years, and places. This has limited their use for germplasm description, but many, if not most, attributes useful for plant improvement programs are of this type. Much of the difficulty encountered in integrating such information from sets of data acquired at different times can be avoided by appropriate data analysis. After standardization by the range or standard deviation within sets, means and variances for each attribute are the same.

As an example, DeLacy et al. (2000) reported on an analysis of a seed multiplication nursery made up of 465 accessions of bread wheat landraces collected in 1992 from 24 sites in three states of Mexico. They were examined in unreplicated hillplots in a screenhouse for 15 morphological, agronomic, and grain quality attributes as part of the routine regeneration process conducted by the CIMMYT Wheat Genetic Resources Program. A pattern analysis (combined use of classification and ordination methods) of the data provided a good description of the accessions and collection sites (Figure 3). Since economically useful attributes were used, the analysis provided relevant information for both germplasm curators and potential users, who now have a description of the accessions from which to choose relevant breeding material.

The data were analyzed using range standardized squared Euclidean distance (rsSED) as the dissimilarity measure. These SEDs are calculated among attributes that are range standardized (Williams, 1976) and employed to ensure each attribute contributes equally to the analysis. Ordination was performed by singular value decomposition (Eckart and Young, 1936) of the Gower complement similarity measure to the rsSED (Gower, 1967; DeLacy et al., 1996). The relationships between accessions and attributes from the ordination were displayed on a biplot (Gabriel, 1971).

Both the accession and attribute plotting points can be interpreted as vectors on the biplot, but since the accessions were investigated in terms of the attributes, attributes were represented as vectors and accessions as points. As the data were centered (i.e., the attribute mean was subtracted from all values for that attribute so the grand mean becomes zero), the origin on the biplot represents average values for all attributes. The percentages on each axis represent the proportion of total variation, measured by the total sum of squares (TotSS), accounted for by each vector. In this case the aim is to represent the original 15 dimensional space defined by the 15 attributes in a low dimensional space. Since not all the variation is modeled, some distortion of the relationships among attributes and accessions will occur when depicted on the biplot.

The attribute vectors are drawn in a positive direction, i.e., in the direction of increasing value for that attribute. The length of each vector is proportional to how well each attribute was modeled, since each vector should be the same

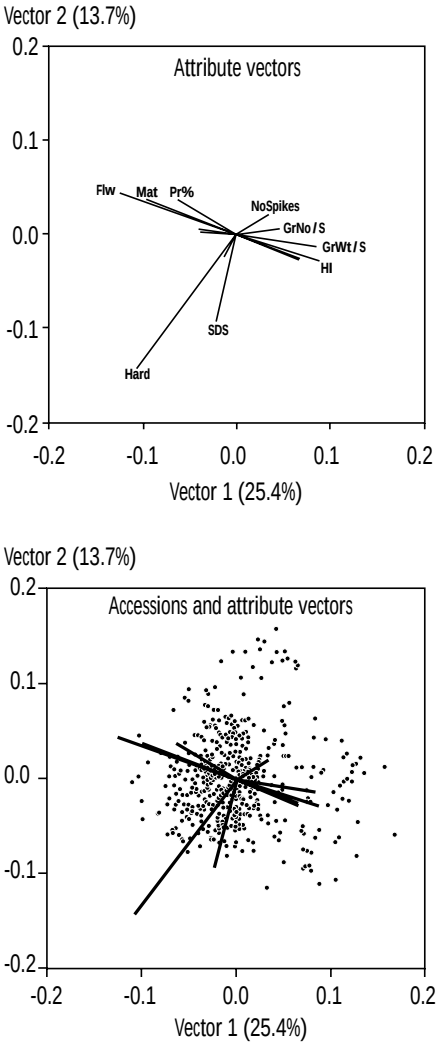

Figure 3. Attribute vectors and accession plotting points for the biplot for vectors 1 and 2 from the ordination based on 15 morphological, agronomic, and grain quality attributes of 465 individual spike accessions of wheat landraces collected from 24 sites covering four states in Mexico.

length if they were all equally well modeled. The angles of the vectors to each other in the biplot represent the phenotypic correlation between the attributes over all values of the accessions for each attribute. An angle of zero indicates a correlation of +1, an angle of 90º a correlation of 0, and an angle of 180º represents a correlation of -1. The length on the attribute vector to the point where a perpendicular dropped from the genotype plotting point to the vector is proportional to the modeled (predicted) value of that genotype for that attribute.

Grain hardness was well modeled, eight attributes (Flw, Mat, Pr%, SDS, GrWt/S, HI, GrSize, GrY; see Table 5) were modeled reasonably well, and six (SpikeS, GlumeS, FlagS, Ht, NoS, GrNo/S) were poorly modeled by vectors 1 and 2 (Figure 3). As the two dimensional representation of the 15 attribute space accounts for 39% of the original variation, some distortion will occur. Grain yield, its components (No/S, GrNo/S, GrWt/S, GrSize), and harvest index are positively correlated and highly negatively correlated with maturity (Flw, Mat), protein content of the grain, size of the flag leaf, and plant height as the two groups of attributes have vectors at close to 180^0 to each other. In contrast, the two quality attributes (Hard, SDS) and glume and spike size are positively correlated with each other (parallel vectors) but independent of the other two groupings of attributes (vectors at 90º).

Accessions plotted to the right in Figure 3 have high yield and high values for yield components, but are early maturing and have low protein percentage in the grain. Clearly those to the left are late, have higher protein percent, but are low yielding. Vector 2 separates those with hard grain and high SDS, at the bottom, from soft wheats with low SDS (Figure 3) at the top. Vector 3 separates those with high grain size, grain weight per spike, SDS, and large glume and flag leaf size (bottom) from those with low values for these attributes and which are hard and have a high number of spikes (top of graph). Hence, accessions occupying different positions in the biplots have different character combinations that can be read directly from the plots, always remembering that some distortion must occur, as not all variation is represented in the low dimensional space. Accessions in different positions on the biplot have different attribute combinations in the "description space."

Augmented use of disease or "special attribute" nurseries

Useful, low-heritability traits can be measured in trials that are routinely planted by the genetic resources program for other purposes, as well as in seed multiplication nurseries with the same value-added characteristic. Thus even nurseries planted for evaluating accessions for disease resistance can be utilized for measuring other traits, the exception being trials where disease is so severe that plants are heavily damaged or dead.

Molecular approaches for identifying useful genetic diversity

Genetic diversity from wheat's wild relatives has already been exploited through wide-crossing to improve disease resistance (e.g., Villareal et al., 1995). Useful characteristics also exist in primitive or landrace varieties, of which there are over 66,000 in the CIMMYT genebank. It would be extremely time-consuming to evaluate all these landraces, wide crosses, and wild relatives for all useful yield traits, such as those described above, in field trials (Figure 2). Potential exists for identifying the loci encoding quantitatively-inherited yield traits using QTL analysis in mapping of delayed backcross generations (Tanksley and Nelson, 1996). When molecular markers linked to traits of interest are identified, they could be used to screen uncharacterized germplasm collections for the same marker and linked alleles. These lines could then be evaluated in controlled experiments to observe how well the molecular marker is linked to phenotypic expression of useful traits. Where there are reasonable associations,

Table 5. Fifteen morphological, agronomic, and grain quality attributes measured on 465 individual spike accessions of wheat landraces collected from four states in Mexico.

Name of attribute	Abbreviation	Description
Flag leaf size	FlagS	Flag leaf length (cm)
Spike size	SpikeS	Spike length (cm)
Glume size	GlumeS	Glume length (cm)
Days to maturity	Mat	Days from sowing
Days to anthesis	Flw	Days from sowing
Height of plant	Ht	Height to tip of glume (cm)
Number of spikelets	No/S	Number of spikelets per spike
Grain number per spike	GrNo/S	Number of grains per spike
Grain weight per spike	GrWt/S	Grams
Grain size	GrSize	1000 kernel weight (g)
Grain weight per plot	GrY	Grams
Harvest index	HI	Grain weight as a proportion of total biomass
Grain hardness	Hard	Percent hardness (NIR analysis, calibrated with particle size index using 0.5 mm sieve in grinder)
Grain protein percentage	Pr%	Percent protein (NIR analysis, calibrated against Kjeldahl N x 5.7)
SDS sedimentation	SDS	Sodium dodecyl sulfate (SDS) sedimentation volume (ml/lg flour)

markers could be used to screen untapped genetic stocks, enabling new sources of genes with potentially useful alleles to be exploited in breeding.

How to use the identified traits

Genetic resources with desirable traits usually need to be tested and improved to be of use in wheat improvement (Figure 2). Most often these resources have many undesirable characteristics, such as extreme disease susceptibility, low yield, and highly specific environmental adaptation, in addition to the needed trait.

These resources therefore need to undergo prebreeding before they can be used in improvement work. Figure 4 demonstrates two prebreeding schemes, each with a different purpose: the open-parent, cyclical crossing program and a backcrossing program aimed at producing isogenic lines. These two program have different purposes and different end results; moreover, the first is progressive, while the second is unprogressive in terms of yield potential.

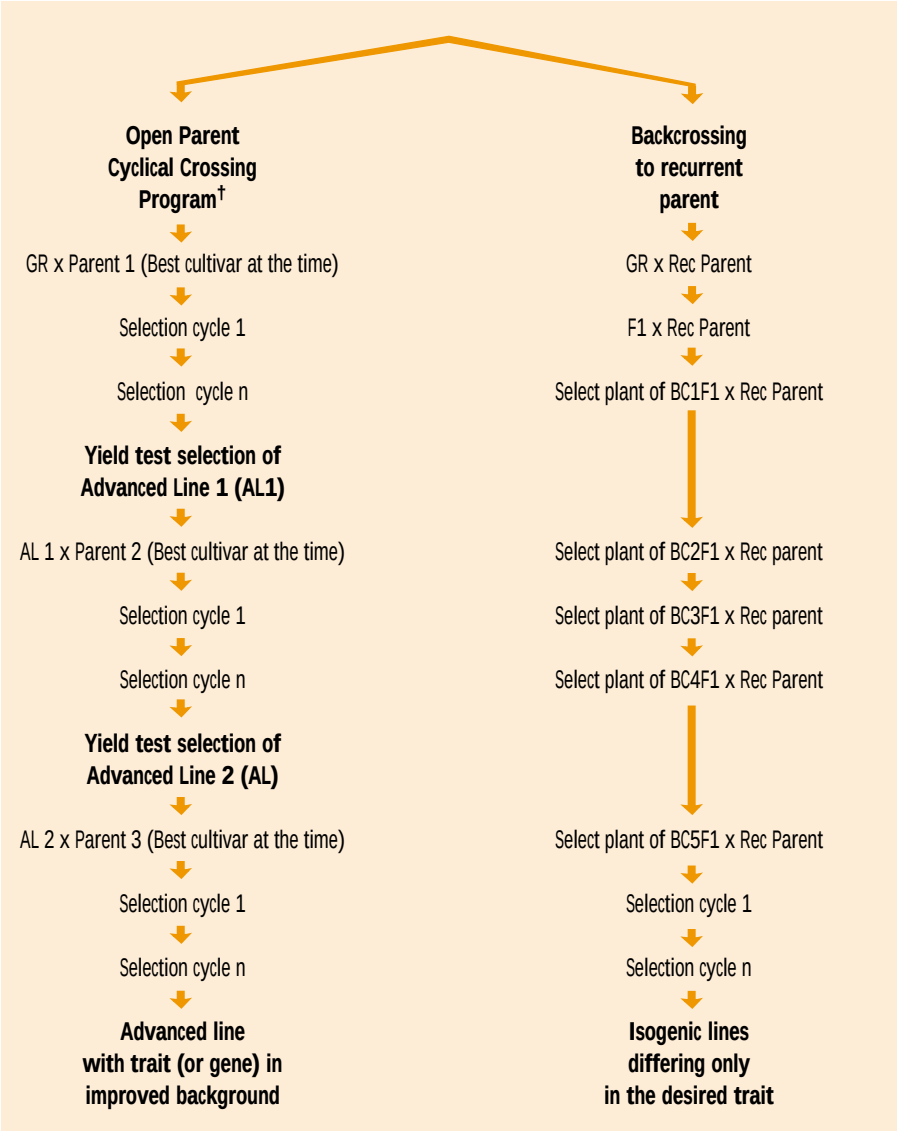

Figure 4. Utilization of genetic resources: prebreeding schemes.
† Source: Rasmusson (2001).

The open-parent, cyclical crossing program described by Rasmusson (2001) is utilized when the introgressing a trait known to be of value. Rasmusson was striving to introgress characters from two-row barley into six-row barley and found that the initial cross yielded germplasm with no putative candidates for cultivar release, with the best lines yielding about 20% less than the improved parent. The second cycle of the program, where the improved parent was the best current cultivar, produced progenies that yielded about 98% of the best parent's yield. The third cycle, again using the best current cultivar as a parent, yielded 112-119% of the checks. Using this scheme, germplasm with the desired trait is produced that could be competitive in a cultivar-release program.

A backcrossing program to generate isogenic lines is applied when the identified trait has as yet no proven value. The recurrent parent is crossed repeatedly to the genetic resource with the desired trait. In each backcross generation, selection is done for the tails of the populations, i.e., lines with the trait and lines without the trait. Lines that differ genetically only for the trait in question are the end result of this program. Additional trials can be conducted to assess the value of the trait, but bearing in mind that the germplasm produced will not outperform the recurrent parent.

Future utilization of genetic resources

As evidenced by the above, genetic resources have played a significant role in wheat improvement and will continue to do so, by providing breeders with the genetic variation they require to effect

future improvements. Variation will be needed 1) to further increase wheat's yield potential; 2) to provide new sources of disease and pest resistance and maintain the yield levels achieved so far; 3) to develop germplasm adapted to more marginal environments; and 4) to improve quality. To date the main contribution of genetic resources has been as new sources of disease and pest resistance, thanks to which achieved yield levels have been maintained.

There are few examples of genetic resources contributing to the three other objectives. One example is the dwarfing genes, especially *Rht1* and *Rht2*, that became available through the Japanese wheat Norin 10, which in turn inherited them from Shiro Daruma, a Japanese landrace (Kihara, 1983). Persistent efforts were required to transfer these dwarfing genes into a genotype of value (Borlaug, 1988; Krull and Borlaug, 1970), which illustrates the difficulty of using genes from unadapted materials. It also shows that desirable characteristics other than apparent ones may result from such germplasm, as evidenced by the fact that while incorporating strong straw to avoid lodging, Krull and Borlaug (1970) obtained better fertility and tillering capacity. It is now obvious that dwarfing genes *Rht1* and *Rht2* have a direct effect on yield over and above the benefits derived from diminished lodging (Gale and Youssefian, 1986).

A survey conducted by Cox (1991) revealed that most introductions to the United States were used to improve disease and pest resistance (Table 6). The only yield-related traits listed in the table are reduced height, stiff straw, large seed, and yield per se. No instances of improving yield in marginal environments are listed and only two where quality was improved: higher protein and gluten strength.

In another report (Fischer, 1996), traits involved in improving yield that were introduced from genetic resources are described. Erect leaf habit was introduced into CIMMYT germplasm from *Triticum sphaerococcum*, and a number of lines were developed through prebreeding. This germplasm was used in both the bread and durum wheat programs and let to the release of one bread wheat (Bacanora 88) and two durum wheat cultivars (Altar 84 and Aconchi 89).

Physiological traits are often identified as having contributed to improving yield potential, but usually in retrospective, after the germplasm has been developed. We need to be more proactive and identify potentially useful traits and then introduce the trait into the improvement program.

Traits to raise yield potential of irrigated wheat

To boost yield in irrigated situations, it is widely believed that genetic improvement must come about through simultaneously increasing both photosynthetic assimilation capacity and partitioning of assimilates to promote high grain number and growth rate (Richards, 1996). However, another way to increase grain number could be to increase the intrinsic fertility of the spike.

Multi-ovary florets is a trait being studied in CIMMYT's wheat germplasm bank. Spikes with this trait may have up to six kernels per flower (Chen et al., 1998), but individual kernel weights tend to be low. The trait is currently being introgressed into high yielding lines with good agronomic traits. Data for the F1 shows that the trait is expressed better in some backgrounds than others. However, average kernel weight of the F1s was in all cases higher than that of the multi-ovary donor and, in many cases, of both parents. Total grain weight per spike was generally higher than that of the parents (Table 7).

High leaf chlorophyll content has been identified in landrace collections: the best genotypes showed substantially greater leaf chlorophyll concentration than the check Seri-M82. While the trait does not guarantee higher leaf photosynthetic rate in all backgrounds, it

Table 6. Contributions to germplasm improvement of introduced genetic resources.

Yield potential	Cases	Resistance	Cases	Marginal environments	Cases	Quality	Cases
Reduced height	15	Strawbreaker	2		None	High protein	2
Yield	6	Powdery mildew	9			Gluten strength	1
Large seed	1	Stripe rust	4				
Stiff straw	1	Leaf rust	12				
		Stem rust	12				
		Septoria leaf blotch	3				
		Bunt	3				
		Soilborne mosaic virus	1				
		Cereal leaf beetle	1				
		Hessian fly	3				
		Snow mold	1				
		Greenbug	1				
		Wheat curl mite	1				

Source: Adapted from Cox (1991).

Table 7. Expression of the multi-ovary trait and yield components in F1 lines, wheat screenhouse, Mexico, 1999.

Line / cross	Yield component: Kernel no. / spike	Kernels / floret	Kernel wt (mg)	Grain wt / spike (g)
Multi-ovary line	124.0	2.17	37.5	4.65
Pastor	**69.3**	**1.00**	**51.5**	**3.57**
Multi-ovary line / Pastor	125.9	1.81	42.1	5.30
Pastor / Multi-ovary line	108.5	1.65	45.0	4.88
Baviacora M 92	**72.7**	**1.00**	**57.5**	**4.18**
Multi-ovary line / Baviacora M 92	84.6	1.04	62.8	5.31
Baviacora M 92 / Multi-ovary line	73.5	1.03	60.0	4.41
Esmeralda M 86	**95.3**	**1.00**	**53.0**	**5.05**
Multi-ovary line / Esmeralda M 86	91.8	1.13	59.1	5.43
Esmeralda M 86 / Yanglin	96.3	1.20	53.8	5.18

has been shown to be associated with increased leaf photosynthetic rate and higher yield in improved durum wheat cultivars grown under irrigated conditions (Pfeiffer, pers. comm.). These two findings suggest that combining higher chlorophyll content with greater spike fertility (for example, due to multi-ovary florets), which creates higher demand for photosynthesis, may help increase yield potential under irrigated conditions.

Traits to raise yield under stress conditions

Wheat yields are reduced by 50-90% of their irrigated potential by drought on at least 60 million ha in the developing world. At CIMMYT attempts are underway to improve drought tolerance by introgressing stress adaptive traits into empirically selected drought tolerant germplasm. Our current conceptual model of a drought resistant cultivar encompasses high expression of the following traits: seed size, coleoptile length, early ground cover, pre-anthesis biomass, stem reserves/remobilization, spike photosynthesis, stomatal conductance, osmotic adjustment, accumulation of abscisic acid, heat tolerance, leaf anatomical traits (such as glaucousness, pubescence, rolling, thickness), high tiller survival, and stay-green (Reynolds et al., 1999). CIMMYT's germplasm collection is being screened, as resources allow it, for high expression of many of these traits.

High stomatal conductance permits leaf cooling through evapotranspiration; this, along with higher leaf chlorophyll content and stay-green, is associated with heat tolerance (Reynolds et al., 1994). Recent studies identified high expression of these traits in bank accessions, and both traits showed high levels of heritability under heat stress (Villhelmsen et al., 2001). As a result, these accessions are currently being crossed into good heat tolerant backgrounds.

Pubescence and glaucousness protect plant organs from excess radiation under stressful conditions (see Loss and Siddique, 1994). Searches are under way for these and a number of other leaf traits, such as leaf rolling, leaf thickness, and upright posture, which may well play similar roles under stress.

Osmotic adjustment (Blum et al., 1999) and stored stem fructans (Blum, 1998) have been implicated in stress tolerance. Searches are underway for high expression of these traits among germplasm bank accessions, although laboratory protocols are required for their identification. High spike photosynthesis is another trait that could contribute to yield under stress but which is very time consuming to measure. For traits that are difficult to measure (and/or that show marked genotype by environment interaction), it is logical to develop genetic markers, which can be used to confirm their presence more unequivocally than by measuring phenotypic expression.

Conclusions

The last 30 years have witnessed an unprecedented level of international wheat germplasm exchange and the development of a greater degree of genetic relatedness among successful cultivars all over the world. The concept of broad adaptation has thus been well vindicated. However, greater genetic relatedness is seen by some as increasing genetic vulnerability to pathogens, although such vulnerability depends more on similarities in resistance genes, which may actually be more diverse now than before. Various new factors (including the growing strength of national breeding programs in the developing world and the advent of breeders' rights) should result in increased diversity among cultivars and may lead to the exploitation of hitherto overlooked specific adaptation in wheat.

This would be especially important if climate change accelerates. Just as increasing nitrogen supply and improving weed control have been almost universal factors driving wheat cultivation in the last 50 years, higher atmospheric concentrations of CO2 and global warming with resulting warmer temperatures could significantly influence breeding objectives in the next 50 years.

To boost yield in irrigated situations, spike fertility must be improved simultaneously with photosynthetic capacity. CIMMYT's wheat germplasm bank has identified a source of multi-ovary florets that have up to six kernels per flower. Other lines from landrace collections have very high chlorophyll concentrations, which may increase photosynthetic capacity. High chlorophyll concentration and high stomatal conductance (which permits leaf cooling) are associated with heat tolerance. Recent studies identified high expression of these traits in bank accessions, and both traits were heritable under heat stress. Searches are underway for drought tolerance traits related to remobilization of stem fructans, awn photosynthesis, osmotic adjustment, and pubescence.

Seed multiplication nurseries can be used for characterizing and evaluating germplasm collections for physiological traits. Characterization data can be analyzed using pattern analysis, which provides a good description of the accessions. The advantage of using these augmented seed nurseries is that cohorts of high(er) yielding lines are identified that can be used directly or examined for "new" traits. Genetic diversity from wheat's wild relatives has been exploited through wide-crossing to improve disease resistance. Further potential exists for identifying quantitative traits using QTL analysis in delayed backcross generations. Once markers linked to traits of interest are identified, germplasm collections could be rapidly screened for unique alleles at these markers.

Genetic resources are fundamental to the world's food security and central to efforts to alleviate poverty. They contribute to the development of sustainable production systems and supplement the natural resource base. Conserved germplasm is especially rich in wild crop relatives, traditional farmer cultivars, and old varieties, which together represent an immense reserve of genetic diversity. Materials conserved both *ex* and *in situ* are a safeguard against genetic erosion and a source of resistance to biotic and abiotic stresses, improved quality, and yield traits for future crop improvement. As D.C. Rasmusson recently stated (pers. comm., 2000), "a little genetic diversity goes a long way."

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CHAPTER 3

Genetic Basis of Physiological Traits

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During the past two decades, molecular tools have aided tremendously in the identification, mapping, and isolation of genes in a wide range of crop species. The vast knowledge generated through the application of molecular markers has enabled scientists to analyze the plant genome and have better insight as to how genes and pathways controlling important biochemical and physiological parameters are regulated. Three areas of biotechnology have had significant impact: the application of molecular markers, tissue culture, and incorporation of genes via plant transformation.

Molecular markers have enabled the identification of genes or genomic regions associated with the expression of qualitative and quantitative traits and made manipulating genomic regions feasible through marker assisted selection. Molecular marker applications have also helped us understand the physiological parameters controlling plant responses to biotic and abiotic stress or, more generally, those involved in plant development. This chapter discusses different types of molecular markers, the basic principles and practical considerations involved in their application in plant improvement, and some contributions they have made to wheat molecular genetics.

The Genome

Although the expression of genes can be modified by environmental factors, the nuclear genome of plant cells carries a genetic blueprint in the form of deoxyribonucleic acid (DNA) that contains information for cell maintenance and replication. The nuclear genome contains the largest amount of DNA and the highest number of genes encoded, but plant cells also contain DNA in their chloroplasts and mitochondria. Nuclear genomes of crop species are estimated to contain thousands of genes, some unique and others in multiple copies. However, the amount of DNA in the nuclear genome represented by transcribed genes is only a fraction of total DNA found in the genome.

Nuclear DNA is packaged and organized into chromosomes along with histones and non-histone proteins. The interactions between DNA and proteins play an important role in gene expression. While DNA encodes genetic information in the form of messenger RNA (mRNA), proteins are involved in the packaging of DNA and in regulating its availability for transcription. Transcribed gene products are transported across the nuclear envelope to be translated into proteins using the cellular apparatus.

Genes are distributed along the chromosomes, and the number of chromosomes a plant cell contains varies among crop species. There is considerable diversity in genome composition and organization of different organisms (Table 1). With the aid of molecular techniques, it has been possible to study and understand the organization of the nuclear genome of several plant species. Plant genome analysis encompasses genome mapping, gene tagging, quantitative trait (QTL) analysis, and synteny mapping.

Table 1. DNA content per haploid genome in different organisms.

Organism	2n	Picograms †	Mega base pairs 10^6bp / 1C	Length (cm)
E. coli	(1)	0.0047	4.2	0.14
Chloroplast (maize)	(c)	0.0002	0.160	0.006
Mitochondrion (maize)	(m)	0.0007	0.570	0.02
Arabidopsis thaliana	10	0.15	150	4.4
Oryza sativa	24	0.45	430	13.1
Triticum aestivum	42	5.96	5,700	173
Zea mays	20	2.6	2,500	75
Homo sapiens	46	3.2	3,900	102

† 1 picogram = 1 pg = 0.965 x 10^9 bp = 29 cm.

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The DNA Molecule

In higher organisms, a DNA molecule consists of a sequence of nuclear acids linked by chemical bonds. Each nucleotide contains a heterocyclic ring composed of carbon and nitrogen atoms (the nitrogenous base), a five-carbon sugar in ring form (a pentose), and a phosphate group. There are two kinds of nitrogenous bases: purines and pyrimidines. Each nucleic acid is composed of only four types of bases: two kinds of purines, known as adenine (A) and guanine (G), and two kinds of pyrimidines, cytosine (C) and thymine (T).

The nitrogenous base is linked to the pentose sugar by glycosidic bonds. When the phosphate group is added to the pentose sugar, the base-sugar-phosphate complex is called a nucleotide. Nucleotides are linked together into a chain by a backbone consisting of an alternating series of sugar and phosphate residues with the bases attached to the sugar molecules. In higher organisms, DNA consists of two strands of nucleic acids that are wrapped around each other in antiparallel form in a double helix. The sides of the two strands are composed of sugar and phosphate molecules, and the bases are inside the double helices. The two strands are held together by hydrogen bonding between the purine of one strand with a pyrimidine of the opposite strand. Base A always pairs with a T via two hydrogen bonds, whereas a G always pairs with a C via three hydrogen bonds. The composition of bases along one strand of the DNA chain is exactly complementary to its partner strand, which allows both strands to carry the same genetic information. This is essential for the self replicating capability of DNA.

The particular order of the bases arranged along the sugar-phosphate backbone is called the DNA sequence. This sequence provides precise genetic instructions for creating a particular organism with its own unique traits. The size of a genome is usually stated as the total number of base pairs in the haploid genome (Table 1).

Genes and Chromosomes

The gene is the basic physical and functional unit of heredity. Each gene is a nucleic acid sequence that carries information encoded to represent a particular polypeptide. Polypeptides provide the structural components of cells and tissues as well as enzymes for essential biochemical functions. The plant genome is estimated to comprise 20,000 to 100,000 genes.

Genes vary widely in length, often extending over thousands of bases, but only about 10% of the genome is known to include protein-coding sequences (exons) of genes. Interspersed within genes are intron sequences, which have no coding function. The rest of the genome is thought to consist of other noncoding regions (such as control sequences and intergenic regions), whose functions are still obscure. The configuration and methyation level of a DNA molecule play a role in gene expression, since expressed regions are generally characterized by a high level of methylation. Some genes have few copies; others may be present in multiple copies per haploid genome. Such repeated sequences may be present in tandem copies at a chromosomal locus or in different chromosomes dispersed throughout the genome.

The vast amount of DNA present in each plant cell is tightly packaged, with the help of histone and non-histone proteins in the nucleus, into microscopic structures known as chromosomes. Genes are scattered along the chromosomes, which vary in number from species to species. During gametic formation the somatic chromosome number is divided in half by cell division (meiosis), which ensures that the zygote (after the male and female gametes unite) will contain the same number of somatic chromosomes as the parents.

The chromosomal form of the nuclear genome varies significantly during cell division. During interphase, the chromatin in the chromosomes remains diffuse and therefore less visible under the microscope, it becomes more condensed and highly visible for cytogenetic manipulation during meiosis and mitosis. Cytological studies of individual chromosomes during metaphase through chromosome banding techniques have helped characterize and identify the individual chromosomes of the wheat karyotype. Moreover, classical cytological studies and current molecular cytogenetic techniques have aided in identifying chromosomal abnormalities and subtle interchanges.

The Wheat Genome

The numerous species of the genus *Triticum* can be classified into three ploidy groups: diploids (2n=2X=14), tetraploids (2n=4X=28), and hexaploids (2n=6X=42). Of the *Triticum* species, cultivated *T. aestivum*, known as bread wheat, is the principal commercial type, whereas *T. turgidum* (durum wheat) is principally used for making pasta. Cultivated bread wheat is an allohexaploid (2n=6X=42), composed of three distinct genomes, A, B and D. Current evidence suggests that it originated from natural hybrids of three diploid wild progenitors native to the Middle East. *Triticum urartu* Tum. is recognized as the donor of the A genome. Although *Aegilops speltoides* was considered the donor of the B genome, current evidence suggest that the real donor is either extinct or an undiscovered species belonging to the *Sitopsis* section of *Aegilops* (Pathak, 1940; Kimber and Athwal, 1972; Miller et al., 1982). *Triticum tauschii*, also

known as *Aegilops tauschii*, is widely recognized to be the donor of the D genome (Kimber and Feldman, 1987).

Among crops, wheat possesses one of the largest (about 16 billion bp per haploid genome) and most complex (hexaploid) genomes, with a high percentage of repetitive sequences (90%), which makes it quite challenging to study and manipulate at the molecular level. However, polyploids have a greater ability to tolerate loss or higher dosages of chromosomes, referred to as aneuploidy. Because of its hexaploid nature and economic importance as a food source, bread wheat is the most cytogenetically studied of the crop species. The complete range of aneuploid lines (nullisomics, monosomics, trisomics, and tetrasomics; Sears, 1953, 1954) and a great diversity of chromosome deletion stocks (Endo and Gill, 1996) have been made available in wheat. These cytogenetic stocks have been utilized in numerous studies aimed at locating genes on chromosomes and chromosome arms, as well as establishing relationships among the chromosomes of hexaploid wheat based on their origin and function.

DNA Markers

Markers are "characters" whose inheritance pattern can be followed at the morphological (e.g., flower color), biochemical (e.g., proteins and/or isozymes), or molecular (DNA markers) levels. These characters are called *markers* because they provide, although indirectly, information about the genetics of other traits of interest in a given organism. The main disadvantage of morphological markers is that they are easily influenced by the environment. In contrast, molecular markers are based on variations in genomic DNA sequences; since they are neutral, they have no phenotypic effect on the plant. The main advantages of molecular markers are that they can be numerous, are not affected by the environment, and can be scored at virtually any stage of plant development.

DNA markers can be based on restriction fragment length polymorphisms (RFLPs) or on the polymerase chain reaction (PCR) technique.

Restriction Fragment Length Polymorphisms

The RFLP technique was the first to be widely used in plant genome analysis. RFLP linkage maps of a number of species including wheat and maize have already been made. In this technique, a DNA sample taken from a particular plant is treated with restriction enzymes. Restriction enzymes recognize unique sequences in the double-stranded DNA and cleave both strands to produce numerous DNA fragments of varying length. These DNA fragments are separated, based on their size, on an agarose matrix in gel electrophoresis, denatured to make the DNA single-stranded, and then blotted onto nylon or nitrocellulose membranes using the Southern transfer technique. The DNA in the membrane is then hybridized with a probe isolated from the same, or a related, plant species, and whose chromosomal location is known. The probe, labelled with radioactivity or chemilluminiscent substances, hybridizes to complementary sequences in the fragmented DNA sample. Because of molecular differences in the plants being studied, the tagged or hybridized fragments will differ in length, which allows the samples to be uniquely characterized as molecular polymorphisms. Since the chromosomal location or "map position" of the probes is known, researchers can trace the length polymorphisms to chromosomal regions. These molecular polymorphisms or molecular markers can then be treated as any other Mendelian difference between contrasting samples.

Although the RFLP technique is time-consuming and somewhat cumbersome compared to more recent marker technologies, it is still extensively used in a wide range of crop species.

Markers Based on Polymerase Chain Reaction

Described in the early 1980s, PCR-based assays have revolutionized molecular marker assay systems. PCR-based techniques are robust, amenable to automation, and widely applied in large-scale marker development or implementation procedures.

PCR-based assays are based on an *in vitro* procedure for the enzymatic synthesis of DNA, in which two oligonucleotide primers hybridize to opposite strands flanking the region of interest in the target DNA (Figure 1). The procedure enables small amounts of specific DNA fragments (which may be mixed with large amounts of contaminating DNA) to be amplified exponentially. In a typical PCR-based assay, the "building blocks" required to synthesize a new strand of DNA are mixed with the template containing the target DNA together with primers in a tube along with thermostable DNA polymerase. They pass through cycles of differential temperatures involving template denaturation, primer annealing, and extending the annealed primers by DNA polymerase. The end result is an exponential accumulation of the target sequence, which can then be resolved on separation matrices such as agarose or acrylamide and viewed as discrete bands after staining.

Several types of PCR-based markers are being used in plant genome analysis:

- Random amplified polymorphic DNA (RAPDs)
- Sequence-tagged sites (STSs)
- Simple sequence repeat (SSR) or microsatellite
- Amplified fragment length polymorphism (AFLP)

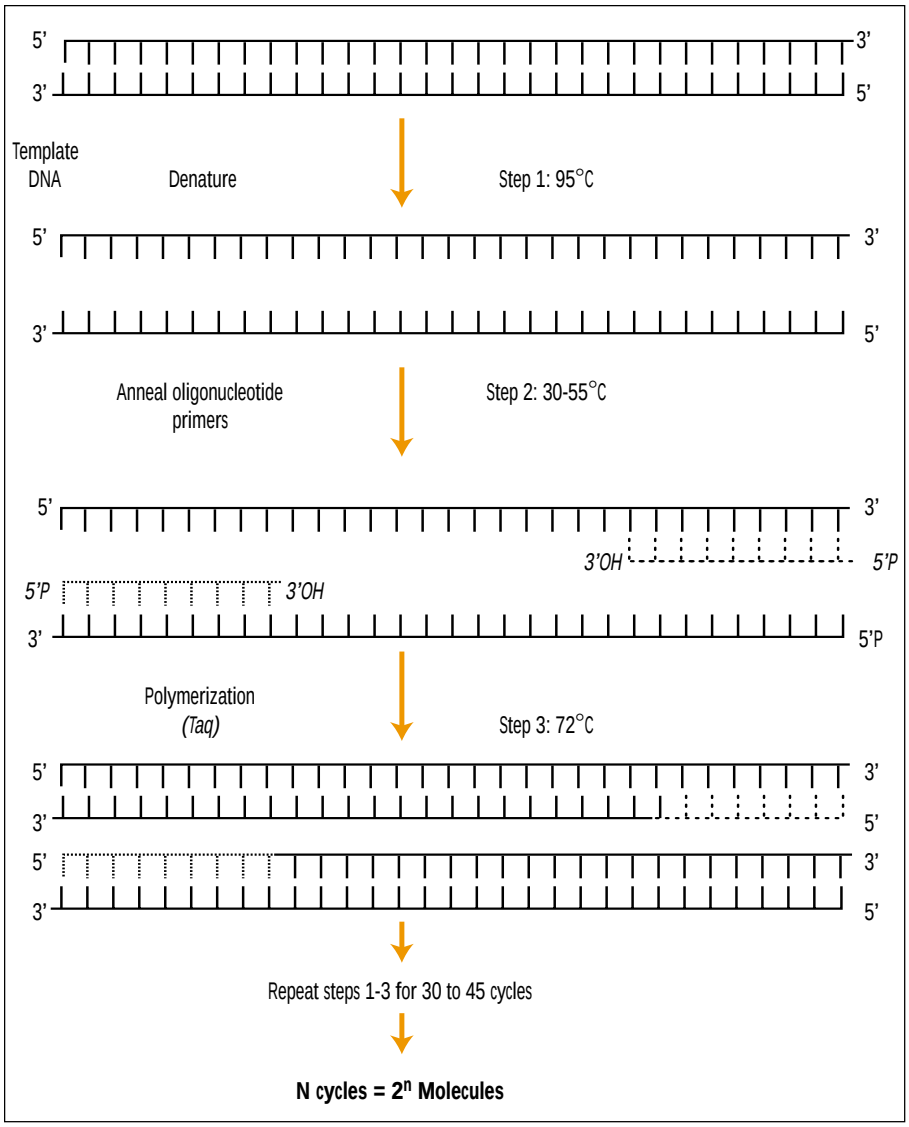

Figure 1. Polymerase chain reaction: DNA amplification.

Random amplified polymorphic DNA

As its name implies, the random amplified polymorphic DNA (RAPD) technique (Williams et al., 1990; Welsh and McClelland, 1990) is used to randomly amplify certain sequences. The primers used contain randomly synthesized oligonucleotides and are usually short (about 10 bp). RAPD polymorphisms are the result of either a nucleotide base change that alters the primer binding site or an insertion or deletion within the amplified regions. The major advantage of RAPDs is that they are suitable for all species because randomly synthesized primers (which are widely available) are not species specific. Disadvantages include the dominant nature of RAPD markers (only presence or absence of a bond, which means that the heterozygous cannot be identified), their randomness, and the resulting lack of repeatability due to non-specificity of the amplification products, specially in species such as wheat, where the genome is very large.

Sequence tagged sites

Sequence tagged sites (STSs) are mapped loci for which all or part of the corresponding DNA sequences have been determined (Olson et al., 1989; Talbert et al., 1994). This information can be used to synthesize PCR primers that amplify all or part of the original sequence. Since the primers are designed to amplify one specific locus and are longer than those used in RAPD analysis, STS assays are more robust and therefore more reproducible and reliable than RAPD analysis. Differences in the length of amplified sequences from different individuals can serve as genetic markers of the locus.

If no polymorphism is detected upon PCR amplification, the amplified fragments can be cut with restriction enzymes to observe length differences among samples, which can then be used as markers. This technique is sometimes referred to as cleaved amplified polymorphic sequences (CAPS).

The STS technique holds great promise for marker-assisted selection schemes, since it can be applied on a large scale and specific loci can be followed through successive plant generations in conventional breeding programs.

Simple sequence repeats

Simple sequence repeats (SSRs), also known as microsatellites, are composed of tandem repeats of two to five nucleotide DNA core sequences such as (AT)n, (GT)n, (ATT)n, or (GACA)n spread throughout eukaryotic genomes (Tautz and Renz, 1984). The DNA sequences flanking microsatellites are generally conserved within individuals of a given species, allowing the design of PCR primers that amplify the intervening SSRs in all genotypes (Weber and May, 1989; Litt and Luty, 1989). Variation in the number of tandem repeats results in different PCR product lengths (Figure 2). These repeats are highly polymorphic, even among closely related cultivars, due to mutations causing variation in the number of repeating units. The main

advantages of SSRs are the co-dominant nature of the observed polymorphisms (which means that homozygous A and B, as well as heterozygous AB, can be identified), the robustness of the assay, and the large number of polymorphisms observed. Their main disadvantage is the significantly high cost involved in sequencing genomic libraries in the development of SSRs.

Amplified fragment length polymorphisms

Amplified fragment length polymorphisms (AFLPs) combine the specificity of RFLP analysis with the robustness of the PCR assay and are designed to amplify a subset of restriction digested DNA (Vos et al., 1995). Usually two restriction enzymes, a rare cutter and a frequent cutter, are used in combination to digest genomic DNA (Figure 3). The DNA fragments thus generated are ligated with double-stranded adaptor sequences. The adapter-ligated fragments are subjected to two rounds of PCR amplifications. In the first round, primers complementary to the adapter sequences, plus an additional nucleotide at the 3' end, are used. A second PCR reaction is performed on the modified fragments with primers having the same sequence used in pre-amplification, plus one to three additional nucleotides, to amplify a subset of the pre-amplified DNA products. The numerous amplified sequences are sorted using high resolution electrophoresis. Differences in the length of the amplified segments are related to differences in the DNA composition of two given individuals. The primary advantage of AFLPs is the large number of fragments that can be compared per analysis.

Utility of DNA Markers

RFLP markers have been used to construct linkage maps for crop species, such as maize, tomato, and rice. Many RFLP markers with tight linkage to genes controlling economically important traits in various crop species have been identified. Once the sequence of an RFLP marker of interest is known, a PCR-based marker (STS) can be developed for large-scale screening (Ribaut et al., 1997). RFLPs are reliable markers, and the same probe can usually be hybridized on different crop genomes, making RFLP markers useful for comparative mapping studies. However, RFLP analysis requires large quantities of quality DNA, and detection of RFLPs by Southern blot hybridization may be laborious and time consuming, which may make this assay undesirable for plant breeding projects with high throughput requirements.

The invention of PCR-based assays has provided the basis for a large number of innovative methods for recognizing DNA polymorphisms among individuals, as described above. For mapping and large-scale screening, SSRs are the most desirable PCR-based markers. Once large numbers of SSRs are available that provide good coverage for a crop genome, large-scale SSR assays can be reliably performed at an early plant development stage, because: 1) a small amount of tissue is required; 2) DNA preparation is faster due to the small amount of template DNA required; and 3) large sample sizes are handled more efficiently. Moreover, SSRs are reliable, co-dominant, abundant, and uniformly dispersed within plant genomes. In July 2000, a

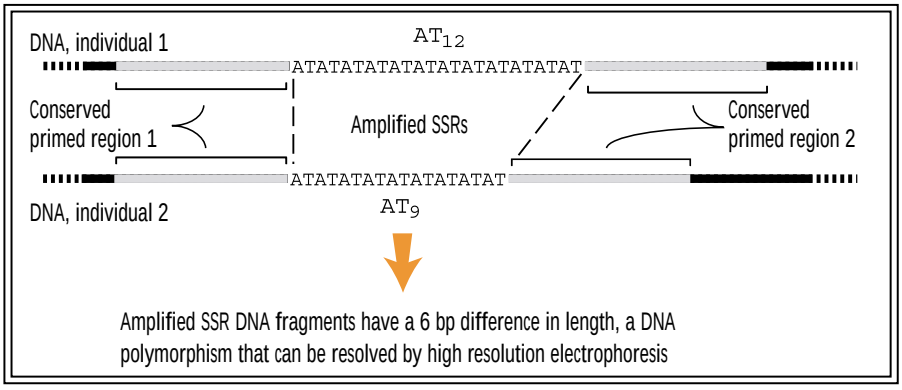

Figure 2. Example of a microsatellite: a dinucleotide repeat showing a polymorphism between two different individuals.

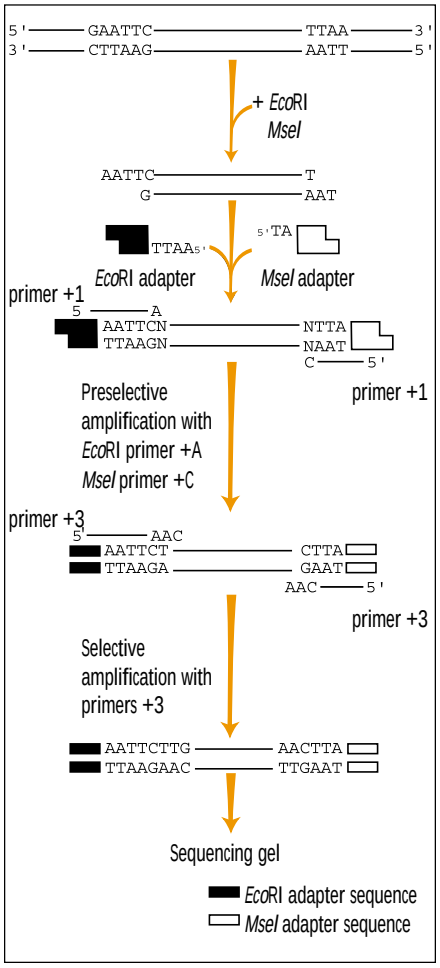

Figure 3. AFLP method.

collection of 500 SSRs became available in wheat, but 1000 to 1500 SSRs would be needed to develop a complete linkage map for QTL identification.

Geneticists today have powerful tools to conduct genomic analysis and trait dissection in crop species. The most suitable marker will depend mainly on the purpose of the investigation and the type of markers available for a particular crop (see a comparison of marker systems in Table 2).

Genomics

The newest area of investigation aimed at understanding the plant genome encompasses genome-wide approaches. "Functional genomics" can be defined as the development and application of genome-wide experimental approaches to assess gene function (Heiter and Boguski, 1997). The ultimate goal of genomics would be to characterize every gene present in a given genome. Approaches being utilized to achieve this goal are large-scale sequencing of expressed sequence tags (ESTs), large-scale functional analysis of plant genes (where thousands of DNA or RNA sequences can be analyzed on microscopic slides), and application of insertional mutagenesis or reverse genetics. These technologies are high throughput and require automation.

Innovative tools such as DNA chips and microarray have been developed to serve the new approaches. DNA chip technology provides efficient access to genetic information using miniaturized, high-density arrays of oligonucleotide probes. A set of oligonucleotides is defined, artificially synthesized, and immobilized on silica wafers or chips to construct a high-density array; each probe has a predefined position in the array. Labeled (fluorescence) nucleic acids from the analyzed plant sample are hybridized on the array, and hybridization intensities are detected by a scanner that reports quantitative assessment of RNA levels in the sample for each gene represented in the array (Lemieux et al., 1998). Microarrays are similar to the DNA chip, except that they use cDNAs (Ex. EST clone inserts). These innovative approaches are expected to provide insight into how the plant genome functions and to identify more genes involved in regulating different pathways in response to stress conditions.

Application of Molecular Markers in Plant Breeding

Conventional plant breeding is based on the selection of superior individuals among segregating progenies of sexual matings. Selection for plant improvement has largely been carried out on the whole-plant or phenotype, which is the result of genotypic and environmental effects. Although conventional plant breeding has made tremendous progress in many crop species, it is often hampered by difficulties in selecting for agronomically important traits, especially when they are influenced by the environment. Moreover, testing procedures may be difficult, unreliable, or expensive, due to the nature of the target traits or the target environment (e.g., abiotic stresses). For those reasons, selection through molecular markers might be an efficient complementary breeding tool, especially when selection is done under unfavorable conditions. If individual genes influencing target traits can be identified and associated with molecular markers, the efficiency of incorporating them into new varieties could be greatly enhanced.

Fingerprinting

A fingerprint specifically and unambiguously identifies a living organism. Identification can be achieved based on polymorphisms determined through molecular markers. In crop species, fingerprinting is a valuable tool for establishing varietal purity, which is important for varietal protection,

Table 2. Characteristics and usefulness of different types of molecular markers in wheat molecular genetics.

	RFLPs	RAPDs	SSRs	STSs	AFLPs
Fingerprinting	++	-/+	+++	+	+++
Genetic diversity	++	+	++	+	++
Qualitative gene tagging	++	++	++	++	++
QTL mapping	++	-/+	++	++	++
MAS	+	-	+++	++	+/++
Comparative mapping	+++	-	-	+	-
Types of probe/primers	gDNA, cDNA	Random 10-mer oligonucleotides	Specific 16-30 -mer oligonucleotides	Specific 20-25 -mer oligonucleotides	Specific adapters and selective primers
Level of polymorphism	Medium	Medium	High	Medium	High
Inheritance	Codominant	Dominant	Codominant	Codominant	Dominant/co-dominant
Technical difficulty	Medium	Low	Low	Medium	Medium/High
Reliability	High	Low	High	High	Medium/High

Source: Modified from Rafalski and Tingey (1993).

currently a concern for the commercial seed industry and public breeding enterprises. Fingerprinting can also be used to estimate the genetic diversity of a set of cultivars or landraces and to establish phylogenetic relationships for evolutionary studies. Other applications of molecular fingerprinting include:

- genomic characterization and identification for propriety purposes;
- identification of superior alleles in genebank accessions (e.g., landraces);
- identification of duplications within genebank accessions to ensure the best use of available resources;
- correlation of genetic diversity with heterotic patterns.

Fingerprinting studies have been reported for specific wheat germplasm using sets of DNA markers including RFLPs, microsatellites, and AFLPs (Barrett and Kidwell, 1998; Bohn et al., 1999; Fahima et al., 1998). The genetic distance among accessions can be evaluated based on fingerprinting. This information allows better characterization of genetic relationships among accessions (e.g., establishment of gene pools) and can be used to identify parental lines with good allelic complementarity.

Genetic Mapping of Target Traits

Genetic dissection of a target trait can be defined as identifying and characterizing the genomic segments or genes involved in its phenotypic expression. Before genetic manipulation using molecular markers, genes or quantitative trait loci (QTLs) must be identified and characterized via a two-step process: 1) construction of a suitable segregating population by crossing two parental lines contrasting for the target trait(s), and 2) identification of markers closely linked to the gene(s) of interest for further allelic manipulation (for a summary of the process, see Figure 4). This provides useful information, such as:

- the number of genes or QTLs significantly involved in the expression of the target trait;
- the effect (additivity, dominance) of the identified genomic regions and their impact on phenotypic expression of the trait;
- the stability of gene expression across environments (QxE); and
- the presence of pleiotropic effects at some target genomic regions.

Unfortunately, the evaluation of epistatic effects remains difficult, due mainly to the reduced number of genotypes used for this kind of genetic dissection.

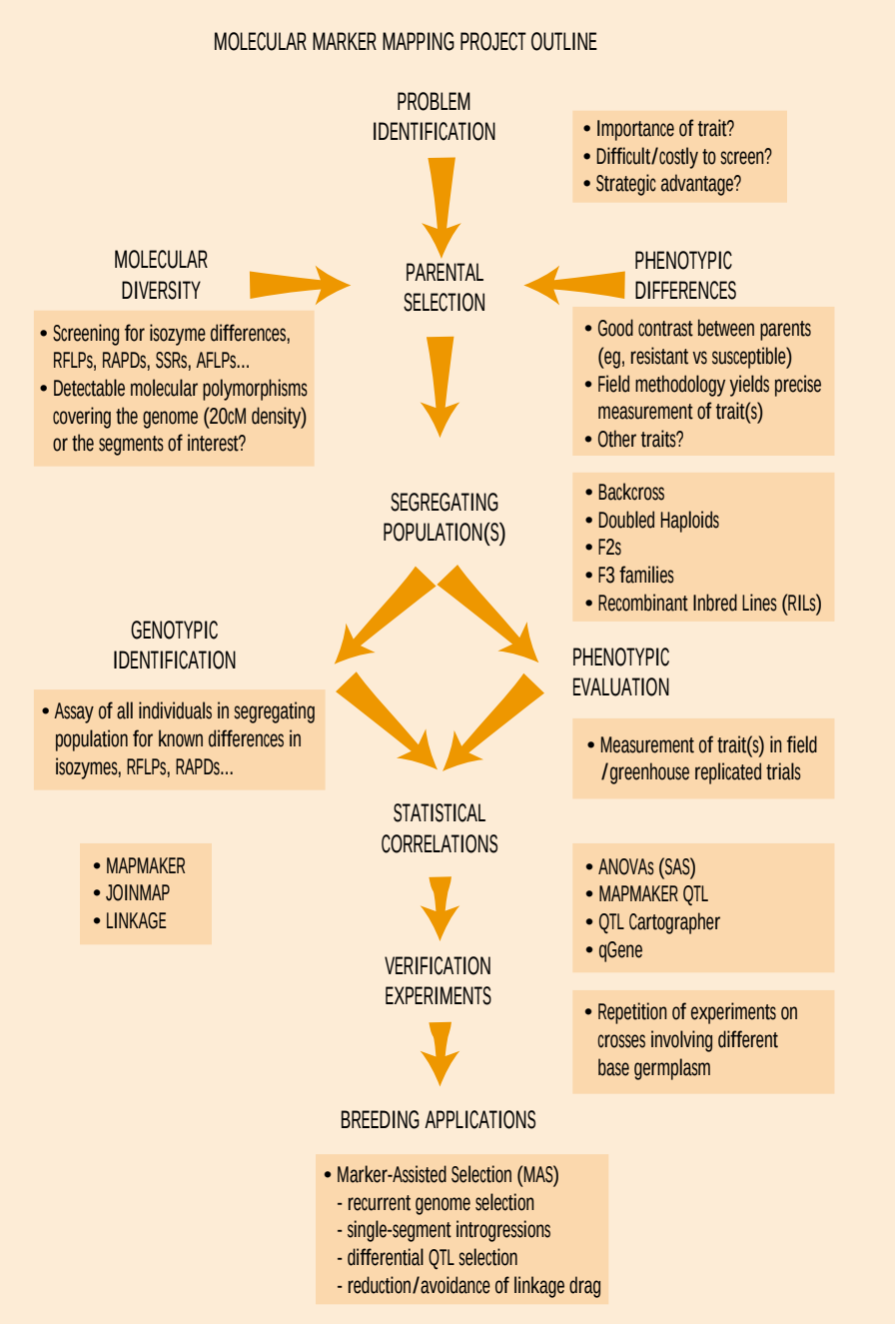

Figure 4. Description of how molecular markers can be used in genetic linkage mapping.

Germplasm for molecular analysis

Segregating populations. The most commonly used materials for genetically dissecting and mapping traits are segregating populations descended from two varieties showing divergence for the target trait(s). Effective marker identification in populations segregating for target traits depends upon laboratory data on the allelic composition of molecular markers at genomic locations distributed evenly within the genome and field evaluation of the trait(s). Based on both types of data, statistical procedures are used to find associations between markers and traits. When target traits are governed or influenced by several genetic factors, a genetic linkage map of the complete genome must be developed and a QTL analysis conducted to associate traits with markers over the complete genome. If the target trait is influenced by one or a few genes, lines can be classified for the trait and bulk segregant analysis used to associate trait alleles to molecular markers.

To develop a complete linkage map, genetically stable populations are advanced through several recombination cycles using self-pollination. In wheat, recombinant inbred lines (RILs) are best suited for such analyses, but a doubled haploid population can be developed to obtain stable, completely homozygous lines for marker analysis and field evaluations. In both cases the size of the segregating population has to be carefully considered, since populations that are too small will not allow precise gene characterization, specially when mapping quantitative traits, and large populations will consume resources unnecessarily. To genetically dissect a polygenic trait, a RIL population of about 200 to 300 families is considered suitable, but the number can be reduced if the trait is controlled by major genes.

Genetic stocks. The allohexaploid nature of bread wheat has significant disadvantages, but also some advantages in genetic analysis. For example, the presence of more than one set of genes allows wheat to tolerate the loss of complete chromosomes or chromosome arms (aneuploidy). Wheat's ability to tolerate aneuploidy has resulted in numerous genetic studies aimed at locating genes to chromosomes. Once genes are located on chromosomes, it is possible to produce detailed linkage maps for individual chromosomes and associate genes with markers. The chromosome constitution of commonly occurring wheat aneuploids is shown in Figure 5.

Aneuploids that lack a complete chromosome or a chromosome arm have in recent years become extremely important in locating biochemical or molecular markers to chromosomes. Initial screening of a new marker against a set of wheat lines, each lacking a different chromosome, will determine the marker's chromosomal location since in the absence of the chromosome carrying the gene, the marker will not be expressed. Although nullisomic (2n = 6x = 40 = 20") plants would be ideal for this analysis, they are difficult to maintain due to their low fertility; consequently, compensating nullisomic tetrasomic lines are utilized in which the absence of one chromosome is compensated for by two extra doses of a related homoeologous chromosome (Sears, 1953).

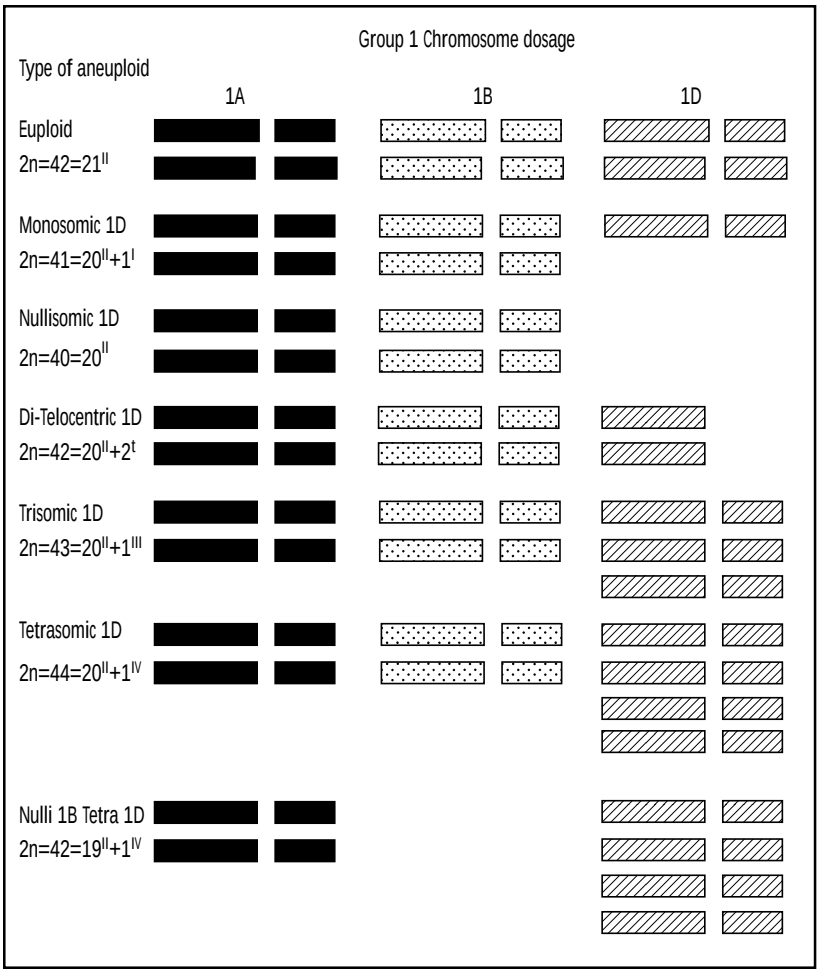

Figure 5. Genetic stocks in wheat.†

† Chromosome constitution assumes complete sets of groups 2-7 chromosomes.

Genetically stable stocks should be used where available to locate complex traits to individual chromosomes. The most suitable stocks are single-chromosome intervarietal substitution lines developed to introduce individual chromosomes from a donor variety into the genetic background of a recipient variety.

To develop intervarietal substitution lines, a series of plants of the recipient variety is needed in which the dosage of individual chromosomes has been reduced from 2 to 1. Known as monosomics, plants missing a single chromosome (2n = 6x = 41 = 20~i x 1~) are the most commonly occurring aneuploids. About 70 monosomic series are now available in different varieties worldwide (Worland, 1988). For simply inherited characters, monosomic series can be used to locate genes using test-cross procedures such as monosomic analysis (Sears, 1953), reciprocal monosomic analysis (McKewan and Kaltsikes, 1970), or backcross reciprocal monosomic analysis (Snape and Law, 1980). For more complex characters, monosomics are used as a base for developing intervarietal substitution lines by backcrossing individual chromosomes from a donor variety into the background of a recipient monosomic (Figure 6a) (Law and Worland, 1973). Once developed, intervarietal substitution lines are stable and true-breeding, and ideal for genetic analysis. By screening a complete series of 21 chromosome substitution lines any gene or trait can be readily located to individual chromosomes (Law and Worland, 1996).

Once the chromosomal location of a gene or trait has been determined using intervarietal substitution lines, these can be used to develop extremely precise genetic stocks known as single-chromosome recombinant lines (Law, 1966; Law and Worland, 1973). These lines are developed by initially producing an F1 between the critical substitution line and its recipient variety. In this F1, recombination is restricted to the single critical chromosome in an otherwise genetically homozygous background. Products of recombination of the critical chromosome are then fixed by crossing the recombining F1 plant onto a plant of the recipient variety monosomic for the critical chromosome. Monosomic progeny are extracted from the backcross and selfed to permit selection of disomic plants carrying a homozygous recombined chromosome (Figure 6b).

An alternative method of fixing recombination products is to pollinate the F1 between the recipient parent and the substitution line with maize pollen to produce haploid progeny that can be doubled with colchicine. Normally about 100 single-chromosome recombinant lines would be produced for the critical chromosome. The lines can then be classified for the trait under investigation in replicated field or growth room experiments. The trait allele can be readily associated with molecular markers by screening the recombinant lines with markers known to be polymorphic between the two parents and located on the critical chromosome.

Linkage map

Construction of a linkage map. During linkage map development, polymorphic molecular markers are used to genotype a segregating population. By statistically evaluating segregating marker alleles and linkages among different marker alleles from previous studies, markers can be placed in "linkage groups." When marker locations in the genome are known (e.g., RFLPs or SSRs), linkage groups can be assigned to chromosomes. When the genome of a crop species has adequate coverage with markers, the number of linkage groups observed should match the number of haploid chromosomes in the genome (i.e., the maize linkage map should have 10 linkage groups, that of wheat should have 21, etc.). Although constructing a linkage map is necessary for identifying genes controlling quantitative traits, a full linkage map is not always required to identify genes and associate them with markers when the target trait is regulated by major genes.

Principles of linkage map construction. To construct a linkage map, first potential parental lines are screened using molecular markers (one or a combination of molecular markers mentioned earlier) to identify DNA polymorphisms between the two. Once a suitable number of markers has been identified, they are used to determine the allelic composition for all genotypes in the segregating population. The segregation of a marker in a given population depends on the type of population. Segregation ratios are based on Mendel's first law of independent assortment. In an F2 population, a dominant marker should segregate 3:1, whereas a codominant marker that would allow the identification of heterozygotes should segregate 1:2:1. If a recombinant inbred line (RIL) or a doubled haploid (DH) population is used, segregation ratios should be 1:1 irrespective of whether the marker is dominant or co-dominant. A Chi-square test can be performed to determine the nature of the segregation of a marker.

After a number of markers has been genotyped across the population, the linkage among them is determined taking into account Mendel's second law of independent assortment (Figure 7). Table 3 presents the expected segregation for two unlinked loci in different populations. If the two loci are linked, significant deviations from the expected segregation ratios can be observed and confirmed statistically by performing Chi-square tests. If the linkage is confirmed, the frequency of recombination between the two loci can be calculated to establish the genetic distance between the two

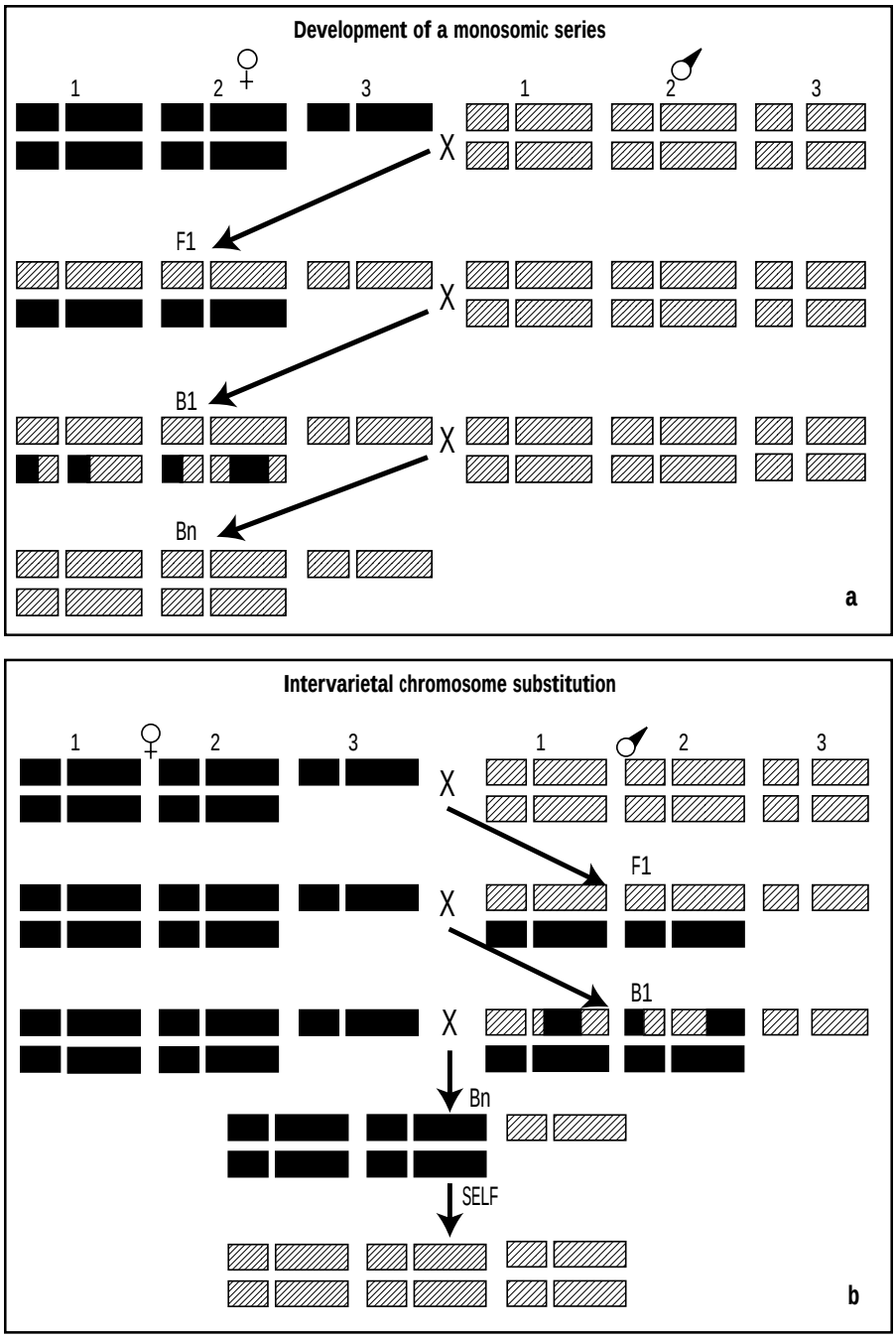

Figure 6. (a) Simplified scheme showing wheat with only 3 of its 21 pairs of homologous chromosomes. By repeatedly backcrossing the donor variety onto the recipient monosomic and selecting monosomic progeny after each backcross, a line monosomic for chromosome 3 is developed in the donor variety. (b) Chromosome 3 of a donor variety is introduced by backcrossing into the recipient variety. Initially the donor variety is crossed onto a chromosome 3 monosomic line in the recipient variety. Monosomic progeny are selected after each cross and backcrossed repeatedly onto the recipient monosomic to reconstitute its genetic background.

markers. When a large number of markers has been screened across a population, it is not feasible to use conventional statistical parameters such as Chi-square tests or computing recombination frequencies to establish linkage among markers. Furthermore, the presence of one recombination event between two adjacent loci would decrease the probability of another recombination event in adjacent loci. Computer programs that take into account all statistical parameters are available for use in linkage map construction.

Gene/QTL identification

Once the genetic map has been constructed, the next step is to find out if marker segregation within the population is associated with segregation of the target trait(s). Effective mapping studies aimed identifying molecular markers associated with target traits depend on two types of data: laboratory data on marker segregation and field data on the segregation of the trait(s). For example, in a population of RILs segregating for disease resistance, if a marker segregates in such a way that when a particular allele of the marker is present in a line, and that line shows disease resistance, a strong association between the marker allele and the trait can be inferred. In other words, the molecular marker has tagged the gene involved in the expression of resistance.

Phenotypic evaluation. Regardless of the type of data being evaluated, the quality of the phenotypic data is crucial for the success of gene/QTL analysis, since laboratory data on marker segregation within a population has to be correlated with field data to identify the QTLs. Therefore, phenotypic evaluation, whether in the field, greenhouse, growth-chamber, or laboratory, must be carefully planned and conducted with adequate replications to reduce the error. To evaluate a segregating population in the

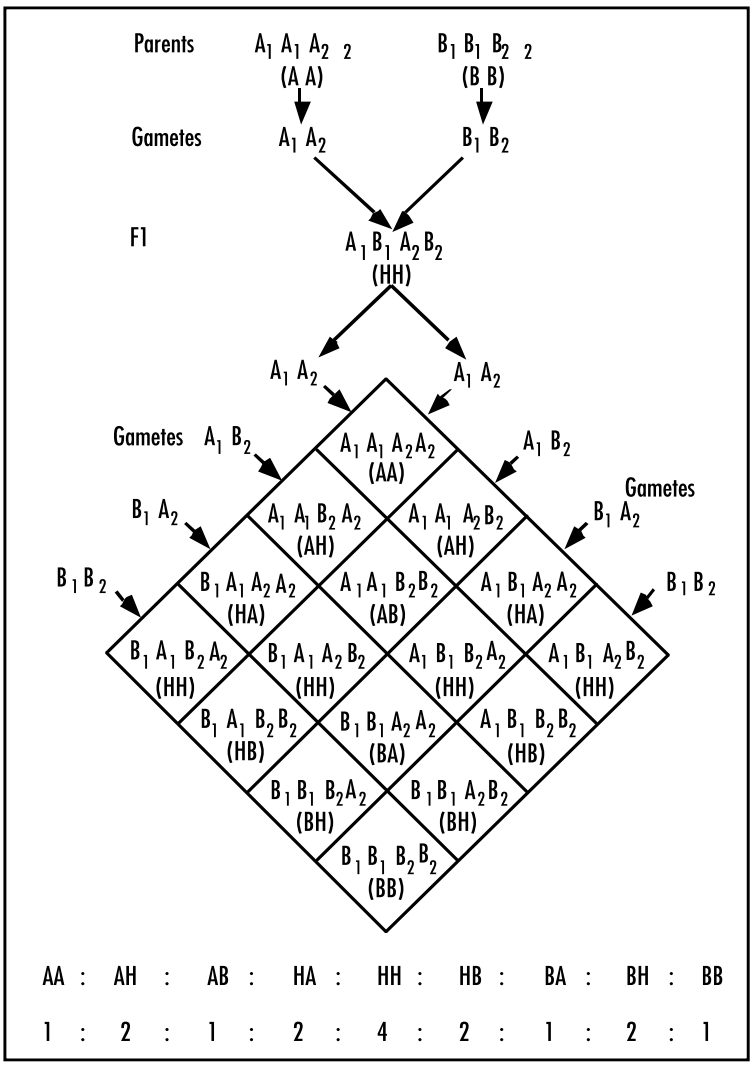

Figure 7. Expected genotypic classes for two codominant independent loci in an F_2 population.

Table 3. Expected allelic segregation of two unlinked loci in various populations.

Population	Dominant markers	Codominant markers
BC_1F_1	1:1:1:1	1:1:1:1
F_2	9:3:3:1	1:2:1:2:4:2:1:2:1
RIL	1:1:1:1	1:1:1:1
DH	1:1:1:1	1:1:1:1

field, we strongly recommend the use of field designs that include several replications, an alpha (0,1) lattice being the most commonly used to produce phenotypic data for QTL analysis. The accuracy of the protocols used in data collection is also very important.

Except for monogenic traits, phenotypic evaluation should not be conducted on a single-plant basis, but always on several plants of a family representing a given genotype, whether it be an F3 family representing an F2 genotype or several plants of a RIL family which, by definition, have the same genotype. As an example, to identify genes involved in the expression of osmotic potential in a segregating population for drought tolerance, the following must be determined:

- at what vegetative stage should leaf tissue be harvested;
- how to harvest genotypes at the same vegetative stage when they might segregate for precocity;
- what time of day to harvest, since temperature changes will induce changes in plant-water status;
- what kind of plant tissue is most suitable for the analysis;
- how to harvest many samples in a short period of time;
- how to avoid changes in water content of the tissue sample between harvest and cell sap extraction;
- how to extract cell sap from different tissue samples in a reproducible fashion;
- how often the osmometer should be calibrated to obtain reproducible measurements;
- how many replicates are needed to ensure, for example, increased accuracy.

Depending on the type of physiological test (e.g., hormone quantification), the number of samples that can be reasonably analyzed might be limited. In this case, the size of the segregating population has to be carefully considered. In a small population (e.g., fewer than 100 F2 plants or than 60 RILs), only genomic regions expressing a large percentage of phenotypic variance might be reliably identified. The complexity of taking

accurate measurements of most physiological traits makes identifying markers linked to genes involved in physiological responses more attractive.

Bulked segregant analysis (BSA). When a trait is regulated by a major gene, bulked segregant analysis (BSA) might be useful for identifying the location of the target genomic region (Michelmore et al., 1991). Bulked segregant analysis has been used to identify DNA sequences linked to a target region in several crop species (Michelmore et al., 1991; Eastwood et al., 1994). Any segregating population originating from a single cross can be used for BSA. Bulked segregants can be made for any locus or genomic region once the segregating population has been constructed.

Phenotypic distribution within a segregating population should indicate whether the trait is regulated by one or a few major genes (e.g., 1:3 distribution) or several minor genes (normal distribution). When the target trait is regulated by major gene(s), the two tails of the distribution can be safely identified through careful phenotypic selection; this material would be suitable for BSA (Figure 8).

The BSA method involves screening two pooled DNA samples from individuals with contrasting traits from a segregating population originating from a single cross. Each pool, or bulk, contains individuals selected to have identical putative genotypes for a particular genomic region (target locus or region) but also random genotypes at loci unlinked to the selected region. Therefore, the two bulked DNA samples differ genetically only in the selected region and present random allelic segregation for all other loci. For example, if markers are to be identified for disease resistance, equal amounts of DNA from the 5-10 most resistant individuals are bulked and taken as a "resistant" pool. Similarly, DNA from the 5-10 most susceptible individuals from the same population is bulked and treated as the "susceptible" pool. The two pools contrasting for the trait are then analyzed to identify markers that distinguish them. Markers that are polymorphic between the pools would most likely be genetically linked to the loci determining the trait used to construct the pools.

Once polymorphic markers for the two pools have been identified, the linkage between a marker and the target locus is confirmed and quantified using the segregating population from which the bulks were generated. It is often necessary to find the marker's genomic location, which also establishes the genomic location of factors controlling the trait of interest. If RFLPs or SSRs are used in the BSA, their genomic location is often known. However, if markers such as RAPDs or AFLPs are used, their genomic locations have to be established using several approaches. If these markers segregate in another population for which a linkage map has already been developed, map locations can be established using this secondary population. Using single-chromosome intervarietal substitution lines is another alternative.

The last and most tedious alternative is to develop a complete linkage map for the cross from which the bulks were

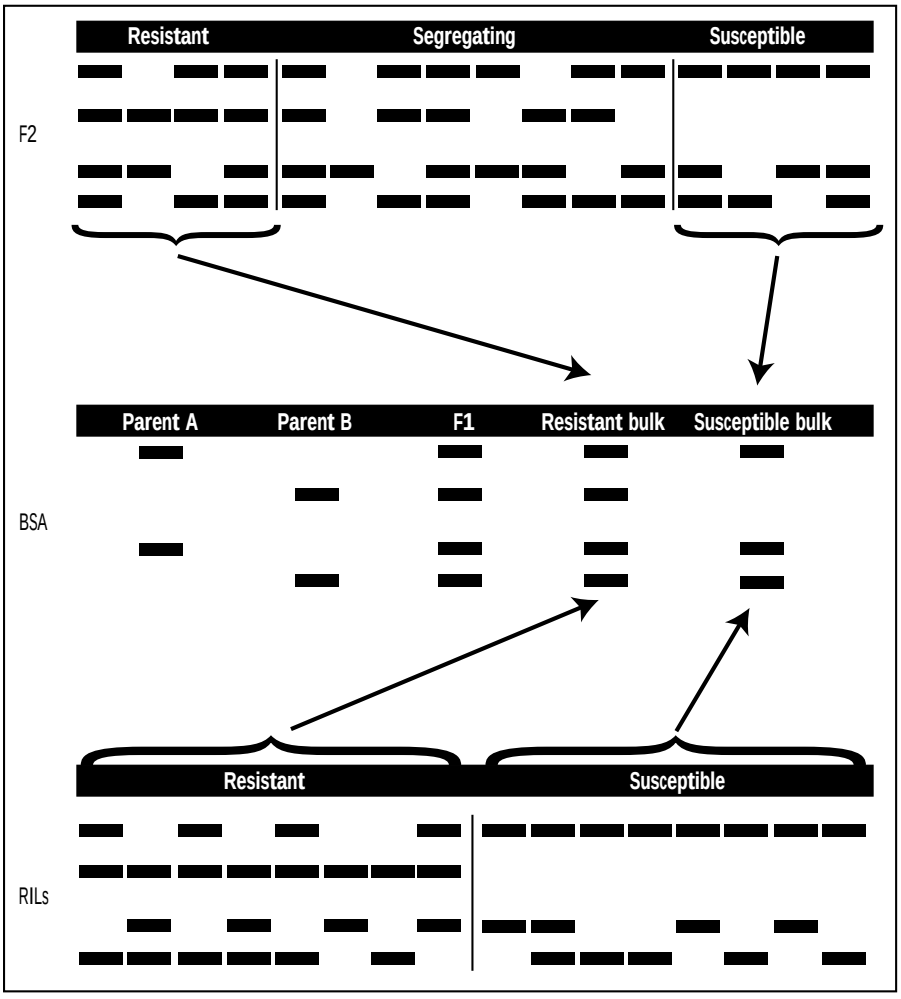

Figure 8. Bulk segregant analysis.

generated. If the linkage between the marker of interest and the target gene is confirmed using the population, the marker may be used for marker-assisted selection. The success of the approach will depend on 1) the genetic divergence between the parents in the target region, 2) the accuracy of phenotypic observations, and 3) the number of major genes involved in the expression of the target trait.

Identification of QTLs. For polygenic traits, phenotypic distribution within a segregating population is usually normal, which implies that several genes are involved in the expression of the target trait, each of them expressing a portion of total phenotypic variance. Bulked segregant analysis is not normally appropriate when target trait(s) are governed by several genes; in this case, constructing a complete linkage map is preferable. If the linkage map is constructed using DNA extracted from F2 plants, field evaluation can be conducted on F3 families derived by self-pollinating each individual F2 plant. Once the linkage map is constructed and phenotypic evaluation conducted, phenotypic correlations are commonly used to associate markers with traits and to genetically dissect complex traits into Mendelian factors. Computer programs are used to assess the correlation between phenotypic values of different genotypes within the segregating population and the allelic composition at each loci used to produce the linkage map. If this correlation is statistically significant at a given locus, the genomic region is assumed to be involved in the expression of the phenotypic trait (Figure 9). The statistical packages used in this procedure can be as simple as an F test or as complex as composite interval mapping.

It is not our purpose in this chapter to describe in detail the different mathematical approaches to QTL identification. However, commonly used approaches can be divided into three categories: simple correlation test, simple interval mapping (SIM, Lander and Botstein, 1989), and composite interval mapping (CIM, Zeng, 1994), and will be described briefly.

The simplest test to identify if there is any statistically significant association between the markers and a phenotypic data is a t –test (2 variables: homozygous parent 1 and homozygous parent 2) or an analysis of variance (F test, 3 variables: homozygous parent 1, homozygous parent 2 and heterozygous). The statistical test is conducted on each molecular marker independently to identify markers associated with the trait.

In SIM, which employs computer programs such as mapmaker/QTL software (Lander and Botstein, 1989) using mixture models and maximum likelihood techniques, a test value can be attributed to each cM on the linkage map. Therefore, a QTL peak (i.e., the point where the highest level of statistical significance is obtained) can be identified at any point on the map, not at a specific marker position, as in the previous approach. The SIM procedure differs from the previous approach in that it considers more than one marker at a time. Although SIM is a more "integrated" method than a simple correlations test, its major limitation is that it does not identify QTLs when they are linked together. For two linked QTL in coupling phase (favorable genetic contribution from the same parental line at two QTLs), SIM will identify only one QTL covering a large chromosome segment and overestimate its impact on trait expression. When two linked QTLs are in repulsion (favorable genetic contribution from different parental lines at two QTLs), SIM may not identify any of the QTLs.

Composite interval mapping, the third approach, takes into account the limitations of SIM (mentioned above). It considers markers as cofactors and has three phases:

- Unlinked markers close to QTL peaks (one marker per QTL) are identified using SIM analysis.
- The analysis is conducted again, but using the identified markers as cofactors to reduce residual variation throughout the genome, thereby eliminating false positive QTLs and identifying "new" minor QTLs.

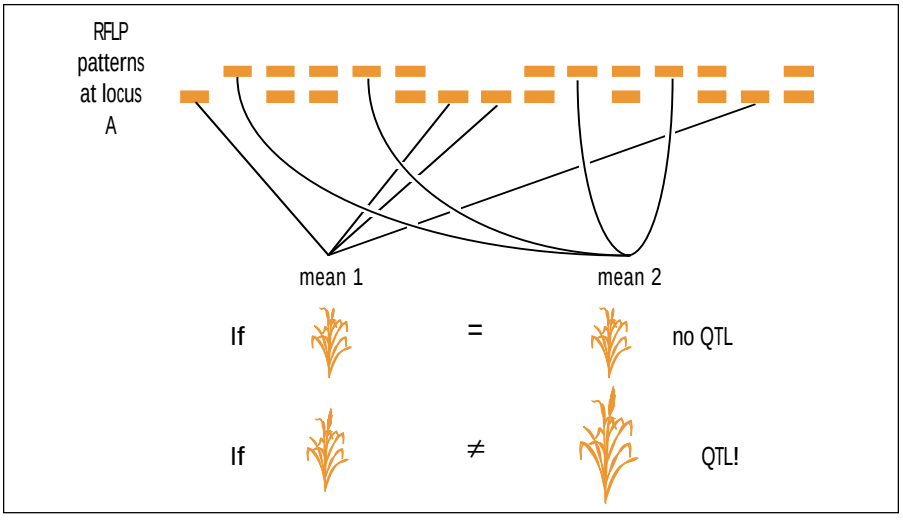

Figure 9. Illustration of a t-test for QTL detection at one RFLP marker.

- At all intervals throughout the genome, markers flanking a tested interval are used as cofactors to block the effects of possible QTLs linked to the interval of interest. The chosen distance between the tested interval and a cofactor is defined as a "window" for testing for the presence of a QTL.

When conducted on a detailed linkage map, CIM allows more precise identification of a QTL in the genome and better identification of coupled QTLs. In addition, it allows analysis of individual field data sets, as well as analysis of combined phenotypic data from different environments (locations, years, or treatments), and therefore also evaluates the QTL by environment interaction (Q x E). However, accurate evaluation of Q x E interactions, which requires top quality molecular and phenotypic data, remains a major constraint for validating marker-assisted selection (MAS) experiments. Moreover, even with new approaches like CIM, there remain clear limitations in evaluating epistatic effects between different regions of a genome.

Characterizing a QTL involves finding its precise location on a linkage map, determining the percentage of phenotypic variance expressed by each QTL independently or by several QTLs together, and quantifying the genetic effect (dominance and additivity) per QTL. Usually, a QTL is defined as major when it explains more then 30% of phenotypic variation. Although major QTLs may be involved in the expression of disease resistance, grain quality, or tolerance to abiotic stresses such as aluminum tolerance, they do not usually regulate the expression of very complex traits such as yield in water limited environments.

Progress in wheat molecular genetics

Use of molecular markers for mapping and gene identification. Progress in gene identification and marker development has been slow in wheat due to its hexaploid nature and the large size of its genome. However, in the recent past, a significant number of genes involved in various functions have been mapped to specific wheat chromosomal regions. Characterizing genes that control flowering in wheat has benefited from chromosome manipulations involving aneuploidy as well as molecular markers.

Using intervarietal chromosome substitution lines and single-chromosome recombinant line populations, genes controlling vernalization response *Vrn1* and *Vrn3* have been located on the long arms of chromosomes 5A and 5D, respectively (Law et al., 1976), and *VrnB1* on chromosome 5B (Zhuang, 1989). Similar procedures have been utilized to identify genes controlling photoperiod response (*Ppd* genes) (Worland and Law, 1986). Plant height, important for determining adaptation and yield in wheat, is genetically complex; so far about 21 genes have been identified to be associated with this trait (McIntosh et al., 1995). A microsatellite marker has recently been developed that is linked to *Rht8* (Korzun et al., 1998).

Efforts have been made to genetically dissect complex physiological traits associated with drought tolerance such as accumulation of abscisic acid in rice and to investigate possible relationship between rice and wheat homeologous loci controlling abscisic acid accumulation (Quarrie et al., 1997). Using single-chromosome recombinant line populations and mapping, Quarrie et al. (1994) located a genetic factor controlling drought-induced abscisic acid production on the long arm of chromosome 5A in wheat. Molecular genetic tools have also been used to study complex traits such as carbohydrate metabolism and the association between abscisic acid concentration and stomatal conductance (Prioul et al., 1997). Comparative RFLP mapping in cultivated and wild wheat (*Triticum dicocoides*) has led to the identification of molecular markers associated with resistance to the herbicide chlorotoluron which is a selective phenylurea herbicide (Krugman et al., 1997). A list of wheat genes that control various physiological and agronomic parameters that have been identified with the use of molecular markers is presented in Table 4.

The existence of numerous sets of wheat near-isogenic lines (NILs) differing in the presence/absence of a resistance allele for various biotic stress factors (diseases and pests) has facilitated the mapping of genes for which such lines exist. Large numbers of genes conferring disease or pest resistance have been identified and associated with molecular markers (reviewed in Hoisington et al., 1999). When the chromosomal location of a particular gene is known from previous genetic studies but no NILs are available, markers mapped to that chromosome (Anderson et al., 1992) can still be used to score parental lines for polymorphisms, construct a single-chromosome map, and determine which markers are close to the gene of interest. This strategy was followed by Dubcovsky et al. (1996) to tag the *Kna1* locus in wheat, which is responsible for higher K+/Na+ accumulation in leaves, a trait correlated with higher salt tolerance.

In wheat, bulked segregant analysis, initially used mostly with RAPDs, can now be used with any type of marker

including AFLPs (Goodwin et al., 1998; Hartl et al., 1998), which have the advantage that a high number of DNA fragments can be amplified with one primer combination. Also, with AFLPs the problem of highly repetitive DNA is overcome by using methylation sensitive endonucleases such as *Pst*I and *SseI*.

Many genes that have been tagged with molecular markers in wheat have been introgressed from alien species (Hoisington et al., 1999). In the case of translocations from wheat's wild relatives known to carry genes for agronomically important traits, markers can be successfully established due to the high level of polymorphisms between the wheat and introgressed genome and the low level of recombination between the translocated segment and the corresponding wheat chromosomes.

Mapping QTLs in wheat. Utilizing a base map and linkage data from a range of other segregating wheat, rye, and barley populations, a consensus map with more than 1000 data points has been developed (Gale et al., 1995). This detailed linkage map has confirmed that the order of genetic loci across the A, B, and D genomes has been conserved (Gale et al., 1995).

A RIL mapping population developed utilizing 'Opata 85' and a synthetic hexaploid from CIMMYT has been used extensively in mapping and genome relationship studies (Van Deynze et al., 1995; Nelson et al., 1995a, b, c). The genetic map of this population, developed by the International Triticeae Mapping Initiative (ITMI), contains over 1000 RFLP loci. Two other published maps are available in wheat (Liu and Tsunewaki, 1991; Cadalen et al., 1997). Linkage maps in wheat have confirmed evolutionary chromosomal translocation rearrangements involving chromosomes 2B, 4A, 5A, 6B, and 7B, which were based on cytological evidence, and have established synteny among closely related grass species such as rice, maize, oats, and wheat (Ahn et al., 1993; Devos et al. 1994; Van Deynze et al., 1995; Borner et al., 1998).

The low number of quantitative traits dissected into their QTLs in wheat is a reflection of the focus on simply inherited traits and the difficulty of building comprehensive linkage maps. Given that the ITMI map is one of the densest and the population from which it was developed is segregating for a number of traits, it has been used to map important traits and several major genes. Known genes include vernalization (*Vrn1* and *Vrn3*), red-coleoptile (*Rc1*), kernel hardness (*Ha*), and powdery mildew (*Pm1* and *Pm2*) genes (Nelson et al., 1995a), as well as genes conferring and suppressing leaf rust resistance (Nelson et al., 1997). Quantitative trait loci have been identified for kernel hardness (Sourdille et al., 1996), Karnal bunt (Nelson et al., 1998), and tan spot (Faris et al., 1997).

Research on developing molecular markers for traits associated with drought tolerance in wheat started recently at CIMMYT. A RIL population is being utilized to identify genomic regions associated with a range of physiological parameters controlling drought tolerance.

Table 4. Genes identified and mapped with molecular markers for physiological and agronomic traits in wheat.

Traits	Genes	Species	Markers	Chromosomes	References
Physiological and agronomic					
Preharvest sprouting	QTL	*Triticum aestivum*	RFLP		Anderson et al., 1993
Vernalization	*Vrn1*		RFLP	5AS	Galiba et al., 1995; Korzun et al., 1997 Kato et al., 1998
	Vrn3		RFLP	5DS	Nelson et al., 1995a
Photoperiod response	*Ppd1*	*T. aestivum*	RFLP	2DS	Worland et al., 1997
	Ppd2	*T. aestivum*	RFLP	2BS	Worland et al., 1997
Dwarfing	*Rht8*		SSR	2DS	Korzun et al.,1998
	Rht12		SSR	5AL	Korzun et al., 1997
Cadmium uptake			RAPD		Penner et al., 1995
Aluminum tolerance	*Alt2*		RFLP RFLP	4D 4DL	Luo and Dvorak, 1996; Riede and Anderson, 1996
Drought induced ABA			RFLP	5A	Quarrie et al., 1994
Na+/K+ discrimination	*Kna1*	*T. aestivum*	RFLP RFLP	4D 4DL	Allen et al., 1995; Dubcovsky et al., 1996
Quality					
Kernel hardness	*Ha Hn* and QTL		RFLP RFLP	5D 5DS, 2A, 2D, 5B, 6D	Nelson et al., 1995a; Sourdille et al., 1996
Grain protein	QTL	*T. turgidum*	RFLP	4BS, 5AL, 6AS, 6BS, 7BS	Blanco et al., 1996
LMW glutenins		*T. turgidum*		1B	D'ovidio and Porceddu, 1996
HMW glutenins	*Glu -D1 -1*	*T. aestivum*	ASA	1DL	D'ovidio and Anderson, 1994
Flour color			RFLP/AFLP	7A	Parker et al., 1998

Marker-Assisted Selection

Based on information provided by mapping and genetic dissection of a given trait, the efficiency of using marker-assisted selection (MAS) can be evaluated and a suitable experiment designed. Molecular markers can be used for 1) tracing a favorable allele (including recessive) across generations, and 2) identifying the most suitable individual among segregating progeny, based on allelic composition across part or the entire genome. For tracing favorable alleles, it is critical to have molecular markers very close to the gene of interest.

Markers can sometimes be identified within genes that have been sequenced. For example, in maize, Opaque-2 mutant gene, which confers high lysine and tryptophan in the kernel, has been sequenced (Schmidt et al., 1990). Microsatellite sequences have been identified within the gene, and primers to amplify the microsatellites have been designed. This is optimal for tracing favorable alleles, since the marker is located within the gene sequence itself and co-segregates with the target gene.

However, in most cases, especially for polygenic traits, target genes have not been characterized at the molecular level. Therefore, the selected genomic regions involved in a marker-assisted selection experiment are chromosome segments or QTLs, identified as described previously. Two polymorphic DNA markers flanking the QTL region are required to follow the presence of elite alleles within the selected QTLs. When a QTL represents a large chromosome segment (more than 20cM), there should be a marker within the QTL to eliminate genotypes presenting a double recombination between the two flanking markers.

Depending on the nature of the genomic region (cloned gene, major or minor QTLs) involved in the expression of a target trait and the number of selected QTLs or genomic regions that need to be manipulated, several MAS schemes can be considered. Optimal strategies, in terms of the breeding approaches and the type of the molecular marker to be used efficiently in a MAS experiment, should also consider, apart from the technical constraints, the objectives of the experiments and the resources available. A favorable allele can be introgressed using the marker in target germplasm through backcrossing (BC), or can be selected in a segregating population whatever the level of recombination present (e.g., F2 or advanced populations). In a"marginal" MAS approach, quite important in open-pollinated crops, the most suitable parental lines for developing new materials through recurrent selection are identified.

Marker-assisted selection strategies

MAS for parental selection. Molecular markers can be used to genotype a set of germplasm, and the data used to estimate the genetic distance among evaluated materials. The degree of heterosis between lines can be predicted based on genetic distance. Moreover, germplasm can be characterized at specific loci known to be involved in the expression of a target trait, such as leaf rust in wheat, provided the germplasm has been well characterized phenotypically and molecular polymorphisms identified in a set of materials that differ in the expression of leaf rust resistance. This allows the identification of lines possessing the most suitable allelic composition for leaf rust at different loci.

Fingerprinting of potential parental lines can be very informative for breeders planning to make new segregating crosses. Although the information provided by molecular markers may not identify the best cross, it does help reduce the number of needed crosses. If by combining phenotypic and genotypic data the number of crosses can be reduced by half, this will greatly increase breeding efficiency.

The backcross-MAS approach. Marker-assisted selection using backcrossing (BC-MAS) has been used extensively—for example, to introgress target alleles when dealing with cloned genes or major QTLs. In a BC scheme, an elite allele at target loci is transferred from a donor to a recipient line. The use of DNA markers, which permit genotyping the progeny at each cycle, increases the speed of the selection process (Tanksley et al., 1989). An excellent example of the introgression of an elite allele at a target gene is the introgression of a transgene into elite maize inbreds (Ragot et al., 1994).

The following parameters must be considered in planning and executing a BC-MAS experiment: the number of target genomic regions involved in the selection, the size of the population screened at each cycle, the number of genotypes selected at each cycle, and the level of line conversion desired. The expected level of conversion is related to the number and distribution of DNA markers at non-selected loci, and to the recombination frequency between the target loci and the two flanking markers. All these parameters interactively have an impact on the number of cycles required to do BC-MAS. With the recent development of reliable PCR-based markers and single-nucleotide polymorphisms (SNP; Gilles et al., 1999), the capacity for screening large populations has been substantially improved (Ribaut et al., 1997).

