

**UNCOVERING THE GENETIC BASIS AND CHARACTERS ASSOCIATION OF WHEAT SPIKE-
RELATED, AGRONOMIC AND QUALITY TRAITS**

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Morgan Echeverry-Solarte

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Morgan Echeverry-Solarte

The Supervisory Committee certifies that this *disquisition* complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

SUPERVISORY COMMITTEE:

Dr. Mohamed Mergoum
Chair

Dr. Elias Elias

Dr. Phillip McClean

Dr. Mohammed Alamri

Dr. Jack Rasmussen

Dr. Edward Deckard

Approved:

3/26/2014
Date

Richard D. Horsley
Department Chair

ABSTRACT

Modern wheat (*Triticum aestivum* L.) cultivars are characterized by having spikes with fusiform architecture and rachis nodes with one spikelet. However, genotypes with supernumerary spikelets (SS) in which rachis nodes have more than one spikelet exist in nature. Although this may be a promising trait that increases yield components, detailed knowledge about the molecular basis of SS trait and their influence on other wheat traits is still lacking. In the present study, a population of 163 recombinant inbred lines (RILs) derived from an elite line and an exotic line with SS was used to identify QTL for SS and other wheat traits. The RILs, seven checks and the two parents were evaluated for seven SS-related, 10 spike-related, 10 agronomic, and eight quality traits over four to six environments in North Dakota. A genetic map of 3,114.2 cM of length with an average distance of 4.6 cM between any two marker loci was developed using 159 RIL and 939 DArT markers. Composite interval mapping identified 221 QTL, out of which 29% were consistent QTL and 19% were major QTL. Most of the QTL were located on the B-genome (44%) followed by the A-genome (37%) and D-genome (19%). The exotic parent with SS contributed 48% of alleles that increased phenotypic values of the traits suggesting the possibility of enriching the breeding germplasm with genes from this genotype. Seven consistent QTL with epistatic interaction were associated to the SS. *QSS.ndsu-2D*, a major QTL for SS, was co-located in a cluster of QTL on 2DS demonstrating either pleiotropic effect or closely linkage with 19 QTL for other wheat traits. Similarly, a major QTL associated with glume pubescences (*QPP.ndsu-1A.1*) was co-located on 1AS with seven QTL for other wheat traits. Major and consistent QTL are targets for further marker assisted selection in wheat breeding programs and/or for research projects aiming of gene cloning.

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There are men who struggle for a day, and they are good. There are others who struggle for a year, and they are better. There are some who struggle many years, and they are better still. But there are those who struggle all their lives, and these are the indispensable ones.”
Bertol Brecht

DEDICATION

Dedicated to my loved one...

Johanna, "La Incondicional", my wife.

Jairo "El Indispensable", my father.

Mireya "Mástil de Hogar", my mother.

Stuart "El Sensato", my brother.

All my loved family

PREFACE

This dissertation consists of five chapters. Chapter 1 includes a general introduction about the dissertation research, along with the objectives, literature review and references. Chapters 2, 3 and 4 are written as three papers to be submitted for publication to the appropriate scientific journals. Therefore, these chapters each include an abstract, introduction, material and methods, results, discussion, conclusions and references. Chapter 5 is the general conclusion. Appendices are included after the end of chapter five.

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LIST OF ABBREVIATIONS

AAL.....	Awns length total averaged
AE.....	Across environments
AFLP	Amplified fragment length polymorphism
ALB.....	Awns length at the bottom of spike
Aless.....	Apical awnleted expression
ALM.....	Awns length at the middle of spike
ALT.....	Awns length at the top of spike
DArT	Diversity arrays technology
DH.....	Days to heading
DM.....	Days to maturity
D-H.....	Double haploids
EST	Expressed sequence tag
FE.....	Flour extraction
GPC.....	Grain protein content
G-SSR.....	Genomic simple sequence repeat
GY	Grain Yield
IL	Introgression line
ISSR.....	Inter-simple sequence repeat
KS.....	Kernels per spike
KSk.....	Kernels per spikelet
KNd	Kernels per node
KVW.....	Kernel Volume weight
Ld	Lodging
MEPT	Mixograph envelope peak time
MMLPT.....	Mixograph MID line peak time
MMLPI.....	Mixograph MID peak integral

Mx.....	General mixogram pattern
Nd.....	Number of nodes
NdD	Node density
NNdISk.....	Number of Nodes with immature spikelets
NdNonSS	Number of nodes with non supernumerary spikeles
NdR	Number of nodes with extended rachilla
NdSS	Number of Nodes with supernumerary spikelets
NIL(s).....	Near isogenic line(s)
NS	Number of spikes per m ⁻²
PH	Plant height
PC	Penetrance of clavate architecture
PP.....	Penetrance of pubescences
PSS	Penetrance of supernumerary spikelets
QTL	Quantitative trait locus/loci
RAPD	Random amplified polymorphic
RIL(s).....	Recombinant Inbred Line(s)
SbLi	Substitution line
SD	Spike density
Sk	Number of spikelets
SkNd.....	Spikelets per node
SL	Spike length
SRAP.....	Sequence-related amplified polymorphism
SS.....	Supernumerary Spikelet(s)
SSR	Simple sequence repeats
TKW.....	Thousand kernel weight
TRAP(s).....	Target region amplified polymorphism

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CHAPTER 1. INTRODUCTION

1.1. General Introduction

Spikes or ears are a type of inflorescence composed of stem tissue with nodes and internodes that support floral leaves. In grasses like wheat (*Triticum aestivum* L.), these structures have a main axis (rachis) bearing spikelets directly attached (sessile) to the nodes and separated by short internodes. Each spikelet is comprised of two glumes and florets attached to a rachilla (Lersten, 1987; Kirby, 2002). Each floret contains two bractlike structures, the outer lemma and the inner palea (on the opposite side of the rachilla), and the enclosed grass flower. This flower carries three stamens with anthers (about 3 mm in length), a pistil bearing two styles with feathery stigma branches, and two lodicules (Lersten, 1987; Kirby, 2002). The lemma may bear a long, medium, or short awn at its tip, or may be entirely awnless. However, the palea is always awnless. The glumes may be pubescent (covered with short, fine hairs) or glabrous (smooth) (Peterson, 1965).