In view of the non-linear relationship between reduction of the donor genome contribution at non-selected loci for different population sizes, in a BC-MAS experiment the number of target genes to be introgressed must first be defined. This facilitates calculating the

population size to be screened at each cycle, considering a target effective population size (defined as the number of individuals with favorable alleles at the target genes from which selection with markers can be carried out on the rest of the genome at non-target loci) of 50-100 genotypes. The effective population size and number of genotypes heterozygous at the target loci are essential for determining the selection model. Once the effective population size is defined, the desired recombination frequency between flanking markers and the target gene should be determined, as well as the number of genotypes selected at each cycle, based on the objectives and constraints of each project. Following this strategy, the number of BCs required to achieve the introgression can be easily predicted based on simulations (Frish et al., 1999).

In case of limited resources, or for concomitant introgression into a large number of recipient lines, as is often necessary in a breeding program, including an MAS step at an advanced BC cycle should be considered. Independently of the BC-MAS scheme considered, it is critical to make some effort to identify the most convenient set of markers.

New MAS strategies

The limitation of MAS for polygenic trait improvement. Despite the success of BC-MAS in certain applications, it has limited utility when several QTLs must be manipulated concomitently. Quantitative traits are difficult to manipulate due to their genetic complexity, principally the number of genes involved in their expression and epistatic interactions between genes (see Ribaut and Hoisington, 1998, for a review). Since several genes are involved in the expression of polygenic traits, they generally have smaller individual effects on plant phenotype. This implies that several regions, or QTLs, must be manipulated at the same time to have a significant impact, and that the phenotypic effect of individual regions is not easily identified. Field trials repeated over multiple years are required to accurately characterize QTL effects and evaluate their stability across environments. Epistatic interactions can induce a skewed evaluation of QTL effects *per se*, and if all the genomic regions involved in the interactions are not incorporated in the selection scheme, they can bias the MAS. In conclusion, although QTL identification has improved significantly, currently used MAS strategies have their limitations (especially related to cost effectiveness) and new ones should be considered.

Single large-scale MAS. In this proposed new approach, the selection of suitable parents and development of new lines are overseen by plant breeders, and DNA markers are used at an early stage of recombination to fix alleles at selected genomic regions (Ribaut and Betran, 1999). The MAS step, conducted only once, is based on the use of reliable PCR-based markers on large segregating populations derived from crosses between elite lines. Parental lines must be outstanding for the target trait and/or environment, and should have good allelic complementarity.

The new strategy offers two major advantages: 1) favorable alleles selected for improving a specific trait are derived from two or more sources of elite parental materials in a complementary scheme, disregarding the "recipient/donor" line concept, and 2) specific genomic segments are fixed only at regions previously identified for each donor line in the selection scheme, with no selection pressure applied outside the targeted genomic regions. This ensures good allelic variability in the rest of the genome for future line development under various conditions. This approach is also relevant for pyramiding favorable alleles at cloned genes or major QTLs in new germplasm.

Pedigree MAS. This approach is especially relevant for crops such as wheat, where pedigrees of elite germplasm are known. Fingerprinting elite wheat materials must be conducted in a set of lines actively used in the breeding program, and in elite materials to be released in subsequent years. Fingerprinting data may be combined with phenotypic data collected during different selection cycles to identify alleles favorable for traits of interest. For example, if an elite line contains alleles for yield performance in a target environment, their frequency should be higher than the expected random frequency in offspring derived from this elite parental line. This shift in allelic frequency reflects phenotypic selection by breeders and may be identified by comparing fingerprinting data of both parents and their offspring. Once the favorable alleles are identified, DNA markers closely linked to the target genomic regions can be used to accelerate fixation of favorable alleles in the next selection step: a new set of elite materials (offspring 1) to derive the next set of elite lines (offspring 2). Such MAS will probably be most efficient when conducted on F2 or F3 segregating populations.

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CHAPTER 4

Managing Experimental Breeding Trials

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The goal of most breeding trials is to assess the performance of a number of genetically diverse breeding materials in such a way that superior lines can be selected that perform better than local checks under specific farming conditions. This must be done without bias under conditions that mimic the conditions where the material will be recommended. If the breeder is going to select material for a specific biotic or abiotic stress, he must conduct the trial in an area that has this stress or condition. This will ensure a higher rate of success than planting only under favorable conditions that do not represent the target environment.

It is also important to manage breeding trials as carefully as possible to minimize experimental error to be able to evaluate differences between materials statistically. Confounding factors need to be kept to a minimum so that the breeder has confidence that his selections will stand up to testing under the client situation. This chapter will look at some of the factors that need to be considered when planning breeding trials.

Choosing the Experimental Site

Selecting the experimental site is critical for success. If a breeder wishes to select materials for salinity tolerance, he must select a site that represents the salinity situation in the areas where the variety will be released. His chances of success are increased by carefully selecting the site for the nursery. This selection will take into consideration the soil, climate, water regime, and biotic and abiotic stresses that will be encountered in the target environment. Factors that need to be considered include:

- Soil factors such as texture, pH, conductivity (salinity and alkalinity), and nutrient status. Soil texture will affect soil physical properties and the permeability and drainage of the soil. Soil pH and conductivity can have a large effect on plant growth, and crops and different cultivars will perform differently under different values for these parameters. Soil nutrient status will affect the yield potential of the germplasm. It is also important to consider soil nutrient status when breeders are specifically looking for tolerance to various nutrient factors such as phosphorus efficient lines or lines tolerant to micronutrient deficiencies.
- Climate is an important consideration especially when looking for abiotic or biotic stress tolerance. Should the breeder select a high or low rainfall area, cool or hot temperatures at the beginning or end of the growing season? Is frost an important consideration or heat during grainfilling?
- Is the crop being recommended for an irrigated or rainfed situation? Is waterlogging or poor drainage a characteristic of the recommendation domain? If selecting for rainfed areas, how do you handle the problem of soil moisture at planting? Do you pre-irrigate the plot so germination is good and then leave the rest of the season to natural rainfall? Is irrigation water available and is it of the quality you need or similar to that of the target environment?
- If the breeder is selecting material for specific biotic stresses, he should select an area where this stress occurs. Incidence of certain diseases and insect pests comes to mind.
- Abiotic stresses such as heat, salinity, and waterlogging also need to be addressed when selecting a site. Are abiotic stresses (for example, salinity or waterlogging) consistent across the selected field? If not, can they be statistically handled by experimental design and layout?

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Another question is whether the experimental area should be on station or in farmers' fields. Most breeding trials have traditionally been conducted on station, mainly because it gives the breeder control over experimental conditions, e.g., land preparation, fertilization, irrigation, crop protection, and security of the trial. However, there is often the problem how representative the station is of the client farmer situation. Unfortunately, many stations are not chosen for their similarity with farmers' fields but rather for convenience or availability. Experiment stations usually represent one or at most a few of the soils or environments faced by farmers.

There is therefore a tradeoff between experimental control and how representative on-station conditions are of the target environment. Probably the best solution is to conduct breeding trials under both situations. The first phase selects promising materials under the control of the station; later the materials are assessed under actual farmer situations. The latter should preferably include farmer participation and experimentation. The extra benefit of farmer participation is the feedback and assessment he can give the breeder. The new emphasis on farmer participatory breeding by some donor and development agencies utilizes this important feedback mechanism.

Influence of Crop Rotations

In many developing countries, especially in subtropical environments, wheat is often grown in double or triple cropping patterns. For example, in Asia, wheat is grown sequentially with rice, cotton, soybeans, and maize in the same calendar year. In Mexico, where the CIMMYT wheat varieties originate, wheat usually follows cotton, maize, or soybeans. It is therefore important that some breeding trials be grown on land with similar cropping patterns, for several reasons:

- The previous crop can strongly influence the harvest date and therefore the planting date for the wheat crop. For example, in Asia, long-maturing "basmati" rice is preferred in some locations because of its high quality and market price, its straw value and the fact that it needs less fertilizer. The next wheat crop will inevitably be planted late. Since there is genetic variability for performance of wheat varieties under late planting, breeding trials should at some point in the selection process be planted late after rice.
- The previous crop can have an effect on soil physical properties. When rice is grown in South Asia, the soil is puddled (wet cultivation) to lower permeability and water use. This profoundly affects the soil structure for the next upland crop, such as wheat. The poor structure following rice affects rooting and soil permeability. Waterlogging is common in wheat following rice, with plants turning yellow due to oxygen stress. Thus a breeder should evaluate his germplasm under these conditions to improve the chances of selecting adaptable varieties for rice-wheat farmers.
- Soil nutrient status is also affected. Some crops like rice are very exhaustive of nitrogen. For others, like potato, soil nutrient status is often better than normal due to high fertilizer application rate, which leaves residual fertility.
- Soil water status can also be affected. Deep-rooted crops such as sugarcane and cotton can deplete the soil profile of water. If the breeder grows his nursery after fallow, he will get different results than if he plants after the previous crop, unless he compensates for the low water status by irrigation.
- The previous crop will have a specific spectrum of pests and diseases. If weeds from the previous crop carry over to the wheat crop, measures must be taken to control the problem. Residues from the previous crop can also influence disease and insect incidence. In many countries combine-harvesting is becoming popular. This system leaves loose residue on the soil surface. In some cases, this residue can harbor diseases. In others, there are benefits to this mulch (especially rainfed areas) in the form of cooler temperatures and better water infiltration and conservation. Thus it is important to screen materials under these conditions.

Preparing the Nursery

Unless germplasm is being selected for reduced or zero-tillage situations, land preparation is a major first step in nursery management. The objective is to prepare the soil to favor wheat germination. Several factors need to be considered:

- Selection of the appropriate plow or harrow for the specific soil to ensure a good tilth, favorable for wheat germination. This may involve a series of steps from deep plowing, to harrowing, to compaction of the surface soil to ensure good seed-soil contact.
- Fields should be leveled as much as possible to reduce variability due to soil moisture, especially if applying irrigation. If germplasm is being selected under rainfed conditions and the fields are sloping, appropriate experimental blocking designs will be needed to reduce experimental variability and error.
- If the nursery is sown in farmers' fields, the plots should be prepared in the same way that farmers do, using the most representative areas (away from field edges, buildings, trees, etc.).

- Care should be taken to control any carryover weeds from the previous crop, to avoid creating a problem in the wheat crop.
- It may be necessary to pre-irrigate the field to ensure adequate soil moisture for germination of the wheat seed.

If farmers in the target area are beginning to use or are already using zero-till planting, strong consideration must be given to managing breeding nurseries in a similar fashion.

Planting Methods

If available, it is better to use commercially available seed drills specifically designed for planting breeding trials (Hege, Wintersteiger, Almaco, etc.). These systems can plant small plots with many different varieties without having to clean the seed drill after each plot. They also ensure consistent planting depth, even distribution of seed in rows, and optimize the chances of good germination, all if which reduce experimental error. If machinery is not available, hand planting is possible, but germination will be less regular and errors may be more common.

The trials should have a sufficient number of rows to eliminate border effects, i.e., the middle three or four rows of wheat to be harvested should be surrounded by at least one row of wheat to prevent bias in the experiment (Figure 1). Border rows will yield more than inner rows due to less competition for water, light, and nutrients. Where there is a lot of variability in plant height in the germplasm, even more space is needed between varieties to prevent experimental bias. This can be achieved by growing more border rows or by leaving a space between the plots.

Statistical Methods and Considerations

Error associated with yield estimates in field trials is increased by spatial variation at the experimental site. Factors causing variation may include uneven application of water and fertilizer, natural differences in soil fertility or water-holding capacity, spatial variation in soil physical properties, etc. Such variation can result in poor precision when estimating treatment effects. It is therefore important to reduce, as much as possible, the residual variation not accounted for by the variety effects. Residual variation can be reduced through the use of an appropriate experimental design (*a priori* approach) and of spatial methods of analysis (*a posteriori* approach), also called nearest neighbor analysis.

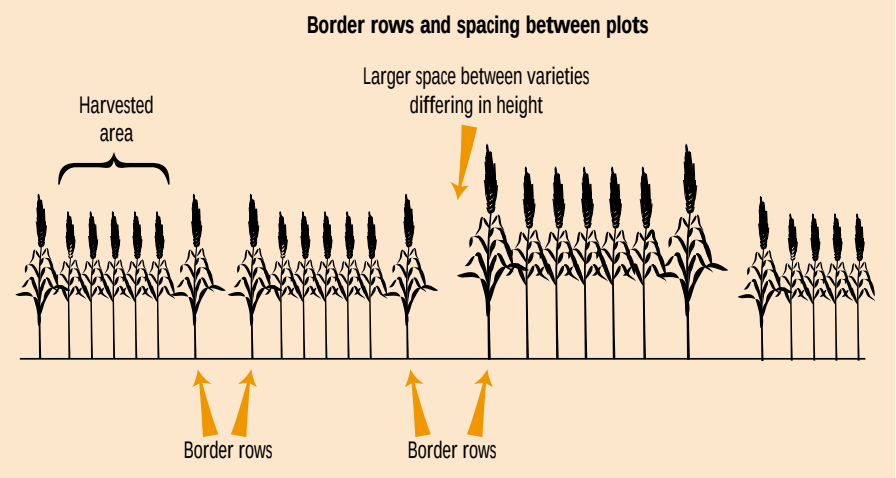

Figure 1. Layout of trials to avoid experimental bias and border effects.

Lattice designs

Most variety trials use complete or incomplete block designs and are analyzed by the traditional analysis of variance. Block designs attempt an *a priori* reduction of the experimental error considering spatial heterogeneity among blocks. If it is probable that environmental heterogeneity exists within an experimental complete block (i.e., replicate), for example in trials with large numbers of treatments, lattice designs can be used to adjust the means. The design of the experiment involves the restricted randomization of entries within sub-blocks so that means can be adjusted according to the variation among sub-blocks (Yates, 1938).

Incomplete block designs (such as alpha lattices) can regularly achieve better yield estimates and consume fewer resources than randomized complete blocks. Such efficient designs must be applied hand in hand with excellent field plot technique to produce accurate estimates of yield within locations. Designs and programs for analyzing lattice designs are included in the statistical package MSTATC, for example. Because they improve precision, lattice designs are highly recommended for field studies. The increased precision resulting from lattice adjusted data may permit trials to be grown with fewer replicates than would otherwise be necessary to establish significant differences. With today's analytical capabilities, it is rarely useful to consider more than two replicates for yield trials, and we believe unreplicated trials (in which only checks are replicated) may prove increasingly useful.

Spatial designs

Given that lattices do not consider the presence of spatial variability within sub-blocks, researchers have to find homogeneous sub-blocks in the field, without knowing their most appropriate shape, dimension, and orientation. The *a posteriori* approach for reducing residual variation can be applied when field variety trials are laid out in a rectangular array of rows and columns with replicates allocated contiguously. Spatial analysis can be performed to improve precision of estimates of variety effects and variety contrasts.

One approach is to adjust a plot for spatial variability by using information from its immediate neighbors. A useful measure for examining soil heterogeneity patterns is spatial autocorrelation of neighboring plots within rows or within columns (i.e., the correlation between residuals separated by various distances). If there is no spatial pattern, all correlations will be low. If there is pattern in the residuals, neighboring residuals will be more similar and so have higher correlation. Gleeson and Cullis (1987) proposed to sequentially fit a class of autoregressive, integrated, moving averages to the plot errors in one direction (rows or columns). This was in the context of randomized complete block experiments. They found that differentiating along the block and then fitting a moving average correlation structure to the residuals in that direction resulted in big gains in trial efficiency. Cullis and Gleeson (1991) extended the previous model to two directions (rows and columns) assuming that, in the field, rows and columns are regularly spaced.

Fertilizer Use

To express the full potential of a variety, sufficient nutrients must be applied to the experimental area. A soil test is useful for determining how much fertilizer to apply. Sufficient nitrogen, phosphorus, and potassium should be applied (as inorganic or organic sources, depending upon availability) to ensure these elements are not limiting. Care should also be taken to apply sufficient quantities of other nutrients, including micronutrients if they are known to be limiting. If varieties are planted when nutrients are limiting, they will be unable to express their true genetic potential, making it difficult to separate out the better lines since they will all yield poorly.

To prevent variation in the field, fertilizer should be applied as uniformly as possible, preferably by using a fertilizer spreader. When a spreader is not available, it is better to divide the fertilizer into smaller parcels or replications and apply each dose carefully to smaller areas. This is easily done for the basal application of fertilizer, but for the topdress application, the dose should be split by replication or plot, and the fertilizer carefully broadcast. If wheat is planted in a bed and furrow system, topdress fertilizer is best applied by machinery.

In farmers' fields, the fertilizer level recommended for assessment should be used and, in some cases, 50% above the recommendation, so the scientist can evaluate the potential of the variety in farmers' circumstances. The exception would be where the breeder is screening material for tolerance to specific nutrients. For example, boron is a known factor in wheat sterility. If a breeder wants to screen wheat lines for tolerance to low boron, he should have two sub-plots, one with applied boron and one without.

Irrigation

In rainfed trials, the experiment should be blocked to ensure uniform moisture distribution within replications. Where irrigation is applied, uniform application is necessary to avoid experimental error. Providing basins for each replication ensures that each plot will receive the same amount of water. In bed and furrow irrigation, replicating across the irrigation run ensures uniform water application within a replication. The advantage of bed and furrow configurations comes from more uniform water distribution, savings in water, and savings in land that would otherwise be needed to construct the basins and water distribution system.

Crop Protection Strategies

Unless the breeding trial is for evaluating germplasm against biotic stresses, crop protection measures should be used to ensure the varieties express their full yield potential. That may mean applying herbicides, insecticides, or fungicides, depending on the stresses present. Care should be taken when choosing the herbicide, since some lines may be susceptible. Labels should be carefully read and, when in doubt, other herbicides should be used or weeds controlled manually. Agronomists in the program should be screening germplasm against various herbicides so that this information is available to breeders.

Since breeders often wish to see the response of the lines to disease and insects, it is rarely necessary to spray for these factors.

Lodging

In the early years of the green revolution, lodging resistance was what enabled plants to break the yield barrier. The old, tall, traditional varieties lodged when fertility improved, and this restricted their yield potential. When new, shorter varieties with stiffer straw were introduced, fertilizer levels could be increased without lodging, which resulted in higher yields. However, even the new varieties lodged at higher fertilizer levels with late irrigation. Lodging is therefore still a major factor limiting yield potential and should be receiving more attention.

Several management methods can be used to reduce lodging and allow the full yield potential of the germplasm to be expressed. The following are some suggested options:

- A major reason for lodging is that the soil around the crown of the stem is wet and will not support the weight of the plant, especially when it is windy. This happens when wheat is planted on the flat and irrigation is given after flowering or during grainfilling. There are few options when wheat is planted on the flat except to apply irrigation in the late afternoon when winds tend to subside or to forego this irrigation. This may cause moisture stress and lead to lower yield, especially due to lower thousand-grain weight.
- Planting wheat on beds, especially with lower seed rates and two rows per 70-cm bed, can result in less lodging. In this system, later irrigation does not saturate the soil around the base of the plant as much as on the flat and so the plant is better supported by the soil. Plants are also exposed to more sunlight, much like border rows in solid stands. This makes the plants stronger and more able to resist lodging. In fact, planting wheat on beds will enable the plants to better express their yield potential than when planted on the flat because of better lodging resistance when more nitrogen is applied or grainfilling irrigation is given.
- Fertilizer timing can also be used to reduce lodging. If all the nitrogen is applied basally, the plants will be luxurious and competition for light will be high. This results in weaker plants more prone to lodging. If nitrogen can be reduced at planting and delayed until just around the first node stage (DC31, Zadoks' scale), it will be utilized more for grain than for excess foliage, and lodging will be less. Experiments have also shown that delaying nitrogen application until this later stage does not sacrifice yield potential but instead, because of less lodging, can give higher yields. Grain protein content will also be higher.
- Seed rate can be manipulated to help reduce lodging. If the plant stand is too thick, independent plants will be competing for light with adjoining plants and be weaker in the stem and more prone to lodging. By reducing the seed rate, lodging can be reduced.

If lodging is a factor in an experiment, it is important to take a good measure of it. Several factors are needed:

- Estimate of the area lodged in the sampled plot. The angle of the stem in relation to the vertical is also important. This datum can be used as a covariate in the analysis.
- The date when lodging occurred is important, though it is better to note the growth stage at which it happened. The timing and cause of lodging can usually be determined by reference to the occurrence of a storm or an irrigation event. Data should be collected as soon as lodging occurs since the angle and extent of lodging may change with time.

If breeders wish to measure the importance of lodging, they may consider growing one set of materials with support (e.g., using netting) and comparing with an unsupported check. Growth hormone sprays that reduce internode length may also reduce lodging and help measure lodging losses.

Harvesting and Sampling

Harvesting the crop and taking reliable samples are critical for breeding trials. If this is not done properly, all efforts to grow a good crop may be wasted. Taking samples as accurately as possible is essential for reducing experimental error. This will allow smaller differences between varietal means to be separated statistically.

In breeding trials, a sample area—rather than the whole plot—is generally used for estimating yield. As mentioned earlier, to reduce bias in the experiment, the border rows are removed and only the inner rows used for yield estimation. It is best to remove the outer rows and half a meter from either end of the plot. The remaining area can be cut and used for measuring yield and yield components. Plants should be cut close to the ground so that accurate harvest index and straw yield can also be determined.

Calculating Yield and Yield Components

Yield components can be calculated or measured individually. Three main harvesting methods are suggested for use when calculating yield components (Table 1). After harvest, yield components can be calculated according to formulas presented in Table 2.

Harvesting steps are similar across the three methodologies (Table 1); the procedure is described below under Method 1.

Method 1: Calculating yield components by harvesting total biomass

- Cut all aboveground biomass in a pre-determined area (A). Avoid border effects by sampling away from edges of plot.
- Sub-sample a set number (e.g., 50 or 100) of spike-bearing culms (i.e., spike, leaves, and stem) randomly from the plot sample and measure fresh weight (sub-sample fresh weight, or SSfw).
- Measure fresh weight of remaining plot sample (plot fresh weight, or Pfw).
- Dry the sub-sample of culms at 70ºC (facilitate by putting culms in a closed bag to avoid loss of grain, etc.) and weigh (sub-sample dry weight, or SSdw).
- Thresh plot sample for fresh grain weight (plot grain weight, or P-GW).
- Count 200 representative kernels and weigh fresh and after drying (200-grain fresh and dry weights, or 200fw/dw).

Method 2: Calculating yield components by harvesting a random sub-sample of culms

Yield components can be determined directly by taking random spike-bearing culms from the crop at physiological maturity.

- Take about 50 culms from rows (or area) to be harvested by grabbing a number of random sub-samples and cutting them off at ground level. All harvested rows should be represented in the sample.
- All culms in the sub-sample are dried at 70ºC.
- After measuring dry weight of the sub-sample (SS-dw), thresh to measure grain dry weight (sub-sample grain weight, or SS-GW).

This method has the advantage that hand harvesting is quick (<5 minutes/plot), and samples can be readily stored for processing when time is available. Note that with this method, measurement of harvest index is statistically independent of measurement of grain yield, whereas that of biomass is not independent of yield.

Table 1. Samples to measure when using three alternative harvesting methods for estimating yield, biomass, and yield components from experimental yield plots.

	Samples to be measured†						
Method	Plot biomass fw (g)	S-sample culms fw (g)	S-sample culms dw (g)	S-sample grain dw (g)	Plot grain fw (g)	200 kernels fw (g)	200 kernels dw (g)
1. Harvest total biomass	X	X	X		X	X	X
2. Harvest sub-sample of culms	X	X	X	X	X	X	
3. Reduced threshing	X	X	X	X		X	X

† S-sample= sub-sample; fw=fresh weight; dw=dry weight.

Table 2. Formulas for calculating yield components using three different harvesting methods.†

	Harvesting method		
Yield component	Method 1	Method 2	Method 3
Harvest index (HI)	P-GW*(200dw/200fw)/P-fw*(SSdw/SSfw)	SS-GW/SSdw	SS-GW/SSdw
Yield (g m^{-2})	[(P-GW*200dw/200fw)+(SSdw*HI)]/A	[(P-GW*200dw/200fw)+SS-GW]/A	Biomass*HI
Biomass (g m^{-2})	[(Pfw+SSfw)*(SSdw/SSfw)]/A	Yield/HI	[(Pfw+SSfw)*(SSdw/SSfw)]/A
1000-GW (TGW) (g)	200dw*5	200dw*5	200dw*5
Grains m^{-2}	Yield/TGW*1000	Yield/TGW*1000	Yield/TGW*1000
Culm dw (g)	SSdw/#culms sampled	SSdw/#culms sampled	SSdw/#culms sampled
Spikes m^{-2}	Biomass/culm-dw	Biomass/culm-dw	Biomass/culm-dw
Grains/spike	Grains m^{-2}/spike m^{-2}	Grains m^{-2}/spike m^{-2}	Grains m^{-2}/spike m^{-2}

† A=plot area harvested (m^{-2}); SS=sub-sample; fw=fresh weight; dw=dry weight; P=plot; GW=grain weight.
Note: Formulas assume that grain dried at 70ºC is at 0% moisture. Grain yield at x% moisture = Yield * [100/(100 – x)] (g/m^2).

Method 3: Calculating yield components with reduced grain-threshing requirement

Measuring grain yield and yield components with this method requires threshing only a sub-sample. The procedure is useful when larger-scale threshing machinery is lacking or when working with species that are difficult to thresh, such as *Triticum dicoccum* or synthetic hexaploid wheats. Measure the samples indicated for Method 3 in Table 1, and follow the relevant sampling procedures described under Methods 1 and 2.

Sampling Biomass in Lodged Crops

It is difficult to cut a given area in a lodged crop, especially if it has been sown by broadcasting. The process is facilitated by folding back spikes and stems to establish a starting reference line before inserting a quadrat. Great care must then be taken to collect only those plants whose crowns fall within the randomly located quadrat. If one side of a quadrat is separated from the other three sides, it is easier to insert the quadrat into the crop. In this case, the free fourth side is used to ensure the frame is properly square by placing it between the two free ends of the 3-sided frame.

Measuring Individual Yield Components in the Field

Plant population

A count of plant population should be made after the maximum number of plants has emerged and before tillering occurs (usually 10-14 days after adequate moisture becomes available for germination).

If plants are sown in rows, then 0.5 m length from each sampling row or from at least six such rows should be counted per plot. If broadcast, then samples of at least 0.5-1.0 m should be taken from each plot. The number of samples required will depend on the degree of variation within the plots, but at least two per plot should be recorded.

The mean plant density may disguise important variability in plant distribution (i.e., gaps that will cause yield reduction). This should be noted and measured by estimating the percent of the plot with missing plants.

Plant population can be used to assess the germination, vigor, and emergence of sown seed, and/or the extent of compensation under conditions advantageous to tillering. It is also needed if early growth per unit area is going to be monitored by successive measurements of growth per plant. Plant population typically varies between 50 and 300 plants/m^2. The number of plants/m^2 has a broad optimum and will vary with variety, climate, and management. However, under good rainfed conditions, 100 plants/m^2 could be considered the minimum for maximum yield, unless the crop is growing on residual moisture, in which case optimum density may be lower.

Spikes/m^2

The number of productive spikes can be measured nondestructively by counting in a given area or length of row, or calculated from sampling as demonstrated above.[3] Spikes per m^2 can be measured most easily before physiological maturity, which also reduces yield loss due to shattering caused by movement in the plots. In broadcast planting, direct measurement can be difficult, especially if crops lodge.

Spikes/m^2 can be used to assess the final number of productive spikes/ m^2 and can be combined with plant population count to assess the extent of tillering. Tillering typically ranges from 1 to 10 per plant. Spikes/m^2 is determined by events occurring from sowing to flowering and is dependent on variety, management, and environment.

Spikelets/spike

Sample a minimum of 10 spikes per plot at random (aim for a total of 30-40 spikes/treatment); select the culms from the base and count the number of spikelets. Take the average based on sample size. Most commonly, count the fully developed or grain-bearing spikelets (or at least those large enough to have at least one grain). Potential spikelet number is obtained by counting all the nodes on the rachis; it can exceed the developed spikelet number because of aborted spikelets at the base or tip of the spikes. Alternatively, under excellent environmental conditions, all potential spikelets may develop into grain-bearing spikelets.

The potential number of spikelets per spike is determined by the time of terminal spikelet formation (in wheat and triticale; barley does not form a terminal spikelet) around first node appearance. Subsequently, primordial spikelets at the base (and, later, at the tip) of the spike may abort because of stress. Normally, 10-25 spikelets form on each spike.

Grains/spikelet

Sample as for spikelets per spike. Count the spikelets, thresh, count grains, and calculate; or less accurately simply calculate from calculated grains per spike and measured spikelet number. When sampling large numbers of samples or plots, time may be saved by randomly counting one side of the spike and multiplying by two.

[3] If measured directly, the procedure and number of sub-samples are as for plant population.

Grains per spikelet is the result of both the number of competent florets/spikelet and kernels/competent floret (or grain set). Values for competent florets per spikelet typically vary from 1.5 to 5.0 and for kernels per competent floret from 0.6 to 0.99.

Grain set

Grain set refers to the percentage of competent or entire florets (florets with fully formed, plump green/yellow anthers at flowering) that actually produce grain (the opposite of percent sterility) and should reflect conditions around anthesis (e.g., pollen fertility, early grain survival), in contrast to grains per spikelet, which can be influenced by earlier conditions as well. However, at maturity, it is difficult to know which florets were competent. It is suggested that the basal two florets of the 6-10 central spikelets are always competent, and therefore a grain set index can be obtained by observing the percentage of such florets with grains. Sample as specified for spikelets or count 10 such spikelets in five random spikes per plot; the total of missed florets is the % sterility (= 100 – **%** grain set).

Alternatively, one can use matched spikes (i.e., spikes showing equal size and development). One spike is sampled at anthesis and the other at maturity, counting (destructively) competent florets at anthesis in one spike (i.e., florets showing normal anther development; non-competent florets will show whitened, flattened anthers with no fertile pollen, whose stamens never elongate) and grains at maturity in the other spike. At least 20 matched spikes per treatment are needed for reasonable accuracy; selection of matched spikes and counting at anthesis are time consuming.

Grain set is an indicator of stress events occurring around anthesis (e.g., drought, temperature extremes, boron deficiency, or genetic sterility, which can interact with the environment). It is a more precise and, hence, more useful measure than grains per spikelet or grains per spike.

Thousand-grain weight at maturity and during grainfilling

To measure thousand grain weight (TGW), count out two random samples of 100 entire grains (i.e., those possessing an embryo). Dry the grains at 70ºC (48 hours should be sufficient) and weigh. This will usually give sufficient accuracy. If weights differ by more than 10%, a third sample of 100 should be taken or recheck the counts.

To study grain growth during grainfilling, maximum accuracy is achieved by selecting groups of a sufficient number of spikes, matched for anthesis date and size, so that one can be randomly sampled from each group on each sampling date. Four to eight such groups (four to eight spikes each date) per treatment should be sufficient for accurately calculating grain growth rate by linear or curvilinear regression. The study can be based on all grains on the spike or in a given position on the spike (e.g., basal florets of central spikelets).

A reduction in TGW may be due to climatic or biological (e.g., pathogen) stress during grainfilling. Kernel weight (calculated as TGW/1000) is usually 20-50 mg. However, lower grain weight may not indicate stress during grainfilling, due to the plasticity of yield components. For example, if the plant population is high (leading to a high number of kernels/m^2), TGW may decrease without seriously affecting yield. TGW tends to be characteristic of a variety, and there are large differences between varieties even under good conditions. Within a variety, kernel weight usually shows a negative linear relationship to mean grainfilling temperature. Potassium deficiency can also result in low thousand grain weight.

Grain or kernel number (per m^2)

Kernel number per m^2 (KNO) is usually calculated by dividing grain yield (GY; in g/m^2) by kernel weight (KW; in mg):[4]

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KNO GY * (1000/KW)
```

Kernel number can also be independently measured by directly determining spike number (SNO/m^2), and kernels per spike (KPS) from at least 20 randomly sampled spikes per plot (aim for 60-100 spikes):

```
KNO=SNO * KPS
```

Kernel number per m^2 acts as a summary of all events up to and a little beyond anthesis. For example, the combined effects of management and climate on plants/m^2, spikes/plant, spikelets/spike, and grains per spikelet are all combined in this single term. Competent floret number (the precursor of kernel number) is also well correlated with spike (inflorescence only) dry weight at anthesis, the relationship being on the order of 100 florets/1.0 g spike (10 mg/floret), although the range across varieties for grain number is 70-140 kernels/g spike dry weight at anthesis.

Under many conditions, yield is a function of KNO, which is particularly dependent on crop growth rate during rapid spike growth (emergence of the second-to-last leaf—or about 1 month before anthesis for spring wheat—until just after anthesis).

[4] Using this calculation, KNO is statistically linked to GY and may give rise to spurious correlations between GY and GNO if GY is not determined accurately.

Yield Estimation and Measurement

Visual estimates

Yield can be estimated by visual assessment (this requires experience and a knowledge of the variety and area) or based on yield components from mid-grainfilling onward.

Yield estimates from yield components

To calculate yield from yield components, first estimate the number of spikes/m^2 from *in situ* counts (outlined above). Next, randomly sample and then count the number of grains/spike. Then, assume a TGW based on the variety and conditions expected during the rest of the grainfilling period (typical TGW under reasonable grainfilling conditions and temperatures: 30-40). Calculate yield with the following equations:

Yield (g/m^2) = spikes/m^2 * grains/spike *(TGW/1000)
Yield (kg/ha) = yield (g/m^2) * 10

Variability of the field or treatment and desired accuracy will determine the number of spike counts made. For greatest accuracy many small samplings are best and feasible when dealing with non-destructive sampling. For example, for a 1-ha drill-planted field, take spike number counts in 20 random but well dispersed 2-row x 50-cm quadrats and combine these counts with kernel number counts in 50 random spikes; a reasonable estimate of kernels/m^2 should result (but be careful to select sampling sites and spikes at random). Counting kernels per spike (one side x 2) while walking between quadrat sites saves time and should take less than 30 minutes. Be sure row spacing is accurately known and/or measure spacing to confirm; replicate the measurements for accurate assessment. For example:

- Average row spacing: 15 cm
- Average spike count (2 row by 50 cm): 40 spikes
- Spike sample area: 2 rows * 15-cm row spacing * 50 cm = 0.15 m^2
- 40 spikes/0.15 m^2 = 266.7 spikes/m^2
- Average kernel count/spike = 21.5
- Kernels/m^2 = 266.7 * 21.5 = S734
- Assume TGW = 40 (based on experience)
- Yield = 5734 * 40/1000 = 229.4 g/m^2 = 2294 kg/ha

Yield estimates from samples

Yield can be estimated as outlined for yield components. Alternatively, borders can be discarded and yield estimated from the remaining plot that is harvested. In some instances, it is not possible (especially in on-farm trials) to harvest the entire plot or to thresh the entire plot sample. In these cases, follow the options outlined above or sample 5 x 1 m^2/field or 2 samples of 1 m^2/plot if there are 3 replicates.

Yield moisture content

The grain trade and farmers usually express yield at a given moisture content (e.g., 10% in Australia, 12 or 14% in Europe on a fresh weight basis). Therefore, conversion factors are required to adjust moisture. Moisture content is calculated as the weight of moisture relative to fresh weight:

[Moisture/(moisture + dry weight)]

The following formulas outline various moisture calculations. Yield and grain moisture calculations:

Field weight of harvested grain = FW (kg)
Harvested area = A (m^2)
Fresh weight of sub-sample = WS
Oven-dry weight of sub-sample = DS

Grain moisture conversions:

Grain moisture content (M%)
M% = [(WS – DS) * 100] /WS
Yield (unknown moisture, GYm)
GYm (t/ha) = (FW * 10)/A
Yield (0% moisture, GY(0%))
GY(0%) = [GYm * (100 - M)]/100
Yield (X% moisture, GY(X%))
GY(X%) = [Y(0%) * 100]/(100 – X)

Throughout the previous discussion, all weights of plant parts (including grain) refer to constant weight after drying at 70°C. However, the American Association of Cereal Chemistry (AACC) defines 0% moisture as that achieved by drying ground grain at 103°C. Therefore, other conversion factors are required in addition to the above to obtain a true 0% moisture reading. To convert the weight of grain dried for 20 hours at 70°C to moisture content as defined by AACC, divide the weight by 1.025 (because grain dried at 70°C has approximately 2.5% moisture). The factor drops to 1.012 as drying time at 70°C increases to 48 hours. This means that, to express grain at 10% moisture, the oven-dry weight (70°C for 24 hours) needs to be multiplied by 1.084 (i.e., 1.00/1.025).

Assessing Crop Residue

Direct measurement

Collect and bulk straw found within five random samples (or two per replicate) of at least 1.0 m^2. Oven dry straw (70°C) and weigh. Convert g/m^2 to t/ha by dividing by 100.

Due to the generally large spatial variation in ground cover, a visual estimate (by an experienced researcher) is often sufficiently accurate using the method described below.

Estimating percent residue cover using the line-transect method[5]

The line-transect method is one of the easiest methods to use for determining the percent residue cover on the soil surface. Accurate measurement is necessary to determine if enough cover is present to comply with the conservation plan.

The following is a step-by-step procedure for using the line-transect method to measure the percentage of residue cover.

Step 1. Use a 100- or 50-m measuring tape for measuring residue cover. Other measuring lengths or even knotted ropes can be used if the appropriate multiplication factor is used to calculate the percentage.

Step 2. Select an area that is representative of the whole field/plot. Avoid end rows or small areas that have been adversely affected by flooding, drought, weed or insect infestations, or other factors that may have substantially reduced yields.

The most accurate reading of residue cover is obtained by taking an average from at least three different representative locations in the field.

Step 3. Anchor one end of tape and stretch it diagonally across the rows so that it crosses several passes by the farming implements. Readings are more accurate when the tape is stretched diagonally across the rows than when taken in a window of residue left by the combine or where the amount of residue is smaller.

Step 4. Measure residue cover by counting the number of "m" marks that are directly over a piece of residue, as shown below. For an accurate reading,

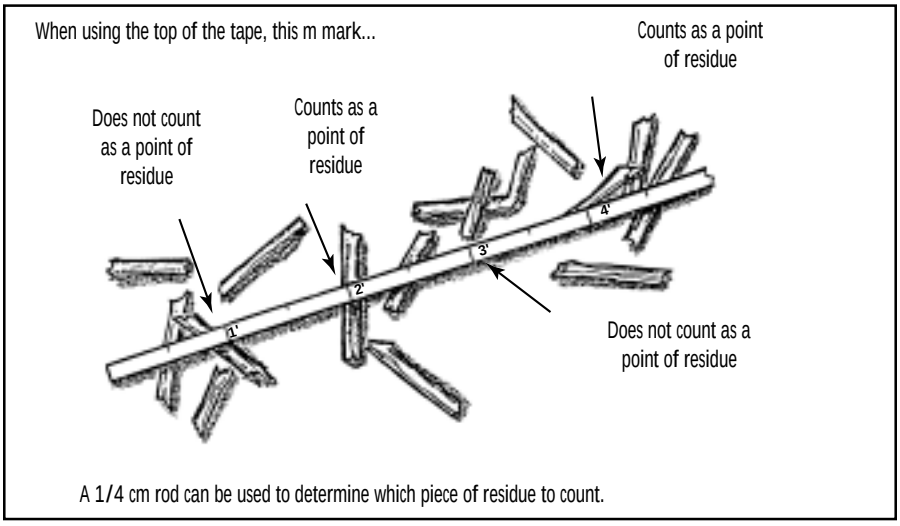
- do not move the tape while counting;
- look at the same side of the tape at each m mark;
- look straight down at the tape and the m mark, and
- count only those m marks that have residue directly under them.

To effectively reduce erosion, a piece of residue must be large enough to dissipate the energy of a raindrop during an intense storm. To be counted, the residue must be larger in diameter than this dot●

A convenient way to determine if a piece of residue is large enough to count is to use a 1/4 cm diameter brazing rod or wooden dowel. Touch the edge of the rod to the m mark. If the residue extends completely beyond all edges of the rod end, count it. If the rod covers the residue completely or if part of the rod end extends beyond the edge of the residue at any point, don't count it because a raindrop falling on this point would strike some unprotected soil.

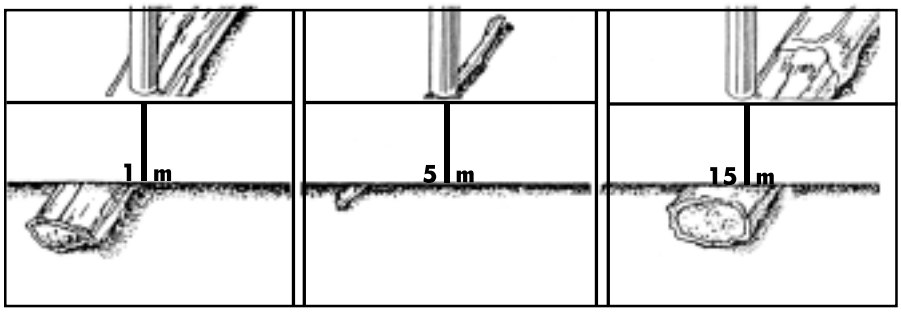
On a 100-m tape, the number of m marks that are directly over residue will be a direct measurement of the percentage of residue cover for the field. On a 50-m tape the number of m marks must be multiplied by 2. If other measurement lengths are used, the appropriate multiplication factor must be used, especially for plot-level measurement.

Residue amount can be used to assess soil cover and thus potential evaporation reduction but, more importantly, erosion control. Assuming uniform distribution, approximately 4 t of wheat straw lying horizontally are required to give 100% ground cover. Straw is also a potential source of disease infection for subsequent crops and may immobilize N during the decomposition process.



A 1/4 cm rod can be used to determine which piece of residue to count.

[5] This step-by-step description of the line-transect method was taken from a fact sheet by David P. Shelton, and Elbert C. Dickey, Extension Agricultural Engineers, University of Nebraska; Robert Kanable, Conservation Agronomist, Soil Conservation Service; Stewart W. Melvin, Extension Agricultural Engineer, Iowa State University; and Charles A. Burr, Extension Agricultural Specialist, University of Missouri. The fact sheet was produced through the cooperative efforts of representatives of Cooperative Extension, Midwest Plant Service, NACD's Conservation Technology Information Center, U.S. Environmental Protection Agency, and the USDA Soil Conservation Service.



Counts
raindrop strikes residue

Does not count
raindrop strikes both soil and residue

Does not count
raindrop strikes both soil and residue

Only those **"m"** marks having a piece of residue directly under them should be counted.

Ancillary data

It is always useful, especially for interpretating the results, to collect other site data. The following are some minimum data sets for consideration.

Climate data. The performance of any crop is very dependent on climate. Usually, there is a weather station close by that can provide this valuable supporting information. The following data would be useful:

- maximum, minimum, and average daily temperature
- precipitation
- radiation data or sunshine hours
- relative humidity (max-min, if possible)

Soil data. Soil data such as the following are useful for interpreting results:

- soil texture
- soil pH
- conductivity (a measure of salinity)
- infiltration (a physical measure of porosity)
- soil moisture holding capacity (permanent wilting point and field capacity)
- soil carbon level (a measure of organic matter)
- available P and K levels
- micronutrients status
- penetrometer measures to assess if plow pan exists
- water table depth
- irrigation water applied, numbers, and, if possible, amounts

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CHAPTER 5

Recent Tools for the Screening of Physiological Traits Determining Yield

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This review presents practical guidelines for breeders of wheat (and other small grain cereals) interested in adopting a physiological approach to crop improvement. Some of the most promising tools for fast, reliable characterization of yield-determining traits will be discussed from an ecophysiological perspective. We will focus on the practical aspects and limitations of using relevant screening tools or selection criteria. The potential contributions of physiological research to plant breeding, as well as its inherent limitations, have been extensively reviewed from a breeding perspective (for example, Jackson et al., 1996). A theoretical framework for identifying yield determinants that are obvious candidates for evaluation has also been established (see below), although it has not been used in practical breeding.

The use of physiological traits as screening tools in breeding is still largely experimental—for different reasons. Sometimes the traits are very indirectly related to yield (Araus, 1996; Richards, 1996) or there is little ecophysiological understanding of the crop, especially when breeding for yield under stress. Nevertheless, breeding for crop escape has been very successful, and phenological changes have been the most important indirect factor in increasing wheat yields under Mediterranean conditions (Slafer et al., 1993; Loss and Siddique, 1994). However, in breeding for crop resistance, the evaluated traits and screening tools are often related to tolerance, not avoidance (see definitions in Larcher, 1995).

An indirect (i.e., physiology-based) breeding strategy could fail to produce yield gains and might even lead to a decrease in yield. Improved plant tolerance, though it protects the crop, can limit yield potential. The target environment where selection is carried out must be defined *a priori*, and the possibility of a negative breeding effort should not be ignored. In fact, plants that show the **most tolerant response during screening may also be the most sensitive**, in terms of yield loss, for example, because they are unable to delay the effect of stress at the cellular level.

The most promising methods allow for quick screening of "integrative" physiological traits (Araus, 1996), so called because they integrate physiological processes either in time (i.e., during the plant cycle) or at the organization level (e.g., whole plant, canopy). Other quick screening methods for evaluating, for example, the photosynthetic performance of plants under stress conditions have been proposed—among them, chlorophyll fluorescence measurements on single leaves. However, under field conditions fluorescence values may only reflect differences in phenology across genotypes. Nevertheless, remote sensing detection of fluorescence spectra at the canopy level could become a promising approach for breeding purposes (Lichtenthaler, 1996).

Identifying Physiological Traits for Use As Selection Criteria

One approach to search for traits that could be used in breeding programs is to identify the physiological processes determining productivity. A crop's yield potential (or harvestable part, Y) over a given period of time can be divided into three major processes (Hay and Walker, 1989). First, the interception of incident solar radiation by the canopy; second, the conversion of intercepted radiant energy to potential chemical energy (i.e., plant dry matter); and third, the partitioning of dry matter between the harvested parts and the rest of the plant. Whereas the first component depends on the canopy's total photosynthetic area, the second relies on the crop's overall photosynthetic efficiency (i.e., total dry matter produced per unit of intercepted radiant energy); the third is harvest index. Total biomass, which is the result

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of the first two components, can be physiologically defined as the result of canopy photosynthesis over time.

Other approaches may be followed, depending on agroecological conditions. For example, under water-limiting (e.g., Mediterranean) conditions, the most widely used framework, proposed by Passioura (1977), allows the study of indices that maximize yield per rainfall unit. Thus economic yield depends on the total water transpired by the crop, water use efficiency, and harvest index. Although these three components are not truly independent, Passioura's is a useful framework for searching for critical traits to improve yield under drought.

Yield can be divided into several integrative components or traits. Yield itself is the most integrative trait, because it is influenced by all factors **(**known and unknown**)** that determine productivity. However, there are many known limitations in a purely empirical breeding approach based only on yield. Therefore, any breeding strategy based on a physiological (i.e., analytical) approach should use screening tools or criteria to evaluate the integrative physiological parameters that determine harvestable yield with a single measurement. Although harvest index has been the most successful trait when modified to improve yield, the other two components of the above equations, which are responsible for total crop biomass, remain **(**basically**)** unchanged. In the following pages we will focus on tools used to evaluate physiological traits determining total biomass. We will discuss two different kinds of tools for screening integrative physiological traits: carbon isotope discrimination (Δ) and indices based on canopy spectral-reflectance.

The Δ not only evaluates genotypic differences in water use efficiency, but can also be affected by the total amount of water transpired by the crop (the first component of Passioura's equation) or by photosynthetic activity (the second component of the yield potential equation). Keeping in mind developing country breeders, we have included under the generic title "surrogates" other screening criteria, such as ash accumulation in different plant parts or criteria related to leaf structure. Though these features are not Δ surrogates in a strict sense, they are always related to yield and are both quicker and cheaper than Δ determinations; furthermore, no large facilities or highly qualified technical support are necessary to use them.

Canopy spectral reflectance is one of the most promising remote sensing techniques (see also Araus, 1996). Although at present the equipment is very expensive, in a few years its cost should drop dramatically. Another remote sensing technique, canopy temperature, provides integrated information on the crop's stomatal conductance at the canopy level (see chapter by Reynolds) (Reynolds et al., 1994) and has the advantage of being low cost. However, its usefulness is limited in severely stressed environments, and genotypic differences in phenology and canopy architecture can further limit its validity.

Carbon Isotope Discrimination

For C_3 plants, discrimination (Δ) of the heavier (^{13}C) stable carbon isotope over the lighter, more abundant (^{12}C) form (99%) during photosynthetic CO_2 fixation is an integrated measure of internal plant physiological properties and external environmental conditions that influence photosynthetic gas exchange (Farquhar et al., 1989). In C_3 cereals such as wheat, Δ is positively related to CO_2 levels in intercellular air spaces (Diagram 1) (Farquhar et al., 1982; Farquhar and Richards, 1984; Ehdaie et al., 1991) and, given a constant leaf-to-air vapor pressure difference, also negatively related to water use efficiency (WUE, measured either as net photosynthesis/transpiration, also called transpiration efficiency, or as plant biomass produced/water transpired) (Farquhar and Richards, 1984; Hubick and Farquhar, 1989). Plants with high WUE would be less able to discriminate against ^{13}C, and thus would accumulate more of the heavy carbon isotope in their tissues than less efficient water users.

When measured in plant dry matter, D provides an (integrated) indication of WUE throughout plant growth (Farquhar et al., 1982, 1989). D has been proposed as a possible screening tool for identifying variations in WUE in wheat (Farquhar and Richards, 1984; Ehdaie et al., 1991; Condon and Richards, 1993) and barley (Hubick and Farquhar, 1989). In fact, the permanent relationships between WUE and D during treatment and the high broad-sense heritability of D in wheat, together with other considerations, indicate that D may be useful for modifying the WUE and yield of water-limited wheat crops (Condon et al., 1987; Condon and Richards, 1992, 1993).

The relationship between Δ and water-use efficiency

Following on the model of Farquhar et al. (1982), Δ in C_3 plants may be defined in its simplest form as:

$$\Delta = a + (b - a)(p_i/p_a),$$

where a is the carbon-13 discrimination caused by diffusion in air (4.4 ‰), b is that caused by carboxylation by the

RuBP carboxylase enzyme (27 ‰), and (pi/p_a) is the intercellular to atmospheric CO_2 partial pressure ratio. Conversely, p_i/p_a may be defined as a function of Δ:

$$p_i/p_a = (\Delta - 4.4)/2.6.$$

If we assume that the temperature of the leaf (or other photosynthetic organ) is close to air temperature, and if daytime relative humidity is also known, WUE (the net assimilation to transpiration ratio) may be defined as a function of p_i/p_a:

$$WUE = p_a(1 - p_i/p_a)/V(1 - RH)1.6,$$

where V is the saturated partial water vapor pressure at a given temperature, RH is relative humidity, and 1.6 is the psychometric constant (Farquhar et al., 1982). Thus, WUE can be estimated from Δ values using the following equation:

$$WUE = pa[1 - (\Delta - 4.4)/22.6]/V(1 - RH)1.6.$$

An agronomic estimation of WUE (considered as the ratio of dry matter accumulated to total water transpired) can be also be derived from Δ using different equations (Hubick et al., 1986; Hubick and Farquhar, 1989; Craufurd et al., 1991; see also Araus et al., 1993).

Methodological considerations

Carbon isotope analysis is performed by mass spectrometry. Although the equipment needed for such testing is expensive and often beyond the capability of many laboratories and research stations, there are commercial firms that do the analyses reliably and at a reasonable price. Before sending the material to be analyzed, it must be ground very finely.

$^{13}C/^{12}C$ ratio values are expressed as carbon isotope composition ($\delta^{13}C$) values, where

$$\delta^{13}C\ (‰) = [(R\ sample/R\ standard)-1] \times 1000,$$

R being the $^{13}C/^{12}C$ ratio. The standard for comparison is a secondary standard calibrated against PeeDee belemnite (PDB) carbonate. Test precision is usually lower than 0.10 ‰. The value of the discrimination (Δ) against ^{13}C is calculated from δ_a and δ_p, where a refers to air and p to plant (Farquhar et al., 1989):

$$\Delta = (\delta_a - \delta_p \Delta / (1 + \delta_p)$$

On the PDB scale, free atmospheric CO_2 has a current deviation, δ_a, of approximately -8.0 ‰ (Farquhar et al., 1989).

Implications for plant breeding

What type of sample to take and when?

Considerable genotypic variations for Δ have been found in bread wheat (Condon and Richards, 1992), barley (Romagosa and Araus, 1991; Acevedo, 1993), and durum wheat (Araus et al., 1993a), but environmental factors may cause even larger changes in the value of Δ, which could compromise the effective use of Δ in breeding programs. After studying wheat Condon and Richards (1992) concluded that assessing genotypic variation in Δ would be most effective under well-watered conditions. In this regard, Richards and Condon (1993) pointed out that under adequate conditions, Δ is highly heritable and exhibits substantial genetic variation and few genotype x environment interactions.

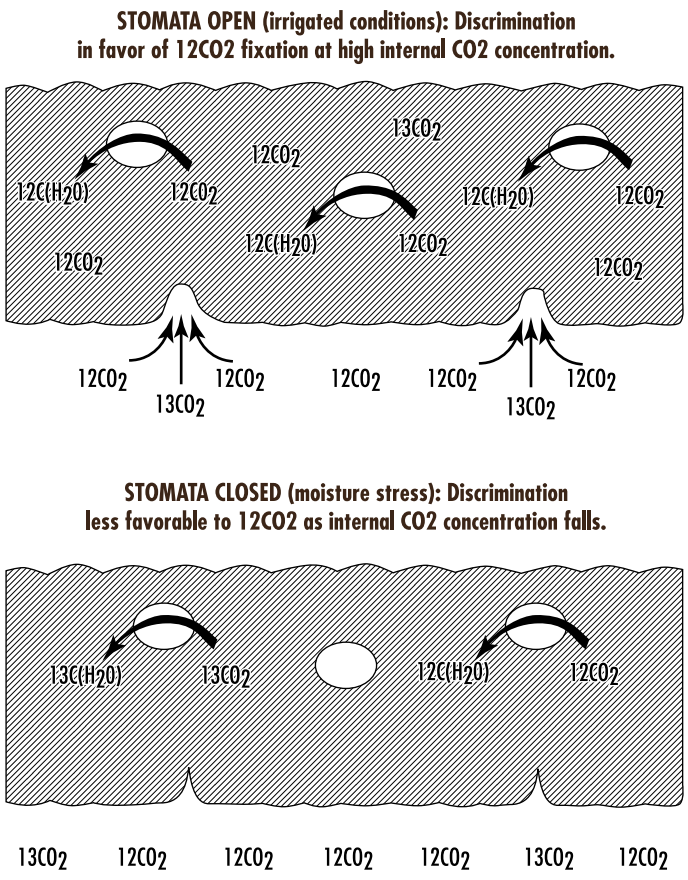

Diagram 1. Carbon isotope discrimination under irrigated and dry conditions.

As an alternative in rainfed environments, Condon and Richards (1992) proposed sampling for Δ at early crop stages, when terminal stress is lacking. However, the information available on rainfed environments often does not support these expectations. Though correlation between Δ and yield is usually weak or non-existent when dry material from seedlings is analyzed (Bort et al., 1998), it increases when upper plant parts are used in Δ analyses (Figure 1). The best genetic correlations between Δ and yield, together with the high broad sense heritability of Δ, have also been reported for the upper parts of durum wheat (Araus et al., 1998b).

The correlation between yield and Δ increases with plant age, perhaps due to the effects of progressive stress (particularly after anthesis) on yield. In fact, Δ usually decreases from the oldest to the youngest plant parts, even under well-watered conditions (Hubick and Farquhar, 1989; Acevedo, 1993; see also Figure 1). Such a decrease may be attributed to stomatal closure in response to declining soil water and/or increasing vapor-pressure deficit during the last period of crop growth (Condon and Richards, 1992; Condon et al., 1992; Araus et al., 1993b). Thus, mature kernels could be the most adequate plant part to sample. Under Mediterranean conditions, for example, the Δ of kernels rather than the Δ of lower plant parts may provide more information on which genotype is less affected by stress during grain filling.

Higher or lower carbon isotope discrimination? In water-limited environments, genotypes with low Δ should have greater biomass and hence potential for higher yields, assuming that all genotypes use the same amount of water for transpiration (Richards, 1996). In fact, selecting for high WUE (Passioura, 1977) or low Δ (Craufurd et al., 1991) has been proposed as an important alternative when defining plant breeding strategies under limited water conditions. However, Δ values often correlate positively with grain yield and/or total biomass in wheat (Condon et al., 1987; Kirda et al., 1992; Araus et al., 1993c, 1997b; Morgan et al., 1993; Sayre et al., 1995) and barley (Romagosa and Araus, 1991; Richards, 1996) under well-irrigated or rainfed conditions (Figure 1).

From an agronomic point of view, a positive relationship between Δ and yield may exist if plants are not using all available soil water. Assuming the same phenology, a genotype with high Δ will

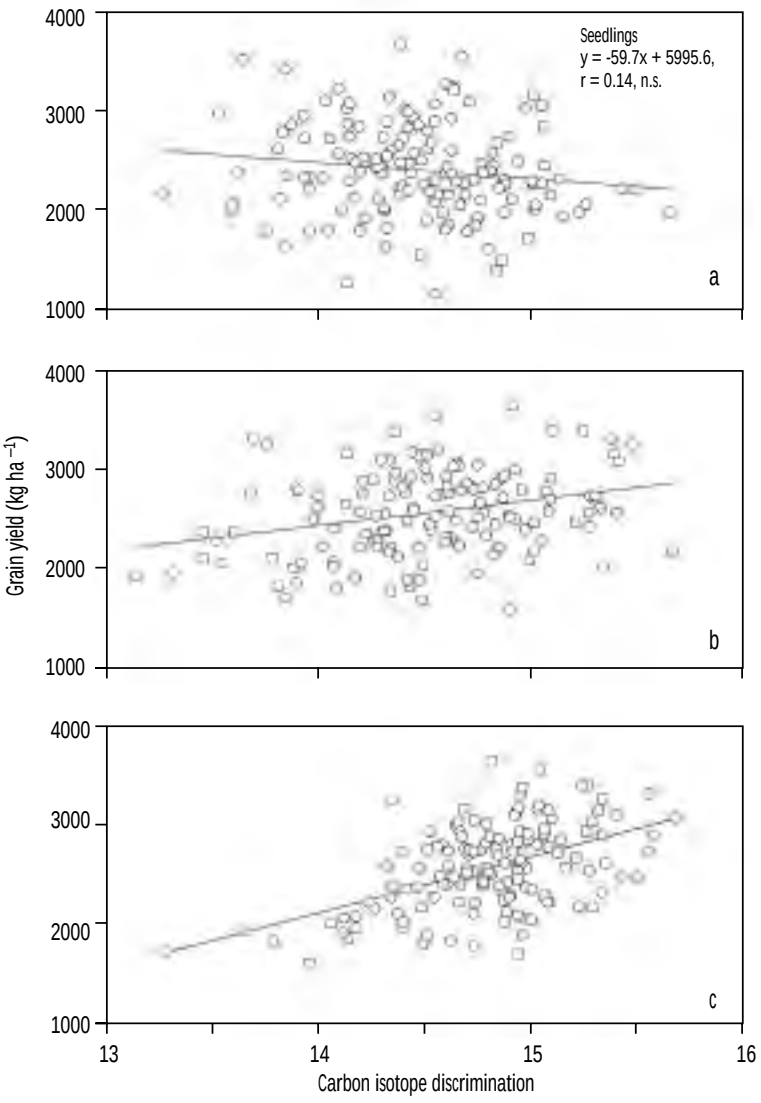

Figure 1. Relationships between grain yield and carbon isotope discrimination (Δ) measured in dry matter of seedlings (a), in the penultimate leaf sampled around heading (b) and in mature kernels (c) for a set of 144 durum wheat genotypes grown under rainfed conditions in the Tel Hadya, northwestern Syria. For more details, see Araus et al. (1997b).

be able to sustain a high level of transpiration. Therefore, Δ can be considered an indicator of WUE, but also depends on the water transpired by the crop, which is in fact the first parameter of Passioura's identity. The positive association between Δ and yield also suggests that variations in stomatal conductance rather than in intrinsic photosynthetic capacity are predominant in determining Δ (Romagosa and Araus, 1991; Condon et al., 1992). Higher Δ is related to a higher level of CO_2 in the cellular air spaces due to greater stomatal conductance (Farquhar and Richards, 1984), which leads to higher photosynthetic rates and, hence, higher yield even in the absence of stress. In this situation, WUE decreases (and Δ increases) because stomatal limitation reduces transpiration more than photosynthesis, even when yield may be positively affected by low stomatal limitation on photosynthetic rate.

Relatively high transpiration levels may have implications for water-limited environments. For example, when water supply can be provided to the crop under drought stress, (e.g., by deep soil moisture extraction), the high-yielding (i.e., high-transpiring) genotype will have the most advantage (Blum, 1993, 1996). In fact, relatively low canopy temperatures resulting from high stomatal conductance and transpiration are typical of the more drought-resistant genotypes (Garrity and O'Toole, 1995; see also Blum, 1996). In addition, when grown at above-optimal temperatures, such as the maximum daily temperatures typical during grain filling, the positive correlation observed between stomatal conductance and yield may also be related to heat avoidance (Reynolds et al., 1994).

Alternatively, mechanisms that prevent water loss, such as inherently lower stomatal conductance, may limit yield potential because of the intercellular levels of CO_2, thus decreasing photosynthesis. These genotypes will consistently show low Δ values (Morgan et al., 1993). In fact, stomata that close only in response to severe water stress may be more useful in terms of yield than stomata that permanently show low conductance values (Jones, 1987). Moreover, selection for low Δ (i.e., high WUE) may favor low-producing genotypes under drought conditions (i.e., drought-susceptible genotypes). Therefore, low Δ may not be a good selection criterion for improving yields in dry environments. Plant production under drought conditions depends not only on WUE but largely on the genotype's capacity to sustain transpiration (Blum, 1993).

Blum (1996) pointed out that when soil moisture is very limited, the high-yielding genotype may be at a disadvantage because of its high stomatal conductance. In fact, it has been reported for wheat and barley that this (crossover) happens if yield is reduced to below 2-3 t ha^{-1} (Ceccarelli and Grando, 1991; Blum, 1993). Other reports on durum wheat and barley do not support the existence of such a crossover in environments with a mean grain yield of 1.5 t ha^{-1} or lower (Romagosa and Araus, 1991; Araus et al., 1998b). In these environments it may still be worth selecting for yield potential, particularly if deep extractable soil moisture is available to provide a yield above that of the crossover of genotype yields (Richards, 1996). Summarizing, the above results support the hypothesis that under Mediterranean conditions high-yielding genotypes, which sustain greater stomatal conductance and transpiration losses during grain filling, can provide higher yields in a wide range of environments with different levels of drought stress.

Optimal yield conditions. Carbon isotope discrimination has also been proposed as a useful trait to select for yield potential (Araus et al., 1993c; Sayre et al., 1995; Araus, 1996). The positive correlation between Δ and yield would exist in the same context as above. A positive relationship between Δ and growth has also been reported for seedlings grown under adequate water conditions (Febrero et al., 1992; Lopez-Castañeda et al., 1995), even when under field conditions cold stress (usual at this early stage) may obscure such a relationship (Bort et al., 1998). Nevertheless, increased early growth and leaf area development may be inherently linked to decreased WUE (Turner, 1993) and thus to higher Δ. Blum (1996) pointed out that the data accumulated on carbon isotope discrimination and yield appears to support a consistent positive relationship between crop yield and photosynthetic capacity for various genetic materials of wheat and other crops (Hall et al., 1994). If selection for high photosynthetic capacity or higher crop productivity brings about an increase in stomatal conductance, then a concomitant increase in Δ (or a decrease in crop WUE) is expected (Blum, 1996).

Role of phenology in genotypical differences in Δ. In the absence of stress, Δ in wheat is independent of phenological differences (Sayre et al., 1995). However, under non-optimal conditions the role of phenology in the relationship between Δ and yield must be considered. Thus, as pointed out before, in Mediterranean environments phenology is the most important factor that accounts for increased wheat yields, as it affects assimilate partitioning, the pattern of water use, and other traits (see references in Slafer et al., 1993; Loss and Siddique, 1994). In addition, some of the genotypical differences in Δ, as well as their positive association with

yield, can be due to phenology. Thus early flowering lines are more likely to have high Δ than later-flowering lines due to the lower transpirative demand, which maintains higher stomatal conductances in the former (Ehdaie et al., 1991; Acevedo, 1993). Summarizing, under Mediterranean conditions early-flowering in wheat and other cereals is related to higher yields, which is in accordance with higher Δ in the earlier genotypes. Alternatively, there is genotypical variability in Δ that cannot be explained by phenology (Condon and Richards, 1993; Richards and Condon, 1993; Araus et al., 1998b) and is just due to differences in accumulated transpiration.

Δ Surrogates

Given the cost and technical skills involved in carbon isotope analysis, different "surrogates" of Δ have been investigated, such as mineral accumulation in vegetative plant parts (Figures 2 and 3) and leaf structure. Instead of being Δ substitutes or surrogates, these selection criteria probably allow the evaluation of traits, other than WUE, that determine yield. Regarding the first criterion, if passive transport driven by transpiration is (at least partially) the mechanism of mineral accumulation in vegetative parts, then mineral content will also be an indicator of the first parameter of Passioura's identity, i.e. total water transpired. The second trait corresponds to structural criteria that indicate the amount of photosynthetic tissue per unit leaf area and is therefore related to photosynthetic capacity. In addition, the mechanisms underlying the physiological association between Δ and either mineral accumulation (Walker and Lance, 1991; Masle et al., 1992) or the amount of photosynthetic tissue (Araus et al., 1997a, b) are not fully understood. However, the empirical relationships of these alternative criteria with Δ and yield may justify their use on a routine basis. Such tools might be used during the early phases of a breeding program, which usually involve large populations. If the facilities or the funds are available, later selections could be based on more precise and accurate, yet expensive, Δ analysis (Mayland et al., 1993). These two alternative criteria are discussed in the following paragraphs. Interestingly, these surrogates can be used in C_4 crops (such as corn or sorghum), where Δ is not as useful for evaluating WUE and yield itself as in C_3 plants (Farquhar et al., 1989; Henderson et al., 1998).

Mineral content in vegetative parts

Potassium, silicon, total mineral, or ash content accumulated in vegetative tissues have been proposed as surrogates of Δ in cereals, forage crops, and soybean (Walker and Lance, 1991; Masle et al., 1992; Mayland et al., 1993; Mian et al., 1996). Masle et al. (1992) reported for all the herbaceous C_3 species they assayed a positive linear relationship between total mineral content of vegetative tissues and the inverse of either WUE or Δ. Therefore, the amount of minerals accumulated by plants in the glasshouse or in the field could be a potentially useful indicator of Δ and WUE (Walker and Lance, 1991; Masle et al., 1992; Mayland et al., 1993).

In theory, total mineral and ash content seem to be better surrogates than the content of any single mineral, such as silicon or potassium (Masle et al., 1992; Mayland et al., 1993). Therefore, estimating plant mineral content, especially ash content, which requires only a muffle furnace, might be an attractive alternative to Δ for preliminary screening of large, genetically diverse populations (Masle et al., 1992; Araus et al., 1998b).

Methodological considerations regarding ash content. Samples must be properly oven-dried and ground. Approximately 1 to 1.5 g of dry matter is placed in a pre-weighed porcelain crucible (empty crucible), the crucible with the sample is weighed (filled crucible), and the sample is burnt in a furnace at 450°C for 12 h. Then the crucible with mineral residue (burnt crucible) is weighed again. Ash content is expressed (on a concentration basis) as a percentage of sample dry weight as follows:

$$\text{Ash content (\%)} = \frac{\text{(burnt crucible weight - empty crucible weight)}}{\text{(filled crucible weight - empty crucible weight)}} \times 100$$

Implications for plant breeding: Choice of environment and type of sample. The positive correlation between ash content and Δ may indicate that plants able to maintain higher stomatal conductance and transpiration will accumulate more ash in a transpirative organ, provided entry and accumulation of minerals in the plant take place (at least partially) through the transpirative stream.

Which are the best growing environments for using this surrogate? Mineral accumulation seems to be better related to Δ and even to yield under well-watered conditions (Masle et al., 1992; Mayland et al., 1993), although these traits can be useful under drought conditions (Figure 2a; Araus et al., 1998b). Measurements taken on plants grown in the greenhouse can give contradictory results (Walker and Lance, 1991). Another question is the kind of sample to use. Later developed leaves (flag or penultimate leaves) are best. Ash accumulated in the flag leaf may be positively related to the Δ of kernels (Araus et al., 1998*b*). Leaves must be mature to let minerals accumulate, but not senescent because minerals can remobilize to other plant parts. Thus, ash content measured at maturity in straw did not correlate with either Δ of mature grains or yield. (Voltas et al., 1998).

Mineral content in mature kernels as a criterion to complement Δ

A negative relationship between ash content (on a dry mass basis) in mature kernels and yield has been reported for barley and durum wheat, under both optimum and non-optimum (i.e., rainfed) conditions (Febrero et al., 1994; Araus et al., 1998b; Voltas et al., 1998). This may be explained by the fact that ash content on a kernel mass basis may be an indirect indicator of total reproductive sink per culm attained at maturity (Araus et al., 1998b). In fact, total kernel mass per spike is the product of two yield components developed last during the crop cycle: number of kernels per spike and kernel mass. Thus, kernel ash has been proposed as a criterion complementary to kernel Δ in assessing genotype differences in cereal yield under Mediterranean conditions (Febrero et al., 1994; Voltas et al., 1998). In theory (Diagram 2), the pattern of ash accumulation in kernels is different from that in vegetative tissues because, unlike mineral accumulation in vegetative tissues, grain filling does not take place via the xylem (driven by transpiration) (Slafer et al., 1993). Such differences in mineral accumulation could explain the complementarity of ash content and Δ in kernels as integrative traits predicting grain yield (Febrero et al., 1994; Voltas et al., 1998).

Summarizing, kernel ash combined with either kernel Δ or leaf ash can be partially complementary (i.e. independent) parameters when assessing differences in grain yield (Febrero et al., 1994; Araus et al., 1998b; Voltas et al., 1998). Selecting for low ash content in kernels, combined with either high Δ in kernels or, alternatively, high ash content in the flag leaf, deserves further attention in wheat breeding (Figure 3). This approach could be particularly interesting if it were coupled with a new analytical technique

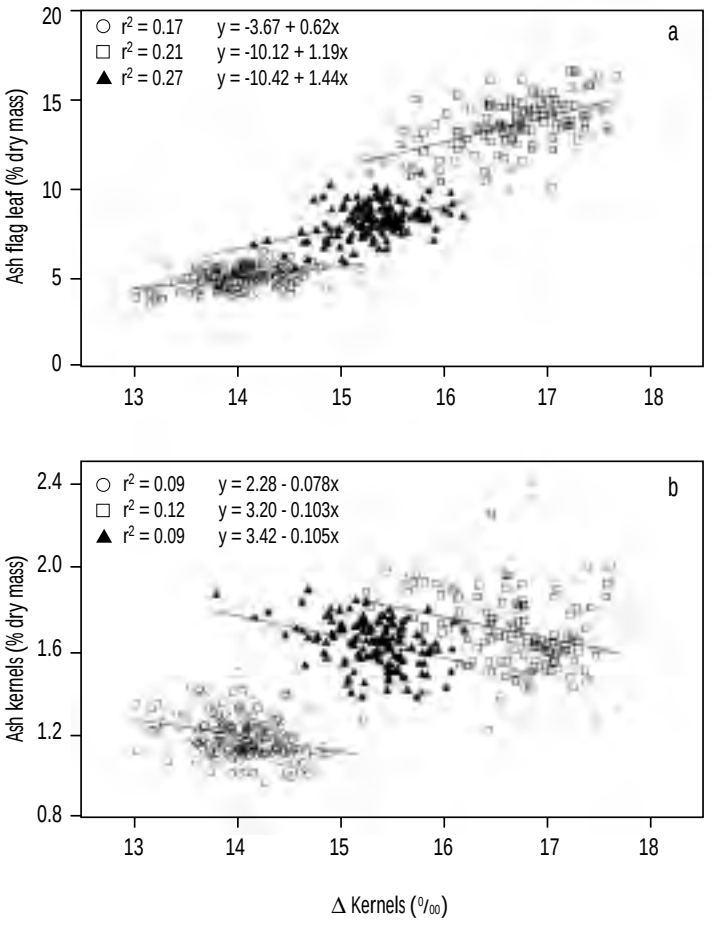

Figure 2. Relationship between carbon isotope discrimination (Δ) in mature kernels and ash content based on dry mass (a) of the flag leaf blades around three weeks after anthesis, and (b) in the same mature kernels. Plants were cultivated in three trials differing in water status: Breda, Tel Hadya rainfed, and Tel Hadya with supplementary irrigation.

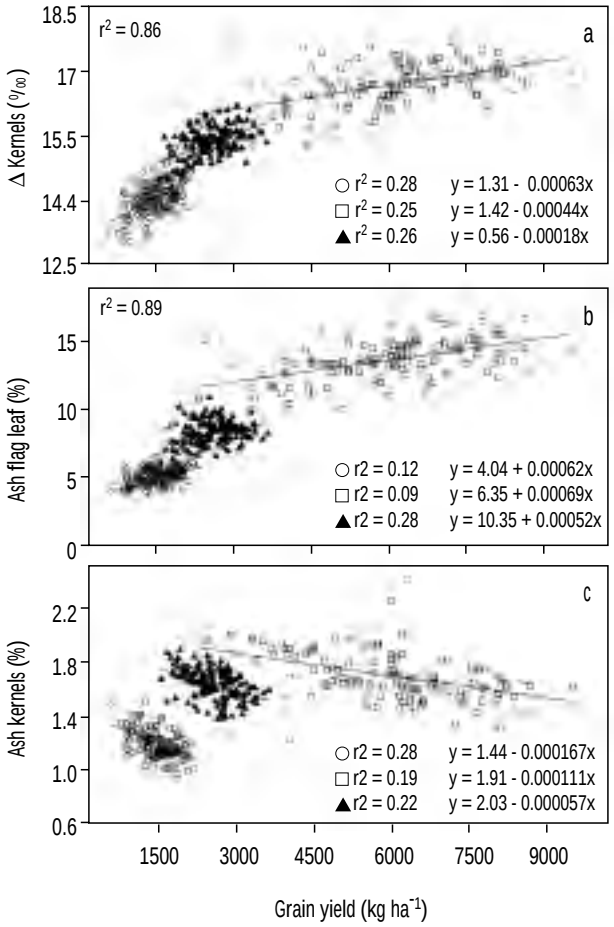

Figure 3. Relationship between grain yield and (a) carbon isotope discrimination (Δ) in mature kernels, (b) ash content (based on dry of the flag leaf around three weeks after anthesis, and (c) ash content of the same mature kernels.

such as near infrared reflectance spectroscopy (NIRS), which would allow a fast, reliable estimation of ash content and Δ in intact kernels (see Araus, 1996).

Leaf structural criteria

Changes in Δ can derive from changes in the balance between leaf stomatal conductance and photosynthetic capacity. In wheat, genotypic variations in Δ seem to derive from differences in both stomatal conductance and photosynthetic capacity, each contributing about the same (Condon et al., 1990; Condon and Richards, 1993; Morgan et al., 1993). If the intrinsic photosynthetic capacity of leaves is increased, Δ could decrease and WUE could be improved, without compromising yield potential (see above). Therefore, a negative relationship between Δ and yield could be expected even in the absence of stress.

Genotypic differences in photosynthetic capacity may depend on the amount of photosynthetic tissue per unit leaf area. Thus, single structural parameters such as dry mass per unit leaf area (LDM, the reciprocal of specific leaf area, also termed specific leaf dry weight, or SLDW) or total nitrogen or chlorophyll content per unit leaf area may be good indicators of the strength of photosynthetic tissue (see references in Araus et al., 1989; Nageswara Rao and Wright, 1994). For example, total chlorophyll content per leaf area may be evaluated in a fast, single and non-destructive way using a portable chlorophyll meter like the SPAD-502 (Soil-Plant Analysis Development Section, Minolta Camera Co., LTd., Japan). Usually the leaf parameter that correlates negatively best with Δ is LDM, followed by SPAD, which indicates that genotypes with thicker and/or more compact leaves have lower Δ. The results suggest that LDM and SPAD measurements can be used as single, rapid indicators of Δ in barley (Araus et al., 1997a) and durum wheat (Araus et al., 1997b) under optimal growing conditions (see also Lopez-Castañeda et al., 1995).

However, some of these correlations may exist under drought conditions and could be useful for breeding, but may be spurious in nature. In fact, growing conditions have a strong direct effect not only on Δ, but also on leaf structure, which in turn could lead to spurious relationships (Araus et al., 1997b). The correlations between Δ and leaf structure, rather than being sustained by a physiological relationship between the amount of photosynthetic tissue and Δ, may sometimes be indirect associations caused by a parallel effect of water status and phenology on leaf structure, grain Δ, and yield (Araus et al., 1997a, b). Summarizing, LDM should be used only in the absence of drought to determine segregating population differences in leaf Δ based on internal photosynthetic capacity. It is worth selecting for higher kernel Δ and grain yield based on higher LDM in rainfed trials, although there probably is no direct physiological basis behind such relationships (Araus et al., 1997b).

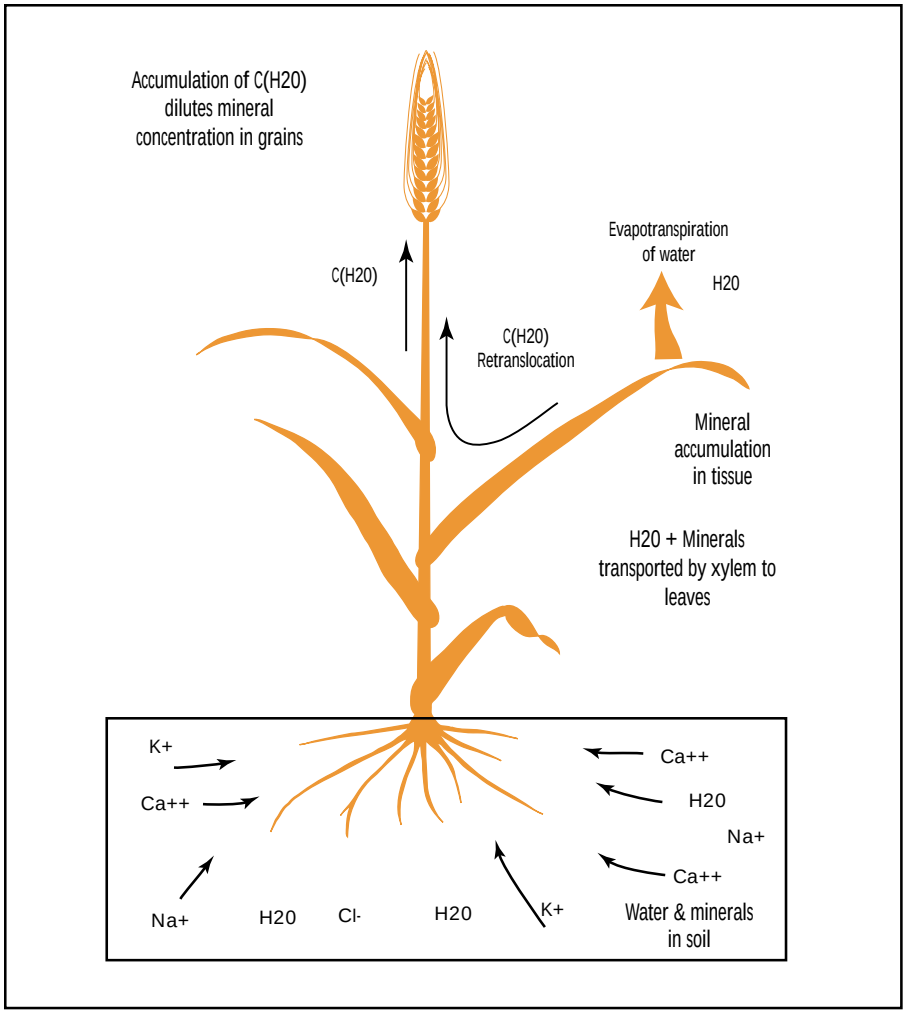

Diagram 2. Accumulation of ash in plants.

Spectral Reflectance Methods

The pattern of light reflection on leaves at different wavelengths through the photosynthetically active radiation (PAR, 400-700 nm) and near infrared radiation (NIR, 700-1200 nm) regions of the electromagnetic spectrum is very different from that of soil and other materials (Diagram 3). Leaf pigments absorb light strongly in the PAR region but not in the NIR, thus reducing the reflection of PAR but not of NIR. Such a pattern of pigment absorption determines the characteristic reflectance signature of leaves (Figure 4). Similarly, the light spectrum reflected by a canopy (either natural or agricultural) differs from that reflected by the bare soil and varies in a way that can be related to the overall area of leaves and other photosynthetic organs in the canopy, as well as to their pigment composition and other physiological factors (Figure 5). Therefore, the measurement of spectra reflected by vegetation canopies provides information that can be used to estimate a large scope of parameters. Some of them are related to the green biomass of the canopy, its photosynthetic size (i.e., total area of leaves and other photosynthetic organs), the amount of PAR absorbed by the canopy, and its photosynthetic potential. Other parameters are more related to the canopy's physiological status at the time of measurement and can be used to assess the extent of some nutrient deficiencies and environmental stresses. The physiological parameters that can be estimated by spectral reflectance techniques include chlorophyll and carotenoid concentrations, photosynthetic radiation use efficiency (PRUE), and water content.

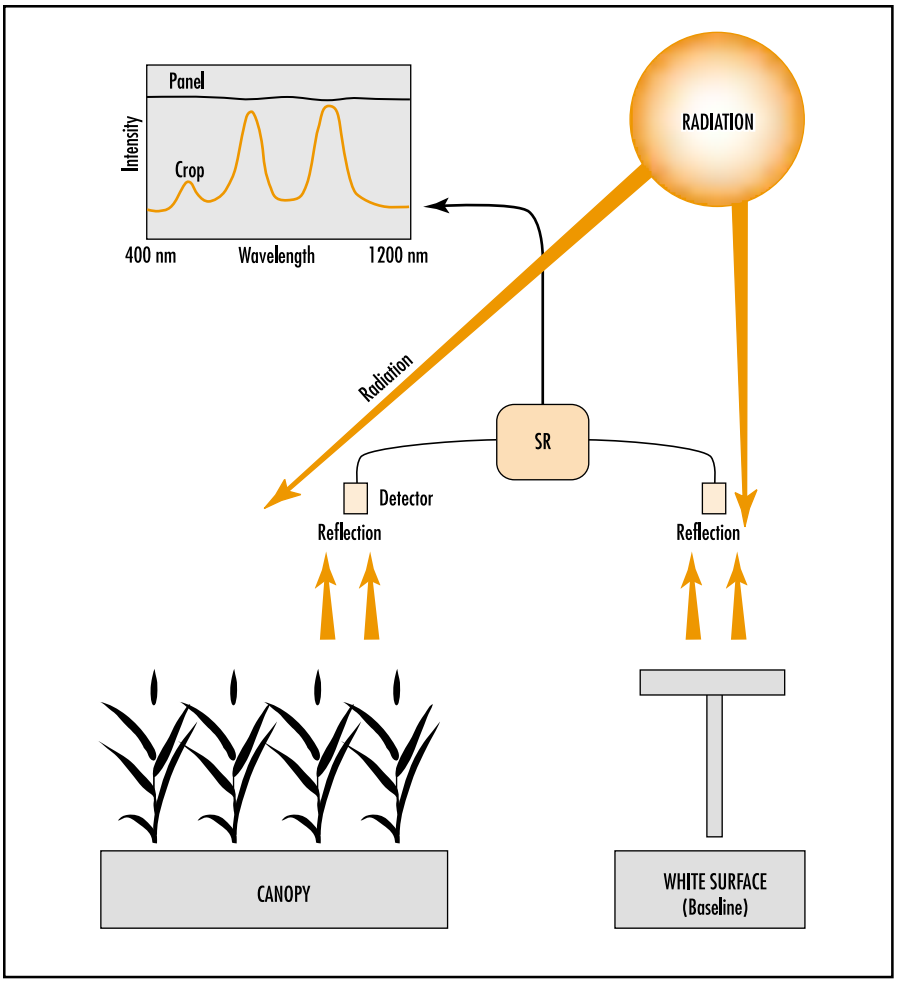

Diagram 3. Spectral reflectance from crop surfaces.

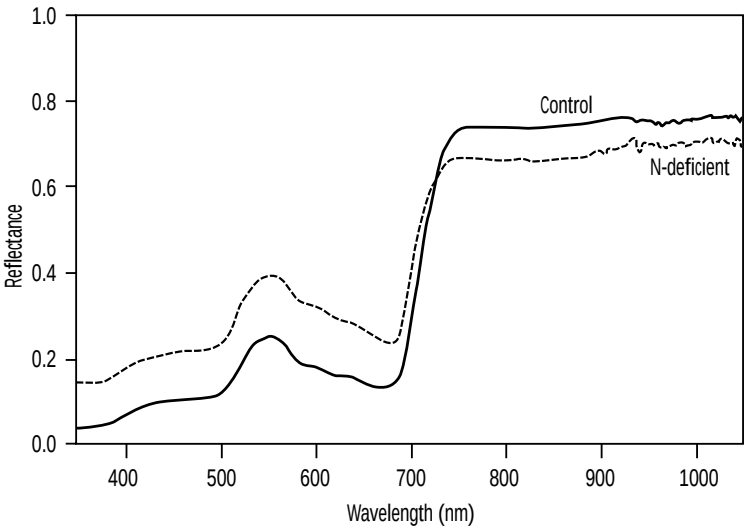

Figure 4. Reflectance signature of two wheat leaves differing in nitrogen status. Note the higher reflectance in the photosynthetically active radiation region of the nitrogen deficient leaf due to lower chlorophyll content in the leaf area.

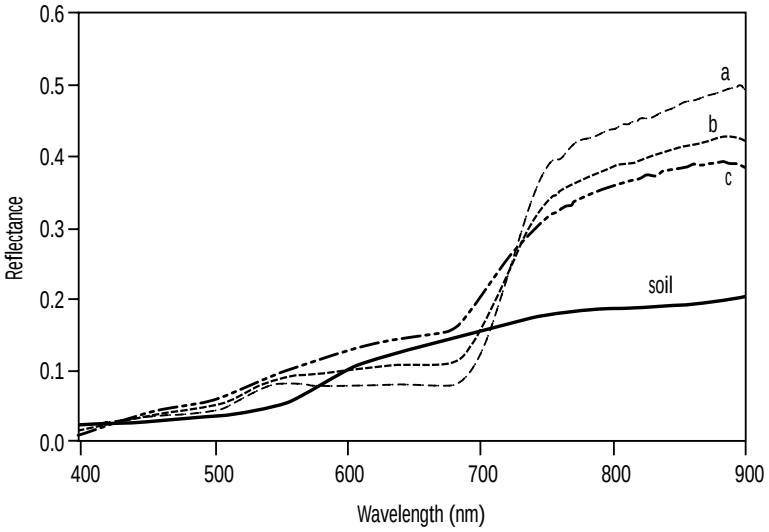

Figure 5. Changes in the pattern of canopy reflectance in a durum wheat plot. Measurements were taken every three days (a, b, c), during the last week of grainfilling, coinciding with fast crop senescence. Note the decrease, during senescence, in the amplitude of the change in reflectance in the red-NIR (around 700 nm) edge. Note also the increase within the PAR region of the reflectance in the red compared to the blue band due to a relatively faster decrease during senescence in chlorophyll compared to carotenoids. Soil reflectance is included for comparison.

Spectral reflectance indices

Spectral reflectance indices are formulations based on simple operations between reflectances at given wavelengths, such as ratios, differences, etc., which are widely used to quantitatively relate changes in reflectance spectra to changes in physiological variables. These indices have the advantage of summing up in a few numbers the large amount of information contained in a reflectance spectrum with narrow waveband resolution.

Originally used in remote sensing by aircraft and satellites, reflectances measured at the ground level are very useful for assessing agrophysiological traits. These traits can be evaluated simultaneously in each sample, at a rate of up to one thousand samples per day, which is much more tedious and time consuming with other methods. This makes spectroradiometric indices ideal for screening for yield potential or for resistance to different stresses.

Sample applications

Perhaps the most widespread application of reflectance indices is for assessing parameters related to canopy greenness. These parameters are related to the canopy's photosynthetic size and include green biomass, leaf area index (LAI) (total one-side leaf area of the crop relative to soil area), green leaf area index (GLAI) (similar to LAI, but includes only functional green leaves), and green area index (GAI) (similar to GLAI, but includes other photosynthetic organs such as green stems). The amount of green area in a canopy determines PAR absorption by photosynthetic organs, which in turn determines the canopy's potential production. The fraction of the incident PAR that is absorbed by the canopy (fPAR) can be estimated from LAI-related parameters or directly from reflectance measurements. Cumulative PAR absorption, which is one of the parameters determining total biomass and thus final yield (see the beginning of this chapter), can be assessed by measuring reflectance periodically during the growth cycle.

Some physiological parameters can also be quantified by spectral indices. Leaf pigments can be detected and quantified based on reflectance spectra and can be used as indicators of several physiological processes. Thus, the canopy's nutritional state can be evaluated through pigment concentration, as chlorophyll (Chl) concentration in leaves is (usually) closely correlated to its nitrogen content. Indices that are good indicators of Chl are (usually) also good indicators of N-content. In addition, plants with low N usually have a high carotenoid (Car) to Chl ratio, which can also be assessed by reflectance indices (Figure 5).

Pigment remote sensing can also be used for assessing the crop's phenological stage (Figure 5) and the occurrence of several stress factors (Blackburn, 1998; Peñuelas, 1998). For example, the Car to Chl ratio can be associated with senescing processes that result from the plant's natural ontogeny pattern or are triggered by different stresses. Also, phenological stages can be associated with different Car/Chl values. Several indices related to changes in pigment composition have been developed and can be used for the remote detection of nutrient deficiencies, environmental stresses, pest attacks, etc. In such contexts, by periodically assessing leaf area, leaf area duration (LAD) can also be used as an indicator of resistance to certain environmental stresses.

The photosynthetic capacity of a canopy can be estimated by using vegetation indices that correlate to the photosynthetic size of the canopy or indices related to the amount of chlorophyll. However, actual photosynthesis may not match

photosynthetic capacity due to the variability of photosynthetic use efficiency of the absorbed radiation, especially when plants are exposed to unfavorable conditions. The photochemical reflectance index (PRI) was developed to detect pigment changes in the xantophyll cycle associated with changes in PRUE (Filella et al., 1996). PRI has been shown to track the changes in PRUE induced by factors such as nutritional status and midday reduction, across different species and functional types.

Another potential application of reflectance indices is remote detection of relative water content (RWC) of plants. Different levels of water stress can be detected indirectly through their effects on vegetation indices related to leaf area, pigment concentration, or photochemical efficiency. In addition, specific indices have been developed for the direct assessment of RWC.

Measurement techniques

Instruments. The instruments required for measuring reflectance spectra are: 1) a field spectroradiometer that analyzes the spectrum of sampled radiation, 2) foreoptics that capture the radiation reflected by a given target, and 3) reference panels, supports, and levels for repeated sampling of incident radiation and radiation reflected by the canopy.

Modern narrow-band spectro-radiometers measure the irradiance at different wavelengths with a bandwidth of about 2 nm through the PAR and NIR regions of the spectrum. Most spectral indices use specific wavebands in the 400-900 nm range; only a few use longer wavelengths, such as the water index, which uses 970 nm (Peñuelas et al., 1993). The use of spectroradiometers with narrow band resolution allows the calculation of several parameters obtained from the first and second derivative of the reflectance spectra against wavelength, which can be used to complement the reflectance indices.

Radiation reflected by the canopy in the PAR and NIR regions is sampled by a foreoptic that limits the field of view to a given solid angle, usually between 10 and 25º. Sampled radiation is conveyed to the spectrum analyser through a fiber optic cable. Light reflected by the canopy is measured with the foreoptic held 1-2 m above the canopy on a fixed or hand-held support, such as a boom **(**Picture 1**)**, and with the help of the required levels or protractors to ensure that all measurements are taken at the same angle between the foreoptics and the sampled surface.

In order to cross-reference the intensity of reflected radiation at each wavelength to the intensity of incident radiation at the same wavelength, all sampled spectra must be converted to reflectance units, i.e., the ratio between the absolute spectrum reflected by the canopy and the absolute spectrum incident on the canopy. Regular measurements of the spectra incident on the canopy are then made. Incident spectra are measured by aiming the foreoptic at a white reference panel in the same orientation to the sun and to the foreoptic as the canopy. Reference panels are commercially available under the name Spectralon (Labsphere, PO Box 70, North Sutton, NH 03260) or they can be made of barium sulphate (Jackson et al., 1992).

Factors affecting the estimation of canopy parameters by spectroradiometrical methods. In addition to canopy variables estimated using spectroradiometrical methods, other factors related to the canopy or external to it will affect the measured

Picture 1. How to place the foreoptic while measuring radiation reflected by a wheat canopy.

reflectance spectra. Variation in canopy structure (such as changes in leaf erectness or appearance of reproductive organs), as well as in the angles between sun, sensor, and target surface, will affect the amount of shadow and/or soil background appearing in the field of view; this can cause non-desired variation in the measured spectra.

There are no standard methods to cope with the variability introduced by interference; most researchers using spectroradiometry adapt the details of their experimental protocols to the particular traits and objectives of their experiments. It is important to fix the measuring conditions used to obtain the spectra. Viewing angle and viewing height, row orientation, and time of day have to be determined when designing an experiment. Disturbance by factors beyond the researcher's control has to be considered when interpreting the results. Not all indices are equally affected by these factors, and indices also differ in their sensitivity to the parameter being measured. Some indices may be more appropriate than others, depending on the aims of the study, canopy characteristics, and measurement conditions.

To minimize the variability induced by sun position, it is preferable to take all measurements at about noon. Nevertheless, the angle of the sun is most important in canopies with low LAI (Kollenkark et al., 1982; Ranson et al., 1985). As for the viewing angle, nadir (sensor looking vertically downward) is perhaps the most commonly used set-up. This is because it has a lower interaction with sun position and row orientation, and delays the time at which spectra become saturated by LAI. On the other hand, nadir viewing is more affected by the soil background. When an off-nadir viewing angle is used, variability due to changes in solar elevation or sensor elevation is minimized if the angle between the sensor azimuth and the sun azimuth is 0-90º (Wardley, 1984).

In a row canopy with low soil cover, the amount of shadow within the canopy varies during the day, depending on the angle between sun azimuth and row orientation. Such angular changes can produce variation in the measured reflectance as great as 100% in red and lower in NIR wavelengths (Kollenkark et al., 1982). Peak variability occurs when the sun is shining down the rows (when sun azimuth equals row orientation), lightening the soil surface and thus giving a higher reflectance reading. For that reason, if reflectance is measured at about noon, rows oriented east to west are more appropriate than rows oriented south to north, especially if soil cover is poor.

Ratio indices are usually less sensitive to changes in viewing geometry and tend to cancel the effects for angular changes (Wardley, 1984). However, they can also be altered because at some wavelengths (such as in red) reflectance is (usually) more intensely altered than at other wavelengths (such as in NIR). Light incident on shaded leaves is poor in the wavelengths that have been absorbed by upper leaves, and their reflected spectra is even poorer. For that reason, the higher the number of shaded leaves that appears in the field of view, the larger the differences in the canopy's reflectance spectra between regions where radiation is absorbed by photosynthetic pigments and regions where it is not. If due to external factors such as viewing angle, sun angle, or wind, the number of shaded leaves in the field of view increases, this will lead to an increase in indices related to green biomass.

The relationship between indices and estimated canopy parameters has been reported to be disturbed by phenological changes that affect crop structure, such as those associated with anthesis in maize (Andrieu and Baret, 1993) or head emergence in wheat (Shibayama et al., 1986). Leaf erectness can also affect canopy reflectance. Model calculations and test results show that radiation reflected perpendicularly from plant canopies is considerably greater from planophile canopies than from erectophile canopies (Jackson and Pinter, 1986). The vertical elements of an erectophile canopy trap reflected radiation within the canopy, while in a more planophile canopy, more radiation is reflected vertically. These structural effects can alter indices used for estimating the same canopy parameter in a different way. For example, Jackson and Pinter (1986) observed that although indices SR and PVI (see later in this chapter) are both used for estimating GLAI, SR was higher in erectophile canopies of wheat, while PVI was higher in planophile canopies. Optical differences in the surface of plant organs, such as different glaucousness (Febrero et al., 1998), can also have some effect on the canopy's reflectance spectra.

Clouds increase the proportion of indirect radiation (i.e., diffuse) to total radiation incident on the canopy; this improves the penetration of light into the canopy. As a result, a greater proportion of incoming radiation is absorbed by photosynthetic pigments; this increases the vegetation indices and leads to an overestimation of green biomass. Wind during the measurements can momentarily alter canopy structure and disturb the relationship between the reflectance spectra and the canopy parameters to be estimated from the spectra (Lord et al., 1985).

Nearby objects, including instruments and the people operating them, can alter the measured spectra by reflecting radiation on the target surface. For that reason, they should be kept as far as possible from the field of view; the instruments should be painted a dark color, and people should wear dark clothes (Kimes et al., 1983).

Taking measurements. Systematic measurements of incident radiation must be made before and during the measurement of reflected radiation to account for possible variation in the incident spectra caused by atmospheric conditions or sun position.

Reference panels should be Lambertian surfaces, that is, they reflect the incident light equally in all directions and for all wavelengths. However, they are not perfect and the intensity of the reflection changes in an important way when panel orientation changes. For that reason, care must be taken to make all incident measurements keeping the panel at the same angle with the foreoptics and with the sun. Changes in the distance from the panel to the foreoptics are less important. This distance is set to ensure that the entire field of view is covered by the panel. Then the reflectance of the canopy samples can be measured making sure that the field of view of the instrument is covered with weed-free canopy and a uniform background, and with plant material homogeneous in structure (Bellairs et al., 1996).

Use of Canopy Reflectance Indices

Assessing the photosynthetic size of canopies using vegetation indices

Vegetation indices (VI) estimate parameters related to the photosynthetic size of a canopy based on the reflectances in the red and near infrared regions. Green biomass, LAI, GAI, GLAI, fPAR, etc., can be estimated through their positive correlation (either linear or logarithmic) with vegetation indices (Wiegand and Richardson, 1990a, b; Baret and Guyot, 1991; Price and Bausch, 1995). Measuring vegetation indices periodically during the crop growing cycle allows the estimation of LAD (which can be used as an indicator of environmental stress tolerance) and the total PAR absorbed by the canopy, which is one of the most important factors for predicting yield (Wiegand and Richardson, 1990).

Vegetation indices take advantage of the great differences in reflectance at red and NIR caused by vegetation. The most widely used VI are the simple ratio (SR) and the normalized difference vegetation index (NDVI), which are defined as:

$$SR = R_{NIR} / R_{Red}, \text{ with a range of 0 to } \infty,$$

where R_{NIR} is the reflectance at NIR and R_{Red} is the reflectance at red.

$$NDVI = (R_{NIR} - R_{Red}) / (R_{NIR} + R_{Red}), \text{ with a range of -1 to 1.}$$

SR and NDVI were originally used with the wide wavebands of former radiometers (for example, 550-670 nm for red and 710-980 nm for near infrared in AVHRR radiometers in satellites of NOAA series). With the high spectral resolution of today's radiometers, wavebands can be much narrower. Hall et al. (1990) used a waveband centered at 770 nm for NIR and another at 660 nm for red, while Peñuelas et al. (1997b) used 900 nm and 680 nm for NIR and red, respectively.

Some authors have reported improvements in NDVI performance after changing the wavebands used in the index. Carter (1998) describes an improved correlation with leaf photosynthetic capacity when using a modified NDVI where R701 (+/-2nm) and R520 (+/-2nm) were used for NIR and red, respectively.

Variations of these indices have been proposed to compensate for the effect of soil background. Thus the soil adjusted vegetation index (SAVI) was defined by Huete (1988) as:

$$SAVI = [(RNIR - RRed) / RNIR + RRed + L)] (1 + L),$$

where the parameter L was adjusted to minimize noise caused by soil for a large range of soil covers. For most crop conditions L=0.5, while for very low soil covers L=1 would be more appropriate, and L=0.25 would be appropriate for very high covers (Huete, 1988).

Other indices include parameters obtained from the soil's reflectance spectrum. One of them is the transformed soil adjusted vegetation index (TSAVI) which was defined by Baret and Guyot (1991) as:

$$TSAVI = a(R_{NIR} - aR_{Red} - b) / [R_{Red} + a(R_{NIR} - b) + 0.08(1+a^2)],$$

where a is the slope and b is the intercept of the linear equation

$$R_{NIRsoil} = a*R_{Red\ soil} + b.$$

An important drawback in estimating LAI by VI is the saturation of the VI with LAI. Saturation of NDVI starts at about LAI=1, and beyond LAI=2 it becomes insensitive to further increases in LAI (Gamon et al., 1995). Perpendicular vegetation index (PVI) partly overcomes the saturation problem inherent to NDVI (Richardson and Wiegand, 1977):

$$PVI = \{(R_{Red.soio} - R_{Red.vegetation})^2 + (R_{NIR.vegetation} - R_{NIR.soil})^2\}^{1/2}$$

Although PVI is more sensitive than NDVI to changes in the viewing

geometry, PVI does not become as clearly saturated as NDVI with changes in GLAI (Shibayama et al., 1986).

Examples of assessing LAI-related parameters by VI can be found in the literature (Baret and Guyot, 1991; Field et al., 1994; Price and Bausch, 1995). Ground level measurement of VI has been used successfully as a tool for assessing early biomass and vigor of different wheat genotypes (Elliott and Regan, 1993; Bellairs et al., 1996). Under experimental conditions of a wheat breeding program, Bellairs et al. (1996) reported young wheat canopies where LAI was less than 1.5, a coefficient determination (r^2) of 0.90-0.95 between biomass and NDVI. As for assessing the intensity of different plant stresses, Peñuelas et al. (1997b) showed that NDVI was a useful tool for measuring agronomic responses of barley to salinity.

A practical use of vegetation indices is for making yield predictions. Yield can be predicted from successive VI measurements taken during the growing season, based on the following assumptions (Wiegand et al., 1991): 1) plant stands integrate the growing conditions experienced and express net assimilation achieved through the canopy, 2) stresses severe enough to affect economic yield will be detectable through their effects on crop development and the persistence of photosynthetically active tissue in the canopy, 3) high economic yields cannot be achieved unless plant canopies fully utilize available solar radiation as the plants enter the reproductive stage, and 4) vegetation indices calculated from remote observations in appropriate wavelengths effectively measure the photosynthetic size of the canopy. Wiegand and Richardson (1990b) reported an r^2 of 0.5 for predicting wheat grain yield from PVI measured on four dates during vegetative growth. Similarly, Rudorff and Batista (1990) reported an r^2 of 0.66 between wheat yield and integrated VI from booting to completely senesced plants. If most uncertainty in yield prediction by VI is site-dependent, then calibrations of yield vs. VI across good and poor growing conditions within production areas can describe the results of past and future growing seasons acceptably (Wiegand et al., 1991).

Remote sensing of pigments

Estimating chlorophyll concentration. Several indices have been developed for estimating Chl concentration using canopy reflectance methods. The simplest indices are just reflectance at 675 and 550 nm. Reflectance at 675 nm (R675) is very sensitive to changes in Chl content. However, the relationship becomes saturated at relatively low Chl values (around 10 $\mu g\ cm^{-2}$) and is a good indicator of chlorophyll content only at very low concentrations. Absorption by Chl at 550 nm is lower than at 675 nm; therefore, the reflectance at this wavelength (R550) is less sensitive to changes in Chl content but is not saturated at such low concentrations, thus covering a range of higher Chl values (Thomas and Gausman, 1977; Jacquemoud and Baret, 1990; Lichtenthaler et al., 1996).

Both R675 and R550 are non-normalized indices that can be affected by external factors (Curran, 1983). Other indices use more than one wavelength. Analyzing wavelengths that were more sensitive to changes in Chla, Chlb, and Cars in soybean leaves grown at different N levels, Chapelle et al. (1992) developed the ratio analysis of reflectance spectra (RARS) indices, RARSa, RARSb and RARSc, which optimized the estimation of Chla, Chlb, and Cars, respectively, in soybean leaves. RARSa = R_{675} / R_{700} showed a determination coefficient of 0.93, with Chla ranging from 0.4 to 27 $\mu g\ cm^{-2}$; RARSb = R_{675} / (R_{650} * R_{700}) showed an r^2 of 0.82, with Chlb ranging from 1 to 7 $\mu g\ cm^{-2}$; and RARSc = R_{760} / R_{500} showed an r^2 of 0.94, with Cars ranging from 1.5 to 6$\mu g\ cm^{-2}$ (Chapelle et al., 1992). Blackburn (1998) reported that using R680 and R800, instead of R675 and R700, in RARSa significantly improved the prediction of Chla in a range of leaves from different species with different degrees of senescence. Other reflectance indices that can be used for estimating pigment concentration are summarized in Table 1.

Leaf chlorophyll content can also be assessed through its relationship with parameters derived from the position of the red edge. The red edge position (REP) is the wavelength in the 680-780 nm range where the change in reflectance when increasing the wavelength from red to NIR reaches its maximum. The REP shifts to slightly longer wavelengths as Chla values increase (Curran et al., 1990; Filella et al., 1995). By obtaining the first and second derivatives of the spectra in this area, several parameters that are good indicators of Chl content can be calculated. Among these parameters are the wavelength of the red edge (λ_{re}), the maximum amplitude in the first derivative of the reflectance spectra (dR_{re}), and the sum of amplitudes between 680 and 780 nm in the first derivative of the reflectance spectra ($\Sigma dR_{680-780}$). These REP-related parameters are suitable indicators of chlorophyll content in a wider and higher range of concentration than R675 and R550, with the additional advantage that they are less affected by external factors such as the geometry, incident intensity, and soil background (Filella and Peñuelas, 1995).

In addition to the wide variety of indices related to absolute Chl concentration, the normalized phaeophytinization index (NPQI) can be used to detect chlorophyll degradation.

$$NPQI = (R_{415} - R_{435}) / (R_{415} + R_{435})$$

(Peñuelas et al., 1995c)

NPQI was introduced as an indicator of pest attacks on apple trees (Peñuelas et al., 1995c). In some cases it also seems to indicate different phenological states in wheat (Casadesús and Araus, unpublished data).

One practical approach for estimating Chl concentration using reflectance indices is to test the performance of more than one index and choose the one most appropriate for the experiment. Another approach is to pool the information contained in a number of indices. In this sense, Filella et al. (1995) were able to assign different reflectance spectra to different N-status classes using a discriminant analysis based on R430, R550, R680, λ_{re}, dR_{re}, and NDPI (defined later in this chapter). Non-destructive portable chlorophyll meters based on absorbance measurements through the leaf provide fast and easy determinations of chlorophyll content and are commercially available at a relatively low price. For example, the SPAD-502 mentioned above calculates the ratio of absorbances at 650 nm λ (chlorophyll absorbance peak) and at 940 nm (non-chlorophyll absorbance) (Monje and Bugbee, 1992). Estimates of chlorophyll using canopy spectral reflectance methods are in general closely related to the amount of chlorophyll per soil area calculated from the reading of portable chlorophyll meters multiplied by the LAI (Filella et al., 1995). Chlorophyll assessment using canopy reflectance methods has the advantage that it directly integrates the chlorophyll content of all the leaves in the canopy. It also offers additional information such as canopy size and content of pigments other than chlorophyll.

Carotenoid to chlorophyll ratios. Estimating the Car: Chl ratio by reflectance indices can be useful for assessing the extent of some plant stresses, given that increases in Cars concentration relative to Chl are often observed when plants are subjected to stress (Young and Britton, 1990).

Both Chl and Car absorb in the blue, but only Chl absorbs in the red. Indices that are combinations of the reflectance in these two regions are correlated to the Car : Chl ratio. The simplest indices are pigment simple ratio (PSR) and normalized difference pigment index (NDPI), which are formulated in an analog way to SR and NDVI and defined to estimate the ratio of total pigments to Chla (Peñuelas et al., 1993):

$$PSR = R_{430} / R_{680}\ ,\ NDPI = (R_{680} - R_{430}) / (R_{680} + R_{430})$$

Both PSR and NDPI are affected by disrupting effects introduced by leaf surface and structure. A new index was developed to avoid such problems: the structural independent pigment index (SIPI), which was defined by Peñuelas et al. (1995a) as:

$$SIPI = (R_{800} - R_{435}) / (R_{415} + R_{435})$$

SIPI uses wavelengths showing the best semi-empirical estimation of the Car : Chla ratio, and its formulation minimizes the disrupting effects of leaf surface and mesophyll structure (Peñuelas et al., 1995a). R800 is used as a reference where neither Cars nor Chl absorb and are only affected by the structure. The best fit between the Cars : Chla ratio and SIPI for a variety of plants with Chla ranging from 0.06 to 54 μg cm^{-2} and Cars from 1 to 16 μg cm^{-2} was exponential, with an r^2 of 0.98 and the form, Cars : Chla = $4.44 - 6.77\exp^{-0.48\ SIPI}$ (Peñuelas et al., 1995a).

Table 1. Reflectance indices for estimating pigment concentration.

Pigment	Definition	Reference
Chl	R_{675}	Jacquemoud and Baret, 1990
	R_{550}	Jacquemoud and Baret, 1990
	$R_{750/550}$	Lichtenthaler et al., 1996 Gitelson and Merzlyak, 1997
	$R_{750/700}$	Lichtenthaler et al., 1996 Gitelson and Merzlyak, 1997
	$NDVI_{green} = (R_{NIR} - R_{540-570}) / R_{NIR} + R_{540-570})$	Gitelson and Merzlyak, 1997
	λ_{re}, dR_{re} and $\Sigma dR_{680-780}$	Filella et al., 1995
Chla	$RARSa = R_{675} / R_{700}$	Chapelle et al., 1992
	$RARSa^* = R_{680} / R_{800}$	Blackburn, 1998
	$PSSRa = R_{800} / R_{675}$	Blackburn, 1998
Chlb	$RARSb = R_{675} / (R_{650} * R_{700})$	Chapelle et al., 1992
	$PSSRb = R_{800} / R_{650}$	Blackburn, 1998
Cars	$RARSc = R_{760} / R_{500}$	Chapelle et al., 1992
Cars/Chla	$SIPI = (R_{800} - R_{435}) / (R_{415} + R_{435})$	Peñuelas et al., 1995a

Indices related to the Cars : Chl ratio change during the crop growing cycle. They are low during vegetative growth and start to increase before the beginning of senescence (Filella et al., 1995). They can be used in assessing the nutritional state of a crop (Filella et al., 1995), shown by high values of the indices when N is low, and for detecting pest attacks (Peñuelas et al., 1995c).

Assessing radiation use efficiency by PRI

Canopy photosynthesis can be roughly estimated based on the estimation of the canopy's photosynthetic size or Chl concentration. However, these parameters are associated with potential canopy photosynthesis, which does not always correspond to actual photosynthesis, especially for plants growing in stressful environments. While VI are correlated with PAR absorption by the canopy (a slowly varying trait, in a range of days to weeks), the photochemical reflectance index (PRI) is correlated with photosynthetic radiation use efficiency (PRUE) of absorbed PAR, a rapidly varying process, in a range of hours.

Part of the PAR absorbed by Chl cannot be used for photosynthesis and is lost mainly through heat dissipation, which is linked to the xanthophyll-de-epoxidation cycle (Demmig-Adams and Adams, 1996). PRI reflects changes in reflectance of around 531 nm, which have been associated with pigment changes in the xanthophylls cycle (Gamon et al., 1992).

PRI was originally defined as physiological reflectance index (Gamon et al., 1992) but later the definition was slightly modified (its sign was changed) and the name of the index was revised as photochemical reflectance index (Peñuelas et al., 1995b). Here PRI refers to the second definition.

$$PRI = (R_{531} - R_{570}) / (R_{531} + R_{570})$$

(Peñuelas et al., 1995b)

PRI is correlated with the de-epoxidation stage of the xanthophylls cycle, with zeaxanthin, and with radiation-use efficiency (Filella et al., 1996). Higher PRI values indicate greater efficiency.

PRI has been shown to track changes in photosynthetic radiation use efficiency induced by different factors such as nutritional state and midday reduction, across different species and functional types (Gamon et al., 1997). However, it does not properly track changes in PRUE if there are structural changes in the canopy associated with stress, such as leaf wilting (Gamon et al., 1992). Also, this index is valid only for fully illuminated canopies and does not perform properly across wide ranges of illumination from shade to sun (Gamon et al., 1997).

Directly assessing plant water status

Some bands of radiation absorption by water exist in the 1300-2500 nm region, but due to its high absorptance in this region, reflectance becomes saturated (i.e., it does not respond to further increases in RWC) even in a canopy with low water content. In the 950-970 nm region, there is some weak absorption of radiation by water that is not saturated for a moderately dry canopy. The reflectance at 970 has been used in the definition of the water index (WI).

$WI = R_{900} / R_{970}$ (Peñuelas et al., 1993, 1997)

In WI, reflectance at 970 nm is taken as a wavelength sensitive to water content, while reflectance at 900 nm is taken as a reference which is similarly affected by canopy and leaf structures but with null absorption by water.

WI has been used to track changes in RWC, leaf water potential, stomatal conductance, and foliage minus air temperature differences when plant water stress is well developed (RWC<0.85) (Peñuelas et al., 1993). Peñuelas et al. (1997a) reported a correlation coefficient of around 0.55 between WI and RWC for a range of species measured at different times of the year in their natural Mediterranean environment. However, WI appears to be quite insensitive until the drying process is well advanced. For that reason, WI can be useful for assessing wild fire risk but has less utility in irrigation scheduling. As for stress detection, Peñuelas et al. (1997b) showed that WI was a good indicator of water status in response to salinity.

NDVI is also affected by the drying process and by structural and color changes in the plants. The ratio of WI and NDVI has a better correlation with RWC increases, especially in species that undergo important changes in NDVI throughout the year (Peñuelas et al., 1997a).

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CHAPTER 6

Economic Issues in Assessing the Role of Physiology in Wheat Breeding Programs

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Wheat breeding can be thought of as an economic activity, in the sense that it involves processes of physical transformation that are characterized by streams of costs and benefits. Decisions taken about the organization and operation of a wheat breeding program (including technical decisions such as the choice of parental materials, crossing techniques, selection methods, and evaluation procedures) are likely to have economic implications. To the extent that changes in the organization or operation of a wheat breeding program affect the streams of costs and benefits, the economic outcomes that can be expected from the program will increase or decrease.

Plant breeders are viewed by many, especially by those in the food production and processing industries, as a resource that can be used to enhance the overall performance of the agricultural sector (Brennan, 1997). Precisely because they have this capacity, plant breeders often face demands on their services that far exceed what they can realistically expect to deliver. In a world of limited resources, plant breeders therefore need some basis for deciding which among the many demands being placed on them should have priority. Although economic factors are often taken into account (explicitly or implicitly) when research priorities are established, the informal and ad hoc manner in which this is done frequently leads to decisions that are far from optimal in an economic sense. Basic economic analysis, grounded in the careful assessment of benefits and costs, can provide the foundation for making those decisions in a more informed and defensible manner.

Assessing Potential Changes to a Wheat Breeding Program

Under what circumstances might it be advisable to incorporate physiology into a wheat breeding program? In assessing the organization and management of an existing breeding program and deciding whether or not changes may be needed to meet a particular objective, it will often be useful to review the following preliminary questions before undertaking formal economic analysis.

Is the problem best addressed through breeding?

Before any changes are made to a breeding program, it is important to determine whether the results being sought could be obtained more quickly and/or cheaply by some other means. For example, if the research objective is to increase protein content in wheat, experience suggests that it will often be better to concentrate on breeding for higher yield, while leaving the challenge of raising protein content to agronomic management. This is because even though cultivars differ in their protein content, prospects for increasing protein content through breeding are limited. Research has shown that protein content is mainly influenced by environment (and by genotype *x* environment interaction), so the genotype effect is generally very small (Bingham, 1979). Furthermore, given the known negative relationship between yield and protein content (O'Brien and Ronalds, 1984), any increases in protein obtained through selecting higher-protein cultivars are likely to result in lower yields.

What level of breeding input is appropriate?

Once it has been decided that the research objective is best addressed through breeding, it is necessary to determine what level of breeding input is appropriate. An appropriate level of breeding input is one that can be justified in terms of the size of the expected benefits. Generally these will be related to the size of the target region: As the size of the target region increases, so will the level of breeding effort that is justified. Brennan (1992)

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and Maredia (1993) have shown that for small target regions, it often will be appropriate only to select from among breeding lines that have been imported from elsewhere. But once the target region increases beyond a certain size, it will be appropriate to establish a full-fledged local crossing program. The precise threshold values needed to justify expansion of an existing breeding program will depend, among other things, on the characteristics of the target environment (area, average yield levels, use of improved varieties, etc.) and the yield gains that can be expected by increasing the breeding input.

What breeding strategy is likely to be most efficient?

Once it has been determined that the problem is best handled through breeding and that the size of the target region justifies a full-fledged crossing program, a breeding strategy must be decided. Wheat breeding can be pursued in many different ways. The initial choice of source materials is of course critical; if the source materials selected for improvement contain a high proportion of favorable alleles for the problem being addressed, the chances of success are greatly enhanced. After choosing the source materials, the breeder must determine how much effort will be put into crossing, as compared to selection and evaluation of the resulting lines. In addition to a wide range of so-called conventional breeding methods, modern breeders also have the option of incorporating biotechnology techniques, such as the use of genetic markers, tissue culture, etc. In deciding what types of selection methods will be optimal, it is important that the decision be driven by what is best for the program and its outcomes, not simply by the availability of new tools or techniques (Brennan, 1997). Innovative technologies that appear to be tremendously appealing in the short run often turn out to be far less attractive over the longer term. For example, Brennan and O'Brien (1991) found that the incorporation of early-generation, small-scale quality testing into an Australian commercial wheat breeding program, while initially attractive, led to a lower economic return for the breeding program (Box A).

On what basis will varieties be released for use by farmers?

The procedures used to evaluate new varieties prior to their release also merit consideration, because evaluation procedures can have important economic implications for breeding programs.

Box A: Incorporating Quality Selection into a Wheat Breeding Program

A recurring question facing any wheat breeding program concerns when to begin selecting for quality characteristics—for example, protein content or gluten level. Opinions differ as to what the appropriate time is for starting quality testing. Some breeders feel that selecting for quality should be left until late in the improvement process, after significant progress has been achieved in raising yield potential. Others feel that selecting for quality in addition to yield should commence early in the breeding process, so that low-quality materials are screened out early on.

Brennan and O'Brien (1991) used an economic framework to evaluate the efficiency of two alternative approaches to the problem. Their study focused on two Australian wheat breeding programs, one which performed early generation quality testing, and another which did not (see table). Both programs used the same amount of labor, and the number of crosses and lines sown in the F_2 generation was identical. The two programs differed mainly in the stage at which quality testing was initiated, which caused different sets of lines to move through each program.

Small-scale tests for quality carried out early in the breeding process (F_2 stage) were found to be less expensive than tests carried out in later generations (F_6 stage). This led to the prediction that early generation testing would prove more cost effective. But when the economic returns of the program doing early generation quality testing were compared to those of the program in which quality testing was introduced at a later stage, they were found to be lower. The costs associated with the program that included early generation testing were slightly higher, but the benefits were markedly lower over the longer term. The program without early generation quality testing was able to concentrate exclusively on yield potential, enabling it to achieve much more rapid rates of yield gains. Admittedly, it also had a lower rate of quality increase, since less selection pressure was placed on quality, but when economic values were assigned to yield levels and quality factors, the additional yield gains more than compensated for the lower levels of quality. The study thus showed that in the absence of a substantial premium for quality, wheat producers and consumers will be disadvantaged if breeding programs opt to select for quality in the early generations at the expense of yield improvement.

Economic returns to early versus late selection for grain quality in wheat.

	Program A†	Program B‡
Expected increase over current varieties (%):		
- yield	4.6	2.3
- quality	0.2	1.1
Total costs‡ (US$ 000)	353	369
Total benefits‡ (US$ 000)	3,710	2,557
Benefit-cost ratio	10.5	6.9

† Quality-testing introduced in F_6 for Program A, and F_2 for Program B.
‡ Discounted to year of crossing at 5% per annum.
Source: Derived from Brennan and O'Brien (1991).

Before a new variety is released, breeders must decide how widely it should be tested and over what period of time, how well it need perform relative to other cultivars that are already in use, and what level of genetic diversity is desirable within and among released varieties.[3] Subjecting experimental varieties to rigorous testing prior to their release increases the likelihood that they will be commercially successful, but extensive testing can also significantly raise overall development costs.

If after reviewing these preliminary questions it still seems worthwhile to proceed, it may be appropriate to undertake more rigorous economic analyses. Although space limitations prevent us from presenting detailed step-by-step instructions, the next two sections provide a broad overview of key economic concepts needed for formally evaluating the desirability of incorporating physiology into a wheat breeding program. They provide a brief description of the basic procedures that would have to be followed.

Key Economic Concepts Relating to Investment Analysis

Basis for economic assessment

The decision of whether or not to incorporate physiology into a wheat breeding program can be approached like any other investment decision. In this respect, the key issue concerns the economic returns that will be generated as a result of the proposed organizational change. The basic economic question that needs to be addressed is really quite simple: What level of investment is justified by the expected benefits, taking into account alternative investment opportunities?

Before any formal economic analysis is undertaken, two important concepts need to be understood: opportunity cost and time value of money.

Opportunity cost. An opportunity cost is the benefit foregone by using a scarce resource for one purpose instead of its next best alternative use (Gittinger 1982). Opportunity costs play an important role in investment analysis, because most investments involve choices between mutually exclusive alternatives. Since the resources available to a breeding program are usually limited, whenever additional emphasis is put on one breeding objective, less emphasis must necessarily be put on other objectives. To return to the example cited earlier, if the decision is taken to target higher protein content, this will probably slow the expected rate of progress in breeding for higher yield. Thus, the opportunity cost of breeding for enhanced protein content will be the progress that would have been achieved (but had to be given up) in breeding for higher yield.

Of course, the tradeoffs may not always be so evident. In plant breeding, targeting one objective does not necessarily mean that progress toward other objectives will be suppressed, at least not directly. The rationale for adding a physiology component in fact may be to achieve the same outcome with greater efficiency. Thus, data from physiological measurements may be used to complement yield trial data; if the additional information improves the breeder's ability to predict cultivar performance in target environments, the need for extensive yield trials may be reduced or even eliminated (Reynolds et al., 1997). But even in cases such as this, the concept of opportunity cost remains valid, because the resources invested in taking the additional physiological measurements presumably could have been used in some other way to generate other types of benefits.

Time value of money. Economic analysis must take into account one important facet of value that stems from human behavior, namely, the time value of money. The time value of money refers to the fact that people place a higher worth on values realized earlier as compared to values realized later (Gittinger, 1982). Asked to choose between receiving $100 today and receiving $100 one year from today, most people would choose today. In economic analysis, the time value of money is taken into account through discounting, whereby costs and benefits expected to occur in the future are assigned lower values.

Discounting is important in any type of investment analysis, which by definition deals with streams of costs and benefits through time. It is particularly important in the analysis of agricultural research investments, given 1) the unequal distribution through time of expected costs and benefits, and 2) the uncertainty about future outcomes of agricultural research. In plant breeding, there are usually long lags—often 10 years or more—between the initial crossing and selection activities (which imply costs) and the eventual adoption of improved varieties by farmers (which generates benefits). Under those circumstances, the expected benefits may be discounted by decision makers, to the extent that the investment may no longer seem attractive (Box B).

3 The important issue of how many varieties should be released may also have to be decided by breeders, although usually this matter is left to some sort of government-appointed varietal certification and release committee.

Box B: Stream of Costs and Benefits Associated with a Breeding Program

To calculate the economic returns to a plant breeding program, it is necessary to estimate the costs and benefits associated with it. Figure 1 illustrates the stream of costs and benefits typically associated with a plant breeding program. During an initial period, net benefits remain negative because research costs are being incurred (for example, in the crossing, selection and evaluation of experimental materials) without any benefits being realized (Morris et al., 1992). Eventually the research produces an improved variety, which after undergoing a certification process is approved for release. Following a lag necessary for the production and distribution of seed, the variety is taken up by farmers, with the rate of adoption typically following an S-shaped (or logistic) curve. Providing the variety leads to improved yields in farmers' fields, the original research investment (made years earlier in most cases) now begins to generate benefits in the form of increased production. The stream of net benefits consequently turns positive, increasing as the area planted to the new variety expands, reaching a maximum at peak adoption, and then declining as the variety is gradually replaced by another, newer variety.

While the relative sizes of costs and benefits are obviously important in evaluating a research investment, their distribution through time is also important. Benefits realized far in the future are considered less valuable than benefits realized in the short term. To accommodate the *time value of money*, research costs and benefits are discounted. Figure 2 illustrates how discounting depresses the value of net benefits realized near the end of the period of analysis relative to those realized near the beginning. Because of the long time lags involved in research such as plant breeding, discounting is an important concept used in analyzing returns to investments in research.

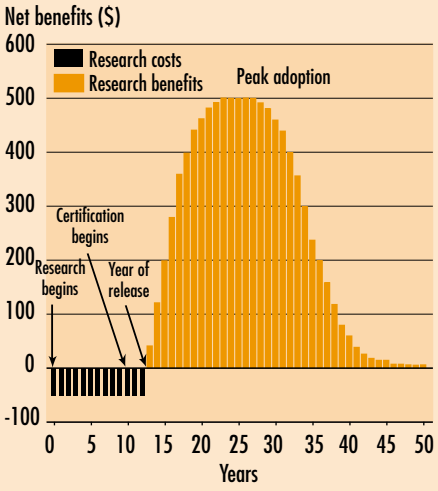

Figure 1. Undiscounted stream of costs and benefits associated with a plant breeding program.
Source: Figure 12 in Morris et al. (1992).

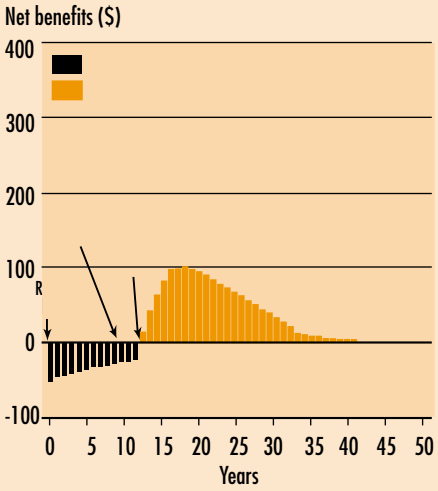

Figure 2. Effects of discounting the stream of costs and benefits associated with a plant breeding program.
Source: Figure 13 in Morris et al. (1992).

Discounting can dramatically alter the attractiveness of any investment opportunity. For example, spending $1 now to bring about a $2 return after 10 years at first glance might seem a sound investment. But with a 12% discount rate (often used to approximate the opportunity cost of capital in developing countries), $2 received 10 years in the future is worth only $\$2/(1.12^{10}) = \$0.64$ at today's prices. Seen in this light, the investment does not appear to be attractive.

Significantly, the effects of discounting are very sensitive to the distribution through time of the expected costs and benefits. If the same $1 investment is expected to generate the same $2 return after only five years rather than ten, the present value of the benefits (i.e., the value at today's prices) is $\$2/(1.12^{5}) = \$1.13$. Depending on whether or not more attractive investment opportunities are available, this return might seem quite attractive.

Approaches to economic analysis

How can economic analysis help a research administrator assess the desirability of establishing a physiology component to the breeding program? Economists would characterize the incorporation of physiology into an existing breeding program as a *marginal change*, since it does not involve reorganization of the entire breeding program, but only an incremental change, or a change "at the margin."

Two alternative approaches can be used to analyze marginal changes: 1) comparing only changes in costs and benefits expected to result from the addition of the physiology component (partial budget analysis), or 2) comparing the costs and benefits of the entire breeding program *with* the physiology component to the costs and benefits of

the entire breeding program *without* the physiology component (whole budget analysis). Which of these two approaches is preferable will depend on the quantity and quality of data that can be collected, the time available for the analysis, and perhaps the level of economic expertise on hand. The results obtained through the two approaches will generally be similar, although they may differ somewhat depending on the level at which the analysis is undertaken.

Whether the approach being used is based on partial budget analysis or whole budget analysis, the key to assessing the economic desirability of the proposed change is to correctly identify the costs and benefits that can be expected to vary—known as the *marginal costs* and *marginal benefits*.

Certain types of cost changes can readily be identified and estimated in advance. For example, if incorporating physiology involves the hiring of a physiologist, then it can safely be predicted that one additional cost will be the physiologist's salary and associated costs. Similarly, if the incorporation of physiology requires the construction of a new laboratory facility with specialized equipment and materials, that cost, too, can easily be foreseen and quantified.

But other types of cost changes will be much more difficult to predict. Decision making in plant breeding programs usually proceeds in a sequential manner, with decisions taken at early stages of selection often leading to unpredictable outcomes that in turn affect decisions taken in subsequent stages. For this reason, determining future cost streams is not always easy. Because of the "snowballing" effect, the cumulative changes in the cost structure of a breeding program over the long run may appear quite different from what could realistically have been anticipated at the time a management decision was originally taken. In studying two wheat breeding programs in Australia, Brennan and O'Brien (1991) found that even though the introduction of early generation quality testing reduced overall breeding costs in the first generation of plants, over the longer term total costs were higher because the sequential cost effect increased costs in subsequent generations (see Box A). The outcomes of research investments being inherently more unpredictable than the outcomes of many other types of investments, careful thought needs to be put into assessing the extent to which future costs are likely to be affected by any marginal changes.

Just as marginal costs are often difficult to predict, so, too, are marginal benefits. With investments that affect the organization and management of a plant breeding program, in some cases it may be relatively easy to identify and estimate expected marginal benefits. That will occur especially when the marginal benefits relate to changes in the value of final outputs of the program (e.g., the acquisition of a new source of germplasm that contributes directly to the development of higher yielding varieties). In other cases, it is extremely difficult to identify and quantify expected marginal benefits. That is particularly so when they will result from changes in current research procedures that are likely to affect the way future research is carried out (e.g., modifying early generation evaluation procedures in ways that are likely to have consequences for the numbers and quality of materials available in later generations).

When the research investment being considered is the addition of a physiology component to the breeding program, identification and quantification of expected marginal costs and benefits may be difficult because so many aspects of the existing program are likely to be affected. Under these circumstances, partial budget analysis based on expected changes in marginal costs and marginal benefits will often be inadequate, and it may be desirable to carry out a more complete economic analysis based on the program's total costs and returns. Brennan (1989a) describes a method for developing detailed estimates of costs and returns for an entire plant breeding program; these detailed estimates can be used to evaluate the economic attractiveness of significant (i.e., non-marginal) changes to the organization of the program. The total-budget approach described by Brennan is based on a comparison of expected costs and returns *without* a physiological component and expected costs and returns *with* the physiological component.

Evaluating the Desirability of Using Physiology in Breeding Programs

Estimating costs and benefits of the current program

Whenever a detailed economic analysis is to be carried out to determine the desirability of incorporating physiology into an existing breeding program, as a starting point it will often be useful to develop broad estimates of the total costs and returns of the current program.

A broad estimate of the current program's costs can be made in a "top-down" fashion based on aggregate budget information about the program's total operating costs, total annual capital costs, total annual salary costs, and total overhead costs. Alternatively, the current program's costs can be broadly estimated in a "bottom-up" fashion based on disaggregated cost data relating to each individual activity of the program (see Brennan and Khan, 1991). Either way, it is important to

include all relevant costs likely to be affected by the proposed change, including costs associated with crossing, evaluation, selection, disease screening, quality evaluation, and regional trials, as well as costs relating to variety release and registration activities.

The extent to which overhead and administrative costs (such as the salaries and support costs of head office personnel, finance officers, and human resources staff; library costs; information technology costs) need to be taken into account depends on whether or not these costs are likely to be affected by the proposed changes. If administrative overheads are likely to remain unaffected, it may be convenient to overlook them.

The benefits generated by a wheat breeding program can be measured at different levels. For researchers working in a breeding program, the benefits include not only improved varieties *per se*, but also scientific benefits such as novel research techniques, specialized laboratory equipment, and original knowledge. For the organization that sponsors the breeding program, especially if it is a profit-oriented private company, the benefits will often be measured in terms of the additional income earned through the sale of improved varieties (whether directly through commercial seed sales or indirectly through royalties or licenses). For society as a whole, the most important benefits that flow from a wheat breeding program tend to be the productivity gains achieved by farmers when they grow improved varieties produced by the program (measured as income increases or as cost reductions).[4] For simplicity, the emphasis in this chapter is on the latter type of benefit (farm-level productivity gains), although our analysis also applies to other types of benefits.

The benefits (or returns) generated by the current program can be broadly estimated based on the outputs from the program. In most cases, these will consist of improved varieties. In order to estimate the economic benefits associated with the adoption of improved varieties, usually it is necessary to answer the following questions:

- To what target regions are the varieties adapted?
- What advantage do the varieties confer (e.g., higher yield, improved quality)?
- What will be the average price of each incremental ton of grain produced, or the average price increase attributable to improved quality?
- What will be the rate and extent of adoption of the varieties following their release?

Once these questions have been answered (to the extent that they can be), it should be possible to estimate the benefits likely to be generated by the proposed change, if only in broad terms. For example, in his study of a public wheat breeding program in New South Wales, Australia, Brennan (1989a,b) made the following estimates:

- the program serves a target region that includes about 1.0 million ha planted to wheat each year, with average yields of 1.7 t/ha;
- each new variety produced by the program generates an average yield increase of 2.25% and an average improvement in the quality index of 1.09%;
- each 1% yield increase is worth $1.11/t, while each 1% improvement in quality is worth $0.81/t;
- on average, adoption of each variety peaks at about 16% of the target area in the seventh year following release; and
- each new variety has a productive life of 20 years.

Based on these broad estimates, Brennan was able to calculate the economic benefits generated by the breeding program, which total approximately $920,000 per year at peak adoption.

Estimating marginal costs and benefits of projected changes

The next task is to estimate the changes to both costs and benefits that would flow from incorporating physiology into the program. To a certain extent, these will necessarily be speculative, and they will in any case depend on the role envisioned for physiology within the larger breeding program.

Estimating future cost changes is frequently complicated by short-run versus long-run issues. In some cases, the physiology component can be expected to lead directly to cost savings, for example when the introduction of early generation screening methods is likely to reduce the number of lines that will have to be evaluated in later generations. Similarly, it has been shown that the introduction of certain tissue culture techniques can dramatically reduce the costs of multiplying experimental materials (Brennan, 1989b) (Box C). In other cases, however, the physiology component can be expected to lead to cost increases, at least in the short run, for example when the physiological tests undertaken can be expected to add measurably to the expense and/or time involved in screening.

[4] Depending on the degree to which farmers sell their products, and depending on the nature of the markets in which they sell their products, these productivity gains may be transmitted in part or in whole to consumers.

Particularly in these cases, it is important to determine whether the additional costs incurred in the short run are likely to lead to even greater cost savings over the longer term. Although additional expenditure in the short run is often justified on the grounds that the long-run payoffs will be large, this is not always the case. In their comparative analysis of two Australian wheat breeding programs, Brennan and O'Brien (1991) found that the slowdown in yield gains that resulted from incorporating early generation quality testing was not sufficiently compensated by the resulting gains in grain quality resulting from that higher selection pressure.

Estimating future benefits is complicated by the difficulty of precisely anticipating research outputs. All research is to some extent speculative, so the outcomes of any research investment can never be known with certainty. Nevertheless, it is usually possible to make educated guesses about the future values of key parameters that will determine the size (and distribution through time) of economic benefits. As stated earlier, the impact of incorporating physiology into an existing wheat breeding program potentially will be reflected in: 1) higher yielding varieties, 2) higher quality varieties, 3) better adapted (and therefore more widely grown) varieties, and/or 4) earlier release of new varieties. To the extent that it is possible to relate the incorporation of physiology to expected changes in the values of these key parameters, it will be possible to arrive at a rough estimate of expected benefits.

In estimating the benefits from a change to the program, it is important to remember that resources are limited, so any gains made in selecting for one objective must come at the expense of gains in selecting for another objective or objectives. These trade-offs must not be overlooked when benefits are being estimated.

Box C: Using Doubled Haploid Tissue Culture in a Wheat Breeding Program

Of the many forms of tissue culture available for use by wheat breeders, one of the most valuable is doubled haploid culture, which involves *in vitro* development of fixed lines from parental material. The technique is attractive because development of each generation of progeny can be initiated before the parents have achieved physiological maturity, thus accelerating the breeding process. Furthermore, it is carried out in the laboratory, rather than in the field, thus reducing the need for costly grow-out trials. By using doubled haploid tissue culturing techniques, wheat breeders can reduce the number of years needed to produce lines for advanced testing, while at the same time saving considerably on field production costs.

Brennan (1989b) examined the potential returns to a conventional wheat breeding program of adopting doubled haploid tissue culture techniques (see table). The anticipated impacts of adopting the techniques were modeled by assuming that: 1) the production of generations F_1 to F_5 would be compressed into just two years, as opposed to the usual five years, and 2) field production costs would be slightly reduced. In addition, it was implicitly assumed that lines produced using doubled haploid culture would be identical to the lines emanating from a conventional breeding program.

Brennan estimated the costs and benefits of the two alternative scenarios (conventional breeding without tissue culture and conventional breeding with tissue culture). His analysis showed that the use of tissue culture to accelerate the production of advanced breeding lines could be expected to generate handsome economic returns. Following the adoption of tissue culture, the net present value increased by more than $600,000 per line, and the benefit-cost ratio rose from 6.9 to 9.0. Thus, by slightly reducing production costs and significantly accelerating the production of advanced generation materials, doubled haploid tissue culture was shown to significantly increase the expected profitability of the breeding program.

Economic returns to the use of tissue culture in wheat breeding.

	Conventional breeding program†	Breeding program with tissue culture‡
Discounted costs ($A 000)‡	550	489
Discounted benefits ($A 000)‡	3,816	4,418
Net present value ($A 000)	3,266	3,929
Benefit-cost ratio	6.9	9.0

† All values in 1986 Australian dollars.
‡ Discounted at 5% per annum.
Source: Brennan (1989b).

Analyzing anticipated future flows of costs and benefits

Once the size of marginal costs and marginal benefits have been estimated, it is necessary to project their distribution through time. The simplest way to do this is by making year-by-year projections of marginal costs and marginal benefits. Given the relatively long period that can elapse between the time a research investment is initiated and the time tangible benefits are first realized in farmers' fields (known as the "research lag"), it is usually desirable to project marginal costs over a period of at least 10 years. The exact duration of the expected research lag will depend on the type of research that is being contemplated. In wheat breeding, some activities can be expected to have a relatively short research lag—for example, 3-4 years to introduce a grain

color characteristic that is controlled by a single gene. Other activities can be expected to have a much longer lag of 10 years or more—for example, incorporating drought tolerance, which is controlled by complex interactions among several different genes.

Once the size and distribution through time of research costs have been estimated, equivalent estimates must be made about expected flows of future benefits. In the case of a wheat breeding program, these will generally depend on the diffusion pattern of the new varieties produced by the program. Varietal diffusion patterns can vary widely, depending on the characteristics of the new variety or varieties, the degree to which farmers recognize and value the new characteristics, the effectiveness of the seed production and distribution system, and other factors. Some improved varieties are adopted very rapidly by a large proportion of farmers, resulting in a short, steep diffusion curve that reaches a ceiling level approaching 100% of the target region. Other improved varieties are adopted much more slowly and only by a relatively small proportion of farmers, resulting in a long, flat diffusion curve that tops out at a ceiling level well below 100% of the target area. Based on the assumptions made about the technology diffusion pattern (known as the "adoption lag"), the size and distribution through time of marginal benefits can be estimated.

Given the two types of lag involved in wheat breeding (research lag and adoption lag), it is advisable to consider an extended period when evaluating the desirability of incorporating physiology into an existing breeding program. As a general rule of thumb, marginal costs and benefits should be projected out over a 30-year period.

Next, the projected flows of costs and benefits must be discounted. Discounting is necessary to take into account the time value of money, i.e., the fact that costs and benefits realized in the future are valued less than costs and benefits realized in the present. To take into account the time value of money, discount factors are applied to future costs and benefits to convert them to their present value.