The spike structures have photosynthetic activity which contributes to grain mass (Grundbacher, 1963; Li et al., 2006), milling and baking traits. In recent years, several studies in common wheat have uncovered the genetic basis of spike-related traits, showing the existence of a large number of QTL controlling these traits (Sourdille et al., 2000b; Li et al., 2002; Börner et al., 2002; Jantasuriyarat et al., 2004; Verma et al., 2005; Marza et al., 2006; Kumar et al., 2007; Li et al., 2007; Ma et al., 2007; Chu et al., 2008; Cui et al., 2012). However, most of these studies did not assess the association effect of these spike-related QTL on agronomic and quality traits of wheat, thus, limiting the knowledge for successful utilization of these QTL in breeding programs. In fact, the improvement of spikes and other structural characteristics to increase yield potential and wheat quality attributes have received less attention of breeders in recent decades, since the incorporation of resistance to biotic and abiotic conditions have become a higher priority for breeding programs (Reynolds et al. 2011).

Reduction of genetic diversity is a major problem in modern wheat breeding which jeopardizes wheat improvement and global food security (Reeves et al., 1997; Hosington et al. 1999; Reif et al., 2005). Natural genetic variation present in *Triticum* has been reduced through the selection imposed by farmers in early times and by the utilization of a reduced number of landraces to develop modern wheat cultivars

by breeders (Reif et al., 2005; Raman et al., 2010). Indeed, most of the recent QTL mapping studies in wheat were conducted in populations derived from closely related elite parental genotypes. Therefore, these studies neither recognize QTL already fixed in this elite material nor discover new allelic variations for incorporation in breeding programs. In wheat, it is possible to find broad genetic diversity in exotic germplasm with variation in spike-related traits that could be utilized to enhance genetic diversity and/or optimize other structural characteristics of wheat. Branched spikes [known as supernumerary spikelets (SS)], pubescent spikes, awnlessness spikes, clavate spikes, gigas spikes, and screwed spikes are examples of spikes variations that could be utilized toward these purposes.

To understand the genetics of SS and several spike-related traits, the NDSU improvement program developed a recombinant inbred line (RIL) population derived from a cross between WCB414, a wheat elite genotype with conventional spikes, and WCB 617, an introduced exotic genotype with SS and pubescent spikes. The population was advanced through single seed descent method to the F₇ generation. This RIL population was used in the present study to assess 35 spike-related, agronomic and quality traits. Subsequently, the parents and the RIL population were genotyped with Diversity Array (DArT) markers (Akbari et al., 2006), which resulted in the identification of a large number of polymorphic markers utilized in this study to construct a genetic map and to identify QTL for the traits evaluated. This evaluation allowed the determination of genetic basis for the traits studied, and established the associations of QTL affecting spike-related traits on agronomic and quality traits. The results of the study are presented in this dissertation in three chapters as follow.

The first chapter of results, titled “**Genome-wide genetic dissection of supernumerary spikelet and related traits in common wheat (*Triticum aestivum* L.)**”, presents the first report on genetics of the SS trait at the whole genome scale. The SS trait was dissected into seven SS-related traits in order to improve the power of QTL detection. Currently, this paper is being submitted to a scientific journal. The second chapter entitled, “**Genome-wide mapping of spike-related and agronomic traits in a common wheat population derived from a supernumerary parent and an elite parent**” describes the association of QTL affecting spike-related traits and agronomic traits in wheat. The QTL mapping of 10 spike-related traits and 10 agronomic traits in the RIL population is described in detail. Likewise, the description of allelic variations for identified QTL and their potential implementation in breeding programs

is discussed. Finally, the third chapter of results is entitled “***Genetic dissection of quality traits in wheat using an elite× exotic RIL population***”. This chapter describes the QTL mapping of 8 quality traits in the population and the associations of these traits with spike-related traits. Likewise, the utility of alleles derived from each parent for increasing the phenotypic values of some quality traits is discussed.

1.2. Objectives

1.2.1. General Objective

To determine the genetic basis of supernumerary-spikelets-related, spike-related, agronomic and quality traits in wheat using a RIL population derived from the cross a wheat elite line and an exotic line with supernumerary spikelet (SS).

1.2.2. Specific Objectives

- To assess the phenotypic variations and correlations of spike-related, agronomic, and quality traits in a RIL population derived from the cross a wheat elite line and an exotic line with SS.
- To determine Quantitative Trait Loci (QTL) associated with supernumerary-spikelet-related traits at whole genome level.
- To determine QTL of spike-related traits and agronomic traits at whole genome level.
- To study the relationships between QTL affecting SS, other spike-related traits and agronomic traits.
- To determine QTL associated with quality traits at whole genome level.
- To study the relationships between QTL affecting SS and quality traits.
- To identify QTL and/or alleles with potential use in breeding programs.

1.3. Literature Review

1.3.1. Branched spikes

1.3.1.1. Natural variation for branched spikes

The genus *Triticum* normally bears one spikelet on each rachis node. However, in nature it is also possible to find a spectrum of spike variations in which a rachis node has more than one spikelet (Sharman, 1967; Martinek and Bednár, 1998). These variations of spikes usually are categorized as branched spikes or supernumerary spikelets (SS). To some extent, branched spikes are variations in spike architecture; however their morphological characteristics as well as their genetics are different from the spikes discussed in the next sections.

Different approaches have been used to classify branched spikes and there is no a final consensus (Sharman, 1967; Koric, 1973; Pennel and Halloran, 1984b; Martinek and Bednár, 1998; Peng et al., 1998; Dobrovolskaya et al., 2009; Aliyeva and Aminov, 2011; Li et al., 2011). Following, we present a synthesis of the categories described by several authors:

- **Vertical Sessile Spikelet (VSS) or “Banana twin spikelets”:** In these spikes, a rachis node has two or three spikelets organized in vertical position (Martinek and Bednár, 1988). Sharman (1967) called this abnormality “Banana” because the spikelet’s positions in the nodes looks like “a miniature bunch of bananas”. These types of spikes have been observed in nullisomic lines for the chromosome 2B of *T. aestivum* (revised by Sharman, 1967). This is a characteristic with dominant and recessive expression (revised by Martinek and Bednár, 1998).
- **Tetrastichon or tetrastichon sessile spikelets (TSS):** In these types of spikes, a rachis node has two or three sessile spikelets organized in horizontal alignment (Martinek and Bednár, 1998).
- **Floribuda:** In these spikes, up to 10 spikelets can share a common rachis node (Dobrovolskaya et al., 2009). Spike fertility is reduced because most of the inflorescence organs are not fully developed due to the reduced space between spikelets (Martinek and Bednár, 1998). The term “multirow spike” (MRS) refer to fertile floribunda types (Martinek and Bednár, 1998);

Dobrovolskaya et al., 2009), in which the central part and upper thirds of the spike have three fertile spikelets per node (Dobrovolskaya et al., 2009).