Discounting is carried out using the following formula:

$$D_n = U_n / ((1+r)^{n-1}),$$

where:

- D = discounted value of cost (benefit) in year n
- U = undiscounted value of cost (benefit) in year n
- r = discount rate
- *n* = year (where n = 1 is the present year, n = 2 is next year, etc.).

Alternatively, discount factors may be applied to future costs and benefits. Standard discount factors are readily available in most handbooks on project analysis and are readily generated by most financial spreadsheet programs.

Calculating measures of project worth

After projected costs and benefits have been discounted, they can be summed to obtain total discounted costs (TDC) and total discounted benefits (TDB). The TDC and TDB can be used to calculate two simple measures for use in assessing the attractiveness of any potential investment:

- net present value (NPV = TDB - TDC), and
- benefit-cost ratio (B/C ratio = TDB / TDC).

If the objective of the economic analysis is simply to determine whether or not the incorporation of physiology will be profitable, then it may be appropriate to proceed with the investment if it can be established that it will add to the overall economic returns generated by the breeding program. This will be the case if the NPV is positive (NPV > 0).

The main advantage of using NPV as a decision criterion is that it is easy to compute. One big disadvantage of the NPV measure, however, is that it fails to take into account the size of the proposed investment; without further investigation, there is no way to tell whether a given NPV was generated by a large investment or by a small one. This limits the usefulness of the NPV as a tool for deciding between alternative investment opportunities, because simply choosing the alternative with the highest NPV may not always be desirable. When asked to choose between investing $100 in one project expected to generate a NPV of $200 and investing $200 in another project expected to generate a NPV of $210, most people would prefer the first project–even though the NPV is lower.

If the objective of the economic analysis is to select among two or more alternative investment opportunities, then it will be preferable to use the B/C ratio as a decision criterion. Since the B/C ratio expresses (discounted) benefits per unit cost, it provides a measure of project worth that is not affected by the size of the project. Choosing the project with the highest B/C ratio, regardless of the size of the absolute size of the project, will ensure the highest possible returns to the investment.

Factoring in non-economic considerations

Measures of project worth such as the NPV and the B/C ratio provide useful information that can help in deciding whether or not to proceed with a proposed investment, but they should not be the sole basis for the decision. Most potential investments are characterized by costs and benefits whose value cannot easily be assessed, meaning they cannot be incorporated into the economic

"bottom line." For this reason, before taking a decision based on conventional measures of project worth such as the NPV and the B/C ratio, it is important to assess the extent to which non-economic considerations should be allowed to influence the final decision. Only after these non-economic considerations have been carefully considered can a balanced judgment be made concerning how to proceed.

Conclusions

With funds for agricultural research becoming increasingly scarce in most countries, research administrators face mounting pressure to ensure that available resources are used efficiently. Although there can be no question that a properly organized and managed physiology component has the potential to add value to wheat breeding activities, this does not necessarily mean that every wheat breeding program should include one.

This chapter has reviewed some basic concepts from investment analysis that can be used to assess the desirability of investing in a physiology component of a breeding program. We have described a series of steps that may be useful in helping to formalize decisions that all too often are still left to the "gut feeling" of scientists and research administrators:

- establish that the problem is appropriately addressed through breeding;
- estimate in rough terms the costs and benefits of the current breeding program;
- identify activities that are likely to change with the incorporation of physiology;
- estimate the economic consequences in terms of changes in costs and benefits;
- calculate economic measures of project worth (NPV and B/C ratio); and
- factor in any non-economic considerations.

Economic analysis is not infallible, so following these steps will not necessarily ensure that the "correct" decision will be taken. And as we have pointed out, the outcomes of research are by nature uncertain, so some of the parameters used in the economic analysis will necessarily be tenuous. But one big advantage of invoking an economic framework of analysis is that it forces decision makers to think somewhat more systematically about the many factors that are likely to influence the outcome of investment decisions; this in turn increases the likelihood of achieving a favorable outcome.

If decisions about the role of physiology in wheat breeding are taken based partly on economic considerations, then changes made to the organization and management of existing breeding programs are likely to lead to genuine improvements in efficiency. Improvements in efficiency in turn enhance the flow of new varieties emanating from the breeding programs, leading to increases in farm-level productivity that will eventually benefit both producers and consumers.

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Breeding for Adaptation to Environmental Factors

CHAPTER 7

Traits to Improve Yield in Dry Environments

R.A. Richards, A.G. Condon, and G.J. Rebetzke[1]

Genetic increases in wheat yields in dry areas have not been as great as in more favorable environments or where irrigation is available. A likely reason for this is that dry environments are characterized by unpredictable and highly variable seasonal rainfall and, hence, highly variable yields. This results in slow genetic advances in breeding programs because genetic variation in yield is masked by large genotype x year and/or genotype x location interactions.

It is interesting, but not surprising, that genetic increases in yield potential made by selection in predictable irrigated environments have resulted in broadly adapted wheats that are often well suited to both favorable and low yielding, rainfed environments. This arises because genetic variation in traits that contribute to high yield in all environments, such as a high harvest index, is greater in predictable environments and, therefore, more likely to be selected under favorable conditions. There is no reason why genetic advance in favorable environments should not continue to contribute to yield in less favorable environments, provided germplasm is widely evaluated in rainfed environments. However, a host of specific adaptations that may be uniquely important in rainfed environments can also be targeted to achieve higher regional yields.

Breeding for specific physiological traits that are expected to impart a yield advantage in dry environments has been notoriously difficult and unsuccessful. Among the reasons for the lack of success is that considerable research effort has been directed towards traits that are unlikely to improve productivity, and traits that have a low heritability or are difficult to measure. There may also be negative correlations between drought adaptive traits and with yield. For example, earlier flowering may result in reduced biomass accumulation or may increase the risk of frost damage.

Traits selected may also be inappropriate for the target region. For example, unless the trait conditioning survival at the seedling stage also conditions response to drought at later stages, breeding for survival during drought at the seedling stage may be totally inappropriate if drought only occurs around flowering or grainfilling. Another reason any attempt to breed for yield under drought is likely to fail is that in many dry environments low rainfall is not always the primary factor responsible for low yields; other factors, such as soil mineral nutrition or soilborne diseases, may be the overriding constraints. Since it is often difficult to identify those other, less obvious factors, this may also limit genetic progress in dry environments.

Only two traits have had a major impact on improving yields in rainfed environments: flowering time and plant height. Genetic manipulation of flowering time has been important to adjust the duration of vegetative growth, reproductive growth, and grain growth in relation to water supply, frost, and evaporative demand. A reduction in plant height has been universally important in increasing the proportion of grain to biomass (harvest index), provided biomass growth is not compromised.

The understanding of physiological and morphological traits that limit yield under drought has improved in recent years, and this has opened up new opportunities. Before discussing these, and how they may be used in a breeding program, it is important, first, to establish how widespread drought may be and in which environments yield may be limited by insufficient water. It is also important to establish, in any environment, whether water is the primary factor limiting yields or whether other factors may override the water limitation.

The Extent of Drought and Its Nature

Globally, CIMMYT recognizes 12 distinct mega-environments (MEs) where wheat is produced. Only three are irrigated (Rajaram et al., 1995). Several of the rainfed environments have high rainfall (>500 mm) but may experience intermittent drought or terminal drought in years when rainfall is below average.

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Three MEs experience frequent droughts and are considered marginal with respect to food production.

There are subclassifications within each of the MEs. For example, ME4, one of the largest rainfed environments, comprising about 33 million hectares, is further subdivided into environments with late drought (e.g., Mediterranean climates), early drought (e.g., Argentina) and regions where wheat is grown on residual moisture (e.g., parts of India following monsoon rains).

Since drought can also be a problem in favorable environments, physiological means of minimizing drought stress or improving water use efficiency may also influence yield in high yielding, rainfed environments. Indeed, the best farmers will often have the best yields as well as experience the most severe droughts because their practices lead to the greatest use of available soil water. Reducing the impact of drought may also be important in irrigated environments if it results in less water being used to achieve high yield (i.e., high water use efficiency).

Is Drought the Primary Determinant of Yield in a Dry Environment?

Low rainfall is usually perceived to be the most important factor resulting in low yields in dry environments. However, this may not always be true. Other factors, such as disease, soil nutritional problems, or even waterlogging at certain times, may limit yields and should probably be overcome as far as possible before applying physiological understanding to improve yields under drought. Because foliar diseases are easily observed, they are targeted in breeding programs. However, other more insidious, not readily identifiable problems may also result in low yields and mistakenly be attributed to drought. There may be nutritional problems, perhaps mineral toxicities due to soil pH; nutrients may be chronically low or even too high; there may be soilborne pathogens such as nematodes, root or crown fungal diseases such as take-all, or rhizoctonia. All of these factors result in either poor root growth or diseased roots, and reduce water uptake, inducing symptoms of drought (Picture 1).

There are a number of ways to determine whether soil-based factors limit yields (Table 1). First, soil tests can be conducted to assess whether a range of disorders such as pH or micro- and macro-nutrient deficiencies or toxicities may limit yields. Second, roots can be examined around mid-tillering to determine the presence of take-all, rhizoctonia, or some nematode species (e.g., cereal cyst nematode). Third, tiller development can be monitored. Tillering in temperate cereals follows a very predictable pattern in favorable conditions and therefore can be used to detect poor plant health. For example, primary tillers appear in the axils of leaves 1, 2, 3 etc., and secondary tillers appear in the axils of primary tiller leaves. Missing tillers under favorable light and macro-nutrient conditions probably indicate some disorder.

Finally, a very effective way to identify limiting factors is to grow a range of probe genotypes, or other cereal species, that are known to vary in their tolerance or resistance to soil mineral disorders or soilborne pathogens (Cooper and Fox, 1996). Relatively greater growth or yield of one or more of the probe genotypes may make it possible to diagnose a particular problem.

Another valuable way to determine whether factors other than drought are more important in limiting yields is to

Table 1. Methods to assess whether biotic or abiotic factors other than drought are limiting yields.†

Tests for soil pH or micro- and macro-nutrient deficiencies or toxicities
Planting and subsequent growth of probe genotypes or species
Tiller development
Examination of roots
Soil moisture availability at harvest
Calculation of water-use efficiency

† Methods are listed in the order in which they should be conducted during the crop cycle.

Picture 1. Droughted sections of fields caused by the root disease take-all and waterlogging during the vegetative period. Limiting the effects of these factors, principally through management, will increase yields in dry environments.

calculate the water use efficiency of the crop and/or determine whether crops are leaving water behind in the soil after harvest. Soil moisture measurements down to at least 1 m will establish the latter. The simplest calculation of water use efficiency is the quotient of grain yield to cumulative rainfall from a month before sowing to physiological maturity. A more accurate value is obtained by using the sum of seasonal rainfall and the difference in soil moisture content at sowing and at harvest from at least the top 1 m of soil. This value will vary depending on the aridity of the environment, the amount of water stored in the soil prior to sowing, and the seasonal distribution of rainfall. Maximum values are around 20 kg ha^{-1} mm^{-1} for cooler environments and may be as low as 10 kg ha^{-1} mm^{-1} in hotter, drier environments. However, comparing moisture content in different soil types and among farmers within a region should identify likely anomalies.

If factors other than drought are found to be the primary determinants of yield, both breeding and management may be important to overcome them. Breeding may be used to increase tolerance or resistance to diseases and to increase tolerance to nutrient disorders, some of which are discussed in this volume. Rotations may be the most effective way to reduce root diseases in the absence of genetic resistance.

Breeding for Yield in Water-Stressed Environments

Yields are likely to continue to increase, although possibly at a slower rate than in the past few decades. Increases in wheat yields in rainfed environments have been achieved during most of this century through the use of conventional breeding methods. Direct selection for yield in target environments will remain the cornerstone of wheat improvement because of the great integrating capacity of final grain weight for genotypic adaptation and the efficiency with which trials can now be conducted. The effectiveness of conventional breeding methods will depend on non-genetic factors such as how precise experiments are, on land and land preparation, sowing and harvesting equipment, trial maintenance, as well as statistical procedures to more precisely discriminate between entries in less than ideal trials or field conditions. Their effectiveness will also depend on trials being conducted in representative regions using standard farming practices and adequate plot sizes to estimate yields.

It is expected that physiological approaches to breeding will become more important. These will develop from an improved understanding of the factors regulating wheat production in the prevailing farming systems, and of wheat physiology and ways to manipulate it in relation to the climate where wheat is grown. This knowledge will make it easier to identify the major limiting factors and ways to overcome them by more precise targeting of physiological traits to reduce the impact of drought and thereby increase yields.

A physiological approach may increase the rate of yield improvement in a number of ways. First, by identifying traits for which there is inadequate genetic variation in breeders' populations. This, in turn, will facilitate the identification of new parental lines to increase variability for key traits. Second, large seasonal variation in yield and G×E may make direct selection for yield ineffective, so that more specific targeting of physiological characters that limit yield and have high heritability may be more effective than direct selection for yield. Third, greater yield stability may be important to minimize yield losses in the driest years and could be achieved through selection for physiological traits. Fourth, selection for physiological traits, particularly in early generations or out of season, may be more cost effective than direct yield selection. Yield trials are expensive to conduct, and if the population can be culled in earlier generations using critical physiological criteria, this will allow either more high yielding, adapted entries to be tested or greater replication to increase selection precision. Fifth, selection may be conducted out of season, which would allow several generations to be completed each year.

If the physiological trait has reasonably high heritability and is not too difficult to select, backcrossing can be very effective for incorporating the trait into an already well adapted cultivar with good grain quality and disease resistance. This will ensure rapid progress in breeding and should result in a high frequency of progeny with high yield, good quality, and appropriate disease resistance.

Trait identification

The most appropriate way to identify traits that may limit wheat yields in dry environments is to use the framework proposed by Passioura (1977). This framework is based on grain yield, not on drought protection or survival under drought, which were popular in the past but largely unsuccessful. Passioura proposed that when water is limiting, grain yield is a function of: 1) the amount of water used by the crop, 2) how efficiently the crop uses water for biomass growth (i.e., water-use efficiency, or above-ground biomass/water use), and 3) the harvest index, i.e., the proportion of grain yield to above-ground biomass. Since each of these components is likely to be largely dependent on the others, an

improvement in any one of them should result in an increase in yield. This identity is shown below.

Grain yield	=	Crop water use	X	Water-use efficiency	X	Harvest index

The local environment must be considered in conjunction with this identity, since traits identified as yield limiting may only be so in specific environments. Indeed, because of seasonal variability in rainfall in dry environments, a particular trait may not even be important every season in a given region. Exceptions to this are traits that are universally important in water-stressed environments—for example, good crop emergence and establishment, and high water use efficiency. Subsequent tables listing traits will indicate whether they are universal or specific to a particular type of environment (e.g., having early or end-of-season drought).

Appropriate flowering time is the single most important factor to maximize yield and adaptation in dry environments. Further genetic modification of flowering time in different regions is likely, since management practices are constantly changing and providing new crop opportunities. For example, new machinery and herbicides may facilitate earlier sowing, or the best adapted cultivars may require different sensitivities to photoperiod or vernalization than currently grown cultivars.

In the rest of this paper we shall tabulate the traits that may be important to increase yield in water-stressed environments. A comprehensive list of traits is provided for consideration. This is because the choice of traits for use in a breeding program will depend on numerous factors such as the nature of drought, trait expression in current cultivars, available genetic variation, and ease of genetic manipulation. We start off with traits that can be selected in early generations, and then list and discuss in some detail traits that may be important to increase either water use, water use efficiency, or harvest index.

Selection in F_2 populations

Table 2 lists physiological traits that, in general, have high heritability and can be visually selected in F_2 populations. All are likely to be important in most rainfed environments. For selection in the F_2, populations should be grown under favorable moisture conditions to maximize genetic variation in morphological traits and to allow the development of diseases so that selection for resistance can also be made.

Water Use

A deep root system is synonymous with drought resistance and with more water uptake from the soil. This trait is, of course, difficult to measure. However, it may be that the root system of current cultivars is adequate and so further improvement may not be necessary. However, information on whether current cultivars extract all available soil water is required to establish this.

If, on the contrary, the root system of current cultivars does need improvement, the simplest way to increase rooting depth and root distribution is to increase the duration of the vegetative period. This may be achieved by sowing earlier or planting later-flowering genotypes. Increased early vigor may result in both faster growth of deep roots and more adventitious roots in the top soil. The latter may be important to mop up water and nutrients before evaporative losses dry the top soil. Appropriate ways to select for greater vigor will be discussed later.

Table 3 lists plant characteristics that may either increase crop water use or root growth, or indicate genotypes that have a deep root system. Phenology and early vigor are listed as traits that increase water use, since they may result in deeper roots. Tiller inhibition is also included in that category, based on evidence that assimilates normally used for the growth of additional tillers could

Table 2. Morphological traits for visual selection in an F_2 population in regions where drought limits yield.

Trait	Heritability	Expected GxE	Universal or environment-specific trait
Selected at flowering			
Flowering time	high	low	specific
Small flag leaves	intermediate	high	universal
Glaucousness	high	low	universal
Awns	high	low	universal
Disease resistance	disease dependent	low	universal
Selected near physiological maturity			
Plant height	high	low	universal
Floret fertility	low	intermediate	universal
Maintenance of green leaf area	low	high	specific
Large seed size	high	intermediate	universal

be used for root growth if a gene for tiller inhibition is present. Osmotic adjustment may also increase root growth and the ability to extract more soil water. However, selection for this trait is not easy at the present time.

Traits that indicate deeper rooting may be used as selection criteria (Table 3). Low canopy temperature or high stomatal conductance (see later), both of which can be measured very simply, may indicate more favorable soil moisture conditions and, hence, a deeper root system. These characteristics could be valuable in selection, but measuring them requires extremely uniform soils to eliminate any subsoil spatial variation.

"Stay-green" leaves may also be an indicator of favorable soil moisture conditions and, therefore, deeper roots. This trait is desirable when, after very dry conditions, there is high probability of further rainfall. Stay-green capability means there would be more photosynthetic tissue for further assimilation and additional soil water extraction. The degree of rolling in the flag leaf, which occurs in unbent leaves of plants growing in dry soils, may also indicate plant water status and, hence, a deep root system. Leaf rolling may be an adaptive feature to avoid leaf senescence and maintain leaf area, which allows further soil water extraction in the event of late rains. However, care must be taken not to to confound these traits with anthesis date.

Water use efficiency

The term water use efficiency (WUE) is generally used to express the ratio of total dry matter to evapotranspiration. An increase in transpiration efficiency (TE, dry matter/transpiration) and/or a reduction in soil evaporation will increase WUE. Both of these components may readily be improved through breeding.

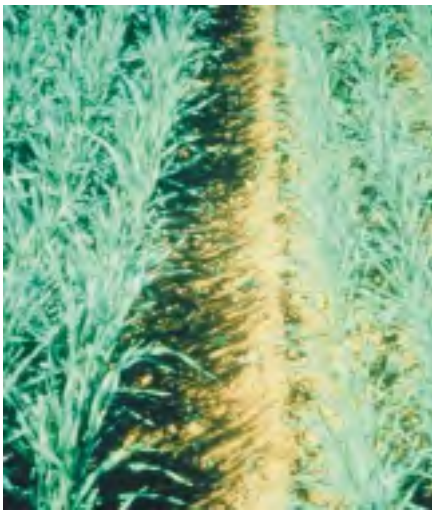
Reducing water evaporation from the soil

Wheat is often grown in environments where rainfall between sowing and stem elongation is frequent. This is typical of Mediterranean environments around the world. In these environments, avoiding water evaporation from the soil is essential to ensure sufficient moisture throughout the crop season. Any increase in early seedling vigor should reduce evaporative losses from the soil surface (Picture 2). The potential for gains is large because 1) as much as half of the growing-season rainfall may be lost through evaporation from the soil, and 2) widely-grown semidwarf wheats have inherently low vigor compared with taller wheats (Richards, 1992). Greater vigor is not likely to be as important in other environments, although if it results in more growth when vapor pressure deficit is low, this will also result in higher TE. If crop duration is short, greater vigor is likely to increase final biomass and yield. Furthermore, greater crop vigor may be an effective way to reduce weed growth and, hence, herbicide use in most environments.

Traits that may contribute to increase seedling vigor are listed in Table 4. The first requirement for maximizing vigor is to establish a high plant population as quickly as possible. This has become particularly important with the widespread adoption of semidwarf cultivars, as they have a shorter coleoptile and slower emergence than standard-height wheats. Short coleoptiles result in poor emergence, which leads to poor crop establishment. This is true, particularly when seeds of semidwarf wheats are sown deeply to seek moisture or are sown into stubble. Better

Table 3. Plant traits that may increase soil water use or root growth, or indicate deep rooting.

	Heritability	Expected GxE	Universal or environment-specific trait
Traits that increase soil water use			
Deeper roots	low	high	specific
Phenology	high	low	specific
Seedling vigor	high	low	specific
Tiller inhibition	high	low	specific
Osmotic adjustment	low	high	specific
Traits that indicate deeper roots			
Canopy temperature	intermediate	high	-
Stomatal conductance	intermediate	high	-
Stay-green	intermediate	high	-
Leaf rolling	intermediate	high	-



Picture 2. Greater seedling vigor, such as that achieved by the crop on the left, will reduce loss of soil water by evaporation from the soil surface and limit weed growth.

emergence is achieved by sowing wheats with long coleoptiles. Increased coleoptile length can be achieved by selection within semidwarf germplasm, but greater progress can be made using parents that are sensitive to gibberellic acid (GA), although short stature also needs to be selected (Rebetzke and Richards, 1999). Such wheats may have a coleoptile up to twice the length of coleoptiles found in current semidwarfs. Wheats with long coleoptiles also tend to have larger early leaves and more rapid rates of emergence, which together contribute to faster leaf area development.

Wheats with large grains also have a higher rate of emergence and larger, more vigorous plants than small-grain wheats. However, if sowing rate is based on weight per unit area, there may be little advantage in sowing large-grain wheats. Of traits that improve early vigor (Table 4), we have found that the breadth of the seedling leaves and the frequency and size of coleoptile tillers are likely to be the most effective. These two traits, together with long coleoptiles, should be selected first. Selection protocols for these are described later.

Other traits that are likely to improve seedling vigor, but which we consider of lower priority, are also shown in Table 4. Although genetic variation exists for each of these traits, they are less likely to influence vigor, are more difficult to select, or initial findings suggest that genetic variation is small. Nevertheless, expression of these lower priority traits may in some cases be improved by selection for the high priority traits. For example, to improve plant establishment by increasing coleoptile length, we recommend substituting the GA-insensitive dwarfing genes *Rht1* (*Rht-Blb*) and *Rht2* (*Rht-Dlb*) with GA-sensitive major or minor genes. Evidence suggests that this will also increase seedling emergence and leaf expansion rates. Furthermore, selection for broad seedling leaves, which integrates both embryo size and specific leaf area, should also increase leaf area ratio.

Improving establishment

The height of semidwarf wheat cultivars is principally due to the GA insensitive *Rht1* and *Rht2* alleles. Although these alleles result in a plant height that maximizes yield, they also limit the length of coleoptiles, which often causes poor establishment (Picture 3). However, wheat plant height can be reduced by the use of either GA-insensitive or GA-sensitive genes. There are GA-sensitive genes that reduce plant height to that of plants that possess GA-insensitive *Rht* genes, yet produce coleoptiles with up to 100% greater length than coleoptiles found in GA-insensitive genetic backgrounds (Rebetzke et al., 1999). Furthermore, plant height and coleoptile length seem unrelated in GA-sensitive backgrounds, which facilitates simultaneous selection for both traits in a breeding population. Our studies show these GA-sensitive dwarfing genes confer the same desirable partitioning characteristics as in current semidwarf wheats, but may also produce greater biomass when soil conditions at sowing are unfavorable (Rebetzke and Richards, 2000).

Genetic studies conducted on different wheat populations have shown that GA-sensitive dwarfing genes have high

Table 4. Traits that may improve plant establishment and early canopy development in wheat.

Traits	Heritability	Expected GxE	Universal or environment-specific trait
Highest priority			
Long coleoptiles	high	low	universal
Broad seedling leaves	high	low	specific
Émbryo size	high	low	specific
Specific leaf area	intermediate	high	specific
Large coleoptile tiller	intermediate	high	specific
Lower priority			
Large grains	high	low	universal
Fast emergence	low	low	specific
Fast leaf expansion rate	intermediate	low	specific
Low temperature tolerance	intermediate	low	specific
Crown depth	intermediate	intermediate	specific
Crown to shoot partitioning	intermediate	low	universal
Leaf area ratio	intermediate	low	specific

Picture 3. Semidwarf GA-sensitive wheats with long coleoptiles establish better under adverse conditions than GA-insensitive semidwarf wheats with the *Rht1* and *Rht2* alleles.

heritability, making it easy to select for them. Coleoptile length is also highly heritable, with long coleoptile selections maintaining their longer coleoptile ranking across a wide range of soil temperatures.

We typically select at 19°C, as large numbers of families can be sown and assessed in just under two weeks. Seeds of uniform size are sown at a uniform depth (10 mm) in a deep, wooden tray containing a fertile potting mix watered to field capacity. Trays are covered with an opaque plastic sheet to exclude light and are then placed at 19°C. After 200°Cd (approximately 10 days, assuming a base temperature of 0°C), the plastic sheet is removed and longer coleoptile progeny identified either visually or by measuring with a ruler (Picture 4). Selected families can then be transplanted into a glasshouse or into the field, provided good care is given to the transplanted seedlings. Plant height can be determined based on primary tillers at maturity, and plants of the desired height retained. High heritability for plant height and coleoptile length indicates that screening for these traits can commence as early as the F_2 generation.

Seedling vigor

Although wheats with high seedling vigor offer considerable promise, there is little genetic variation for this characteristic among currently grown semidwarf wheat cultivars. Characterization of seedling vigor differences between wheat and barley (López-Castañeda et al., 1996) has shown that barley achieves almost double the leaf area of wheat primarily because of its earlier emergence (around 1 day), larger embryo, and greater specific leaf area (leaf area-to-leaf mass ratio). Barley often produces large coleoptile tillers that emerge before the primary tillers and contribute to increased vigor. Exhaustive screening of international wheat collections has revealed two populations containing suitable genetic variation for leaf area measured early in the season (Richards and Lukacs, 2001). As in barley, the greater early vigor of these wheats arises from their larger embryo and specific leaf area. Pyramiding these two independent characteristics has produced progeny with even greater vigor than the original wheat parents (Richards, 1996). Large coleoptile tillers are also frequent in these high vigor progeny.

Picture 4. Wooden tray containing seedlings grown in the dark to screen for coleoptile length.

Our experiments show that heritability for early leaf area is small. However, leaf breadth averaged across the first two leaves is highly heritable and has a strong genetic correlation with leaf area (Rebetzke and Richards, 1999a). The combination of higher heritability and strong genetic correlation suggests that selection for early leaf area development using leaf breadth as a measure of leaf area is as good as selecting for leaf area itself. Measurement of leaf width has other advantages: it is rapid and non-destructive, and can be done on seedlings. The larger heritability of leaf width indicates that it is less sensitive to G×E interaction than leaf area. Nonetheless, genetic variance for leaf width and, to a greater extent, for plant leaf area and biomass is greatest in the cooler months when wheat is typically sown in farmers' fields. Families showing greater seedling vigor in our *ex situ* tray assessments also show greater vigor in the field. This is also true for coleoptile tiller assessments, although the appearance of the coleoptile tiller seems to be somewhat dependent on soil fertility and soil strength.

The procedure for assessing lines or families for early vigor is a simple one. It is essential to start with good quality seed of each line. Because seed size can have a big effect on early vigor, it is necessary to discard seeds that are either too small or too large, or to weigh individual seeds for later covariance adjustment. We measure seed size either visually based on seed length × breadth assessments or by weighing seed on a balance. Seed may also be passed through different sized screens.

Seeds are planted at uniform depth in a deep tray containing a fertile potting mix and then watered. Time of emergence is scored by measuring seedling height (from the soil surface to the tip of the first leaf) when approximately 90% of seedlings have emerged. Leaf width of

the first two leaves is measured with a ruler upon full expansion of the second leaf (usually at 2.4 leaves). Leaf length and leaf number are also measured at this time. Coleoptile tillers are simply assessed by recording their presence or absence; if present, the length of the tiller is recorded. More detailed assessment of coleoptile tillers might include the time of tiller emergence from the soil. The best plants or families are then transplanted and used for hybridization or seed increase. Picture 5 shows seedlings growing in wooden trays at the time of selection.

Field observations indicate that wheats containing GA-insensitive genes for reduced height produce smaller leaf areas and plant dry weights early in the season (Richards, 1992). Preliminary data also indicate that the potential of larger embryos and greater SLA to increase seedling vigor may be somewhat restricted when expressed in these GA-insensitive *Rht* genetic backgrounds. We believe that the expression of seedling vigor using larger embryos and greater SLA would be enhanced in genetic backgrounds containing GA-sensitive dwarfing genes. Furthermore, longer coleoptiles and greater seedling vigor would provide a "package" that is better suited to production in less favorable environments.

Greater transpiration efficiency

Transpiration efficiency (the ratio of dry matter to transpiration), the other component of water use efficiency, is also amenable to improvement. There are numerous ways to increase TE in wheat; the more important ones are given in Table 5. Perhaps the simplest way to improve TE is to ensure that the period of maximum biomass increase occurs during the coolest period. This capitalizes on the fact that less water is required for growth when it is cool. To achieve this may require a change in planting time so that full canopy closure is achieved by the onset of the coolest period. It may also require wheats with a different phenology. Since this is such a simple way to improve TE, opportunities to alter management practices for earlier sowing, including dry seeding, should be explored where practical. Selection for increased vigor outlined earlier should also result in a higher leaf area index and, hence, more light interception and growth when it is cool, resulting in higher TE.

Other ways to improve TE are to increase surface reflectance, thereby lowering surface temperatures of photosynthetic tissue. Selection for glaucousness and, possibly, pubescence are two ways to achieve this. Also, smaller photosynthetic surfaces are more effective at dissipating heat than larger surfaces, if it is dry and hot. Selection for awns that maintain photosynthetic activity and for a small erect upper canopy of leaves may be an

Picture 5. Measuring leaf breadth of seedlings grown in trays to screen for early vigor.

Table 5. Traits that can be selected to improve transpiration efficiency in wheat.

Traits	Heritability	Expected GxE	Universal or environment-specific trait
Phenology	high	low	specific
Seedling vigor	high	low	specific
Carbon isotope discrimination	high	low	universal
Ash content	?	high	
NIR	?	?	
Stomatal conductance	intermediate	high	
SPAD	intermediate	intermediate	
SLA	intermediate	intermediate	
Canopy temperature	intermediate	intermediate	
Glaucousness	high	low	universal
Pubescence	high	low	universal
Residual transpiration	?	high	universal
Leaf size and habit	?	high	universal

effective way of achieving this. Some transpiration occurs at night through incompletely closed stomata and through the cuticle. This is unlikely to be large in most environments, but it could result in up to 0.5 mm of water per day being lost from a crop at night if the vapor pressure deficit is high. Cuticular loss during the day may also be important. Substantial genetic variation for cuticular transpiration has been found in wheat, and a relatively simple procedure to select for it has been established (Clarke and McCaig, 1982).

Carbon isotope discrimination

Carbon isotope discrimination (Δ) is a measure of the ratio of the stable isotopes of carbon ($^{13}C/^{12}C$) in plant dry matter relative to the value of the $^{13}C/^{12}C$ ratio in the air that plants use in photosynthesis. About 1% of the CO_2 in the atmosphere contains ^{13}C. Because $^{13}CO_2$ is a larger molecule than $^{12}CO_2$, plant species such as wheat and barley with the C_3 photosynthetic pathway discriminate against $^{13}CO_2$ during photosynthesis. As a result there is relatively less ^{13}C in plant dry matter than in the atmosphere. The extent of discrimination against ^{13}C varies among genotypes.

The processes that influence the extent of ^{13}C discrimination are also important in determining the TE of leaf gas exchange. Thus Δ provides a relative measure of leaf-level TE among genotypes or breeding lines. Discrimination is less (i.e., the value of Δ is low) when TE is high. In terms of leaf gas exchange, TE is a ratio which describes how much CO_2 is assimilated in photosynthesis per unit water lost in transpiration. The rate of CO_2 uptake into the leaf is determined by 1) the "sucking power" of the leaf for CO_2, i.e., the amount of photosynthetic machinery per unit leaf area, and 2) how easily CO_2 can move into the leaf, which is determined by the stomatal conductance. Since CO_2 and water are exchanged through the same stomatal pores, stomatal conductance is also important in determining the rate of transpiration. The other major determinant of transpiration rate is the evaporative demand on the leaf (i.e., the "sucking power" of the air for water), which is most precisely measured as the vapor pressure gradient between the leaf and the air.

We have shown that the Δ of plant material is closely related to TE integrated over the life of the plant material sampled (Farquhar and Richards, 1984; Condon et al., 1992). We are now using this technique to breed wheats with higher TE. Lines developed so far using backcrossing have higher yields than the recurrent parents in water-stressed environments.

There are several properties of Δ that make it appealing as a potential breeding tool (Hall et al., 1994). Most importantly, Δ is a lot easier and faster to measure than TE itself. Measuring Δ of plant dry matter sampled from a collection of genotypes provides an estimate of variation in leaf-level TE integrated over the time that the dry matter was laid down. We have established that the heritability of Δ is high and that Δ can be measured on freshly sampled dry matter or on samples that have been stored indefinitely.

Our experience indicates that assessing lines or families for Δ is best done using plants grown in the field. In essence, leaves of similar age are sampled from field plants at full tillering before the onset of drought, when vapor pressure deficit is low. Sampling plant material later is less reliable because of possible differences in phenology, soil water availability, and translocation of assimilates formed earlier. We have found heritability of single-plant Δ to be low; hence, it is advisable to take samples from several plants.

To do this we sow F_3 (or later generation) families in replicated short rows (Picture 6). Plant material is sampled at full tillering, before stem elongation, by cutting along the row at

Picture 6. Short rows of F3 families from which leaf material will be harvested to screen for carbon isotope discrimination.

about 5 cm above the soil surface. The plant tops (mainly leaf material with minimal sheath and stem) are placed in paper bags for oven drying at about 70ºC. Because they are cut above the apex, the plants are able to recover, and plant height, flowering time, and disease reactions can be assessed. If Δ analyses are completed before flowering, it is also possible to identify lines that could be used in further crossing (e.g., in a backcrossing program). Once the sampled material is oven dried, it can be stored indefinitely. Prior to Δ analysis the material should be ground as finely as possible. It is useful to re-dry the samples before grinding to remove residual moisture. Thorough mixing of the ground sample is also advisable to minimize subsampling errors, since only about 5 mg of the sample is used for carbon isotope analysis.

For temperate cereals, including wheat, the utility of Δ in breeding is likely to vary depending on the extent to which yield is limited by water supply and also at what stage of the crop cycle water stress occurs. In wheat, Δ is a "conservative" trait associated with somewhat slower water use and possibly slower growth rate. Consequently, in environments where water is largely non-limiting, low Δ (high TE) has been associated with relatively low yield potential in wheat (Condon et al., 1987). The implication is that selection for high Δ in these environments may prove useful in identifying lines with high yield potential (see chapter by Fischer). Selection for high Δ may also be effective for yield improvement in rainfed environments where crop growth to anthesis is sustained by current rainfall or where drought is relieved well before anthesis. In these environments high Δ should be associated with more dry matter at maturity.

In environments where crop growth is heavily dependent on moisture stored in the soil profile from rain that falls outside the main crop growth phase, selection for low Δ (rather than high Δ) is likely to be effective for yield improvement. In these environments, where transpiration makes up a high proportion of total crop water use, high TE promotes conservation of soil water, which sustains yield determining processes in the period leading up to and after anthesis. In stored-moisture environments, profligate high Δ genotypes are more likely to exhaust the soil water supply before this critical phase.

The fact that the direction of selection based on Δ may vary depending on the target environment may be seen as a disadvantage of Δ. Another disadvantage is that its measurement requires specialized equipment (a ratio mass spectrometer) and therefore is relatively expensive (US$5-15 per sample at commercial analytical laboratories). For this reason several "surrogates" for Δ have been proposed that may be suitable for use at various stages of a breeding program. These surrogates have been shown either to be correlated with Δ itself or to provide estimates of the important plant processes that determine genotypic variation for Δ.

Surrogates that may help cull the populations that are related to Δ, for reasons not yet clear, are ash content of dry matter (Masle et al., 1992) and near-infrared reflectance of bulk tissue (Clark et al., 1995). Furthermore, measuring traits related to the determinants of Δ (viz., stomatal conductance and photosynthetic capacity) may also be effective for culling populations during breeding. The measurement of leaf stomatal conductance, particularly with a new, portable and fast viscous-flow porometer (Picture 7; Rawson, 1996), or an infrared thermometer to measure canopy temperature (see chapter by Reynolds), which in itself is a function of stomatal conductance, may be advantageous. Possible surrogates for photosynthetic capacity are leaf chlorophyll content measured using, for example, the Minolta 'SPAD Meter' (Araus et al., 1997), or specific leaf area (the leaf area per unit leaf dry weight), which often reflects the amount of photosynthetic machinery per unit leaf area (Wright et al., 1988).

Picture 7. Viscous-flow porometer used to measure leaf stomatal conductance. It takes between 10 and 20 seconds to do single measurements on healthy wheat plants.

Harvest Index

Measuring harvest index (HI) is simpler than measuring water use and water use efficiency. Above-ground biomass and grain yield of a "grab" sample at maturity together provide a simple measure of HI in a breeding program. Furthermore, when comparing genotypes, the measurement of HI is generally more robust than either of its two components (yield and above-ground biomass) and genotype ranking for HI is relatively stable, provided plants are grown under favorable conditions. However, like water use and water use efficiency, the genetic manipulation of HI in variable rainfed environments is not simple, given that two separate factors determine HI in crops that experience drought and, hence, two factors can be genetically manipulated to maximize HI to achieve a high grain yield. The first determinant of HI is independent of drought, i.e., HI in the absence of drought in a given environment. The second determinant of HI is drought dependent, i.e., it depends largely on water availability during grainfilling.

Drought-independent harvest index

Traits that result in a high HI under optimal conditions are likely to contribute to high yield in all environments, provided there is no sacrifice in biomass. This is the advantage semidwarf wheat varieties have over standard height varieties and is the reason semidwarfs have been so successful in both favorable and less favorable environments. A high drought independent HI in a given environment is a prerequisite to high yield under drought, as it determines the genetic potential in that environment. In brief, the drought independent HI is a function of differential partitioning of dry matter to reproductive and non-reproductive organs. Thus, incorporating genes that contribute to height reduction and to early flowering is a simple and effective way to increase HI as they result in less growth of vegetative organs. Traits that increase the drought independent HI are listed in Table 6. Factors contributing to a greater HI under favorable conditions are discussed elsewhere (in this volume and in Richards, 1996).

Drought-dependent harvest index

Only when the HI of a given genotype in the absence of drought in the target environment is already high, does the genetic improvement of the drought dependent HI become important. Drought dependent HI is a function of post-anthesis water use. If post-anthesis water use as a proportion of total water use is large, HI will be large. If soil water is finite, conserving soil water before flowering so that it can be used for grainfilling should increase HI. Achieving high grain yield will then depend on the balance between growth before and after anthesis. Getting this balance right is difficult. For example, too little growth before anthesis will limit total dry matter yield but maximize HI, whereas too much growth before anthesis will ensure that total dry matter yield is maximized, but could result in a low HI.

Water use is a function of evaporative demand and leaf area. There is little that can be done to alter evaporative demand, although crop phenology may be altered to change the timing of crop growth. However, there are a number of traits that may be manipulated to genetically reduce leaf area development. These may regulate water use and therefore effectively increase the drought-dependent HI. Table 6 lists traits that achieve this.

Phenology is a major determinant of drought independent HI, as it can determine the amount of pre-anthesis and post-anthesis water use. For example, if flowering occurs a few days earlier, this may mean an extra 5-10 mm of soil water for post-anthesis use (drought escape) and, hence, a higher HI and, perhaps, greater yield. However, if earlier flowering also results in less pre-anthesis growth, yield may not be greater despite the higher HI. Seasonal variation in yields is generally large in water stressed environments, and sowing

Table 6. Traits that improve the harvest index of wheat.

Trait	Heritability	Expected GxE	Universal or environment-specific trait
Drought-independent			
Phenology	high	low	specific
Height and peduncle length	high	low	universal
Tiller inhibition	high	low	specific
Assimilate retranslocation	intermediate	high	universal
Drought-dependent			
Phenology	high	low	specific
Tiller inhibition	high	low	specific
Xylem vessel diameter	intermediate	low	specific
Leaf conductance	intermediate	intermediate	specific
Stay-green/leaf rolling	intermediate	intermediate	specific
Assimilate retranslocation	intermediate	high	universal

earlier flowering cultivars may not always be advantageous. After decades of breeding and yield testing, it is likely that anthesis time of successful varieties in a given region is close to optimum. Nevertheless, earlier flowering will result in higher water use efficiency in environments where temperatures increase after anthesis. Combining earlier flowering with greater vigor or frost resistance may also assist in improving HI and yield.

A reduction in tillering may contribute to a higher HI both in the presence and absence of drought because of the lower number of sterile tillers. Reduced tillering may also contribute to a higher HI under drought because a smaller leaf area before anthesis increases the likelihood of less transpiration and more water being available for grainfilling. Narrower xylem vessels in the seminal roots would have a similar effect. Seminal roots are responsible for uptake of water in the deeper soil layers, and reducing the diameter of xylem vessels in the roots should increase the hydraulic resistance. This in turn slows water use before anthesis if it is dry, making more soil water available for grainfilling. An important feature of reducing xylem vessel diameter is that it is likely to be advantageous under dry conditions but neutral in favorable conditions, as the nodal roots, which are in the topsoil, will supply the crop with its water requirement. Other ways to reduce pre-anthesis transpiration may be to select for smaller uppermost leaves, including the flag leaf, or for lower stomatal conductance, and/or lower nighttime leaf conductance.

There is substantial genetic variation among wheats for tillering, xylem vessel diameter, leaf dimensions, and stomatal or cuticular water loss. In the case of tillering, there is a major gene on chromosome 1AS that inhibits tillering (Richards, 1988). The penetrance of this gene varies with climate and genetic background, and so it provides substantial scope for the regulation of tillering and, therefore, leaf area.

Measuring xylem vessel diameter is not difficult: a cross-section of the seminal roots is made using a tightly sprung clip to hold the tissue. It is then stained with toluidine blue. The diameter of the largest vessel can be measured quickly under a microscope (Richards and Passioura, 1981). This is best done on wheat seedlings with two to three leaves. Selected plants can later be grown out for hybridization or seed increase. Genotypic variation and ways to select for stomatal conductance and/or nighttime leaf conductance were discussed previously.

Manipulating pre- and post-anthesis water use is one way to increase the drought dependent HI, but there are others. Surplus assimilates are stored in stems around the time of anthesis. These are in the form of water soluble carbohydrates in wheat and can amount to 25% of the total above-ground dry weight at anthesis. Assimilates are translocated to the developing kernels during grainfilling and, if it is dry, may form up to 100% of the final grain yield. This redistribution of dry matter can be very important to increase HI.

There appears to be substantial genetic variation in wheat for the storage and remobilization of assimilates. Effective selection techniques have not yet been developed, although novel procedures using senescence agents have been tried (Blum et al., 1983). An effective way to identify the best parents is to determine the weight loss in stems between anthesis and maturity in genotypes grown in bordered field plots. This must be determined on a ground area basis and should provide an estimate of assimilate remobilization. Morphological traits could also be effective. For example, the tiller inhibition gene results in thicker stems. Also, there is variation for the size and anatomy of the internode cavity, which may be important for assimilate storage.

Some dry environments have a high probability of rainfall during the grainfilling period. For these, it may be possible to extend the duration of grainfilling, thereby achieving a higher HI. An extended grainfilling period would allow time for further translocation of assimilates to the grain. Genetic means exist to delay leaf senescence and extend the duration of grainfilling. Selection for leaf rolling is effective in shedding radiant energy and likely to result in cooler leaf temperatures and less transpiration, while leaf senescence tolerance or “stay-green” capability may be selected visually.

Concluding Remarks

Making genetic gains in yield under drought is not an easy task. Even the most assured methods (i.e., empirical breeding where plot yield is the unit of selection) are difficult and slow because of the unpredictability of drought and the large seasonal variation. Using a physiological approach (where the underlying physiological limitations to yield under drought are known) to more precisely target the yield limiting factors also has a substantial risk in a breeding program. No single trait is likely to be universally important, and the fact that there are few examples of success is evidence of the difficulties inherent in this approach. However, if successful, the resulting benefits would probably be large.

Identifying yield limiting traits and applying them effectively in a breeding program are major challenges because of the different types of drought and seasonal variation in the severity of

drought. Also, both predictable and unpredictable pleiotropic effects of different traits are likely, which adds a further degree of complexity. Another challenge lies in trait validation, given that a character may be important in one year but not in the next. Thus, there is no certainty that targeting so-called important traits will be effective from one season to the next. For this reason it is important that a physiological approach complement empirical breeding programs.

A physiological approach has substantial potential to improve yields and yield stability over and above that attainable through empirical breeding alone. First, it may identify key traits that currently limit yield in dry environments and therefore identify outstanding parental germplasm with extreme expression of the trait that would not normally be found in a breeding program. Second, it has the potential to more effectively cull large populations so that only the most elite lines are yield tested. Often this can be done out of season, making it possible to advance more than one generation per year. If the trait is correlated with yield, it may be more effective to select for the trait itself rather than for yield in early generations, owing to large G×E interactions for yield. Thus, trait selection has the potential to make breeding more efficient. Some traits may be difficult to measure. However, this often motivates a breeding program to devise fast and effective ways to select for the trait.

In the end success may also be difficult to measure. This will not be the case where backcrossing the trait into an adapted background results in better cultivars. But it will not always be clear if a physiological approach has been successful when it is used in a pedigree breeding program. A physiological approach will stimulate more detailed thinking and a deeper understanding of crop growth in relation to the prevailing environment and, hence, a deeper appreciation of the underlying factors influencing yield. It will also bring about a broadening of the germplasm base, the creation of novel germplasm, and more efficient ways of manipulating and evaluating populations. As with an empirical breeding program, it requires a long term investment.

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CHAPTER 8

Salinity Tolerance

K.N. Singh and R. Chatrath[1]

Natural soil salinity predates human civilization. When early man, looking for better sources of livelihood, moved to arid lands along the riverbanks, he resorted to irrigated agriculture. With the practice of irrigation began salinity, the first man-made environmental problem. The earliest written account of salt lands dates back to 2400 BC and was recorded in the Tigris-Euphrates alluvial plains of Iraq (Russel et al., 1965). The first time salt lands were associated with irrigation was in northeastern Sumer, in the vicinity of modern Telloh. Salinity is thought to have been partially responsible for the breakdown of the ancient Sumarian civilization (Jacobson and Adams, 1988).

Systematically recorded research on salt-affected soils is only a century old. In the Indian Subcontinent, the British spread irrigation and constructed a large network of irrigation canals. This initiated the process of secondary salinization, and several regions started reporting salt-related problems. Salinity also emerged in the Deccan Plateau, with the commissioning of the Nira Irrigation Project in Maharashtra. Many other areas of India became waterlogged and saline during the post-independence period due to the rapid commissioning of several large and medium-size irrigation projects.

Distribution of Saline Soils

Salt-affected lands occur in practically all climatic regions, from the humid tropics to the polar regions. Saline soils can be found at different altitudes, from below sea level (e.g., around the Dead Sea) to mountains rising above 5000 meters, such as the Tibetan Plateau or the Rocky Mountains. The occurrence of saline soils is not limited to desert conditions; the problem has been reported in the tropical belts of Africa and Latin America, and even in the polar regions, particularly Antarctica.

Areas affected by soil salinity are not well defined, since detailed maps are available for only a few. Consequently, global estimates vary widely (Flowers et al., 1986). Of nearly 160 million hectares of cultivated land under irrigation worldwide, about one-third is already affected by salt (Figure 1), which makes salinity a major constraint to food production. It is the single largest soil toxicity problem in tropical Asia (Greenland, 1984). In the wheat growing areas of India, the combination of salt-affected soils and poor quality groundwater (Figure 2) severely limits productivity.

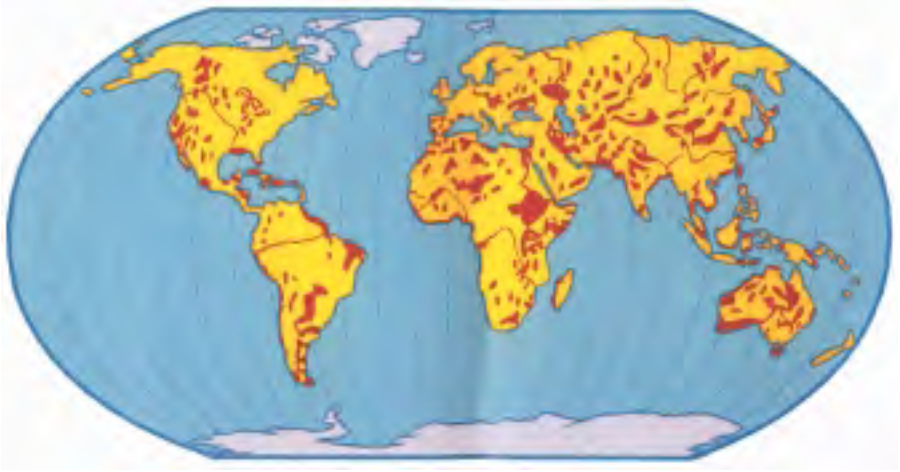

Figure 1. Global distribution of salt-affected soils.

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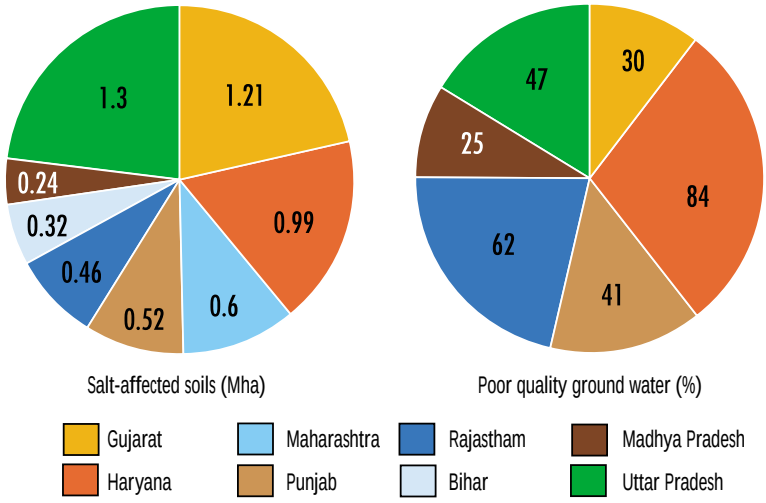

Figure 2. Distribution of salt-affected soils and poor quality ground water in wheat-growing states of India.

Some salt is introduced into the soil with every round of irrigation. Part of the salt is leached below the root zone, but part remains in it. The gradual buildup of salt in previously salt-free topsoil is referred to as secondary salinity. The productive life of the land is limited as a result of secondary salinity. When salt concentration in the soil forces production below the economic threshold, cultivation soon becomes impossible.

The expanded use of saline water for irrigation, together with poor management practices, aggravates the problem (Framji, 1976). The immense potential of salt-affected soils for much needed production of food, fiber, fuel, and forage crops is now more relevant than ever; production demands are increasing due to the growing population, and there is scant possibility of bringing new land under cultivation. This points up the urgent need to increase productivity of salt-affected soils and help innumerable low-income small farmers to improve their lot.

Classifying Salt-Affected Soils

High salinity levels can damage soil structure. The action of Na+ ions, when they occupy the cation exchange complex of clay particles, makes the soil more compact, thereby hampering soil aeration. As a result, plants in saline soils not only suffer from high Na levels, but are also affected by some degree of hypoxia. It is possible to leach out salt deposited in the root zone through extensive irrigation. However, in heavy textured soils leaching either through irrigation or rainfall is minimal due to the soil's poor infiltration characteristics. In alkaline soils such crusts are formed when soils dry out and harden; as a result, tender seedlings are unable to emerge. This usually happens if immediately after sowing there is a period of rain followed by a dry spell.

The diagnostic parameters for classifying saline soils are electrical conductivity (EC) of the soil solution, which detects osmotic problems, and exchangeable sodium percentage, indicative of a physical dispersion problem. Salt-affected soils are classified as shown in Table 1.

Effect of Salinity / Alkalinity on Plants

Crop species show a spectrum of responses to salt, although all have their growth and, eventually, their yield reduced by salt. Salt effects are the combined result of the complex interaction among different morphological, physiological, and biochemical processes.

Morphological effects

Morphological symptoms are indications of the injurious effects of salt stress. The extent of inhibitory or adverse effects can be known only by making critical comparisons with plants growing under comparable conditions in normal soils.

Salinity may directly or indirectly inhibit cell division and enlargement in the plant's growing point. Reduced shoot growth caused by salinity originates in growing tissues, not in mature photosynthetic tissues (Munns et al., 1982). As a result, leaves and stems of the affected plants appear stunted. Chloride induces elongation of the palisade cells, which leads to leaves becoming succulent. Salt stress hastens phenological development induces early flowering in wheat (Maas and Poss, 1989; Francois et al., 1986; Bernal and Bingham, 1973; Rawson, 1988). It also

Table 1. Saline soil classification system used by the United States Salinity Laboratory.

	EC † ≤ 4 mmhos cm^{-1}	EC > 4 mmhos cm^{-1}
ESP ‡ ≤15%	Non-saline, non-sodic soil §	Saline soil
ESP>15%	Sodic soil	Saline sodic soil

† EC = electrical conductivity.
‡ ESP = exchangeable sodium percentage.
§ The pH of saline soils is generally less than 8.5; of saline-sodic soils, about 8.5; and of sodic soils, more than 8.5.
Source: USDA (1954).

reduces dry matter content, increases root : shoot ratio, and diminishes leaf size in wheat. As a result, grain yield is reduced. This is attributed to the reduced numbers of seeds, spikelets, and tillers, as well as low grain weight.

Under sodic conditions growth is drastically affected due mainly to gross nutritional deficiencies or imbalances and to a root system severely restricted by poor soil physical conditions and high alkalinity. Restricted growth is typically accompanied by delayed flowering in sensitive varieties.

Physiological effects

Salt stress affects many aspects of plant metabolism and, as a result, growth is reduced. Excess salt in the soil solution may adversely affect plant growth either through osmotic inhibition (Bernstein and Hayward, 1958) of water uptake by roots or by specific ion effects. Specific ion effects may cause direct toxicity or, alternatively, the insolubility or competitive absorption of ions may affect the plant's nutritional balance. These effects may be associated with enzyme activity, hormonal imbalance, or morphological modifications. It should be noted that the relative role of osmotic and specific ion phenomena in explaining the observed effects is disputed.

Even at low salinity levels, external salt concentration is much greater than that of nutrient ions, so that a considerable concentration of ions may reach the xylem. Being the actively transpiring parts of the plant, the leaves accumulate salt, which leads to their premature death (Munns and Termat, 1986). Photosynthesis is reduced because it is affected by leaf expansion rate, leaf area, and leaf duration, as well as by photosynthesis and respiration per unit leaf area. Growth may be indirectly affected given that salts decrease the amount of photosynthates, water, and other growth factors reaching the growing region. This decrease may be due to stomatal closure or the direct effect of salt on the photosynthetic apparatus. Transport of photosynthates in the phloem may also be inhibited. Although photosynthesis is reduced in wheat, this is thought to be caused more by inhibition of oxygen release rather than by stomatal closure (Passera and Albuzio, 1997).

Mineral uptake by roots is affected as a result of imbalance in the availability of different ions. In wheat the cause of reduced growth was attributed more to the reduced rate of transport of essential nutrients to the shoot (Termaat and Munns, 1986). Although salinity may upset cation nutrition, imbalances tend to be restricted when mixed salts are present.

Proportions of Ca in the medium that are adequate under non-saline conditions become inadequate under saline conditions. In sodic soils, increases in exchangeable sodium are accompanied by decreases in exchangeable Ca and Mg, leading to Ca and/or Mg deficiencies: When Ca and Mg concentrations in the soil solution fall below critical levels, K uptake also decreases, affecting nutritional balance.

Soil alkalinity severely affects zinc solubility and drastically reduces its availability to plants. Iron availability may also be adversely affected. Buildup of organic matter may accentuate zinc deficiency in sodic soils through the formation of a chemical complex that ties up zinc. Therefore, increased nutrient levels through fertilizer application may appear to increase salt tolerance under conditions of salinity-induced nutritional deficiency (Bernstein et al., 1974).

Growth inhibition by salt also occurs due to the diversion of energy from growth to maintenance (Nieman and Maas, 1978). The latter may include the regulation of ion concentration in various organs and, within the cell, the synthesis of organic solutes for osmoregulation or protection of macromolecules, and for maintenance of membrane integrity. Osmoregulation ensures that adequate turgor is maintained in the cell. Organic compounds that accumulate in the cytoplasm may function as osmotica and in protecting the conformation of macromolecules in the changing ionic environment (Borowitzak, 1981; Wyn Jones and Pollard, 1983).

Since salt damage has a broad physiological spectrum affecting many metabolic processes, it is difficult to assess the contribution of individual processes to plant death or to the final damage done to the plant. One approach for evaluating the contribution of individual processes to salt damage has been to compare crop varieties that show differential responses to salt stress, varieties being genetically closer to each other than are different species. In studying two wheat cultivars, one regarded as sensitive and the other as resistant to salinity, it is concluded that osmotic stress is not the major factor discriminating between the two lines; rather, susceptibility to specific ions may be what causes the difference. Short-season varieties have been found to have higher Na and Cl contents than long-season varieties. This has been suggested by Bernal et al. (1974) to be the cause of the poor performance of short-season varieties.

Biochemical effects

Given that the physiological approach for identifying traits that confer stress resistance so far has not been very successful (i.e. there is no single physiological trait that is strongly associated with salt tolerance) (Yeo et al., 1990), studying the pattern of protein synthesis under salt stress may help to identify a protein(s) associated with

stress. Under saline conditions there is a change in the pattern of gene expression, and both qualitative and quantitative changes in protein synthesis.

Although it is generally agreed that salt stress brings about quantitative changes in protein synthesis, there is some controversy as to whether salinity activates specialized genes that are involved in salt tolerance. In comparing a salt tolerant amphiploid of the bread wheat Chinese Spring and *Thinophyrum elongatum*, Gulick and Dvorak (1987) did not find that novel mRNAs were present in the tolerant amphiploid and absent in Chinese Spring following salt stress. Therefore, it appears that variation for salt tolerance, at least between closely related species or varieties, may be attributed to allelic variation at the gene level, which gives rise to quantitative changes in the levels of expression. Therefore, salt tolerance does not appear to be conferred by unique gene(s).

Salinity also changes the levels of plant hormones, such as abscisic acid (Moorby and Besford, 1983) and cytokinin. It has been suggested that salt affects cellular and nuclear volume, induces endopolyploidy, and induces nucleic acid and protein synthesis (Leopold and Willing, 1984). Several steps involved in protein synthesis are very sensitive to changes in the ionic environment and may result in impairment of protein metabolism (Wyn Jones et al., 1979).

Mechanisms of Salt Tolerance

A salt tolerance breeding program cannot be successful in the absence of data on the physiological mechanisms by which plants cope with salinity stress. A precise understanding of the mechanism of expressed tolerance can help to resolve the genetic base of the trait to complement knowledge on the inheritance and dominance pattern of salt tolerance.

Mechanisms examined to investigate salt tolerance include ion transport and localization, osmoregulation and ion-induced metabolic shifts, and effects on photosynthesis and transpiration. The mechanisms that operate to impart tolerance vary with the level of salt stress.

Control of salt uptake

Under conditions of low salinity, tolerance in a plant can be due to minimized salt uptake (i.e., exclusion). This is achieved through the following mechanisms:

- Selectivity of the carriers and ion channels that are responsible, respectively, for the active and passive transport of ions across the roots and into the xylem. This has been reviewed by Rains (1972), Flowers et al. (1977), and Wyn Jones (1980).
- Better water use efficiency, as this minimizes the potential salt load per unit of new growth, finally reducing the salt concentration in the tissue (Flowers et al., 1988).
- Vigorous growth and/or continual replacement of lost leaves results in dilution of salt concentration in the plant (Yeo and Flowers, 1984).

Reducing damage under excessive ion uptake

Due to the effect of transpiration and xylem ionic concentration, the salt concentration in leaves rises so fast that it causes damage. Plants use the following mechanisms to accommodate the salt load without reducing leaf photosynthetic activity:

- Localization of saline ions in old leaves so as to protect the relatively new and actively transpiring leaves (Yeo and Flowers, 1982).
- Compartmentalization of ions within the leaf to avoid water deficit in the leaf (Oertli, 1988).
- Removal of excessive salt from the leaf through specialized structures (Fahn, 1979).

Osmotic adjustments

Under high salinity levels, osmotic adjustment to the external water potential is required. The plant has to accumulate or synthesize compounds that are osmotically active, for example, through:

- Better nutrient acquisition and high ion selectivity.
- Synthesis of organic solutes, such as sugars and organic acids, proline, glycinebetaine, sorbitol, etc., to adjust the osmotic potential of the cytoplasm and vacuole.
- Compartmentalization of salt ions in the vacuoles (Jeschke, 1984).

Strategies for Breeding for Salt Tolerance

Breeding for salt tolerance is a formidable task, and slow progress may be due to a combination of many factors:

- Incomplete knowledge of the effects of salinity on plants.
- Inadequate means of detecting and measuring salinity.
- Ineffective selection methods.
- Poor understanding of the interactions of salinity as it affects the plant.
- The vague or nonspecific effects (other than on plant growth) of moderate salt stress.
- Interactions of the ionic and osmotic properties of salt in plants.
- Changes in salt tolerance with plant development.

Some prerequisites for improving salt tolerance are:

- Availability of suitable genetic variability in the cultivated species or their wild relatives.
- Method of screening large numbers of genotypes for salt tolerance.
- A suitable breeding methodology.

Genetic diversity for salt tolerance

Genetic variation for salt tolerance, as defined by parameters such as survival and yield, has been reported in many crop species, including wheat. Variation for salt tolerance has been found in world collections of bread wheat (Quershi et al., 1980; Kingsbury and Epstein, 1984; Sayed, 1985), and potential exists for small improvements using conventional breeding methods (Rana, 1986). Singh and Chatrath (1992) screened and found variation for salt tolerance in tissue-culture-derived wheat lines developed at CIMMYT. The chromosomal locations of genes controlling tolerance to several mineral stresses have been identified, and genes controlling tolerance are located on all seven homoeologous chromosome groups of the Triticeae. However, group 4 and group 5 chromosomes are predominant for most stresses (Forster, 1994).

Screening and selecting for salt tolerance

The physiological effects of salinity are not fully known, measuring salt tolerance is difficult, and little is known about genes involved in salt tolerance. Since salinity imposes an environmental restraint on plant growth, quantitative parameters of growth and yield reductions can be measured based on the principles of biometrics and quantitative genetics. Reductions in yield and growth are the only measure of salinity stress.

These difficulties raise the problem of how to measure the development of salt tolerance in plants. However, they can be overcome. Thus, breeding for salinity tolerance will provide plant materials to aid plant physiologists understand the mechanism of salt tolerance.

Field and microplot studies

Though a variety of methods have been used for screening for salt tolerance, there is a need to develop a simple, convenient, and rapid screening technique. A large number of genotypes can easily be screened at the germination stage in a small space after just four weeks of growth. However, the tolerance of a genotype varies with the stage of growth. So before utilizing the genotypes in a breeding program or recommending them for sowing in saline soils, they should be re-tested in the field.

It has been reported that genotypes evaluated for salt tolerance in one medium may show a differential response to the same salinity levels in other media. Final selection should therefore be carried out under field conditions similar to those for which the genotype will be recommended. Moreover, environmental conditions in the screening field should also correspond to the growing field; salinity was observed to produce differential effects under different environmental conditions such as temperature and humidity (Salim, 1989).

Selecting salt tolerant plants in saline fields and microplots (Picture 1) is basic, but there are many problems associated with this type of screening. Soil salinity varies substantially with time, location, and soil depth. At high salt concentrations, certain ions may have specific toxic effects on plants, and the physical structure of soils may be changed by salt-soil chemical interactions. Saline irrigation water should be composed of a realistic mixture of salts, not just NaCl. Salt imbalance is the most common deficiency in screening studies and varietal assessments.

Sodium absorption ratios (SAR) must be considered in soils that have substantial clay content. The effects of salt on plants in soils with high SAR and permeability problems are substantially different from those on plants in non-sodic saline soils.

Another problem that may complicate selection is that salt tolerant plants may actually have higher root zone salinity, since their greater growth and water use concentrate the salt through exclusion processes. Plants that happen to be

Picture 1. Screening for salt tolerant wheat genotypes in microplots under controlled salt stress conditions.

located in slightly less saline areas may thus appear more tolerant, but in reality are not. Screening for salt tolerance can be improved if selections are based on plant response to soil salinity and water content measurements.

More refined control of water application and use of saline waters is important. Drip and sprinkler systems are easily modified and can be applied according to breeding objectives because water application can be closely controlled and monitored. High frequency irrigation decreases the variability in soil moisture content and soil water salinity. Use of the Triple Line Source Sprinkler, developed at Servicio de Investigación Agraria, Zaragoza, Spain, was however not successful due to foliar uptake of salt. Their studies later indicated that drip irrigation system was found to be more practical and successful.

Greenhouse and laboratory methods

Greenhouse and laboratory techniques are usually used for screening at germination or early vegetative stages of growth. The seedling stage is generally the most sensitive phase of plant development, and almost all work on salt tolerance in different crop species reported previously (Kingsbury and Epstein, 1984, Norlyn and Epstein, 1984, Allen et al., 1985, Sayed, 1985, Singh and Rana, 1989, Cramer et al., 1991) has included plant assessment at this stage.

Pot culture and solution culture are common greenhouse techniques. Screening under controlled conditions in glasshouse or laboratory can overcome the problem of environmental interactions. Such techniques are popular because large numbers of genotypes can be screened in smaller spaces and less time. However, these systems may not be useful in selecting for traits associated with root-soil interactions. A wide variety of criteria and media are used for screening, including simple devices such as germination dishes or more advanced techniques such as tissue culture and recombinant DNA.

Germination under high osmotic stress has been advocated as a method for screening for salt tolerance (Fryxell, 1954). However, it is now clearly known that there is no correlation between tolerance at germination and at later growth stages. Also, screening during germination could eliminate valuable genetic sources of tolerance. Thus it is best to select for salt tolerance during germination and emergence independently from seedling or later growth stages. This is because different genes may be involved in various mechanisms of salt tolerance as the plant develops.

The emergence of seedlings from sand cultures has been reported as a useful technique for determining emergence potential under salt stress (Sexton and Gerard, 1982). Salinized agar gel, nutrient film technique, and other screening techniques have used root growth as the criteria. But root growth is not a reliable indicator of salt tolerance because root growth at low salinities is far less sensitive to salinity than vegetative growth. Resistance to leaf chlorosis as a selection criteria is based on the fact that absence of chlorosis generally indicates varieties that are better salt excluders (Ream and Furr, 1976; Shannon, 1978).

Biochemical techniques

Screening based on morphological traits is easier than using physiological markers, but salt glands and hairs are the only morphological markers that have been associated with increased salt tolerance The use of biochemical markers, such as proline, glycinebetaine, sugar accumulation, and Na : K ratio, has not shown a consistent trend and hence is unreliable. This is probably because salt tolerance is the combined result of many plant features, both morphological and physiological.

Procedures for Breeding under Salt Stress

The introduction of crops to new areas has played a major role in varietal improvement. The majority of traditional varieties grown on the problem soils of South and Southeast Asia appear to have originated in India and Thailand. The importance of plant introductions has recently been recognized, and plant materials have been exchanged at both national and international levels. International organizations have available for different crops germplasm with tolerance to salinity, alkalinity, and acidity.

It is important to screen and evaluate the full spectrum of genetic variability available for tolerance to salt stress. Landraces of self-pollinated crops contain a heterogeneous mixture of highly homogeneous lines. It is therefore necessary to select within the landrace to develop a pure breeding line. Kharchia Local, a highly salt tolerant landrace, is prevalent in the Kharchi area of Rajasthan in India.

When variability is limited or unavailable, it can be generated by making crosses between salt tolerant donors and high yielding varieties. Breeding methods used are the pedigree method, the bulk method, backcross breeding, and recurrent selection. The pedigree method was used to transfer the salt tolerance of Kharchia 65 by hybridizing it with a popular high yielding wheat variety WL 71 I which led to the development of India's first systematically bred salt tolerant variety KRL I-4 (Picture 2) at Central Soil Salinity Research Institute, Karnal. This method has also been used successfully in rice.

When variability is restricted, it may also be increased by inducing polyploidy. It has been observed that allopolyploid crop species are more tolerant to both alkaline and saline soil conditions than their diploid counterparts. It was inferred that favorable genome interactions and the availability of a wide range of genetic variation arising from allopolyploidy enable amphiploids to attain a level of tolerance that diploid parents are incapable of attaining (Rana et al., 1980).

Soil stress varies widely over both time and space; as a result, population size needs to be increased. In such cases the pedigree method is less efficient than the "modified bulk method" where only desirable genotypes are selected and bulked. Segregating materials are bulk evaluated and selected under stress conditions for several successive generations. When the desired level of tolerance is achieved, plant selections are made and the pedigree method is used for further selection. Final testing is done in the target environment.

Backcrossing is also used to transfer one or two major genes from the donor parent. In wheat phosphorus use efficiency characteristics of two varieties were transferred to high yielding wheat varieties. Plants in segregating generations have been selected under low soil pH and low soil phosphorus. Efforts were also made to transfer genes for P use efficiency from rye to wheat using the backcross method (Rosa, 1988).

In crop plants, adaptability and productivity are usually negatively correlated. Recurrent selection can be employed to break undesirable linkages and to increase the frequency of favorable combinations, particularly when the trait has additive genetic effects. Mutation breeding can be resorted to if tolerance to salt stress is inadequate; however, mutagenesis has rarely been applied for developing tolerance to problem soils.

Picture 2. Salt tolerant Indian wheat variety KRL 1-4.

Improving Breeding Efficiency

Wide hybridization in wheat

Thinopyrum bessarabicum is a perennial species within the graminaceous tribe Triticeae and is more salt resistant than the annual *Triticum aestivum* (bread wheat). The amphidiploid produced (Forster and Miller, 1985) by hybridizing the bread wheat Chinese Spring and *Th. bessarabicum* was found to be more resistant in terms of survival and ability to produce grain at moderate salinity (250 mol ma), than Chinese Spring or even Kharchia. The greater resistance of the amphidiploid was attributed to its inheritance of more efficient exclusion of Na+ and Cl^- from younger leaves and reproductive tissue (Gorham et al., 1986). This technique has yet to be fully utilized by breeders, given that the amphidiploid expresses much of the *Thinopyrum* genome, which has many other undesirable qualities. Transferring only the relevant part of the genome should be considered.

It is also known that bread wheat (AABBDD) expresses more K+/Na+ selectivity than tetraploid (AABB) wheats, as K+/Na+ was found to be associated with chromosome 4 of the D genome (Gorham et al., 1987). Although there appears to be little allelic variation for this character in hexaploid wheat, variation may exist in *Aegilops squarrosa*, donor of the D genome, and other relatives of wheat carrying this genome. Studies using wheat tetrasomic lines (2x=44) and wheat/*Agropyron junceum* disomic lines (2x=44) have shown that chromosomes 2A, 2B, and 2D of wheat and 2J of *A. junceum* carry genes that confer salt susceptibility. However, chromosome 2J also appears to carry genes for salt tolerance (Forster et al., 1988). These findings suggest the existence of genes with major effects that might be exploited to increase salt tolerance.

Tissue culture

It may be possible to select cells and protoplasts that show salt resistance, and regenerate salt-resistant plants from them. The main advantages of this approach are the large numbers of individual cells that can be handled, and the ease of screening. There is also potential for increasing the frequency of genetic variation through somaclonal variation.

Since selection based on tissue culture is at the cellular level, it is important to know whether salt tolerance observed at the cellular level is reflected in the whole plant. It is also a matter of great interest to determine whether the salt tolerance induced in cells taken from salt sensitive species will be transmitted to regenerated whole plants. Nabors et al. (1980) regenerated plants from salt tolerant tobacco callus tissue that was found to be tolerant; however, they did not obtain unequivocal evidence for genetic control.

Quantitative trait loci approach

The existence of genetic variation for tolerance to salt stress in wheat and its relatives is clear, but its complex inheritance hinders both its genetic dissection and the incorporation of the relevant genes into commercial varieties. However, the recent and continuing development of marker-based genetic maps (e.g., RFLPs and subsequent PCR-based molecular markers) of major crop species offers a way to overcome these difficulties, as the maps, in principle, allow the partitioning of quantitative variation into effects associated with defined chromosomal locations; this is known as quantitative trait loci (QTL) analysis. Foolad and Jones (1993) made a few attempts to apply this methodology to the salt tolerance response in tomatoes, and Lebreton et al. (1995), to the drought tolerance response in maize.

Wheat x maize doubled haploids

Developing new salt tolerant wheats via the doubled haploid system using wheat x maize crosses saves time and is more efficient than using conventional breeding. Wheat x maize sexual hybridization is, to date, the most efficient technique for wheat polyhaploid production (Laurie and Bennett, 1988). Since maize is insensitive to the Kr alleles of wheat, embryo recovery frequencies across different wheat genotypes are high. Haploid wheat embryos result from the elimination of maize chromosomes in the zygote or during the first three cell divisions. The germinated embryos are later transferred to pots and treated with colchicine for chromosome doubling at the four-tiller stage.

Doubled haploids (KTDH series) developed at John Innes Centre, Norwich, UK, involving Kharchia and TW 161, a sodium excluding line, have been evaluated in natural salt-affected fields and under controlled microplot conditions at CSSRI, Karnal. The best performers, KTDH 54, KTDH 6, and KTDH 7, are tall and late maturing under Indian conditions. Other doubled haploid lines developed by crossing salt tolerant Kharchia, KRL 14, and KRL 3-4 lines with high yielding Indian wheat varieties (Picture 3) are being multiplied for field testing.

Conclusions

New, biological, and genetic techniques should be vigorously applied to research efforts aimed at developing salt tolerant crop varieties. Genetic and physiological approaches have received a fraction of the attention devoted to research and development on, for example, land reclamation, drainage, and irrigation with good quality water. The use of tolerant varieties is likely to make positive contributions to solving salinity problems.

A discreet choice of species with genetic potential for salt tolerance has to be made, and the available germplasm of each species should be collected and systematically screened for salt tolerance based on physiological factors. Rapid and reliable screening techniques should be developed considering the selection procedure and the specific type of salinity stress.

When there is little or no diversity for salt tolerance in the genepool, exotic and wild genotypes should be explored. Other alternatives that may increase genetic diversity are somatic hybridization, mutation breeding, and, eventually, genetic engineering.

Picture 3. Haploid seedlings being developed using wheat x maize intergeneric crosses.

Inheritance studies of new lines and ecotypes isolated for tolerance should be conducted. The inheritance and dominance pattern needs to be identified based on a precise understanding of the mechanisms of the expressed tolerance. Studies on the heritability of salt tolerance derived from wild relatives backcrossed to the cultivated species, and the development of isolines for particular physiological traits contributing to salt tolerance are also important.

Newer techniques, such as selection at the cellular level, somaclonal variation, protoplasmic fusion, and mutation breeding, may contribute to the development of salt tolerant varieties.

Selection based on the Na and K percentage in plants grown on sodic soils may prove more useful than selection based on quantitative characters.

Although wheat yields under salt stress conditions cannot be expected to equal those in a highly productive environment, it seems quite feasible to develop wheat varieties that will withstand moderate increases in soil salinity and alkalinity with little yield reduction. Basic research into the mechanisms of salt tolerance and sensitivity based on the physiological, metabolic, biochemical, structural, and ultra-structural features that enable salt tolerant plants to exist and even thrive under stress conditions must continue.

Exploitable genotypic differences in plant growth and yield under saline and alkaline soils conditions are now well established: Breeding efforts aimed at combining superior salt tolerance with acceptable yield levels are thus on firmer ground than research directed at developing tolerance to other abiotic stresses. Progress in developing salt tolerant varieties of wheat is just the beginning of a process that we hope will end in a significant contribution to human welfare.

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CHAPTER 9

Cold Tolerance

N.N. Sãulescu[1] and H.-J. Braun[2]

Wheat is grown across a wide range of environments and is considered to have the broadest adaptation of all cereal crop species (Briggle and Curtis, 1987). This broad adaptation is due, to a large extent, to wheat's cold tolerance, i.e. the ability to withstand temperatures much lower than 1-4ºC, considered the minimum temperature for growth (Figure 1).

In a general sense, cold tolerance in wheat should refer to performance at temperatures lower than the optimum for growth (about 20ºC), and there are definitely differences in the growth rate of cultivars at low temperatures and, consequently, in their adaptation to cool climate. However, the term "cold tolerance" is most frequently used to describe a plant's response to freezing temperatures, which have more dramatic effects on the crop. Most often, freezing temperatures affect autumn-sown wheat during winter. Freezing tolerance refers to the broader term of "winter hardiness," an attribute of autumn-sown cereals that is responsible for differences in "winter survival" or "overwintering."

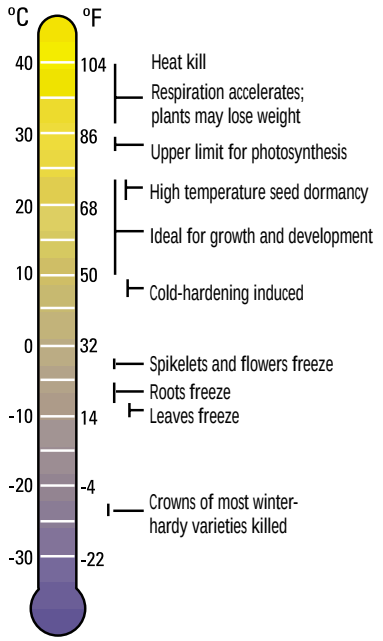

Figure 1. General range of favorable and unfavorable (stress) temperatures for wheat.

Winter survival is defined by Blum (1988) as "the final integrated plant response to a multitude of stresses involved during and after freezing stress, including both external-physical and biotic stresses." Even if plants are not winter-killed, they can be affected by freezing temperatures that may damage the leaf, causing reduction in leaf area, delayed growth, and plant debilitation. Considerable variation for winter hardiness exists among cultivars, which justifies dedicating extensive efforts to this breeding objective (Pictures 1 and 2).

Less often, freezing temperatures can occur during late frosts in spring, causing leaf or spike injury. Unhardened leaves can tolerate -4 to -8ºC (Gusta and Chen, 1987), but the reproductive tissue of the developing ear is considerably less resistant to freezing and may be injured at -1.8ºC (Single and Marcellos, 1974). Differences in what is generally called "frost tolerance" are less pronounced, although waxy or hairy lemma, palea, and awns are thought to delay formation of ice in the tissue. Due to the limited genetic variation for frost tolerance, breeding efforts have been directed mostly to escaping frost by selecting for later flowering.

Picture 1. Differential winter damage in head rows.

Picture 2. Differential winter damage in plots.

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It should be noted that low, non-freezing temperatures (below 10ºC) at the critical stage of meiosis can also have dramatic effects on wheat by causing male-sterility and, consequently, low yields. Genetic differences in the response to this stress are known to exist (Qian et al., 1986, Saulescu et al., 1997) but, because of its relatively rare occurrence, little effort is directed towards breeding for tolerance other than selecting for an appropriate flowering time that allows plants to escape the stress.

Stress Factors Involved in Winterkill

The reasons for winterkill in wheat, as well as the extent of the damage, vary greatly from region to region and from year to year. The main factors causing winterkill (alone or in combination) are related to low temperature *per se* (such as extreme air or soil temperatures, below the critical temperature of a particular wheat cultivar):

- inadequate hardening, due to late emergence in autumn or a sudden drop in temperature;
- long periods of cold-induced desiccation (Gusta et al., 1997a);
- prolonged periods of low sub-zero temperatures; in particular, mid-winter temperatures below -15ºC result in the rapid loss of winter hardiness (Gusta et al., 1997b);
- alternate freezing and thawing, which causes increased injury from ice crystal growth with each freeze (Olien, 1969).

Another factor responsible for winterkill is ice encasement, a major cause of plant death in areas of high rainfall and fluctuating temperatures during winter (Andrews et al., 1974). Ice has high thermal conductivity and can aggravate the effect of low temperatures. It also has low gas permeability and may, in extreme cases, smother or suffocate plants by depriving them of oxygen (Poltarev et al., 1992).

Finally, low temperatures or snow can cause indirect damage through:

- frost heaving due to the formation of ice in the soil. The ice pushes the plants upward, breaking and exposing the roots;
- snow mold, caused by fungi in areas with long-lasting snow cover. The most damaging fungus affecting winter survival is pink snow mold (*Microdochium nivale* (Fries) Samuel and Hallet), previously known as *Fusarium nivale* (Fr) Ces. (Hömmö, 1994). Although *Microdochium nivale* cannot survive freezing, it is tolerant to low temperatures and severely damages plants in the 0-5ºC temperature range. Other, less important fungi causing snow mold are *Typhula* spp., the pathogen for speckled snow mold or typhula blight, and *Sclerotinia borealis*, which causes sclerotinia snow mold.

The relative importance of stress factors causing winterkill can vary greatly among regions. In the Ukraine, an analysis of data from the last 100 years showed that winterkill was caused by low temperatures in 35% of cases, by alternate freezing and thawing in 26% of cases, and by ice encasement in 22% of years when significant winter damage occurred (Poltarev et al., 1992). Wisniewski et al. (1997) stated that the critical factors that affect winter survival in Poland are low temperature, freeze-induced desiccation, and infection by pathogenic fungi.

Gusta et al. (1997a) reported that the main factors responsible for winterkill in the Great Plains of North America are long periods of cold-induced desiccation, poor acclimation conditions in autumn, and unpredictable timing and duration of extremely cold temperatures, whereas the primary cause of winterkill in western Canada is freeze-induced desiccation. For eastern North America, Olien (1967) found that winterkill is most likely to occur during low temperature stress following a midwinter thaw, when the crown tissues have high moisture content. Correct evaluation of the frequency with which these or other factors can affect winter survival in the target area is essential for making a better choice of parents and testing procedures in a breeding program; this can also improve resource allocation efficiency.

Wheat plants can cope with each of the above mentioned winter stress factors through different genetic and physiological mechanisms. For example, a plant's freezing tolerance and snow mold resistance are based on different genetic mechanisms (Hömmö, 1994). However, the basic process behind most events leading to winterkill is freezing, or formation of ice in plant tissues (Figure 2). Freezing damage is in general not a consequence of low temperature *per se*, but rather the result of cellular dehydration brought about by extra-cellular ice crystallization. Cellular membranes have been recognized as the primary sites of freezing injury (Hincha and Schmitt, 1994).

Freezing tolerance is defined as the ability of plants to survive ice formation in extracellular tissues without significant damage to membranes or other cell components.[3] It is the result of physiological, chemical, and physical

[3] For the sake of clarification, it should be noted that intracellular ice formation is always lethal. The chemical potential in the intracellular solution must be equal to the chemical potential of the external solution or the ice. This equilibrium is attained through removal of intracellular water. To avoid cellular dehydration under freezing stress, the osmotic potential of intracelluar solutions is increased as the osmotic potential of extracellular solution decreases. For a detailed discussion of the physiological processes during freezing stress, see Blum (1988).

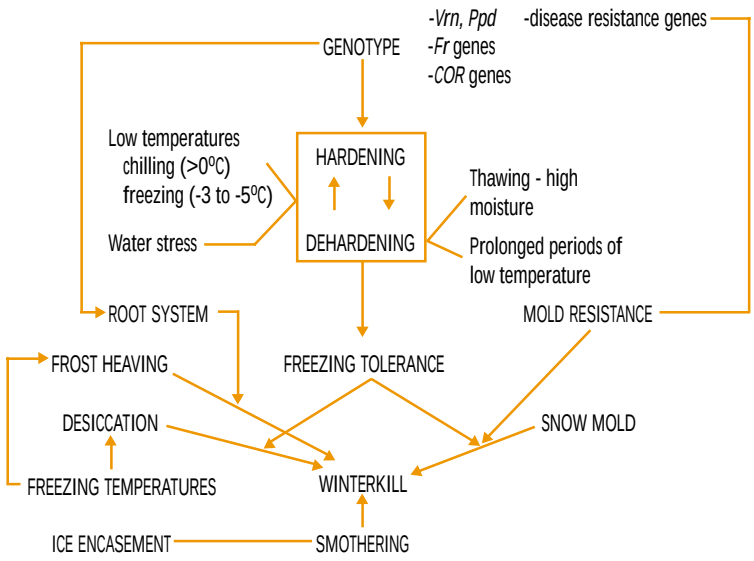

Figure 2. Diagram of the processes involved in winterkill and winter hardiness.

reactions, and of changes in plant cell structure that take place at appropriate developmental stages, under suitable environmental conditions. This process is called hardening or acclimation.

Acclimation proceeds in two stages, depending on the sequential action of chilling (>0ºC) and freezing (-3 to -5ºC) temperatures. A decrease of water potential in tissues, due to decreased osmotic potential (because of sugar accumulation in vacuoles), is the most important feature in the first stage of plant acclimation. It is correlated with a significant increase in the abscisic acid (ABA) level and results in modification of protein synthesis. There are great differences among cereals regarding the above 0ºC temperature at which acclimation is initiated. Winter rye starts at much warmer temperatures, which makes its acclimation period longer than that of winter wheat. Spring wheat and spring barley do not initiate acclimation at temperatures above 2ºC (Gusta et al., 1997a). Reversible modifications of membrane properties, which result in further decrease of water potential in parenchymatic tissue, seem to play a main role in the frost-dependent stage of acclimation (Kacperska, 1994).

Low temperatures act as the primary inducing factor, but stresses other than low temperature (water stress, wind, etc.) can also induce a certain level of freezing tolerance. Low temperature itself or secondary factors (ABA, sucrose fatty acids, and water status), produced in response to the primary signal, can result in conformational changes in either membrane and/or proteins and/or an ABA hormone receptor, and this, in turn, can result in the regulation of genes involved in cold acclimation (Gusta et al., 1997a). Several genes have been identified in various plants as low-temperature-inducible (lti) or cold regulated (COR). These genes may have a cryoprotective effect on cellular membranes (Thomashow, 1993).

The fact that frost-resistant cultivars harden faster and deharden more slowly than frost-susceptible genotypes suggests that acclimation may have different threshold induction temperatures in cultivars differing in cold tolerance. In barley, a higher degree of frost resistance is associated with a higher threshold induction temperature for the accumulation of COR proteins (Rizza et al., 1994).

Freezing tolerance is not a static condition, for it changes with time, temperature, soil and plant moisture, nutrition, and physiological age and status. It depends largely on the cold acclimation or hardening processes. Indeed, differences in freezing tolerance of unhardened plants of different cultivars are negligible, while considerable differences can be detected after full hardening. The hardening process can be stopped, reversed, and restarted. Generally, under natural conditions, the dynamics of freezing tolerance are characterized by three stages (Prasil et al. 1994):

- a hardening period, in autumn, when cold tolerance is acquired;
- a period of tolerance maintenance, when the critical or lethal temperature varies, depending on temperature fluctuations in winter;
- a dehardening period, generally at the end of winter, when plants lose their cold tolerance.

Each stage is influenced not only by genotypical (vernalization requirement and photoperiodic response) or developmental (age of plants) factors, but also by environmental ones. For example, if water content is altered by flooding or desiccation, the cold hardiness of winter cereals changes dramatically (Metcalfe et al., 1970). Similarly, prolonged periods of low sub-zero temperatures decrease the freezing tolerance of winter wheat seedlings significantly. The most cold tolerant, fully hardened winter wheats can tolerate -15ºC for only around six days, and survive at -18ºC for only 24 h and at -23ºC for only 12 h (Gusta et al., 1982). After being exposed to low temperatures for a longer period, the seedlings' acquired freezing tolerance is greatly reduced, and they are killed at much higher temperatures than in early winter.

Cultivars with similar freezing tolerance in early winter vary greatly in their

ability to cope with long periods of sub-freezing temperatures. The duration and intensity of sub-zero temperature is therefore a main factor determining the loss of freezing tolerance and consequent winterkill in winter wheat. (Gusta et al., 1997a). Freezing tolerance is also known to be influenced by plant nutrition (Freyman and Kaldy, 1979), herbicides (Freyman and Hamman, 1979), viral infection (Paliwal and Andrews, 1979), and seed-borne diseases such as common bunt (*Tilletia foetida* and *T. caries)* (Veisz, 1997). This is why, to detect genotypic differences in freezing tolerance, it is important to keep all environmental factors as uniform as possible.

Traits Associated with Freezing Tolerance in Wheat

Freezing tolerance is the result of complex physiological mechanisms involving many cell and plant traits. Numerous studies have shown that the genetic control of cold tolerance is complex and can be regarded as polygenic. As many as 15 out of 21 chromosomes in wheat have been found to influence tolerance to low temperatures (Stushnoff et al., 1984). Nevertheless, major genes such as *Fr1*, closely linked but separable from the *Vrn1* gene on chromosome 5A, and *Fr2*, linked but easily separated from *Vrn3* on chromosome 5D, were shown to have a large effect on low temperature response (Snape et al., 1997).

The association between freezing tolerance and vernalization requirements can therefore be partially explained by the linkage between major genes controlling freezing tolerance and two genes that control growth habit. In addition, the vernalization genes have been identified as key developmental factors responsible for the duration of expression of low-temperature-induced structural genes (Fowler et al., 1996b). Recent data show that the regulatory influence exerted by the vernalization genes over low-temperature-induced structural gene expression occurs at the transcriptional level (Fowler et al., 1996a).

Winter wheats initiate hardening at higher temperatures than spring wheats, and the latter harden only to a very limited extent. Similarly, in spring, completely vernalized winter wheats only reharden to the level of spring cereals. Obviously, a high level of hardening can only be achieved in "dormant," not rapidly developing, plants; therefore, a strong association exists between the degree of vernalization and the degree of freezing tolerance that can be achieved in winter cereal seedlings (Roberts, 1990a). On the other hand, several northern European wheat cultivars with very long vernalization requirements only have moderate freezing tolerance (Gusta et al., 1997a). Braun (1997) found a highly significant correlation (r=0.67-0.77) between growth habit and freezing tolerance, which suggests that only 45-60% of the variation for cold hardiness can be attributed to the differences in vernalization requirements.

Freezing tolerance was found to be associated with prostrate growth type. A gene controlling prostrate growth was found to be closely linked with *Fr1* and *Vrn1* on chromosome 5A (Roberts, 1990b). Genetic linkage is probably not the only explanation for the observed association, since a prostrate plant is also less exposed to low temperatures and desiccation, and better protected by snow.

It is worth mentioning that prostrate growth type, found in dormant juvenile plants in autumn, can also occur in cultivars with low vernalization requirements but high photoperiod response. In wheat such cultivars are usually only moderately winter hardy, but in barley some of the most cold tolerant cultivars are known to be day-length sensitive, with a low vernalization requirement.