- **Tibetan Tripe-spikelet wheat:** In this landraces, three spikelets are found on each rachis node. The trait is genetically stable and resembles to six-row barley. In one spike, it is possible to find more than 50 spikelets (Yang et al., 2005; Li et al., 2011).
- **Spike branching, miracle spikes, wonder wheat, or mummy wheat:** In these spikes, miniature spikes are produced from the lowest buds (Sharman, 1944, 1967). Potentially, one of these spikes can produce 120 spikelets and up to 150 kernels (Percival, 1921; Bonnet, 1966). Miracle spikes were originally recognized in turgidum wheat. For several years, wide crosses between branched-tetraploid wheat and hexaploid wheat were used to incorporate these spikes in hexaploid lines (Martinek and Bednár, 1998). However, Koric (1973) discovered that these spike types exist in hexaploid wheat and named this wheat as *Triticum aestivum ramifera*. Usually, wheat plants with miracle spikes have variation in the expression of branching in their primary stem and tillers. Therefore, it is possible to find branching spikes as well as conventional spikes in the same plant. This phenomenon is called 'hetero-branching'. Apparently, other factors, such as plant density, determine this condition (Denčić, 1988; Huang and Yen, 1988; Klindworth et al., 1990a; Martinek and Bednár, 1998).
- **Vavilovoid or Pulled out spikes:** These spikes are similar to miracle spikes. However, in vavilovoid spikes, the rachillas are exceptionally long and each spikelet resembles "a telescope pulled out" (Sharman, 1967). This spike variation is usually present in the hexaploid wheat *Triticum vavilovi* Jakubz and *Triticum jakubzineri* Udacz. Et Schachm (Sharman, 1967, Aliyeva and Aminov, 2011).
- **Forked, Y-shaped heads:** Sharman (1967) describes this spike type as the rarest of spike abnormalities. According to the author, in this type of spike a bifurcation of the rachis axis produces a "Y shape with two identical arms.

In branched spikes, two spikelet configurations have been described: 1) extra sessile spikelets at a rachis node, which are predominant in hexaploid wheat; and 2) spikes with additional spikelets

extended on rachillas, which are predominant in tetraploid wheat (Pennell and Halloran, 1984a, 1984b). Pennell and Halloran (1983) demonstrated that both configurations are controlled by one recessive gene (see section 1.3.1.3) and suggest using the term SS to include both types of spikelet organization. Since then, different authors have followed this suggestion (Pennell and Halloran, 1984a, 1984b; Peng et al., 1998; Dobrovolskaya et al., 2009; Sun et al., 2009; Aliyeva and Aminov, 2011).

The number of SS is influenced by the environment. Sharman (1944) reported that branched germplasm is expressed under short days and low temperatures. Pennell and Halloran (1984a, 1984b) demonstrated that generally, a strong vernalization response and winter sowing, in addition to short photoperiods and low temperature, are conducive for the expression of SS. However, interactions between these factors were observed. For instance, the lines with strong or moderate vernalization response have an increment in the number of SS when they are grown under 24-h photoperiod and low temperature. Likewise, the lines with weak vernalization response have an increment in the number of SS when they are grown under short photoperiods and low temperature (Pennell and Halloran, 1984a).

Genes also play an important role in the expression and stability of SS. According to Pennell and Halloran (1984a, 1984b), in branched stable lines, a high expression of SS is exhibited in different environments; meanwhile, in branched instable lines, the highest values of SS are observed only under conducive environments. These observations suggest differences in genetic constitution between lines, or differential gene × gene interaction in each line. Further discussion of the genetics of branched spikes is provided in this chapter.

1.3.1.2. Associations between branched spikes and other wheat characteristics

SS have been presented as one way to increase the yield potential of wheat due to the high number of florets producing caryopsis (Pennell and Halloran, 1983, 1984b, Hucl and Fowler, 1992; Peng et al., 1998). However, branched varieties are far from meeting this expectation. Salunke and Asana (1971) when compared one branched variety and one normal-ear type variety (“Kalyan Sona”) found that although the production of dry matter was similar in both lines, the branched-spike produced a very small kernel number per spike, resulting in reduced grain yield. Similarly, Rawson and Ruwali (1972) compared the grain yield of one branched variety of wheat and two checks under irrigated conditions. Among these checks was an adapted cultivar (“Kalyan Sona”), and the other one was an exotic cultivar (“Late

Mexico”). Their results showed that the branched line yield less compared to “Kalyan Sona,” but exceeded the performance of the exotic cultivar. Likewise, they reported sterility in many of the spikelets in this line, resulting in low number of kernels per spike. Hucl and Fowler (1992) compared two varieties of spring wheat with a tetraploid line named “Branched Spike Wheat” (BSW) and observed that the branched cultivar produced lower number of spikes per m⁻² and had the lowest grain yield regardless of the environment and seeding rate. Interestingly, under low precipitation rates during the growing season, Hucl and Fowler (1992) found that the branched lines had reduced spikelet formation as well as the kernel number.

Despite the poor grain yield performance of the branched genotypes, some authors state that grain yield can be improved in some SS lines. For instance, Saluke and Asana (1971) suggested that the grain yield in branched lines can be increased if the fertility of the florets is improved. According to Rawson and Ruwali (1972), spikelet sterility is a characteristic associated with the whole plant physiology and not only related with the spike development. Similarly, Pennell and Halloran (1984a, 1984b) suggested that lines with a stable and high expression of SS could be used to incorporate this trait in commercial wheat varieties.

Associations between branched spikes and other agronomic traits (other than grain yield) have also been studied. Koric (1973) reported a large number of kernels per spike in germplasm with SS but had low thousand kernel weight (TKW). Pennell and Halloran (1983), however, could not find any relationship between SS and plant height, TKW and days to emergence. Millet (1986, 1987) reported strong associations between heading date and spikelet number in a multi-spikelet line. On the other hand, in the specific case of the Tibetan Triple-Spikelet wheat, some lines have excellent seed production (>120 seeds per spike), and normal TKW (Yang et al., 2005). Recently, Zhang et al. (2012) developed four near isogenic lines (NIL) with variations in the alleles for two genes involved in the production of SS. They found that the NIL with SS had higher grain number, number of spikelets and fertile florets, but lower grain weight as well as late maturity. Finally, there is hardly any report which has attempted to study the associations of SS and quality traits, such as flour yield, grain protein content and gluten strength. To our best knowledge, the results reported in this study are the first of this type.

1.3.1.3. Genetics of branched spikes

The genetics of branched spikes has been studied for almost a century. In tetraploid wheat, Percival (1921) and Sharman (1942, 1967) described that the branched –headed character is controlled by a recessive gene. Sharman (1944), suggested the symbol *bh* for the recessive allele that produce the branched phenotype. However, after the discovery of branched germplasm in hexaploid wheat (Koric, 1973), different inheritance patterns have been suggested. Table 1-1 summarizes the main genetics studies since 1973.

Koric (1973) reported that the branched spikes of hexaploid wheat are controlled by a complex of genes. Three important factors, *Ramifera* (*Rm*), *Tetrastichon* (*Ts*) and *Normalizator* (*Nr*) were reported. The factor *Rm* and *Ts* work in complementary action in the formation of the branched spike; meanwhile, the *Nr* is a repressor of the branched phenotype. Therefore, the branched phenotype is possible only when the inhibitor is silenced or absent.

Pennel and Halloran (1983) reported a detailed study of inheritance of SS in hexaploid wheat. Crosses were made between hexaploid wheat line with SS and three commercial hexaploid semi-dwarf cultivars ('Condor', 'Egret', and 'Phoenix'). They attempted to perform similar crosses with a branched tetraploid wheat line, but most of the F₁ seed did not germinate due to the unbalanced chromosome number in the gametes of the pentaploid hybrid. In hexaploid wheat, the analysis of F₁, F₂, and backcrosses showed that this trait is controlled by two independent recessive genes and one independent repressor gene. The recessive condition was evidenced by the absence of SS in the F₁ generation as well as in the F₁ of the first backcross when the recurrent parents were 'Condor' and 'Egret'. In F₂ a ratio 15: 1 was observed, which fit with the presence of two recessive genes. Interestingly, in the commercial cultivar 'Phoenix', the presence of one recessive gene for SS was observed, which was confirmed by the presence of SS in the F₁ plants of the first backcross when 'Phoenix' was the recurrent parent. The authors suggested that 'Phoenix' differed from the parental line with SS in only one of the genes involved in SS characteristic. Most likely, this gene is a suppressor which inhibited the expression of SS in 'Phoenix'. Additional evidence of this gene repressor was observed in the failure to fit a 3:1 (Normal: SS) ratio in the F₁ of backcrosses when the SS was the recurrent parent. The authors observed that the cultivar 'Phoenix' had a large number of spikelets and nodes per spikes in relation to the other

commercial cultivars studied. They suggested that these characteristics can be related to the presence of some of the branched genes. This suggestion opens the door to the speculation that some of the higher yielding wheat cultivars have the presence of some of the branched alleles suppressed by one gene.

Millet (1986, 1987) crossed the multispikelet line “Noa” with the conventional-spike-type line “Mara” and with monosomic lines derived from “Mara.” “Mara” also carried day-length insensitive allele *Ppd1* on its 2D chromosome, which causes early heading date. Phenologic and morphologic studies stated that “Noa” had a longer spike development phase, higher initial number of spikelet primordia, reduced rate of spikelet production and later heading than “Mara.” The monosomic 2D line of “Mara” was the only monosomic line with a reduced final number of spikelets (Millet, 1987). Likewise, this monosomic line increased the duration of the spikelet development phase, causing a decrease in the rate of spikelet production (rate determined as the ratio between the produced number spikelets after double ridge stage and the duration of the spikelet phase). The monosomic F_1 produced from the cross of the monosomic 2D line and “Noa” had increased number of spikelets and later heading date compared to the euploid F_1 . This result demonstrated that the chromosomes 2D of ‘Noa’ carries recessive alleles for the number of spikelets per spike and delayed heading date. Chromosomes 5D and 7A were also reported to have minor effects on spikelet number (Millet, 1987).

In common wheat, Denčić (1988) studied the F_1 and F_2 progenies of three crosses involving branched germplasm (normal spike x branched spike; normal spike x tetrastichon spike; and tetrastichon spike x branched spike). The progeny analysis of these crosses suggested the presence of an inhibitor of the branched phenotype (*N*) and two additional genes (*R* and *T*). Contrary with previous studies, Denčić (1988) suggested that *R* and *T* had a complementary gene action which determined the branched or tetrastichon phenotype. Thus, in absence of the inhibitor, the genotypes *RRtt* or *rrTT* determine spike with tetrastichon characteristics; while the genotypes *R_T_* determine spike with branched characteristics.

Huang and Yen (1988) found four *bh* genes associated with branchness through the progeny analysis of F_1 and F_2 of 18 crosses between branched lines and non-branching lines of common wheat. According to these authors, the four genes are independent, recessively inherited and with different contribution to the phenotype. The analyses of six reciprocal crosses showed that when the non-branched phenotype is used as male, the frequency of branched spikes in F_2 is less than the frequency

observed when the non-branched phenotype is used as female. This result suggested the influence of cytoplasmic genes on branched spikes in common wheat. On the other hand, Huang and Yen (1988) classified the branched spikes in long branch (LB), short branch (SB), lateral supernumerary spikelet (LSS), opposite supernumerary spikelet (OSS) and normal multispikelet (NMS). They found that under different environments and/or genetic backgrounds, one genotype can express LB, SB, LSS or NMS. In the case of OSS Huang and Yen (1988) suggested that this type of spike is controlled by another gene system.

Table 1-1. Summary of the main genetic studies for SS in wheat during the 1983 and 2012 period

Reference	Wheat sp.	Plant material	Inheritance suggested	Location
Pennel and Halloran (1983)	Hexaploid	F ₁ , F ₂ , backcross	Recessive: Two genes and one repressor.	N.A
Millet (1986, 1987)	Hexaploid	F ₁ , F ₂ , backcross, monosomic lines	Recessive Major effect on 2D; Minor effect on 5D and 7A.	2D, 5D, 7A
Denčić (1988)	Hexaploid	F ₁ , F ₂	Recessive and cytoplasmic: Two genes with complementary action and one repressor gene.	NA
Huang and Yen (1988)	Hexaploid	F ₁ , F ₂ , backcross, reciprocal crosses	Recessive: four independent genes with different contribution on the phenotype.	NA
Klindworth et al. (1990a)	Tetraploid	F ₁ to F ₆ , backcross	Quantitative: A single major gene and several minor genes	NA
Klindworth et al. (1990b)	Tetraploid and D genome	'Langdon' D-genome disomic SbLi	Recessive	Tetraploid: 2A and 2B Hexaploid: 2D
Peng et al. (1998)	Hexaploid	Monosomic analysis	Recessive, polygenic	2D, 4A, 4B and 5A
Sun et al. (2009a)	Hexaploid wheat and rye	F ₁ , F ₂ , F ₃	Two dominant genes with complementary action	N/A
Dobrovolskaya et al. (2009)	Hexaploid	F ₂	Recessive. One gene	2D
Yang et al. (2005, 2009)	Hexaploid	F ₁ , F ₂ , backcross, F ₂ generation	Recessive. Two genes	2A
Aliyeva and Aminov (2011)	Complex hybrid	F ₁ , F ₂ , backcross	Recessive	N/A
Zhang et al.(2012)	Hexaploid, Gene introduced from durum	NILs	Recessive. Two genes	N/A