Both high vernalization requirement and day-length response, as well as other mechanisms that cause "winter dormancy" and delay development to the generative stage (differentiation of the meristem), are generally associated with lateness. In an experiment with winter oat, populations selected for higher levels of winter hardiness were also later maturing and often taller than desired (Marshall, 1976). A similar association has also been observed in wheat. This association can create difficulties in breeding early and short winter hardy cultivars. However, no effect of height alone on winter hardiness was found in a comparison of winter wheat height isolines derived from the cultivar Yogo (Allen et al., 1986). This suggests that the association between winter hardiness and plant height may depend on the height genes involved and on genetic background.

Cell length, as measured in stomatal guard cells, was also found to be associated with cold hardiness, as was leaf length and height of hardened plants (Limin and Fowler, 1994; Roberts, 1990b). Although the association is not very strong, these traits have the advantage of being easily determined on individual plants.

Hardening induces significant changes in many of the plant's biochemical and physiological characters. Phenotypic differences in these characters are often associated with freezing tolerance. For example, tissue water content (Limin and Fowler, 1994), accumulation of simple sugars or polysaccharides (Olien et al., 1986), free proline accumulation in leaves and shoots (Dörffling et al., 1990), and accumulation of specific cold-regulated proteins (Houde et al.,

1992) in hardened plants were found to be correlated with freezing tolerance.

Genetic linkage is the most likely explanation for the association between certain gliadin blocks, as identified by electrophoresis, and freezing tolerance (Sasek et al., 1984). Such associations can be useful only in specific crosses, involving specific parents. Despite the large number of correlations with other traits, none is high enough to justify replacing direct freezing tests.

Breeding Approaches

Handling a complex trait such as winter hardiness in a breeding program is a difficult task, due to the large number of genes involved and the numerous interactions with the environment. But the main difficulty in breeding cold tolerant wheat is that high freezing tolerance is generally associated with lower yields and later maturity.

Many traits that are associated with freezing tolerance, such as delayed spring growth or small cells, can have negative effects on yield, especially in rainfed environments where rapid growth in early spring and earliness are important to avoid late drought and high temperatures. Besides, every additional breeding objective will slow down genetic progress for all other traits of interest. Therefore, the breeding objective should not be to maximize winter hardiness, but to develop cultivars with the minimum winter hardiness necessary for a given target area. As Fowler et al. (1981) pointed out, in general the most successful winter wheat cultivars have only marginally greater winter hardiness than the minimum required for the area in which they are grown.

Definition of the minimum hardiness required for a given region is not a simple job. It should be based on assessment of the winterkill risk, based both on weather data and information about cultivar performance in the area. A careful analysis of historical weather data, including minimum temperatures and time of their occurrence, is useful, but not sufficient. The same low temperature can have rather different effects on wheat plants, depending on prior temperature regimes and other factors, which determine the level of hardening achieved. Some of the crop models, such as CERES, have a sub-routine that simulates hardening and winterkill, and these could be used for a more correct estimation of the winterkill risk.

A simpler, but very useful approach, is to use winterkill data of cultivars with varying levels of winter hardiness that have been cultivated in the target area for a long time and for which long-term records for overwintering are available. In general, it is not difficult to identify a cultivar grown for many years in a region with only occasional and not very serious winterkill. A frequency of 1 out of 10 years with some winterkill can generally be considered acceptable. But the accepted risk can be higher or lower, depending on economical, social, and other factors. If such a cultivar is identified, it is the best definition of the minimum level of hardiness required for the area and should be used as a check in all cold hardiness tests. Additional checks should be used to provide a range of hardiness.

When wheat cultivars with higher levels of freezing tolerance are selected to lower the risk of winter damage, it should be remembered that the resulting yield penalty in years without freezing stress may be far greater than the advantage of better winter survival in years with severe winters.

Obviously, the breeding strategy for developing winter hardiness will depend on the ratio between the hardiness level in the gene pool used and the minimum necessary for the target area. If most parents used in crosses have a winter hardiness equal to or higher than the accepted minimum, maintaining this level is a relatively easy task that can be accomplished through the application of mild selection pressure against the rare, less hardy segregants. On the other hand, if a large number of parents are not winter hardy enough, as is the case in programs that use spring x winter crosses, higher selection pressure is advisable, beginning with the early generations, to increase chances of recovering an acceptable level of hardiness. Early generation selection against spring growth habit, as suggested by Braun (1997), can be very efficient.

Breeding for winter hardiness is much more difficult in areas marginal for winter wheat cropping where the minimum required hardiness is at or above the maximum available cold hardiness potential. As Grafius (1981) pointed out, there “has been a notable absence of improvement in the maximum cold hardiness potential of cereals in this century”, and this “inability of plant breeders to increase maximum cold tolerance levels suggests that all of the available cold tolerance genes had been previously concentrated in hardy land races within winter cereal species.”[4] Recovering this maximum level of hardiness in higher yielding genotypes is only possible by applying very high selection pressure in large segregating populations.

[4] Cereals differ greatly in their ability to survive low temperatures. The most cold tolerant rye cultivars are killed at around –34ºC, wheat cultivars at around –23ºC, and barley at around –18ºC.

Transgressive segregation for freezing tolerance has only been recorded in crosses among medium or less hardy parents, but not among the most hardy parents. There are hopes that interspecific hybridization can bring in new genes from species with higher freezing tolerance (such as rye), but to date no such transfer has been successful for common wheat.

Durum wheat generally has much lower winter hardiness than bread wheat, so breeding for freezing tolerance is more difficult. However, for areas where winter durums are superior to spring durums, breeding for winter hardiness has to be a high priority. The best winter hardiness is found in cultivars derived from interspecific crosses with bread wheat, and such crosses, as well as transgressive segregation in intraspecific crosses, will probably allow further progress in this respect.

Methods and Techniques

Field testing

Whenever winter conditions differentiate genotypes for winter survival, evaluation of winter hardiness in the field is desirable. Field evaluation allows large-scale, inexpensive characterization of breeding materials against the full range of factors affecting winter survival, whereas controlled freeze tests measure only low temperature tolerance. For this reason, most breeding programs, regardless of available resources, favor field testing to measure winter survival (Fowler et al., 1993), despite the disadvantages described below.

Levitt (1972) defined a "test winter," or "differential winter," as a winter severe enough to kill the most tender plants and damage those of intermediate hardiness to various degrees. Unfortunately, from a plant breeder's point of view, winters with "good" differentiation among genotypes for their winter hardiness are infrequent, even in areas that require a high level of cold tolerance.

It should be stressed that winterkill is often not only the result of low temperature stress, but also of the interaction of a range of factors, which most likely will not all occur in a given year or location. Therefore, multilocational testing can give better information on winter hardiness, especially if locations are selected to provide higher probability of winterkill.

Years with milder winters than a "test winter" may sometimes exert some selection pressure for winter hardiness, based on leaf damage and color (Picture 3). Although the correlation with actual winter hardiness is not very high, scores based on leaf damage are helpful for discarding the less cold tolerant lines, provided notes are taken when symptoms are most visible (a very short period, only 2-3 days) in spring, before active growth begins.

An additional problem of field testing for winter hardiness is the great variability within a field due to non-uniform snow cover, soil preparation, planting depth, soil and plant moisture, etc. To cope with this problem, the following are highly recommended:

- Plant one or two check cultivars of known winter hardiness every few rows.
- Take notes on replicated nurseries, preferably in small plots. Marshall et al. (1981) consider short (0.5-1.5 m long), replicated, single-row plots as the most efficient and reliable method of selecting under field conditions.
- Make every effort to improve uniformity in the field (especially soil preparation). It has even been suggested that the top soil be completely replaced with a homogeneous mixture, carefully leveled over a coarse base to provide uniform drainage.
- Use special data-handling procedures that allow controlling and reducing environmental errors.

Fowler and Gusta (1979) developed a field survival index (FSI) based on the relative winter hardiness of winter wheat cultivars tested in more than 60 trials over a five-year period. The FSI uses:

Picture 3. Leaf damage and discoloring after a mild winter.

- only data from plots with partial winterkill;
- differences in percent winterkill among entries in a block, rather than actual percent winterkill in each plot;
- a moving average.

Although calculations and efforts involved in determining the FSI may seem tedious, the index provides a very robust measure for comparing the winter hardiness of cultivars. Other approaches such as a "nearest neighbor analysis" may also be useful.

Enhancing winter stress in the field

The probability of differential winterkilling in a natural winter environment can be increased by using simple procedures:

- planting wheat on ridges, 20-30 cm high, from which snow is usually blown out, leaving plants more exposed to low temperatures and desiccation (Nam et al., 1982);
- planting wheat in wooden or cement boxes placed above the ground, preferably in an open field, to allow lower temperatures to be reached at the crown level, producing higher winterkilling than in field planted wheat;
- leaving plots without snow cover by temporarily covering them during snowfalls, or by gently removing newly fallen snow from the plots.

As in normal field testing, use of several checks of known winter hardiness, repeated every few plots or rows, is highly recommended.

Artificial testing

The irregular occurrence of natural conditions that satisfactorily differentiate genotypes has led many plant breeders to develop artificial techniques for assessing the freezing resistance of their materials. Already in 1956, Dexter concluded that the results of such tests were generally well correlated with field assessments of winter hardiness, and recent methodological refinements have improved the correlation. On the other hand, Fowler et al. (1981, 1993) concluded that, although controlled environments should allow more rigid control of freezing conditions, comparative studies suggested that field trials usually provide more repeatable results and have lower experimental errors. The decision on which method to apply should largely depend on how frequently field testing results in "good" differentiation and on the equipment available for screening under artificial conditions. Wherever possible, both methods should be applied.

Most methods used in wheat breeding programs are direct, i.e., they are based on exposing plants or seedlings to controlled freezing in artificial climate facilities, such as freezing cabinets, growth chambers, etc. However, there are indirect methods in which plants are not exposed to freezing; instead, their freezing tolerance is estimated based on biochemical changes induced by hardening or on the presence of molecular markers associated with genes involved in controlling winter hardiness (Table 1).

Direct freezing tests

Many methods have been suggested and are used in winter wheat breeding programs around the world for artificial testing of freezing tolerance. They differ in the way plants are sown and prepared for testing, in the hardening and freezing procedures, and in the way freezing damage is assessed.

In many cases, sowing is done in boxes, flats, or pots, which makes it easy to handle, regardless of weather conditions, but is relatively laborious. The other choice is to pick up plants from breeding plots in the field. This approach is more economical, but picking plants from the field is dependent on weather conditions and can be hampered by snow cover or frozen soil.

Table 1. Approaches used for testing freezing tolerance in wheat.

	Hardening	Exposure to freezing		Assessment
Direct	- Natural in the field - In growth chambers - Combined	- Field (regular or special locations) - Field enhanced (ridges, boxes above ground, snow removal) - Freezing cabinets - Immersed in a refrigerated solution	- Plants in boxes - Plants from field, transplanted (in boxes, rootrainers, moist sand) - Crowns (in polyethylene bags, tubes, sand) - Seedlings	- Plant survival - Leaf damage - Root regrowth - Cell membrane damage (electrolyte leakage) - Tissue viability - Tissue electrical conductivity - Fluorescence - Enzyme activity
Indirect	- Natural in the field - In growth chambers - Combined	No		- Tissue water content - Free proline - COR proteins - Tissue electrical resistance of: - Seedlings - Crowns
	No	No		- Molecular markers

Hardening is most easily done under natural conditions, either by placing the boxes or pots outdoors or by picking up hardened plants from the field (Picture 4). The main disadvantage of this approach is the lack of control over the hardening level. Results from such freezing tests are not reproducible, and the test temperature must be adjusted for each test, according to the hardening level, to properly differentiate genotypes.

Hardening in growth chambers under controlled temperature and light regimes can achieve a controlled level of hardening, but is expensive and requires space in growth chambers for about 30 days. A workable compromise is to use natural field conditions for the first stage of hardening and then transfer the boxes or transplanted plants to growth chambers with a controlled environment for the second stage of hardening, which may take only 24-130 h. It is important that plants be collected before they are covered by snow.

Freezing response shows significant cultivar x hardening-duration interactions (Jenkins and Roffey, 1974). Therefore, ideally, the frost resistance of a genotype should be assessed over a range of hardening and freezing regimes; however, this is not practicable when testing large numbers of early-generation selections. Several alternative hardening regimes can be selected, depending on the breeding requirements:

- natural hardening, which better reflects the situation in farmers' fields. Many years of testing are needed to characterize freezing tolerance of a genotype using this method;
- an "average hardening" regime, representative of most years in the area. Various hardening regimes are used by different testing programs (see Table 2);
- striving for maximum level of hardening, corresponding to "potential freezing tolerance" or "static freezing resistance."[5]

Picture 4. Hardening of plants sown in wooden boxes, using a vegetation house.

There are several options for exposing wheat plants to low temperatures. Often boxes or pots in which wheat has been planted are placed directly in freezing chambers. This has the advantage of not disturbing the plants before stress exposure, but requires larger freezing cabinets and a longer exposure period, due to thermal inertia of the large amount of soil.

Most important for plant survival, the crown meristem must be able to produce new roots and tillers, so some methods expose just the crowns to freezing temperatures. Crowns are prepared by trimming the upper part of the plant to 2-3 cm above the crown and the roots to 0.5-1 cm (Figure 3). Crowns are then put into plastic bags, vials, tubes, moist sand, etc. (Fowler et al. 1981, Gusta et al. 1978). To avoid the work involved in trimming, other methods expose plants that have been transplanted from the field into small boxes, trays, or some type of supporting device (e.g., roottrainers) with a small amount of moist sand or soil (Poltarev, 1990; O'Connor et al., 1993; Ryabchun et al., 1995). Using young seedlings has the advantage of reducing test duration and the amount of soil needed, but, as differential survival of seedlings is more difficult to obtain, evaluation is usually based on leaf damage (Figure 4). Larsson (1986) found a very good correlation between seedling leaf damage and field winter hardiness.

Most methods use freezing cabinets with controlled air temperature. However, to achieve better temperature control, Jenkins and Roffey (1974) used a refrigerated bath with ethylene glycol, in which pots with plants were immersed. Most authors recommend a

[5] Ryabchun et al. (1995) recommend adding 36 h at -5ºC, 56 h at -7ºC, 24 h at -9ºC, and 14 h at -10ºC of artificial hardening to the level achieved through natural hardening in the field by 14-25 November under conditions in Kharkov, Ukraine.

Table 2. Main characteristics of methods used for assessing freezing tolerance in cereals.

Author	Planting	Hardening	Exposure	Freezing	Recovery	Assessment
Jenkins and Roffey (1974)	In paper pots, 1.9 cm diameter and 6.4 cm deep	In growth chambers at 8/5 ºC for around 30 days	Pots placed in glass tubes, immersed in a refrigerated bath with 40% ethylene glycol	Solution chilled 2ºC/h to -4.5 At -4.5 ºC for 7 1/2 h; Decreased 2ºC/h to -9 At -9 ºC for 11 h	Solution heated to +1 ºC in 7 h	Electrical resistance of leaves, by clipping 2 platinum electrodes 2 cm apart through lamina of first leaf
Fowler et al. (1981)	Plants from the field	In the field	Crowns above (trimmed 3 cm and 0.5 cm below the crown) placed in aluminum dishes with moist sand	Equilibrated 12 h at -3 ºC Decreased 2ºC/h to 5 test temp separated by 2 ºC intervals Dishes removed when test temp. is reached	At 0 ºC for 15 h Planted in soil-perlite-peat At 15 ºC, 3 weeks	Plant survival
Larsson (1986)	In plastic boxes with mixture of peat and sand Grown 2 weeks in glasshouse	In growth chambers at +1ºC, 20-30 days	Seedlings in plastic boxes	Decreased 1ºC/h to 5 test temp. separated by 1.3ºC intervals		Foliar damage on primary leaves
Poltarev (1990)	Plants from the field Transferred in boxes or trays	In the field	Plants in boxes or trays	Decreased 2-3 ºC/h to 2 test temp. at 2 ºC interval	Increased 2-3 ºC/h 15-16 days at 20-22 ºC or 3 days at 24-26 ºC	Plant survival
O'Connor et al. (1993)	Plants from the field transplanted to folding rootrainers.	In the field	Plants in rootrainers	Decreased 2ºC/h to 8 test temp. separated by 2 ºC intervals	Thawing at 4 ºC for 15-20 h Recovery at 17 ºC for 3 weeks	Plant survival
Ryabchun et al. (1995)	Plants from the field Transferred in wooden boxes or special trays	In the field + artificial 36h at -5ºC 56h at -7ºC 24h at -9ºC 14h at -10ºC	Plants in boxes or trays	Decreased 1ºC/h Exposed for 24 h at -16, -18, -20 and -22 ºC	Increased 2-3 ºC/h to -2ºC, then 1ºC/h Crowns planted in soil at +20ºC for 15-16 days	Plant survival
Fedoulov (1997)	In wooden boxes	In natural field conditions + artificial 24h at -5ºC	Plants in wooden boxes	Decreased 1ºC/h Exposed for 24 h at -17 to -20 ºC	Increased 1ºC/h 24 h at +5 ºC 21 days in greenhouse	Plant survival
Tischner et al. (1997)	In wooden boxes (38x26x11 cm)	Artificial 7 days at +3 to -3ºC 4 days at -4ºC	Plants in wooden boxes	24 h at -15ºC	In phytotron	Plant survival
Dencic et al. (1997)	In pots 20 cm deep	In the field + 24h at 0ºC	Plants in pots	24h at -15ºC 96h at -17 ºC	120h at +5 to +7ºC	Plant survival + leaf damage

gradual decrease in temperature (by 1-3ºC/h), but direct exposure to the test temperature can also be used (Dencic, 1997; Tischner et al., 1997).

Difficulty in reproducing cold acclimation conditions severely limits the resolution of controlled-freeze tests that employ a single minimum (test) temperature. Therefore, it is best to use of a series of test temperatures, usually separated by 2 ºC intervals, to determine the LT_{50}, i.e., the lowest test temperature at which 50% or more of the plants of a wheat genotype survive freezing (Fowler and Limin, 1997).

As stated above, cold tolerance of winter cereals is reduced by prolonged exposure to sub-lethal temperatures and, consequently, both minimum temperature and exposure time are important variables in controlled-freeze test procedures. For economic reasons, most methods prefer shorter exposures to lower temperatures, but longer exposures might be advantageous if thermal inertia is large or if freezing cabinets have limitations in reaching lower temperatures. Thomas et al. (1988) recommended prolonged

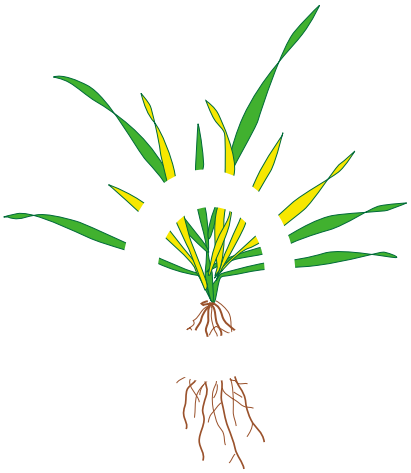

Figure 3. Crown prepared for artificial freezing test by trimming the upper part of the plant to 2-3 cm above the crown and the roots to 0.5-1.0 cm.

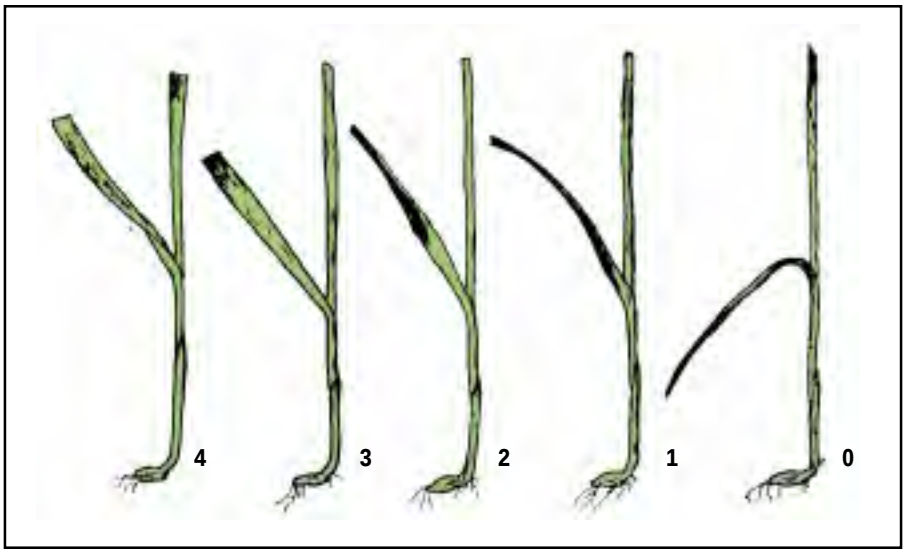

Figure 4. Grading freezing injuries in seedlings.
Source: Larsson (1986).

freezing of dark-hardened seedlings for rating and selecting winter wheats for winter survival.

There are small variations among methods for the recovery procedures. Most authors recommend a gradual increase in temperature until thawing, followed by a 2-3 week recovery period at 15-22 ºC. To reduce this long period when greenhouse facilities are used, Poltarev (1990) recommends transferring the plants at higher temperatures (24-26 ºC) where survival can be observed after only 3 days, provided great care is taken to avoid desiccation.

After the recovery period, freezing damage is usually assessed either by plant survival counts or by visually scoring leaf damage (Pictures 5 and 6). However, such indices are to some extent subjective, have high experimental errors, and involve a considerable delay between freezing and survival assessment. Many techniques have been proposed to evaluate the effect of freezing on plants by measuring other traits, such as:

- electrical conductivity of solutions resulting from exosmosis after freezing. Electrical conductivity, which depends on electrolyte content, is measured using a solution analyzer after freezing (initial EC) and again the next day, after killing tissue by immersing test tubes in a 80 ºC water bath for 1 h (final EC). Percent electrolyte leakage at each temperature is defined as: EL (%) = (initial EC / final EC) x 100. Lethal temperatures are determined by fitting data with a sigmoidal response curve and using the inflection point of the sigmoidal response curve to predict the lethal temperature (Fry et al., 1993);
- electrical resistance of plant tissue (Jenkins and Roffey, 1974);
- chlorophyll fluorescence analysis is rapid, sensitive, non-destructive to the plant, relatively cheap, and able to detect injury before visible symptoms occur. Onset of chilling injury is accompanied by a decrease in chlorophyll fluorescence *in vivo*; if the chilling treatment is prolonged, chlorophyll fluorescence eventually declines to zero (Wilson and Greaves, 1990). Unfortunately, the need for costly equipment limits the use of this type of analysis;
- tissue viability, estimated by using chemicals (such as tetrazolium or acid fuxine) that change color in the presence of oxidation reactions. This approach is very time consuming and labor intensive (Poltarev, 1990);
- enzyme activity (Bolduc et al., 1978).

Picture 6. Differential recovery of crowns submitted to artificial freezing.

Picture 5. Differential damage in plants grown in boxes, after artificial freezing.

These methods for assessing freezing damage have lower coefficients of variation and produce results without delay after freezing, but require special equipment and more laboratory work. This probably explains why most breeding programs still rely on determining plant survival.

Table 2 summarizes the main characteristics of methods used for directly evaluating freezing tolerance in wheat. This could serve as inspiration for adapting a method fitted to available equipment and conditions.

Cultivars can be compared not only for their hardening potential or maximum freezing tolerance, but also for the stability of their freezing tolerance and the ability to reharden (Prasil et al., 1994), which, in some areas, may be equally important. Three approaches have been used for evaluating these traits:

1. repeating freezing tests several times during the winter, based on the assumption that plants are naturally subjected to variable conditions leading to dehardening and rehardening;
2. exposing plants to controlled thawing and rehardening before the freezing test. For example, at the Odessa Institute, hardened plants are subjected to thawing at 10-12ºC for 120 h with continuous light, rehardened at -2 to -4ºC for 24 h and then frozen at -12ºC for 24 h (Litvinenko and Musich, 1997);
3. estimating the stability of the hardening condition based on the time needed to fulfill the vernalization requirements. To estimate the time needed to complete vernalization, Poltarev et al. (1992) recommended planting the genotypes in several boxes in the field and then transferring them to a greenhouse at about 20ºC and continuous light, at 47, 55, 62, 68, 74, and 82 days after planting. Growing point development is evaluated after one month in the greenhouse, and the number of plants that show spikelet differentiation is recorded.

The authors have established a close correlation between the length of time to complete vernalization and the stability of freezing tolerance. Even if this correlation is not general (see Gusta et al., 1997a), it can be useful in selecting for stability of freezing tolerance, especially when segregants with low and very low vernalization requirements are common in a breeding program.

Indirect freezing tests

Many scientists have tried to avoid problems related to direct freezing of plants (expensive freezing cabinets, high experimental error) by suggesting indirect methods that estimate the level of hardening instead of freezing damage.

Water content in plants is reduced during hardening, especially in hardier genotypes. Water content after hardening was found to be correlated with winter survival (Fowler et al., 1981).

Proline is thought to play a protective role in plants subjected to several stresses, including frost. Considerable amounts of free proline accumulate in leaves and shoots during cold hardening, and proline accumulation is positively correlated to genotype-specific frost tolerance (Dörffling et al., 1990). Measuring proline content after hardening can therefore provide information about potential freezing tolerance of cultivars.

There is a close correlation between the degree of freezing tolerance and the accumulation of a specific cold-regulated (COR) wheat protein (WCS120). The corresponding antibody discriminates between frost-resistant and frost-susceptible wheat cultivars (Houde et al., 1992). Therefore this protein can be used as a molecular marker to select for freezing tolerance.

Tissue electrical resistance of eight-day-old seedlings was found to be correlated with freezing tolerance. Used as a selection tool, it is very convenient in breeding schemes that employ artificial climate for accelerating generations (Musich, 1987; Litvinenko and Musich, 1997).

Restricted fragment length polymorphisms (RFLPs) and other molecular markers may also be used to detect the presence of alleles having positive effects on winter hardiness.

Although very attractive, indirect methods generally describe only some of the mechanisms involved in the control of genotypic differences in freezing tolerance, and therefore can probably exploit only part of the genetic potential available in a breeding program. Besides, they are generally more expensive and therefore restricted to stronger research programs.

Conclusions

Little progress has been made in breeding for increased tolerance to low temperature stress since the introduction of the winter wheat variety Minhardi at the beginning of this century (Grafius, 1981). However, this statement refers to the absolute minimum temperature wheat plants can survive. Most of the winter wheat growing areas in the world do not require wheat varieties with such a high level of winter hardiness. Consequently, the main breeding objective in many winter wheat breeding programs is not to lose the winter hardiness level present in commercial cultivars, rather than to increase it.

This target is often reached through routine field screening. The costs related to screening in controlled environments or using other indirect methods are probably one of the reasons why the measurement of winter survival in the field is still the standard procedure for most winter wheat breeding programs. With the identification of genes that

control frost resistance and the development of markers, it is likely that some of the problems related to field testing and/or controlled environment screening will be overcome. However, field testing will remain for some time to come the final measure of the winter hardiness of a wheat cultivar.

To increase the efficiency of breeding for winter hardiness in wheat, we recommend:

- identifying priorities among stress factors involved in winterkill across the target area. Evaluate long-term data on temperature fluctuation, snow cover, diseases, etc.;
- estimating the minimum freezing tolerance needed in the target area to reduce the risk of significant winter damage to an acceptable level;
- establishing a set of check cultivars, preferably with a long growing history in the region, representing the maximum and minimum winterkill risk assumed;
- taking every opportunity to select in the field to reduce the frequency of winter-tender lines;
- adapting an artificial freezing procedure suitable to the available facilities and potential. Standardize planting, hardening, freezing, recovery, and assessment procedures to increase reproducibility; and
- creating a database on winter hardiness of potential parents and advanced lines. Avoid crosses where none of the parents has the desired level of hardiness.

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CHAPTER 10

Heat Tolerance

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Wheat is the most widely grown cereal in temperate environments, and is also cultivated in many tropical cropping systems, where it is often grown as the winter season crop in rotation with a number of other crops—for example, with maize in Africa, rice in Asia, and soybean in Latin America (Figure 1). Among the numerous advantages of cultivating wheat in this niche are that it is stress tolerant, relatively high yielding, and easy to transport and store.

There are also disadvantages linked to growing wheat in tropical areas; foremost among them are the different types of high temperature stress that affect the crop. Perhaps the greatest challenge to understanding the physiological problems associated with heat stress is to encompass the diversity of hot environments all over the world (Figure 2). Continual heat stress affects approximately 7 million ha of wheat in developing countries, while terminal heat stress is a problem in 40% of temperate environments, which cover 36 million ha. Continual heat stress is defined by a mean daily temperature of over 17.5°C in the coolest month of the season (Fischer and Byerlee, 1991), and over 50 countries (importing more than 20 million tons of wheat per year) experience this type of stress throughout the wheat cycle.

When consulted, representatives of national agricultural research systems (NARSs) from the major wheat-growing regions in the developing world identified heat stress as one of their top research priorities (CIMMYT, 1995).

CIMMYT/NARS Collaboration on Heat Tolerance

Breeding efforts by a number of national wheat breeding programs has resulted in the release of germplasm adapted to warm growing environments, such as in Egypt and Sudan (AbdElShafi and Ageeb, 1994), India (Tandon, 1994),

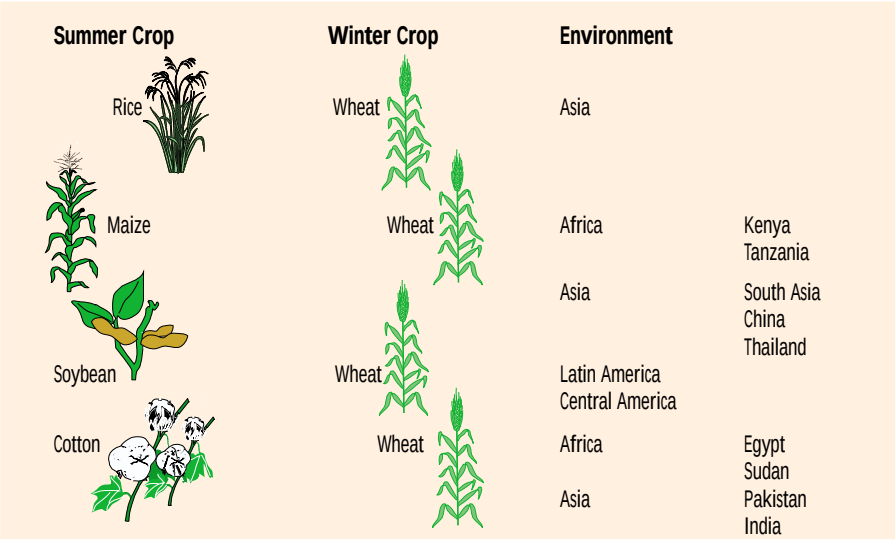

Figure 1. Wheat in tropical cropping systems.

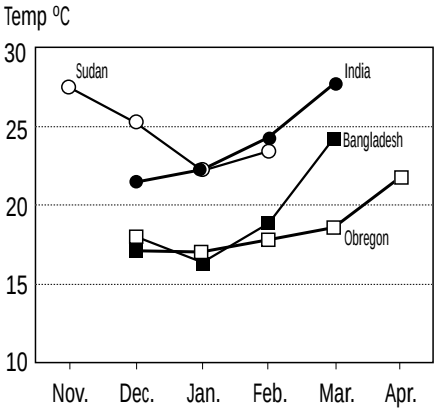

Figure 2. Typical average temperatures during the wheat cropping cycle for three types of hot environments (Wad Medani, Sudan; Dharwad, India; Dinajpur, Bangladesh) and one temperate environment (Ciudad Obregon, Mexico).

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Bangladesh (Razzaque et al., 1994), and Uruguay (Pedretti and Kohli, 1991). CIMMYT has been actively involved in many of these regions (Kohli et al., 1991; Ortiz-Ferrara et al., 1994). Collaboration between CIMMYT and NARS on physiological aspects of heat tolerance in wheat started in 1990, with the establishment of a network involving wheat scientists in Bangladesh, Brazil, Egypt, India, Nigeria, Sudan, and Thailand.

Collaborative experiments conducted by network scientists were named the International Heat Stress Genotype Experiment (IHSGE) (Reynolds et al., 1992; 1994; 1997; 1998; Reynolds, 1994). The IHSGE was grown in wheat-growing areas classified by CIMMYT as heat stressed, i.e., CIMMYT mega-environment 5 (ME5). The main objectives of the IHSGE were to establish the degree of genotype by environment interaction (GxE) in ME5, evaluate potential physiological screening techniques by observing genetic diversity for traits and their association with heat tolerance, and improve our understanding of the physiological and genetic basis of heat tolerance.

There were three main outcomes of the study. First, cluster analyses of over 40 hot site x year combinations indicated that most interaction between sites and genotypes was accounted for by relative humidity (RH). Hence low RH sites (e.g., Sudan, Mexico, and India) and high RH sites (e.g., Bangladesh and Brazil) showed less GxE within RH groups than when comparison was done across RH groups (Reynolds et al., 1998; Vargas et al., 1998). This kind of analysis indicates that breeding for these two broad environments should be undertaken as separate objectives. Second, data collected on IHSGE lines in the low RH sites showed consistent association between yield and a number of morphological traits (Table 1). Third, physiological data collected in Mexico showed that several parameters were associated with performance at international low RH sites (Table 2).

The information emanating from the IHSGE may be useful in establishing indirect selection criteria for heat tolerance. The application of some of these traits to breeding will be discussed in subsequent sections, but first we will present a brief review of some of the physiological traits associated with heat stress.

Table 1. Genetic correlations between morphological traits and wheat yields for 10 varieties averaged over 16 low relative humidity environments in ME5, IHSGE 1990-94.

Trait	Genetic correlation
Final biomass (above ground)	0.88**
Grains/m^2	0.77**
Grains/spike	0.67*
Harvest index	0.51
Kernel weight	-0.10
Spikes/m^2	0.0
Days to anthesis	0.83**
Days to maturity	0.81**
Plant height	0.20
% ground cover (anthesis)	0.67*
Biomass at anthesis	0.35
Plant dry weight (5-leaf stage)	-0.45
% ground cover (5-leaf stage)	-0.30
Plants/m^2	-0.15

* Denotes significance at ≤ 0.05, ** significance at ≤ 0.01.

Table 2. Genetic correlations (Rg) for physiological parameters measured in Tlaltizapan, Mexico, and wheat yields for 10 varieties averaged over 16 low relative humidity environments, IHSGE 1990-94.

Physiological trait	R(g)
Canopy temperature depression	0.86**
Membrane thermostability	0.81**
Leaf chlorophyll (grainfilling)	0.72**
Leaf conductance (heading)	0.63*
Photosynthesis (heading)	0.63*

* Denotes significance at ≤ 0.05, ** significance at ≤ 0.01.

Physiological Traits Associated with Heat Tolerance

Genetic diversity for heat tolerance in cultivated wheat is well established (Midmore et al., 1984; Rawson, 1986; Wardlaw et al., 1989; Al-Khatib and Paulsen, 1990; Reynolds et al., 1994). Photo-assimilation is more likely to be yield-limiting under heat stress than in temperate environments, especially as stress typically intensifies during grainfilling, when demand for assimilates is greatest. This is borne out by the observation that under stress, total above-ground biomass typically shows a stronger association with yield than with partitioning, i.e., harvest index (Table 1); the situation is usually reversed under temperate conditions.

Hence traits affecting radiation use efficiency (such as early ground cover, stay-green, and photosynthetic rate) could be expected to be important under heat stress. Although early ground cover seems to be important in an agronomic context (Rawson, 1988; Badaruddin et al., 1999), variation in this trait among genotypes does not seem to be associated with heat tolerance (Table 1). The stay-green trait has been used widely in breeding for heat tolerance, partly as an indicator of disease resistance (Kohli et al., 1991). Physiological evidence indicates that loss of chlorophyll during grainfilling is associated with reduced yield in the field (Reynolds et al., 1994). Studies in controlled environments have revealed genetic variability in photosynthetic rate among wheat cultivars when exposed to high temperatures (Wardlaw et al., 1980; Blum, 1986).

Such differences in photosynthesis under heat stress have been shown to be associated with a loss of chlorophyll and a change in the a:b chlorophyll ratio due to premature leaf senescence (Al-Khatib and Paulsen, 1984; Harding et al., 1990). Studies at CIMMYT comparing 16 diverse semi-dwarf wheats demonstrated genetic variability for photosynthetic rate under heat-stressed field conditions

(Reynolds et al., 2000). In addition, both canopy temperature depression (CTD) and flag-leaf stomatal conductance, as well as photosynthetic rate, were highly correlated with field performance at a number of international locations (Reynolds et al., 1994). Besides being a function of stomatal conductance (Amani et al., 1996), CTD itself is a mechanism of heat escape, as suggested, for example, by Cornish et al. (1991) in cotton.

Conductometric measurement of solute leakage from cells was used in several studies to estimate heat damage to the plasma membranes. Genetic variation in membrane thermostability (MT) has been inferred using conductometric measurements in various field-grown crops, including spring wheat (Blum and Ebercon, 1981). Shanahan et al. (1990) obtained a significant increase in yield of spring wheat in hot locations by selection of membrane-thermostable lines, as determined by measurements on flag leaves at anthesis. By applying the MT test on winter wheat seedlings, Saadalla et al. (1990) found a high correlation in MT between seedlings and flag leaves at anthesis for genotypes grown under controlled environmental conditions. Measurements of MT of 16 spring wheat cultivars were compared internationally with performance at several heat-stressed locations. Variation in MT of both field-acclimated flag leaves and seedlings grown in controlled conditions was associated with heat tolerance in warm wheat-growing regions (Reynolds et al., 1994). Other studies have confirmed genetic variation of these materials and indicated high heritability for the trait (Fokar et al., 1998).

Although the physiological basis for the association of MT with heat tolerance has not been determined, plasma membranes are known to be more heat tolerant than the photosynthetic thylakoid membranes, for example (Berry and Bjorkman, 1980). While loss of membrane integrity may be the cause of ion leakage from the cell, this phenomenon could also be caused by thermally induced inhibition of membrane-bound enzymes responsible for maintaining chemical gradients in the cell. Direct evidence for a biochemical limitation to heat tolerance in wheat comes from studies of the enzymes involved in grainfilling, specifically soluble starch synthase, which is deactivated at high temperatures (Keeling et al., 1994). If conversion of sucrose to starch is a limitation to yield under heat stress, this would explain the increased levels of carbohydrates in vegetative tissue of wheat observed when grainfilling was limited by heat stress (Spiertz, 1978).

Several other processes are clearly affected by high temperatures, but not discussed in depth here, since either genetic variation has not been shown to be associated with performance, or they do not lend themselves to simple screening. There is some evidence that meiosis is adversely affected at high temperatures (Saini et al., 1983). Respiration costs are higher as temperature increases, leading eventually to carbon starvation because assimilation cannot keep pace with respiratory losses (Levitt, 1980). However, this apparently wasteful process would seem unavoidable, at least in current germplasm, as evidenced by positive associations observed between dark respiration at high temperatures and heat tolerance of sorghum lines (Gerik and Eastin, 1985). On the other hand, high rates of dark respiration in grains may be severely detrimental to yield (Wardlaw et al., 1980).

Heat shock proteins are synthesized at very high rates under high temperature stress and are thought to have a protective role under stress; nonetheless, their role in determining genetic differences in heat tolerance has not been established. Chlorophyll fluorescence may be more promising as a screening trait, given that associations between heat tolerance and lower fluorescence signals have been reported in a number of crops, including wheat (Moffat et al., 1990). Although screening protocols are yet to be thoroughly evaluated, preliminary evidence with CIMMYT lines has indicated that fluorescence parameters may lend themselves to screening for heat tolerance (Balota et al., 1996).

While there is still no definitive picture of the physiological basis of reduced growth rates under heat stress, many drought-adaptive traits may be useful under heat stress. Examples would include leaf glaucousness to reduce the heat load, awn photosynthesis when high temperatures reduce assimilation rate of the leaves, and early escape from heat stress. Heat stress is almost certainly a component of drought stress, since one of the principal effects of drought is to reduce evaporative cooling from the plant surface. Nonetheless, not all traits conferring heat tolerance are also associated with genetic variability for drought tolerance, a good example being membrane thermostability (Blum, 1988). In addition, wheat germplasm that typically performs well under heat stress is not necessarily useful under drought (S. Rajaram, pers. comm.).

Physiological Approaches to Breeding for Heat Tolerance

Different physiological mechanisms may contribute to heat tolerance in the field—for example, heat tolerant metabolism as indicated by higher photosynthetic rates, stay-green, and membrane thermostability, or heat avoidance as indicated by canopy temperature depression. Breeding programs may measure such traits to assist in the selection of heat tolerant parents, segregating generations, or advanced lines (Figure 3). Based on field data collected in the IHSGE, a number of physiological traits that had been presented in the literature were evaluated as potential selection criteria (Table 3). While useful as indicators, these conclusions are by no means definitive for two important reasons.

For one thing, the results cannot be extrapolated with any certainty to environments outside the test site. Also, the data were for the most part measured on unrelated fixed lines and as such do not necessarily imply that selection for these traits would result in genetic gains in yield among the progeny of a cross. To establish the potential genetic gains associated with indirect selection criteria, similar experiments need to be conducted with randomly derived sister lines using a number of relevant crosses and heritability of traits established, as outlined in the introduction of this book.

Evidence for applying three traits (namely, canopy temperature depression, leaf conductance, and membrane thermostability) in selecting for heat tolerance is presented in the following sections; sufficient evidence has been collected on these traits to suggest their potential as breeding tools. Nonetheless, if these techniques have not been evaluated in a given breeding environment, they should first be evaluated, as outlined in chapter one, before being applied to mainstream breeding operations.

Canopy temperature depression

As discussed earlier, experimental data have shown a clear association of CTD with yield in both warm and temperate environments. CTD shows high genetic correlation with yield and high values of proportion of direct response to selection (Reynolds et al., 1998), indicating that the trait is heritable and therefore amenable to early generation selection. Since an integrated CTD value can be measured almost instantaneously on scores of plants in a small breeding plot (thus reducing error normally associated with traits measured on individual plants), work has been conducted to evaluate its potential as an indirect selection criterion for genetic gains in yield. CTD is affected by many physiological factors, which makes it a powerful

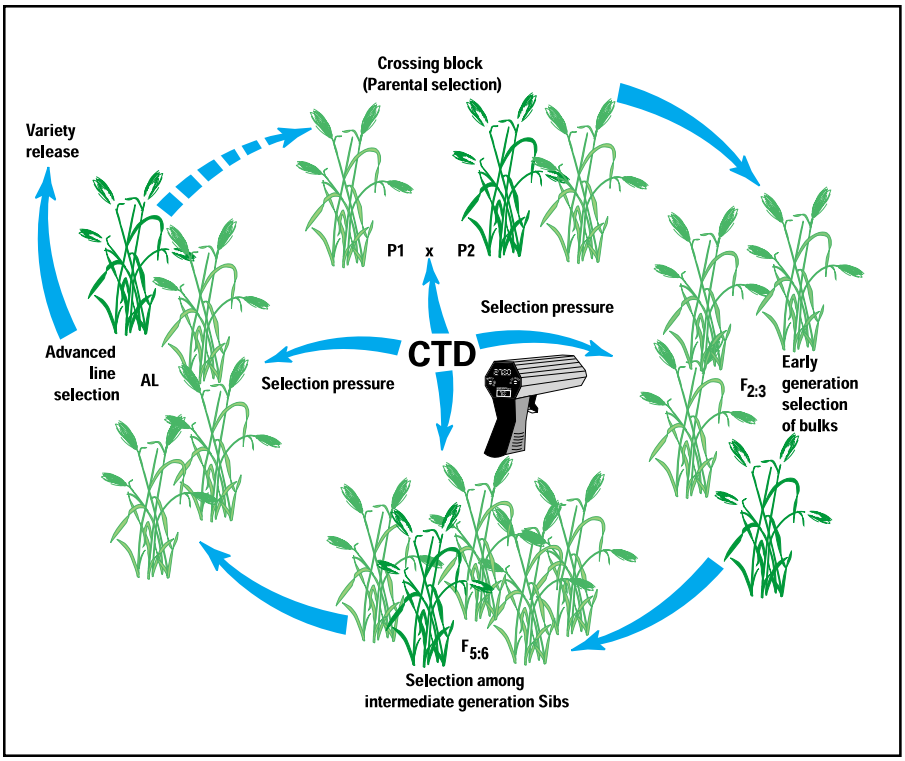

Figure 3. Potential use of canopy temperature depression in a breeding program.

Table 3. Summary of heat stress mechanisms previously reported for wheat and their association with yield in the IHSGE.

Reported heat stress mechanism	Accounting for genetic variation in yield in IHSGE
Accelerated development (Midmore et al., 1984)	Yes, lateness associated with higher yield in many environments
Stand establishment (Rawson, 1988)	No, poor correlation with early growth
Evaporative cooling (Idso et al., 1984)	Yes, strong correlation of CTD with yield
Inhibition of meiosis (Saini et al., 1983; Zeng et al., 1985)	No, sterility not observed. Grain:spikelet ratio not correlated with yield
Sensitive growth phase (Fischer, 1985; Shpiler and Blum, 1991)	Partial least squares analysis confirmed spike growth sensitivity, especially to high night temperatures (Vargas et al., 1988)
Photosynthesis/chlorosis (Al-Khatib and Paulsen, 1990; Shpiler and Blum, 1991)	Yes, high association of photosynthesis and stay-green with yield in field plots
Thylakoid thermostability (Moffatt et al., 1990)	Preliminary data on IHSGE lines confirms association of chlorophyll fluorescence with yield (Balota et al., 1996)
Membrane thermostability (MT) (Shanahan et al., 1990)	Yes, MT measured on seedlings and flag leaves associated with yield at several sites
Inhibition of starch synthase (Bhullar and Jenner, 1986; Rijven, 1986)	No clear evidence, but yield not associated with TGW

integrative trait, but its use may be limited by its sensitivity to environmental factors (Figure 4).

Factors affecting expression of CTD. Leaf temperatures are depressed below air temperature when water evaporates from their surface. One of the factors determining evapotranspiration is stomatal conductance, which itself is regulated by the rate of carbon fixation. To maintain high rates of photosynthesis, a genotype must have an effective vascular system for transpiration of water, as well as for transport of nutrients and assimilates. Since CTD is directly or indirectly affected by a number of physiological processes, it is a good indicator of a genotype's fitness in a given environment. CTD also seems to be affected by the ability of a genotype to partition assimilates to yield, indicated by the fact that CTD frequently shows a better association with yield and grain number than it does with total above-ground biomass (Table 4).

For a given genotype, CTD is a function of a number of environmental factors (Figure 4), principally soil water status, air temperature, relative humidity, and incident radiation. The trait is best expressed at high vapor pressure deficit conditions associated with low relative humidity and warm air temperature (Amani et al., 1996). For these reasons, CTD is not a useful selection trait in generally cool and/or humid conditions, and is quite sensitive to environmental fluxes. Therefore, it is important to measure the trait when it is best expressed—that is, on warm, relatively still, cloudless days. Some environmental flux during the measurement period is inevitable, but correcting data against reference plots, spatial designs, use of replication, and repetition of data collection during the crop cycle can compensate for this.

When measuring CTD, care should be taken to view the plot so as to avoid including soil temperature. If a plot is sown in rows, it is best to stand to one side of it so that the thermometer is pointed at an angle to the rows. If ground cover is low (e.g., leaf area index of less than 2-3), it is best to point the thermometer at a low angle to the horizontal to minimize the likelihood of viewing soil (Figure 5).

Association of CTD with performance. Measurements of CTD made on 23 wheat lines at CIMMYT's subtropical experiment station (Tlaltizapan, Mexico) showed a high correlation with yield measured on the same plots (Figure 6). Sixteen of the same cultivars were yield tested at a number of hot wheat-growing regions internationally, and their performance compared with CTD measurements made in Mexico (Table 5). In some cases, CTD was associated with over 50% of yield variability of the same lines at sites in Brazil, Sudan, India, and Egypt, clearly indicating the promise of CTD as an indirect selection criterion for yield.

In subsequent work, crosses were made between parents contrasting in CTD to generate homozygous sister lines. These were evaluated for both CTD and yield in warm and temperate environments. Populations of randomly derived F_5 sister lines from two crosses showed a clear association of CTD with yield potential in both warm and temperate environments (Figure 7; Table 6), with CTD explaining up to 50% of yield variation.

While heritability of CTD has not been thoroughly evaluated, preliminary data suggest moderate heritability values for the trait. When comparing traits measured on $F_{2:5}$ bulks with subsequent yields in $F_{5:7}$ lines, performance was

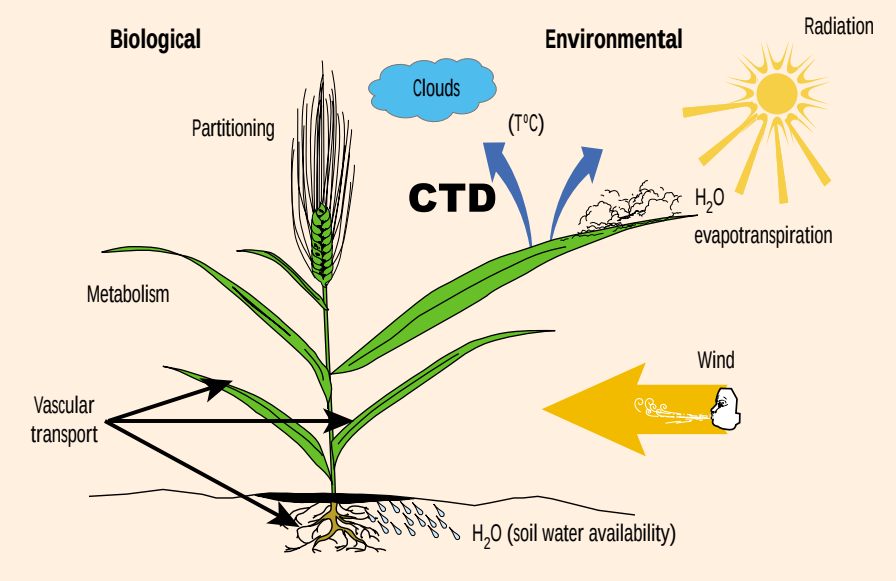

Figure 4. Factors affecting canopy temperature depression (CTD) in plants.

Table 4. Association of CTD with traits of 60 advanced lines, Ciudad Obregon (March-sown), Mexico, 1995.

Trait	Correlation coefficient with CTD
Yield	0.60**
Biomass	0.40**
Harvest index	0.14
Kernel weight	-0.32*
Grains m^{-2}	0.62**
Spikes/m^2	0.33*
Grains/spike	0.40**
Days to maturity	0.10
Days to flowering	0.42**
Height	0.10

* Denotes significance at ≤ 0.05, ** significance at ≤ 0.01.

better predicted by CTD than it was by yield, when both were measured on bulks (Reynolds et al., 1997).

CTD as an efficient means of evaluating advanced lines. In addition to the work on early and intermediate generation breeding lines, experiments were also conducted at CIMMYT with advanced lines to assess the power of CTD as a tool for predicting performance (Reynolds et al., 1997; 1998). Sixty advanced lines (ALs) of diverse genetic backgrounds were selected for superior performance under hot conditions using late sowings in Ciudad Obregon, Mexico. The 60 ALs were multiplied and grown as replicated yield trials in the 1995-96 spring wheat cycle at 15 warm environments: 4 in Mexico, 4 in Sudan, 3 in Bangladesh, 3 in India, and 1 in Nigeria. Physiological traits were measured on yield plots and on smaller 3-row plots in the selection environment, i.e., a March-sown trial in Obregon. Yield and CTD in the selection environment were compared with performance of ALs averaged

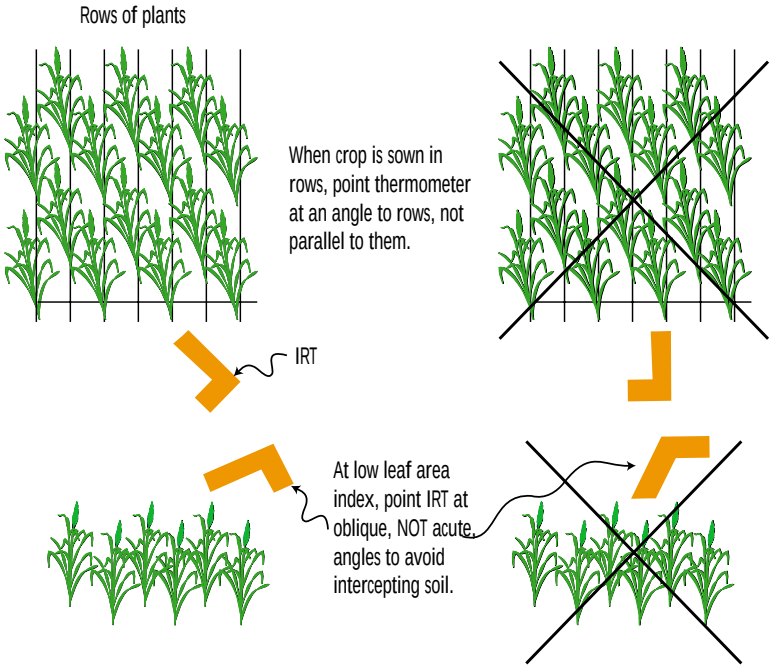

Figure 5. How to view a plot to avoid including soil temperature when measuring canopy temperature depression with an infrared thermometer (IRT).

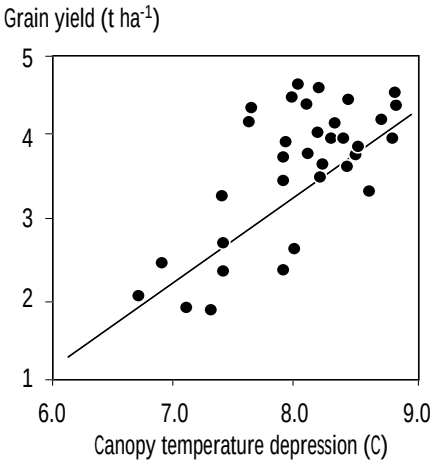

Figure 7. Regression of yield on CTD measured after heading for 40 recombinant inbred lines from a cross between lines contrasting in heat tolerance (Seri 82* Siete Cerros 66), Tlaltizapan, Mexico, 1995-96.
Source: Reynolds et al. (1998).

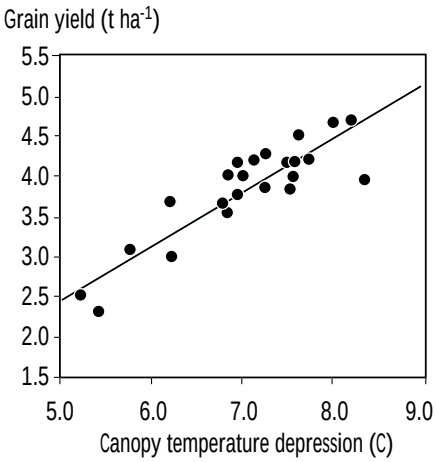

Figure 6. Relationship of mean grain yield to mean CTD for 23 genotypes, averaged over two sowings, Tlaltizapan, Mexico, 1992-93.
Source: Amani et al. (1996).

Table 5. Correlation coefficients between yield, averaged over two cycles at six locations of the IHSGE (1990-92), and CTD of 16 wheat lines measured at different stages of development, December and February sowings, Tlaltizapan, Mexico, 1992-93.

	CTD December			CTD February		
Location	Pre-anthesis	Anthesis	Post-anthesis	Pre-anthesis	Anthesis	Post-anthesis
Brazil	0.45	0.60*	0.50*	0.68**	0.52*	0.68**
Egypt	0.73**	0.91**	0.91*	0.82**	0.79**	0.78**
India	0.33	0.56*	0.62**	0.60**	0.37	0.64**
Sudan	0.71**	0.91**	0.88**	0.77**	0.75**	0.71**
Tlaltizapan	0.66**	0.84**	0.78**	0.50*	0.53*	0.43
Average correlation	0.58	0.76	0.74	0.67	0.59	0.65

* Denotes significance at ≤ 0.05, ** significance at ≤ 0.01.
Source: Reynolds et al. 1994.

across the 15 environments. CTD measured in the selection environment explained at least as much of the variability in performance across all warm sites as yield itself (Table 7).

In this study, several other physiological and morphological traits were evaluated along with CTD. While some also showed significant association with yield (e.g., leaf chlorophyll, leaf conductance, spike number, biomass, and flowering date), no other single trait was consistently as well associated with performance as CTD (Reynolds et al., 1997; 1998). Data also indicated that CTD measured in 3-row plots was as good a predictor of yield as CTD measured in yield plots, suggesting that the technique would be amenable to selection in smaller plots.

Stomatal conductance

Canopy temperature depression is highly suitable for selecting physiologically superior lines in warm, low relative humidity environments where high evaporative demand leads to leaf cooling of up to 10 °C below ambient temperatures. This permits differences among genotypes to be detected relatively easily using infrared thermometry. However, such differences cannot be detected in high relative humidity environments because the effect of evaporative cooling of leaves is negligible. Nonetheless, leaves maintain their stomata open to permit the uptake of CO_2, and differences in the rate of CO_2 fixation may lead to differences in leaf conductance that can be measured using a porometer.

Porometry can be used to screen individual plants, unlike CTD, which can only be estimated on a canopy. The heritability of stomatal conductance is reasonably high, with reported values typically in the range of 0.5 to 0.8 (Vilhelmsen et al., 2001; Rebetzke, pers. comm.); genetic correlation with yield is also high (Table 2). Plants can be assessed for leaf conductance using a viscous flow porometer that is newly available on the market (Thermoline and CSIRO, Australia). This instrument can give a relative measure of stomatal conductance in a few seconds (Rebetzke et al., 1996), making it possible to identify physiologically superior genotypes from within bulks.

For reliable results, more than one reading of stomatal conductance should be taken per plot or per plant. Single-leaf readings always have associated errors that may be caused by environmental fluxes, leaf position, and the fact that leaves may show diurnal and cyclical patterns in stomatal behavior. When crops are irrigated, it is best to take measurements shortly after irrigation to avoid effects of soil heterogeneity that may affect water availability. It is advisable in preliminary studies to measure leaf conductance at different times of day and during different stages of the crop cycle to ascertain when differences between genotypes are best expressed.

Since CTD and leaf conductance show an association with each other and with yield (Amani et al., 1996), the possibility of combining selection for both traits is attractive. For example, CTD could be used to select among early generation bulks that are heterogeneous and may still be segregating. Porometry can be used to identify the best genotypes from among the plants making up the bulk (Figure 8). Work in Mexico where leaf conductance was measured on individual plants in a F2:5 bulk indicated the utility of this approach (Figure 9; Gutiérrez-Rodríguez et al., 2000).

Membrane thermostability

Although resistance to high temperatures involves several complex tolerance and avoidance mechanisms, the membrane is thought to be a site of primary physiological injury by heat (Blum, 1988), and measurement of solute leakage from tissue can be used to estimate damage to membranes. Since membrane thermostability is reasonably heritable (Fokar et al., 1998) and shows high genetic correlation with yield (Table 2), it has potential application in breeding, but does require a laboratory methodology to make measurements.

Laboratory methodology. Membrane thermostability (MT) can be measured on leaf tissue taken at almost any phenological stage, from 10-day-old seedlings to grainfilling. Plants must be heat-acclimated either *in situ* if growing conditions are warm enough, or by putting them in a controlled environment for 48 hours at approximately 35/15°C max/min. At least four leaves should be sampled per plot to ensure that tissue is representative, and 10 or more if the

Table 6. Association of CTD with yield potential of homozygous sister lines from two crosses, sown in warm (1995-96) and temperate environments (1996-97).

	Correlation coefficient of CTD with yield	
	Cross 1	Cross 2
Site	Seri 82* Siete Cerros	Seri 82* Fang 60
Tlaltizapan (warm)	0.64**	0.39*
Ciudad Obregon (warm)	-	0.55**
Ciudad Obregon (temperate)	0.64**	0.51**

* Denotes significance at ≤ 0.05, ** significance at ≤ 0.01.

Table 7. Phenotypic correlations between mean yield of 60 advanced lines at international sites and CTD and yield measured in Ciudad Obregon (March-sown), Mexico, 1995-96.

	Average yield	
Trait	n=11†	n=15
Yield	0.62**	0.59**
CTD 3-row plot	0.66**	0.56**
CTD 5-row plot	0.65**	0.58**

** Denotes significance at ≤ 0.01.

† 11 locations with least G*E determined by cluster analysis for crossover interaction.

plot contains segregating lines or lines that are genetically heterogeneous. Leaves should be randomly collected and placed with their cut ends immersed in water in stoppered glass jars. All jars should be placed in a cold box for transportation from the field to the laboratory.

In the laboratory, the middle portions of leaves can be isolated, quickly washed with de-ionized water, and completely re-hydrated by keeping them in de-ionized water overnight in a refrigerator. To measure MT, 1-cm sections of each leaf can be cut for both the control and heat-shock treatments. To measure MT on seedlings, fungicide-treated seed should be germinated on moistened paper and grown in an environmental growth chamber at 10-20 °C. The oldest leaves of 10-day-old seedlings can be used; however, seedlings must be acclimated before measuring MT. For this purpose, approximately 10 seedlings are placed in a covered water bath with their roots immersed in water maintained at 35°C for 48 h.

Once acclimated, plant material (flag leaves or seedlings) should be washed with de-ionized water and divided into vials containing 17 mL de-ionized water. Half of the vials are maintained at 46.5°C (flag leaves) or 49°C (seedlings) for 60 min in a water bath. The second set of vials is used as controls and maintained at room temperature for the same time periods. After the treatment periods, the heat-treated and control samples are held at 6°C overnight. A first conductometric reading is made at 25°C and a second (also at 25°C) after autoclaving for 20 min at 120°C and 0.10 MPa. MT is expressed as relative injury (RI) using the following:

$$RI\% = (1-(1-T_1/T_2)/(1-C_1/C_2)) \times 100,$$

where T is treatment, C is control, and 1 and 2 refer to the first and second readings of conductance, i.e., before and after autoclaving.

Measuring MT on seedlings vs flag leaves. At CIMMYT experiments were conducted on 16 lines of the IHSGE using both seedlings and flag leaves (Reynolds et al., 1994). The MT trait was favorably correlated with yield in a number of heat stressed international environments, using both methodologies (Table 8). When comparing MT for seedlings versus field-grown flag leaves, there was a significant positive correlation (r^2 = 0.67, n = 16) indicating that the MT determined at the two

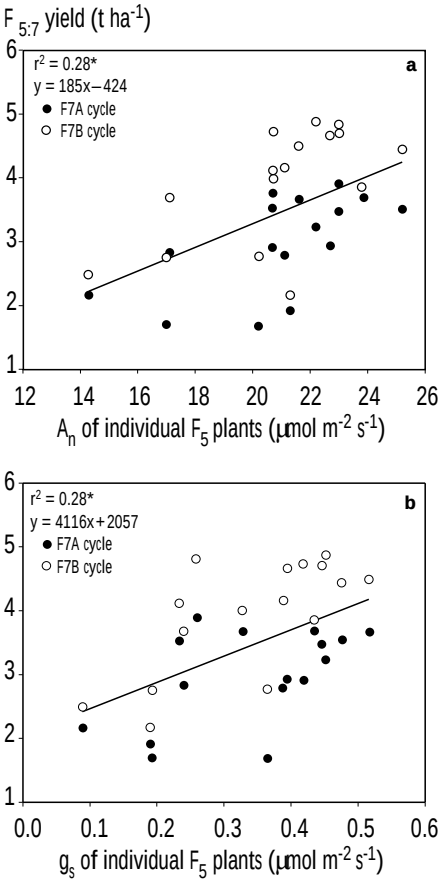

Figure 9. (a) Relationship between $F_{5:7}$ grain yield and leaf photosynthesis rate (A_n) of individual F_5 plants. (b) Relationship between $F_{5:7}$ grain yield and stomatal conductance (g_s) of individual F_5 plants.
* significant at p = 0.05.

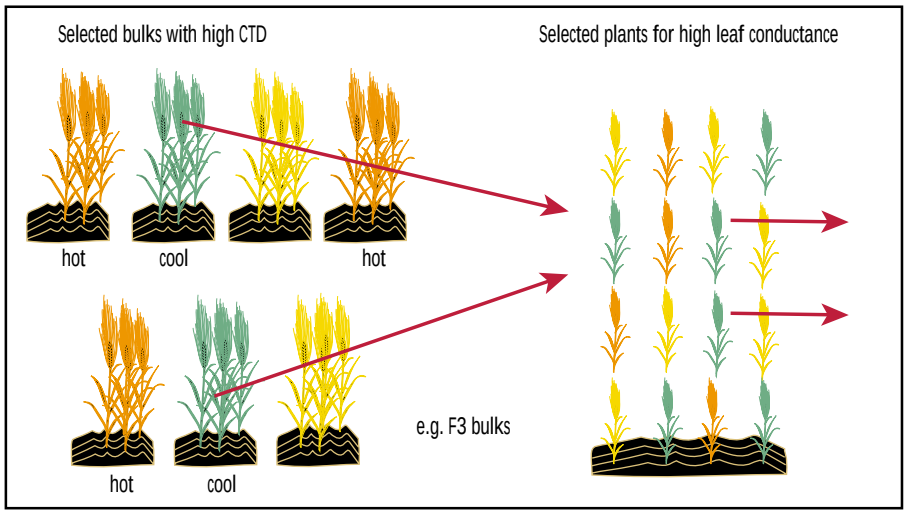

Figure 8. Using canopy temperature depression (CTD) and leaf conductance in early generation selection.

Table 8. Spearman correlation coefficients between yield, averaged over two cycles at each of six locations of the IHSGE (1990-92), and membrane relative injury of 16 wheat genotypes measured using two different methods.

Heat-stressed location	Flag leaf (field grown)	Seedlings (chamber grown)
Tlaltizapan Dec.	-0.65**	-0.40
Tlaltizapan Feb.	-0.31	-0.01
Brazil	-0.59*	-0.57*
Egypt	-0.69**	-0.64**
India	-0.66**	-0.57*
Sudan	-0.69**	-0.58*
Average correlation	-0.60	-0.46

* and ** refer to P < 0.05 and P < 0.01, respectively.

development stages was well associated. These data support the idea that using seedlings raised under artificial conditions for screening MT may be a viable alternative to using field-grown tissue. The use of seedlings is preferable logistically because the conditions of plant acclimation can be controlled, which is not possible in the field. The importance of this point is illustrated indirectly by the data.

Using the seedling procedure, the three repetitions of the experiment were measured for MT on three subsequent days. While the interaction of genotype with repetition was not significant, the main effect of repetition (i.e., day of experiment) was highly significant (data not shown). Even under controlled conditions, unintentional discrepancies either in procedure or day-to-day variability of conditions influenced absolute values of MT. Since it would not be practical for a breeding program to assess MT on all the germplasm of interest in one experimental run, a methodology involving controlled conditions would seem preferable.

Another advantage of using seedlings rather than more mature tissue is that MT is unlikely to be affected by phenology at such an early stage of development. In these experiments there was a range in anthesis and maturity dates among the genotypes. Instead of measuring MT on the precise date of anthesis for each genotype, MT values were measured on all flag leaves on the same calendar date and subsequently adjusted using the number of days between measurement of MT and anthesis as a covariate.

Genetic Diversity for Heat Tolerance Traits

While genetic diversity for heat tolerance has been shown to exist among conventional wheat cultivars (Rawson, 1986; Wardlaw et al., 1989; Al-Khatib and Paulsen, 1990; Reynolds et al., 1994), progress would be limited if new sources of genetic diversity were not exploited. Materials that could be exploited fall into two broad categories: landraces that can be used directly in conventional breeding efforts and wild species with compatible genomes from which genes can be introduced into cultivated wheats using wide crossing approaches.

Genetic diversity for heat tolerance has been shown to exist in wild *Triticum* and *Aegilops* species by Edhaie and Waines (1992), who tested accessions from Afghanistan, Iran, Iraq, Israel, Jordan, Syria, Lebanon, Turkey, and the USSR. Interestingly, all of the heat tolerant accessions came from only three regions: eastern Israel, western Jordan, and southwestern Syria. The authors suggest that a search among the bread and durum wheat landraces from these regions may provide genotypes with a high degree of heat tolerance that could be incorporated into modern wheat backgrounds.

Some work has been conducted to identify new sources of heat tolerance traits among accessions in the CIMMYT wheat genebank. For example, high leaf chlorophyll content has been identified in Mexican landrace collections where the best genotypes showed substantially greater leaf chlorophyll concentration than the check Seri-M82. While high leaf chlorophyll content does not guarantee heat tolerance, the stay-green trait has been associated with heat tolerance in fixed lines (Reynolds et al., 2000), and high chlorophyll was associated with heat tolerance of sister lines in some wheat crosses (Reynolds et al., 1997).

High stomatal conductance (which may permit leaf cooling through evapotranspiration) has started to be examined in accessions from CIMMYT's genebank collections, under heat stressed conditions. For reasons discussed earlier, measuring stomatal conductance as an indication of heat tolerance/escape is more suitable than measuring CTD, since it can be evaluated relatively easily on individual plants, a necessary efficiency when screening very large numbers of accessions from a germplasm bank. Apart from identifying genetic diversity for the trait, preliminary work also indicated reasonable levels of broad-sense and realized heritability (60-75%) for the trait (Vilhelmsen et al., 2001).

Molecular approaches may be helpful for identifying useful genetic diversity expressed in the progeny of wide crosses. Genetic diversity from wheat wild relatives has already been exploited through wide crossing to introduce disease resistance (e.g., Villareal et al., 1995). Potential exists for identifying the loci encoding other quantitatively inherited traits associated with abiotic stress tolerance using QTL analysis in mapping of delayed backcross generations (Tanksley and Nelson, 1996).

Agronomic Strategies for Ameliorating the Effects of Heat Stress

Optimal crop growth requires a non-limiting supply of water, nutrients, and radiation; as temperatures rise, the demand for growth resources increases due to higher rates of metabolism, development, and evapotranspiration (Rawson, 1988). When growth resources are limited by heat stress, the size of plant organs such as leaves, tillers, and spikes is reduced (Fischer, 1984). The apparent sensitivity of metabolic processes to heat stress in the field (Reynolds et al., 1998; 2000), coupled with the reduced length of life cycle at high temperature (Midmore et al., 1984), explains why grain yield is strongly associated with total plant biomass in hot environments. These interactions make crop management practices critical to sustaining wheat yields in warm environments.

A few studies have shown the benefits of specific management practices under stress. For example, the application of farmyard manure (FYM) has been reported to improve soil physical and

chemical conditions, and to help conserve soil moisture (Sattar and Gaur, 1989; Gill and Meelu, 1982; Tran-Thuc-Son et al., 1995). A one-time application of FYM (10-15 t ha^{-1}) increased wheat yields for up to three successive crop cycles, when applied in conjunction with inorganic N fertilizers, and for up to four years with the addition of P fertilizers under hot and humid conditions in Bangladesh (Mian et al., 1985). Under high-temperature conditions, volatilization of N fertilizers such as NH_3 is more likely, and further decreases wheat yields compared with the application of equivalent N in organic forms such as FYM (Tran-Thuc-Son et al., 1995).

Straw mulch is another agronomic input with the potential to ameliorate stress by reducing evaporation of soil moisture and increasing infiltration rate (Lal, 1975). Straw mulch has also been reported to lower soil temperature (Benoit and Kirkhoun, 1963) and to impede seedling emergence, a negative effect (Chopra and Chaudhary, 1980). Surface soil temperatures can exceed air temperature by 10 to 15°C if the soil surface is bare and radiation intensity is high; straw mulch in such conditions may increase seedling emergence and survival (Fischer, 1984). Given that wheat growth under warm conditions is highly sensitive to management, judicious combinations of management practices could substantially benefit performance by improving crop establishment and the availability of water and nutrients during subsequent growth stages.

A collaborative study was conducted by CIMMYT and the national wheat research programs of Sudan and Bangladesh to provide information from warm environments on the response of wheat to management factors such as mulching and application of FYM, and to elevated levels of inorganic fertilizer and increased irrigation frequency (Badaruddin et al., 1999). The research was conducted to determine whether modifications to recommended crop management practices could significantly improve grain yield. Control treatments represented recommended practices and gave yields of 3.6 t ha^{-1}, averaged across all environments. Considering main effects, FYM (10 t ha^{-1}) gave the highest yield response (14%), and approximately equivalent levels of NPK the lowest (5.5%), suggesting that organic fertilizer provided growth factors in addition to nutrient content. Mulch and extra irrigation increased yield in the hot, low relative humidity environments (i.e., Sudan and Mexico), but not in Bangladesh, which is hot and humid.

In Mexico, extra inputs were more beneficial under hotter, spring-sown conditions than in winter sowings. Comparison of heat tolerant (Glennson 81) and heat sensitive (Pavon 76) genotypes showed that the heat tolerant genotype was generally more responsive to additional inputs. Improved performance in response to inputs was generally associated with better stand establishment and with significant increases in plant height, grain m^{-2}, and above-ground biomass; in Mexico it was also related to higher canopy temperature depression and light interception.

These results clearly indicate that wheat yields in warm environments can be raised significantly by modifying agronomic practices. Overall, the application of animal manure had the largest and most consistent effect on yield. Some of the benefits associated with extra organic matter may also be provided by practicing residue retention and reduced tillage. Such integrated approaches to crop and soil management in abiotically stressed environments are becoming increasingly relevant in light of diminishing water supplies in many agro-ecosystems.

This study did not attempt to analyze the economic basis of management factors, only to establish their biological value. Nonetheless, data indicate that recommended levels of fertilizer, whether organic or otherwise, were not generally sufficient to meet crop requirements. Average yield responses to NPK and FYM at a given site were as much as 17% and 24%, respectively, suggesting that in hot regions even economic yields might be improved through better crop nutrition.

The economic basis of increasing irrigation frequency is more complex for two reasons. First, irrigation schemes such as the one in the Gezira of central Sudan lack the flexibility to permit farmers to irrigate at will. Water is usually available only at set times in a given area as water is passed systematically through the whole irrigation scheme. Second, water availability is declining in many regions of the world, so the expectation of raising economic returns through increased irrigation may not be fulfilled if water prices rise dramatically. As mentioned previously, it may be possible to obtain the benefits of mulching and, perhaps, increase soil organic matter through a combination of residue retention and reduced tillage practices. Nonetheless, significant investment will be required on the part of national agricultural research systems and their governments, or agricultural development agencies sponsored by industrialized countries, if such practices are to become a reality in the developing world.

Conclusions

While patterns of heat stress may vary widely between wheat growing regions, a major factor explaining genotype by environment interaction has been shown to be relative humidity (RH). In low RH environments, lack of physiological heat tolerance is the major yield constraint, while in high RH environments, disease pressure may be an additional and possibly more serious limitation. Canopy temperature depression seems to be a potentially powerful indirect selection criterion in low RH environments, while stomatal conductance and membrane thermostability may be applied in all hot environments. However, genetic gains to

selection should be tested in any new environment using locally adapted germplasm (as outlined in the introductory chapter) before the use of physiological traits as indirect selection criteria is incorporated into mainstream breeding.

Where germplasm collections are available, accessions from abiotically stressed regions should be screened for heat tolerance characteristics as a means of introducing new sources of genetic diversity into the breeding program. In addition to genetic improvement, agronomic strategies (such as residue retention to lower soil surface temperatures and increase soil organic matter) are also a means of increasing productivity in warm environments.

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CHAPTER 11

Waterlogging Tolerance

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More than one third of the world's irrigated areas suffer occasional or more frequent waterlogging (Donmann and Houston, 1967). Waterlogging has been shown to limit wheat yields in many regions of the world; an area estimated at 10 million ha is waterlogged each year in developing countries (Sayre et al., 1994). Waterlogging occurs when rainfall or irrigation water collects on the soil surface for prolonged periods without infiltrating the soil. Soil characteristics that contribute to waterlogging include soil physical properties that allow formation of a crust on the soil surface or of a pan in the subsoil. Waterlogging can also occur when the amount of water added through rainfall or irrigation is more than what can percolate into the soil within one or two days.

Waterlogging occurs in many wheat growing regions around the world, especially irrigated and high rainfall environments. In irrigated regions, the main culprit seems to be the lack of proper drainage systems. Irrigation facilities do not allow easy drainage of excess water, sometimes due to poorly kept irrigation canals from which water seeps out. Major examples are the Indian Subcontinent, certain river basins in China, and the Nile River Delta in Egypt. In the northern Indo-Gangetic Plains of India alone, 2.5 million ha of wheat are affected by irregular waterlogging (Sharma and Swarup, 1988).

The effects of waterlogging are most widespread in the irrigated rice-wheat regions of South and Southeast Asia (i.e., China, Vietnam, Thailand, Bangladesh, Nepal, India, and Pakistan) and in the southern United States (i.e., Georgia, Mississippi, and Louisiana). A common denominator in these countries is that rice rotations are practiced on much of the land. Soils are generally puddled to restrict water percolation and create flooded conditions for rice cultivation. Due to soil puddling, wheat that follows rice in the drier season is planted under less than optimal soil physical conditions. The soil pan that was created intentionally for rice cultivation is often left undisturbed and may create a barrier for water movement, causing waterlogging when excessive irrigation or rainfall occurs.

In South Asia, wheat is a relatively new option within rice rotation schemes. Some farmers, accustomed to applying generous amounts of water to rice, tend to over-irrigate their wheat crop. Additionally, many rice-wheat soils are silt or loam and susceptible to crusting, which creates waterlogging by restricting percolation from the surface. Declines in organic matter in the topsoil of South Asia are well documented and also contribute to poor soil physical quality (FAO, 1994; Hobbs and Morris, 1996; Nagarajan, 1998).

Waterlogging can affect other irrigated areas in Asia besides the rice-wheat growing regions. Wheat-producing areas in Egypt, Sudan, and Nigeria also suffer regularly from waterlogging. In parts of Africa and Latin America, heavy rainfall combined with heavy clay soils creates waterlogging that limits wheat production. In the traditional wheat-growing regions in the Ethiopian highlands, downpours are heavy and prolonged during the rainy season. Hence waterlogging is a common occurrence at the beginning of the wheat cycle. The situation is further exacerbated by the black vertisols in Ethiopia, consisting of heavy clays that inhibit infiltration, swell, and crack severely. Waterlogging limits wheat yields in Australia due to rising groundwater (Grieve et al., 1986; McDonald and Gardner, 1987; Meyer and Barrs, 1988).

Conditions and Symptoms Associated with Waterlogging

Except at sowing or during early germination, waterlogging will not generally destroy wheat plants nor affect plant establishment (Musgrave, 1994). The major morphological and biochemical effects will be discussed in detail later, but under mild waterlogging wheat plant growth is

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usually stunted, bottom leaves senesce, tiller survival is reduced, and florets may become sterile.

High temperatures tend to exacerbate the negative effects of waterlogging. When aerobic soil conditions re-occur, plant growth resumes slowly. Consequently, wheat yields are affected.

An entire field will rarely be waterlogged; waterlogging is usually restricted to the lower lying areas of a field (Picture 1). Waterlogging occurs when the soil is fully saturated, and standing water replaces the air in the soil pore spaces. There is a lack of oxygen in the soil, restricting aerobic respiration by growing roots and other living organisms. Soil chemical properties change when anaerobic conditions persist for several days, increasing the availability of some major or minor elements while decreasing the availability of others. Plant transpiration is affected until wheat roots recover (when soil aerobic conditions recur) or adapt to the anaerobic conditions. However, extended waterlogging will result in root death. Waterlogging also limits the wheat plant's nutrient uptake by reducing plant transpiration and diminishing root function.

Another effect of waterlogging is to stimulate the production of certain plant hormones. In anaerobic conditions these hormones are released from the roots in greater concentrations and may affect leaf and root responses. Ethylene is produced both by the roots and by microorganisms in waterlogged soils. The hormonal effects of ethylene released under waterlogging are attracting a great deal of interest. Water acts as a barrier to the escape of ethylene produced in roots and other submerged tissue. Ethylene is known to be a trigger (not a promoter) of leaf senescence (Dong et al., 1983).

Waterlogging during sowing or germination generally kills the seed or seedling. The seedling's radicle and roots do not adapt readily to waterlogging or are more susceptible to seedling diseases that may follow (Belford et al., 1985). Generally, the wheat plant's tolerance to waterlogging increases as it ages, and the detrimental effect on yield decreases (Meyer and Barrs, 1988). Once the wheat crop is established, many genotypes can withstand waterlogging up to 10 days with no yield loss, if the wheat leaves are not submerged. Wheat crops can make an amazing recovery following early waterlogging stress, if supplied with extra nitrogen.

In waterlogged soils and in the roots of plants growing in them, exceptionally high levels of ethylene may build up, given that ethylene diffuses more slowly in water than in aerated soil. The first response of a wheat plant to anaerobic conditions involves its biochemical pathways as a response to the lack of respiration by root cells. Various hormones are stimulated and transported to the leaves, causing early senescence of older leaves within days (Dong et al., 1983; Dong and Yu, 1984). Seminal roots are generally killed or their growth greatly restricted (Huang and Johnson, 1995).

However, some wheat genotypes have nodal or adventitious roots that begin aerenchyma cell formation. Aerenchyma is tissue that can carry oxygen from the leaves to the roots under anaerobic conditions to maintain root respiration, though on a more limited basis than in aerobic conditions. The process is accelerated if temperatures are elevated. Genetic variability for this trait has been documented in the literature (Cao et al., 1995).

Winter wheat areas may also be prone to waterlogging. Winter wheats are sometimes grazed and allowed to re-grow for grain production in the spring. Trampling of saturated pasture soils by cattle can cause restricted water movement and waterlogging.

The literature documents some tolerance of winter wheats to waterlogging (Musgrave, 1994). Yet this may not be true tolerance, since the colder soil temperatures associated with waterlogging in winter-wheat-growing areas reduce the amount of oxygen required for root respiration. Thus yield reductions associated with waterlogging in colder areas are not as great as those in the more temperate and tropical areas of the world. On the other hand, some studies show soil oxygen decline under waterlogging is rapid at most temperature ranges (Trought and Drew, 1982). It should also be noted that winter wheats are longer maturing and hence less sensitive to waterlogging than the earlier maturing spring wheats (Gardner and Flood, 1993).

Picture 1. Non-uniform waterlogging in a wheat field in Bangladesh.

The literature contains many references on the possible genetic variability for tolerance of wheat to waterlogging, hypoxia, or anoxia. This chapter will review the physiological and biochemical causes of wheat yield reductions due to waterlogging. It will also explore the different options for screening wheat for waterlogging, plus the advantages of incorporating waterlogging tolerance into a breeding program. Agronomic practices developed through research or being used by farmers to alleviate the detrimental effects of waterlogging are also included in this chapter.

Effect of Waterlogging on Soil Chemistry

Decreases in yield brought about by waterlogging may be caused by numerous factors acting upon the wheat plants, such as changes in soil chemistry. As an example, denitrification of soil nitrogen as a result of waterlogging may affect the amount of nitrogen that concentrates and accumulates in the upper leaves of the plants, which will eventually have a negative effect on grain yield. Table 1 shows a list of soil chemical responses and the corresponding bibliographic references that can be consulted for further information on each.

Genetic Improvement of Waterlogging Tolerance

Some studies have suggested that the waterlogging tolerance trait is highly heritable (Cao et al., 1995; Boru, 1996); others demonstrated there is little variability for waterlogging tolerance among durum wheat lines (Tesemma et al., 1991). Some authors have found that the trait is controlled by a single gene (Cao et al., 1992; Cao et al., 1995), while others maintain it is polygenic (Hamachi et al., 1989; Boru, 1996). Closely related species of wheat may be sources of waterlogging tolerance (Cao and Cai, 1991; Taeb et al., 1993; Cai et al., 1994); however, there may be other sources of tolerance within wheat. Boru (1996) concluded that there were four genes involved in waterlogging tolerance: one major gene, two intermediate ones, and one minor gene. Triticale has proved to be superior to bread wheat in tolerance to waterlogging (Johnson et al., 1991a). The Chinese have reported considerably more work on breeding waterlogging tolerant lines in the literature than any other country.

Screening techniques in the laboratory or the field are well documented in the literature. While waterlogging tolerance is directly related to the ability to quickly form roots with aerenchyma cells under anaerobic conditions, there may be concurrent tolerance to Mn toxicity (Wagatsuma et al., 1990). Tolerance to Mn toxicity seems to be secondary to the formation of aerenchyma cell in roots for extending tolerance. Wagatsuma et al. (1990) also determined that when any tolerance was expressed, it was not due to the ability of plant roots to tolerate low O_2 levels.

One study showed differences in nodal roots and aerenchyma cell formation among wheat and triticale varieties (Thomson et al., 1992). Those lines with increased nodal and aerenchyma forming abilities endured waterlogging with fewer detrimental effects. Data suggest that waterlogging tolerance may be related to the ability to produce more crown roots and more aerenchyma in those roots, to maintain stomatal opening, and to more quickly resume seminal root growth and stomatal opening when aerobic conditions recur (Huang et al., 1994). Boru (1996) showed that aerenchyma cell formation and yield were highly correlated in lines that survived severe waterlogging. Their cortical tissue had dissolved to form the aerenchyma; in contrast, sensitive genotypes expressed little or no aerenchyma formation after waterlogging.

Techniques for screening for waterlogging tolerance

The authors feel that screening for waterlogging is best done in the field, using simple designs, rather than in less realistic laboratory conditions.

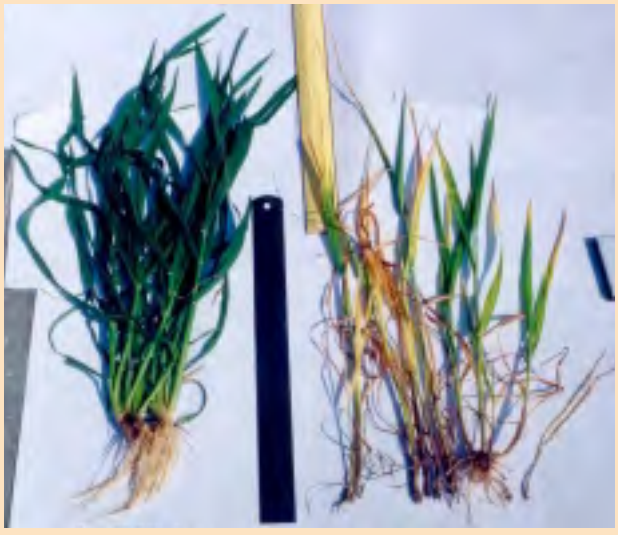
Table 1. Soil chemical responses to waterlogging as reported in the literature.

Chemical response	Reference
Increased Mn concentration that could be toxic to plant growth	Sparrow and Uren, 1987; Wagatsuma et al., 1990
Decreased soil oxygen; generally greater at warmer temperatures	Belford et al., 1985
Decreased Mo availability; Mo application in waterlogged, acid soils retained plastid pigments, cyclic phosphorylation, and CO_2 fixation within wheat plants	Salcheva et al., 1984
Denitrification of both organic and inorganic soil N	Feigenbaum et al., 1984; Singh et al., 1988; Mascagni and Sabbe, 1991; Humphrey et al., 1991
Mineral (Fe) coating of epidermal surface of roots under waterlogging	Ding and Musgrave, 1995
Volatile fatty acids and phenolic compounds accumulated in soils high in organic matter affect root metabolism and growth	Lynch, 1978; Jackson and St. John, 1980

Specific Physiological Responses of Wheat to Waterlogging as Reported in the Literature

1. Chlorosis of lower leaves (Sparrow and Uren, 1987; van Ginkel et al., 1992) (Picture 2).
2. Early senescence of lower leaves (Dong et al., 1983; Dong and Yu, 1984).
3. Decreased plant height (Sharma and Swarup, 1989; Wu et al., 1992).
4. Delayed ear emergence (Sharma and Swarup, 1989).
5. Reduced root and shoot growth (Huang and Johnson, 1995).
6. Lower number of spike-bearing tillers (Belford et al., 1985; Sharma and Swarup, 1989; Wu et al., 1992) (Picture 3).
7. Fewer grains per spikelet and reduced kernel weight (Belford et al., 1985; Musgrave, 1994; van Ginkel et al., 1992).
8. Reduced diameter of metaxylem and protoxylem vessels of the nodal roots (Huang et al., 1994).
9. Enhanced formation of aerenchyma cells in the cortical tissue of both seminal and nodal roots (Huang et al., 1994; Boru, 1996).
10. Leakage of cell electrolytes (Wang et al., 1996a).
11. Reduced uptake of N, P, K, Ca, Mg, and Zn while increasing Na, Fe, and Mn absorption under alkaline soil conditions (Sharma and Swarup, 1989; Stieger and Feller, 1994a).
12. Reduced root respiration (Wu et al., 1992; Wang et al., 1996b).
13. In wheat oxygen concentrations between 33 and 66 $\mu g\ m^2\ s^{-1}$ were categorized as deficient and $< 33\ \mu g\ m^2\ s^{-1}$ as critical. Roots were significantly reduced by the small amount of oxygen available, especially at lower depths. Temperature also influenced root reduction, with 15^o C appearing to be the best soil temperature for root growth (Box et al., 1991).
14. Decreases in wheat yields of 37-45% due to waterlogging have been observed (Musgrave, 1994; Wu et al., 1992; Cai et al., 1994; van Ginkel et al., 1992; Boru, 1996). Wheat yield depression was due to reduced kernel number and weight rather than to an effect on stand establishment.
15. Waterlogging was shown in one study to cause only slight suppression of flag-leaf photosynthesis and leaf conductance in waterlogging intolerant wheat lines (Musgrave, 1994). Other studies showed overall lowered rates of plant photosynthesis, stomatal conductance, and transpiration (Dong and Yu, 1984).
16. Root carbohydrate supply was shown in some studies not to be a limiting factor for root growth and respiration (Huang and Johnson, 1995).
17. Anoxia (waterlogging) inhibited the transport of sugars from the shoots to the roots by more than 79% in seedlings. However, there are interactions between temperature and other environmental factors that could affect interpretation of data on tolerance of wheat to anoxia, which explains the lack of consistent results in the literature (Waters et al., 1991).
18. Data collected on wheat under waterlogged conditions (i.e., deficient in oxygen) in the field and glasshouse showed that the biosynthesis of new tissue was more inhibited than the supply of substrates for growth (Attwell et al., 1985).
19. Flower sterility associated with waterlogging is linked to lower transpiration and, hence, to less uptake of boron (and other nutrients) (Somrith, 1988; Saifuzzaman and Meisner, 1996; Rawson et al., 1996; Misra et al., 1992; Kalidas, 1992; and Subedi, 1992).
20. Ethylene production increases and acts as a trigger (not promoter) of accelerated wheat plant senescence (Dong et al., 1983). Exogenous cytokinins applied to wheat seedlings at the onset of waterlogging delayed degradation of chlorophyll and other biochemical processes (Dong and Yu, 1984). Enhancement of ACC (1-aminocyclopropane-1-carboxylic acid), its precursor, and ethylene was more pronounced in older leaves than in younger ones during waterlogging (Dong et al., 1986).
21. Less nitrogen concentrates and accumulates in the upper leaves of waterlogged wheat, probably due to the denitrification of soil nitrogen (McDonald and Gardner, 1987).
22. Nitrogen remobilization from lower leaves is accelerated on flooded soils and explains their chlorosis (Stieger and Feller, 1994b).
23. Reduced rooting depth and increased root porosity (Yu et al., 1969).

Picture 2. Lower leaf chlorosis.



Picture 3. Waterlogging reduces the number of spike-bearing tillers.

Retaining water on the surface is easier to achieve on heavy soils than on lighter soils. To administer waterlogging stress on heavy soils, wheat lines can be irrigated such that water is retained at or slightly above the soil surface from emergence to the boot stage (van Ginkel et al., 1992; Sayre et al., 1994). In the former study, carried out using extreme waterlogging stress, only three genotypes were shown to be tolerant out of a total of 1,344 lines. In lighter soils, waterlogging may be more difficult to induce.

Our experience shows that even "over-watering" (i.e., keeping the soil slightly at or above field capacity at various growth periods) can induce waterlogging that is adequate for screening wheat lines.

Evaluating differences among varieties in leaf chlorosis or withering after 15 days of waterlogging has been shown to be a quick method for assessing tolerance. Using this method the number of green leaves remaining on the main stem was correlated with the number of fertile grains in the main ear and grain weight per plant (Cai and Cao, 1990; van Ginkel et al., 1992). Field studies in Mexico and Bangladesh on hundreds of CIMMYT wheat lines over years have shown clear evidence of variability to waterlogging tolerance. Fields were kept flooded from emergence to boot stage. Percentage foliar chlorosis at heading and simple agronomic scores during grainfilling appeared to be highly correlated with yield in large plots. Many lines can be screened rapidly using this simple methodology (van Ginkel et al., 1992).

Studies in Japan showed that assessing leaf senescence in early generations was useful for screening for waterlogging tolerance (Hamachi et al., 1989). Wiengweera et al. (1997) used a "stagnant" nutrient solution in agar closely resembling waterlogged soil for rapid screening of wheat seedlings in the lab. Studies in China indicated that an index based on the number of grains per ear and one thousand grain weight was effective for evaluating waterlogging tolerance (Lin et al., 1994). Musgrave (1994) found that flag leaf photosynthesis in winter wheat correlated well with grain weight under waterlogging.

Although waterlogging during early seed germination and seedling growth is very detrimental to the wheat crop, studies have shown there are genetic differences in the ability of wheat genotypes to withstand early waterlogging stress (Johnson et al., 1991b). Mineral (Fe) coating of rice roots (showing oxygen release from the roots) is highly correlated to rice yields, but the trait was negatively correlated to wheat yields in 12 cultivars grown under waterlogged conditions (Ding and Musgrave, 1995).

Since tiller production decreases during waterlogging, tiller production, shoot dry matter, and root penetration were used for screening Triticeae species for tolerance. When these criteria were used, many wild species expressed a level of tolerance to waterlogging that was better than that of wheat (Taeb et al., 1993).

Agronomic Practices Known to Reduce Waterlogging

Setting planting dates to coincide with reduced rainfall patterns is one way to avoid waterlogging (Aggarwal et al., 1987). However, this may not always be possible due to rotation restrictions, and may be associated with lower yields due to sub-optimal climatic conditions.

Application of nitrogen fertilizer after waterlogging has been shown to reduce the detrimental effects of this stress (Trought and Drew, 1980a; Swarup and Sharma, 1993; de Oliveira, 1991). Waterlogging under optimum soil nutrient (N) supply conditions resulted in less growth restriction than under a sub-optimal nutrient supply (Guyot and Prioul, 1985). Further studies provided evidence that doubling the concentration of nutrients supplied to the plants under waterlogging reduced the rate of decline in photosynthetic rate, chlorophyll content, and number of nodal roots, while improving shoot N status and growth (Huang et al., 1994).

Singh et al. (1992) found that the use of green manures, straw, and animal manures increased the availability of Fe and Mn several fold under flooded conditions. Organic manures can also improve soil physical factors and reduce soil surface crusting, enhance plant rooting, and alleviate the effects of pan formation on yields. Therefore the use of manures is considered beneficial in waterlogging-prone environments.

Seed treatments such as calcium peroxide (Thomson et al., 1983) were tried with mixed results for alleviating the detrimental effects of waterlogging during germination or early seedling growth.

Several cultivation and sowing techniques have been shown to give yield increases under waterlogged conditions. For example, Rasmussen (1988) found that direct drilled wheat was more sensitive to waterlogging between germination and emergence than conventionally plowed wheat. The furrow or bed planting system has significant yield advantages, even when there is no waterlogging. Furrows also make it possible to drain fields or keep a large portion of the root system out of waterlogged soils (Abebe et al., 1991; Tedia et al., 1994).

Shifting from basin flooding to furrow or sprinkler irrigation on waterlogging-prone soils has been shown to reduce the problem significantly (Melhuish et al., 1991). Surface seeded wheat (sown on top of uncultivated, saturated soil) showed the least sensitivity to waterlogging compared to wheat sown in conventionally plowed and chiseled soil (Table 2).

Screening for Waterlogging Tolerance under Bangladesh Conditions

Identification of waterlogging tolerant wheat genotypes began during the 1993 wheat season in Bangladesh. In the northwestern part of the country, screening of wheat genotypes for waterlogging tolerance spanned four seasons. The soil type at the Dinajpur Wheat Research Centre experiment station is deep, sandy loam. Over three seasons (1993-95), 162 wheat genotypes were subjected to waterlogging by irrigating at 10-day intervals from 10 to 100 days after sowing (DAS). The field was flooded and the land submerged for 24-36 hours in each of the 10 irrigations.

However, under those soil conditions (sandy loam), our treatments were closer to "over-irrigation" than true waterlogging, since the water percolated quickly within 36 hours of waterlogging. Five replications were used during the first two seasons, and three replications were practiced in the other two seasons to collect data on 2.5-m plots consisting of three rows, 20 cm wide. The experiments were sown at normal sowing time (third week of November). Seeding rate was 120 kg/ha, and fertilizer rates were as recommended (100: 60: 40: 20: kg/ha of NPKS).

Table 2. Wheat plant population under waterlogged conditions at sowing and early germination in different tillage systems.

Tillage and sowing system	Wheat plant population (plants m^{-2})
Conventional tillage: broadcast sowing	136a[†]
Chisel tillage: broadcast sowing	142a
Zero tillage: surface seeding	225b

[†] LSD among the rows are designated by letters.
Source: Unpublished field data from Bangladesh (Badaruddin, 1997).

Additional N fertilizer was top-dressed just after the second irrigation, as recommended in Bangladesh (33:0:0).

In contrast to previous years, in which waterlogging resembled "over-watering," in the 1996 season 64 wheat genotypes were subjected to true waterlogging as in a rice field; the field was irrigated consecutively for three days at three growth stages: crown root initiation, booting, and grainfilling. Crop growth was severely affected during this season, and most genotypes did not produce sufficient spikes for sampling to record spikelet/spike, grains/spike and one thousand grain weight (TGW). Some genotypes produced only a few small spikes with hardly any grain.

Twenty-one waterlogging tolerant and twenty waterlogging sensitive wheat genotypes were identified from lines subjected to various modes of waterlogging over the years. As an example, the scores of lines tolerant and sensitive to waterlogging in 1994 are presented in Table 3.

Another experiment with eight waterlogging treatments (including a control) was conducted in central Bangladesh during the 1996 growing season. Soils were heavy 2:1 montmorillanitic. Waterlogging treatments were imposed at 10 (T2), 20 (T3), 30 (T4), 40 (T5), 50 (T6), 60 (T7), and 70 (T8) DAS, which correspond to Zadoks' growth stages 12, 21, 31, 42, 52, 63, and 73. Control was normal irrigation (T1). Water left standing for four days in the treatment plots was considered waterlogging in these soils. The control plot received three normal irrigations. The objective of this experiment was to observe the effect of waterlogging on seed set and yield, as well as to determine which crop growth stages are critically related to poor seed set and yield in wheat under simulated waterlogging conditions compared with the other years and locations.

Table 3. Characteristics of selected waterlogging tolerant and sensitive wheat genotypes grown under varying waterlogging conditions, WRC, Nashipur, Dinajpur, Bangladesh, 1994.

Genotypes	Avg. grain yield (kg ha^{-1})	Avg. TGW (g)	Avg. grains $spikelet^{-1}$	Visual sterility (%)	Leaf yellowing[†] (1-5)	Plant vigor[‡] (1-5)
Waterlogging tolerant						
MOZ-2 (Bangladesh)	4,333	49.8	1.80	0	1	5
BAW-451 (Bangladesh)	4,233	30.1	2.67	0	1	5
BR-16 (Brazil)	3,767	42.1	1.90	17	1	5
IAS58/4/KAL/BB/CJ/3/ALD/5/VEE CM88971-9Y-0M-0Y-3M-0Y	3,700	49.9	1.86	34	3	5
MOZ-1 (Bangladesh)	3,533	49.1	1.64	0	1	5
Waterlogging sensitive						
HD 22629 (India)	1,167	42.6	1.92	68	3	2
BAW-905 = K 9162 (Bangladesh)	1,233	42.8	1.93	12	4	1
K 8962	1,233	38.4	2.20	16	4	1
Aestivum Roelz W9047	1,300	35.3	2.24	0	3	3
FLN/ACC//ANA/3/DOVE CM65720-3Y-1M-1Y-1M	1,367	38.6	1.85	74	3	2

[†] Recorded in the field at 65 DAS using 1 to 5 scale, where 1 = yellowing of lower leaves and 5 = of flag leaves.
[‡] Judged using a 1 to 5 scale at 65 DAS, where 1 = very poor growth and 5 = excellent plant vigor.

There was no significant influence of waterlogging on spikes per unit area (Figure 1), which is consistent with the literature. Grains m^{-2} was used as an indicator of wheat seed set (Meisner et al., 1992). Waterlogging affected seed set. Misra et al. (1992) also reported that waterlogging affected seed set in wheat in Nepal. Seed set was most affected when waterlogging was imposed at 30 DAS (T4), followed by 10 DAS (T2). The highest number of grains m^{-2} was obtained in the control treatment (T1), followed by waterlogging treatments T6, T5, and T8 (Figure 2). Waterlogging stress during Zadoks' 31 was identified as being most critical for seed set in wheat, followed by Zadoks' 12. This is consistent with the data of van Ginkel et al. (1992). The wheat crop was found to be sensitive to waterlogging stress, though to a lesser degree, during Zadoks' 21 and 63.

One thousand grain weight was not affected by imposing waterlogging treatments at different growth stages (Figure 3) in our experiment. Differences in grain weight did not occur because waterlogging treatments were applied at and before anthesis but not from grainfilling onward. Other studies show contrasting results (van Ginkel et al., 1992).

Wheat grain yields differed with waterlogging treatments (Figure 4). Luxmoore et al. (1973) also observed negative effects on wheat grain yields when waterlogging was imposed for 30 days during grainfilling at 15 and 25^{0}C soil temperatures, which reduced grain yield by 20 and 70%, respectively. Waterlogging reduced wheat yields due to poor seed set and fewer spikes per unit area.

Conclusions

Realistic but cautiously optimistic conclusions can be drawn based on the above review of the literature and on data from the case study in Bangladesh. Waterlogging is a widespread problem in the irrigated and high rainfall wheat-growing regions of the world. Despite the breadth of the problem, understanding of the basic soil and plant processes involved in waterlogging tolerance is improving. The good news is that there is genetic variability for waterlogging tolerance within wheat, and that the genetics of waterlogging tolerance appears to be relatively simple, with medium to high heritabilities. Therefore, the prospects are good that varieties suitable for areas suffering from waterlogging stress can be bred and/or identified.

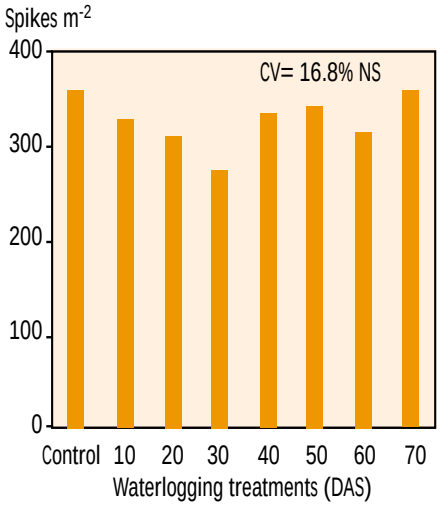


Figure 1. Waterlogging at different growth stages (days after sowing-DAS) affected spikes per unit area in 1995-96 at Joydebpur, Bangladesh.

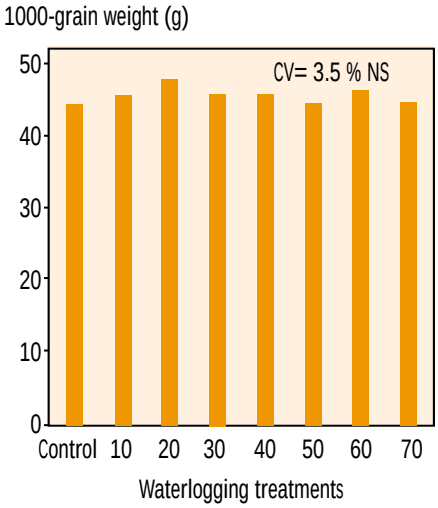


Figure 3. Waterlogging at different growth stages (days after sowing-DAS) affected 1000-grain weight in 1995-96 at Joydebpur, Bangladesh.

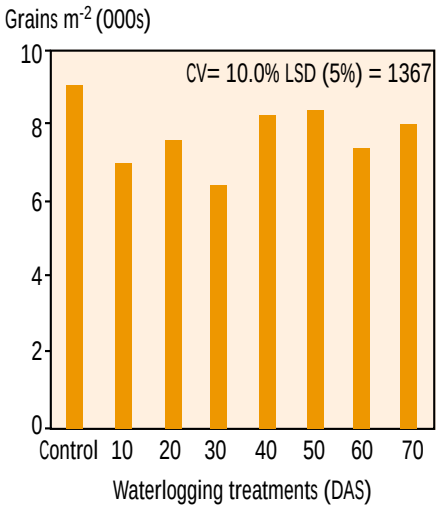


Figure 2. Waterlogging at different growth stages (days after sowing-DAS) affected grains per unit area in 1995-96 at Joydebpur, Bangladesh.

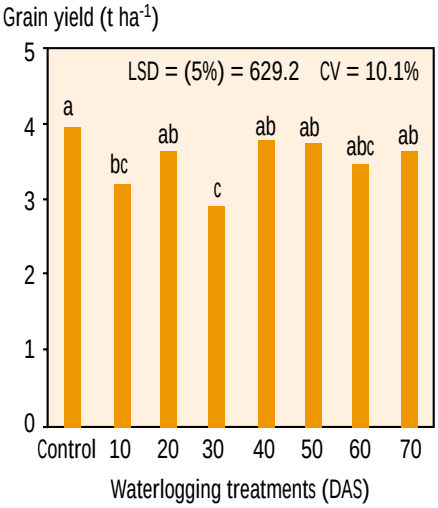


Figure 4. Waterlogging at different growth stages (days after sowing-DAS) affected grain yield in 1995-96 at Joydebpur, Bangladesh.

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CHAPTER 12

Preharvest Sprouting Tolerance

R.M. Trethowan[1]

Rainfall during or just prior to harvest can cause wheat grain to germinate while still on the spike (Figure 1). This phenomenon, called preharvest sprouting (PHS), reduces yield, lowers test weight, and adversely affects the milling and baking quality of harvested grain. Farmers receive lower prices for sprouted grain and, in severe cases, their harvests may be downgraded to animal feed. The occurrence of PHS is generally erratic and as difficult to predict as rainfall in most wheat-growing areas. Researchers have, however, been able to provide farmers in areas prone to PHS with a degree of protection. This chapter attempts to outline the role of physiology and plant breeding in the wider effort to develop strategies to combat this intractable problem.

Extent of the Problem

Rainfall can cause extensive PHS damage in most wheat-growing regions; however, some areas are more prone than others to its occurrence. Although many of these regions are found in developed countries, significant areas of the developing world are also affected. Northern Europe, the Pacific Northwest of the United States, and the wheat growing areas of central Canada and northeastern Australia periodically suffer PHS damage. In developing countries the Southern Cone of South America, encompassing parts of Chile, Argentina, and Brazil, and the wheat-growing areas of eastern Africa are prone to PHS. The damage is more pronounced in regions where white-grained wheat is grown. Red-grained wheat is more tolerant to the problem, since there is an association between grain color and grain dormancy, the primary mechanism of PHS tolerance (Gale, 1989).

Damage Caused

As its name indicates, preharvest sprouting begins before the grain is harvested, while it still on the spike. The process is set in motion by rainfall, during which the seed imbibes water. This causes germination to begin as starch reserves in the grain endosperm are hydrolized through the action of germinative enzymes called amylases. The embryo swells and grows as it consumes the hydrolized carbohydrate reserve.

The test weight and flour milling yield of sprouted grain are considerably lower than those of non-sprouted wheat. Bread produced from sprouted grain has poor loaf volume and crumb structure, and is unsuitable for marketing (Figure 2). The quality of flat breads and chapatis is less affected by the use of sprouted grain, but their texture is nevertheless impaired, resulting in a less favorable product. Sprouted grain causes discoloration of Chinese noodles and spaghetti, lowering the value of these products as well.

Figure 1. Two spikes damaged by pre-harvest sprouting (left) and a sound spike (right).

Figure 2. Bread produced from sprouted grain (left) and from sound grain (right).

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Tolerance Mechanisms

The primary mechanism of PHS tolerance is grain embryo dormancy. Dormant seeds will imbibe water and yet not germinate. Grain dormancy is, however, influenced greatly by environmental conditions prior to and during grain maturation. High temperatures during this period can reduce the expression of dormancy, as can cool conditions following rainfall (Plett and Larter, 1986; Trethowan, 1995). The expression of dormancy is also linked to seed-coat color (Gale, 1989). Red-seeded wheats are generally more dormant than white-seeded types. Crosses between dormant red and non-dormant white-seeded wheat can produce dormant white-seeded progeny; however, the level of dormancy in these progeny is always lower than that of the original red-seeded parent (DePauw and McCaig, 1987). This suggests that the expression of dormancy is governed by epistasis between the seed color and dormancy loci.

The seed coat or husk (in the case of barley) may also influence PHS because of the differences in water permeability (Trethowan et al., 1993). This mechanism involves a physical barrier that keeps water from entering the seed, thereby reducing the effects of PHS following light rains.

Similarly, the bracts or floral structure of the wheat spike may physically impede the entry of water into the grain. The bracts may also chemically inhibit germination through the release of water-soluble inhibitory chemicals (Trethowan et al., 1993). Some evidence suggests that awnless wheat has an advantage under rainfall pressure because awnless spikes shed water quickly (King, 1989). In contrast, awns collect water, thereby maintaining a higher level of humidity in the spike.

The combination of grain dormancy with seed- or bract-based physical or chemical tolerances will greatly enhance the overall tolerance of wheat to PHS.

Screening Methods and Physiological Tools

It is difficult to effectively screen for PHS tolerance in the field because of rainfall variability in most environments. Variable maturity dates characteristic of genetic materials in most plant breeding programs also confound interpretation of dormancy in segregating and advanced lines under naturally occurring rainfall. Rain-simulation facilities have been developed by some researchers to remove the confounding effects of the environment after physiological maturity (Mares, 1989). When using rain simulation, it is critical that all materials be harvested at the same stage of development (harvest ripeness), stabilized at the same moisture content (usually 12%), and stored at low temperatures (-20ºC or lower) prior to evaluation in the rain simulator. Low temperature storage ensures that all enzymatic activity in the seed is halted. Spikes from plants with different maturity dates can then be evaluated together in the rain simulator.