SS in tetraploid wheat were studied by Klindworth et al. (1990a). A SS wheat cultivar was crossed with the normal-spike “Langdon” durum, and the F_1 was backcrossed to each parent. The authors classified the spikes in three basic types: normal spikes, four-rowed spikes and ramified spikes. The analysis of segregation in F_3 and BC_1F_2 families indicated that the SS gene is controlled by a major recessive gene and numerous minor genes, suggesting that the SS is quantitatively inherited. In addition, the stability observed through generations in the types of branchness (four rowed or ramified) could not be explained by environmental conditions. If the plants were homozygous dominant for the major gene, then the phenotype should have normal spikes. However, if the plant was homozygous recessive for the major gene, the phenotype observed is ramified although minor genes can affect its expression. Heterozygous plants for the major gene results in normal spikes, but high frequency of the minor genes can produce SS. In a subsequent study, Klindworth et al. (1990b) used a set of “Langdon” D-genome disomic substitution lines, to precisely identify the chromosomal location of SS genes. A major gene was identified on chromosome 2A in durum, and one minor gene was identified on chromosome 2B. Additional experiments with a “Langdon” 2A telosomic line located the major gene on the short arm of the chromosome 2A. Interestingly, Klindworth et al. (1990b) noticed that 2D monosomic addition lines had inhibitory effect on the expression of SS. This effect was larger in the 2D monosomic addition lines derived from “Langdon” 2D(2A) than “Langdon” 2D(2B). Considering that D chromosomes come from “Chinese spring” wheat, they concluded that an inhibitor for SS is located on chromosome 2D in hexaploid wheat. On the other hand, further studies with molecular markers confirmed the presence of a recessive locus for the branched head (*bh*) phenotype on the short arm of chromosome arm 2A in durum wheat which was associated with the microsatellite marker *Xgwm425* (Haque et al., 2012).

Peng et al. (1998) found that genes for branched spikes were present on chromosomes 2D, 4A, 4B and 5A in the hexaploid wheat line “Yupi Branching”. They used monosomic lines as Miller (1986, 1987) did previously. These monosomic lines were derived from “Chinese spring” wheat which was crossed with “Yupi Branching”. In this study, the disomic F_1 hybrids did not express the branched character; therefore, they concluded that all the genes for branchness were recessives. They found plants with branched spikes in 2D, 4A, 4B and 5A in F_2 monosomic populations; although the largest number of branched plants was observed in the 2D F_2 populations. According to the authors this result suggests that

2D carries the genes with larger effect on branched spikes. On the other hand, the number of branched plants in the disomic and monosomic subpopulations indicated that the chromosomes 4A and 5A are strong effective *bh* genes, and 4B is the location for a weak effective gene for this trait. Subsequent studies demonstrated that branching gene located on chromosome 5A and 4B increased the rachis node number and spike length of normal spikes (Peng et al., 2000).

Sun et al. (2009a) reported different conclusions on the genetics of SS in wheat. They worked with the SS line “518885”, an atypical germplasm derived from the cross between the octoploid Triticale and common wheat line “Fei 5056”. According to the authors, the line “518885” has SS but not branched. It is important to note that Sun et al. (2009a) reported other system of classifications of SS: spikes containing over 28 spikelets are classified as SS, while with less than 28 spikelets per spike are classified as normal spike. The authors did not explain if the SS phenotype is produced by a large number of rachis nodes bearing one spikelet or by each rachis node bearing more than one spikelet (sessile-type spikelet configuration). The line “518885” was crossed with seven commercial lines of common wheat in order to study the inheritance of SS. Their results showed that the SS character is controlled by two dominant genes with complementary action (epistasis). Moreover, it was also suggested that these genes are derived from rye (*Secale cereale*) and not from wheat (Su et al., 2009a).

Dobrovolskaya et al. (2009) report was the first molecular mapping of genes study addressing the SS character. They worked with the floribunda type (MRS, see section 1.3.1.1) of wheat, and with the “monstrosum ear” from rye. Two F₂ populations were developed in order to map the MRS trait: The population-I was the result from the cross between “Rüc163-1-02” and “Sol149-1-02”, whereas population-II was developed from the cross between “Rüc167-1-02” and “Sol149-1-02”. The Rüc lines are MRS types, meanwhile the line “Sol149-1-02” is a conventional wheat type. The two populations were genotyped with a set of SSR markers belonging to chromosomes 2A, 2B, 2D and 4A. In both F₂ populations, the results showed a 3:1 (Normal: MRS) ratios. Consequently, the trait was controlled by one recessive gene. In both populations, the SS traits co-segregated with the microsatellite locus *Xwmc453* on chromosome 2D. The authors named the mutant allele *mrs1* and the wild-type allele *Mrs1*. Subsequent analysis with chromosome deletion bin lines delimited the physical location of the gene *Mrs1*

into the distal half of chromosome 2DS. A consensus map 2S showed that this gene (*Mrs1*) is located in a gene rich region named 2S0.8 (Erayman et al., 2004).

Yang et al. (2005) and Li et al. (2011) described the genetics of another type of spike with variation in the spikelets number: the Tibetan triple-spikelet wheat. Yang et al. (2005) reported that this trait was controlled by two independent recessive genes, which they called *Ts1* and *Ts2*. In a subsequent study, Li et al. (2011) identified one major QTL linked to the triple spikelet trait on the chromosome 2A using two F₂ populations derived from the crosses of the Tibetan triple spike line “TTSW-5” with two conventional wheat lines “Jian 3” and “Chuanmai 55”. This QTL was named qTS2A-1 and explained 33.1% of the phenotypic variation. Unfortunately, the authors were not able to detect other gene controlling the triple-spikelet phenotype and suggested more saturation of this molecular map in order to find minor genes associated to this trait. The genetics of another atypical spike variation was studied by Aliyeva and Aminov (2011). They studied a new branched spike form named 166-Schakheli. This line was obtained from the cross between a hexaploid wheat line 171AC [(*T. durum* Desf. X Ae. *Tauschii* Coss.) x *S. cereale* L. ssp. *segetale* Zhuk] x *T. aestivum* L. “Chinese Spring”] and durum wheat variety *T. durum* Desf. “Bereketli-95”. The spikes of the line 166-Schakheli resemble the vavilovii type (see section 1.3.1.1) but differ in more extended rachillas. The authors of this study found that the branched trait was recessive and the gene is dose independent.