Temperature, humidity, and spike wetting are strictly controlled in the simulator, and spikes are evaluated for visible germination after a fixed number of days. This method is very effective for identifying dormant progeny when dormancy is present in the physiologically mature grain. However, the expression of dormancy at maturity is often suppressed by rainfall during the three weeks prior to maturity. Some researchers use rain shelters during this period to protect field-grown plants from the confounding effects of rainfall (Trethowan, 1995). However, temperature fluctuations during the later stages of grainfilling cannot be controlled in the field.

Rain simulators and rain shelters are effective in controlling some of the environmental factors that influence the expression of grain dormancy; however, such equipment is expensive to build or buy, and many scientists cannot afford it. Grain dormancy, the primary mechanism of tolerance, can be measured more simply by hand-threshing mature grain and measuring germination in a petri dish using filter paper as the water-adsorbent medium. The rate of germination is then compared to that of non-dormant remnant seed germinated in the same way. Differences between the two treatments indicate the possible presence or absence of dormancy. Seed should be washed in a surface sterilant such as 20% chlorox solution and rinsed in distilled water prior to placement in the petri dish (Trethowan et al., 1993). The effect of environmental fluctuations before maturity can be minimized by planting and evaluating the same material on several dates.

CIMMYT's bread wheat breeding program, based in Mexico, uses a field screening technique to evaluate many thousands of lines. Materials are sown in the field during the dry season in January, which causes plants to ripen at the height of the rainy season in July/August. Physiological maturity (PM) is scored, and spikes are harvested from each plot a fixed number of days post PM. Grains are then threshed and evaluated visually for germination. The correlation between this technique and the rain simulator is very high (Trethowan et al., 1996). This methodology is very much dependent upon the stability of the screening environment. The CIMMYT test site is situated at high altitude (2600 masl), and rainfall is a daily event during crop maturation time.

Overhead sprinkler irrigation of field-grown materials may also be effective in inducing high levels of PHS damage (Trethowan et al., 1994). This method has the advantage of providing spike wetting at the most desirable times; however, maintaining effective humidity in the canopy to induce the desired levels of germination post wetting is again a function of the test environment.

Molecular markers linked to grain dormancy are being developed. Once available, these markers will greatly facilitate the development of PHS tolerant wheat. The most obvious benefit will be the ability to track dormancy genes away from the confounding effects of the environment. Furthermore, genetic engineering techniques may in future provide a way of silencing the a-amylase genes, thereby providing an immediate solution to this problem (Gale, 1989). The new "terminator" technology, now subject to a US patent, may also provide a comprehensive solution to the problem of PHS. This technology, still in development, produces non-viable seed through the insertion of a lethal-gene and promotor which causes seed death during late embryogenesis (AgBiotech Reporter, 1998). This method ensures that farmers will return to the seed company each year to buy their seed. The hidden benefit for farmers in sprouting-prone areas is that, regardless of rainfall, the grain will not germinate.

Some researchers use the Hagberg falling number test (AACC, 1983) to measure enzymatic activity in the harvested grain. This test measures the level of starch degradation caused by germinative enzymes and is therefore strongly correlated to PHS. The test is a relatively fast and easy one; however, grain must be harvested in sufficient quantity and milled to produce a minimum of 7 g of flour before the test can be implemented. Other dyed-substrate tests are available which link color change in a starch substrate to amylase activity (Meredith and Pomeranz, 1985). These tests are quick and easy to perform and require grain halves or at most 1 g of ground wheat.

Other tools that can be used to evaluate PHS damage include the Amylograph (Brabender OHG, Germany: AACC Method No. 22-10) and the Rapid Visco Analyzer (Dengate, 1984). These tools are used by the cereal chemist to measure dough and starch pasting properties of harvested wheat. The tests provide the most comprehensive assessments of PHS available; however, they require large quantities of flour, particularly in the case of the Amylograph, and are relatively time-consuming.

Conclusions

The methods for assessing PHS tolerance described in this chapter have a wide range of potential uses. These range from breeders' early generation testing for grain dormancy using germination tests in petri dishes, to the cereal chemist's comprehensive quantification of the effects of sprouting on physical dough properties using the Amylograph. Assessments can be carried out using expensive equipment such as rain simulators and rain shelters, or can be more cheaply conducted through germination tests or dyed-substrate assays for detecting the presence or absence of germinative enzymes. The strong environmental influence on the expression of grain dormancy makes molecular markers a favorable option, particularly when screening for the more subtle variations in dormancy expression found in white-grained wheats.

There are, therefore, a variety of options available to the researcher looking to solve this intractable problem. These options will allow plant breeders to progress towards developing new and better cultivars, regardless of the practical limitations imposed upon them by their current resource base.

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CHAPTER 13

Selection Traits for Improving Yield Potential

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The yield potential of wheat (Yp) is usually defined as the yield of grain produced when the crop has no water or nutrient limitations, nor constraints arising from pests, diseases, weeds, or lodging. It is useful to breed for increased yield potential, as it appears to be a necessary, although perhaps not sufficient, condition in wheat to achieve superior on-farm yields under many less favorable conditions, even where water or nitrogen stress levels reduce yield to as little as 30% of Yp. Besides, growing genotypes under optimal conditions tends to maximize genetic variance for many traits and minimize error variance relative to genetic variance, such that broad sense heritability is maximized and selection facilitated.

This chapter will focus on Yp in spring wheats autumn-sown at low latitudes, the conditions under which most wheat in the developing world is grown. It will briefly look at the causal basis of variation for Yp in such wheat from the environmental point of view and, more thoroughly, from the genetic one. It will then cover traits associated with Yp that are potential indirect selection criteria, and discuss how they could be measured or subjected to screening. Because of our poor understanding of past Yp progress, it is unlikely all traits of relevance to Yp will be covered and unwise to automatically extrapolate relationships between a trait and higher Yp levels. Nevertheless, at least to the extent that future breeding populations will resemble those studied to date, traits with strong associations to Yp should be good candidates for culling-type selection designed to maintain, if not increase, Yp.

Environment and Yield Potential

By definition, Yp is determined by the genotype interacting with its environment—in this case, the environment as determined by radiation, temperature, and photoperiod. Other environmental factors, such as wind, vapor pressure deficit of the air, atmospheric pollution, and subtle soil characteristics, are beyond the control of the agronomist attempting to optimize the crop environment, and may have smaller effects. Despite this, numerous experiments involving multiple locations or environmental manipulation at a given location indicate that wheat Yp is positively related to daily solar radiation, negatively and strongly related to daily mean temperature, and negatively and less strongly related to photoperiod length.

The solar radiation effect appears to arise because dry matter accumulation rate in the crop is a close-to-linear function of the radiation intercepted by the crop canopy (for much of the crop cycle under optimal conditions, the canopy is large enough to intercept essentially all [> 95%] of the incident radiation). The second effect appears to arise because higher temperature and longer days speed development and reduce crop duration (for vernalization responsive wheats this situation is somewhat more complex, since higher temperatures may actually delay early development).

Experiments also show that Yp is more sensitive to growth and development effects at certain stages of development. In particular, the 20-30 days (depending on temperature) leading up to and just after anthesis are critical for effects on the number of kernels (KNO, kernels or grains m^{-2}), while the grainfilling period is critical for effects on kernel weight (KW, individual kernel or grain mass, mg). Overall for environmental effects on Yp, the period before and just after anthesis and associated KNO variation, is generally more critical than the grainfilling period and KW variation.

Various other aspects of radiation (proportion direct and diffuse, sun angle, maximum intensity) and temperature (daily and absolute maximum and minimum, diurnal range, frost) may have additional but smaller effects on Yp.

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There are no reports of direct yield response to either high wind or vapor pressure deficit, but negative responses have been suggested. Atmospheric pollution—ozone, in particular—can reduce yield. Anoxia associated with transient waterlogging and, perhaps, flood irrigation, and high mechanical impedance could have subtle negative effects on yield despite apparent optimal soil management.

All these external factors affecting Yp vary substantially and, to some extent, independently in the world's wheat-growing locations (representing combinations of latitude, longitude, and altitude). They can also vary at the same location because of year and sowing date effects. Experience over many years has usually led to identification at each location of the optimum flowering or anthesis date, and, accordingly, the optimum sowing date. Nevertheless, the climate varies sufficiently that Yp with the current best-adapted cultivar planted on the optimum sowing date ranges from 5 to 15 t/ha across locations, and at least 30% across any 5-year period at any location. As a rule of thumb, Yp (in t/ha at 10% moisture) with today's best cultivars is given by:

$$Yp = 8 + 4PTQ - 0.15T - 0.07\ PTQ*T \qquad (1)$$

In equation (1), PTQ is the mean daily solar radiation (MJ m^{-2}) divided by the mean daily temperature in ºC minus 4.5 ºC during the 30-day period up to the end of anthesis (usually between 1 and 2 MJ m^{-2} $ºC^{-1}$), and T is the mean daily temperature during grainfilling (usually 15-22 ºC). This is derived from relationships in Sayre et al. (1997).

Genotype and Yield Potential

Genetic variation for Yp at a particular location and, especially, historic progress in Yp must reflect genetic variation for the efficiency with which the crop interacts with the external yield-controlling factors discussed above. Curiously, however, such reasoning has not usually facilitated the identification of genotypic traits associated with high Yp. Traditionally, crop scientists have sought to understand yield progress through the trait changes seen in historic sets of cultivars exhibiting yield progress due to breeding, a so-called retrospective approach. From this point of view, Feil (1992) and Slafer et al. (1994) have thoroughly reviewed genetic improvement of yield potential of small grain cereals and wheat, respectively. A second, somewhat distinct approach has been to predict from physiological understanding the ideal plant type for maximum yield and then to construct this plant in order to test and hopefully validate the physiological hypothesis (Donald 1968). A variation on this approach identifies single traits, or ideotraits, for improvement which are amenable to testing through the development of near-isogenic lines. Donald's ideotype approach was reviewed by Sedgley (1991) and Marshall (1991), while Austin (1994) gives a more recent, physiological view of designing crops for higher yield. The results of both retrospective and ideotype studies will therefore be reviewed only briefly here. The reader is directed to Reynolds et al. (1996, 1999) for additional and recent detail on Yp in wheat, and to Cassman (1994) for a useful consideration of the parallel situation in rice.

Crop phenology

The above comments on the identification of optimum flowering dates notwithstanding, the retrospective approach has sometimes revealed shifts in crop phenology associated with yield improvement. In the case of wheat, modern varieties have tended to have a reduced germination-to-anthesis period (AD in days), although some recent CIMMYT cultivars appear to be reversing this trend. Of course, a shorter germination-to-anthesis period may be desirable from a cropping systems perspective, even if Yp does not increase. Variation among the phenological sub-periods within the germination-to-anthesis period has not been studied in this context, but could be a significant determinant of Yp (Fischer, 1996). Durum wheats and triticales appear to have a longer post-anthesis period, and some calculations of Yp suggest that in bread wheat, yield cannot increase substantially without an extension of this period. Phasic development is discussed in more detail in the chapter by Slafer.

Reduced stature and harvest index. Many studies have shown that in wheat the use of major dwarfing genes, most notably those from Norin-10, has brought about significant and largely unanticipated increases in Yp, quite independently of the increase in lodging resistance. This is reflected in an increase in harvest index (HI, % of final grain biomass) but no change in final biomass. In wheat, reduced height due to minor genes also appears to increase harvest index and Yp, a process that has been exploited by breeders throughout this century.

However, in the last 20 years, we seem to have arrived at the optimum plant height (70-100 cm) for maximum Yp, below which biomass decreases faster than harvest index increases (Figure 1)

(Fischer and Quail, 1990; Miralles and Slafer, 1995). There are reports of yield increases associated with greater biomass (e.g., Waddington et al., 1986), but the most recent study of CIMMYT progress confirms the importance of HI increase, even in the absence of height reduction (Sayre et al., 1997). Curiously, HI does not appear to have increased much in temperate maize, despite large genetic gains in yield (Tollenaar, 1994).

Kernel number per square meter. Yield progress in cereals has been strongly associated with increased KNO, and wheat is no exception. In wheat this appears to be directly related to the reduction in stature due to major dwarfing genes and, probably, to minor genes as well. Increased KNO is likely due to reduced competition between the growing spike and the growing stem in the few weeks preceding anthesis and, hence, greater partitioning of assimilate to the spikes (Fischer and Stockman, 1986; Slafer et al., 1990). Consequently, shorter wheats have a greater portion of their anthesis biomass invested in spikes, which can be defined as the spike index at anthesis (SIA), and higher spike weight (g m^{-2}) at anthesis.

With a similar number of competent florets and kernels per unit of spike weight at anthesis (Fischer, 1983), short wheats have a greater KNO because of the extra spike weight. These extra kernels can be filled apparently because KW has remained unchanged or has decreased only slightly with progress. The approach to understanding KNO determination outlined here is taken from Fischer (1983, 1985), who points out that crop dry matter accumulation during the critical spike growth period leading up to flowering (given by the product of crop growth rate [CGR, g m^{-2} d^{-1}] and duration of this period) is another major determinant of KNO variation.

However, CGR appears to differ little among wheat cultivars (Calderini et al., 1997; Abbate et al., 1998; Fischer et al., 1998), nor does duration of the spike growth period appear to show cultivar variation (Fischer, unpublished data; Abbate et al., 1998). Although differences in seedling vigor are noted in wheat cultivars and can affect early CGR, they are not relevant to CGR during the critical spike growth period because under optimal conditions all cultivars reach full interception of incident radiation before its onset.

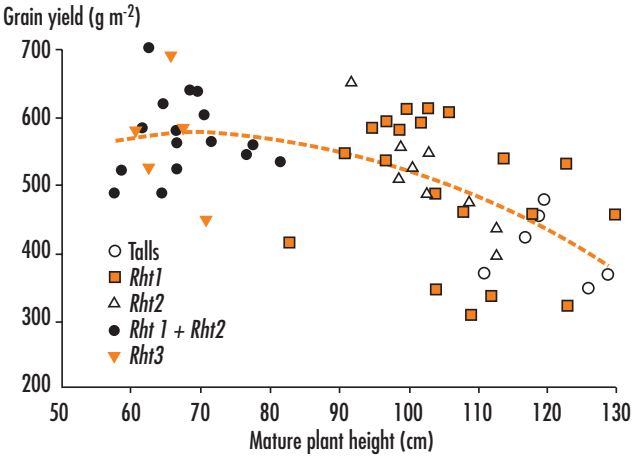

Figure 1. Relationship between grain yield and mature plant height.†
Source: Fischer and Quail (1990).
† The line is the quadratic fit between grain yield (Y) and height (X) ignoring dwarfing gene class: $Y = 290 + 8.2X - 0.058X^2$ ($R^2 = 0.384$).

The continuing increases in KNO and, to some extent, HI in the most recent short cultivars, where variation in stature probably does not appear to be involved, have not been elucidated in detail. However, there is some evidence that other factors besides SIA are involved. This is clearly the case in modern CIMMYT spring durum wheats, where spike sterility is a significant factor in Yp variation. In other words, there is significant variation in grains per unit spike weight as reflected in the grain set index (GSI = % of the two basal florets in the 10 central spikelets that bear grains). GSI appears to exceed 95% in modern bread wheats studied (in the absence of such subtle factors as frost or boron deficiency), but can be as low as 50% in early dwarf durums and 80% in the most modern ones (Fischer, unpublished data).

In a second example, Abbate et al. (1998) show that the most recent Argentinian wheat cultivars have a higher KNO because they produce more kernels per unit of spike dry weight at flowering. This trait, which would combine partitioning within the growing spike, floret survival, and grain setting and survival (GSI), merits further consideration.

Numerical components of yield. A numerical approach to understanding genetic progress in KNO (and Yp itself) has often been attempted but usually without success in wheat. Variation in spikes m^{-2}, spikelets per spike, and grains per spikelet and per spike forms the basis of this approach. However, there appears to be no preferred route to higher KNO among these components, and selection for any one of them alone invariably leads to compensation in the others, as might be expected if some other factor, such as dry matter supply, is what ultimately limits KNO. Studies purporting to have found relations of yield with spikes m^{-2} have often involved low planting densities or

small plots, which favor spreading, high-tillering types; this is not observed in a crop community where plants compete intensely for light resources most of the time.

Although kernel weight is the last yield component to be determined, it is also subject to compensatory effects, with a strong tendency toward negative genotypic correlations between KNO and KW in unselected materials. This tendency has been countered by breeders to the extent that yield progress is associated with no significant change in KW. Not surprisingly, KW is more sensitive in modern wheats than older ones to source manipulation during grainfilling (Fischer and HilleRisLambers, 1978; Kruk et al., 1997).

Physiological activities

As field instrumentation has improved, a number of retrospective studies have attempted to look directly at physiological activities. A good example was the study of recent Yp progress in eight CIMMYT bread wheats (Fischer et al., 1998). Results revealed that yield progress across these short wheats released between 1962 and 1988 was closely associated with increased stomatal conductance (g_s in mmoles m^{-2} s^{-1}) and significantly associated with increased maximum leaf photosynthetic rate (maximum photosynthetic activity, or Amax, in μmoles $m^{-2}s^{-1}$). In addition, increased yield was found to be associated with cooler canopies (CTD = canopy temperature depression, ^{0}C), which could be predicted from the changes in g_s, and with increased grain C_{13} content (D in ^{o}%), which is predicted by the g_s and Amax changes. Over the 26-year breeding period, yield increased 27%, g_s 63%, and Amax 23%, while CTD fell 0.6 ^{0}C. Leaf greenness or chlorophyll also tended to increase, but this was not necessarily related to enhanced stay-green, which was not measured in this study. Enhanced stay-green is, however, clearly a feature of modern maize hybrids (Tollenaar, 1994).

Other studies conducted on modern spring wheat cultivars tend to corroborate various aspects of these remarkable results (Condon et al.,1987; Blum, 1990; Araus et al., 1993; Reynolds et al., 1994a; Amani et al., 1996; Reynolds et al., 2000). A recent analysis of residual samples from the same eight bread wheat cultivars showed a close relationship between yield progress and flag leaf O_{18} isotope content, in agreement with a theory based on the above-mentioned changes in g_s (Barbour et al., 2000). Chlorophyll fluorescence has been used to indicate the status of the leaf photosynthetic system but apparently has not been applied to study yield progress in wheat.

Other physiological activities that have been examined include respiration and translocation and, at a deeper level, enzyme levels. Respiration is a major component of the plant's carbon balance, and there is some suggestion that its efficiency can been changed by breeding (see Austin, 1994), but not in wheat. Translocation is a critical process little studied for cultivar differences, although there have been comparisons of the contribution to grainfilling by translocation from pre-anthesis reserves. However, this contribution, as a proportion of grain yield, is low under optimal conditions.

Biochemical studies of yield progress have not received much attention since the excitement 25 years ago surrounding nitrate reductase level as a key determinant of yield. Recently, oxygen-radical-scavenging enzymes in the leaf have been implicated in improved photosynthetic activity and yield progress in maize (Tollenaar, 1994). Despite the dearth of information on changes in enzyme activities, identification of desirable alterations in enzymes is undoubtedly an essential first step to genetic engineering for higher Yp.

Morphological traits

The second major approach to yield progress—namely, ideotype postulation—tends to focus on visual morphological traits. In his famous paper on the subject, Donald (1968) emphasized the design of "communal" ideotypes to reduce interplant competition and thereby transfer resources used in competition to enhance yield (Figure 2). His wheat ideotype for high yield had a single short culm, with small erect leaves and a large awned spike, giving a high harvest index. While the extreme non-tillering uniculm plant type, which does exist in wheat, has received little support from others and has not been extensively tested, a case can be made, based on the poor survival rate of later tillers in a normal, well-managed crop, for a reduced tillering

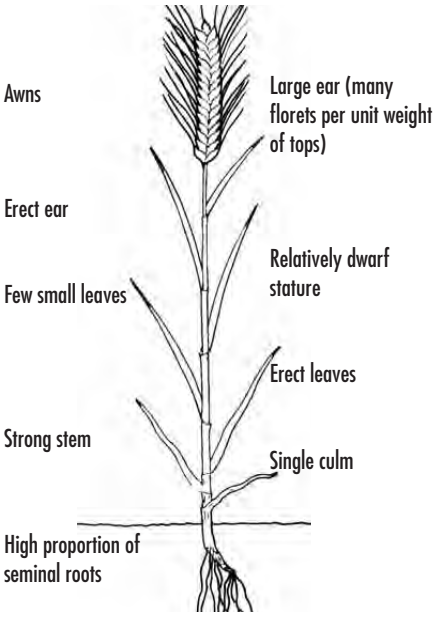

Figure 2. Design for a wheat ideotype.
Source: Adapted from Donald (1968).

type. Although to date there is no strong evidence to support this concept, Reynolds et al. (1994b) noted Yp associations with some indices of apparent "communalism."

There is evidence that modern cultivars have more erect leaves (Feil, 1992; Tollenaar, 1994). Explicit studies on the benefits of erect leaves in wheat and other crops, however, have produced variable results (Evans, 1993; Araus et al., 1993), despite models indicating higher canopy photosynthesis with erectophile canopies (provided there is full light interception and sun elevation is relatively high). CIMMYT has been involved in several of these efforts (CIMMYT 1978a, 1978b; Vanavichit 1990; Araus et al., 1993). As for leaf size, a case can be made for the desirability of smaller later leaves under optimal conditions (Fischer, 1996), but the trait has not been tested. Awnedness may be unnecessary or even undesirable under optimal conditions, but there is no clear consensus on this either (Evans, 1993). Near isogenic lines and populations have often been used to investigate the importance of these morphological traits. Dwarfing has already been discussed and is essential for high Yp.

Promising Putative Yp Selection Traits

The preceding discussion does not offer a very convincing picture of our understanding of the physiological basis of Yp improvement and of traits strongly correlated with Yp, yet such a correlation is surely a prerequisite for an indirect yield selection trait. Nevertheless, there are some promising traits that need further study or that may be useful if examined in combination, for to date little has been done or said about possible interactions between traits. Most traits (apart from phenological ones) mentioned in the above discussion of Yp determination are listed in Table 1.

For a trait to be useful, not only must it show significant genetic correlation with yield, but it must also have a reasonable heritability (rated separately for each trait in Table 1), the level of uncertainty notwithstanding. Not rated, but important, is the likely amount of desirable genetic variability (in the yield positive direction); this is assumed to be present in all cases. Table 1 does include two other important features: a relative assessment of the cost of measuring the trait and a comment on whether genotypic differences in the trait can be measured in a spaced planting, typical of early generations but atypical of crops. This latter issue is important and fully discussed in Donald (1981). Key references to the traits shown in Table 1 are given throughout the text.

Lest Table 1 give too optimistic a picture of the value of indirect selection criteria, an example of seemingly promising traits needs to be considered. Syme (1972), measuring many traits on well-watered spaced plants in a glasshouse, showed that three traits—namely, harvest index, rate of leaf production, and kernel weight—predicted with high accuracy (r^2 = 0.785) the mean yields of the 49 entries in CIMMYT's 5th International Spring Wheat Yield Nursery (ISWYN) across 63 sites all over the world. Favoring this remarkable result is the fact that cultivar mean yields in the ISWYN showed a 2-fold range and were strongly influenced in those early years by three traits (days to ear emergence, height, and kernel weight), all of which were accurately expressed in the glasshouse. In fact, the single glasshouse trait explaining most ISWYN mean yield variation was harvest index (r^2 = 0.719) for it was influenced by both height and maturity.

Needless to say, no one today selects explicitly for harvest index, and the challenge facing breeders is much more difficult. First, genotypes cluster within height and maturity categories that have broadly optimal values for lower latitude locations; thus cultivar mean yield varies much less. Second, the breeder seeks increased yield across years at a location or group of similar locations, and this is probably a more difficult target to predict. Third, the Syme (1972) study was done with fixed cultivars. An attempt to repeat the study dealing with all three of these weaknesses (Quail et al., 1989) was not very successful, but is commended to the reader as an example of a comprehensive test of indirect selection traits for Yp. The review by Bhatt (1980) dealing with problems and opportunities surrounding indirect selection for yield in wheat and that by Austin (1993) are also suggested.

Description of Trait and Screening Methods

Harvest index

Further rationale supporting harvest index as a selection criterion is given by Donald and Hamblin (1976) and Fischer and Kertesz (1976); both studies emphasize the fact that HI can be assessed in spaced plants. Many subsequent studies have tested HI selection in segregating populations with variable effects on grain yield (e.g., Whan et al., 1981; Naas, 1983; Ellison et al., 1985). Some have limited relevance

Table 1. Putative indirect selection criteria for yield potential, their association with yield potential, their heritability, the possibility of measurement on spaced plants, and the cost of measurement. The target of trait selection, yield potential itself, is included for reference.

Trait	Correlation with yield potential (Yp)	Heritability	Can be measured on spaced plant	Cost to characterize genotype	Comment
Growth and partitioning					
Crop growth rate (CGR)	Zero in current material	unknown	no	very high	Refers to CGR during the critical spike growth phase
Harvest index (HI)	moderate-high	low-moderate	yes	high	Integrates many components
Spike index at anthesis (SIA)	moderate	unknown	yes	very high	Integrates pre-anthesis partitioning to spikes
Kernels per unit spike weight (KPSDW)	moderate	unknown	unknown	very high	Integrates partitioning within the spike, floret survival, and grain setting and survival
Leaf activity					
Stomatal conductance (g)	moderate	moderate	yes	high	May be integrative trait, reflecting inter alia sink strength
Leaf resistance to air flow (LR)	moderate	moderate	yes	moderate	Indirect measure of g; fast, robust, low-cost instrument
Canopy temp. depression(CTD)	low- moderate	unknown	no	low-moderate	Indirect measure of g; possible to reduce cost via airborne IRT
Oxygen 18 discrimination (DO_{18})	moderate	unknown	probably yes	high	Indirect measure of g but needs mass spectrometer
Photosynthetic activity (Amax)	low- moderate	low	unknown	high	Expensive instrument
Chlorophyll fluorescence	low- moderate	moderate	probably yes	high	Expensive instrument
Carbon 13 discrimination (DC_{13})	low- moderate	moderate	yes	high	Measure of –Amax/g; needs mass spectrometer
Leaf greenness (SPAD) content; associated Amax	low	unknown	probably yes	low	Measure of aerial leaf chlorophyll and N
Specific leaf weight (SLW)	low	low- moderate	unknown	low	
Yield components					
Tiller number m^{-2} (TNO)	zero	moderate-high	yes	low	Refers to maximum tiller number around stem elongation
Spike number^{-2} (SNO)	zero-low	low- moderate	no	moderate	
Spikelets per spike	zero	moderate-high	yes	low	No. of spikelet positions largely unaffected by competition
Grains per spike (GPS)	zero-low	moderate	no	low-moderate	
Grains per spikelet	zero-low	unknown	no	low	
Grain set index (GSI)	unknown, maybe moderate in durum	unknown	unknown	low-moderate	Refers to grains formed in competent florets, namely basal ones
Kernel weight (KW)	zero	high	yes	low	
Kernel number m^{-2} (KNO)	high	low- moderate	no	high	Usually calculated from yield
Morphology					
Mature plant height (HT)	low in range 70-100 cm	very high	yes	low	
Leaf erectness	unknown-low	moderate-low	yes	low- moderate	
Leaf size	unknown	moderate	yes	low	
Awnedness	unknown	very high	yes	very low	
Yield potential	very high	low	no	high	Even Yp cannot be measured without error

because yield was not measured under optimal conditions or in plots large enough to avoid significant bias. These topics are discussed thoroughly in Quail et al. (1989), who themselves found selection for reduced stature in F3 to be somewhat more efficient than selection for high HI, but both were far superior to selection based on yield per F3 plant. Few subsequent studies appear to have been done, but the fact remains that no cultivar with a high Yp has an HI below 40% (e.g., Sayre et al., 1997).

Harvest index is a simple measure involving cutting at ground level, bundling, drying, weighing, threshing, and weighing the grain. The resources needed to obtain an HI could be reduced by mechanizing cutting and binding. Also, allowing plants to dry while standing in the field would reduce the procedure to cutting, weighing, threshing, and weighing, all in rapid succession, possibly in the field. A modification used in rainless northwestern Mexico is to cut and allow the bundle to dry in the field in the hot sun; a few days are necessary to bring materials to a constant moisture content. A similar modification is to leave the bundle in the field in a rainproof metal container (e.g., a disused sea container), which serves as a cheap field dryer.

With open-air, field drying of bundles from genetically identical, single-spaced plants in Mexico, the standard deviation of plant HI was 4%, and the time invested, 5 person-minutes per plant (R.A. Fischer, unpublished data). If artificial drying is necessary, simply taking one or a few central culms from the plant (or plot) may be more efficient. Fischer and Kertesz (1976) showed that main culm HI actually gave a better prediction of Yp than whole plant HI.

Recently A. van Herwaarden (pers. comm.) has suggested a simple, rapid, indirect measure of harvest index. It is based on the relationship between the harvest index of a bundle of mature spike-bearing culms and the balance point of the bundle. It warrants further investigation.

Partitioning at anthesis

Quail et al. (1989) failed to demonstrate spike index at anthesis as a selection criterion, but it showed some promise in a later study by Siddique and Whan (1994). High SIA would seem to be an excellent index of efficient partitioning. It is measured by cutting, drying (artificial is essential), and weighing. Unfortunately, the procedure is destructive, so it can only be done on some culms in a spaced plant. A certain amount of time is necessary, not so much for the procedure, but for visiting the field to judge when each particular genotype has reached an appropriate sampling stage, since the ratio changes with the stage of development. The first appearance of anthesis in a culm is probably the most convenient sampling date.[2]

Measuring kernels per unit spike dry weight is even more time consuming because matched culms or plants must be sampled, one at anthesis and one later, when the number of kernels can be determined. Because of this, measuring kernels per unit spike dry weight is likely to be done only during detailed physiological studies. It is possible, however, to determine the number of kernels per unit chaff weight by sampling spikes at maturity, weighing, threshing, weighing (grain), and counting. Chaff weight at maturity exceeds spike weight at anthesis.

Although this apparently reflects source-sink balance and mineral accumulation in the transpiring spike, ranking for kernels per unit spike dry weight determined this way may be unaffected. It is also evident that the potential or actual total grain weight per unit spike weight at anthesis (or chaff weight at maturity) is an important efficiency index: modern awned cultivars appear to have a potential grain yield of about 4 times the spike weight at anthesis (Fischer and HilleRisLambers, 1978). Clearly these partitioning traits, fundamental to genetic variation in sink size, deserve further study, but not perhaps in a selection study at this stage.

One component that reflects some of these events at anthesis and that can be quickly noted at maturity is grain set index. Grains missing from the two basal florets of the 10 central spikelets can be counted in five random spikes; these florets are always competent at anthesis (Figure 3). A total of more than 10 missing would mean greater than 10%

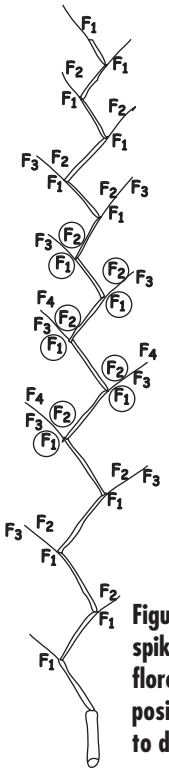

Figure 3. Schematic wheat spike showing competent florets (F_1, F_2, F_3, F_4) and floret positions to be counted (circled) to determine grain set.

[2] For detailed physiological studies, a somewhat later sampling date, when the spike has finished growing, but before the grains gain significant weight, would seem more appropriate. However, grain weight increases rapidly as a % of spike weight, and the sampling window is very narrow.

floret sterility, and would likely indicate grain-setting problems and inefficiencies incompatible with achieving maximum Yp.

Leaf activity traits

Stomatal conductance (g_s), maximum photosynthetic activity (Amax), chlorophyll fluorescence**,** and leaf content traits (leaf chlorophyll, chlorophyll a:b ratio, leaf N, specific leaf dry weight per unit area) may be physiologically related. Leaf resistance to air flow (LR), canopy temperature depression (CTD), and O_{18} discrimination (DO18) are good indirect measures of g_s; C_{13} isotope discrimination (DC13) measures the balance between g and Amax, and SPAD indirectly measures leaf chlorophyll and N content. Chlorophyll fluorescence appears to measure metabolic imbalance in photosynthesis (Araus et al., 1998). This discussion will focus on direct and indirect measurement of g_s and leaf contents. Although Amax and chlorophyll fluorescence determination may contribute additional information, they are not considered further because of their high cost. However, the reader is referred to Gutierrez-Rodriguez (2000) where almost all these techniques were tested as selection criteria in F_5 progeny lines. Carbon isotope determination is also expensive, but is being used in some special breeding programs (e.g., Rebetzke et al., 2001). It has the advantage that materials (usually dry grain, sometimes dry plant parts) can be drawn from more than one plant, and thoroughly mixed before subsampling, grinding appropriately, and measuring in a mass spectrometer. Also, materials can stored for later determination.

Instruments used to measure leaf activity traits

Diffusion porometer. This instrument, well described by McDermitt (1990), is used to determine g_s but may measure only one leaf surface or both surfaces together. In the first case, separate measurement of the other surface is needed to give total leaf conductance. In wheat the abaxial surface (which at leaf insertion to the stem faces away from the shoot axis) usually has a lower g_s than the adaxial surface, but there is evidence (Rawson et al., 1976; Condon et al., 1987) that it is more sensitive to the physiological status of the leaf and plant, and perhaps to yield-determining factors. For this reason, it is possible that measuring only abaxial g_s is a more efficient use of time than measuring both abaxial and adaxial g_s.

Commonly a central sunlit portion of the uppermost fully expanded leaf is chosen for measuring. After a few weeks before flowering, this is always the flag leaf. Within this defined class, there is still obvious variation—for example, depending on which surface is sunlit and on the angle of incidence of the solar beam. Stomata respond rapidly to such factors as shade, touch, and elevated CO_2, so each measurement must be executed quickly. It is usual to measure several leaves to characterize a plant or plot, but the sampling strategy needs careful consideration.

Because stomata are so sensitive, in the field they appear to be changing continuously in response to subtle weather factors. Thus when comparing genotypes, it is more efficient to keep the number of leaves sampled per genotype per block down to a low number, even as low as one, so that the block is completed quickly (see also Clarke and Clarke, 1996). If greater accuracy is needed, measuring across the block can be repeated. Assuming that stomata in all genotypes respond similarly to subtle weather variations, this strategy minimizes the error due to temporal changes, which often appears to be a much greater source of measurement error than spatial variation within and between plots. Thus speed is important for determining g_s and not only for cost-saving purposes.

In this regard, diffusion porometers have improved so much that today a single reading can be completed in 30 seconds. However, for rapidity of measurement it is the viscous airflow porometer (for LR) and the infrared thermometer (for CTD) which excel.

Viscous airflow porometer. A recently-designed, hand-held airflow porometer with electronic timing takes less than 5 seconds per leaf (Rebetzke et al., 2000a). The airflow porometer times how long a given amount of air takes to pass through the leaf from one side to the other under pressure, giving a resistance measure (LR) in hundredths of a second that is proportional to the reciprocal of air permeability or porosity. The major resistance to this air movement is not the leaf air spaces but the stomatal pores on each surface, which act as resistance in series to air flow.

Theory suggests that diffusive conductance is related to porosity by a power function, so that leaf g_s will be linearly related to –log LR (Fischer et al., 1977; A.G. Condon, pers. comm.). Rebetzke et al. (2000) found that a linear relationship between g_s and 1/LR was adequate over the range of leaf conductances (200-1000 mmoles m^{-2} s^{-1}) that they encountered. This will be an imperfect relationship. LR measures the two surfaces in series and is dominated by the least open abaxial surface, whereas for leaf g_s the surfaces are

in parallel and the more open adaxial surface dominates. This, however, could favor LR over g as a yield predictor.

Early work at CIMMYT suggests that LR can be determined on spaced plants (Fischer et al., 1981), and Wall (CIMMYT, 1979) found leaf porosity to be the most useful selection criterion in F2 spaced plants, far superior to yield per plant or visual plant score. A follow-up selection study in Australia was unable to confirm this promise for LR (Quail et al., 1989), but recently Fischer et al. (1998) found that yield progress in modern short Mexican cultivars (1962 to 1988) is closely related to decreased LR (and increased g_s). The latter study also revealed how sensitive this relationship was to the conditions under which LR and g_s were determined. This sensitivity could not be related to irrigation timing, stage of development, or obvious weather parameters. It may explain why earlier selection studies had variable success with selection based on stomatal conductance, and it demands further study, with emphasis on careful monitoring of the environment. The fast instrument described has already facilitated determination of heritability in progeny populations (Rebetzke et al., 2000).

Infrared thermometer. Using the infrared thermometer for measuring canopy temperature as a surrogate for g_s represented a further step forward in measurement speed and efficiency (Hatfield, 1990). Canopy temperature is best expressed as CTD, i.e., the extent to which the canopy is cooler than the air, as it almost always is in irrigated wheat. Certain infrared thermometers measure CTD directly, and all thermometers determine the temperature averaged across a view of leaves, several 100 cm^2 or more in area, depending on distance from the plot. The temperature reading is obtained in less than a second and with an accuracy of about 0.1 ^{0}C.

Although the relationship of CTD to g_s is well validated in theory and practice, and the study of modern Mexican cultivars revealed a relationship between this trait and yield, the relationship is even more capricious on a daily basis than that between g_s and yield (Amani et al., 1996; Fischer et al., 1998). These studies also revealed that CTD ranking was not noticeably affected by the appearance of spikes at the top of the crop canopy and, hence, in the instrument's field of view, their temperature presumably being little different from that of leaves.

Recent studies at CIMMYT suggest that indirect yield selection via CTD selection in segregating populations has promise (Reynolds et al., 1998). Infrared imagery can be collected from airplanes so that whole nurseries could be measured in the few seconds the plane flies over (Reynolds et al., 1999). Difficulties have to be resolved with regard to angle of view, plot sampling, and processing the vast amount of canopy temperature data that could be collected from an airborne platform, but they are not insurmountable. With ready access to such a system, data could be collected very inexpensively on genotypes in any plot larger than about 1 m^2. However, we need to understand the conditions that determine when canopy temperature measurements give their strongest relationships with yield, because a fly-over with an airborne platform has high fixed costs.

SPAD meter. This robust and relatively inexpensive field instrument rapidly and non-destructively measures leaf greenness. Leaf greenness provides an indirect estimate of leaf chlorophyll and N content, although the relationships can be affected by independent variation in other leaf features, such as specific leaf weight (Peng et al., 1993), and by stage of development especially after flowering when SPAD reading decreases with time. It might be expected that the SPAD reading is related to certain leaf activities, and that successive readings would provide a simple way of quantifying the stay-green trait.

In modern Mexican bread wheats, SPAD reading, despite significant cultivar by year interaction, was correlated with aspects of Amax (Fischer et al., 1998), while in durum wheats, it was clearly correlated with both Amax and yield progress (CIMMYT, unpublished). Also related to leaf chlorophyll are observations that high Amax associated with adaptation to high radiation environments is related to a high chlorophyll a : chlorophyll b ratio (Austin, 1994). Unfortunately, there is no quick way of screening for a:b ratio, but the SPAD meter and leaf greenness warrant testing in selection studies. If SPAD readings prove useful, it is possible that the meter could be used to guide an even faster visual rating system.

Plant height

Plant stature or height is easy to measure or score upon termination of stem elongation, soon after anthesis. Rules need to be made regarding its definition (height to the spike tip is most commonly used) and to deal with the variability between individual culms. Recent results have tended to suggest that the actual genes (various major and many minor ones) conferring reduced stature are not as critical as the stature itself. Nevertheless, the presence of some major genes can be detected at the seedling stage fairly readily and non-destructively through the absence of an elongation response to gibberellic acid (Gale and Gregory, 1977).

Leaf angle

The critical aspect of leaf angle is the angle that the lamina makes with the sun's rays, but this changes throughout the day. Usually it is leaf angle with respect to an upwards vertical that is measured; using the vertical is probably easier than the horizontal because, after stem elongation commences, the emerging leaves and then the stem itself provide a vertical axis against which angle can be measured. When the leaf lamina is unbent, the angle is that at its point of insertion on the stem; this angle could be as low as 20^0 for a very erect genotype. When leaves bend and twist, as is common soon after their emergence, the situation is much more complex, and direct measurement is very tedious. An indirect measure is given by calculating the extinction coefficient (k) from measuring the leaf area index (LAI) and the fraction of light intercepted by the canopy (I/I_0):

$$k = (\ln(I_0/I))/LAI \qquad (2)$$

The coefficient k can be shown to be largely determined by average leaf angle, since erect wheat canopies have values as low as 0.3. This means that an LAI of 10 is needed for 95% light interception by leaves. In selection studies it would seem that the only feasible option is to visually score leaf angle, perhaps guided by initial measurement of angles or determination of k, and/or the preparation of photographic standards. Leaf angle in particular needs to be assessed preferably in plots under optimal growing conditions where the contrasts between truly erectophile and planophile canopies are most obvious. The trait should be determined when it is most critical to performance, usually in the period after full light interception and before anthesis. Visual scoring was adopted during a major effort to develop and test leaf angle isogenic populations at CIMMYT (see CIMMYT, 1978a).

Leaf size

In contrast to leaf angle, leaf size is easier to measure, and non-destructive measurements at a defined leaf position are usually adopted. Phenotypic correlations between leaves at different positions on the main stem are high but not perfect, and the flag leaf area may differ notably from earlier leaves (Rawson et al., 1983). Mechanical considerations would seem to suggest that short (and small) leaves are a necessary but insufficient condition for erect leaf lamina. Independently of lamina angle, small leaves, because of greater penumbral effects, tend to have the same beneficial effect theoretically with respect to light distribution as erect ones.

Other morphological aspects (awnedness, tillering) that have been mentioned can be readily assessed visually in spaced plants. However, the reduced tillering trait is quite sensitive to environmental conditions; consequently, they should be controlled as much as possible.

Conclusion

The application of indirect selection criteria in wheat breeding is a broad area of endeavor and littered with unsatisfactory results in the past. Indirect selection for Yp may be easier (by definition, fewer environmental constraints, no disease, relatively lower error) but it may also be more difficult (no single dominant environmental constraint, small relative yield differences). Research on indirect selection must, however, continue, for the goal is vital and the prospects have improved relative to empirical selection. While the latter has become more difficult, understanding and instrumentation for indirect selection have improved. To be successful, indirect selection must ultimately be validated for cost effectiveness in breeders' programs, compared to conventional breeding methods, or as a marginal complement to them. This must involve the breeders from the outset, as pointed out yet again in a recent survey of the goals and attitudes of physiologists and breeders by Jackson et al. (1996).

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CHAPTER 14

Manipulating Wheat Development to Improve Adaptation

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Crop development, though a continuous process, may be divided into three major components: the vegetative, reproductive, and grainfilling stages. The duration of each stage and of the whole life cycle, as well as the number of primordia initiated, are determined by interactions between genetic and environmental factors. These responses largely determine a crop's adaptability to a range of environmental conditions.

This chapter describes wheat's main developmental responses to environmental factors and highlights opportunities for improving wheat's adaptation to particular conditions. It also discusses how to use developmental responses to further increase yield potential. Breeders can capitalize on this knowledge by using different factors to make crops fit the targeted growing season.

Also included in this chapter is a summarized, simplified view of wheat phasic development, but a more comprehensive description can be found in two recent reviews of the interactions among environmental factors (Slafer and Rawson, 1994a) and inter-relationships between phasic and morphological development (Slafer and Miralles, 1998).

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Wheat Adaptation

Wheat is cultivated throughout the world (from South America and southern Oceania to North America and the northern parts of Europe and Asia, from sea level to about 3000 m), and its wide adaptability is based on complex developmental responses to environmental factors. As wheat has adapted to different regions, its development patterns have been modified to suit particular environmental conditions, the key issue being that anthesis must occur when the risk of frost is small. Thus an important feature of wheat adaptability lies in its ability to sense the seasons so that development is accelerated or delayed depending on the environment.

Different types of wheat—spring, winter, and Mediterranean—are adapted to the cold, harsh-winter temperate, mild-winter temperate, and tropical regions where they are grown (Figure 1).

Spring types

In cold regions, many wheat plants could not survive the winter to produce a reasonable yield; wheat is thus normally sown in spring. Spring type wheats sense how advanced spring is and accelerate their developmental rate accordingly. The length of day (or night) is the environmental factor they sense best, as it invariably increases from the beginning of winter to the beginning of summer. Photoperiod sensitivity may therefore help to delay anthesis in early sowings and accelerate development in late sowings.

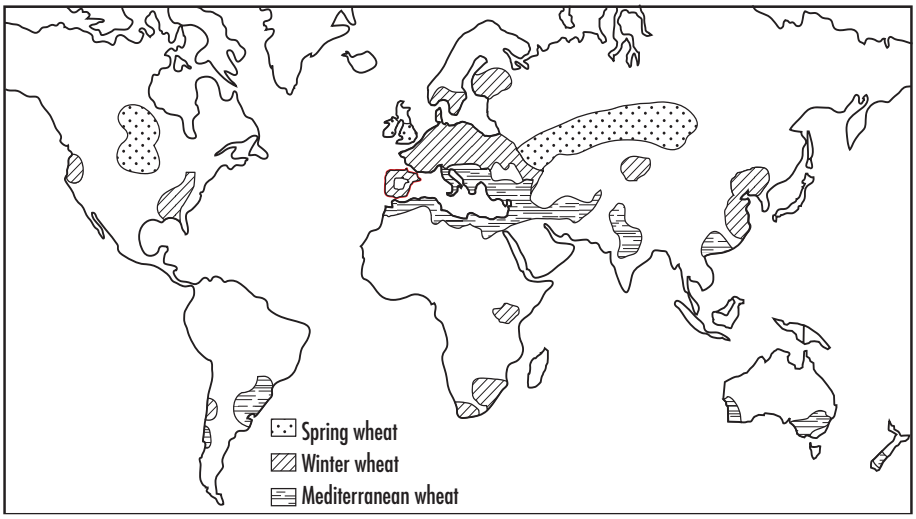


Figure 1. Distribution of different wheat types in countries with more than 5% of their arable land under wheat.
Source: Adapted from Bunting et al. (1982).

Winter types

Winters are quite severe in harsh-winter temperate regions, but not cold enough to keep crops from surviving. Autumn sowing means the wheat crop has a long growing season and relatively early anthesis, having produced much biomass by then. In these regions plants must sense the season independently of daylength. Winter wheat plants must be exposed to low temperatures before their reproductive phase can begin, and autumn-sown plants do not initiate their reproductive period until winter has ended. Winter wheats sense a period of low temperatures and accelerate development thanks to vernalization sensitivity. The adaptive role of this sensitivity is highlighted in these regions, since it prevents inflorescence initiation in autumn, when photoperiod and temperatures are similar to spring.

Mediterranean types

In mild-winter temperate regions (such as Australia and Argentina, and Mediterranean areas), where wheat may be sown in winter, strong photoperiod sensitivity or slight vernalization sensitivity guarantees that the crop will flower shortly after the onset of a period with low or no frost risk. Genotypes with slight vernalization requirements are frequently referred to "intermediate, semi-winter, or Mediterranean types."

High temperatures and few rains are major constraints in tropical regions, so the wheat growing season must fit within the humid season, with plants flowering towards the end of it. In the tropics wheats do not need vernalization and are normally photoperiod insensitive, since the humid season does not always coincide with appropriate photoperiods.

How Wheat Adapts to Different Environments

Environmental signals

Wheat adapts its growing cycle to the best environmental conditions by sensing the right season (through vernalization and photoperiod sensitivities) and regulating flowering time based on temperature *per se*, to drive growth associated with development (Slafer and Rawson, 1994a). Thus the main environmental signals are temperature and daylength.

Temperature affects wheat development in two markedly distinct ways. First, the development rate is accelerated (and the time a developmental phase lasts is shortened) due to increased temperatures, in a wide range of thermal conditions. This general biological effect of temperature is probably caused by the activation of enzymatic processes. Second, wheat development may be accelerated by exposure to a period of relatively low (vernalizing) temperatures (vernalization response is thought to occur in the shoot apex). In sub-optimal temperatures, the relationship between development rate and temperature is linear, and progress towards flowering may be quantified in thermal time units.

Vernalizing temperatures are defined by their effects rather than as particular thermal values. In the literature there is variation in the temperature ranges at which vernalization is most effective. Although this variation may reflect methodological differences, it most likely reflects genetic variation in thermal thresholds at which vernalization takes place. The vernalizing stimulus may be perceived by seeds imbibed in the soil (immediately after sowing and before seedling emergence), by young green plants (during the vegetative stage), and even by grains in the spike of the mother plant, if exposed during grainfilling to low temperatures. Most characterization has been done in seedlings. A generalized pattern of the most effective vernalization temperatures is shown in Figure 2.

Depending on the cultivar (and vernalizing conditions other than temperature), the maximum effectiveness has a lower threshold of between 1 and 4°C and an upper threshold of between 6 and 10°C (Figure 2). Temperatures higher than the latter—and as high as 18°C—are still vernalizing, but with reduced effectiveness.

Daylength is the most reliable environmental signal because it invariably changes with the season. The actual daylength for any particular site and date can be calculated easily if the latitude is known. In calculating actual daylength for plant responses, the length of the day includes periods of twilight. This is why the annual average for a particular site is always greater than 12 h (and daylength on 21 March and 21 September is also greater than 12 h). This factor is markedly affected by latitude: the further north or south from the equator, the greater the daylength variation during the year.

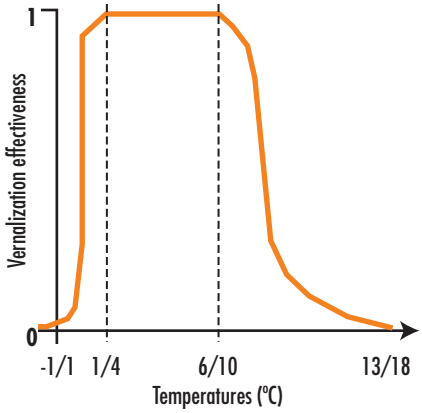


Figure 2. Vernalization effectiveness of different temperatures. A range of values is provided for each point identified on the abscissa, probably reflecting genetic variation for these parameters.

Response to environmental factors

General response of time to heading. Heading is the first unequivocal external sign that a plant has reached the reproductive stage. Since heading occurs quite close to anthesis, the effects of environmental factors on time to heading are key determinants of wheat adaptability. For these reasons, and because it is easy to assess, time to heading is the most common variable for determining the effect of genetic and environmental factors on wheat development.

Sensitivity to temperature. Time from sowing to heading is universally (i.e., in all cultivars, all vegetative and reproductive phases during time to heading are sensitive) affected by temperature (Angus et al., 1981; Del Pozzo et al., 1987; Porter et al., 1987; Slafer and Savin, 1991; Slafer and Rawson, 1995a). It is widely recognized that time to heading shortens, in curvilinear fashion, as temperature increases (Figure 3a). However, the reduction in the time elapsed to reach heading is the result of an accelerated rate of development in response to increased temperatures. The relationship between this rate and temperature is almost invariably linear (Figure 3b; see also Slafer and Rawson, 1995a).

Figure 3 is just a schematic example, but a substantial amount of published data confirms its general shape (e.g., Gallagher, 1979; Angus et al., 1981; Monteith, 1981; Rickman et al., 1983; Morrison et al., 1989; Slafer and Savin, 1991; Slafer and Rawson, 1995a). Thus, rates of progress towards heading increase linearly with temperature from a theoretical threshold at which the rate is zero (it would take infinite time to reach anthesis at that temperature) to an optimum value at which the rate is maximized (and time to heading is minimized), and beyond which higher temperatures frequently reduce the rate of progress towards flowering (once again lengthening the period to heading). The thermal thresholds within which the rate of development increases linearly with temperature are the base and optimum temperatures (Figure 3).

Since the relationship is linear, there is only one slope for the entire interval between base and optimum temperatures. The reciprocal of the slope is thermal time (degree days) needed to reach heading at the designated base temperature; there is thus only one value of thermal time regardless of the temperature, provided the plants are exposed to thermal regimes between the base and optimum temperatures.

In practice, thermal time is simply calculated as the sum of daily effective temperatures (mean minus base temperature, the latter being the abscissa intercept of the relationship). By means of thermal time, development events can be expressed fairly independently of fluctuations in temperature.

Sensitivity to vernalization and photoperiod. Wheat genotypes may vary widely in their sensitivity to photoperiod and vernalization. Some genotypes are virtually insensitive, others have quantitative responsiveness (which may vary considerably), and still others show qualitative responses (Figure 4a). Although all these responses are possible, most cultivars exhibit a quantitative type of response. The slope of the curve (Figure 4b) indicates sensitivity to either vernalization or photoperiod, i.e., the degree of increase in the rate of development (reduction in time) per unit increase in vernalizing or photoperiod stimulus. This parameter varies widely among genotypes and is probably the reason wheat adapts so well to so many different climates.

Although, for the sake of clarity, the three examples in Figure 4a have the same optimum values of length of vernalization exposure or photoperiod, there is genetic variation for them (see examples in Slafer and Rawson, 1994a). Similarly, the example does not provide evidence for genetic variation in intrinsic earliness, but cultivars do differ in this trait.

In passing, it should be noted that the term "optimum" is used in developmental studies in reference to the values of environmental factors that maximize

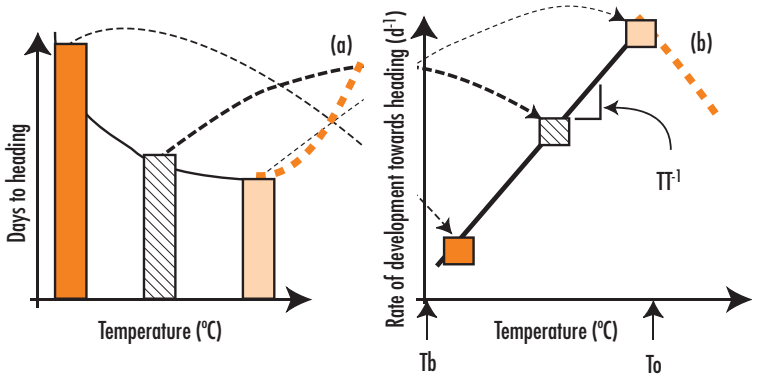


Figure 3. Relationship between calendar time to heading (a) or its reciprocal, rate of development towards heading, (b) and temperature. Strong linearity of the relationship between base (Tb) and optimum (To) temperatures. The slope ([°C d]$^{-1}$) is the reciprocal of thermal time (TT^{-1}) required for heading using estimated Tb for any temperature between Tb and To. Heavy lines represent the most common trends for sub-optimum (solid line) and supra-optimum (dashed line) temperatures.

the rate of development (optimum temperature, optimum vernalization and optimum photoperiod), which does not at all mean that these conditions optimize yield (Slafer, 1996). In fact plants growing under optimum thermal and photoperiodic conditions would likely yield very poorly as they would experience an excessively short season.

Which phases are sensitive to each factor?

Although development is a continuous succession of changes progressing towards maturity, to facilitate understanding of the processes involved, it is frequently defined as a sequence of discrete phenological events controlled by external factors, each event causing important changes in the morphology and/or function of some organs (Landsberg, 1977). Thus, key developmental events marking the limits of phenophases must be identified. The most accepted markers of developmental progress from sowing to maturity are seedling emergence, floral initiation, terminal spikelet initiation, and anthesis (Figure 5a, b, c). These developmental stages limit the following phases.

Pre-emergence development. The crop is established during this vegetative phase, and the shoot apex initiates leaf primordia after seed imbibition. When soil moisture does not limit germination, development rate depends only on temperature *per se*. The length of this phase thus depends on soil thermal conditions at sowing depth and on sowing depth itself (the deeper the sowing the longer seedlings take to emerge at a given temperature). There is no evidence that vernalization affects the rate of development until seedling emergence and since daylength is perceived by the leaves (and the signal transmitted to the apex; Evans, 1987), it does not affect the length of this initial phase.

Vegetative development. All the leaves (and potential tillers) on the main shoot are initiated. This phase continues until the apex becomes reproductive (marking the end of the phase). Leaves start to appear at a regular thermal interval (known as phyllochron) and tillering begins; the appearance of the first tiller coincides with the appearance of the fourth leaf, and the subsequent primary tillers appear at regular one-phyllochron intervals (e.g., Masle, 1985). The theoretical relationship between leaf and tiller appearance most frequently holds for this phase (since plants are small and not very demanding, available resources usually match demand).

The rate of development during this phase is sensitive to all three major environmental factors. Although not strictly true physiologically (see examples in Slafer and Rawson, 1994a), it may be assumed that vernalization requirements must be satisfied before a cultivar becomes responsive to photoperiod. While temperature similarly affects the rate of development towards floral initiation and the rate of leaf initiation, final leaf number is hardly affected by temperature *per se* (Slafer and Rawson, 1994b). On the other hand, vernalization and photoperiod affect the rate of development much more markedly than the rate of leaf initiation. Therefore, the longer the phase (due to lack of satisfaction of vernalization or photoperiod requirements), the higher the final leaf number (Halloran, 1977; Kirby, 1992; Rawson, 1993; Rawson and Richards, 1993; Evans and Blundell, 1994; Slafer and Rawson, 1995b,c).

Early reproductive development. All the spikelets and many florets are initiated. Leaves continue to appear, and tillering usually reaches its maximum rate. However, this rate is hardly the one theoretically expected from the relationship with leaf appearance, because intra- and/or inter-plant competition for resources begins, reducing the assimilates available for growth of all tillers that could appear. Depending on the length of the phase and on agronomic conditions, the maximum number of tillers may be reached by the end of this phase.

Although it is frequently assumed that vernalization affects only the length of the vegetative period (Halse and Weir, 1970; Flood and Halloran, 1986; Roberts

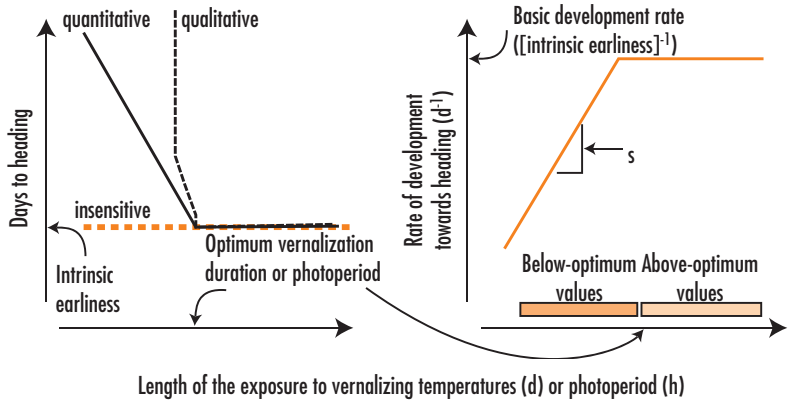
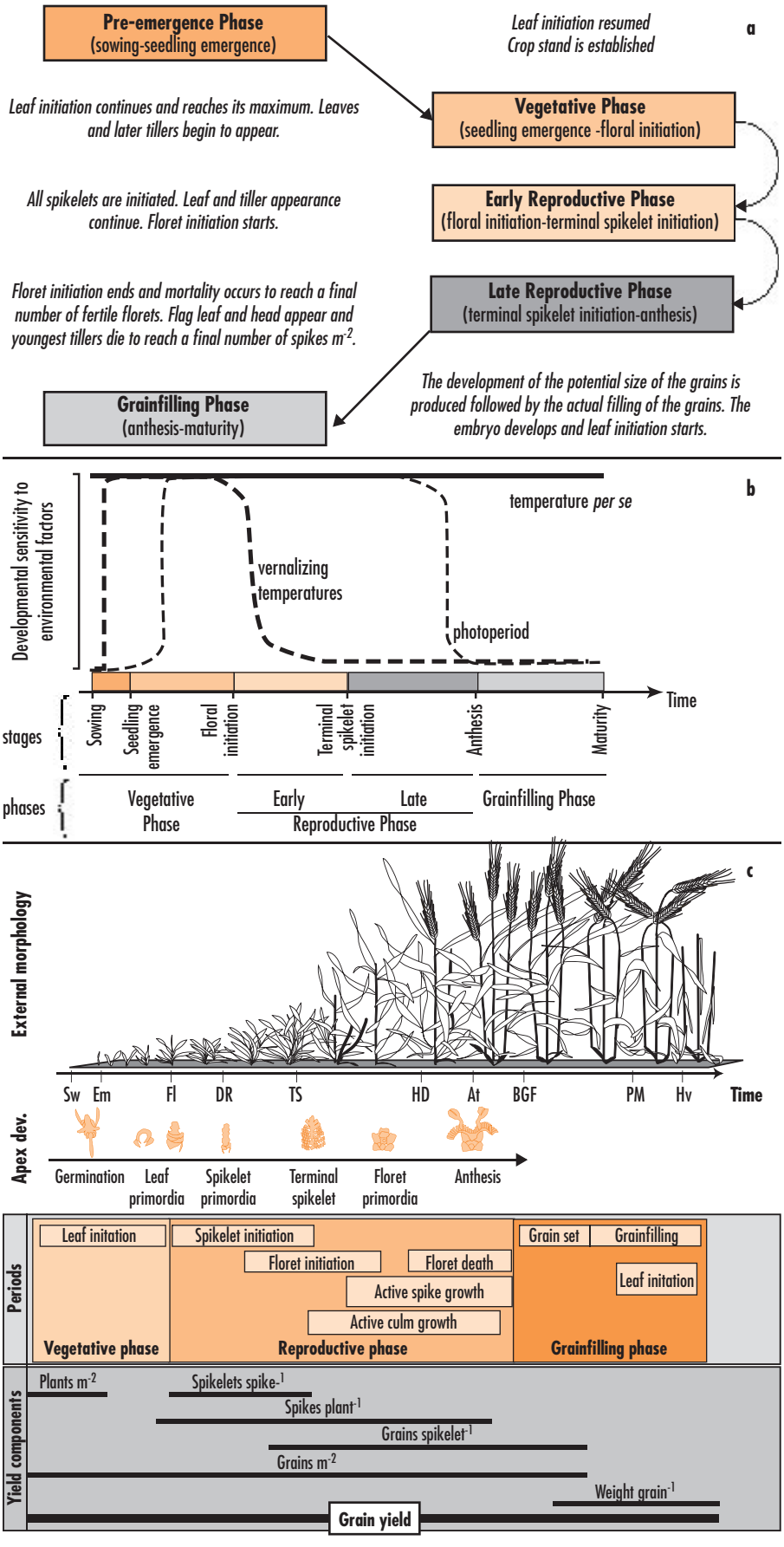


Figure 4. Response of wheat development to vernalization or photoperiod. (a) Possible responses of days to heading. (b) Changes in rate of development for a quantitative response, highlighting the fastest or basic development rate under above-optimum photoperiod and vernalization. The slope of the relationship under below-optimum values represents sensitivity (s) to these factors.
Source: Adapted from Slafer (1996).



et al., 1988; Ritchie, 1991), several authors (e.g., Halloran and Pennel, 1982; Fischer, 1984; Stapper, 1984; Masle et al., 1989; Manupeerapan et al., 1992; Slafer and Rawson, 1994a) have recognized that vernalization may also affect the duration of early reproductive development, though usually less than the vegetative phase. Sensitivity to photoperiod and temperature *per se* are widely acknowledged in this phase (see examples in Slafer and Rawson, 1994a). In keeping with the discussion of final leaf number, when the rate of development of this phase is accelerated by photoperiod and vernalization, the shorter period results in fewer spikelets being initiated. This is not necessarily true when the length of the phase is affected by temperature *per se*, since it also substantially affects the rate of spikelet initiation (Slafer and Rawson, 1994b).

Late reproductive development. The number of fertile florets is determined simultaneously with active growth of stems and spikes. Leaves continue to emerge until the flag leaf (initiated last) appears. Stems first and spikes later grow actively, dramatically increasing the demand for assimilates, which in turn markedly increases competition for resources. Growth becomes most sensitive to changes in resource

Figure 5. (a) Phenophases of wheat development from sowing to maturity. (b) Major events occurring at each phase and relative sensitivity to vernalizing temperatures, photoperiod, and temperature per se of different phenophases in a sensitive cultivar. Time scale is arbitrary. (c) Stages of wheat development: sowing (Sw), emergence (Em), floral initiation (FI), first double ridge appearance (DR), terminal spikelet initiation (TS), heading (HD), anthesis (At), beginning of grainfilling (BGF), physiological maturity (PM), and harvest (Hv). Apex development shown under each stage.
Source: (c) Adapted from Slafer and Rawson (1994a).

availability and yield most reduced if the crop is exposed to stress. The period from terminal spikelet initiation to anthesis is thus considered crucial in determining yield potential.

Due to increased competition, resource availability becomes insufficient to maintain all young tillers, and some of them die, normally in reverse order to when they appeared, and the number of tillers per m^2 is reduced from its peak to final number of spike-bearing tillers per m^2, normally set by the time of heading. At the end of the early reproductive phase, when the terminal spikelet is initiated, the final number of spikelets per spike is determined and several floret primordia initiated, particularly in spikelets in the middle third of the spike. From then on, floret initiation increases rapidly to its peak value, more or less coinciding with full flag-leaf expansion, when apparently no further florets are initiated (Kirby, 1988; Miralles, 1997).

From booting to heading/anthesis, many florets degenerate during stem and spike growth, and only a few floret primordia become fertile and are fertilized by anthesis. Most wheats requiring vernalization, if grown in the right season, have by this time satisfied their requirements. Thus, even if vernalization sensitivity of this phase can be proven experimentally (Masle et al., 1989; Slafer and Rawson, 1994a), it may be assumed insensitive to vernalization under reasonable agronomic conditions (e.g., a wheat cultivar with strong winter habit should not be sown in spring). However, photoperiod may keep influencing the length of the late reproductive phase (Allison and Daynard, 1976; Rahman and Wilson, 1977; Masle et al., 1989; Connor et al., 1992; Manupeerapan et al., 1992; Slafer and Rawson, 1996); this effect may be direct (Slafer and Rawson, 1997), rather than simply mediated by a higher final leaf number due to exposure to short photoperiods during the vegetative phase. As discussed above, temperature *per se* affects the rate of development of all phases, and the fact that the time from terminal spikelet initiation to anthesis is reduced by increasing temperatures has been well documented (see examples in Slafer and Rawson, 1994a).

Post-anthesis development, or grainfilling. Grains develop their potential size and grow to their maximum dry weight by maturity. Most endosperm cells develop during early grainfilling; they are the actual sinks for the accumulation of assimilates during the next phase of active grainfilling, when grains grow and gain weight linearly with thermal time. At the end of the phase, grain growth declines until grains reach their maximum dry weight. During this phase the embryo is formed and the shoot apex initiates the first (normally, of four) leaf primordia.

The rate of post-anthesis development towards maturity in wheat is insensitive to photoperiod and vernalization and only appears to respond positively to temperature *per se*. Thus, the length of the grainfilling phase is quite conservative in terms of thermal time, unless severe water stress occurs. This will virtually end grainfilling, regardless of how many degree days have elapsed since anthesis. Since grain growth is most frequently limited by sink size (Slafer and Savin, 1994), accelerated development due to higher temperatures will reduce final grain weight (Sofield et al., 1977; Chowdhury and Wardlaw, 1978; Slafer and Miralles, 1992) much more than total protein content (since nitrogen is mostly source limited). Thus, higher temperatures during grainfilling will reduce yield-increasing protein percentage.

A more comprehensive explanation of these (and other) markers and the main features of each phase can be found in Slafer and Rawson (1994a) and Slafer and Miralles (1998).

Genes affecting physiological responses

Although the rate of development may respond markedly to vernalizing temperatures, daylength, and temperature *per se*, photoperiod and vernalization sensitivities apparently account for most genetic variation for this trait. In other words, most differences among wheat cultivars in time to heading (or in the length of any phase from seedling emergence to anthesis) can be ascribed to differences in their sensitivity to photoperiod and/or vernalization.

Temperature *per se* has universal impact on the rate of development in all wheat cultivars, which would seem to imply there are no genetic differences in sensitivity to temperature *per se*. However, "residual" differences are frequently found among genotypes, once their vernalization and photoperiod requirements have been satisfied. While these differences are usually less marked than differences in photoperiod and vernalization sensitivities, they are nonetheless both statistically and agronomically significant.

Residual differences have long been thought to reflect the impact of a third group of genetic factors determining differences in "basic development rate" or "intrinsic earliness," also termed earliness *per se* (e.g., Major, 1980; Flood and Halloran, 1984; Masle et al., 1989; Worland et al., 1994). Although reasons have been put forward for considering intrinsic earliness genes as responsive to temperature *per se* (in which case differences in intrinsic earliness would be better defined as differences in temperature sensitivity) (e.g., Slafer, 1996), for simplicity's sake we will use the term intrinsic earliness in this chapter.

Genetic control of the rate of development in wheat is complex enough that almost any development pattern is possible in the duration of the seedling emergence to anthesis period (Slafer and Rawson, 1994a), which means that almost any length of time to heading can be achieved through genetic improvement. The three groups of genes (photoperiod-sensitive, vernalization-sensitive, and intrinsic earliness genes; Worland, 1996) combine to determine the precise time of anthesis in a specific environment.

Bread wheat is a hexaploid species, i.e., it has three sets of genetic material (the A, B, and D genomes) and seven homologous groups. While photoperiod-sensitive and vernalization-sensitive genes appear to be located in certain homologous groups, intrinsic earliness genes are apparently distributed among different groups. Evidence for the location of these genes:

- *Photoperiod sensitivity genes* (*Ppd1*/*ppd1*, *Ppd2*/*ppd2* and *Ppd3*/*ppd3*) are located on the short arms of homologous group 2; dominant alleles (*Ppd*) confer insensitivity, and recessive alleles (*ppd*) sensitivity. Chromosomes 2D, 2B, and 2A carry the *Ppd1*/*ppd1*, *Ppd2*/*ppd2*, and *Ppd3*/*ppd3* genes, respectively (Welsh et al., 1973; Scarth and Law, 1983; Sharp and Soltes-Rak, 1988). *Ppd1*/*ppd1* genes are believed to have the strongest effects, and *Ppd2*/*ppd2* and *Ppd3*/*ppd3* progressively milder effects.
- *Vernalization sensitivity genes* (*Vrn1*/*vrn1*, *Vrn2*/*vrn2*, and *Vrn3*/*vrn3*) are located on the long arms of homologous group 5. As with photoperiod-sensitive genes, sensitivity is conferred by recessive alleles and insensitivity by dominant alleles. The *Vrn1*/*vrn1* gene, considered responsible for the strongest responses, is located in chromosome 5A; *Vrn2*/*vrn2* (also termed *Vrn4*/*vrn4*; Snape, 1996) and *Vrn3*/*vrn3* were found in chromosomes 5B and 5D, respectively (Law et al., 1975; Maistrenko, 1980; Hoogendoorn, 1985). A *Vrn5*/*vrn5* gene has been reported to be located in chromosome 7B (Snape, 1996). Wheats with strong winter habit reportedly possess the three recessive alleles (*vrn1*, *vrn2*, and *vrn3*), but spring wheats may have different combinations of recessive and dominant alleles (Pugsley, 1972), which means that some may respond to vernalization (Slafer and Rawson 1994a).
- *Intrinsic earliness genes*, unlike photoperiod and vernalization sensitivity genes, are not well documented (likely because their impact on time to flowering is less than that of *Ppd* or *Vrn* genes; also, they behave more like "minor" genes), having received much less attention. However, there is some evidence they are located on several chromosomes, including the long arms of homologous group 2 (Scarth and Law, 1983; Hoogendoorn, 1985). For example, in wheat these genetic factors have been reported on chromosomes 2B (Scarth and Law, 1983), 3A, 4B, 4D, 6B, and 7B (Hoogendoorn, 1985), 2A and 5B (Major and Whelan, 1985) 7B (Flood and Halloran, 1983), 6D (Law, 1987) and 3A (Miura and Worland, 1994). In barley they are distributed throughout the genome (Laurie et al., 1995).

There are no apparent associations among these genes, so that a particular genotype may possess any combination of alleles for photoperiod sensitivity, vernalization sensitivity, and intrinsic earliness genes.

Improving adaptation

Selecting for improvied adaptation appears simple, since it may be accomplished by including time to heading in the traits considered when selecting progeny. If a breeding program is local, targeting the region is simpler than when a program attempts to release cultivars to be grown over large areas (Figure 6).

In the first case, mechanisms controlling the rate of development towards heading may be disregarded, since the priority is to obtain cultivars that reach heading within a certain time and will not be distributed except in areas with characteristics similar to those of the breeding program (not only environmentally but also agronomically influencing time to heading, such as sowing time). There would also be little interest in choosing parents based on specific sensitivities.

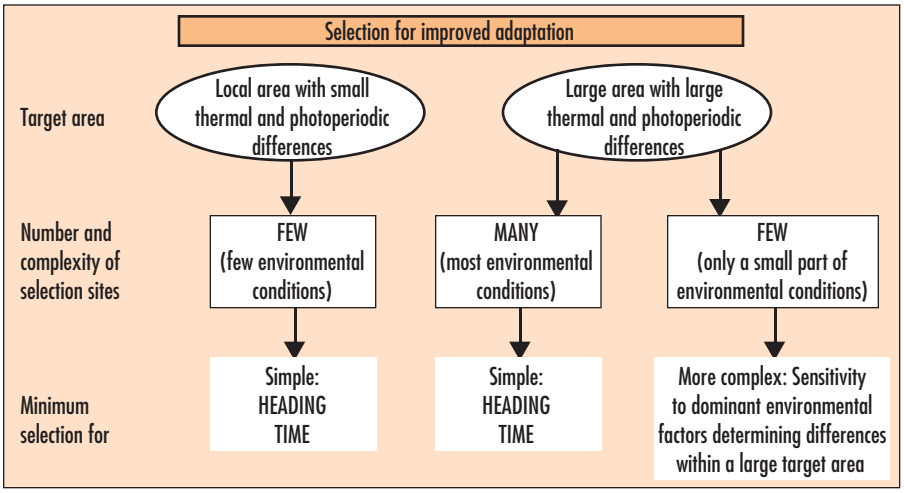


Figure 6. Alternatives for selecting to improve adaptation depending on diversity of the target area and selection sites.

If a second generation per year is obtained by growing the plots in an inappropriate season or a completely different environment, we recommend applying the least amount of selection pressure possible for time to heading, since the environment would be too different from the target environment and variation in time to heading might not be related to time in an appropriate growing season. For example, a vernalization-sensitive line may show optimum time to heading in a normal growing season, but if the second generation is grown in warmer conditions, the line may appear unsuitably long and be wrongly discarded.

When the program's target environment is extensive, the lines should be adapted to most environments they will be exposed to. Empirically this may be done by running the program simultaneously in many sites representing the range of conditions under which the released cultivars will be grown. In this case, it may be sensible, when choosing parents, to consider not only their time to heading in particular circumstances, but also their genetic sensitivities to major environmental factors governing rate of development (and the range of environmental factors in the target region). For some areas the requirements of plasticity given by vernalization- or photoperiod-sensitive genes could be predicted; in those cases choosing parents carrying the required genetic information may help to increase the likelihood of obtaining a reasonable number of well adapted lines that could be selected for yield or other targeted characteristics to provide the best possible cultivar.

In fact, knowing what limits yield in modern cultivars grown in the region may also help identify the best possible combination of genetic sensitivities so that not only adaptation may be improved but also yield potential. Figure 4 shows how different phases are sensitive to different factors; therefore it may be possible to customize cultivars that reach heading or anthesis at a specific time and have a certain combination of durations for component phases. As sources and sinks are predominantly formed in different phases, it is speculated that manipulation of genetic sensitivity to photoperiod and vernalization, together with appropriate combinations of intrinsic earliness genes, may present additional opportunities for further increasing yield potential.

Identification of key phenological phases

To visualize phenological changes occuring during plant development, an accurate identification of the different stages is necessary. Although external morphological observations can give a general idea of phenology, microscopic observation of apex morphology is much more accurate in determining the stages of development.

In the field, development wheat plots can be monitored periodically, and plant samples taken at random.The few stages (described above) that limit phenological phases are mostly seen externally, with the exception of floral initiation and terminal spikelet initiation. The former cannot be unequivocally determined by simple observation (i.e., it is not marked by a clear morphological change in the apex) and is many times replaced by the observation of the first double ridge, which is the first sign that the plant is undoubtedly floral, and occurs a bit later than floral initiation (see Slafer and Rawson, 1994a). Determination of both double ridge and terminal spikelet initiation requires dissecting the apex and the use of an optic magnifier. (See in Figure 7 how the apex looks at different stages, from vegetative stage to terminal spikelet initiation.)

A plant is taken and leaves are extracted by means of a sharp cutting instrument; the apex can be observed under the most recently formed leaves. Important events that mark the onset of phenological stages can be seen without major difficulty, after practice has been gained. When apex dissection cannot be practiced, external morphological observation of important events can sometimes be useful. For example, the beginning of stem elongation (frequently coinciding with terminal spikelet initiation, see above) can be determined by the "perception" of the first nodes.

Using Phasic Development to Further Increase Yield Potential

Crop development has been long used as the most powerful trait to genetically improve adaptation, and that has been the

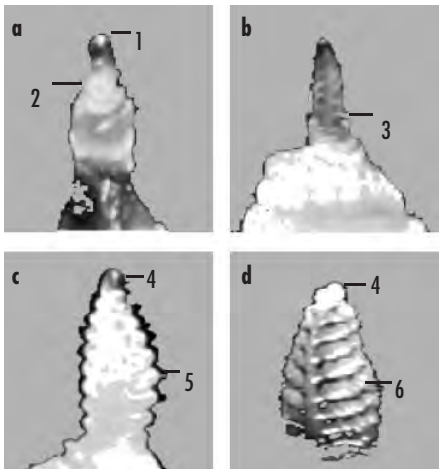


Figure 7. Stages of apex development. (a) Apex still within vegetative phase; (b, c, and d) reproductive stages. (b) Apex some time after floral initiation, at spikelet initiation; first double ridges microscopically visible. (c) Spikelet primoria just before glume differentation. (d) Floret initiation within central spikelets during terminal spikelet formation.
1: shoot apex; 2: leaf primordium; 3: double ridge; 4: site of terminal spikelet; 5: spikelet primordium; 6: floret primordium.

main target of this chapter. In fact, by improving adaptation, yield potential for a particular area has been concomitantly increased, given that improved adaptation essentially means optimized flowering time for that environment (optimized is used here in terms of flowering times that maximize yields). However, there is no clear evidence that wheat development could be manipulated to further increase yield potential in traditional wheat growing regions, where timing of anthesis has already been optimized.

Physiological traits may help breeders to further increase yield potential, breaking barriers that have lately become apparent (Reynolds et al., 1996). We are not convinced that phasic development can be manipulated to increase yield potential independently of changes in timing of anthesis, but are working on hypotheses that may prove that it could. A detailed explanation is given in Slafer et al. (1996), and only a simplified summary is included here.

Many authors have suggested that there are associations between particular developmental stages and yield components (Figure 5b) (e.g., Rawson, 1970, 1971; Rawson and Bagga, 1979), and associations between the duration of phases and absolute yield (e.g., Rawson, 1988a, 1988b; Craufurd and Cartwright, 1989), so it may be important to be able to manipulate the duration of these phases to indirectly manipulate yield components (Slafer et al., 1996). In addition, it appears that a growing season of a given length can be achieved with component phases of different duration (Slafer and Rawson, 1994a). Thus it is hypothesized that manipulating development without greatly modifying the length of the entire growing period could bring about increases in yield potential.

For example, a longer late reproductive phase would increase the amount of biomass accumulated during stem and spike growth, and the final number of grains would probably increase as floret abortion and/or tiller death decreases. This would result in an increased number of grains per unit land area to be filled. Another example would be to attempt to extend the grainfilling phase, which would increase the availability of assimilates for satisfying the demand of growing grains, where it is not fully satisfied at present (see discussion in Kruk et al., 1997).

Genetic variation in the length of the late reproductive phase is controlled by photoperiod-sensitive and intrinsic earliness genes. Since the responses to photoperiod during pre- and post-terminal spikelet initiation phases appear to be independent (see discussion in Slafer and Rawson, 1996), and there is clear evidence of substantial genetic variation in this response (e.g., Slafer and Rawson, 1994a; Slafer and Rawson, 1996), attempting to lengthen the late reproductive phase at the expense of either the vegetative or early reproductive phase may be possible. If control of intrinsic earliness during a particular phase is independent of control of other phases (which appears likely; see Halloran and Pennel, 1982; Slafer, 1996), it would change the partitioning of a particular time to anthesis among the vegetative, early reproductive, and late reproductive phases.

As temperature *per se* appears to be the only environmental factor affecting length of post-anthesis, it may be manipulated only through genetically improving intrinsic earliness. Genetic variation for the length of this phase is widely acknowledged (Slafer and Rawson, 1994a).

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Breeding for Nutritional and Soil Factors

CHAPTER 15

Acid Soils and Aluminum Toxicity

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Acid soils limit crop production on 30-40% of the world's arable land and up to 70% of the world's potentially arable land (Haug, 1983). Although the poor fertility of acid soils is due to a combination of mineral toxicities (aluminum and manganese) and deficiencies (phosphorus, calcium, magnesium, and molybdenum), Al toxicity is the single most important factor, being a major constraint for crop production on 67% of the total acid soil area (Eswaran et al., 1997). Therefore, this chapter will focus on Al toxicity.

Although aluminum toxicity can be ameliorated by surface application of lime, this is often not economically or physically feasible. Hence, combining the use of Al tolerant cultivars with liming is often the most effective strategy for improving crop production on acid soils. To breed genotypes with improved Al tolerance, reliable, efficient screening methods must be available to the researcher. Several screening methods have been employed for this purpose, from genotype screening in the laboratory to soil bioassays and field evaluations.

Plant species differ in their Al tolerance; some are inherently more tolerant than others—for example, cassava (*Manihot esculenta* Crantz), cowpea (*Vigna unguiculata* L. Walp), groundnut (*Arachis hypogea*), pigeon pea (*Cajanus cajan* L. Millsp.), potato (*Solanum tuberosum*), rice (*Oryza sativa* L.), and rye (*Secale cereale* L.) (Little, 1988). Rye is one of the most stress tolerant species in the Triticeae family. Several studies comparing Al tolerance in rye with that of other cereals have shown that rye has the highest tolerance, followed by triticale (X *Triticosecale* Wittmack), wheat (*Triticum aestivum* L.), and barley (*Hordeum vulgare* L.) (Mugwira et al., 1976, 1978; Aniol and Madej, 1996).

Information on the mechanisms of Al tolerance, and the genetic control and chromosome location of Al tolerance genes in different crop species are not only of interest for those crops, but also for other, more or less closely related species. With the identification of molecular markers closely linked to Al tolerance genes in, for example, rye, marker assisted selection (MAS) strategies can be applied to introgress rye Al tolerance genes into wheat.

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Global Expanse of Acid Soils

There are several estimates of the extent of acid soils in the world. According to van Wambeke (1976), acid soils occupy 1,455 million ha (11%) of the world's land, while Haug (1983) estimated that 30-40% of the world's arable soils and up to 70% of potentially arable land are acidic. Von Uexkull and Mutert (1995) estimated the global expanse of acid soils (defined as soils with pH <5.5 in their surface layers) to be 3,950 million ha, or approximately 30% of total ice-free land in the world. This is in accordance with Eswaran et al. (1997), who estimated that globally around 26% of total ice-free land is constrained for crop production by soil acidity. Acid soils occur mainly in two global belts (Figure 1): the northern belt, with cold, humid temperate climate, and the southern tropical belt, with warmer, humid conditions (von Uexkull and Mutert, 1995).

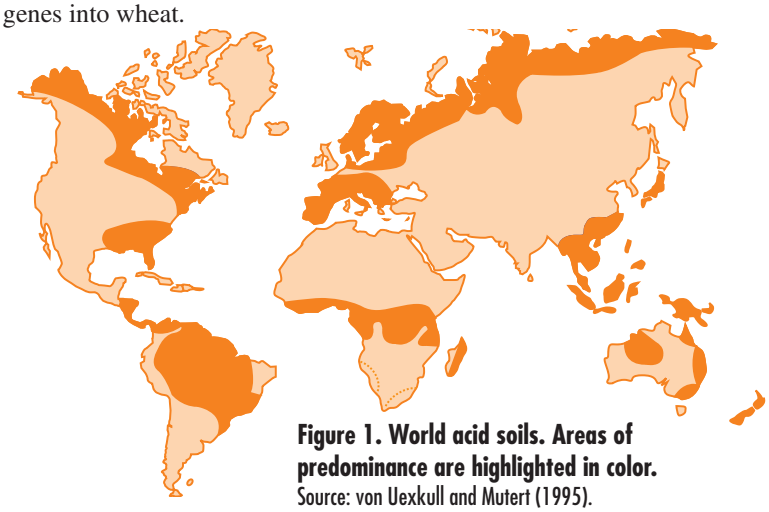

Figure 1. World acid soils. Areas of predominance are highlighted in color.
Source: von Uexkull and Mutert (1995).

Most acid soils are forests and woodlands (66.3%, or 2,621 million ha), while 17.7% (699 million ha) are covered by savanna, prairie, and steppe vegetation. Only 212 million ha (5.4%) of the world's acid soils are cropped (von Uexkull and Mutert, 1995). The acid soils of the world comprise large areas of potentially arable land, of which the savanna region of Brazil, called Los Cerrados, is a good example. Its total area is approximately 205 million ha, of which about 112 million ha are potentially arable. Much of the remainder could be used for forest plantations and improved pastures for animal production. Similar areas are found in Colombia, Venezuela, Central Africa, and Southeast Asia (Borlaug and Dowswell, 1997).

The Chemistry of Soil Acidity

Development of acid soils

Soil acidity is determined by the amount of hydrogen (H^+) activity in soil solution and influenced by edaphic, climatic, and biological factors. Soils that develop from granite parent materials acidify at a faster rate than soils developed from calcareous parent materials. Sandy soils with relatively few clay particles acidify more rapidly due to their smaller reservoir of alkaline cations and higher leaching potential. High rainfall affects the rate of soil acidification depending on the rate of water percolation through the soil profile. Soil acidification is intensified by the removal of cations through the harvesting of crops and by acid precipitation from polluted air (Ulrich et al., 1980). Organic matter decaying to form carbonic acid and other weak acids also contributes to acidification (Carver and Ownby, 1995).

Aluminum is the most abundant metal in the earth's crust, comprising approximately 8% by weight (FitzPatrick, 1986); it is also a major constituent in a wide array of primary and secondary minerals. Under acid soil conditions, these primary and secondary minerals dissolve to a limited extent, releasing Al into the soil solution, where it may be hydrolyzed and contribute to soil acidity. Among the most important soil cations that hydrolyze and contribute significantly to soil acidity are Al^{3+} and Fe^{3+} (Thomas and Hargrove, 1984). Soil acidification is often accelerated by certain cropping practices such as repeated applications of nitrogen in amounts that exceed crop uptake (Adams, 1984). Net H^+ production occurs through natural processes such as nitrification of ammonical nitrogen.

Neutralizing soil acidity

Acidity and Al toxicity in surface soil can be ameliorated through liming. A liming material is defined as a material whose Ca and Mg compounds are capable of neutralizing soil acidity (Barber, 1984). The bulk of agricultural lime comes from ground limestone, and can be calcite ($CaCO_3$), dolomite ($CaCO_3$, $MgCO_3$), or a mixture of the two. Other materials are used to neutralize soil acidity, including marl, slag from iron and steel making, flue dust from cement plants, and refuse from sugar beet factories, paper mills, calcium carbide plants, rock wool plants, and water softening plants (Thomas and Hargrove, 1984). However, total use of these materials is relatively small, and they are generally applied only in areas close to their source.

Lime is usually broadcast on the soil surface and then mixed with the soil during tillage operations. In water, $CaCO_3$ dissolves and hydrolyzes to form OH^- ions that can subsequently react with both H^+ ions formed from hydrolysis of Al^{3+} and exchangeable Al^{3+} (Thomas and Hargrove, 1984). Other compromising factors are NO_3^- uptake and the subsequent release of OH^- (which can neutralize part of the H^+), NO_3^- denitrification, and NH_3 volatilization. Management practices that optimize N-use efficiency and ultimately reduce the amount of NO_3^- lost through leaching could slow the rate of acidification (Carver and Ownby, 1995).

Factors of Acid Soil Infertility

Acid soils are phytotoxic as a result of nutritional disorders, deficiencies, or unavailability of essential nutrients such as calcium, magnesium, molybdenum, and phosphorus, and toxicity of aluminum, manganese, and hydrogen activity (Foy et al., 1978; Foy, 1984; Carver and Ownby, 1995; Jayasundara et al., 1998). The solubility of soil compounds and, therefore, nutrient availability to plants is related to soil pH (Figure 2).

Aluminum toxicity

Aluminum toxicity is considered the most important growth-limiting factor for plants in acid soils (Foy et al., 1978; Foy, 1984; Carver and Ownby, 1995; Jayasundara et al., 1998). The primary response to aluminum stress occurs in the roots (Foy et al., 1978; Foy, 1984, Taylor, 1988, Jayasundra et al., 1998). Aluminum-injured roots are stubby and brittle. Root tips and lateral roots thicken and turn brown. The root system as a whole is affected, with many stubby lateral roots and no fine branching. Such roots are inefficient in absorbing nutrients and water (Foy et al., 1978).

The main symptom of Al toxicity is rapid inhibition of root growth. A number of mechanisms may cause this, including Al interactions within the cell wall,

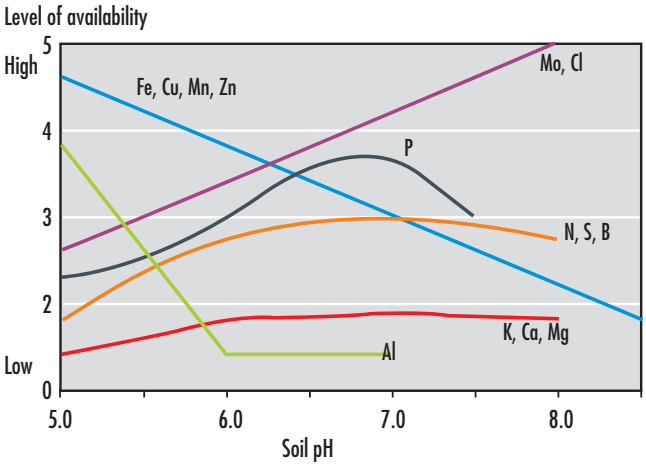

Figure 2. Relationship between level of availability of different elements and soil pH.
Source: Goedert et al. (1997).

the plasma membrane, or the root symplasm (Taylor, 1988; Marschner, 1991; Horst, 1995; Kochian, 1995). According to Ryan et al. (1993), the root apex is the critical site for Al toxicity. They demonstrated in maize (*Zea mays* L.) that for root growth to be inhibited, the terminal 2 to 3 mm of the root (root cap and meristem) must be exposed to Al. Application of Al to the next 3 mm of the root (elongation zone) did not result in significant root growth inhibition.

Ryan et al. (1993) investigated whether the root cap affords protection from the inhibitory effects of Al and found that decapped roots were no more susceptible to Al-induced growth inhibition than intact roots. They concluded that the root cap does not provide protection from Al damage. This is in disagreement with other studies that suggest the root cap provides protection from Al through its involvement in signal perception and hormone distribution (Bennet and Breen, 1991).

Aluminum Tolerance Mechanisms

Aluminum tolerance can be divided into mechanisms that facilitate Al exclusion from the root apex (external tolerance mechanisms) and mechanisms that confer the ability to tolerate Al in the plant symplasm (internal tolerance mechanisms) (Taylor, 1988; Carver and Ownby, 1995; Kochian, 1995). Due to the common assumption that most Al in the root is apoplasmic and that penetration of Al into the symplasm in general is very low, the amount of research addressing internal tolerance mechanisms is limited compared to research on external mechanisms. However, it has been demonstrated that 50-70% of total Al might be present in the symplasm (Tice et al., 1992) and that Al can be present in the symplasm after only 30 minutes' exposure to a solution containing Al (Lazof et al., 1994).

Several external tolerance mechanisms have been suggested, of which the most important are: 1) exudation of organic acids (Hue et al., 1986; Suhayda and Haug, 1986; Miyasaka et al., 1991; Delhaize et al., 1993; Basu et al., 1994b; Ryan et al., 1995; Pellet et al.,1995; de la Fuente et al., 1997); 2) immobilization at the cell wall (Mugwira and Elgawhary, 1979; Blamey et al., 1990; Taylor, 1991; Kochian, 1995); 3) exudation of phosphate (Taylor, 1991; Ryan et al., 1993; Pellet et al., 1997); 4) active Al efflux across the plasma membrane (Zhang and Taylor, 1989; 1991; Taylor, 1991); 5) production of root mucilage (Horst et al., 1982; Henderson and Ownby, 1991); 6) Al exclusion via alterations in rhizosphere pH (Foy et al., 1965; Mugwira et al., 1976; Mugwira and Patel, 1977; Foy, 1988; Taylor, 1988; Taylor, 1991; Kochian, 1995; Pellet et al., 1997), and 7) selective permeability of the plasma membrane (Wagatsuma and Akiba, 1989; Taylor, 1991).

The most important internal tolerance mechanisms are Al-binding proteins, chelation in the cytosol, compartmentation in the vacuole, evolution of Al tolerant enzymes, and elevated enzyme activity (Taylor, 1991). Substantial experimental evidence supports the synthesis of Al-binding proteins (Aniol, 1984b; Picton et al., 1991; Rincon and Gonzalez, 1991; Delhaize et al., 1991; Basu et al., 1994a; Somers and Gustafson, 1995; Somers et al., 1996; Basu et al., 1997).

Genetic Mechanisms of Aluminum Tolerance

Acidity in the surface soil can be corrected by applying agricultural lime. When the subsoil layers are acidic, amelioration of the surface layer will not allow the plant roots to penetrate the acid layer and reach critical water and nutrient supplies below it. Selection and development of genotypes with enhanced tolerance to acid soils and toxic levels of Al is the only reasonable solution to this problem.

The genetics and chromosome localization of aluminum tolerance genes have been extensively studied in cereal crops, with emphasis on wheat. For chromosome manipulation in wheat and triticale breeding, it is important to know which wheat and rye chromosomes carry genes for aluminum tolerance (Aniol and Gustafson, 1984).

Genetic control in wheat

Slootmaker (1974), who first roughly located genes for acid soil tolerance in wheat, found the D genome to be most important, followed by the A genome and the B genome. Aniol and Gustafson (1984) identified Al tolerance genes on chromosome arms 6AL, 7AS, 4BL, 2DL, 3DL, 4DL, and 7D, confirming that Al tolerance genes are mainly located in the A and D genome. Aniol (1990) found genes controlling Al tolerance on 2DL, 4DL, and 5AS.

According to Kerridge and Kronstad (1968), a single dominant gene is responsible for Al tolerance in a cross between the two wheat varieties Druchamp and Brevor, but additional genes are present in Atlas 66. Aniol (1984a) concluded that several genes are responsible for Al tolerance in wheat. This is consistent with Lafever and Campbell (1978) and Campbell and Lafever (1981), who found that Al tolerance in wheat is not simply inherited and that the expression of Al tolerance is additive, with high heritability.

Genetic studies conducted by Camargo (1981) demonstrated that Al tolerance in Atlas 66 is determined by a complex genetic mechanism involving at least two dominant major genes and perhaps some minor genes. Previous studies had identified a gene on chromosome 5D but Berzonsky (1992) concluded that Al tolerance in Atlas 66 is determined not only by dominant genes located on the D genome but also by genes on the A and/or B genomes. Studying different crosses, Rajaram et al. (1991) found the presence of two complementary dominant genes in one parent and one recessive gene in two other parents. Camargo (1984) also reported recessive Al tolerance in wheat.

Other studies have shown Al tolerance to be a simply inherited trait based on a single major dominant gene (Delhaize et al., 1993; Somers and Gustafson, 1995; Somers et al., 1996; Basu et al., 1997). Recently, an RFLP clone on chromosome 4DL has been linked to a gene conferring tolerance to Al in the wheat variety BH1146 from Brazil (Riede and Anderson, 1996).

Genetic control in rye

Rye is one of the most stress tolerant species in the Triticeae family (Little, 1988), and several studies comparing Al tolerance in rye with other cereals demonstrate that on average rye has the highest Al tolerance, followed by triticale, wheat, and barley (Slootmaker, 1974, Mugwira et al., 1976; Mugwira et al., 1978; Manyowa et al., 1988; Aniol and Madej, 1996; Hede et al., 2001a).

Genes for Al tolerance in rye have been located on chromosomes 3R, 4R, and 6RS (Aniol and Gustafson, 1984). Manyowa et al. (1988) found that Al tolerance in rye is controlled by factors on more than one chromosome, though predominantly on chromosome 5R. As has been found in other species, aluminum tolerance in rye seems to be a dominant character (Aniol and Madej, 1996). Gallego and Benito (1997) investigated the segregation of Al tolerance genes and several isozyme loci in a population segregating for Al tolerance and found that Al tolerance in rye is controlled by at least two major dominant and independent loci. Two isozyme loci (*Aco1* and *Ndh2*) were linked to a segregating aluminum tolerance gene on chromosome 6R. Evaluating the segregation ratios in several rye populations, Hede et al. (2001b) found Al tolerance in rye to be controlled by several dominant alleles with different effects at two or three independent loci. A major QTL was identified on chromosome 4R through the application of molecular markers to a specific cross. It accounted for 48% of total phenotypic variation and was linked to an RFLP marker with a distance of 2 cM.

Genetic control in triticale

Many triticales have a high degree of tolerance to Al, but not as much as rye itself (Mugwira et al., 1976; Mugwira et al., 1978; Hede et al., 2001c). Apparently certain wheat genes suppress the expression of Al tolerance genes from rye, yet others allow expression of rye Al tolerance. Aniol and Gustafson (1984) demonstrated that the expression of Al tolerance from 6R depends on which wheat chromosome is substituted. Gustafson and Ross (1990) found suppressors of rye Al tolerance on chromosome arms 4AL, 5AL, 6AL, 7BS, 7BL, and 3DS. Similarly, activators of rye Al tolerance were present on the chromosome arms 2AL, 5AS, 6BS, 1DS, 1DL, 2DL, 4DL, 5DS, 5DL, 6DL, 7DS, and 7DL.

Genetic control in barley

Barley is regarded the most Al sensitive of the small-grain cereals. Genetic analyses have shown that tolerance to acid soil in barley is inherited as a single dominant gene (Stølen and Andersen, 1978) plus multiple alleles (Minella and Sorrells, 1992). Stølen and Andersen (1978) found that tolerance to high soil acidity is controlled by a single dominant gene, designated *Pht*, on chromosome 4. Reid (1971) found Al

tolerance in barley cultivars Dayton and Smooth Awn 86 to be controlled by a single dominant gene, designated *Alp*. Minella and Sorrells (1997) studied the inheritance and chromosome location of *Alp* and found that the *Alp* gene is distally located from the centromere on chromosome 4, suggesting that tolerance to low pH (*Pht*) and aluminum tolerance (*Alp*) are controlled by the same locus.

Genetic Resources to Enhance Al Tolerance

Plant genetic resources are a rich source of valuable traits that could be used to improve crop species. Aluminum tolerance in wheat can be enhanced by incorporating tolerance genes present in the primary, secondary, and tertiary genepools of the Triticeae family. Wheat's primary genepool includes hexaploid, tetraploid, and diploid wheats. It is relatively easy to transfer genes within species of the primary genepool.

Aegilops species and rye belong to the secondary genepool, while triticale is classified between the primary and secondary pools. Most species in the secondary genepool are fairly easy to cross with wheat, although there can be problems with the expression of alien genes in a wheat background. The tertiary genepool consists of annual and perennial forage grasses that are difficult to tap into without the use of specialized techniques.

Significant variation for response to aluminum has been identified in wheat. An efficient way of identifying Al tolerant accessions in a genebank collection is to evaluate those from areas with highly acidic soils and aluminum problems. Some of the most Al tolerant wheats and ryes (such as BH1146 and Blanco) come from Brazil, where vast expanses of land have acid soils.

Hede et al. (1996) investigated whether as a result of natural and human selection, wheat landraces collected from acid-soil regions are more likely to be Al tolerant than those collected from regions with neutral or basic soils. They evaluated the aluminum tolerance of Mexican landraces collected from soils with different acidity levels and found that landraces from acid-soil regions were not more Al tolerant than those from regions with basic or neutral soil; rather, the opposite was true. The most likely explanation is either that the acidity of soils in Mexico is not strong enough to have selected wheats with higher tolerance, or that there was no genetic diversity for Al tolerance in lines brought into Mexico from Europe.

Thus the most appropriate germplasm for increasing aluminum tolerance is likely to be found in areas of extremely low pH (e.g., Brazil). Rye could be used as an indicator; if rye with high Al tolerance is found in an area, wheat with good tolerance will probably also occur there. The wheat landrace Barbela, grown for centuries in certain acid-soil regions of Portugal, was outyielded only by the local rye. Barbela was found to have high levels of Al tolerance; it also has small rye-chromosome segments representing up to 5% of a chromosome (Ribeiro-Carvalho et al., 1997). Barbela may be a good source of Al tolerance genes, especially since it has not yet been utilized in wheat improvement. A similar situation was found in Ecuador, where a highly Al tolerant rye was reported (Baier et al., 1996); it might be worthwhile to screen the wheat landraces from that region for Al tolerance.

Rye has a large amount of variation that could be transferred to wheat. Triticale (a cross between rye and wheat) could serve as a bridging parent to transfer Al tolerance genes from rye to wheat. Slootmaker (1974) studied a number of *Aegilops* species for Al tolerance and found they possess little variation for the trait. He concluded that the reason for this lack of variation is that soils in the center of origin of *Aegilops* (the Fertile Crescent in Asia Minor) are not acid. Nevertheless, *Ae. umbellulata* has been found to have useful levels of aluminum tolerance (Mujeeb-Kazi, pers. comm.).

Relatively little is known about the reaction to acid soil of the species in the tertiary genepool, which are also extremely difficult to utilize in wheat improvement. The preference is thus to utilize species of the primary genepool. However, it may not be possible to achieve greater Al tolerance than found in BH1146 utilizing genetic resources from the primary genepool. The variation found in the secondary and tertiary genepools has great potential for improving the levels of Al tolerance in wheat. The potential payoff in terms of improved Al tolerance may make it worthwhile to use extraordinary means to access the genes conferring such tolerance.

Strategies for Screening for Aluminum Tolerance

Different screening methods have been used to evaluate Al tolerance: cell and tissue culture (Conner and Meredith, 1985), nutrient solution culture (Baier et al., 1995), soil bioassays (Stølen and Andersen, 1978; Ring et al., 1993), and field evaluations (Johnson et al., 1997). Laboratory- and greenhouse-based techniques for screening for Al tolerance are widely used because they are quick, highly accurate, non-destructive, and can be applied at early developmental plant stages. Field-based techniques are more laborious (Carver and Ownby, 1995).

Nutrient solution culture

By far the most common screening medium for Al tolerance is solution culture, which provides easy access to the root system, strict control over nutrient availability and pH, and non-destructive measurements of tolerance (Carver and Ownby, 1995). Different assays have been applied to identify Al tolerant and Al sensitive genotypes, of which the most widely used are hematoxylin staining of root tips and root growth measurement (Baier et al., 1995; Carver and Ownby, 1995). Plant parameters such as root and top dry weight, height, tiller number, and number of spikelets per ear have also been used to evaluate Al tolerance (Mugwira et al., 1976; Mugwira et al., 1978; Manyowa et al., 1988).

Aluminum-induced callose (1,3-b-D-Glucan) synthesis after short Al treatment in nutrient solution has been reported to correlate well with Al tolerance (Zhang et al., 1994; Basu et al., 1997; Horst et al., 1997). Results obtained using the nutrient solution technique have proven to be highly relevant to acidic field conditions. Genotypes classified as Al tolerant based on the nutrient solution evaluation very often show improved agronomic performance under acid soil and Al stress (Carver et al., 1988; Rajaram and Villegas 1990; Ruiz-Torres et al., 1992; Rengel and Jurkic, 1993; Baier et al., 1995).

Hematoxylin staining method

The hematoxylin staining method is an extremely powerful tool for observing tolerance without laborious quantitative measurements. The hematoxylin dye forms complexes with tissue Al that has been immobilized as $AlPO_4$ by phosphate on or immediately below the root surface (Ownby, 1993).

There are several variations of the hematoxylin method. Polle et al. (1978) used the hematoxylin-staining pattern of root tips as an indicator of Al tolerance. As the intensity of staining increases, reflecting a higher level of Al uptake, the level of tolerance decreases. Another procedure using hematoxylin, the modified-pulse method, evaluates Al tolerance based on the ability of Al tolerant seedlings to continue root growth after a short pulse treatment with high Al concentrations (Aniol, 1984a). Aluminum sensitive seedlings do not show root re-growth because their apical meristem has been damaged. This method can be applied to determine Al tolerance through either measuring root regrowth (Aniol and Gustafson, 1984; Gustafson and Ross, 1990; Gallego and Benito, 1997) or evaluating seedlings on a 1 to 3 scale (tolerant, medium tolerant, and susceptible) based on their ability to present root regrowth (Rajaram and Villegas, 1990; Riede and Anderson, 1996).

Laboratory protocol for the hematoxylin method (modified pulse method)

1. Sterilize seeds by placing in a 3% sodium hypoclorite solution for 5 minutes and rinsing thoroughly with water (Figure 3.1).
2. Place seeds on moist filter paper in petri dishes for 84 hours at 7 °C and leave to germinate at room temperature (18-20 °C) for approximately 24 hours (Figure 3.2).
3. Place seedlings with similar root lengths (5-10 mm) and endosperm size on a polyethylene net fixed on lucite frames; attach styrofoam blocks to the frames with rubber bands so they will float (Figure 3.3).
4. Place frames in plastic containers with nutrient solution (4 mM $CaCl_2$, 6.5 mM KNO_3, 2.5 mM $MgCl_2$, 0.1 mM $(NH_4)_2SO_4$, and 0.4 mM NH_4NO_3) maintained at pH 7. Place plastic containers in a water bath kept at 25 °C. Grow seedlings in the nutrient solution for 32 hours. Continuously aerate the nutrient solution during the whole process (Figure 3.4).
5. Transfer frames with seedlings to a nutrient solution containing Al (pH 4.0) and keep in solution for 17 hours (Figure 3.5).
6. Thoroughly wash roots with water and stain with a 0.2 % hematoxylin aqueous solution for 15 minutes. Wash off excess dye.
7. Return seedlings to nutrient solution for 24 hours (Figure 3.4).
8. Remove seedlings from trays; measure root growth or rate seedlings as tolerant (T), susceptible (S), or moderately tolerant (MT). Seedlings with all roots showing continued growth are rated T, whereas seedlings with no roots showing re-growth are rated S. Seedlings showing re-growth on some roots are rated MT (Figure 3.6).

Root growth method

The root growth method considers two Al tolerance parameters: root growth (RG) and a root tolerance index (RTI) (Baier et al., 1995). The RG parameter is measured root growth under Al stress while RTI is root growth under Al stress compared to root growth without Al stress. A low-ionic-strength nutrient solution combined with a low Al concentration is used, as evidence suggests that Al tolerance studies should be conducted using solutions containing ionic strength and Al activity approximating soil composition (Blamey et al., 1991). Assessment of Al tolerance based on root growth and RTI has been used extensively in genetic and molecular studies (Somers and Gustafson, 1995; Baier et al., 1996; Riede and Anderson, 1996; Somers et al., 1996).

Laboratory protocol for the root growth method

1. Sterilize seeds by placing in a 3% sodium hypoclorite solution for 5 minutes and rinsing thoroughly with water (Figure 3.1).

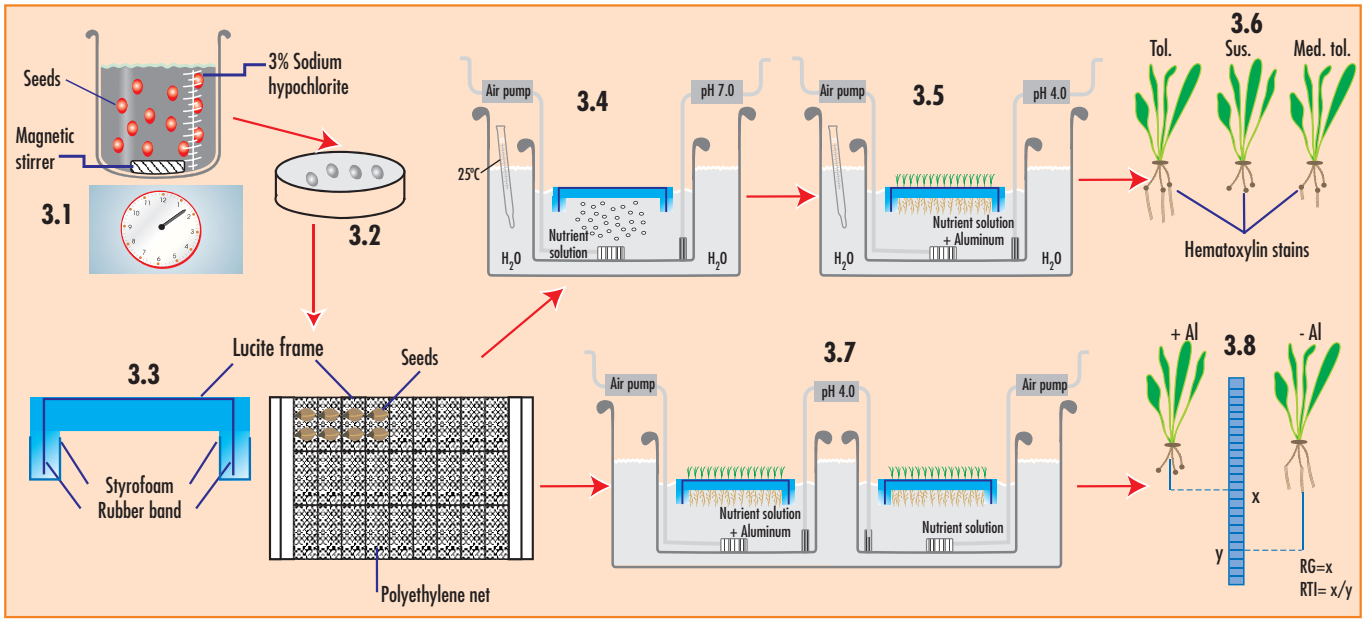

Figure 3. Laboratory procedures for nutrient solution methods.

2. Place seeds on moist filter paper in petri dishes for 84 hours at 7 °C and leave to germinate at room temperature (18-20 °C) for approximately 24 hours (Figure 3.2).
3. Place seedlings with similar root lengths (5-10 mm) and endosperm size on a polyethylene net fixed on lucite frames; attach styrofoam blocks to the frames with rubber bands so they will float (Figure 3.3).
4. Place frames in plastic containers with a low-ionic-strength nutrient solution (400 µM $CaCl_2$, 650 µM KNO_3, 250 µM $MgCl_2$, 10 µM $(NH_4)_2SO_4$, 40 µM (NH_4NO_3) containing Al and maintained at pH 4.0. As a control, a similar experiment but without Al in the medium is conducted at the same time. Replace solutions daily to minimize changes in pH and aluminum concentration. Leave containers for four days (preferentially in a growth chamber maintained at 25 °C, 16-hour day/ 8-hour night, and 70% relative humidity) (Figure 3.7).
5. Remove seedlings from trays; measure the longest primary root of each seedling and average, combining data within each genotype. RG is calculated as the mean root growth of seedlings after four days in a solution containing Al, while RTI is calculated as the ratio between RG and the mean root growth of seedlings after four days' growth in a solution without Al (control) (Figure 3.8).

Comparing the hematoxylin and root growth methods

The optimal Al concentration for screening genotypes depends on the plant species being evaluated. Since rye is more Al tolerant than wheat, the optimum Al concentration for rye is higher than that for wheat. However, optimum Al concentration also depends on the purpose of screening. If it is part of an on-going breeding program and the aim is simply to identify the most Al tolerant plants, higher Al concentrations can be applied. However, if the purpose is to quantitatively characterize the Al tolerance of genotypes, a lower Al concentration has to be applied to better separate the germplasm. Hede et al. (2001a) found that with the hematoxylin method, the most appropriate Al concentration for separating rye populations was 50 mg/l. At higher Al concentrations very few rye plants showed root re-growth. With the root growth method, the best separation of genotypes was achieved using the lowest Al concentration (4 mg Al/l).

Several studies have demonstrated that the primary response to Al stress occurs in the roots. Root growth under Al stress is therefore used as an indirect estimate of Al tolerance in several screening techniques. However, Al tolerance as measured by root growth under Al stress is a combination of Al tolerance (Al tolerance alleles) and root vigor. Thus a relative scale like RTI should be a better indicator of root performance under Al stress, since it can eliminate genotype-specific differences in root growth and standardize comparisons between genotypes (Baier et al., 1995). Since RTI is the relative growth of the genotype in Al solution compared to its potential growth without Al, this parameter is a measure of Al tolerance alone.

To improve precision of the root growth method, Baier et al. (1995) suggest using seedlings with similar vigor, achieved by selecting seedlings with similar sized

endosperm and initial root length. Since seed age is also very important for plant and root vigor, seeds should be regenerated before evaluating for Al tolerance and other traits that may be affected by seed age. This ensures that differences in root growth are not due to differences in vigor caused by seed age (Hede et al., 2001a).

Hede et al. (2001a) compared the hematoxylin and root growth methods to determine whether they identify the same Al tolerant genotypes. Using the root growth method and evaluating genotypes in a solution with and without Al, five different classes of root growth under Al stress were identified, each with a specific combination of root vigor and Al tolerance (Figure 4).

Class 1 and 4 are both very Al tolerant because their RTI is close to, or equal to, 1. However, due to differences in root vigor, classes 1 and 4 will differ in RG values. An experiment considering RG only will never be able to identify class 4 as Al tolerant. Class 2 is a combination of high root vigor and intermediate Al tolerance. This combination results in high RG values but intermediate RTI values. Class 3 has a combination of intermediate root vigor and intermediate Al tolerance, resulting in intermediate RG and RTI values. Class 5 is Al susceptible with a combination of low root vigor and low Al tolerance, resulting in low RG and RTI values.

Measuring RG only will select genotypes with good root growth under Al stress. However, they are not necessarily the most Al tolerant genotypes, defined as those with the most favorable Al tolerance alleles. For example, the RG parameter will characterize classes 3 and 4 as equally Al tolerant, although the RTI reveals that class 4 is actually more tolerant (Figure 4).

Hede et al. (2001a) concluded that the only way to separate the effects of root vigor and Al tolerance is to include a non-stressed control in the experiment. However, due to its quick, inexpensive, and easy screening protocol, the hematoxylin method is still a very efficient way to evaluate large numbers of seedlings from segregating populations derived from elite germplasm. The root growth method (including the RTI parameter), in which the root length of every single plant has to be measured before and after Al treatment, is more laborious than the hematoxylin method. The extra cost of the root growth method is justified when screening new or exotic germplasm, such as genebank accessions. The RTI parameter will identify genotypes that may have superior alleles for Al tolerance, even though their genetic background may lack desirable agronomic characters such as plant and root vigor (e.g., class 4 genotypes).

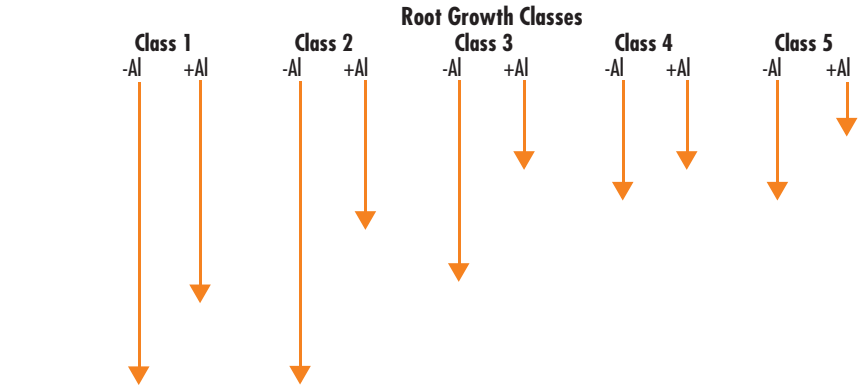

Figure 4. Five classes of root growth without Al stress (-Al) and with Al stress (+Al), and how the specific combination of root vigor and Al tolerance of each type translates into the Al tolerance parameters root growth and root tolerance index.
Source: Hede et al. (2001a).

Cell and tissue culture

The application of cell and tissue culture may offer a way of screening for Al tolerance, given that Al resistance can be expressed at the cellular level (Taylor, 1995). However, this methodology has not yet been substantially explored in wheat, possibly due to the technical difficulty of culturing cells in a low pH, Al-toxic medium (Carver and Ownby, 1995) and there are only a few examples in the literature in which cell and tissue culture was applied (Conner and Meredith, 1985; Parrot and Bouton, 1990).

Soil bioassays

Soil bioassays are not necessarily done on soils from the targeted production area, but screening in a soil representative of the targeted area could be a critical intermediate step, after screening in nutrient solution but before more tedious and expensive field evaluations (Carver and Ownby, 1995). Soil bioassays have a distinct advantage over nutrient solution culture when Al tolerance may be influenced by soil dependent external factors (Ring et al., 1993). The use of soil media has received

less attention than solution media for Al tolerance evaluation, and relatively few examples of its use can be found in the literature (Slootmaker, 1974; Stølen and Andersen, 1978).

Field evaluation

The ultimate and most direct method of evaluating for Al tolerance is by measuring economic yield (forage or grain) under field conditions. Field evaluation is normally conducted in two duplicate tests: one in an unamended and naturally acid plot, and the other in a lime-amended plot. The data are reported as the ratio of grain yield in the unamended plot to that in the lime-amended plot to adjust for differences in yield potential without acid soil stress (Carver and Ownby, 1995; Johnson et al., 1997).

The two most important problems observed when evaluating for Al tolerance in the field are the presence of fungal pathogens such as take-all (incited by *Gaeumannomyces graminis* var. *tritici*), in which infection is often favored by the application of lime to low pH soils (Johnson et al., 1997), or spatial variability of pH in the surface and sub-surface soil layers (Carver and Ownby, 1995). There are several examples of evaluating for Al tolerance in the field, but they are more expensive and laborious (Stølen and Andersen, 1978; Ruiz-Torres et al., 1992; Baier et al., 1995; Johnson et al., 1997).

Conclusions

The global demand for wheat and other crop species is increasing as a consequence of rapid population growth. Projections by the International Food Policy Research Institute (IFPRI) indicate that the world demand for wheat will rise from 552 million tons in 1993 to 775 millions tons by 2020 (Rosegrant et al., 1997). This is a total increase of about 40%, or 2% annually. To meet this future demand, productivity in both favorable and marginal environments needs to increase. Most opportunities for opening new agricultural land have already been exploited. However, opening the Brazilian Cerrados and similar areas in Latin America, Central Africa, and Southeast Asia could contribute greatly to raising world food production in the future.

To crop these large areas, a program aimed at developing Al tolerant cultivars and sound cropping practices must be implemented. The first step would be to identify Al tolerant genotypes through an efficient screening and evaluation process. Field evaluation, the ultimate and final test, is rather laborious, since the presence of acid soils in the field often is not very homogeneous. A number of quick and highly efficient laboratory techniques have thus been developed. As discussed earlier, which screening technique to use is strongly determined by the type of germplasm to be evaluated and the screening objective.

Due to its low cost and simple nature, the hematoxylin method is very efficient when working with large populations derived from well-adapted germplasm. However, when evaluating germplasm with superior Al tolerance alleles, but a poor agronomic background, and inferior plant and root vigor, the root growth method with the RTI parameter is the preferred screening method. The frequency of genotypes with superior Al tolerance alleles but poor root vigor is often high in exotic germplasm. Once identified, the Al tolerance alleles may be introgressed into a genotype possessing desirable agronomic characteristics via a backcrossing program using the inexpensive and less laborious hematoxylin method. The root growth method, including the RTI parameter, is also the most suitable approach for genetic and molecular studies requiring a precise quantitative response for Al tolerance.

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CHAPTER 16

Genotypic Variation for Zinc Efficiency

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Zinc deficiency is a common micronutrient deficiency in wheat and other cereals. It is estimated that about 50% of soils used for cereal production in the world have low levels of plant available Zn (Graham and Welch, 1996). Wheat shows substantial decreases in growth and grain yield under Zn-deficient field conditions (Graham et al., 1992; Cakmak et al., 1996a). Zinc deficiency in soils also reduces Zn concentration in wheat grain and diminishes its nutritional quality. Approximately 40% of world's population suffers from micronutrient deficiencies (the so-called "hidden hunger"), including Zn deficiency (Bouis, 1996; Graham and Welch, 1996). High consumption of cereal-based foods with low Zn content is considered to be one of the major reasons for the widespread occurrence of Zn deficiency in humans, especially in developing countries.

In bread and durum wheat, there is a wide range of genotypic variation in response to Zn deficiency (Graham et al., 1992; Cakmak et al., 1997a; 1998). Such large variation is promising for developing wheat genotypes that make more efficient use of Zn, which constitutes a sustainable solution to the problem of Zn deficiency. Zinc-efficient genotypes show better growth and higher grain yield under Zn-deficient conditions than other genotypes.

Breeding wheat for Zn efficiency is a process that involves taking into account many inter-related and complex issues. This chapter gives a broad review of these issues by concentrating on: 1) the occurrence, distribution, prediction, and correction of Zn deficiency, 2) the extent of genotypic variation for Zn efficiency, 3) the description of mechanisms involved in the expression of Zn efficiency, 4) methods useful in screening genotypes for Zn efficiency, 5) the genetics of Zn efficiency, and 6) Zn and phytic acid bioavailability in grain.

Zinc efficiency, as used in this paper, is defined as the ability of a genotype to grow and yield better than other genotypes in soils deficient in Zn (Graham, 1984).

Distribution of Zinc-Deficient Soils

Zinc deficiency is widespread throughout the world, occurring in different climate regions and almost all countries (Sillanpää, 1982; Sillanpää and Vlek, 1985). Usually, Zn deficiency in crop plants occurs either in acid and highly leached soils with low Zn content or in calcareous soils with Zn that for the most part is not available to plants. Zinc deficiency occurs more frequently in calcareous soils with high pH such as those found in arid and semiarid regions, and in the Mediterranean region. Based on the analysis of 298 (Sillanpää and Vlek, 1985) and 1511 (Eyüpoglu et al., 1994) soil samples, Zn deficiency has been identified as the most widespread micronutrient deficiency in Turkey, particularly in Central Anatolia. Soils in this region are highly alkaline, organic matter content is low (mostly below 1.5%), and annual precipitation is 300-400 mm (Cakmak et al., 1996a). Zinc deficiency has also been reported in Australia (Graham et al., 1992) and India (Takkar et al., 1989) in a wide range of soil types and crop plants.[3]

Soil and Climatic Factors Causing Zinc Deficiency in Plants

Soil pH is the most decisive factor affecting availability of Zn to plant roots. Increases in soil pH stimulate Zn adsorption to surfaces of various soil constituents, such as metal oxides and clay minerals; this results in substantial decreases in solubility and, hence, reduced availability of Zn to plants (Brümmer et al., 1988; Barrow, 1993). High pH decreases desorption of Zn from soil surfaces, which also limits availability of Zn to plants (Dang et al., 1993). The desorption rate of Zn from soil surfaces is essential for maintaining a continuous supply of Zn to plants.

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3 For detailed information on the distribution of Zn deficiency throughout the world, see Welch et al. (1991) and Takkar and Walker (1993).

Precipitation of Zn in high pH soils also lowers Zn availability to plant roots. At high pH, Zn can precipitate in the form of $ZnCO_3$, $Zn(OH)_2$, and Zn_2SiO_4 (Ma and Lindsay, 1993). Zinc concentration in soil solution is, therefore, largely dependent on pH. At pH 5.0, the concentration of Zn in soil solution is about 10^{-4} M, and at pH 8.0 it is 10^{-10} M (Lindsay, 1991). Asher (1987) reported that adequate Zn concentration in soil solution for a number of crop species ranges between $6x10^{-8}$ M and $8x10^{-6}$ M. However, in most cases calcareous soils cannot maintain this level of Zn concentration in solution, which increases the risk of Zn deficiency.

Liming of acid soils to increase pH raises the risk of Zn deficiency in plants. An increase in soil pH from 5.2 to 6.8 by liming results in about a 10-fold decrease in Zn concentration in plants (Parker and Walker, 1986).

Other factors that contribute to Zn deficiency in plants are low organic matter, low soil water regime, low soil temperature, and high light intensity (Moraghan and Mascagni, 1991; Marschner, 1993). Zinc transport from soil solution to roots occurs mostly by diffusion (Wilkinson et al., 1968). Since diffusion of Zn in soils is highly dependent on soil moisture content, Zn nutrition of plants may be at risk in arid and semiarid regions where soils, particularly topsoil, are usually deficient in water for long periods during the growing season. Accordingly, it has been shown that wheat yield reductions in Zn-deficient calcareous soils are more severe under rainfed than irrigated conditions (Table 1; Ekiz et al., 1998).

Zinc diffusion rate is also influenced by organic matter. An adequate level of organic matter is believed to increase solubility and Zn diffusion rate in soils (Sharma and Deb, 1988; Moraghan and Mascagni, 1991). In field experiments with wheat, Zn uptake was found to be positively correlated with the level of soil organic matter (Sillanpää, 1982; Hamilton et al., 1993). In addition, the occurrence of Zn deficiency in plants can be accelerated in areas where topsoil is removed by land leveling, erosion, or terracing (Martens and Westermann, 1991).

Increased phosphorus fertilization can induce Zn deficiency in wheat. A broadcast application of 160 kg P ha^{-1} significantly reduced Zn concentration in wheat (Wagar et al., 1986). High supply of P can reduce colonization of roots by vesicular-arbuscular (VA) mycorrhizae and, hence, Zn uptake by roots in wheat (Singh et al., 1986). The role of VA mycorrhizae in Zn uptake in wheat plants is well documented (Swamvinathan and Verma, 1979; Marschner, 1993). High P supply can also induce Zn deficiency by decreasing the physiological availability of Zn at the cellular level (Cakmak and Marschner, 1987).

Zinc deficiency is more prevalent under low soil temperature conditions. This effect is due to reductions in root growth, VA mycorrhizal infection, Zn uptake by roots, and Zn translocation into shoots (Moraghan and Mascagni, 1991). High light intensity is another climatic factor that promotes the development of Zn deficiency symptoms in wheat, particularly in durum wheat (Cakmak et al., 1996b). This light effect can be explained by Zn deficiency-induced photooxidation in leaves, and therefore it is pronounced at low air temperatures (Cakmak et al., 1995).

Table 1. Effects of different Zn applications on grain yield of wheat cultivars grown in a Zn-deficient calcareous soil under rainfed and irrigated conditions. +Zn refers to the mean of the grain yields obtained with Zn applications of 7, 14 and 21 kg Zn ha^{-1}, as the differences between the Zn doses were not significant.

	Rainfed			Irrigated		
	Grain yield		Increase	Grain yield		Increase
Cultivars	- Zn	+Zn	in yield	- Zn	+Zn	in yield
	kg ha^{-1}		%	kg ha^{-1}		%
Bread wheat						
Gerek 79	3100	4460	44	4070	5250	29
Bezostaja-1	1900	4150	118	3190	5080	59
Durum wheat						
Kunduru 1149	330	2470	648	1550	3580	130
Cakmak 79	170	760	347	630	2870	355

Source: Ekiz et al. (1998).

Predicting Zinc Deficiency through Soil Analyses

As reviewed by Sims and Johnson (1991), several soil testing procedures are available for predicting Zn availability in soils and response of plants to Zn fertilization. Among these tests, the DTPA (diethylenetriamine pentaacetic acid) method is widely used for predicting plant-available Zn in soils, particularly in calcareous soils (Lindsay and Norvell, 1978). Increases in DTPA-extractable Zn in soils from 0.1 mg kg^{-1} soil to 0.6 mg kg^{-1} soil are associated with increases in grain yield and Zn concentration in plants (Sillanpää, 1982; Cakmak et al., 1996a). Field experiments at six different locations in Central Anatolia have shown that wheat grown in calcareous soils containing less

than 0.4 mg kg^{-1} DTPA-extractable Zn can respond to soil Zn applications (23 kg Zn ha^{-1}) with increases in grain yield (Cakmak et al., 1996a). In 20 field experiments on farmers' fields in India, Zn fertilization of wheat at the rate of 5 kg Zn ha^{-1} enhanced grain yield in locations where DTPA-extractable Zn was below 0.6 mg kg^{-1} soil (Dwivedi and Tiwari, 1992). In contrast, Zn fertilization had no significant effect on grain yield of wheat and barley in Saskatchewan in soils containing less than 0.5 mg kg^{-1} soil of DTPA-extractable Zn (Singh et al., 1987).

These contrasting results may be due to differences in soil conditions or genotypic differences in root-induced changes in Zn solubility and uptake (see "Screening for Zinc Efficiency" on p. 188). According to Brennan (1996), the critical DTPA-extractable Zn concentration for wheat-growing acidic soils in Australia is 0.25 mg kg^{-1}; Zn application to soils having more than 0.25 mg extractable Zn per kilogram of soil is not effective for increasing wheat yields.

Correcting Zinc Deficiency

Soil applications of zinc

Zinc deficiency can be corrected by applying Zn to soils or plant foliage as well as by treating seeds with Zn. Both organic and inorganic Zn fertilizers are used to correct Zn deficiency. Zinc sulphate ($ZnSO_4$) is the most common source of Zn fertilizer because of its high solubility in water, existence in both crystalline and granular forms, and its low cost compared to synthetic Zn chelates such as ZnEDTA (Martens and Westermann, 1991; Mordvedt and Gilkes, 1993). In most instances, Zn deficiency in plants can be corrected by broadcast applications of Zn at 4.5 to 34 kg ha^{-1} as $ZnSO_4$ (Martens and Westermann, 1991). Differences in rates of Zn application depend mainly on variations in DTPA-extractable Zn in soils and the severity of Zn deficiency symptoms. Ekiz et al. (1998) found that broadcast applications of $ZnSO_4$ at the rate of 7 kg Zn ha^{-1} to severely Zn-deficient calcareous soils significantly enhanced wheat yield, but that increasing applied Zn from 7 to 21 kg ha^{-1} did not result in an additional increase in grain yield. Compared to ZnO, $ZnSO_4$ seems to be more effective in improving Zn nutrition of wheat plants under deficient supply of Zn (Sharma at al., 1988).

Foliar applications of zinc

Foliar applications of Zn are effective in correcting Zn deficiency in plants. As reported by Martens and Westermann (1991), 0.5 to 1.0 kg Zn ha^{-1} as $ZnSO_4$ or 0.2 kg Zn ha^{-1} as ZnEDTA is often used to correct Zn deficiency in plants. Depending on growth stage of wheat, foliar applications of ZnEDTA and $ZnSO_4$ at the rate of 400-450 g Zn ha^{-1} are either equally effective or ZnEDTA is superior to $ZnSO_4$ in correcting Zn deficiency in field-grown wheat (Brennan, 1991).

However, soil applications of Zn are more effective than foliar applications in correcting Zn deficiency (Yılmaz et al., 1997). In field experiments with four wheat cultivars grown in Zn-deficient soil (DTPA-Zn: 0.1 mg kg^{-1} soil) different Zn application methods were tested for their effectiveness in correcting Zn deficiency (Table 2) (Yılmaz et al., 1997). The highest increase in grain yield was obtained from soil, soil+leaf, and seed+leaf applications, while seed only and leaf only applications were less effective. Soil application alone was considered the most economical method on a long-term basis. Soil+leaf applications can be effective when both high grain yield and high Zn concentration in grain are desired (Yılmaz et al., 1997). Since Zn applied to soils has a great residual effect, it is not necessary to apply Zn every year (Martens and Westermann, 1991).

Sowing seeds with higher zinc content

There is increasing evidence that sowing seeds containing higher amounts of Zn can be a practical solution to alleviate wheat yield depression under Zn-deficient conditions. In greenhouse

Table 2. Effects of different zinc application methods on grain yield of bread wheat cultivars Gerek-79, Dagdas-94, and Bezostaja-1 and durum wheat cultivar Kunduru-1149 grown in a Zn-deficient calcareous soil.

Application methods	Gerek-79	Dagdas-94	Bezostaja-1	Kunduru-1149	Mean	Increase by Zn application
			kg ha^{-1}			%
Control†	738	633	805	56	558	-
Soil‡	2700	2225	2340	903	2042	265
Seed§	2052	1997	1958	772	1695	204
Leaf#	1472	1365	1555	617	1253	124
Soil + Leaf††	2712	1955	2330	818	1954	250
Seed + Leaf‡‡	2768	2100	2380	987	2059	268

† Control (no Zn application).
‡ 23 kg Zn ha^{-1} as $ZnSO_4$.
§ 1 liter of 30% $ZnSO_4$ for 10 kg seed.
# 2x220 g Zn ha^{-1} as $ZnSO_4$ in 450 liters during tillering and stem elongation.
†† Combination of methods 2 and 4.
‡‡ Combination of methods 3 and 4.
Source: Yılmaz et al. (1997).

experiments with low seed-Zn (250 ng Zn per seed) and high seed-Zn (700 ng Zn per seed), wheat plants derived from the high seed-Zn have better seedling vigor and grain yield (Rengel and Graham, 1995a,b). In a field with Zn-deficient soils, wheat seeds with low Zn content (355 ng Zn seed^{-1}) had a significantly lower grain yield compared to seeds with medium (800 ng Zn seed^{-1}) and high (1465 ng Zn seed^{-1}) Zn content (Table 3).

Grain yield differences of 18% and 116% are of such magnitude that genotypic differences in Zn efficiency are masked when seeds with great differences in Zn content are used. **It is therefore absolutely crucial to use seeds from the same source or at least with similar Zn content in experiments related to screening genotypes for Zn efficiency**. However, high seed-Zn content could not match the effect of soil Zn application on grain yield (Table 3). Nevertheless, the use of seed with high Zn content could provide a practical solution to the problem, especially where farmers are not aware of Zn deficiency, and Zn applications are not practiced.

Cellular Functions of Zinc

In Zn-deficient plants, a number of critical cellular functions and processes are impaired, leading to severe reductions in growth and development (Brown et al., 1993). The adverse effects of Zn deficiency occur more distinctly in meristematic tissues, where intensive cell division and elongation take place. There is a high specific requirement for Zn in meristematic tissues, especially for maintenance of protein synthesis (Kitagishi et al., 1987; Cakmak et al., 1989).

More than 300 enzymes are Zn dependent (Coleman, 1992). Zinc plays catalytic, co-catalytic, and structural roles in these enzymes. The enzyme superoxide dismutase (SOD) has attracted much attention in recent years. An SOD isoenzyme, copper-zinc SOD (CuZn-SOD), is required for protecting plant cells against toxic superoxide radical ($O_2^{\cdot-}$); activity of this enzyme is very low in plants under Zn-deficient conditions (Cakmak and Marschner, 1988b,c; 1993). Measurement of CuZn-SOD is suggested as a tool for screening cereals for Zn efficiency (see “Internal utilization” on p. 192).

Zinc plays a decisive role in DNA and RNA metabolism, chromatin structure, and gene expression (Vallee and Falchuk, 1993). Several Zn-containing proteins are known to be involved in DNA replication and gene transcription processes (Klug and Rhodes, 1987). The role of Zn in regulating gene expression is currently an exciting research area.

The structural and functional integrity of cellular membranes is greatly affected by the lack of Zn, which plays both structural and protective roles in membrane integrity (Welch et al., 1982). Zinc can stabilize membranes by binding to phospholipids and sulphydryl groups of cell membranes, thereby protecting these compounds against oxidative damage (Marschner, 1995; Cakmak and Marschner, 1988b,c; Welch and Norwell, 1993; Rengel, 1995a). When these compounds undergo oxidative damage, membrane integrity of Zn-deficient plant cells is impaired, and leakage of ions from Zn-deficient root cells is increased (Welch et al., 1982). Several plant species, including wheat, showed a dramatic increase in the exudation of several organic compounds, such as carbohydrates and amino acids, under Zn deficient conditions (Table 4; Cakmak and Marschner, 1988a). Due to the increased leakage of carbon containing compounds in the rhizosphere, Zn-deficient plants may be susceptible to root diseases such as *Fusarium graminearum* (Sparrow and Graham, 1988), *Gaeumannomyces graminis* (Brennan, 1992), and *Rhizoctonia solani* (Thongbai et al., 1993).

Zinc deficiency also affects metabolism of phytohormones, especially indoleacetic acid (IAA). The concentration of IAA is reduced by Zn deficiency, and this reduction is accompanied by decreases in shoot elongation (Skoog, 1940; Cakmak et al.,

Table 3. Effect of seed zinc content on grain yield in two cropping seasons under rainfed conditions in a Zn-deficient soil with (+Zn=23 kg Zn ha^{-1}) and without Zn (-Zn) fertilization.

	1994-1995 cropping season		1995-1996 cropping season	
Seed Zn content	-Zn	+Zn	-Zn	+Zn
ng seed^{-1}	kg grain ha^{-1}			
355	480	2720	2490	3180
800	920	3170	2930	3180
1465	1040	2840	2950	3220

Source: Yılmaz et al. (1998)

Table 4. Effect of zinc nutritional status of wheat plants on root exudation of amino acids, sugars, and phenolics.

Zn supply	Amino acids	Sugars	Phenolics
	μg g^{-1} root dry wt.6h^{-1}		
- Zn (deficient)	48±3	615±61	80±6
+ Zn (sufficient)	21±2	315±72	34±6

Source: Cakmak and Marschner (1988a).

1989). According to Suge et al. (1986), besides IAA, concentration of gibberelin-like substances are also decreased in Zn-deficient plants.

Diagnosing Zinc Deficiency

Visible symptoms of zinc deficiency

Diagnosing Zn deficiency based on visible symptoms is not always easy. Symptom development is highly dependent on climatic conditions and varies greatly among plant species. In some instances, diagnosis of Zn deficiency can be complicated by the simultaneous occurrence of toxicity of a nutrient such as P (Webb and Loneragan, 1988), intense light-induced chlorophyll damage, (Marschner and Cakmak, 1989; Cakmak et al., 1995), and virus infection (Bergmann, 1992).

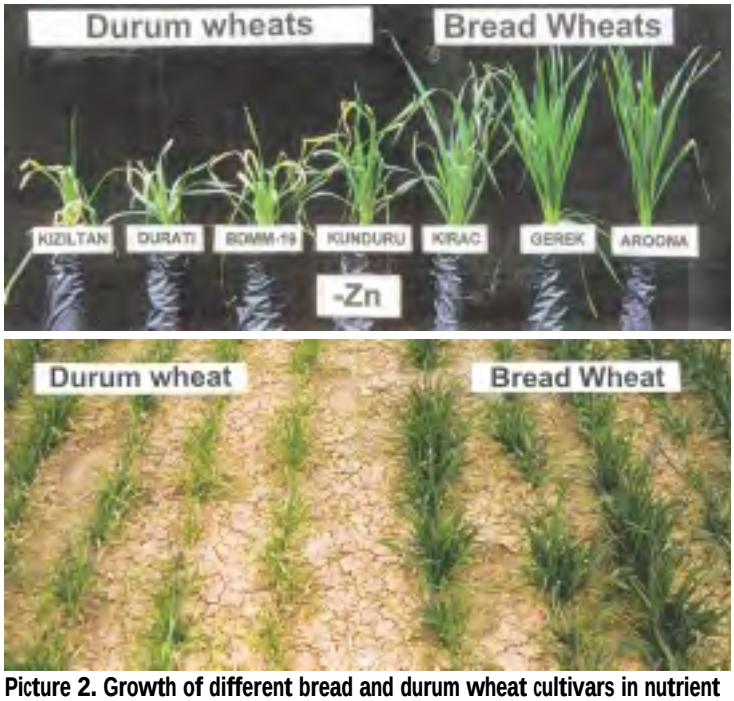
The first and most characteristic reaction of wheat plants to Zn deficiency is a decrease in shoot elongation and leaf size. These symptoms are followed by development of whitish-brown patches and then necrotic lesions on the leaf blades, predominantly in the middle and older leaves (Picture 1; Cakmak et al., 1996a,b). As severity intensifies, necrotic lesions on leaves spread, and leaf blades often collapse in the middle and take on a "scorched" appearance. In most cases, the basal part of those same leaves remains green. Young leaves are small in size and become yellowish green in color, but show no necrotic lesions. Similar visible symptoms of Zn deficiency in wheat have been reported by Dang et al. (1993) and Rengel and Graham (1995c).

The development period and severity of Zn deficiency symptoms vary greatly between durum and bread wheats (Picture 2). Decreases in shoot elongation and appearance of necrotic patches on leaves occur more rapidly and severely in durum wheats than in bread wheats (Cakmak et al., 1994, 1997a; Rengel and Graham, 1995 c,d).

Critical zinc concentrations in plants

Critical Zn concentration is influenced by plant development stage and leaf age (Table 5). In most cases, the critical Zn concentration in leaves or

Picture 1. Development of Zn deficiency necrotic lesions on wheat leaves.



Picture 2. Growth of different bread and durum wheat cultivars in nutrient solution (above) or Zn-deficient calcareous soil (below) without Zn supply.

Table 5. Critical concentrations of zinc in different tissues and development stages of wheat.

Leaf	Stage	Zn concentration	Reference
		mg kg^{-1} dry wt	
Youngest leaf blade	tillering	11	Reuter and Robinson (1986)
Youngest leaf blade	post anthesis	7	Reuter and Robinson (1986)
Youngest leaf	anthesis	16	Dong et al. (1993)
Youngest leaf	tillering	17	Riley et al. (1992)
Youngest leaf	milk stage	7	Riley et al. (1992)
Mature leaf	-	17	Rashid and Fox (1992)
Whole shoots	tillering	10-15	Graham et al. (1992)
Whole shoots	tillering	10-15	Cakmak et al. (1997a)
Grains	mature	15	Viets et al. (1966)
Grains	mature	15	Rashid and Fox (1992)

whole shoots varies considerably among cereals and among cultivars of a given cereal, such as wheat. Under Zn-deficient conditions, wheat cultivars show distinct differences in sensitivity to Zn deficiency and in yield reductions caused by the deficiency. However, such genotypic differences are not related to Zn concentration in leaves or in shoots (Graham et al., 1992; Cakmak et al., 1997a; 1998). Total Zn concentration is therefore not always a reliable parameter for diagnosing Zn deficiency in wheat. Apparently, genotypes containing similar total Zn concentration under Zn-deficient conditions may differ in use of Zn at the cellular level. Therefore, measuring the activity of Zn-containing enzymes can be a better tool for diagnosing the Zn nutritional status of plants, as shown in wheat by measuring the activity of carbonic anhydrase (Rengel, 1995b) and superoxide dismutase (Cakmak et al., 1997b) in leaves.

Measuring Zn in grains is suggested to be indicative of Zn nutritional status of plants. According to Rashid and Fox (1992) the critical Zn concentration in wheat is 17 mg kg^{-1} dry weight for recently maturated leaves of seedlings and 15 mg kg^{-1} dry weight for grains. Viets (1966) suggested that the critical level of Zn in grains is 15 mg kg^{-1} dry weight. Several authors (Graham and Rengel, 1993; Cakmak et al., 1997a) have found that Zn concentration in grain is not always a good indicator of the Zn nutritional status of plants under Zn-deficient conditions.

Screening for Zinc Efficiency

Currently, the development of genotypes with high Zn efficiency is attracting increasing interest worldwide. Zinc-efficient genotypes may provide a number of benefits, such as reductions in the use of fertilizers, improvements in seedling vigor, resistance to pathogens, minimization of yield losses, increased yields, and enhancement of grain nutritional quality (Graham and Rengel, 1993; Bouis, 1996; Graham and Welch, 1996).

Genotypes are usually selected for Zn use efficiency based on the expression of Zn deficiency symptoms in leaves and decreases in shoot dry matter or grain yields. Zinc efficiency can be calculated as the ratio of yield (shoot dry matter or grain yield) produced under Zn deficiency (-Zn) to yield produced with Zn fertilization (+Zn), as follows (Graham, 1984):

[Zn efficiency = (yield at –Zn/yield at +Zn) x 100]

When comparing Zn-efficiency ratios of cultivars with similar yield capacity under Zn-deficient conditions, it should be kept in mind that cultivars with the smallest response to Zn fertilizer will have the highest Zn-efficiency ratio. Cultivars with low response to Zn application are often landraces (M. Kalayci et al., unpublished).

Various mechanisms have been studied to explain differences in Zn efficiency. The results reported in the literature suggest that more than one mechanism is involved in the expression of Zn efficiency in plants (Graham and Rengel, 1993; Cakmak et al., 1998). A better understanding of the morphological, physiological, and genetic bases of Zn efficiency is required for developing rapid and reliable screening procedures to be used in identifying and breeding genotypes with high Zn efficiency (Table 6).

Leaf symptoms

Zinc efficiency of genotypes can be determined based on phenotypic manifestations such as severity of visible deficiency symptoms on leaves. Zinc deficiency symptoms seem to be a useful criterion to identify Zn-efficient genotypes. The severity of zinc deficiency symptoms (i.e., intensity of whitish-brown necrotic patches on leaves, Picture 1) can be evaluated using a scale from 1 (severe symptoms) to 5 (slight or no symptoms). Actually, a good correlation between the severity (score) of Zn deficiency symptoms and calculated Zn efficiency ratios has been found in cultivars of wheat, barley, oat, and rye (Cakmak et al., 1997b; Schlegel et al., 1998). However, caution should be exercised when assessing Zn efficiency based on leaf symptoms (see “Visible symptoms of zinc deficiency” on p. 187), and using them together with other criteria such as the Zn efficiency ratio and total Zn content is recommended (see below). Scoring Zn deficiency symptoms is quick and easy,

Table 6. Plant traits used in screening wheat genotypes for zinc efficiency.

Plant trait	Growth conditions†	Reference
Scoring Zn deficiency symptoms	N, G, F	Cakmak et al. (1997a, b), Schlegel et al. (1997)
Shoot/root dry weight	N	Rengel and Graham (1995c); Cakmak et al. (1996b)
Fine roots with diameter ≤0.02	N, G	Dong et al. (1995); Rengel and Wheal (1997a)
Synthesis of a 34-kDa polypeptide	N	Rengel and Hawkesford (1997)
Zn amount (content) per shoot	F	Graham et al. (1992); Cakmak et al. (1997a)
Zn uptake (content) per shoot	G	Cakmak et al. (1997b, c)
Zn uptake (content) per plant	N	Cakmak et al. (1996b)
Zn uptake per root dry weight	N	Cakmak et al. (1997b); Rengel and Wheal (1997b)
Zn translocation into shoot	N	Rengel and Graham (1995d); Cakmak et al. (1996b)
^{65}Zn translocation into shoot	N	Cakmak et al. (1997b)
SH-groups of plasma membranes	N	Rengel (1995b)
Release of phytosderophores	N	Cakmak et al. (1994); Walter et al. (1994)
Carbonic anhydrase	N	Rengel (1995a)
Superoxide dismutase	G	Cakmak et al. (1998)

† N: Nutrient solution, G: Greenhouse, F: Field.

and allows screening large numbers of genotypes with no special equipment.

Shoot and root growth

In Zn-deficient nutrient solution, Zn-efficient wheat cultivars are characterized by higher shoot dry matter production and a better shoot:root dry weight ratio compared to Zn-inefficient cultivars (Rengel and Graham, 1995c; Cakmak et al., 1996b). However, we found that Zn-efficient genotypes have lower shoot:root dry weight ratios than Zn-inefficient genotypes in Zn-deficient calcareous soils (Cakmak et al., unpublished results).

Genotypic differences in zinc uptake

In general, Zn-efficient wheat genotypes show either enhanced Zn uptake efficiency by roots, or enhanced Zn utilization efficiency within the plant, or both. Enhanced Zn uptake might be affected by root surface area, root colonization by VA mycorrhizae, reduction in rhizosphere pH, release of Zn-mobilizing compounds such as phytosiderophores (PS) from roots, and induction of polypeptides involved in Zn uptake and transport across the plasma membranes.

In field experiments on Zn-deficient calcareous soils, Zn efficiency is positively correlated with total amount (uptake) of Zn in shoots (Graham et al., 1992; Cakmak et al., 1997a). Total amount of Zn per plant can be calculated as:

Total plant dry weight (kg) x Zn concentration (mg Zn kg^{-1} dry weight)

Under deficient supply of Zn in nutrient solution, bread wheat cultivars with fewer Zn deficiency symptoms contained about 42% more Zn per shoot (or 29% more Zn per plant) than durum wheat cultivars showing very severe Zn deficiency symptoms (Cakmak et al., 1996b). Despite their higher Zn accumulation capacity per plant, Zn-efficient genotypes do not contain more Zn per unit dry weight of plants (see "Zinc concentration in tissues" on p. 190). However, under sufficient Zn supply, Zn-efficient and Zn-inefficient cultivars are not different in total Zn uptake (Cakmak et al., 1997a). Recently, Rengel and Wheal (1997b) clearly demonstrated in short-term uptake experiments that differences in Zn efficiency between wheat cultivars are closely related to differences in Zn uptake capacity (Table 7). Increases in Zn uptake capacity of Zn-efficient genotypes can also be demonstrated by measuring uptake of labelled Zn (^{65}Zn) per root dry weight in nutrient solution experiments (Cakmak et al. 1997b; Rengel et al., 1998).

Root-to-shoot zinc translocation

Under Zn-deficient conditions, Zn-efficient genotypes translocate more Zn from roots to shoots than Zn-inefficient genotypes, but not under Zn-sufficient conditions (Rengel and Graham, 1995d; Cakmak et al., 1996b). The ability of genotypes to translocate Zn from roots to shoots can be estimated as mg Zn $shoot^{-1}$/mg Zn whole $plant^{-1}$ or µg Zn $shoot^{-1}$/g root dry wt.

In rye, which is very Zn-efficient, shoot growth and grain yield are not affected by Zn deficiency or Zn fertilization (Cakmak et al., 1997a; 1998). The high Zn efficiency of rye is closely related to its ability to absorb Zn and translocate it to shoots at much greater rates than other cereals (Cakmak et al., 1997a, b).

These results indicate that enhancements of Zn uptake by roots and its translocation into shoots under deficient supply of Zn are of major importance for expression of Zn efficiency. Determining total Zn uptake per shoot or per plant is

Table 7. Maximum rate of net zinc uptake (Imax) by wheat cultivars Excalibur (Zn-efficient bread wheat), Gatcher (moderately Zn-efficient bread wheat), and Durati (Zn-inefficient durum wheat) grown in nutrient solution.

Cultivar	Zn uptake rate, Imax
	µg Zn g^{-1} root dry $wt.h^{-1}$
Excalibur	0.36±0.04
Gatcher	0.24±0.03
Durati	0.10±0.02

Source: Rengel and Wheal (1997b).

recommended in screening studies. Zinc uptake capacity of genotypes is not always a good parameter for determining Zn efficiency when calculated per root dry weight instead of per plant (Rengel and Graham, 1995d).

Zinc concentration in tissues

Although Zn-efficient genotypes possess higher Zn uptake capacity, they do not necessarily have higher Zn concentration (amount of Zn per unit dry weight) in leaf or shoot tissue, or grain (Graham et al., 1992). Zinc-inefficient wheat genotypes may even contain higher Zn concentrations in leaves or grains than Zn-efficient genotypes (Rengel and Graham, 1996; Cakmak et al., 1997a, b; 1998). Enhanced Zn uptake by efficient genotypes under Zn deficiency improves dry matter production and results in corresponding decreases of Zn concentration in tissues to concentrations similar to those present in Zn-inefficient genotypes ("dilution by growth," Marschner, 1995). In Zn-deficient soil, the most Zn-efficient rye has lower Zn tissue concentration (mg Zn kg^{-1} tissue) at heading than a Zn-inefficient durum wheat. However, total amount of Zn per shoot is about four times higher in rye than in durum wheat, since rye produces four times more shoot dry matter yield under Zn deficiency (Cakmak et al., 1997a). Therefore, we do not recommend using Zn concentration in leaves, shoots, or grains as criteria to select for Zn-efficient wheat cultivars.

Root morphological properties

Mycorrhizae play an important role in Zn uptake (Marschner, 1993; 1995). However, to our knowledge, genotypic differences in mycorrhizal colonization under Zn-deficient conditions are not reported in the literature. We recently showed that Zn-efficient and Zn-inefficient wheat cultivars grown in Zn-deficient soils do not differ in mycorrhizal colonization of roots at flowering at 0-30 cm soil depth (unpublished results).

Dong et al. (1995) and, more recently, Rengel and Wheal (1997a) showed that Zn-efficient wheat cultivars possess more root surface area and a greater proportion of fine roots (≤0.02 mm in diameter) than Zn-inefficient wheat cultivars (Table 8). Obviously, a greater proportion of fine roots in the total root biomass would allow more efficient contact with soil constituents, which in turn would contribute to efficient Zn uptake. Dong et al. (1995) and Rengel and Wheal (1997a) used the same bread wheat and durum wheat cultivars in their studies. Testing more bread and durum wheat cultivars and alien species is necessary before any root properties can be recommended for use as selection criteria in an applied breeding program.

Membrane effects on zinc uptake

Increased Zn uptake capacity of Zn-efficient genotypes has been related to induced *de novo* synthesis of the 34-kDa polypeptide in plasma membranes of root cells (Rengel and Hawkesford, 1997). Synthesis of this polypeptide occurs only under Zn deficiency conditions in Zn-efficient bread wheats, but not in Zn-inefficient durum wheats. There is some correlation between increase in Zn uptake and increase in 34-kDa polypeptide synthesis in the presence of Zn deficiency. Rengel and Hawkesford (1997) suggest that this polypeptide might be a structural or regulatory unit of a putative plasma membrane Zn transporter and may therefore be connected with the Zn uptake process. There is promise in the use of such specific polypeptides for screening genotypes for Zn efficiency.

Table 8. Length of roots (diameter ≤0.2 mm) and average root surface area of 24-day-old wheat cultivars Excalibur (Zn-efficient bread wheat), Gatcher (moderately Zn-efficient bread wheat), and Durati (Zn-inefficient durum wheat) grown in nutrient solution.

Cultivar	Root length	Average root surface area
	m $plant^{-1}$	mm^2 $plant^{-1}$
Excalibur	2.1	3350
Gatcher	1.6	2300
Durati	1.3	1950

Source: Data calculated from Rengel and Wheal (1997a).

The higher Zn uptake capacity of Zn-efficient genotypes might be attributable to a greater amount of sulfhydryl groups in root-cell plasma membranes, particularly in ion transport-related proteins (Rengel, 1995a; Rengel and Wheal, 1997b). Maintenance of higher levels of sulphydryl groups in ion transport proteins or ion channels of plasma membranes is considered important for Zn absorption into cells (Kochian, 1993; Welch, 1995).

Phytosiderophores

Release of phytosiderophores (PS) may be an adaptive response of graminaceous species to Zn and Fe deficiency. Phytosiderophores, also called phytometallophores (Welch, 1995), are non-proteinogenic amino acids released from roots of graminaceous species under deficiency of Fe (Takagi, 1976; Marschner et al., 1986) and Zn (Zhang et al., 1989). Phytosiderophores released from roots are highly effective in complexing and mobilizing Zn in calcareous soils (Treeby et al., 1989); they are involved in mobilizing Zn from root apoplast of wheat plants (Zhang et al. 1991) and, possibly, in solubility and long-distance translocation of Zn within plants (Figure 1; Welch, 1995).

Under Fe deficiency, the rate of PS release differs between and within cereal species. This release is closely related to

genotypic differences in tolerance to Fe deficiency in cereals (Takagi et al., 1984; Marschner et al., 1986, Jolley and Brown, 1989). Differences in Zn efficiency between durum and bread wheat cultivars are closely correlated to the rate of PS release (Table 9; Figure 2; Cakmak et al., 1994; Walter et al., 1994). Under Zn deficiency, the rate of PS release is about 6-8 times lower in Zn-inefficient durum wheats than in Zn-efficient bread wheats (Table 9; Cakmak et al., 1996c). Zinc-efficient cultivars also contain higher amounts of PS (mainly deoxymugineic acid) in root tissues under Zn deficiency (Figure 2). Enhanced PS synthesis and increased PS release from roots have been suggested as mechanisms that help wild grasses to adapt to severely Zn-deficient calcareous soils (Cakmak et al., 1996d).

Based on these results, selection of genotypes with high capacity to synthesize and release PS appears to be a promising screening method. However, PS release rate is not always positively related to differences in Zn efficiency among bread wheat genotypes (Erenoglu et al., 1996; Cakmak et al., 1997b). In addition, the rate of PS release of rye cultivars is not significantly higher than that of Zn-efficient or Zn-inefficient bread wheat cultivars (Table 9). Wheat releases PS 2'-deoxymugineic acid (DMA) and rye releases hydroxymugineic acid (HMA) (Mori, 1994; Cakmak et al., 1996d). Considering the exceptionally high Zn efficiency of rye (Cakmak et al., 1997a), it can be argued that compared to other PSs, HMA may be more efficient in Zn solubilization and mobilization both in the rhizosphere and within plants. This point needs to be further investigated.

Despite insignificant differences in their capacity to release PS, Zn-efficient and Zn-inefficient genotypes may differ in their capacity to absorb either Zn-complexed PS or Zn from Zn-PS complexes (Figure 1). Recently it was shown that the rate of PS release of two maize genotypes did not differ, while their uptake and root-to-shoot translocation of Zn-complexed PS (Zn-DMA) differed greatly (von Wiren et al., 1996).

The importance of PS in enhancing Zn uptake has been questioned, since PSs show greater affinity for Fe than Zn, and Fe exists in soils in larger amounts than Zn (Murakami et al., 1989; Ma and Nomoto, 1996). However, it should be stressed that in many instances small changes in Zn concentration in plant tissues (around 1 to 2 mg Zn kg^{-1} dry weight) seem to be decisive in improving plants under Zn-deficient conditions (Jones, 1991; Cakmak et al., 1997a). Thus

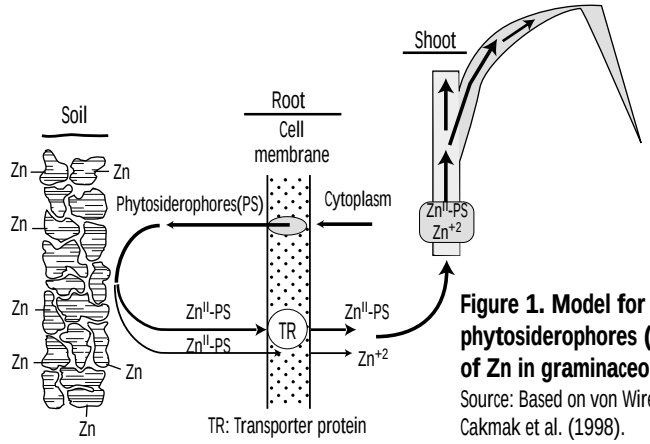


Figure 1. Model for release of phytosiderophores (PS) and uptake of Zn in graminaceous species.
Source: Based on von Wiren et al. (1996) and Cakmak et al. (1998).

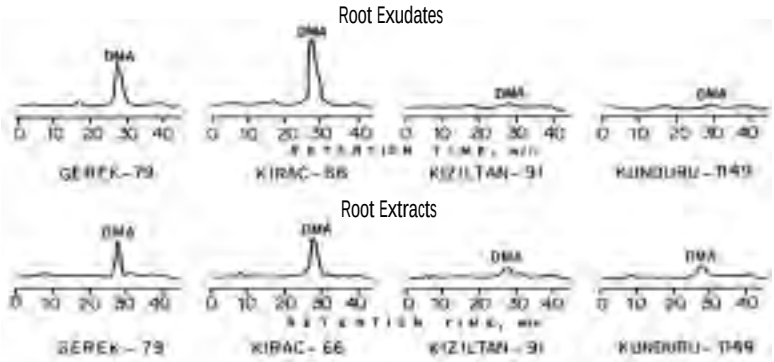


Figure 2. High pressure liquid chromatograms of root exudates and root extracts of Zn-deficient bread wheats Gerek-79 and Kırac-66 and Zn-deficient durum wheats Kızıltan-91 and Kunduru-1149.
Source: Cakmak et al. (1996b).

Table 9. Effect of zinc deficiency on the rate of phytosiderophore release from roots of various cereals grown for 14 days in nutrient solution without Zn supply.

Cereals	Leaf symptoms of Zn deficiency†	Release of phytosiderophores
		µmol 48 plants^{-1} 3h^{-1}
Secale cereale		
Aslım	5	11.4±2.3
Triticale		
Presto	5	8.0±3.3
Triticum aestivum		
Dagdas-94	3	9.3±1.8
Gerek-79	3	9.2±1.1
BDME-10	2	8.1±1.8
Partizanka Niska	2	5.5±1.0
Bul-63-68-7	2	7.2±2.4
Triticum durum		
Kızıltan-91	1	1.7±0.1
Kunduru-1149	1	1.2±0.1

† Leaf symptoms of Zn deficiency noted in Zn deficient calcareous soils in Central Anatolia: 1= very severe, 2=severe, 3= mild, 4= slight and 5= very slight or absent.
Source: Cakmak et al. (1997b).

just a small contribution of PS to Zn uptake by plants can greatly affect plant growth under Zn deficiency. As discussed below, PS can also influence Zn utilization at the cellular level.

In conclusion, measuring PS release in root exudates and also Zn uptake by the roots from Zn-PS complexes or directly in the form of the Zn-PS complex should be considered in evaluating genotypes for Zn efficiency. Collecting root exudates and measuring PS in root exudates are easy to do and can be applied to plants grown in nutrient solution. The rate of PS release can be measured either directly by using high pressure liquid chromatography (HPLC) or indirectly using Zn loaded resin (for methods see Zhang et al., 1989; Cakmak et al., 1994, 1996c; Walter et al., 1994).

Internal utilization

As mentioned before, the amount of Zn per unit dry weight of shoots or leaves is not different between Zn-efficient and Zn-inefficient genotypes under Zn-deficient conditions. Therefore, it can be argued that genotypic differences in Zn efficiency might also be related to differences in the internal utilization of Zn. Zinc-efficient genotypes might contain a higher proportion of Zn that readily participates in metabolic reactions and binds to critical cell compounds, such as Zn-requiring enzymes. Thus under Zn-deficient conditions and at same or similar Zn concentrations in leaves, Zn-efficient wheat genotypes demonstrate higher activity of Zn-requiring enzymes than Zn-inefficient wheat genotypes, as shown for carbonic anhydrase (Rengel, 1995b) and superoxide dismutase (SOD) (Cakmak et al., 1997b). These results indicate better Zn utilization at the cellular level in Zn-efficient genotypes. Higher SOD activity in Zn-efficient genotypes may better protect them against oxidative attack by toxic O_2 radicals. Despite time- and equipment-related constraints, enzyme analyses can contribute valuable information to gaining a better understanding of efficiency mechanisms and identifying genotypes with high Zn efficiency.

The reasons for higher Zn utilization in Zn-efficient genotypes are not known. Zinc-efficient genotypes may possess higher amounts of chelators that bind Zn and increase its physiological availability at the cellular level. Examples of such Zn chelators in plant tissues are PS, nicotianamine, and S-containing amino acids, such as histidine and methionine (Stephan et al., 1994; Welch, 1995; Cakmak et al., 1998). These compounds may be important in Zn translocation from roots into shoots or in Zn retranslocation from older tissues to apical shoot meristems. Further studies are needed to clarify the relevance of these chelators to the expression of Zn efficiency.

Considerations for field and pot screening

Selection under field conditions for tolerance to low levels of available micronutrients is in general more difficult than selection for tolerance to micronutrient toxicities. This is because toxicities are likely to occur every year, whereas micronutrient deficiencies—including Zn—are not due to the absolute lack of a micronutrient, but rather to its poor availability in soils. Screening under field conditions is further complicated by the irregular distribution of Zn within a plot. Also important is that soils low in available Zn cannot be used for several years after Zn fertilizer has been applied. These factors influence year-to-year variability, as well as spatial variability within a field.

In areas where the climate is relatively similar over years and field conditions are relatively homogeneous over large areas, field screening is an efficient and relatively cheap way of screening large numbers of genotypes. Yield tests under low Zn field conditions are the final test of the Zn efficiency of a given genotype. However, both deficiency symptoms and yield are influenced by many factors, which may cause high experimental error or increase genotype x environment and genotype x environment x year interactions.

Alternatively, screening in pots is fast, cheap, and overcomes problems of soil heterogeneity. Using 23 wheat cultivars we recently demonstrated a close relationship between Zn efficiencies in field and greenhouse (Figure 3). Most cultivars grown in the same Zn-deficient soil in field and greenhouse behaved similarly in both environments. However, in soils having both Zn deficiency and boron toxicity the correlation between Zn efficiencies in field and greenhouse was poor (Figure 3). In a greenhouse experiment with Konya soil, surface soil (0-30 cm) with lower B toxicity (around 9 mg soluble B kg^{-1} soil) was used, while in the field roots were exposed to higher B toxicity in deeper soil (around 20-30 mg soluble B kg^{-1} soil at 60-90 cm). These results indicate the suitability of screening cultivars for Zn efficiency in pot experiments using Zn-deficient soil without additional nutrient stress (Figure 3). However, growing conditions are less realistic, and the ranking is not always closely correlated to grain yield obtained in field trials. In addition, pots should be big enough to avoid root binding, which can affect results (Graham and Welch, 1996). Greenhouse experiments can, however, act as a primary screening to reduce the number of genotypes that will be tested under field conditions.

Inheritance of Zinc Efficiency in Bread Wheat

Wheat genotypes vary in their ability to produce grain in Zn-deficient soils; this can be exploited by breeders to improve wheat for Zn efficiency (Shukla and Raj, 1974; Graham et al., 1992; Cakmak et al., 1997a). Most research related to Zn deficiency in wheat and other crops has concentrated on the physiological aspects of Zn uptake, or has compared wheat genotypes in their relative efficiency to grow in Zn-deficient soils. Little information is available on how Zn efficiency is inherited.

Several mechanisms that are most likely controlled by several genes may affect Zn uptake. In contrast, micronutrient use efficiency seems to be controlled by a single, major gene (Graham and Welch, 1996). Results from a diallel analysis in rice suggested that the genes controlling Zn efficiency are additive and, to a lesser degree, dominant (Majumder et al., 1990). In maize, four additive genes were reported to affect Zn concentration in the ear leaf (El-Bendary et al., 1993). However, we are not aware of reports on the inheritance of Zn efficiency in hexaploid wheat. Our unpublished data from a diallel experiment comparing seven wheat cultivars differing in Zn efficiency with their F1 derivatives suggest that in bread wheat, genes controlling Zn efficiency are dominant (Table 10). Although we are far from fully understanding how Zn efficiency is inherited in wheat, progress has been made through phenotypic selection.

Although Zn deficiency in soils is common in Turkey, it was recognized as a wheat production constraint less than a decade ago. Data from two-year yield trials in the Central Anatolian Plateau showed a strong correlation between grain yield and Zn efficiency (M. Kalaycı et al., unpublished). Bread wheat cultivars derived from landrace populations (e.g., Yayla 305, Sertak 52, and Ak 702) are well adapted to Zn-deficient conditions (Figure 4). However, recently developed cultivars Gerek 79, ES 90-3, and Kirgiz outyielded Yayla 305 by 6%, 12%, and 15% respectively. All widely grown and/or recently released winter wheat cultivars (e.g., Gerek 79, Dagdas, Kirgiz, and Gun 91) are Zn efficient. However, it should be stressed that modern cultivars have much higher yield potential in soils with no Zn deficiency (M. Kalayci et al., unpublished).

Table 10. Mean, minimum, and maximum score for zinc deficiency symptoms of seven bread wheat cultivars and sixteen F1 lines derived from a diallel cross with seven parents.

	Zn deficiency scores† of parents			Parent cultivars						
				1	2	3	4	5	6	7
Parents	Mean§	Min	Max	Zinc deficiency scores of F1 obtained from a diallel‡						
1. Arapahoe	1.4	1.0	2.3	1.4#						
2. Sn64//SKE/2*ANE/3/SX/4/BEZ/5/JUN	1.5	1.2	2.2	1.5††	1.5					
3. Dagdas	2.4	1.5	3.5	2.5	2.0	2.4				
4. F134/Nac	3.2	2.5	4.0	3.5	2.5	-	3.2			
5. F4105W-2-1	3.3	2.8	4.0	-	3.0	2.5	2.5	3.3		
6. Gun	3.4	2.5	4.0	-	3.5	3.0	3.5	4.5	3.4	
7. Katia	3.4	3.0	4.0	4.0	3.0	-	4.5	-	4.5	3.4

† 1 = severe deficiency symptoms, 5 = no symptoms.
§ Mean of 8 replications.
†† Mean of 2 replications.
‡ Only 16 of the 21 possible combinations were obtained.
# Diagonal gives mean score of respective parent.

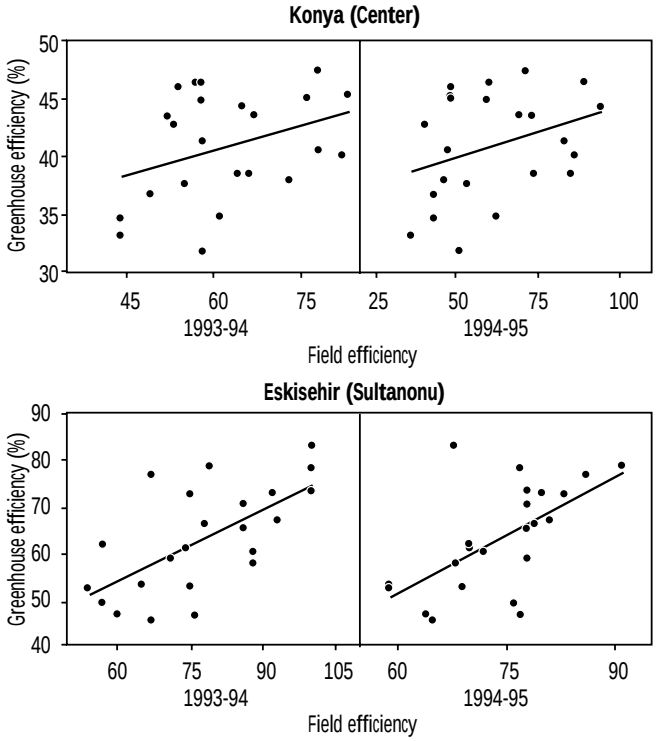


Figure 3. Relationship between Zn efficiency values obtained in greenhouse and field for two locations. Soils (0-30 cm) in Eskisehir have Zn deficiency (DTPA-Zn: 0.09 mg kg^{-1} soil) and no B toxicity (soluble B: lower than 1 mg kg^{-1} soil). Soils (0-30 cm) in Konya have both Zn deficiency (DTPA-Zn: 0.10 mg kg^{-1} soil) and B toxicity (soluble B: 9 mg kg^{-1} soil).

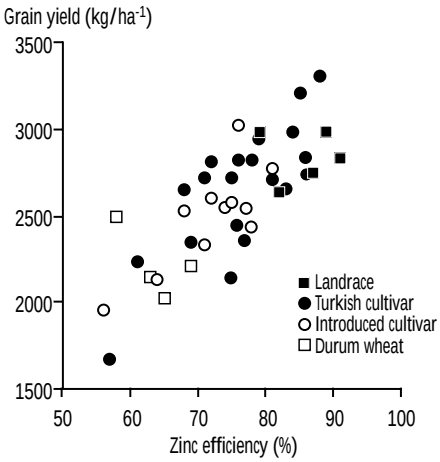


Figure 4. Grain yield and zinc efficiency of 40 wheat cultivars across two years at Eskisehir, Turkey.
Source: M. Kalaycı (unpublished data).

Besides Turkish winter wheat cultivars, winter wheat accessions from Bulgaria and Romania were found to be highly tolerant to Zn deficiency, whereas winter wheats from the Great Plains of the United States are mostly sensitive. Table 11 shows the 10 best- and 10 worst-performing accessions among 155 entries tested for Zn efficiency in the winter wheat crossing block of the International Winter Wheat Improvement Program in the 1994/95 season (Torun, 1997). The generally poor Zn efficiency of cultivars from the Great Plains may explain why, despite similar climatic conditions, only one cultivar (Bolal) of the thousands of introductions tested from the Great Plains was released for the Central Anatolian Plateau. This suggests that when introductions from areas with similar environmental conditions lack adaptation, the possible cause may be micronutrient disorders.

In experiments comparing wheat and rye cultivars for Zn efficiency, rye cultivars were always more Zn efficient than the best bread wheat (Graham, 1988; Cakmak et al., 1997a,b; Schlegel et al., 1997, 1998). The existence of large numbers of wheat/rye translocation, substitution, or addition lines has led researchers to concentrate on studies dealing with the effect of rye chromosomes on Zn efficiency in bread wheats. Using wheat/rye addition lines, Graham (1988) found that several loci on chromosomes 2R, 3R, and 7/4R enhanced Zn-efficiency. Cakmak et al. (1997c) reported that 1R, 2R, and 7R had positive effects on Zn efficiency, but 3R was found to have a negative effect on Zn uptake. Based on available results, rye chromosomes 1R, 2R, and 7R are most likely to carry genes that enhance Zn efficiency, with genes on 1RS and 7RS being most effective (Cakmak et al., 1997c; Schlegel and Cakmak, 1997).

Genes controlling Zn efficiency in rye are expressed in triticale to a similar extent as in rye (Cakmak et al., 1997c). In studies with wheat amphiploids, Schlegel et al. (1997; 1998) found that chromosomes L1 from *Agropyron intermedium* and V7 from *Haynaldia villosa*, both belonging to homoeologous group 7, increased Zn efficiency in wheat. This suggests that in screening alien species for new sources of Zn efficiency, researchers should start with chromosomes belonging to homoeologous group 7.

Results from wheat/rye translocation lines indicate that particular translocations involving the short arm of 1R enhance Zn efficiency. These translocations would be an ideal source of Zn efficiency because they are present in many wheat cultivars. However, several lines carrying the 1B/1R translocation, e.g., Seri 82 and BDME10 = Ctk/Vee, were found to be sensitive to Zn deficiency (Schlegel at al., 1997), suggesting that epistatic effects are important. The 5RL/4A translocation,

Table 11. Leaf symptoms and total zinc content (per shoot) of the ten best and worst accessions among 155 entries in the crossing block of the International Winter Wheat Improvement program, 1994-95. Plants were grown in a Zn-deficient calcareous soil under greenhouse conditions.

Cross	Origin	Symptom severity†	Zn content
			µg shoot^{-1}
F4549-W1-1	Romania	3	4.73
602-156-22	Bulgaria	4	4.27
JUWELL/LLV32//2*FL80	Romania	3	4.16
F134.71/NAC	Mexico	4	3.97
AGRI/BJY//VEE	Mexico-Turkey	2	3.94
KATIA1	Bulgaria	4	3.75
KSK//INIA/LFN/3/CALIBASAN	Turkey	3	3.70
4206/3/911B8.10/K351//SAD1/MEXIPAK	Bulgaria	4	3.69
SADOVA-1	Bulgaria	4	3.69
DAGDAS	Turkey	4	3.50
1D13.1/MLT	Mexico	2	1.84
F362 K 2.121	Romania	3	1.82
NE7060/VG3 N89L771	USA	1	1.82
NZT/BEZ//ALD/4/NAD//TMP/CI12406/3/	USA-Mexico	1	1.77
ES 14	USA	3	1.76
KS73H530/VEE	USA	1	1.74
63-122-77-2/NO66//LOV2/3/KVZ/HYS/4/	USA	1	1.72
KS82142	USA	3	1.66
PYN//TAM101/AMIGO	USA	1	1.63
SU92/CI13465//PGFN/3/PHO/4/YMH/TO	USA-Mexico	1	1.63

† Symptom severity: 1: very severe - 5: no symptoms.
Source: Torun (1997).

which increases the copper use efficiency in wheat, does not effect Zn efficiency (Graham et al, 1992), indicating independent gene action for Zn and Cu efficiency.

Few results are available on Zn efficiency of alien species. Substantial genotypic variation was observed among *Aegilops* spp. and *T. dicoccoides* accessions, but no species showed Zn efficiency comparable to that of rye (Cakmak et al., unpublished). No information is available regarding inheritance of Zn deficiency in durum wheat. Accessions of all tetraploid species evaluated so far, including *T. durum*, *T. dicoccoides*, and *T. polonicum*, are much more sensitive to Zn deficiency than *T. monococcum* and bread wheats (Cakmak et al., unpublished). This would suggest that genes controlling Zn efficiency might be located in the A genome of *T. monococcum*; it also indicates the possible existence of suppressor genes in the B genome of tetraploid species. Whether these genes are suppressed by genes in the D genome of bread wheat or whether the D genome also carries genes enhancing Zn efficiency is not known at this time.

Although the genetic variability for Zn efficiency in bread wheats and its presumably simple inheritance should allow progress towards Zn efficiency, a breakthrough is more likely to come from introgressing genes from rye. Schlegel et al. (1997) pointed out several advantages of this approach. Wheat/rye translocations have been widely used in wheat breeding (Rabinovich, 1998) and are genetically and meiotically stable. If deleterious genes can be removed from the translocated rye segment, the genes encoding for Zn efficiency will likely be maintained in the wheat genome without undergoing recombination during crossing and backcrossing, since rye segments introduced into the wheat genome do not normally recombine with the wheat chromosomes. Molecular markers for the genes controlling Zn efficiency would greatly enhance selection efficiency, but no marker has been reported so far. The real breakthrough in increasing Zn efficiency of wheat may come when genes are found in alien species and cloned. Transformation systems to introgress these genes into wheat are now in place.

Genetic variability for Zn concentration in grain is relatively low, and we are not aware of any study on the inheritance of this trait in wheat.

Phytic Acid and Bioavailability of Zinc

Improved Zn efficiency is usually not accompanied by higher Zn concentration (density) in grain; in fact, Zn-inefficient durum genotypes may have higher Zn concentrations in grain than Zn-efficient ones (Graham et al., 1992; Cakmak et al., 1997a). Lack of higher Zn concentration in grains of Zn-efficient genotypes can be attributed at least in part to a dilution effect as the result of greater shoot dry matter production and higher grain yield.

Wheat grains are inherently rich in compounds, such as fiber and phytic acid, that depress utilization/absorption of Zn in human cells (Welch, 1993). Phytic acid (or its salt, phytate) is the main storage form of phosphorus in cereal grains. Around 75% of total P in wheat grains is stored as phytic acid (Raboy et al., 1991), particularly in the germ and aleurone layers (O'Dell et al., 1972). Because phytic acid has high Zn-binding and -complexing ability, it hampers zinc's biological availability in diets (Welch, 1993). In animal experiments, the addition of phytate to diets reduced Zn bioavailability and animal growth, while removing phytate from diets by the addition of phytate-degrading enzymes improved Zn bioavailability and animal growth (Lei et al., 1993). In other studies, widespread occurrence of Zn deficiency in humans in rural regions of Iran was attributed to high consumption of cereal-based foods rich in phytic acid (Halsted et al., 1972; Prasad, 1984).

The phytate:Zn molar ratios in foods can be used for predicting Zn bioavailability in diets and the risk that Zn deficiency will occur. Based on animal experiments, ratios above 20 are associated with reduced Zn absorption and increased risk for Zn deficiency (Oberleas and Harland, 1981; Solomons, 1982). Therefore, to increase bioavailability of Zn in grains 1) Zn concentration in grains can be increased, 2) the concentration of phytic acid can be lowered, or 3) concentration of promoters of Zn bioavailability such as S-containing amino acids (i.e., methionine, histidine, and lysine) in grains can be enhanced (Welch, 1993; Graham and Welch, 1996). For example, adding methionine to animal diets increases Zn absorption in animal cells (Hause et al., 1996). Currently, the short-term means of increasing Zn bioavailability in grains is through the application of higher amounts of Zn fertilizers. The long-term solution is to develop new genotypes with higher Zn concentration and promoters that affect Zn bioavailabilty.

There is large variability for phytic acid concentration in seed among wheat (Raboy et al., 1991) and triticale (Feil and Fossati, 1997) genotypes. These results indicate that breeding genotypes for lower phytic acid is feasible and may be the solution to phytate-induced nutritional problems in humans. However, it is still possible that reducing phytic acid levels in seed may have not only beneficial but also adverse effects on seed quality and human health (see Raboy et al., 1991 for more detail; Feil and Fossati, 1997).

Conclusions

Given the widespread occurrence of Zn deficiency in plants, particularly wheat, it is of great importance to breed bread wheat genotypes with high Zn efficiency. High Zn efficiency in wheat seems to be linked to various morphological and physiological plant traits. Particular plant traits involved in expression of Zn efficiency are root surface area, release of Zn-mobilizing PS from roots, affinity of membranes to absorb and transport Zn, and better utilization of Zn at the cellular level.

Great genetic variability for Zn efficiency exists within and among cereals and alien species of wheat, and breeders are able to enhance Zn efficiency in modern bread wheat cultivars. Genes encoding for Zn efficiency in bread wheat seem to be dominant. Markers are not available at this time. Among cereals, rye is most efficient, followed by triticale, barley, and bread and durum wheat. Genes transferred from rye into bread wheat are expressed in wheat/rye addition and translocation lines. Therefore, breeding efforts should concentrate on transferring rye genes into a wheat background.

Successful breeding for Zn efficiency in wheat depends on the existence of quick and reliable screening methods. Efforts aimed at developing suitable screening methods have, so far, concentrated on different morphological and physiological plant traits. Evaluating severity of Zn deficiency symptoms on leaves, together with the Zn efficiency ratio (yield at -Zn/yield at +Zn), appears to be a reasonable approach for reliably screening large numbers of genotypes for Zn efficiency within a short time.

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CHAPTER 17

Nitrogen and Phosphorus Use Efficiency[1]

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The improvement of nutrient use efficiency in wheat cropping systems can be achieved through two main strategies: by adopting more efficient crop management practices (such as nutrient rate, timing, source, and placement) and breeding more nutrient use efficient cultivars. Although both are important, this paper will focus on improving nutrient use efficiency (specifically, of nitrogen and phosphorus) through plant breeding. More detailed guidelines on how to improve nitrogen use efficiency in wheat through crop management have been described elsewhere (Ortiz-Monasterio, 2001).

It is important to clearly define nutrient use efficiency before describing methods for improving it. We have found the definition proposed by Moll et al. (1982) useful in looking at genetic differences in nitrogen use efficiency among wheat cultivars. (Though the concept was developed using nitrogen as an example, it can also be applied to phosphorus.) These authors define nitrogen and phosphorus use efficiency in wheat as grain yield per unit of nutrient supplied (from the soil and/or fertilizer). They divide nutrient use efficiency into two components: uptake, or the ability of the plant to extract the nutrient from the soil, and utilization efficiency, or the ability of the plant to convert the absorbed nutrient into grain yield. Hence

Nutrient use efficiency = Uptake efficiency x Utilization efficiency

$$\frac{Gw}{Ns} = \frac{Nt}{Ns} \times \frac{Gw}{Nt} \quad (1)$$

where Gw = grain dry weight, Nt = total above-ground plant nutrient at maturity, and Ns = nutrient supplied. All units are in g m^{-2}. Utilization efficiency can also be subdivided into two components, as suggested by Ortiz-Monasterio et al., 1997a, and expressed as follows:

Utilization efficiency = Harvest index x Nutrient biomass production efficiency

$$\frac{Gw}{Nt} = \frac{Gw}{Tw} \times \frac{Tw}{Nt} \quad (2)$$

where Tw = total above-ground plant dry weight at maturity.

Utilization efficiency can also be expressed as:

Utilization efficiency = Harvest index x Inverse of total nutrient concentration in the plant

$$\frac{Gw}{Nt} = \frac{Gw}{Tw} \times \frac{1}{Nct} \quad (3)$$

where Nct = total nutrient concentration in the plant as a percentage.

The definition for nitrogen use efficiency proposed by Moll et. al. (1982) can be used for both low and high input situations. However, there are other nutrient efficiency classification systems that take into account performance both in the presence and in the absence of nutrient stress as, for example, the system proposed by Gerloff (1977), which separates cultivars into four groups based on their response to P. The groups are 1) efficient, responder; 2) inefficient, responder; 3) efficient, non-responder, and; 4) inefficient, non-responder. An efficient cultivar has higher yield than the other cultivars under low nutrient supply, while a responder cultivar has higher yield under high nutrient supply. This classification groups cultivars based on performance under low (efficient vs. inefficient) and high (responder vs. non-responder) nutrient supply, and allows the identification of those cultivars with adaptation to a range of soil nutrient conditions.

CIMMYT and its predecessor have been generating wheat germplasm for the developing world since the 1940s. CIMMYT bread wheats were first and most rapidly adopted in irrigated areas of the developing world (e.g., the Yaqui Valley in Mexico, the Indian Punjab, and the Pakistani Punjab) (Byerlee, 1996). Fertilizer is widely applied (sometimes at sub-optimal levels) by farmers in those areas as a way to correct nutrient deficiencies. However, in other target environments farmers do not apply

[1] This chapter does not attempt to make an exhaustive review of the literature but rather presents practical information based on CIMMYT Wheat Program experience working on nitrogen and phosphorus use efficiency.

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fertilizers because they cannot afford them or because inputs are simply not available. It is thus essential that CIMMYT wheats be widely adapted and able to grow in different (low and high) soil nutrient situations.

In this chapter we will discuss how studying the individual components of nutrient use efficiency (uptake vs. utilization) under different nutrient levels can help us gain a better understanding of the opportunities and limitations of breeding for nitrogen and phosporus use efficiency.

Nitrogen

With the adoption of the input-responsive and lodging tolerant semidwarf wheat cultivars that launched the green revolution in the 1960s, the use of nitrogen fertilizer rapidly increased, as did yields. Thanks to the introduction of the new genetic material, the amount of grain produced per unit of N applied has increased significantly (Figure 1).

We have documented the changes in the nitrogen use efficiency of CIMMYT bread wheats developed between 1950 and 1985 under medium to high levels of N fertility. Results show that more recent CIMMYT cultivars outyield both earlier semidwarfs and old tall cultivars at all nitrogen levels (Ortiz-Monasterio et al., 1997a). This indicates that the current strategy of selecting and evaluating under medium to high N levels has resulted in germplasm that produces higher yield when grown under low or high levels of N fertility. CIMMYT bread wheats from 1950 to 1985 gradually became not only more **responsive** to N inputs, but also more **efficient** in their use, according to Gerloff's classification (1977). As a result, CIMMYT bread wheats do not require more N than the old tall cultivars; in fact, they often need less N to produce the same yield. In addition, since CIMMYT bread wheats are more responsive to N application, the optimum economic rate is higher than that for the old tall cultivars (Ortiz-Monasterio et al., 1997a).

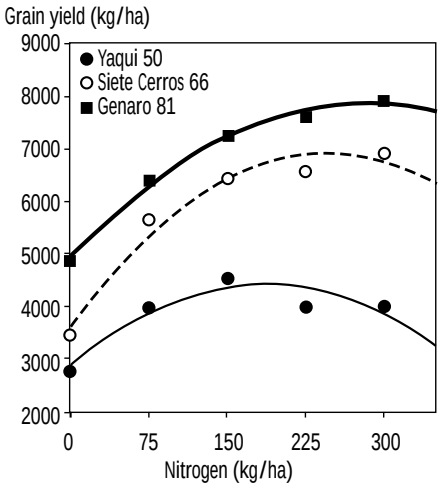


Figure 1. Response of tall (Yaqui 50) and semidwarf spring wheat cultivars to increasing levels of nitrogen fertilizer.

Although our current breeding strategy has been successful in addressing the needs of both low input and high input wheat-producing environments, we are interested in identifying alternative selection methods that might be even more successful. To that end, we characterized relevant CIMMYT germplasm for two main components of nitrogen use efficiency: nitrogen uptake and utilization efficiency. We found that there is genetic diversity for both traits.

Our work and that of others has shown that the level of N in the soil plays a very important role in the expression of uptake and utilization efficiency (Dhugga and Waines, 1989; Ortiz-Monasterio et al. 1997a). However, the effect of different soil N levels on the expression of a given component of nitrogen use efficiency in spring wheat may be affected by genotype and/or location. Dhugga and Waines (1989) found better expression of uptake efficiency under high soil N and better expression of utilization efficiency under low N. In contrast, Ortiz-Monasterio et al. (1997a) found better expression of uptake efficiency under low N conditions and better expression of utilization efficiency under high N conditions. These findings notwithstanding, available information has shown that the level of soil N may be manipulated together with genetic variability to develop cultivars with improved performance under both low and high input conditions (Ortiz-Monasterio et al., 1997a; van Ginkel et al., 2001).

Nitrogen uptake vs. utilization efficiency

In view of the above, an important aspect of our current research is to identify the best selection strategies for developing genotypes that produce higher grain yields as a result of their improved uptake and/or utilization efficiency. The question is which component to emphasize.

Utilization efficiency has ecological appeal, since it means either higher yields with the same nutrient levels in the plant or the same yield with lower nutrient levels in the plant, which requires fewer resources. As indicated earlier, utilization efficiency can be broken down into harvest index and biomass production efficiency. If we analyze which component has been most associated with utilization efficiency gains in the past, we find that most progress has been associated with improvements in harvest index (HI) rather than in biomass production efficiency (Eq. 2). However, Fischer (1981) and Calderini et al. (1995) suggest that the possibilities of further improving HI as a way to increase grain yield are limited.

There are two main routes for making further progress in grain yield through better utilization efficiency: 1) to

increase grain yield while maintaining or reducing nutrient concentration in the plant, and 2) to reduce total nutrient concentration in the plant while increasing or maintaining grain yield (Eq. 2). Most CIMMYT high yielding wheats grown under a wide range of N levels tend to have, on average, a nitrogen harvest index of around 75%. In other words, 75% of the plant's total N is found in the grain at maturity. This means that cultivars with higher utilization efficiency, which is not associated with HI (assuming a constant HI), will have lower protein concentration in the grain. This can negatively affect the grain's bread making quality and nutritional value, unless the percent protein reduction is compensated for by a proportional improvement in protein quality.

We should point out that bread making quality, which is a key issue for breeding programs in the developed world, is now gaining significance for breeding programs in developing countries. The original focus of many wheat breeding programs in developing countries—i.e., generating sufficient yield increases to feed their rising populations—has expanded to include fulfilling farmers' need to produce high quality grain that competes well on the market.

The nutritional value of wheat grain is another issue that is gaining in significance due to its perceived potential to better the nutrition of developing country populations. The nutrient content of wheat grain is negatively affected by lower protein concentration in the grain. Studies in Mexico and Argentina have shown that protein concentration in the grain has decreased as grain yield has increased throughout the history of breeding (Ortiz-Monasterio et al., 1997b; Calderini et al., 1995). This reduction in protein N has been associated with higher utilization efficiency. Thus an important challenge for breeding programs in both developed and developing countries will be to continue to improve nitrogen use efficiency and, at the same time, maintain or improve the bread making quality and/or nutrient content of wheat grain.

A similar dilemma arises when uptake efficiency, the other component of nutrient use efficiency, is implemented as a strategy to improve grain yield. For resource poor farmers who cannot afford fertilizers and grow wheat under low input conditions, the development of cultivars with high N uptake efficiency may not be desirable because it may accelerate soil nutrient mining. In contrast, in high input environments, high uptake efficiency is a very desirable trait because residual soil N (soil N not absorbed by the crop) may either leach through the soil to pollute waterways with soil nitrates or escape into the atmosphere as N_2, N_2O, NO_x, or NH_3.

Nitrate leaching has been well documented in many developed countries (CAST, 1985; Keeney, 1986). The problem tends to be associated with the application, especially in sandy soils, of more nitrogen than is required for producing maximum yield. Until recently, total N fertilizer use in the world was almost evenly divided between developed and developing countries from a total of 80 Tg y^{-1} (FAO, 1990). However, the use of N fertilizer has been accelerating in the developing world. Of the 60-90% increase in global application of N fertilizer estimated to take place by 2025, two thirds will occur in developing countries (Galloway, 1995).

There are wheat production systems in the developing world where very high rates of N fertilizer are already being applied—for example, in certain wheat-growing areas of Mexico and Egypt. In the high input wheat systems of northwestern Mexico, where farmers apply an average of 250 kg N/ha, researchers have recorded large N leaching losses (Riley et al., 2000) and high emissions of greenhouse gases into the atmosphere (Matson et al., 1998). If cultivars and crop management systems remain as they are now, as N rates increase, the problems of N leaching and greenhouse gas emissions (N_2O), common in many industrialized countries, will also become widespread in the high input areas of developing countries.

Strategy for improving nitrogen use efficiency

Grain yields of CIMMYT bread wheats developed between 1950 and 1985 have gradually increased. We evaluated these wheats at N levels commonly applied by farmers in irrigated areas of the developing world (75-150 kg N/ha) and found that 50% of the yield gains was associated with higher nitrogen uptake efficiency and the other 50% with better utilization efficiency (Ortiz-Monasterio et al., 1997a). This clearly shows that improvements in both uptake and utilization efficiency have been important in the past and most likely will continue to be in the future.

Hence, it is important to select and evaluate for nitrogen use efficiency under both low and high nutrient conditions; this allows the researcher to identify genotypes that perform well under nutrient stress (low input) (efficient) and genotypes that respond well to high input conditions (responder) (Picture 1).

In a study that evaluated five N selection treatments (low, medium, high, alternating high-low, and alternating low-high N levels), we found that the highest yielding germplasm in medium or high input environments was obtained by alternately selecting (from F_2 to F_7) under high and low N conditions. No differences between N selection treatments were observed when the resulting lines were evaluated in low N environments (van Ginkel et al., 2001).

Picture 1. Varieties with and without nitrogen application, Ciudad Obregon, Sonora, Mexico. (Photo: J.I. Ortiz-Monasterio.)

We conclude that the relative importance of both uptake and utilization efficiency will vary according to the needs of different production systems. Given that wide adaptation is a primary objective in breeding CIMMYT germplasm, we will continue to improve both components.

Phosphorus

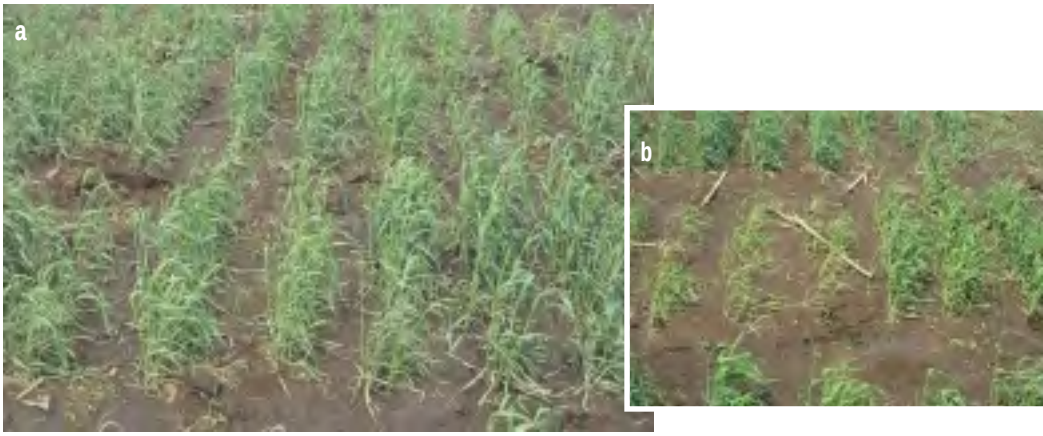
Many soils have large reserves of total phosphorus, but low levels of "available" phosphorus. Al-Abbas and Barber (1964) reported that total soil P is often 100 times higher than the fraction of soil P available to crop plants. Our objective in breeding for P efficient and responsive cultivars has been to identify wheat cultivars that can access P not usually available to the average cultivar under low P conditions (P efficiency), but also respond to P applications (P responsiveness).

As in the case of N, CIMMYT has been breeding under medium to high levels of P in the soil. Preliminary results suggest that phosphorus use efficiency in CIMMYT bread wheat cultivars between 1950 and 1992 has improved under low as well as high levels of P fertility (Ortiz-Monasterio et al., unpublished data). Again using Gerloff's (1977) definition, CIMMYT bread wheat germplasm has become more efficient as well as more responsive to P applications during that time period.

There is little information on the contribution of uptake and utilization to total P use efficiency in wheat. In a recent CIMMYT study, the relative importance of uptake and utilization in spring wheat was evaluated in two different environments: a rainfed area with Andisols in the central highlands of Mexico and an irrigated, low-altitude area with Vertisols in northwestern Mexico. Uptake and utilization were characterized in a set of CIMMYT lines. Results showed that in an acid Andisol with no Al toxicity, uptake was more important than utilization in explaining P use efficiency. In contrast, in the same group of genotypes utilization efficiency was more important when evaluated in an alkaline Vertisol (Manske et al., 2000a). In these two different environments it was shown that there was genetic diversity for both uptake and utilization efficiency in the CIMMYT material tested.

This study shows that, as in the case of N, the environment where a given set of genotypes is evaluated plays a very important role in the expression of P uptake and utilization efficiency. However, in the case of P, what influenced the expression of uptake vs. utilization was not low P vs. high P, but rather the effect of location. At this point it is not clear how much of the location effect is due to soil effects and how much to above-ground effects (radiation, temperature, etc.) (Manske, 1997). Also to be determined is why the same genetic material expresses genetic diversity for uptake efficiency in some environments but not in others.

Evaluating germplasm under both low and high nutrient conditions allows the identification of genotypes that perform well under nutrient stress (low input) and genotypes that are responsive to high input conditions (Picture 2). Preliminary data suggest that evaluating advanced



Picture 2. Screening plots for phosphorus use efficiency, Patzcuaro, Michoacan, Mexico. In both pictures, plants on the right received 80 kg P/ha, while the ones on the left received none. (a) P use efficient genotype. (b) P use inefficient genotype. (Photos: J.I. Ortiz-Monasterio.)

genetic materials under low P conditions is useful for identifying exceptional germplasm for P stress conditions. When advanced genetic materials are evaluated only under high input conditions, this sometimes results in genotypes that are outstanding under low P conditions, but intermediate under high input conditions. This germplasm might be discarded if it is tested only under high input conditions (since only the top 10-15% of the lines are selected) and its performance under high input conditions is intermediate (Trethowan et al., unpublished data). Hence the importance of selecting and evaluating under both low and high nutrient conditions. More definite data will be available once a CIMMYT study is completed in which germplasm is selected under low vs. high and under alternating low and high P levels, as was done in the N study.

In acid soils P deficiency is often accompanied by Al and Mn toxicity, especially when soil pH is below 5.4. Evidence available so far indicates that genes controlling adaptation to Al and Mn toxicity and tolerance to P deficiency appear to be independently inherited and recombinable (Polle and Konzak, 1990). Therefore the recommendation is that screening for P use efficiency be done first in soils without Al or Mn toxicity, if possible. Once elite materials have been selected for P use efficiency in the field, they can be screened for Al and/or Mn toxicity either in the field or in hydroponics (see chapter by Hede and Skovmand).

We suggest that screening for P uptake efficiency under nutrient culture conditions be avoided until a satisfactory correlation between performance in the field and in nutrient cultures has been shown. This is particularly important for P, given that very little of the crop's P requirement is provided by mass flow (transpiration flow). Diffusion is more important, but difficult to simulate in solution culture. It is generally recognized that nutrient culture should be limited as a screening environment primarily because of the low correlation of the results with those of field tests. Nutrient cultures cannot simulate the soil-plant interface properly.

Phosphorus uptake vs. utilization efficiency

Phosphorus utilization efficiency (grain yield per unit P in the plant) is dependent on the plant's internal P requirement. Increased harvest index, P harvest index, and low P concentration in grain may improve P utilization efficiency (Jones et al., 1989; Batten, 1992).

Most CIMMYT high yielding wheats have a P harvest index of about 80% under irrigated conditions. As in the case of N, breeding for higher P utilization efficiency, given the small margin to breed for higher HI, will result in lower P concentration in the grain. Selection for wheat genotypes that remove small amounts of P from the soil due to their low P grain concentration can contribute to sustainable land use (Schulthess et al., 1997). Genotypic differences in grain P concentration are fairly consistent across environments (Schulthess et al., 1997). If breeders in Australia, which is a major exporter of wheat grain but has soils that are poor in P availability, can reduce the P concentration in the grain of wheat cultivars, farmers will have to purchase substantially less P to replace the P exported with the grain.

However, the strategy of reducing P concentration in the grain has a limit. There is evidence that excessively low values of P concentration in the grain affects seed vigor, particularly in P deficient soils. A study on a set of historically important CIMMYT semidwarf bread wheats showed that P concentration in the grain decreased significantly over the years as a result of breeding (Manske, 1997). Similar information is available from a wheat breeding program in Argentina (Calderini et al., 1995). As in the case of N, this reduction in P concentration in the grain is associated with gains in utilization efficiency.

Most nitrogen absorbed by plants comes from mass flow (i.e., soil water moves towards the roots as the plant loses water through transpiration), but phosphorus is absorbed mainly by diffusion through gradients created by root absorption. Phosphate concentrations in soil solution are small ($<0.05\ \mu\ g^{-1}$) compared to nitrate-N concentrations ($100\ \mu\ g^{-1}$), and very little phosphate is moved to the roots by capillary water movement. The amount of P extracted is limited by P concentration at the root-soil interface, which means that wheat roots have to grow to come into contact with new soil from which they can extract phosphate. Root length is thus a major determinant of the absorbing surface area.

Wheat genotypes with greater root length density are able to take up more phosphorus (Manske et al., 2000b). When P supply is low, the correlation between root length density and P uptake or grain yield is usually 0.50-0.60, but with adequate P supply this correlation is lower. In some environments, P uptake can be more important than utilization efficiency. In areas where uptake is the main component associated with P use efficiency, P uptake efficiency holds great promise for improving P use efficiency, since soils with relatively high levels of total P in the soil often have low levels of available P.

Strategies for improving phosphorus use efficiency

Different approaches can be used to enhance P uptake (Polle and Konzak, 1990; Johansen et al., 1995).

- *Increasing the root surface/soil contact area.* This can be achieved by modifying root morphology. For a constant level of root biomass, roots with higher specific root length (i.e., roots with smaller diameter) can cover a larger surface area. A second approach for achieving the same objective is through increased hair root development. Root fineness or branching is an important determinant of P uptake efficiency in wheat (Jones et al. 1989). This route seems promising given that there is evidence of large genetic variability for this trait in wheat. However, the time consuming and labor intensive methodologies currently in use limit its application in breeding programs where large numbers of genotypes need to be screened.
- *Increasing the effective root area.* Root symbiosis with arbuscular mycorrhizal fungi (AMF) has been shown to enhance P absorption by increasing the effective root area (Hayman and Mosse, 1971). AMF infection improves P influx (P uptake per unit root length). On the other hand, the information available discussing the genetic diversity present among wheat cultivars to associate with vesicular-arbuscular mycorrhiza (VAM) is not consistent (Vlek et al., 1996). There are reports that show differences in mycorrhizal association among wheat cultivars (Vlek et al. 1996). In contrast, extensive screening of CIMMYT's spring wheat cultivars for mycorrhizal association found very small differences among genotypes; the differences were not strongly associated with higher P absorption (Manske et al., 2000b).
- *Increasing nutrient availability through rhizosphere modification.* Root exudates, ranging from protons to complex organic molecules, can influence nutrient availability and uptake. Phosphatases have been reported to transform poorly available organic phosphorus, which usually accounts for 40-50% of a plant's total P supply, into inorganic forms available to the plant (Randall, 1995). There are genotypic differences in root phosphatase excreted or bound at the root surface (McLachlan, 1980). Our work in an Andisol showed an association between acid phosphatases and P uptake in different wheat and triticale cultivars (Portilla-Cruz et al., 1998).

As in the case of N, most opportunities for breeding for higher utilization efficiency probably lie in improving biomass production efficiency (BPE) rather than HI. In this case biomass production will have to either increase with the current levels of P in the plant or be maintained with a lower concentration of P in the plant. Utilization efficiency is associated with the efficiency with which plants use absorbed P; this, in turn, is a function of 1) how efficiently P is distributed to the functional sites and 2) the P requirement of the cells at those sites (Loneragan, 1978).

Calculating Nutrient Uptake Efficiency

As defined earlier, uptake efficiency refers to the ability of the crop to extract or absorb nutrients from the soil.

Uptake efficiency = Total above-ground nutrient in the plant at maturity (Nt) / Nutrient supplied (Ns)

Uptake efficiency can be measured at any stage of development, but particularly useful information can be collected at anthesis and physiological maturity. Follow the steps described below to measure uptake efficiency.

First, a biomass sample is collected by either harvesting all the above-ground biomass in a given area (a minimum of 0.5 m^2 is suggested) or harvesting a predetermined, representative number of plants (a minimum of 50 stems is suggested) at random. Detailed methods for doing this type of sampling at different stages of development are described by Bell and Fischer (1994).

If the sample is collected right before or shortly after anthesis, there is no need to separate the grain from the rest of the plant for N or P analysis. However, if the sample is collected at or around physiological maturity, it is important to separate the grain from the rest of the biomass for N analysis. This is because there is a large difference in % nutrient concentration between the grain and non-grain biomass (leaves, stems, chaff). In well fertilized spring wheat crops under irrigated conditions, we have observed values of approximately 2% N in the grain and 0.8% N in non-grain biomass. Therefore it is best to take a weighted average to calculate total nutrient in the plant, using the following formula:

Total above-ground nutrient in the plant at maturity (Nt) =
[Grain weight at 0% moisture (g m^{-2}) x Nutrient concentration in the grain (%)] +
[Non-grain biomass at 0% moisture (g m^{-2}) x Nutrient concentration in non-grain biomass (%)]

Total nutrient in the plant is then divided by the amount of nutrient supplied (g m^{-2}) as fertilizer. If soil samples are collected and the amount of soil available nutrient is known, this can be added to the amount supplied as fertilizer.

Nutrient absorption is dependent on root characteristics, especially for immobile plant nutrients in the soil, such as phosphorus. Methods for measuring root traits in wheat are explicitly explained in the chapter by Manske et al.

Calculating Nutrient Utilization Efficiency

Nutrient utilization efficiency is defined as a crop's ability to convert the absorbed nutrients into grain yield.

Utilization efficiency = Tw/Nt

where Tw = total above-ground plant dry weight at maturity and Nt = total above-ground plant nutrient at maturity. To measure uptake efficiency, certain information needs to be collected. First, calculate the harvest index (HI), as follows:

HI = Gw/Tw

where Gw = grain weight at 0% moisture and Tw = total plant biomass at 0% moisture.

This can be done either on an area or a plant basis, as suggested by Bell and Fischer (1994). Finally, biomass produccion efficiency (BPE) is calculated as:

BPE = Gw/Nt

Conclusions

Bread wheat breeding work at CIMMYT has shown that selection and evaluation of genetic material under medium to high nitrogen levels results in genetic gains expressed when this material is tested under low, medium, or high nitrogen levels. In other words, selecting for high yield potential under optimum conditions has resulted in germplasm with higher nitrogen use efficiency under low, medium, or high nitrogen fertility conditions. Now there is evidence that breeding under alternating low and high nitrogen levels may produce germplasm that is even more efficient and responsive to nitrogen.

It is clear that nutrient use efficiency and nutrient responsiveness are under genetic control. Some researchers consider these traits as two different breeding objectives, but it has been shown that they are not incompatible. One of the best pieces of evidence for this are the results achieved by bread wheat breeders at CIMMYT. During the last several decades, CIMMYT has been breeding wheat under medium to high levels of nitrogen and phosphorus and has developed cultivars that are not only more responsive to nitrogen and phosphorus, but also more efficient in their use.

To characterize and better understand the mechanisms associated with higher N and P use efficiency:

- Use the definition of N and P use efficiency suggested by Moll et al. (1982).
- Distinguish between efficiency and responsiveness. This will require that all germplasm be evaluated under low as well as high N and P conditions.
- Establish the importance of uptake vs. utilization efficiency in the target environment.
- Understand the mechanisms associated with higher uptake (more roots, phosphatases, etc.) or utilization efficiency (biomass production efficiency vs HI). If these mechanisms are well understood, they can be used as selection criteria.
- Once genetic markers for genes controlling these traits are identified, selection for these traits could be done in the laboratory.

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CHAPTER 18

Techniques for Measuring Genetic Diversity in Roots

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Physiologists, agronomists, and breeders have dedicated relatively little attention to research on root systems, mainly because root studies require much time and intensive labor. Nonetheless, root traits have a significant role to play in the development of new wheat genotypes with improved input efficiency and adaptability, especially for marginal environments.

This chapter describes methods for conducting root research in the field; most have the advantage of not requiring large investments in equipment. The main focus is on: 1) areas of root research in wheat; 2) genetic diversity and heritability of root traits; 3) examples of successful root research; 4) root traits potentially useful in genetic improvement, and 5) screening methods and trait measurement techniques.

Areas of Root Research

Root research can be divided into three main fields of study: root ecology, root physiology, and selection of root traits for genetic improvement. Root ecology studies deal with environmental factors that influence root growth and are nearly always combined with other ecological research. Important ecological factors are soil bulk density, soil pH, soil water, and nutrient availability in the soil. Root physiology, an integral part of plant physiology, deals with physiological processes in the roots, such as cell division, nutrient and water uptake, and root-to-shoot transport mechanisms. Measurable root traits for genetic improvement are architecture, morphology, number, weight, volume and diameter, root length density, root hair density, root:shoot ratio, infection by vesicular-arbuscular or arbuscular mycorrhizal fungi [(V)AMF], and root exudates.

Genetic differences in root traits are essential in the following areas of wheat research:

- Nutrient uptake efficiency
- Drought tolerance
- Tolerance to mineral toxicity
- Lodging resistance
- Simulation of nutrient and water uptake

Morphology of Wheat Roots

Cereals have two types of roots: seminal roots (also called primary roots), which develop in the embryonic hypocotyl of germinating seed, and adventitious roots (also called nodal, secondary, or crown roots), which emerge from the base of the apical culm and tillers. The architecture of wheat roots has been described in detail by Manske and Vlek (2002).

Seminal roots constitute 1-14% of the entire root system. They grow and function throughout the vegetative period, and penetrate the soil earlier and deeper than adventitious roots. In theory, the number of seminal roots may be as high as 10, but not all root primordia develop. In wheat, three to six seminal roots normally emerge from the seed, but there is genetic variation for this trait (Robertson et al., 1979). Seed size and seminal root number are positively correlated, though not in all genotypes (O'Toole and Bland, 1987).

Adventitious roots grow mostly in the upper soil layers, and their number depends mainly on the plant's tillering ability. The number of adventitious roots and tillering are thus positively correlated (Hockett, 1986). The ratio of seminal to adventitious roots is altered by the degree of tillering and, later, by interplant competition.

High-input wheats are characterized by a low number of tillers and a high harvest index, and depend mainly on seminal roots. In contrast, adventitious roots are essential to low-input wheat genotypes, which develop larger root systems to explore the greatest volume of soil possible.

Results at CIMMYT showed that the number of tillers was positively correlated with phosphorus acquisition and grain yield among semidwarf bread wheats grown in P-deficient acid soils (Manske et al., 2000a). Phosphorus

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uptake was not affected by tiller number when phosphorus was amply available.

Roots hairs and extramatrical hyphae of vesicular-arbuscular or arbuscular mycorrhizal fungi [(V)AMF] enlarge the effective surface of roots considerably. Thin protrusions from epidermal root cells, root hairs are 0.003–0.007 mm in diameter and 3-13 mm in length, with a normal lifespan of a few days. They emerge just behind the root tip, in the area of root elongation. The (V)AMF hyphae are 10 times finer than root hairs. The average radius of wheat roots is 0.07-0.15 mm. Root length density varies between 2 and 10 cm cm^{-3} soil depending on plant development stage, soil depth, and environmental factors.

The size of a wheat plant's complete root system depends on the environment. The roots' horizontal spread is usually 30-60 cm (Russell, 1977). Roots may be abundant at soil depths of more than 100 cm, with some reaching beyond 200 cm. However, about 70% of total root length is found within the top 0-30 cm of soil (Figure 1). This is because roots grow towards areas of higher nutrient and water concentrations (but avoid toxic levels), where root branching can increase.

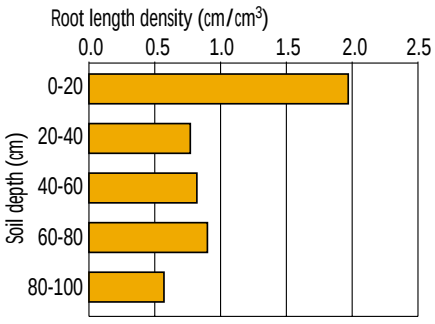


Figure 1. Distribution of root length density in different soil layers; mean of 12 semidwarf bread wheats grown on residual soil moisture in Cd. Obregon, Mexico.

Heritability and Genetic Diversity of Root Traits

Knowledge of heritability and inheritance patterns of morphological root traits in wheat is still restricted, but indicates they are controlled by a polygenic system. Root systems are largely influenced by additive genes that may allow breeding progress to be made by selecting for root quantity and depth of penetration (Monyo and Whittington, 1970). Over 32% of total phenotypic variability for the root:shoot ratio is conditioned by additive gene effects (Kazemi et al., 1979). Although this additive portion of total variance is not especially high, the authors suggest that lines with favorable root:shoot ratios may be obtained by direct selection in early generations of wheat crosses. Moderate heritability values for total root length (0.62) and root branching (0.42) have been reported (Monyo and Whittington, 1970). Narrow sense heritability for root length ranged between 0.38 and 0.46. Many researchers have found the number of seminal roots to be highly heritable (Tiwari et al., 1974; Mac Key, 1973).

Genetic diversity for root traits in bread wheat (Mac Key, 1973) and durum wheat (Motzo et al., 1993) is well documented. Considerable variation in the degree of root branching has been found among wheat cultivars (O'Brien, 1979). Many landraces and wild species of wheat possess large root systems, but tend to lodge due to their height (Manske, 1989; Vlek et al., 1996). The extent of rooting can be modified through selection during breeding, regardless of dwarfing genes or shoot dry matter (Mac Key, 1973; Gale and Youssefian, 1985). McCaig and Morgan (1993) found no significant relationship between dwarfing genes and root dry matter.

At CIMMYT, selecting for tolerance to acid soils, low phosphorus conditions, and aluminum toxicity has resulted in superior semidwarf bread wheats with higher grain yield, improved phosphorus uptake, and greater root length density compared with outstanding tall cultivars from Brazil (Table 1)(Manske et al., 1996; Egle et al., 1999).

Impact of Root Traits on Wheat Growth