Recently, Zhang et al. (2012) reported the development of NIL for two recessive genes (*Sb*) related to SS located on chromosome 2A and 2D. These genes were identified in hexaploid wheat line “Fen33” (a SS line resulted from the interspecies hybridization of durum x common wheat (reviewed by Zhang et al., 2012). To develop these NIL, “Fen33” was the donor parent of *Sb* genes; meanwhile, the conventional wheat line “Taishan” was the recurrent parent. Four NIL were obtained: 1) double recessive (DR), *sb1sb1 sb2sb2*; 2) double dominant (DD), *Sb1Sb1 Sb2Sb2*; 3) single recessive 1 (SR1), *Sb1Sb1 sb2sb2*; and 4) single recessive 2 (SR2), *sb1sb1 Sb2Sb2*. Genetic studies using these NIL showed that NILs with only DR alleles at both genes loci had SS suggesting that the production of SS results from the interaction of both genes. DR genotype had a high number of grains, spikelets, and fertile flowers, but a low grain set and grain weight. Both SR types had normal spikes. Interestingly, SR1 needed several days

for heading and anthesis; while, SR2 reduced the number of fertile florets and grains. Therefore, the Sb genes have pleiotropic effects on these traits and/or they are linked to genes associated to them.

1.3.2. Spike size traits

1.3.2.1. Natural variation for spike architecture

Spike architecture includes traits determining spike compactness, such as spike length and number of rachis nodes. The spikes of common wheat differ greatly in form and degree of compactness. Spikes usually are classified in four general categories that incorporate length and width: 1) fusiform, in which the spikes taper toward the apex or from the middle toward both base and apex, 2) oblong, in which the spikes are uniform in width and thickness, but tend to be longer than wide, 3) clavate, in which the spikes are larger and more dense at the apex, and 4) elliptical, in which the spikes are short and uniformly rounded at both the base and apex but are flattened on the sides (Briggle and Reitz, 1963).

Differences in spike architecture are the result, in part, of differences in density (Briggle and Reitz 1963). Most of authors define spike density as the number of spikelets per longitude of spike (Sourdille et al., 2000b; Jantasuriyarat et al., 2004; Ma et al., 2007, Cui et al., 2012). However, other approaches have been used to categorize the spike density. For instance, Briggle and Reitz (1963) consider spike density as the distance in mm occupied by 10 internodes of the rachis measured from the center of the spike. Considering this definition, these authors suggested three spike density categories: 1) lax, in which 10 internodes occupy from 50 to 75 mm; 2) mid-dense, in which 10 internodes occupy from 35 to 60 mm; and 3) dense, in which 10 internodes occupy from 20 to 45 mm.

Differences in spike morphology have been recognized as a criterion of further classification of bread wheat. Currently, scientists have described six subspecies of bread wheat : 1) *T. Spelta* L. [*Triticum aestivum* (L.) Thell. ssp. *Spelta* (L.)]; 2) *T. Compactum* Host. or club wheat [*T. aestivum* (L.) Thell. ssp. *compactum* (Host.) Mk., *T. vulgare compactum* Alef., *T. sativum compactum ssp compactum* Asch. and Graeb.]; 3) *T. sphaerococcum* Perc. [*Triticum aestivum* (L.) Thell. Ssp. *Sphaerococcum* (Perc) Mk]; 4) *T. macha* Dek. and Men. [*Triticum aestivum* (L.) Thell. ssp. *macha* (Dek. and Men) Mk. *T. tuballicum* Dek., *T. imeriticum* Dek.]; 5) *T. Vavilovi* (Tum.) Jakubz. [*Triticum aestivum* (L.) Thell ssp. *vavilovi* (Tum.) Sears]; and 6) *T. aestivum* L. [*Triticum aestivum* (L) Thell ssp. *vulgare* (Vull) Mk., *T. vulgare* Vill., *T. vulgare* Host.,

T. hybericum L., *T. sativum* Lamk., *T. sativum* Pers.] (Miller, 1987). The differences between these hexaploid forms are caused partially by genes affecting the gross spike architecture (see section 1.3.2.3). The spikes of spelt wheat are long and lax, and their spikelets are well separated from each other on the rachis. These spikes are 10 to 15 cm long with 16 to 23 spikelets (Percival, 1921). Club wheat is characterized by the short, stiff, and compact spikes. Usually, the club spikes are of uniform density and oblong with a spike length of 3.5 to 6 cm with 17 to 25 closely packed spikelets (Percival, 1921). *T. sphaerococcum* is characterized by short and dense spikes and small near-hemispherical grains (Miller, 1987). *T. macha* is distinguished by laterally compressed wide fragile spikes (Miller, 1987). *T. vavilovi* has branched spikes with a fragile rachis, and glumes attached to the grain (Peterson, 1965) (for more details see section 1.3.2.3). Finally, in the case of *T. aestivum aestivum* or common wheat, the spikes range in length from 6 to 18 cm, with an average of 20 spikelets per spike (Percival, 1921). A broad variety of spike architectures resulted from variations in spike length, number of spikelets, density, immature spikelets, etc. are present in common wheat.

1.3.2.2. Associations among spike architecture and other wheat traits

Compared to cultivated common wheat (ssp. *aestivum*), knowledge of agronomic and quality performance of other five subspecies is lacking in general. The *spelt* wheat is tall with lodging problems and low yield (Bertin et al., 2001). In terms of quality, spelt has high flour protein content and short dough-mix times indicating weak gluten (Wilson et al., 2008). Club wheat (*Triticum aestivum* ssp. *compactum*) is a type of soft white wheat with a significant role worldwide in drylands (Johnson et al., 2008). Club spikes are one-half the length of common wheat spikes. However, the spikelet number of club wheat is not different than non-club types. This sub-species has small grains compared to common wheat, but the decrease in spike size is compensated by an increase in the seed number (Zwer et al., 1995). Thus, the yields reached by the club wheat are closely comparable to the yields produced by common wheat. The limited spaces within the club spikes constrain a full kernel development; resulting in a lower test weight (Steven et al., 1994). Rarely, other subspecies of wheat have been studied in terms of agronomic and quality traits. Overall, we know that *T. vavilovi* has good performance in dry growing conditions (Peterson, 1965) and, *T. macha* is cultivated in Georgia with salt tolerance (Badridze et al., 2009).