Good root growth is a prerequisite for improved shoot growth and higher yields, especially in marginal environments (Manske and Vlek, 2002). Complex interactions between roots and soil take place in the rhizosphere, which commonly includes 20-30% of topsoil volume. The extent of soil exploration is often dependent on water and nutrient availability. Plants may respond to nutrient and water stress by altering root

Table 1. Grain yield, P uptake, and root length density, means of 8 semidwarf and 8 tall bread wheats from Brazil, grown without and with P fertilization (0 or 80 kg P_2O_5 ha^{-1}), with irrigation in a calcareous Aridisol.

	High P regime **		Low P regime	
	Semidwarf	Tall	Semidwarf	Tall
Yield (12% moisture) (kg ha^{-1})	**6240** b	4598 a	**4539** b	3169 a
P uptake into total above ground biomass (kg P ha^{-1})	**22.5** b	19.2 a	**11.3** b	9.5 a
Root length density (cm/cm3)	**10.4** b	8.7 a	**7.7** b	6.6 a

** a<b for LSD P=0.05, separately calculated for P levels.
Source: Manske (unpublished data).

branching and extension rates (Figure 2), rate of uptake per unit root length or weight, partitioning between roots and shoots, root exudates and (V)AMF infection, and by a lower demand for nutrients and water. Each of these parameters can be altered by selection and breeding. Wheat exhibits remarkable plasticity in root growth, which adjusts to soil nutrient and water status (Cholick et al., 1977; Vlek et al., 1996). The partitioning of assimilates between roots and shoots appears to be highly dependent on genotype (Sadhu and Bhaduri, 1984).

Nutrient use efficiency

Root system geometry is essential to improving nutrient uptake and maximizing roots per unit soil volume in areas where nutrients and water are available. Root length is a major determinant of the absorbing surface area. Wheat genotypes with higher root length density are able to take up more phosphorus (Manske et al., 2000a). Root fineness or branching is also an important determinant of P uptake efficiency in wheat (Jones et al., 1989).

Under low phosphorus conditions, the correlation between root length density and P uptake or grain yield is usually 0.50-0.60, but decreases when P supply is adequate. The benefit of high root length density generally diminishes in irrigated wheat with high fertilizer input. If the demand for carbohydrates in large root systems is not compensated for by improved P and water acquisition (as happens when there is adequate water and nutrient supply), the roots themselves may limit yield.

Under high P and good water supply, root hairs are rudimentary, whereas under deficient conditions long root hairs are abundant (Foehse and Jungk, 1983). Root hair length and density modify P depletion profiles in the rhizosphere (Gahoonia and Nielsen, 1996). Root hair density scored in plants derived from pot cultures in quartz sand varied considerably among 19 bread wheat genotypes. Root hair density scores were positively correlated with P uptake at anthesis when plants were grown in a P deficient calcareous Aridisol in northwestern Mexico.

Vesicular-arbuscular or arbuscular mycorrhizal fungi [(V)AMF] are essential for uptaking nutrients that are relatively immobile in soil, such as P (Hayman and Mosse, 1971), Cu (Gildon and Tinker, 1983), and Zn (Swaminathan and Verma, 1979). Screening experiments with wheat have shown considerable genotypic variability in (V)AMF colonization and efficiency (Bertheau et al., 1980; Manske, 1990a; Kalpulnik and Kushnir, 1991; Hetrick et al., 1992). Both the extent of (V)AMF infection and the degree of benefit from (V)AMF are heritable traits (Manske, 1990b; Vlek et al., 1996).

Botanically, mycorrhiza is the symbiotic association between soil-borne fungi and roots of higher plants. Endomycorrhizal fungi are obligate symbiotic fungi that cannot as yet be cultured on artificial media. Extramatrical hyphae grow from the roots into the rhizosphere and can explore an area around the roots that far exceeds that available to root hairs. It is the ability of these hyphae to absorb relatively immobile elements (P, Zn, Cu) in this additional soil that enables endomycorrhizal fungi to benefit plants, especially those with poorly developed root systems.

Efficiency of (V)AMF infection is influenced by soil type. In a P-fixing Andisol, P uptake into wheat shoots and P uptake per unit root length was positively correlated with (V)AMF infection rate. In a calcareous Aridisol these correlations were negative. The role of (V)AMF in calcareous soils is still questionable (Bolan, 1991). In both soils, P uptake was positively correlated with root length density (Table 2).

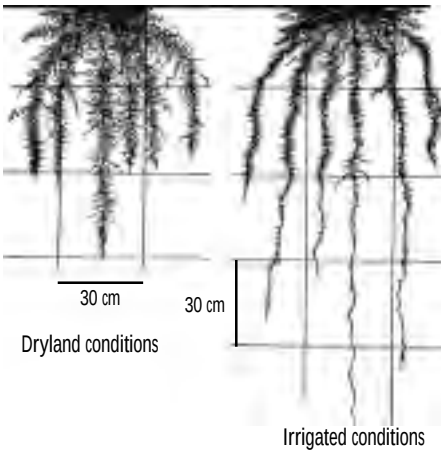


Figure 2. Wheat root system for dryland and irrigated conditions.
Source: Weaver (1926).

Table 2. Correlation between P uptake, root length density, AMF infection rate, and P uptake per root length of semidwarf bread wheats grown in field-inserted pots with calcareous Aridisol from Cd. Obregon and acid Andisol from Patzcuaro, Mexico.

		Calcareous Aridisol			
		P uptake into above-ground biomass	Root length density	AMF infection rate	P uptake per root length
Acid Andisol	P uptake into above-ground biomass		0.71	-0.73	0.83
	Root length density	0.86			
	AMF infection rate				-0.67
	P uptake per root length			0.82	

Source: Buddendiek (1998).

Wheat genotypes may have differential capacities to utilize poorly available soil P. Genotypic differences in root phosphatase excreted or bound at the root surface have been identified (McLachlan, 1980). Organic P in the soil, which commonly accounts for 40-50% of the total P supply of plants, can be utilized by root phosphatase (Helal, 1990). The acid phosphatase activity of 42 bread wheat genotypes from the CIMMYT collection that were screened in nutrient solution was positively correlated with P uptake efficiency in genotypes grown in the field in a P deficient Andisol.

Drought tolerance

Traits associated with high water uptake efficiency are similar to those associated with nutrient uptake efficiency. However, soil water status is more dynamic in nature. Thus, a well-developed root system combines the ability to reach residual moisture deep in the soil profile with a high degree of plasticity that allows it to adjust to rapid changes in topsoil water status. Wheat grown under residual moisture depends on deeper roots to access soil moisture from the deep soil profile (Jordan et al., 1983; Mian et al., 1993). In rainfed wheat, root length densities are much higher in drier years (Hamblin et al., 1990). Varietal differences in rooting depth of wheat were demonstrated by Hurd (1968). In a study conducted at CIMMYT, most drought tolerant semidwarf bread wheats (Pastor, Synthetic 2, Sujata, and Nesser) formed more roots in deeper soil layers, whereas the non-tolerant checks (Tevee 2, Pavon, and CRC) had fewer roots in deep soil (Manske et al., 2000b).

Root Parameters and Methods for Their Determination

Advances in our knowledge of root traits discussed so far have been due largely to the use of relatively simple, but time-consuming methods. Recently developed techniques (Table 3), though often more precise and quicker, are expensive and/or not suitable for field studies on many genotypes. The older techniques were comprehensively reviewed by Böhm (1979).

Table 3. Methods of root research.

Non-sophisticated methods feasible for root research in plant breeding	New, sophisticated methods, expensive and not feasible for many genotypes	Methods feasible only for detailed studies of ecological and physiology root research
Core break method	Minirhizothron	Trench profile method
Electrical capacitance method	Radioactive tracer methods	Profile wall method
Measuring root-pulling strength	Non-radioactive tracers	Glass wall method
Root angle method		Rhizothron
Mesh bag, container method		Excavation method
Sampling methods (spate, root box, root auger)		Split-root technique
Root washing techniques		
Root parameters for determination after washing: Root number, weight, surface, volume, diameter, root length density, root/ shoot ratio		
Image analyzer methods		
Root hairs, arbuscular mycorrhizae		
Root studies in nutrient solution		

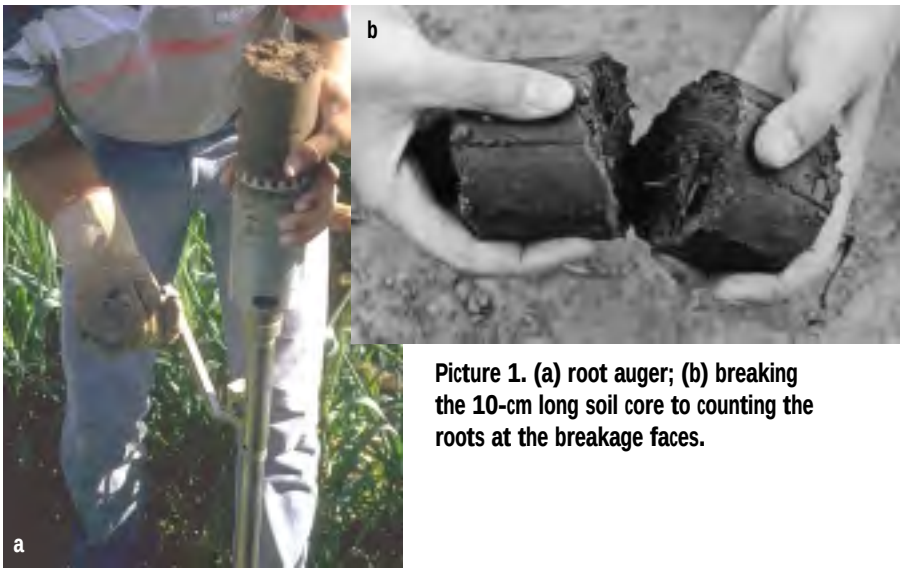
Methods used in root research can be termed as descriptive or quantitative. Many descriptive methods are used to estimate root quantities in a time- and labor-efficient manner. They are non-destructive and provide only limited information about roots of field-grown wheat.

Descriptive methods used to measure root distribution

The core break method. A root auger is used to take soil-root cores in the field. The bi-partite root auger (for example, from FA. Eijkelcamp) (diameter: 8 cm; length: 15 cm; volume: 750 cm^3) is suitable for hard, heavy soils. Introduce the auger into the ground using an impact-absorbing hammer with a nylon head (ø 70 mm; weight: 2.3 kg). Now put the complete auger cylinder into the soil and then extract by rotating clockwise. Invert the auger and turn the crank handle to force the soil core out of the cylinder (Picture 1a).

The amount of roots in a sample (root length density or root mass) is estimated by breaking the soil core horizontally through the middle and counting the roots exposed on both faces of the breakage (Picture 1b). Every exposed root is counted, regardless of its length. The number of roots visible on the surface of a broken sample is expressed as a surface-covering figure. Accuracy increases when the sample is broken in more than one place. Each exposed surface has only one surface-covering figure. An average of all figures is calculated for more reliable results. Counting is greatly facilitated if the two breakage faces are wetted with a sprayer to make the roots more visible (Köpke, 1979).

The exposed surface must be compared with a reference, consisting of a number of circles having the same diameter as the root auger, to depict the increasing root intensity and estimate a constant C for



Picture 1. (a) root auger; (b) breaking the 10-cm long soil core to counting the roots at the breakage faces.

root length density (cm root length/cm^3 soil). The root length density is then estimated by multiplying root intersections by constant C. Most studies using the core break method have been conducted on gramineaceous species. Bland (1989) demonstrated the use of the core break method in wheat.

The mesh bag method. The dynamics of root growth and root turnover can be studied in root-free soil placed in mesh bags (nylon stockings can be used). The mesh bags are placed in holes in the field and removed at established intervals. Roots that have invaded the mesh bags are measured and used as an index to calculate root productivity (Fabiao et al., 1985). The disadvantage of this method is that the soil is disturbed when the bags are placed in it, which can alter root growth (Fitter, 1982).

The electrical capacitance method. Root volume and root length density can be estimated by the electrical capacitance method (Dalton, 1995). This *in-situ* method is based on measuring the electrical capacitance of an equivalent parallel resistance-capacitance circuit formed by the interface between soil-water and the plant root surface. The method is adequate for measuring the root surface of field-grown plants quickly without using labor-intensive procedures such as root sampling, washing, and counting. However, this technique does not measure the spatial distribution of roots in a soil profile.

Van Beem (1996) developed such a technique for maize (Figure 3). The negative electrode of a capacitance meter (a BK Precision 810A instrument set at the 200 nF level) was attached to the maize stem exactly 6 cm above the soil surface. The positive electrode was attached to a copper ground rod, inserted 15 cm deep into the soil, 5 cm from the base of the maize stem. Capacitance readings were taken at maximum soil moisture after irrigation, 5 seconds after the meter was turned on.

Figure 3. Electrical capacitance measurement of root size in a maize plant.
Source: van Beem (1996).

It is more complicated to take these measurements on wheat because each plant has several tillers. An apparatus developed at CIMMYT measures the capacitance of all wheat stems and their roots growing in a 30-cm planting row, but more research is needed before it can be used routinely.

Rhizothron and minirhizothron. Root growth can be observed by using a root periscope (minirhizothron) to peer through a glass or acrylic tube inserted in the soil. This technique involves the use of cameras coupled with endoscopes or miniaturized color video cameras, and thus is more costly (Upchurch and Ritchie, 1983). A large computer system and special software are needed to analyze large numbers of root images quantitatively. The system can provide images of small root hairs and may be used for fractal analysis of root systems.

Several authors have compared this new technique with the unsophisticated soil-core root-counting method. Root length densities derived from the two methods are not always well correlated (Majdi et al., 1992; Volkmar, 1993; Box and Ramseur, 1993). Usually, the number of roots encountering the tube (barrier-soil interface) exceeds the number of roots passing through an analogous area of soil. Roots encountering vertical tubes tend to grow along the tube.

The wax layer method. The ability of cereal roots to penetrate compacted soils helps them avoid drought stress. Root penetration can be screened by using wax-petrolatum layers with defined resistance strength (Yu et al., 1995).

The root angle method. Deep rooting is associated with drought tolerance of wheat grown under residual moisture.

The gravitropic response of roots axes determines the shape and spatial distribution of root systems in cereals. The plagiogravitropic response of roots depends on the growth angle of the root axes, which is controlled by genetic and environmental factors.

Genotypic characteristics of the vertical distribution of wheat roots in the field can be estimated based on the growth angle of seminal roots of wheat seedlings grown in baskets. The baskets can be buried in the field or used as pots in the greenhouse. A wheat grain is sown 1 cm deep with the tip of the embryo at the center of the soil-filled basket. After several weeks, the angle between the vertical axis and the line connecting the grain and the mesh of the baskets through which the root appeared is determined (Figure 4).

Root growth angle is negatively correlated with root length density in the top 0-10 cm of soil, but positively correlated with root length density at a depth of 10-30 cm (Nakamoto and Oyanagi, 1994; Oyanagi et al., 1993). This method thus promises to determine genetic differences in deep-rooting ability, a trait important for drought tolerance. However, more root data from the field need to be compared to establish its reliability.

Root pulling strength. Root traits contribute to lodging resistance in wheat, as do dwarfness, resistance, and stem elasticity. The spreading angle of root systems (Pinthus, 1967), root-clump weight (Thompson, 1968), root tensile strength (Spahr, 1960) and root pulling strength (Ortmann et al., 1968) are all related to lodging resistance. Simple root-pulling machines have been constructed for use in maize (Ortmann et al., 1968). Vertical pulling strength is measured with a tensiometer. Homogeneous field soil is required for reliable measurements with this method. Despite its simplicity, this method is rarely used in field studies.

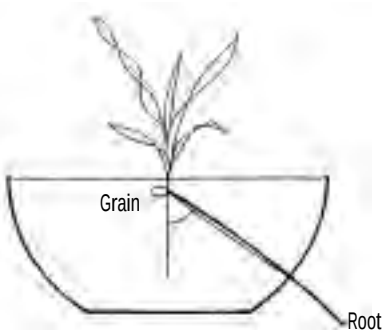


Figure 4. Determination of root growth angle by growing a wheat plant in a basket.

Quantitative methods

Assessing root parameters from roots sampled in the field. This approach has been widely applied to measure root length density, depth, and lateral extent of root systems. Roots are separated from the soil to determine root fresh weight, dry weight, (V)AMF infection, and, if needed, nutrient content. Recovering roots from the field involves taking samples, removing roots from the soil, separating roots from organic debris, storing the roots, and determining and calculating several root parameters. With this approach the root system can only be observed at one point in time, unlike excavation, which allows an accurate description of the morphology of the whole root system.

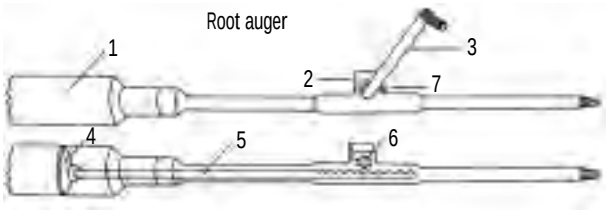
Sampling roots in the field. Roots from a defined volume of soil can be extracted in the form of monoliths and/or soil cores. Root core samples can be taken with a root auger, as previously described (Figure 5). Soil monoliths can be collected by driving a root box (metal frame) into the soil or by using spates.

The root box supports the monolith, which is important if the soil lacks sufficient structure.

Metal boxes of the required size are easy to build. Root boxes are open at the bottom and the top. An extra sheet of metal is added to reinforce the upper rim so it will resist being hammered into the soil (Picture 2). Although an impact-absorbing hammer with a nylon head (2 kg, Δ 70 mm) is preferred for driving the root box into the soil, a rubber or wooden hammer—but not a metal one—can also be used. Heavy loamy soils must have the right soil moisture content for root sampling (not too dry, not too wet). Sandy soil should be wet; otherwise, parts of the monolith may be lost from the bottom.

The size of the root box depends on the crop and field design. Cereal crops should be planted in rows, not broadcast, which causes high root variation. The box should cover the width of one row completely. The sampling area consists of two symmetrical halves on either side of the row. The ideal depth of a root box is 20-30 cm, and its length should be 30-50 cm. Usually, 70-80% of the total root system is found within the top 20 cm of soil.

Picture 2. Root box with extracted root-soil monolith together with wheat shoots.



1: Cylindric auger body
2: Pinion casing
3: Crank handle
4: Pressure plate
5: Pressure plate shaft containing rack
6: Pinion
7: Lubrication nipple

Figure 5. Root auger.

Sampling wheat roots with an auger is another option (Kumar et al., 1993). Its advantage is that the soil is minimally disturbed, but sample volume is small. Samples are normally taken at random, but how many samples per plot should be taken to obtain reliable root information is difficult to determine. Böhm (1979) recommended five bore holes per plot. The position of the sampling sites within or between the wheat rows is also very important, and different methods are used. Gajri and Prihar (1985) employed two sampling sites, on the plant row and midway between the rows. Gregory et al. (1978) sampled wheat roots between plant rows. Gajri et al. (1994) analyzed horizontal root distribution between wheat rows to identify the ideal site for augering.

Excavating the root/soil sample in a root box is much faster than using a root auger (Vepraskas and Hoyt, 1988), but the auger allows deeper sampling. However, spatial variability rises as soil depth increases. The root box and the root auger can be combined as follows. The first 0-20 cm are sampled with a root box, which gives the best accuracy in the top soil, and the root auger is used deeper in the profile. At CIMMYT, six bore holes were sufficient for 1-m depth. The CV for root length density was about 27%, and significant differences between wheat cultivars were observed.

Samples can be stored for 2-3 weeks if protected against the rain and high temperature. Plastic bags may be used, but they should have holes and must be kept open to allow ventilation and keep the samples from getting moldy.

Removing roots from the soil. Böhm (1979) reviewed various washing and floating procedures, as well as chemical dispersing agents. Especially in the case of heavy, loamy soil, samples should be soaked overnight in a saturated NaCl or soap solution to increase buoyancy and disperse soil aggregates.

The simplest and often the most economic technique of separating roots from soil is to wash them by hand. First the soil-root sample is suspended in water and poured into sieves where roots are retained and collected for further cleaning. The sieve mesh should not be too fine (or the procedure becomes very time consuming) or too coarse (or it will not retain very fine roots). Ordinary plastic sieves (10-mm mesh size) are adequate. The roots are then washed by hand under a water jet or spray. This technique can be used in remote field sites when large amounts of soil/root samples cannot be transported to the laboratory. The roots may even be washed in irrigation channels or nearby rivers. In this case, the roots can be washed in baskets that act as sieves.

A root-washing machine can be effective when manual labor is in short supply, but often takes longer, especially with larger soil volumes. A machine cannot wash roots better than can be done by hand, and using tweezers to separate debris from the roots is still necessary. Smucker et al. (1982) devised a root washing apparatus, termed a hydropneumatic elutriation device. The device has a high-kinetic-energy first stage in which water jets erode the soil from the roots and a second low-kinetic-energy flotation stage in which the roots are deposited in a submerged sieve. The procedure is quick (3-10 min for one core sample), has a high root recovery rate, and does not sever laterals. Its biggest advantage is greatly improved consistency in sample handling. Root washers with four or eight tubes that can wash several samples at a time are available on the market; one to four people can use the machine simultaneously.

Separating roots from organic debris. Roots must be separated manually when there is an appreciable amount of organic debris. Distinguishing between living and dead roots (debris) is usually done subjectively based on criteria such as color, but methods for objectively detecting live tissue have been established. For example, a tetrazolium dye technique suitable for estimating the proportion of live material was reported by Joslin and Henderson (1984), and Ward et al. (1978) used congo red for quantitatively estimating living wheat-root lengths in soil cores. These methods notwithstanding, many workers still prefer to subjectively separate large numbers of root samples by sorting through them manually.

Storing washed roots. Washed roots may be stored before further processing, for example, in small plastic bags containing 50% alcohol in a refrigerator at 7°C (freezing destroys root structures).

Assessing root parameters after washing. Total root length or root length density are measured on representative subsamples of the whole (washed) root system, especially for plant species with fine roots, such as wheat. At CIMMYT, the following method was developed for representative subsampling: a washed root system, free from organic debris, is cut with scissors into 1-2 cm pieces that are placed in a small bowl of water and mixed. The water is poured through a sieve to collect the root pieces, and total fresh weight is determined. Subsamples are extracted and weighed, and root length measured.

The simplest way of measuring fresh weight is by blotting roots with blotting paper. Since the accuracy of this method is affected by how the sample is handled, the same person should always do the procedure. To determine dry weight, clean roots are dried in an oven at 60-70ºC (higher temperatures could pulverize the roots) for about 24 h.

Measuring root length, diameter, and surface area. Root length per unit of soil volume (root length density) is one of the best parameters for studying water and nutrient uptake by plants (Nye and Tinker, 1969; Claassen and Barber, 1974). The line-intercept method developed by Newman (1966) and modified by Tennant (1975) counts the total number of intersections between roots and the vertical and horizontal lines of a grid drawn on a plastic petri dish (Picture 3). Root length can be estimated by the equation

$$R = \pi AN / 2H,$$

where R is the total length of the roots in area A of the petri dish, and N is the number of intersections between the roots and random horizontal grid lines of total length H. For a grid of indeterminate dimensions, the intersection counts can be converted to centimeters using the equation

Root length (R) = 11/14 x number of intersections (N) x grid unit

As proposed by Tennant (1975), 11/14 in the equation can be combined with the grid unit to obtain a length conversion factor. The factor for 1 cm is 0.786. If ½ inch (1.27 cm) is used as the grid unit, each intersection count represents 1 cm of root length (because 1.270 x 0.786 = 1.00, exactly 0.998), and the equation for calculating the total root length per sample is

R = N x fresh weight of total sample/ fresh weight of counted subsample

The size of the grid and of the subsample used for counting depends on the amount of roots to be measured. According to Köpke (1979), the number of intersections counted in a root sample should not be above 400, to keep the operator from tiring, or below 50, so accuracy will not be affected. For wheat, 200-300 gives maximum accuracy. Subsample size should be 0.1 g for coarse roots and 0.05 g for fine roots. Depending on location and soil uniformity in the field, the coefficient of variation for root length density ranged between 20 and 35%.

Picture 3. Plastic petri dish with grid line, grid unit $^1/_2$ inch (1.269 cm).

Image analysis. Instead of counting roots tediously by hand, one can use image analysis systems, which have become available on the market in the last few years. An image analysis system is a multipurpose instrument that measures leaf area, numbers of objects (seeds), different portions of an area (leaf necrosis due to disease), and, through root analysis, root length, diameter, and surface. It usually consists of a computer, a video image monitor, and a video camera or scanner.

The correlation between image analysis and the manual line-intercept method depends on calibration and the type of imaging system used. The digital method often underestimates root length because it does not detect small, fine root systems (Farrell et al., 1993). However, the technology is progressing rapidly, and the latest image analyzer systems have improved resolution and lighting systems that can distinguish between fine and coarse roots, and correct for overlapping roots (Arsenault et al., 1995).

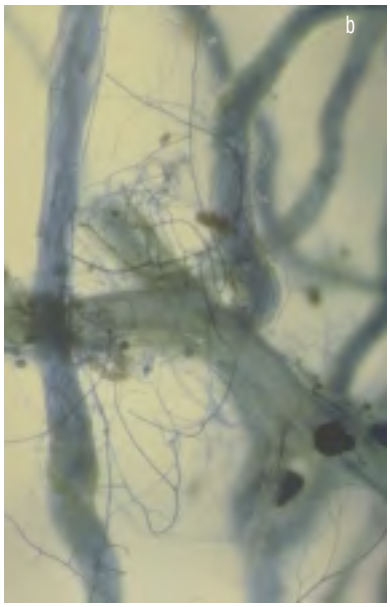
Root radius. Average root radius can be calculated from root fresh weight and total root length. Assuming that the wheat root contains almost 90% water, the specific weight is almost 1, and the formula for obtaining the average root radius is

$$r = (\text{root fresh weight}/\text{total root length}/\pi)^{-1/2}$$

Shoot:root ratio. A common parameter used to study the relation between above- and below-ground plant growth is the root:shoot ratio. The root length:shoot dry weight ratio gives more information than root weight:shoot weight, whether fresh or dry. Relationships between root length and plant development stages can be observed.

Root hair density. Quantifying root hairs (Picture 4) is difficult because root length density varies considerably within the same root system. Some root units, usually older ones, have no hairs, while others, usually younger roots, have dense hairs. At CIMMYT, a scoring method was used to determine genotypic differences in root hair density in wheat: Hundreds of intersections between roots and grid lines in a petri dish were scored at random using a microscope (0 = no root hairs to 5 = very dense). Average root hair density for each sample was calculated. Though the number of root hairs can usually be counted, this is not possible in wheat, because there are too many. The diameter and length of the hairs can be measured by means of a binocular or a microscope.

Vesicular-arbuscular or arbuscular mycorrhizal fungi [(V)AMF]. Hyphae of endomycorrhizal fungi develop mycelia, arbuscules, and/or vesicles in roots. In addition, extramatrical hyphae grow from the root into the rhizosphere soil (Picture 5) and can explore an area around the root which far exceeds that available to root hairs. To quantify AM infection, the root sample (usually a



Picture 4. Under the microscope: (a) root hairs, and (b) extramatrical hyphae of (V)AM fungi and wheat roots.

representative subsample) is cleaned and stained (Philipps and Hayman, 1970), and examined under a microscope. A modified staining method (Giovannetti and Mosse, 1980) may be used that stains the root without the use of phenol, which is toxic and carcinogenic.

Studies using containers and mesh bags

Root properties can be assessed on wheat plants grown in containers, but the resulting data should be viewed with caution, since the container is often too small for unrestricted root growth, and roots concentrate at the container wall. Root data obtained from mesh bags buried in the soil give a more reliable picture of the field situation. However, here also, taking root samples directly from field soil may be a better alternative.

Root studies in nutrient solution

Root systems grown in nutrient solutions can be observed and studied *in situ* without time-consuming washing and cleaning. This method allows a large number of plants to be studied simultaneously within a short time. However, growth conditions in nutrient solutions differ greatly from conditions in soil and water, and results from these studies must be compared with results of field experiments. Mian et al. (1994) found that wheat genotypes with large root systems in hydroponic culture may also produce more roots in the field.

Aluminum toxicity in acid soils inhibits root growth by preventing cell division, which results in reduced root penetration in the soil and leads to reduced nutrient and water uptake. At CIMMYT, wheat genotypes are routinely screened for tolerance to aluminum toxicity (Kohli and Rajaram, 1988). Hundreds of wheat seedlings are screened simultaneously in nutrient solution with 46 ppm of aluminum, which irreversibly damages the root meristem of susceptible genotypes. After four days' growth, the roots are stained with 0.2% hematoxylin solution and transferred to an aluminum-free solution. Selection of tolerant genotypes is based on root regrowth beyond the hematoxylin-stained cell layers (Rajaram and Villegas, 1990).

Conclusions

Wheat breeders and physiologists who decide to study wheat roots should be aware that it can be very labor intensive. This is the reason most breeding programs have largely avoided root studies. Nonetheless, assessing root traits is essential in some cases (e.g., to improve nutrient acquisition, drought tolerance, and other adaptation mechanisms).

Methods exist to study root traits indirectly for different purposes, for example, to determine genotypic differences in phosphorus uptake efficiency in wheat. Root angle and wax layer methods may provide knowledge about deep rooting of wheat for drought tolerance. Root volume studied in nutrient solution may indicate how roots grow in the field. Wheat grown in nutrient solution is routinely used at CIMMYT to screen for aluminum tolerance. The electrical capacitance of root systems is a promising method for indirectly studying root dimensions in the field. However, this method needs to be improved before it can be used routinely.

Since direct assessment of root traits cannot be avoided in many cases, techniques for conducting such studies are presented here. Most of them are labor intensive, but not sophisticated, and easy to use in the field. Actually, sampling roots in the field and washing and counting them by hand have an advantage in regions where labor costs are low. In other cases, manual labor may be the best option, and may compare favorably with many new techniques.

Root washing machines and new, improved image analysis systems are affordable and practical in most research situations. Their biggest advantages are greatly improved consistency in handling and measuring samples and higher labor cost effectiveness. However, these field methods do root analysis only on a small number of genotypes, for example, for identifying parental lines. Screening large numbers of wheat lines in

segregating populations is only practicable if root analysis is done indirectly, for instance, in nutrient solution. More in-depth studies, such as (V)AMF infection, root geometry, root morphology, and the minirhizothron technique are not practical for use in selection.

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CHAPTER 19

Micronutrients

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Little effort has been made to apply modern breeding techniques to adapt crop plants to soils of poor nutritional status, even though this is genetically feasible. Rather, the use of fertilizers to solve soil nutrient problems agronomically has encouraged plant breeders to concentrate on other objectives such as yield, climatic adaptation, disease resistance, and quality.

Some nutritional problems, however, do not appear to be easily resolved, and a case for breeding adapted varieties can be made in those instances. The most obvious problems are those of nutrient toxicities, where the cost of removal is much greater than that of applying fertilizer to address deficiencies; hence breeding solutions are more practical and indeed necessary. Furthermore, fertilizers are ineffective with micronutrient deficiencies such as iron and manganese, which are induced by high pH; agronomic solutions may thus not be satisfactory, and a genetic solution is necessary.

Soils with chronic micronutrient deficiencies are often high-pH, calcareous soils in seasonally dry climates, but may include deep sands in any climate. Although micronutrient fertilizers are often strikingly beneficial, the plant's yield potential can only be reached if its roots penetrate the chemically inhospitable subsoils to access water stored there. Tolerance to micronutrient deficiencies (that is, micronutrient efficiency; see definition below) is generally manifested by greater uptake from deficient soil. Roots must find micronutrients in their immediate environment to continue to grow, to express disease resistance, and to access water stored in the subsoil.

In this chapter we outline the case for breeding crop plants for traits that confer adaptation to nutrient deficiencies in soils. In this context a deficient soil is one that contains reasonable amounts of the limiting nutrient; however, it is relatively unavailable to the common cultivars of the crop in question. We then discuss the genetics of micronutrient traits and the status of screening techniques that can be used in breeding programs.

Definition of Nutrient Use Efficiency

We define nutrient use efficiency (for each element separately) as a genotype's ability to produce high yield in a soil whose nutrient content is limiting for a standard genotype. This agronomic definition is meaningful to a plant breeder selecting genetic material in the field. Often a nutrient-efficient genotype in infertile environments will also have high yield potential in fertile soils. Nutrient efficiency may be achieved physiologically by one or more mechanisms:

- better root system geometry;
- faster specific rate of absorption at low concentrations (low K_m);
- chemical modification of the root-soil interface to solubilize more of the limiting nutrient;
- improved internal redistribution of the nutrient;
- superior nutrient utilization, or a lower functional requirement for the nutrient in the cell.

In the field the plant breeder cannot readily identify the operative mechanism(s), but selection would be more precise if he/she knew which ones they are. Examples of this are given in the text, but for the greater part, efficiency is inferred from yield measurements, nutrient content, or symptom expression.

The Case for a Breeding Program

Plant breeding is a numbers game, and any new objective, such as micronutrient use efficiency, represents a considerable escalation of the breeder's work or else a diversion of effort away from traditional targets such as quality and disease resistance. Thus there must be a strong case for a new breeding objective before it can be added to the research agenda. The lack of compelling arguments is the reason little effort has been made until

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now to adapt crop plants to micronutrient-deficient soils. To argue for breeding to improve nutritional characters, it is necessary to show:

- a need for it as pressing as for other breeding objectives;
- there is reasonable genetic potential to be exploited;
- it is agronomically, economically, and ecologically feasible.

Graham (1984, 1987, 1988a, b) demonstrated that there is genetic diversity for micronutrient characters within wheat, and further argued that nearly all soils, no matter how poor, had sufficient amounts of micronutrients stored in the profile. The problem was usually their lack of availability due partly to soil chemistry, but equally to poor genotypic adaptation. This brings us back to the agronomic arguments.

Twelve years ago, Ascher and Graham (1993) bulldozed off the topsoil and dug up the subsoil from grave-sized pits on 10 soil types scattered across South Australia. Various nutrient treatments were applied to the subsoils as they were returned to the pits in their original layers. The topsoils were then replaced and the sites sown by each farmer as part of the field. The farmers applied all the usual treatments and fertilizers, including in some cases micronutrients, without disturbing the treated subsoil below. Responses to the nutrients were immediate and often spectacular, though in general there was little response to physical disturbance only or to gypsum, which underlines the chemical nature of the problem.

It is important to note that these responses (at the five most trace-element-deficient sites) continue to the present, and with the original nitrogen most likely lost by now, the residual responses are probably due to phosphorus and trace elements (Picture 1). Indeed, the micronutrient treatment seems to have relatively greater residual value as time passes. It appears that root channels developed to depth in the early years have been re-utilized annually to the present. In pot studies we have shown that wheat roots grow poorly in subsoil even when fertilized with nitrogen and phosphorus. Although we are experimenting with methods of deep placement of micronutrients with highly unconventional and expensive machinery, we believe a better approach to this problem is to breed wheats with roots that will penetrate subsoils with low availability of phosphorus and micronutrients.

Immediately relevant to this argument is the picture emerging from physiological studies of roots spanning four decades. From the papers of Haynes and Robbins (1948), Epstein (1972), Pollard et al. (1977), Bowling et al. (1978), Graham et al. (1981), Welch et al. (1982), Nable and Loneragan (1984), Loneragan et al. (1987) and Holloway (1991), it appears that the elements phosphorus, zinc, boron, calcium, and manganese are all required in the immediate external root environment for healthy growth, membrane function, and cell integrity. In particular, phosphorus and zinc deficiencies in the external environment promote leaking of cell contents such as sugars, amides, and amino acids (Graham et al., 1981), which are chemotactic stimuli to pathogenic organisms.

Phosphorus is phloem mobile, but the other elements are not, or are poorly so; this means that the root tips cannot be adequately supplied from elsewhere in the root system, such as for example, from roots that come into contact with a fertilizer band. Moreover, in the case of zinc, a high internal zinc content did not prevent leakiness due to a deficiency of zinc external to the membrane (Welch et al., 1982). It follows that the roots of those wheat genotypes that have a greater capacity to mobilize nutrients strongly bound to soil particles in the rhizosphere will be better able to penetrate the infertile, high pH subsoil. It follows too from the above that roots

Picture 1. Grave-sized barley plots in a farmer's field at Marion Bay, 1992. The areas around the graves represent completely undisturbed subsoil. The middle plot shows a huge response to subsoil N, P, and micronutrients, even after seven seasons and despite regular topsoil fertilization by the farmer. (Photo: J. Ascher-Ellis.)

that are far from a fertilizer band and have leaky membranes are at greater risk from pathogens.

Recent studies have clearly linked trace element deficiencies with enhanced susceptibility to particular pathogens (Graham and Webb, 1991). Manganese-deficient wheat plants are more susceptible to *Gaeumannomyces graminis* var. *tritici*, the take-all fungus (Graham and Rovira, 1984; Huber and Wilhelm, 1988), and to the foliar pathogen, *Erysiphe graminis* (Graham, 1990). Zinc deficiency decreased the resistance of wheat to *Fusarium graminearum*, the crown rot fungus (Sparrow and Graham, 1988) and to *Rhizoctonia solani* (Thongbai et al., 1993a, b), the causal agent of bare patch. The implication is that nutrient-efficient genotypes growing in deficient soil, enjoy better nutrient status and should have greater resistance to these pathogens; this has been confirmed by Wilhelm et al. (1990), Pedler (1994) and Rengel et al. (1993) for the manganese efficiency /take-all system and by Grewal et al. (1996) for the zinc efficiency/crown rot system. Collectively, these results suggest causality in the concurrence in South Australia of some of the world's most severe root disease and micronutrient deficiency problems.

Topsoil drying also affects wheat production on infertile soils of the seasonally humid zone by causing loss of fertilizer efficiency. Most micronutrients are in the topsoil by virtue of fertilizer additions and nutrient cycling. Leaching of the heavy metals is negligible (Jones and Belling, 1967). When the topsoil dries as a result of a week or two of dry weather in spring, roots in the nutrient zone are largely deactivated and the plant must rely on deeper roots or retranslocation for further nutrition. With phloem-immobile micronutrients and inefficient genotypes, deficiency can result. This occurs in the field (Grundon, 1980), where copper deficiency from topsoil drying at early boot stage can cause severe sterility problems; similarly in a pot trial Ascher and Graham (see Table 5, Graham, 1990). These authors showed that Cu deficiency could be induced in wheat plants growing in tall pots if an otherwise adequate Cu supply in the topsoil was rendered unavailable by soil drying and there was insufficient available Cu in the subsoil. This problem may, however, be overcome by using copper-efficient genotypes such as triticale (Grundon and Best, 1981).

Two further advantages accrue to micronutrient efficient varieties if by virtue of their efficiency they also accumulate more of the limiting nutrient in the grain: 1) they improve human nutrition if consumed (iron, zinc, especially), and 2) seedling vigor is markedly better when the seed is resown on deficient soils. A final advantage: the degree of micronutrient efficiency currently known to exist in wheat germplasm, if deployed in modern varieties, would overcome subclinical deficiency commonly unrecognized by farmers or their advisers.

The Case for High Nutrient Reserves in Seed

Reserves of nutrients in the seed must be sufficient to sustain growth until the root system has developed sufficiently to supply nutrients from the growth medium. During plant establishment nutrients are supplied partly from seed reserves and partly from the soil. High levels of seed nutrients are particularly important in soils with low nutrient availability, since a larger root system is required for the soil to supply the needs of the crop. Low nutritional status of seeds has been reported to reduce plant growth under conditions of low nutrient availability (Marcar and Graham, 1986; Asher, 1987; Rengel and Graham, 1995a, b; Moussavi-Nik, 1997).

The nutrient status of seed has been shown to affect both seed viability and seedling vigor. There are reports in the literature of minimum seed concentrations of nutrients below which seedlings will not grow normally (Ascher and Graham, 1993). Seedling nutrition has been shown to be an important factor in plant susceptibility to pathogens (Graham, 1983; Graham and Webb, 1991; Pedler, 1994; Streeter, 1998); seed nutrient levels that fail to maintain adequate nutritional status of seedlings in infertile soils may reduce the plant's resistance to some seedling diseases. The importance of seed nutrient status in confounding the screening procedures for micronutrient use efficiency traits is dealt with in a later section.

Mineral Nutrient Quality of Grain

The mineral content of grain contributes not only to seedling vigor in the next generation but—equally important—to the nutrition of humans and animals. This is particularly so for micronutrients, since over half of the world's population is deficient in micronutrients, and cereal grains, the major component of the diet of those at risk, also contribute most of the minerals in their diet. Of primary concern in human nutrition are iron and zinc, and secondary concerns are provitamin A and the minerals iodine, calcium, selenium and copper (Graham and Welch, 1996).

Zinc is an element of special interest as it is commonly deficient in soils (50% globally), plants, animals, and humans.

Much can be achieved through the whole food chain by fertilizing the soil with zinc (Graham and Welch, 1996), but because of topsoil drying and the role of subsoil fertility, the case has been made elsewhere in this chapter for breeding zinc-efficient wheats, probably involving three or four genes per genome.

Where zinc efficiency translates into higher zinc concentration in the seeds, as it can, we have a win-win benefit for producers and consumers, and an additional mechanism for better yields in subsequent crops on deficient soils: zinc-dense seed. In some cases, however, zinc-efficient cultivars may enhance yield so much that the concentration of zinc in grain is diluted by extra dry matter (Graham et al., 1992). The evidence available indicates there are genes (independent of those controlling the agronomic zinc efficiency trait) that control the transport of zinc to the grain from vegetative parts during grain filling. Since the heavy metal nutrient cations iron, zinc, copper, manganese, cobalt, and nickel are insoluble at the high pH of the phloem sap, these transport genes probably code for natural chelators that hold these metals in solution in the phloem sap so they may be transported to the grain. One such gene, identified in tomato, codes for the synthesis of nicotianamine, which is essential for the transport of iron in that plant. In a nicotianamine-deficient recessive mutant, Chloranova, this trait is controlled at a single locus (Ripperger and Schreiber, 1982).

Genetic variation for iron and zinc concentration in grain has been explored in wheat by Ortiz-Monasterio and associates at CIMMYT. The range in concentration from the lowest to the highest for both elements is 3-5, and there is a potential advantage over current high yielding varieties of a factor of 1.5-3 (Graham et al., 1997). Although high levels of iron and zinc are not co-inherited, there are genotypes higher in both, and among them are high yielding advanced lines.

Household resource allocation studies (Bouis, 1994) suggest that doubling the iron content in grain would significantly improve iron deficiency in humans, provided the iron in iron-dense varieties was bioavailable. The bioavailability of the iron in high-iron beans (and rice) has already been tested on iron-deficient rats and proven to be as available (percent of total) as that in low or standard types (Welch et al., 1999). Tests are under way in humans. Since beans are high in absorption inhibitors such as phytate and tannins, it is likely that an equally favorable result will be established for wheat in the near future.

Genetics of Micronutrient Traits

The first genetic study of a micronutrient efficiency factor was conducted by Weiss (1943). He showed that iron efficiency in soybeans was due to a single dominant gene that controls the reducing power of the root surface. Since this pioneering study, several minor additive genes have been discovered to contribute to iron efficiency in soybeans (Fehr, 1982). This situation of one major and several minor genes is generally the case with the micronutrients. Reports to about 1970 were reviewed by Epstein (1972), who noted that boron efficiency was apparently under simple genetic control in tomato and celery, as were iron efficiency in maize and tomato, and magnesium efficiency in celery. More recently iron efficiency in tomato has been shown to be controlled by a major gene, coding for an iron-transporting amine, nicotianamine, and a string of minor genes (Brown and Wann, 1982; Ripperger and Schreiber, 1982).

Copper efficiency

Copper efficiency in rye appears to be a dominant trait controlled at a single locus on the long arm of chromosome 5R (Graham, 1984). Copper efficiency has been transferred from rye to wheat by translocating part of 5RL to a chromosome of wheat. The translocated 5RL chromosome segment carrying the copper-efficiency trait Ce-1 onto the 4Aβ (now 4Bβ) chromosome confers on plants a much greater ability to mobilize and absorb copper ions tightly bound to the soil (Graham, 1984). Several such translocations exist but the 5RL/4A translocation appears to be the most satisfactory agronomic type (Picture 2); it has been successfully incorporated into

Picture 2. The copper-efficient 5R/4A plants on the right grow well and show good seed set in soil that is too copper-deficient for the control genotype (2R/4A).

cultivars adapted to South Australia (Graham et al., 1987). The 5RL chromosome arm also confers copper efficiency in triticale, unless a copper-inefficient rye is used in the cross. Triticales generally show agronomically useful copper efficiency, intermediate between wheat and rye, and have been used for this reason on many sandy or peaty copper-deficient soils in South Australia (Graham, 1987), and on deficient clayey soils in Queensland to counter the effects of topsoil drying (Grundon, 1980).

Work with these 5R materials has shown that copper efficiency in rye is not clearly linked to zinc or manganese efficiency. Thus independent and relatively specific genes are involved, and neither root system geometry nor size appears to be critical (Holloway, 1996).

Although rye has a much longer and finer root system than wheat, triticales generally do not, yet they are usually more efficient for all three elements (Graham, 1984; Graham et al., 1987; Harry, 1982; Cooper et al., 1988). Studies of rye addition lines suggest that 6R contributes a little to efficiency for all three elements, perhaps by way of a root geometry feature, but the major genes are elsewhere. Manganese efficiency is located on 2R, a conclusion supported by the poor performance on manganese-deficient soils of cv. Coorong, an Armadillo-type triticale lacking 2R. By way of contrast, zinc efficiency in rye does not appear to be clear-cut from our studies of rye addition lines of wheat, and may be spread across four or five chromosomes: 2R, 3R, 7/4R and, to a lesser extent, as we've said, 5RL and 6R (Table 6, Graham, 1988a). Cakmak et al. (1997) have additionally linked Zn efficiency to 1R.

Zinc efficiency

Studies of addition lines have shown that Cu, Zn, and Mn efficiency in rye are independent traits, carried on different chromosomes (Graham, 1984). Copper and Mn efficiency in rye and Mn efficiency in barley appear to be controlled by single major genes (Graham, 1984; McCarthy et al., 1988), as is Fe efficiency in soybeans (Weiss, 1943). Boron and Mg efficiency in celery (Pope and Munger, 1953a,b) and B efficiency in tomato (Wall and Andrus, 1962) likewise appear to be controlled at a single locus (all reported to be dominant).

However, less is known of the genetics of Zn efficiency. Several loci on as many different chromosomes are involved in Zn efficiency in rye, and a few genes are involved in Zn efficiency in rice. The largest single screening exercise was done on 3,703 lines of paddy rice (Ponnamperuma, 1976; IRRI, 1979); 388 lines were judged to be tolerant and a similar range of responses was observed. Following diallel analysis, a recent report suggested that the genetic effects responsible for the Zn efficiency trait in rice are mostly additive, and to a lesser extent dominant (Majumder et al., 1990).

Soybean varieties differ in their response to Zn fertilizer (Rao et al., 1977; Rose et al., 1981; Saxena and Chandel, 1992). This may be a consequence of differential efficiency of Zn absorption; the distribution of F3 lines from the cross between Zn-efficient and Zn-inefficient genotypes (330 F3 lines tested) suggested that only a few genes control the Zn efficiency trait (Hartwig et al., 1991).

The various mechanisms of Zn efficiency in wheat are likely to be additive (as shown for rice, Majumder et al., 1990), which suggests that in a breeding program stepwise compounding of genetic information should be greatly emphasized (see Rengel and Jurkic, 1992). Such pyramiding into one locally adapted crop cultivar of a number of Zn efficiency mechanisms that are expressed at different levels of the plant organism (molecular, physiological, structural, or developmental; see Rengel, 1992) might

Picture 3. Varying degrees of tolerance to zinc deficiency in paired plots of barley lines growing in zinc-deficient soil, Horsham, 1998. One plot of each pair was treated with zinc fertilizer granules drilled with the seed and, later, a foliar spray. (Photo: J. Lewis.)

follow the approaches of Yeo and Flowers (1986). In such a breeding program, genotypes having genes controlling a particular mechanism of Zn efficiency may be very important even though they themselves may not show phenotypically high overall Zn efficiency. It would be advantageous to use local genotypes because this would expedite the development of cultivars with improved Zn efficiency without severely disrupting the broad adaptation already achieved (Picture 3).

Durati, a very sensitive durum wheat in the heavy black clay soils of New South Wales was also poor in our light sandy soils. It is therefore a valuable indicator line. Kamilaroi is a derivative of Durati (Durati x Leeds) which not only incorporates yield, quality and disease resistance from Leeds but also zinc efficiency on the heavy black earths of New South Wales. However, in South Australia on light sands, Kamilaroi appears worse than Durati for zinc efficiency. Zinc deficiency in the black earths is a complex phenomenon involving very high levels of native soil phosphorus and manganese that appear to aggravate the low zinc status. Thus zinc efficiency on these soils may not be so much a "foraging capacity of the roots" but a better discrimination for zinc over manganese and phosphate. In South Australia phosphorus and manganese are relatively low. We therefore recognize different types of zinc efficiency.

Besides diversity for yielding ability on zinc-deficient soils, there may be genetic control over zinc concentrations in tissue and grain (Graham et al., 1992). Excalibur and Warigal 5RL are generally superior to the other lines (30 tested in all) in zinc concentration in leaves and zinc uptake at tillering. However, Excalibur had low grain concentration, a condition apparently linked to its high yield since grain zinc content (g/ha) was high. There is a distinct trend, as with grain nitrogen, for a lower grain zinc concentration with increasing yield (across genotypes). This is undesirable both because of lower seedling vigor when low zinc seed is used for resowing and because wheat is generally considered to be too low in zinc for adequate human nutrition when it forms a high proportion of the diet (Welch and House, 1983; Graham and Welch, 1996). However, Warigal stood out as having the highest grain zinc concentration and content under zinc-deficient conditions. It is highly likely that grain zinc concentrations could be improved by breeding. It should be noted, however, that grain zinc concentration responds dramatically to fertilization under these conditions.

Manganese efficiency

Remarkable diversity for manganese efficiency exists within wheat, especially in hexaploids and durums and this may be further supplemented, if warranted, with efficiency genes from rye.

Manganese efficiency in barley appears to be simply inherited, taking the evidence of the cross of Weeah (efficient) and Galleon (inefficient) (Graham, 1988b) and similar evidence of the cross between Weeah (efficient) and WI2585 (inefficient, McCarthy et al., 1988). This major gene has recently been mapped to 4H (see later section). However, another line WA73S276 that has common parentage with Weeah, has markedly more efficiency than Weeah, suggesting that other loci may be involved. Moreover, an important parent in the barley breeding program at the Waite Institute, CI3576 from Alexandria, is exceptionally susceptible to manganese deficiency, and so is a high percentage of its progeny. At this stage we are not sure whether this is some type of semi-dominant manganese ***in***efficiency trait, or an effect of tight linkage to another trait.

A low percentage of wheat cultivars also have exceptional sensitivity to manganese deficiency, the genetic basis of which is also unclear. However, recent studies (Khabaz-Saberi et al., 1998) has shown that Mn efficiency in durum wheat is contolled by two additive genes. It is likely that these are in homeologous positions in the two genomes, and from our experience with barley (single), rye (single) and durum wheat (2 genes), we can safely predict three major loci in bread wheat. Minor genes are almost certain to be involved in all species, but in the southern Australian breeding programs, much progress still needs to be made with these major genes.

The rankings for zinc and manganese efficiencies are somewhat inversely correlated (see Graham, 1990). Zinc-efficient Excalibur has poor manganese efficiency, and Bayonet, Millewa, and Takari are reasonably zinc efficient but acutely manganese inefficient. Others are reasonably efficient for both (Aroona, Machete) and some quite inefficient for both elements (Durati, Kamilaroi, Songlen, Gatcher). Durati

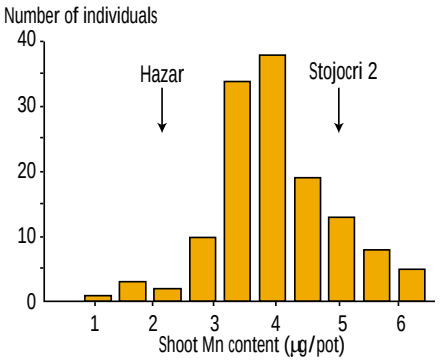

Figure 1. Frequency diagram of shoot manganese content (mg/pot) of F2 individuals from the cross of Stojocri 2 x Hazar durum wheats, when grown in manganese-deficient Wangary soil for 4 weeks.
Source: Khabaz-Saberi et al. (1998).

and Kamilaroi are poor for manganese, zinc, and copper. Indeed, most durums tested are poor for micronutrient efficiencies. Zinc efficiency in wheat, as earlier discussed for rye, appears also to be independent of copper efficiency.

Boron efficiency and toxicity tolerance

Both boron deficiency and boron toxicity are common nutritional imbalances of many crops, including wheat. Boron deficiency occurs mainly in highly leached soils of the humid zones while boron toxicity is more common in lower rainfall regions where limited leaching results in boron accumulating in the subsoil. Deficiency and toxicity affect different physiological processes and different tissues in wheat. Boron deficiency primarily affects pollen development and pollination, while boron toxicity affects the growth of all tissues at all stages of development. A high level of genetic variation has been identified in wheat in response to both boron deficiency (Rerkasem and Jamjod, 1997) and boron toxicity (Moody et al., 1988). As the two imbalances are expressed at different stages of development contrasting screening methods have been developed to assist selection of required genotypes (Picture 4).

Picture 4. Symptoms of boron toxicity on wheat and barley leaves. Barley is generally more sensitive and the symptoms more distinctive, making a sensitive variety like Stirling a good indicator line. (Photo: J. Coppi.)

Identification of boron deficiency and boron toxicity can be undertaken, with varying degrees of success, by recognition of plant symptoms indicative of the disorders and by plant and soil analysis. As mentioned above, the major effect of boron deficiency of wheat is on development and function of pollen, thus sterility may occur in plants without foliar symptoms. The symptoms of boron toxicity of wheat consist of regions of chlorosis and necrosis developing from the tips and along the margins of the oldest leaves. While the symptoms for wheat are not readily distinguishable from symptoms of other stresses, boron toxicity symptoms of barley are very distinctive and consist of black spots developing within the necrotic lesions at the tips and margins of leaves. If boron toxicity is suspected, including a few plots of barley within a wheat trial will provide a rapid, low-cost means of diagnosing the problem.

Tissue analysis, using tissues such as youngest emerged leaf blades (YEBs), will give an indication of the boron status of the plant, and can be used to compare between genotypes in a high boron situation, but does not readily differentiate between efficient and inefficient genotypes under low boron supply. Boron concentrations in YEBs of less than 2 mg/kg might indicate deficiency, while concentrations of greater than about 20 mg/kg in YEBs and 3 mg/kg in mature grain indicate a likelihood of boron toxicity.

Boron can be extracted from soil by a number of methods and the amount extracted will depend on the method and soil type. The most common methods used are hotwater and hot 0.01M $CaCl_2$ extractions. As the amounts extracted vary according to time, soil type and method, the absolute values only provide a rough guide to the amount of boron available to plants. A hot-water extractable boron concentration in soil of less than 0.5 mg/kg might indicate boron deficiency, while a concentration greater than 15-20 mg/kg is associated with toxicity. As high concentrations of boron in low rainfall environments are generally found in the sub-soil, the soil profile should be sampled to 1 m.

Additional methods of diagnosing the nutritional problem include application of the nutrient, in the case of a deficiency, and inclusion of probe genotypes in trials. Many Australian wheat varieties have been characterized for response to high concentrations of boron and several, including Halberd, Frame, and Spear that are tolerant, while Hartog is very sensitive. Appropriate probe genotypes for boron deficiency, identified in Thailand, include SW41 (inefficient) and Fang 60 (efficient).

Severe boron deficiency can result in complete sterility of an inefficient wheat genotype that produced healthy vegetative growth with a similar concentration of boron in the flag leaf and the ear as an efficient genotype with a high level of seed set. As no seedling or vegetative response has been identified that is correlated with seed set, it is necessary to select for efficiency at low boron supply during the reproductive stage.

Boron deficiency can be induced in sand culture in pots or in field trials. In both situations the response of the genotypes under test can be compared to an adequate boron control (application of 1 kg boron/ha, as borax), or to well characterized check genotypes (e.g., SW41 and Fang 60). The response is described by the grain set index, which is calculated as the number of grains in the primary and secondary florets of the 10 middle spikelets of the primary spike, expressed as a percentage of the potential (i.e., 20 grains). Restricting the number of grains to the primary and secondary florets, rather than counting total grain set, minimizes confounding effects such as moisture stress and other nutritional imbalances that influence the development of grain in the higher order florets.

Screening Techniques

Undoubtedly, the ideal is to understand the mechanisms involved and to select for the desired characters by means of their gene products. This may be phytosiderophore release as in the case of iron efficiency in wheat (Marschner et al., 1986), binding affinity in the membrane (Km), root geometry, or composition of simple root exudates that may control nutrient availability in the rhizosphere. If iron efficiency were a problem in wheat, it would appear a simple matter to select for greater ability of the roots to release deoxymugeneic acid under standard conditions of iron stress (as Marschner et al., 1986, have defined). The all-important advantage of this approach is that we are no longer dependent on measuring yield with all its potential for interactions (as the integration over time of gene expression on the limiting factor), but are measuring directly the intensity of expression of the efficiency alleles present in that genotype.

Field testing

Selection in terms of yield is always imprecise and fraught with difficulties: almost everything in the genome contributes to yield either directly or indirectly. If the selection pressure is great enough (that is, deficiency is severe and the primary limiting factor), then efficient genotypes will be selected based on yield, but the possibility that strong interactions will cloud selection is always there. Graham et al. (1992) reported inconsistent results between two sites for two lines of barley, one of which, Schooner, was able to respond to late (October) rains by virtue of its later maturity and, under warm soil conditions, it benefited from improved availability of native zinc in the soil. In this case, we believe the results from Lameroo, the other site, are more typical and reflect better the true zinc efficiency of the lines. Mid-season harvests help to support such interpretations.

Site selection. One of the most difficult problems with field studies is selecting an even site with a level of deficiency that will differentiate varieties for efficiency. Appropriate extraction techniques are unavailable, and soil analysis is poor in predicting trace element deficiencies. Field history is a valuable resource, but analyzing plant tissue taken from the wheat crop prior to the experiment is perhaps the most reliable tool for selecting a site. However, trace element deficiency is extremely dependent on environmental conditions (e.g., temperature, light intensity, and rainfall) and careful attention to tissue concentrations in previous crops may not ensure a deficiency in the experiment in a given year.

It is important that adequate levels of all other nutrients be applied as a basal fertilizer to all plots to avoid nutrient imbalances and interactions of other nutrient deficiencies with genotype. One of the most difficult interactions to avoid has been that of boron toxicity in our zinc deficiency trials. Genotypes that are more susceptible to zinc deficiency exhibit greater symptoms of boron toxicity.

Two-level assessment. Our main approach to screening is to use plus-and-minus plot pairs to calibrate the performance of a genotype in deficient soil against its own potential with the limiting element supplied. Our primary efficiency index then becomes:

$$100 \cdot \frac{GY-}{GY+} \text{ or sometimes } 100 \cdot \frac{\text{veg Y-}}{\text{veg Y+}}$$

where GY = grain yield, and veg Y = vegetative yield.

This is the parameter we call micronutrient use efficiency. However, we do not rely on this quotient alone, given that for various purposes, the lines with the highest GY- may be of interest when they have outstanding yield potential but still respond significantly to zinc (e.g., Excalibur; see Graham et al., 1992). Also of independent interest is GY+, the potential yield for a belt-and-braces approach, that is, a combination of nutrient and genotype that frequently outyields either alone (Graham, 1988a). We argue that 100 · GY-/GY+ is probably the best basis for identifying parental material for a breeding program, whereas GY- may be of most immediate interest to producers when the problem is subsoil deficiency or topsoil drying, or when they are not willing to use micronutrients or are unaware of the deficiency.

It is obvious that a genotype can be characterized better by a yield response curve generated by increasing rates of fertilizer than by simple plus-and-minus treatments. However, in such studies only a few lines can be reasonably handled before the task becomes too large.

Particularly in field experiments, the increased area means increased spatial variability, a serious problem with micronutrients; this variability appears to be intrinsically greater than that for macronutrients (see Table 8, Graham, 1990). Moreover, the critical comparison between any given two rates (whether it be the plus-and-minus pair or any other pair) will be spread by randomization requirements over greater distances, with other treatments in between. Therefore, provided the site chosen has the degree of deficiency to be targeted in the breeding program, the paired-plot system has the advantage that the extreme proximity of the two treatments allows the most precise determination of 100 · GY-/GY+ and that minimal size permits the comparison of the greatest number of lines.

This is the system we have used most in South Australia, but its justification depends on both the selection of relevant sites and on a perception of the number and nature of the genes involved. Paull (1990) found a number of genes involved in resistance to boron toxicity and proposed a model (to which Figure 2 is analogous for deficiencies)

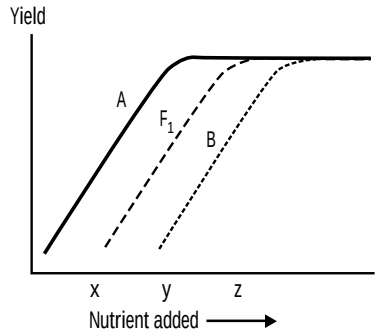

Figure 2. Model of the response to added micronutrient of two parents and their F_1 progeny showing how if screening them at a single level of stress, the genetic interpretation could be: at Z, A is dominant; at Y, partial dominance; at X, B is dominant (sensitive parent).
Source: On analogy with Paull (1990).

to account for the variable expression of dominance at different selection pressures. In his work, to select for/against all possible segregants in a tetragenic system, he selected at three different levels of stress.

In deficiency work, it is common to discuss the relative merits of the genotypes with yield-fertilizer rate responses like those in Figure 2. Genotype A is desirable because it reaches its potential at the lowest level of supply. But with micronutrients, it is common to add 10-100 times as much fertilizer as is absorbed by the crop, perhaps for several years (Fe, Mn excepted). Thus, the lowest rate of nutrient to achieve the yield potential is not determined by the paired-plot system; it can be argued that this is not critical and we need only a point safely on the yield plateau and a point at nil nutrient supply, provided the latter results in a meaningful degree of deficiency for the region targeted in the breeding program (for example, y in Figure 2). In this way we have justified our paired-plot approach in the past, and until such time as we have a better idea of the genes and mechanisms involved, it seems the most practical approach for our purposes.

Using the paired-plot system. A typical field experiment (using zinc as an example) currently consists of 36 genotypes x ± zinc x 4 replications, with border plots, laid out as a split-plot randomized block design. The experiment is sown with all main plots entered in pairs; one of each pair (chosen at random) receives zinc. Our seed and fertilizer drill uses a magazine system for delivering seed to a cone seeder, and zinc granules are delivered along with the seed via the magazine. The fertilizer box thus contains only basal nutrients (all other necessary nutrients, especially in our environment, nitrogen, phosphorus, sulphur, copper, manganese, molybdenum, cobalt) and does not need to be changed. The zinc granules are commercial zinc oxysulphate (~ 30% zinc) used at several times the commercial rates (11-14 g per 4.5 m^2 plot) because its effectiveness is less than when coated on macronutrient granules (ammonium phosphate), the current commercial practice. The soil-applied zinc is supplemented with a foliar spray of zinc sulphate at tillering (equivalent to 200 g zinc/ha). (For manganese studies, manganese oxysulphate granules are used followed by one or two foliar sprays at 1 kg manganese/ha. For copper experiments, we have used copper sulphate granules and foliar sprays at 0.2 kg copper/ha).

Mid-season harvests are taken from 0.5 m^2 quadrants, partly to guard against loss of information due to end-of-season events (drought, hail, heavy late rains, sheep or cattle getting through the fence), and given that we escape the above, grain yield is measured by a small-plot harvester.

Because of the large spatial variation in the soils we study, advantage is taken of modern spatial statistical analyses, which have proved to be more efficient, that is, higher F, for the genotype x zinc interaction. Generally, a higher F is found in this analysis than from the simple split-plot randomized block factorial analysis.

The spatial ANOVA process is iterative and can identify non-treatment variation associated with the position of a plot or subplot in the field-plan array. We have identified variation due to the effect of tractor-wheel compaction on some plots and not on others, direction of sowing and harvest in relation to slope (up-down, left-right), prevailing wind (direction of head-bending), and depth of sowing. Removing variance of this type from the residual increases the significance of that due to treatment (Gilmour et al., 1997).

In addition to grain yield data analyses and the similar treatment of mid-season vegetative yields, a further index of considerable value is generated, after chemical analysis of the tissues and grain, by calculating the total uptake of zinc into vegetative growth and/or grain. The most efficient genotypes are so because they absorb more zinc and maintain higher zinc concentrations in vegetative tissues and often, but not always, in grain. The same is true for Cu and Mn. Uptake, being the product of yield x zinc concentration, frequently shows greater variation among genotypes than yield. (Compared with yield, variation in concentration is relatively small but usually significant). Uptake reflects in some measure the expression of the nutrient use efficiency trait integrated over time from sowing to harvest (Picture 5).

Single-level assessment. Single-level assessment is conducted without control plots supplied with the limiting nutrient, which are replaced for purpose of interpretation by plots of a check genotype. A typical design that proved useful in our manganese program (Graham et al., 1983) involved 196 plots and only two complete replications of the 72 barley lines tested. The remaining 52 plots were check plots of one cultivar located every fourth plot in a regular array.

The purpose of this experiment was to identify genotypes as nutrient-efficient as the check cultivar (or better) by using an acutely manganese-deficient site. The results can be analyzed by spatial analysis techniques (ASREML) and by comparing test plot yield to the check genotype yield surface for the site generated from the array of check plot yields. More simply, plot yields can be compared to the arithmetic mean yield of adjacent check plots. The check plot yield array has proved highly efficient at defining site variability and

Picture 5. Paired-plot screening of wheat breeding material for zinc efficiency, Horsham, 1998-99 (Photo J. Lewis). Plus and minus zinc plots of the one entry are sown side by side, with zinc applied to one plot chosen at random. The trial consists of 30 lines sown in four replications in a randomized complete block design, with the data subjected later to spatial ANOVA (Graham et al., 1992).

Picture 6. Single-plot screening of barley breeding material for manganese efficiency, Wangary, 1981. No Mn fertilizer was used but a Mn-efficient check was sown every fourth column throughout, with single plots of 72 test lines sown in between (Graham et al., 1983). Two replicates. In the column to the left of center, the four plots are (starting from the front) efficient, inefficient, inefficient, efficient. A column of an efficient check runs the length of these plots on the left; parts of three other check plot columns can be seen to the far left and right. (Photo: R. Graham.)

fertility trends. Consequently, where a wide range of performance is expected among the entries, efficient selection may be achieved with entries appearing only twice in the matrix. Quite a number of borderline genotypes may not be convincingly placed in either the efficient or inefficient group, but usually this is not important. The efficiency of selection in this simple design was underlined by the meaningful genetic relationships discovered in both the efficient and inefficient groups (Graham et al., 1983) (Picture 6).

Measurements. In field work measurements of grain yield should be supported by observations that assess efficiency at seedling and mid-season growth stages. Atypical weather can confound measurements at a single stage of growth (such as late rains that favor late-maturing genotypes). Chlorosis, vigor, and delayed maturity (heading date) can be scored by eye. However, quadrat sampling (or 1 m of row) at mid-season can quantify vegetative yield and, equally important, provide plant material for analysis. Total uptake (dry matter yield x concentration) is a valuable index of nutrient efficiency that integrates root system vigor and efficiency with shoot requirement. Genotypic differences in critical concentration are recognized (Ulrich and Ohki, 1966) but in our experience are relatively small compared with the total uptake for defining nutrient efficiency.

Collecting and analyzing plant samples. A reliable measurement in assessing our field experiments has been to analyze whole plants and youngest expanded leaf blades (YEBs) by inductively coupled plasma (ICP) spectroscopy (Zarcinas et al., 1987). It is important that plant material be as free from contamination (for example, dust, soil particles, galvanized products such as gates and tools, cigarettes, and some paper bags) as possible. We recommend the use of plastic gloves for handling both YEB and whole plant collection. When collecting whole plants, plants are cut approximately 1 cm above ground level to minimize contamination with soil. They are then stored in suitable paper bags and dried overnight at 80 ºC.

In our experience manganese efficiency may vary 10-42%, with grain yields of -Mn plots from 0.1 to 0.8 t/ha. Durati, Takari, and Millewa are so inefficient and the +Mn fertilizer treatment (soil + foliar) so ineffective that the +Mn plots were still deficient (young leaves 12 mg/kg Mn) and perhaps yielded barely half their potential (Graham, 1990). Although not a great problem, this results in higher efficiency indices than the inefficient lines warrant, making the extent of diversity for this character actually greater than measured.

At the manganese-deficient site, the resistance of Aroona, Machete, and Millewa to the take-all fungus was in line with their manganese efficiency at the vegetative stage (Graham, 1990; Pedler, 1994). Machete improved its ranking from 19th at tillering to 7th at grain harvest, while Gj*Wq faded from 2nd to 21st. While only a few lines changed ranking markedly through the season, this meant that selecting the top five at tillering would have netted only three of the top five at maturity, a point in favor of selection in the field (Picture 7).

Screening in controlled environments

Soil cultures. Using soil in pots requires less effort to set up and maintain than solution cultures, and the work is less labor-intensive than field sites. The same soil requirements apply to screening in field or in pots, but in pot work the experimenter obtains, by thorough mixing, a uniform soil for each genotype tested, albeit in a quite atypical environment. The latter is particularly important. Nutrient stresses are frequently associated with low soil temperatures, and uncontrolled soil temperatures in the glasshouse can be extremely unrealistic. For example, it is often difficult to produce manganese deficiency in glasshouse conditions, even with soil that is severely deficient in the field, and low temperature baths or

Picture 7. Paired-plot screening of parents and breeding lines for manganese efficiency, Wangary, 1987. Manganese was delivered as Mn oxysulfate granules with the seed and 1-2 foliar sprays of Mn sulfate solution applied mid-season as required. (Photo: R. Graham.)

controlled-environment rooms are necessary to keep temperatures below 15 ºC. The results of Fox (1978) are an excellent example of the genotype x climate x nutrient interaction preventing the correct interpretation of nutrient efficiency tests.

The size of pots has also been shown to be an important consideration when screening for nutrient efficiency. While it is tempting to use small pots in the growth chamber to allow the screening of as many lines as possible, it has been shown that for manganese efficiency, pot capacity should not be less than 0.5 kg soil for two wheat seedlings grown for 28 days. Pot size should roughly double for each week of growth beyond 28 days (Huang et al., 1996).

We have done considerable work in pots, usually vegetative growth studies, but we have occasionally found the rankings quite disparate with field-based grain-yield rankings, which suggests mid-season or later effects can be important. Examples found in Marcar and Graham (1987), Rerkasem et al. (1990) and in our recent screening for zinc efficiency in wheat (R.D. Graham et al., unpublished) suggest that field screening is important. In screening for manganese efficiency in barley, only one of two major gene loci contributing half of the trait each could be screened for in pots. The other, it seems, much be screened for in the field.

Screening for copper, zinc, and manganese efficiency in pots seems to be satisfactory (Graham and Pearce, 1979; Grewal and Graham, 1997; Rengel and Graham, 1995a; Huang et al., 1996), except as mentioned above. When screening for manganese efficiency in pots, both the storage, temperature, and moisture effects on manganese availability and the screening technique (Longnecker et al., 1991; Webb et al., 1993a; Huang et al., 1996) are critical. The mechanism of manganese efficiency has proved quite elusive, but empirical screening has been effective enough to lead to the development of a molecular marker for the major gene involved (see later).

Screening for boron toxicity and deficiency in pots has been effective. High boron supply results in chlorotic and necrotic lesions at the tips of the older leaves, reduced plant vigor, restricted tillering, poor root elongation, and elevated concentrations of boron in all plant tissues. Tolerant genotypes maintain lower boron concentrations in tissues, develop less severe symptoms of toxicity, and produce more vigorous shoot and root growth than sensitive genotypes. These differences in response have been used to develop efficient screening systems that have assisted in breeding boron tolerant wheat varieties (Moody et al., 1988) and enabled the identification of major genes controlling boron tolerance and their locations on chromosomes 4A and 7B (Paull, 1990; Chantachume et al., 1994).

There is a strong correlation between the response of wheat to high boron concentrations during seedling growth and at later stages of development. This has enabled the development of several rapid seedling assays to identify boron tolerant genotypes. One method involves growing seedlings in a glasshouse. Additional boron in the form of boric acid is uniformly mixed through fertile clay-loam soil to give an extractable boron concentration in the range of 50-80 mg/kg. Plants are watered well for several weeks, until they are established, and then less frequently. During this second phase, roots of the more tolerant plants will be able to extract moisture from deeper in the soil profile, and these plants will continue to grow. Roots of the sensitive genotypes do not grow in the high boron soil, and the plants appear stunted. Tolerant genotypes also develop less severe symptoms of boron toxicity.

Solution cultures. Simple solution cultures can rarely be used to select nutrient efficiency factors that operate on some feature of the root-soil interface. Efficiency factors operating within the root surface may be screened for in solution: characteristics of the absorption isotherm, xylem loading, short- and long-distance translocation, and efficiency of nutrient utilization (for example, carbon fixed per unit of nutrient absorbed). Such micronutrient efficiency factors operating internally (e.g., boron translocation in tomato; Wall and Andrus, 1962) are also dealt with by soil techniques.

Special adaptations that operate in soil may be successfully observed in solution cultures under certain conditions. Brown and Ambler (1973) used strong iron chelates in solution to study the reducing power of the root, since the reduction Fe^{3+} to Fe^{2+} was necessary to break the ligand-Fe^{3+} bond and free ionic iron for absorption. Clark et al. (1982) used solutions modified with high phosphate, nitrate, and calcium carbonate to induce iron stress in susceptible sorghum genotypes.

Iron and manganese efficiencies may be studied in solutions containing suspensions of insoluble iron hydroxide or manganese dioxide, both of which require reduction for dissolution; this process is promoted by proton extrusion (see Brown, 1978; Romheld and Marschner, 1981; Uren, 1982). These insoluble higher oxides/hydroxides may also be precipitated on chromatography paper (Uren, 1982) and roots made to grow along the wet paper surface, a system that is a compromise between soil and solution culture. Effective reduction and dissolution of the dark-

colored oxide by the root is detected by a white depletion zone on either side of the efficient root. For effective screening of genotypes, differences must be quantified, requiring good quality control over the precipitation process and test conditions.

Higher iron concentrations are on occasions found in chlorotic tissues than in green tissues (Brown, 1956), and up to 100 times more iron may be found in roots than in shoots of iron-deficient plants (Brown, 1978). These observations show that there are efficiency mechanisms operating within the plants that may be tested for in solution cultures.

Flowing culture systems add another dimension to solution culture approaches. With this technique, it is possible to define for each genotype the lowest solution-phase concentration of an element that can sustain maximal growth rates. This is clearly a genetically controlled character (Asher, 1981). Such systems, while too expensive for routine screening, provide information about physiological mechanisms to aid in developing rapid screening tests.

Solution techniques have been most widely and successfully used in screening for tolerance to mineral toxicities and in elucidating the mechanisms and genetics involved. Screening and selecting for aluminum tolerance, for example, has been efficiently carried out in solution cultures (Furlani et al., 1982; Foy et al., 1978; Reid, 1976).

The relatively new technique of chelate-buffered nutrient solution culture has potential in screening for micronutrient traits (Webb et al., 1993b; Huang et al., 1994a,b; Rengel and Graham, 1996). However, caution and more development are needed in terms of activities of ions in solution and interpretation of results.

Boron tolerance. The effect of boron on root growth has been utilized to develop a rapid, objective assay to identify genotypes tolerant to boron toxicity (Chantachume et al., 1994). Seeds are imbibed in petri dishes at 2-4 ºC for 2 days, then at 18-20 ºC for 1 day. Large, rectangular filter papers or absorbent paper towels are soaked in a solution of boric acid (concentration in the range 5-10 μM), 0.0025 μM zinc sulphate, 0.5 μM calcium nitrate, and basal nutrients, and then allowed to drain for 1 min. The seeds are placed in a row across the top third of the paper, with the embryos facing the bottom. The towels are rolled up into a cylinder, enclosed in aluminum foil and stood on end with the embryos facing down and stored at 15 ºC for 12 days. The towels are then unrolled and the lengths of the longest roots are measured. Tolerant genotypes develop longer roots than sensitive genotypes. Tolerant and sensitive controls should be included in each filter paper, and reference should be made to a control filter paper without boron to compare relative root lengths.

In a modification of this method by Campbell et al. (1998), seeds were sown on a fine mesh over a "lunchbox" containing the boron solution, with aeration. Tolerant and sensitive genotypes are again distinguished on the basis of root growth. This method has the advantage of being less labor-intensive and less expensive because filter paper and aluminum foil are not required; however, correlations with other screens may not be quite as good. Nevertheless, the ranking of well characterized genotypes is consistent among the alternative screening methods and with the concentration of boron in shoots and grain when grown in fields with high concentrations in the sub-soil. The root length screen can be combined with a measure of coleoptile length where this is important (Picture 8).

Importance of seed quality in screening for micronutrient efficiency

Seed with high mineral content has been shown to be associated with early seedling vigor; this early advantage due to nutrient content of seed may still be observed at maturity as increased grain yield, larger seed size, and more grains per plant (Longnecker et al., 1991; Rengel and Graham, 1995a, b), even where nutrient supply is non-limiting for plant growth. The nutrient content of seed is dependent on soil type, nutrient availability, species, and to a lesser extent, variety and season. Longnecker and Uren (1990) showed for both barley and white lupin that seasonal effects had less influence on seed Mn content than choice of site.

It has already been demonstrated that there is genetic potential for wheat genotypes to grow at sub-optimal levels

Picture 8. Rapid screening for boron tolerance on wheat seedlings in solution culture.† (Photo: ETU, University of Adelaide.)

† Boron concentration: 100 mg/liter, as boric acid (Campbell et al., 1998).

of micronutrient supply. Since the addition of micronutrients has in many cases decreased the incidence of diseases in wheat plants (Graham, 1983; Graham and Webb, 1991) and since differences in nutrient content of seed are also under genetic control (Moussavi-Nik, 1997), there exists a large overlap of factors that may confound the performance of genotypes.

Nutrient content of seed has also been shown to affect the susceptibility of wheat to diseases, for example, McCay-Buis et al. (1995) demonstrated that wheat plants grown from seed with higher manganese content (1.83-2.28 µg seed^{-1}) were more vigorous, had less take all, and produced more grain than plants grown from seed with lower manganese content (1.26-1.86 µg seed^{-1}). J.L. Cooke (thesis in preparation) reported less damage due to *Rhizoctonia* in wheat plants that had been grown from seed with high zinc content (0.66-0.74 µg seed^{-1}) than in wheat plants grown from seed with low zinc content (0.24-0.26 µg seed^{-1}) (Picture 9).

For durum wheat cultivars differing in manganese efficiency, Khabaz-Saberi et al. (2000) developed a correlation between seed manganese content and the amount of added Mn required in Wangary soil to assess the Mn efficiency in a pot bioassay. This correlation differed for the manganese-efficient durum wheat cultivar Stojocri 2 compared to the manganese-inefficient Hazar.

When comparing genotypes for dry matter production, grain yield, grain quality, disease resistance, and nutrient efficiency, it is important that the quality of seed sown be similar for all genotypes to avoid confounding effects. However, seed size and nutrient distribution within the seed has been shown to be under genetic control (Moussavi-Nik, 1997). This means that seed of genotypes collected from the same site (soil type) and season may differ notably in nutrient content. In an extensive series of experiments involving 11 wheat genotypes, seed for each genotype was selected from eight localities in South Australia and tested at eight locations over two contrasting climates.

Picture 9. Seedling vigor of zinc-inefficient Gatcher wheat growing in zinc-deficient soil, showing the importance of seed zinc content to establishment (Rengel and Graham, 1995a).

Moussavi-Nik (1997) demonstrated that genotype effects accounted for the largest portion of treatment variance, while seed source effects accounted for from almost none to 44% of the treatment variation and were significant in 9 of 16 of the experiments conducted. Generally the interaction between genotype and seed source contributed less to treatment variation than seed source main effects. Significant seed source or genotype x seed source effects occurred over the whole range of environments tested, from the highest to the lowest yielding. The seed selected from all locations had adequate nutrient content for all elements investigated; the eight sites used for assessing the effects of seed source were all adequate for trace elements. In spite of this, there were four significant associations of increasing zinc content of seed resulting in increased grain yield and one association of sodium seed content decreasing grain yield.

A Breeder's Approach

Knowledge of the mechanisms and inheritance of trace element efficiency simplifies the breeding and selection of improved lines because the choice of parents and the screening methods can be more targeted and objective. However, lack of this information must not deter a breeder when trace element deficiencies are known problems in his target area. Significant advances can be made while the genetics is still being investigated.

Potential parents for trace element efficiency can be sourced from the literature, by reputation and, particularly, from cultivars that continue to be grown by farmers in trace element deficient areas, despite the fact that crop evaluation trials indicate that these cultivars have been superseded by new, higher yielding cultivars.

Since trace element efficiency is only one objective in an overall breeding program, it should be incorporated into the breeding effort and fitted in with strategies and methodologies used for selecting for disease resistance, yield, adaptation, and quality. Once potential parents have been chosen and appropriate crosses made, an important part of the strategy is to set up nurseries which with plant or line selection will enhance the frequency of genes in the breeding population for trace element efficiency.

A critical consideration in such nurseries is seed source, given that the trace element content of seeds can greatly affect early plant vigor and even final grain yield (previous section). Seed low in Mn content, harvested from Mn deficient sites, is best for screening for Mn efficiency; however, such seed may not be available in a normal breeding program. It is important therefore to use seed as close to the same Mn content as practical, and as low as possible (probably not seed produced on a research station, where soil fertility tends to be higher than on farms). All comparisons should be made using seed from the same original nursery. To reduce the effects of seed Mn content on selection, the Mn selection nurseries should be grown in soils with an acute deficiency. In South Australia selection sites for Mn efficiency have a highly calcareous soil in which Mn is tightly bound and poorly available to inefficient plants.

The flow chart in Figure 4 details how selecting for Mn efficiency is incorporated into the wheat breeding program in South Australia, as a model for any micronutrient efficiency trait. Note that it is no different from incorporating selection for resistance to a root disease such as cereal cyst nematode, where much of the screening is done in specially chosen field sites.

Early generation screening

In early generations populations expected to segregate for Mn efficiency are grown in spaced-plant nurseries in soil known to be acutely deficient in Mn. Bulk F2 and/or F3 populations are planted in long rows using a precision seeder with 10 cm between seeds in a row. Rows are 30 cm apart. This arrangement allows for easy observation of single plants.

In these soils even small changes in available manganese can cause large differences in plant growth response. To be able to get some indication of this and to take it into account when selecting in the nursery, indicator rows are sown at regular intervals throughout the nursery, usually as a pair every 7th and 8th row. Cultivar Yarralinka, recognized as being very manganese efficient, and Millewa, a very inefficient variety, are planted as paired rows. Contrasting growth between these two control cultivars indicates very deficient areas where meaningful selection of single plants from adjacent rows can be carried out. Where the growth between the two rows is similar, the Mn deficiency is not such a limiting factor or there are other more limiting factors and selection for Mn efficiency will not be effective.

Long rows are better than short plots because they will traverse across the variability that occurs and there will nearly always be sections of each row where selection can be practiced. Single plants or single heads are selected and these are treated in a routine way in the breeding program to select for other attributes. Whether the selections from each cross are bulked or whether they are kept as separate progeny, the population resulting from this nursery is enhanced for Mn efficiency genes. This selection process can be repeated.

Later generation screening

In later generations lines should be evaluated for yield in locations, soils, and farmers' fields where Mn problems are expected as part of the range of sites used in assessing adaptation. Thus Mn efficiency will be measured as part of the region's overall genotype x environment effects. In areas where there are strong abiotic factors limiting yield, the breeder should be testing and selecting in the presence of the stress and selecting for stress tolerance rather than for yield *per se*. Such sites are not usually found on research stations that have been chosen and/or managed for yield potential and that are free of abiotic stresses.

To get a measure of Mn deficiency directly (which the breeder needs to know to do further selection and crossing), lines are assessed in a known Mn-deficient site in replicated split-plot experiments, with genotypes as the main plots and different levels of Mn fertilizer as the subplots. Fertilizers (P and N) are applied at normal recommendations, and there are nil and applied Mn subplots. On Mn fertilized subplots an initial dose is applied to the soil at seeding as manganese oxysulphate; further applications are made as foliar sprays of manganese sulphate at tillering and again during stem elongation if needed.

Control cultivars include Yarralinka and Millewa, as well as cultivars being grown commercially in the area. The performance of this pair indicates the responsiveness and success of the experiment. Yield data are spatially analyzed using ASREML software, and mean yields with and without applied Mn are plotted against each other (Figure 3).

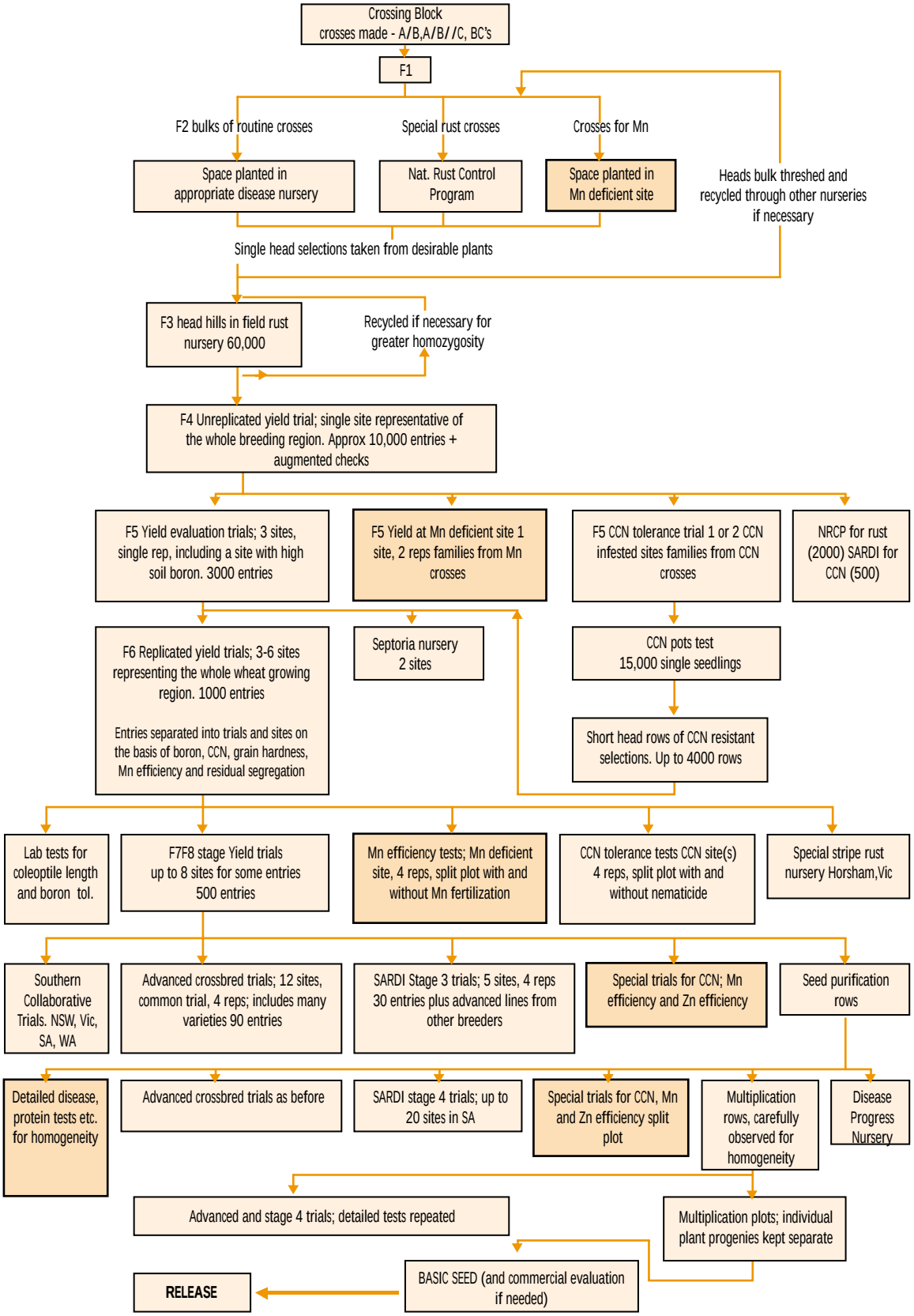

Figure 4. The Roseworthy modified pedigree wheat breeding program, describing the integration of breeding for cereal cyst nematode (CCN) resistance and manganese efficiency within the program. Heavy boxes mark specific activities for Mn efficiency.

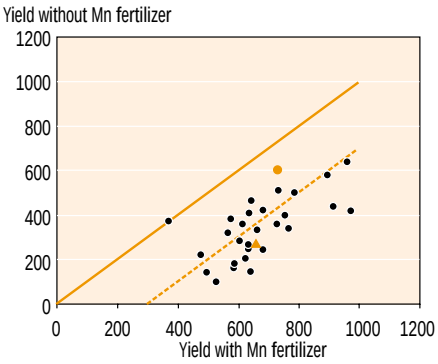

Figure 3. The regression of yield of genotypes without manganese fertilizer on yield with Mn fertilizer, in a field trial on Mn-deficient calcareous sand at Marion Bay.

Use of Molecular Markers in Screening for Micronutrient Efficiency

Molecular markers simplify selection for micronutrient efficient genotypes

Selection of micronutrient-efficient lines from segregating populations is desirable but difficult to do in either field trials or glasshouse and growth-chamber pot bio-assays. The primary reason for this is the requirement for specific growing conditions to maximize genotype differentiation. This is not always attainable in the field and, consequently, screening in field trials is expensive and may not be successful in some seasons such as droughts. Even in the glasshouse or growth chamber where day length, temperature and lighting can be controlled, variation in expression of the trait can be affected by other factors, such as the nutrient status of the seed (Uren et al., 1988) and the length of time and conditions in which the soil used to grow the plants was stored (Webb et al., 1993a).

Another difficulty to overcome is the problem of differentiating heterozygotes, even those which may be intermediate in phenotype, from homozygotes. Our studies have shown that this is not easily done, even where the trait is primarily controlled by a single gene that can express (depending on environment) as dominant, semi-dominant, or recessive, as appears to be the case for manganese efficiency in barley (Pallotta et al., 1999). Separating genotypes in situations where several genes control the trait, as is likely to be the case for hexaploid wheat, is exceedingly difficult and requires extensive progeny testing.

Identification of micronutrient efficient genotypes in early generations of crosses is virtually impossible since each individual plant represents a different genotype. In addition, genotypes should be tested at both low and sufficient micronutrient availability to compensate for genetic variation in plant response due to segregation of genes independent of the nutrient effect.

Where traits are difficult to reliably assay, as are micronutrient efficiency traits, there is a strong case for the use of marker assisted selection (MAS). Our group has identified several RFLPs (restriction fragment length polymorphisms) closely linked to a major gene (*Mel* 1) controlling manganese efficiency in barley (Pallotta et al., 1999); these are currently being used for early generation selection and to facilitate rapid backcrossing of the trait into elite breeding lines.

A second major locus in barley, *Mel* 2, has just been mapped. Work is under way to genetically characterize and map the manganese efficiency trait in the durum wheat cultivar 'Stojocri.' Results indicate the trait is semi-dominant, as was found in our studies on barley and segregation in an F_2 population fitting a two-gene model (Khabaz-Saberi et al., 1998). Genetic variation for manganese and zinc efficiency has been reported in hexaploid wheats (Graham, 1988b; Graham et al., 1992; Grewal and Graham, 1997). Efficient wheats have a yield advantage when grown in environments where zinc or manganese is limiting. Mapping genes controlling these traits in both species would assist the efficient breeding for these traits.

Marker-assisted selection is especially useful in mainstream breeding programs to accelerate backcrossing. The trait of interest can be followed in successive backcross generations by MAS with no need for phenotyping, given that the overall performance of the recurrent parent is usually well known. Two breeding schemes are shown in Figures 5 and 6, for the backcross and intercross programs. Marker-assisted selection can substantially reduce the time required for variety development.

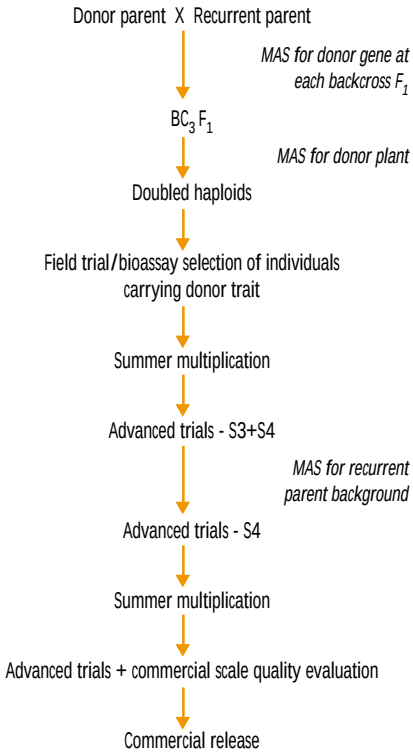

Figure 5. The flow-chart indicates where MAS (marker-assisted selection) can be used in a backcrossing program. The scheme facilitates the rapid incorporation of one or more desired traits into an elite background.

Doubled haploids aid in screening for micronutrient-efficient cultivars

Marker-assisted selection is especially useful when combined with doubled haploid (DH) technology. If molecular marker technology is unavailable, the use of DH populations provides an option for improved selection of micronutrient-efficient lines. Doubled haploid populations make early generation screening by traditional methods more accurate because all progeny of each DH line are genetically identical and true-breeding, enabling replication of tests, which is desirable.

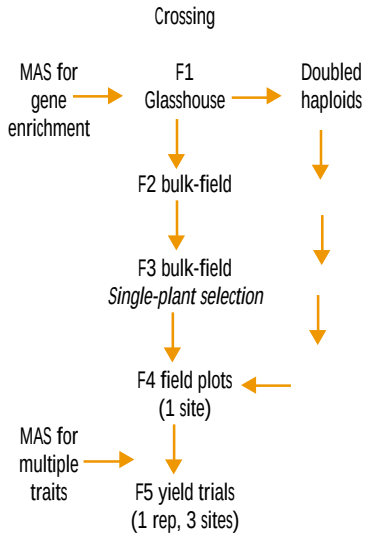

Figure 6. The flow chart indicates where marker-assisted selection (MAS) can be used in an intercross breeding program. MAS would be used where either a backcross or a topcross is involved.

However, precautions need to be taken to reduce the variations in kernel size, the position of the kernel in the spike, and the conditions under which different spikes of the same plant mature. It is necessary to carefully control the ripening conditions of the primary DH plants and to select for uniformity of kernel size.

Doubled haploid populations offer other advantages over recombinant inbred populations when molecular markers are available, particularly if the molecular marker for a trait is a co-dominant marker because the need for progeny testing is eliminated. Doubled haploids in combination with molecular markers provide a powerful tool for early generation screening of micronutrient-efficient genotypes.

Molecular marker-assisted selection can be used to pre-screen the F_2 plants used for DH production and increase the proportion of DH lines carrying the desirable gene(s). This is illustrated in Figure 7 for a single gene for micronutrient uptake efficiency where F_2 putative donor plants are tested for the presence of molecular markers closely linked to the efficiency gene. Selection of F_2 donor plants may be restricted to homozygotes only (homozygote selection) or may also include heterozygotes (allele enrichment). A comparison between homozygote donor plant selection, allele enrichment, and no MAS is made in Table 1, which tabulates the proportion of F_2s and F_2-derived DHs expected when selecting for independently segregating genes at 1, 2, 5, or 10 gene loci.

It can be seen from Table 1 that with homozygote selection, 100% of the DHs produced will possess the desired alleles irrespective of the number of gene loci under selection. However, the proportion of donor plant F_2s which are homozygous at each locus decreases exponentially with increasing number of loci so that the probability of selecting five genes homozygous for the desired allele would be 9.77×10^{-4}, or less than 1/1,000. This is actually less favorable than using no donor plant selection at all, where 3.13×10^{-2}, or around 3% of the DHs derived from unselected F_2s, would be expected to carry all five favorable alleles.

With allele enrichment, the proportion of F_2 donors carrying at least one favorable allele from each of five genes would be 24%. It would be expected that 14% of DHs produced from these selected donors would carry all five desirable able alleles. That is, approximately 1 in 4 F_2 plants can be selected as donors for DH production and among these 14% will carry the desirable allele at each of the five gene loci. Allele enrichment is clearly a more efficient procedure for combining DH technology and MAS than homozygote selection, particularly when larger numbers of genes are being screened.

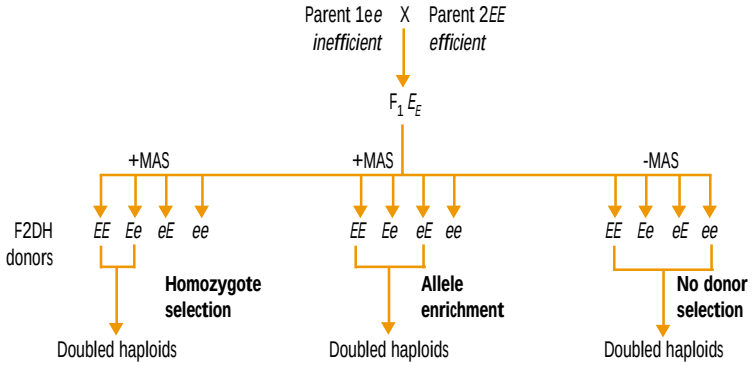

Figure 7. Selection of F_2 donor plants with and without marker-assisted selection (MAS).

Doubled haploids for the elucidation of molecular markers

Doubled haploids are also very useful for genetic and mapping studies of micronutrient efficiency traits, as all lines in a DH population represent the result of a single meiotic event, whereas

F_2s are the summation of a male plus a female meiosis. The DH lines therefore reflect the results of recombination precisely without the necessity for statistical inference.

In both wheat and barley DHs are being widely used for mapping many different genetic traits. In addition to the barley manganese efficiency genes described above, a DH population derived from a cross between two zinc-efficient bread wheat lines, cultivar 'Trident' and breeding line 88ZWK043, exhibited transgressive segregation in a pot bioassay for zinc efficiency; segregation data suggests several genes control the trait in this cross.

Conclusions

Genotypic variation exists for tolerance to practically every abiotic stress in every crop investigated. The level of tolerance available in elite germplasm is agronomically valuable and justifies breeding efforts in most cases, not just those for which there are no other successful agronomic solutions. Inheritance varies from simple to quantitative, and both genotype and soil type, as well as climate and season, affect the expression of a trait. Often these traits are still manageable in a breeding program, and the G × E interaction is not prohibitive of the effort.

Screening for deficiency tolerance traits is, however, much more difficult than for toxicity tolerance traits, owing to quite sophisticated inducible systems that respond to deficiency in the plant by mobilizing nutrient bound in the soil that is not otherwise available. Strong expression of these systems is the objective. However, in view of the difficulty in developing fast methods for screening for efficiency (such as seedling selection in pots) or their limited ability to reflect field screening, molecular marker assisted selection is considered important for success in practical breeding programs, and a number of major gene loci already identified make this possible.

Table 1. Selection strategies for combining doubled haploid (DH) technology with marker assisted selection. Probability of obtaining F_2 donor plants homozygous or heterozygous for the desired allele(s) at *n* loci and the proportion of DHs expected to be homozygous for these alleles when only homozygotes are used as DH donor plants (homozygote selection), when either homozygotes or heterozygotes are used as DH donor plants (allele enrichment), or when selection is not practiced on donor plants.

	Homozygote selection		Allele enrichment		No donor selection
No. of gene loci	F_2s homozygous for desired allele at each locus	DHs homozygous for desired allele at each locus	F_2s homo- or hetero-zygous for desired allele at each locus	DHs homozygous for desired allele at each locus	DHs homozygous for desired allele at each locus
	$(1/4)^n$	$(1)^n$	$(3/4)^n$	$(2/3)^n$	$(1/2)^n$
1	0.25	1	0.75	0.67	0.50
2	6.25×10^{-2}	1	0.56	0.45	0.25
5	9.77×10^{-4}	1	0.24	0.14	3.13×10^{-2}
10	9.54×10^{-7}	1	5.6×10^{-2}	1.7×10^{-2}	9.77×10^{-4}

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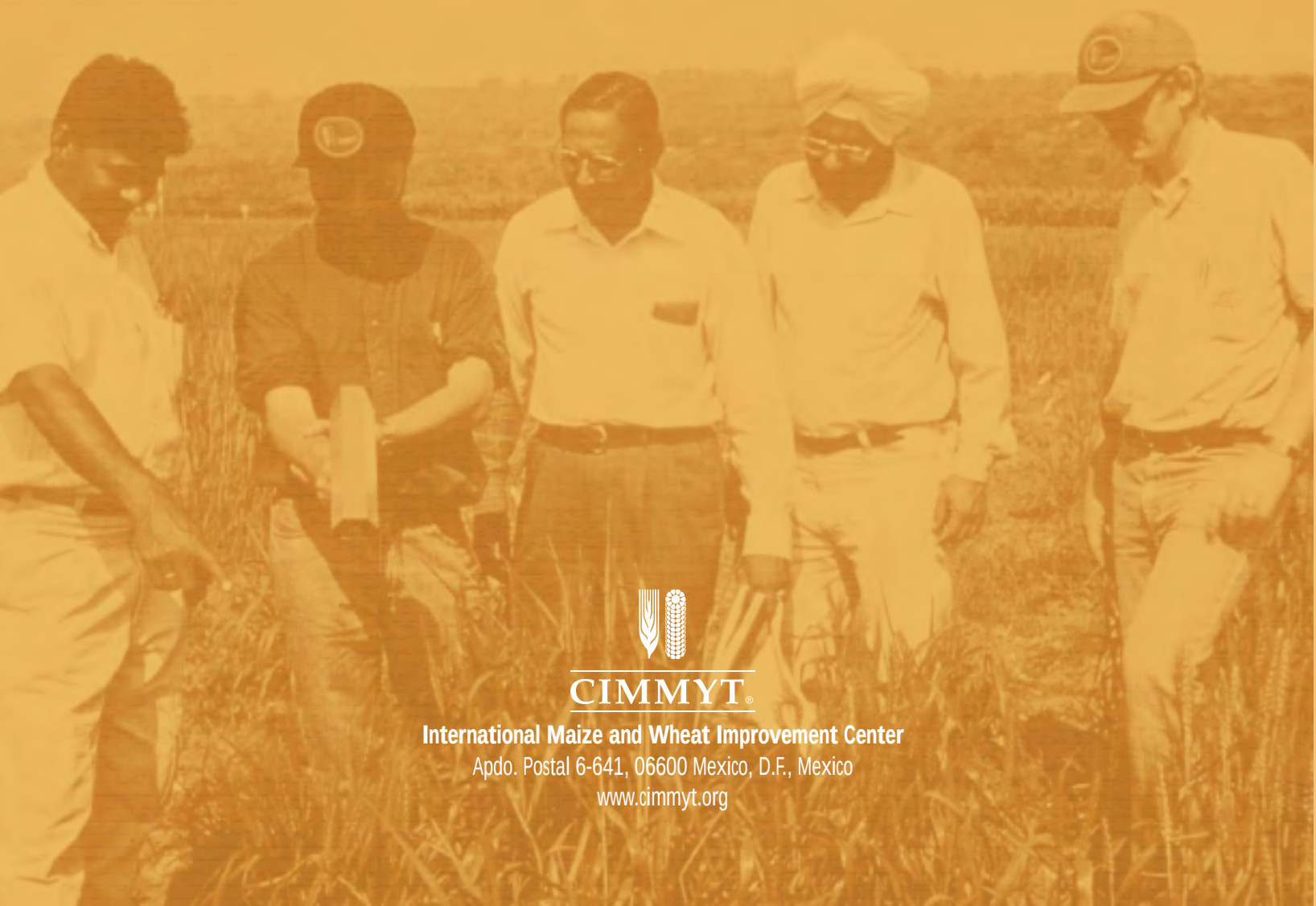

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