In common wheat, spike characteristics determine the number of kernels per spike; therefore, in part, they are influencing grain yield (Cui et al., 2012). Spike length is positively associated with grain yield, grain weight per spike, grain-weight, plant height, culm length, and no. of spikelets per spike (Kato et al., 2000; Börner et al., 2002). Meanwhile, spike length is negatively associated with the tiller number per plant (Kato et al., 2000). There are contradictory conclusions about associations of spike length and days to heading. Kato et al. (2000) found negative correlation between these traits, while Börner et al. (2002) reports a positive association. On the other hand, spikelets per spike is positively associated with yield, plant height, culm length, grain weight per spike and grain weight (Kato et al., 2000). However, the number of spikelets per spike is negatively correlated with days to heading (Kato et al., 2000). Both spike length and number of spikelets per spikes are positively associated (Kato et al., 2000). In terms of quality, Steve et al. (1994) reported no relationship between spike-related traits (spike length and number of spikelets) and test weight. However, they found negative correlation between test weight and kernels per spike and kernels per spikelet.

The association between the number of spikelets per spike and photoperiod has been studied in detail. Rahman and Wilson (1977) demonstrate that several wheat cultivars increase their number of spikelets as the photoperiod decreased from 24 to 8 hours. Same authors found that the length of spikelet formation and rate of spikelet initiation are the most important factors behind number of spikelets per spike. They found a positive correlation ($r = 0.55$) between spikelet number and length of the spikelet phase (starting from the initiation of floral structure to the formation of terminal spikelet), but a negative correlation between the rate of spike initiation and length of spike phase ($r = -0.93$). Rahman and Wilson (1977) hypothesized that the rate of spike initiation and the length of spike phase can be controlled by separated factors (such as hormones). Thus, favorable conditions for one of the factors inhibit the activity of the other factor. This compensatory mechanism (feedback) keeps an adequate number of spikelets per spike because in nature, the cultivars with too few or too many spikelets are at disadvantage (Rhaman and Wilson 1977).

Recently, Cui et al. (2102) reported an association between spike-related characteristics in two RIL populations. They collected information on spike length, spike density, spikelet number, basal sterile spikelet number, tops sterile spikelet number, sterile spikelet number in total, and fertile spikelet number.

For most of these traits, they found weak correlations. The most important associations were: 1) positive correlation of spike length with spikelet number and fertile spikelet number; 2) positive correlation of spikelet number and the number of fertile spikelets; 3) positive association of spike density with number of spikelets per spike and number of fertile spikelets; 4) negative association of spike density and spike length; 5) positive association of sterile spikelet number with top sterile spikelet number and basal sterile spikelet number; and 6) negative correlation between sterile spikelet number and the number of fertile spikelets.

1.3.2.3. Genetics of spike architecture

In wheat, four major complexes of genes controlling spike architecture were reported: Q, C, S₁ and V. These genes determine the phenotype of the subspecies *Triticum aestivum* ssp. *aestivum* (QQccS₁S₁), *T. aestivum* ssp. *spelta* (qqccS₁S₁), *T. aestivum* ssp. *compactum* (QQCCS₁S₁), *T. aestivum* ssp. *macha* (qqCCS₁S₁), and *T. aestivum* ssp. *sphaerococcum* (QQccS₁S₁) (Miller, 1987; Jantasuriyarat et al., 2004) (see section 1.3.1.1). *T. aestivum* ssp. *vavilovii* is an exception because it is produced by the V factor (Miller, 1987). The complex Q gene is located on the chromosome arm 5AL and determines whether a spike is normal or speltoid. The allele *q* is considered as the speltoid gene. Therefore, wheat cultivars with the dominant Q allele are shorter than those with the recessive allele, but they have compact spikes, non-brittle rachis, and free-threshing seeds (Jantasuriyarat et al., 2004). The complex C is located close to the centromere of chromosome 2D and determines if a spike is lax or compacted. Thus, the dominant allele C produces club wheat (*Triticum compactum* group). The genes C inhibit the free-threshing character from the complex Q. Therefore, at least for tenacious glumes, the C genes are dominant over Q genes (Jantasuriyarat et al., 2004). The complex S₁ is located on the short arm of chromosome 3D, and it is associated with *T. aestivum* ssp. *sphaerococcum*. On the other hand, the complex V of *T. aestivum* ssp. *vavilovii* is located on chromosome 5A.

In common wheat, the evidence collected during the last 15 years suggests that variation in traits such as spike length and spike density is also caused by genes other than Q, C, S₁ and V. The allelic variation on these gene complexes is reduced in this subspecies, and differences in spike morphology cannot always be explained by these complexes (Sourdille et al., 2000b; Cui et al., 2012). Instead, it is considered that spike length, number of spikelets (in non-branch spikes) and spike density in common

wheat is controlled by multiple QTLs distributed throughout the wheat genome (Sourdille et al., 2000b; Li et al., 2002; Börner et al., 2002; Jantasuriyarat et al., 2004; Verma et al., 2005; Marza et al., 2006; Kumar et al., 2007; Li et al., 2007; Ma et al., 2007; Chu et al., 2008; Cui et al., 2012). These QTLs are highly influenced by environment. Table 1-2 shows a summary of QTL associated with spike-dimensions traits in common wheat (updated from Kumar, 2009).

Table 1-2. Summary of QTL information on studies for spike architecture related traits (adapted from Kumar, 2009)

Trait	QTL Location	Pop.	Marker	Reference
Spike Length	1AL, 2BS, 2DS, 4AS, 5AL	D-H	RFLP, SSR	Sourdille et al. (2000b)
	1BS, 4AL, 4AS, 5AL	RIL	RFLP, SSR	Börner et al. (2002)
	1AL, 1BS, 4AL, 7AL	RIL	RFLP, SSR	Li et al. (2002)
	1BS, 4AL, 4DL, 7AS	RIL	RFLP, SSR	Jantasuriyarat et al. (2004)
	2DS, 4D	D-H	SSR	Verma et al. (2005)
	1AL, 1AS, 1B, 2BL, 2BS, 3BL, 4BL, 5BL, 7AS, 7BL	RIL	AFLP, SSR	Marza et al. (2006)
	1AS, 1BL, 1DL, 2BL, 2DL, 2DS, 4AL, 5AL, 5DL	RIL	RFLP SSR	Kumar et al. (2007)
	1AS, 2DS, 4AS, 5AS, 5BL, 7DS	RIL and IF2	RAPD, SSR	Ma et al. (2007)
	3DS, 4AL, 5AL	D-H	SSR	Chu et al. (2008)
	1A, 2D, 6A	D-H	AFLP, RAPD, SSR	Heidari et al. (2011)
Spikelets/spike	1B, 1D, 2A, 2B, 3B, 4A, 5A, 5B, 6B, 6D, 7A, 7D	RIL	G-SSR, EST-SSR, ISSR, STS, SRAP and RAPD	Cui et al. (2012)
	5AS, 5AL	Substitution lines for 5A	RFLP	Kato et al. (2000)

(Continues)

Table 1-2. Previous QTL studies for spike architecture related traits (continued)

Trait	QTL Location	Pop.	Marker	Reference
Spikelets/spike	2AS, 2BS, 5AL	D-H	RFLP, SSR	Sourdille et al. (2000b)
	2DS, 7AL	RILs	RFLP, SSR	Li et al. (2002)
	3AS, 3DL, 4AL, 7AL	RILs	RFLP, SSR	Jantasuriyarat et al. (2004)
	2BS, 2DS, 4AL, 4DS, 5AL, 6AS, 6AL	RIL	RFLP SSR	Kumar et al. (2007)
	1BL, 2DL, 3BL, 5AL, 5BL, 7AS, 7DS	RIL and IF2	RAPD, SSR	Ma et al. (2007)
	5DL	RILs	SSR, EST-SSR, ISSR	Li et al. (2007)
	4DL	D-H	SSR	Chu et al. (2008)
	1A, 2A, 4B	D-H	AFLP, RAPD, SSR	Heidari et al. (2011)
	1AS, 1B, 1DL, 2B, 3B, 4AL, 4D, 5B 5D, 6A, 6B, 6D, 7B	RIL	G-SSR, EST-SSR, ISSR, STS, SRAP and RAPD	Cui et al. (2012)
Spike Density	1AL, 2AL, 2BS, 2DS, 4AS, 5AL	D-H	RFLP, SSR	Sourdille et al. (2000b)
	1BS, 2BL, 4AL, 5AL, 6AS	RIL	RFLP, SSR	Jantasuriyarat et al. (2004)
	1B, 4AL, 7BS, 7DL	RIL	AFLP, SSR	Marza et al. (2006)
	4AL	RIL	SSR, EST	Kiriwi et al. (2007)

(Continues)

Table 1-2. Previous QTL studies for spike architecture related traits (continued)

Trait	QTL Location	Pop.	Marker	Reference
Spike Density	1AS, 2DS, 4AS, 5AS, 5AL, 5BL, 7DS	RIL and IF2	RAPD, SSR	Ma et al. (2007)
	5AL, 5BL	D-H	SSR	Chu et al. (2008)
	2A, 2D, 4B, 6A	D-H	AFLP, RAPD, SSR	Heidari et al. (2011)
	1A, 2A, 2B, 2D, 3A, 3B, 4A, 4D, 5A, 5D, 6D, 7D	RIL	G-SSR, EST-SSR, ISSR, STS, SRAP and RAPD	Cui et al. (2012)

1.3.3. Spike awnedness

1.3.3.1. Natural variation of spike awnedness

The awns in cereals are considered rudimentary leaves (Grundbacher, 1963), and awnedness refers to the presence or absence of awns in the spikes. Börner et al. (2005) found associations between geographical origin and the presence/absence of awns. According to these authors, the absence of awns (awnless) is a common characteristic in cooler, temperate climatic regions of northern and central Europe as well as northern America. On the other hand, the presence of awns (awned) is typical of Mediterranean areas, northern Africa, and Middle East.

The physiological importance of the awns has been recognized through decades. For instances, Olugbemi et al. (1976) demonstrated that the awns intercept 9% of the incident visible light and contribute 12.2% to canopy gross photosynthesis. Li et al. (2006) demonstrated the photosynthetic importance of the awns during dough-development stage and maturity. According to these authors, at these stages, the number of chloroplast and photosynthetic activity in the flag leaf are reduced, while the awns increase the number of these organelles as well their photosynthesis. Li et al. (2006) also demonstrated that the activity of the enzyme PECase (EC 4.1.1.3.1) is high in the awns than in the flag leaves. These authors suggested that this enzyme provided a new source of substrates for carbohydrate synthesis in the awns at grain filling, as well as a CO₂ recycling activity which is desirable under drought conditions.

Considering awnedness, wheat varieties have been classified differently. Watkins and Ellerton (1940), suggested the following classification of the *Triticum* varieties as: 1) bearded or fully awned; 2) tipped 1, in which very short awn tips at the base and center of the spike are observed, but in the apical region are awns with a length of 1 cm in average found; 3) tipped 2, which has short tips in all the spike; 4) half-awned, in which the awns gradually increase in length toward the spike apex; 5) Hooded bearded, in which the awns are short and curved; 6) Hooded beardless, which are similar to hooded bearded spike but with shorter awns; 7) Beardless, in which awns tips are hardly observed.

However, Briggie and Reitz (1963) classified the varieties as: 1) awnless or without awns; 2) apically awnleted, in which the apex of the spike has awnlets of 1 to 15 mm long; 3) awnleted, in which

awnlets of 3 to 40 mm are observed in all the spike, but increase in length toward the apex; and 4) awned, in which all lemmas have a defined awn.

1.3.3.2. Associations between spike awnedness and other wheat traits

The impact of wheat awns on agronomic and quality traits have been studied by comparing awned with awnleted (spikes containing tip awns) and awnless wheat lines (Atkins and Norris, 1955; Grundbacher, 1963; McNeal et al., 1969; McKenzie, 1972; Olugbemi et al., 1976; Weyhrich et al., 1994). Most of the studies reported an advantage in test weight and kernel weight in favor of the awned cultivars compared to the awnless or awnleted. Thus, an awned variety produces more starch for the kernels compared to awnless or awnleted varieties. In addition, under semi-arid conditions, the awns photosynthesize and translocate photosynthates to the kernel for several days (Grundbacher, 1963). Similarly, Börner et al. (2005) observed a high frequency of awned germplasm in regions with severe drought stress (Mediterranean areas, northern Africa and Middle East)

The relationships between awns and yield have sometimes resulted in controversial reports. McKenzie (1972) reported that yield in awnleted spikes was superior to the awned yield under both irrigated and not irrigated conditions. These results were contradictory to most of the previous conclusions (Atkins and Norris, 1955; Grundbacher, 1963; McNeal et al., 1969). However, later on, Weyhrich et al. (1994) reported that the effects of awn suppression on grain yield in hard red winter wheat appeared to be genotype-dependent. This conclusion could explain some of the controversial results obtained in the past decades. However, additional research using modern approaches is needed to clarify these issues.

Few studies have been conducted to study the relationship between awns and wheat quality. McNeal et al. (1969) reported a paradox in the breeding for awns in wheat. In general, producers prefer awned cultivars while the milling industry prefers awnless cultivars. According to their results, the awnleted wheat is useful for the milling industry since it produced 3.7% more flour than the awned population. At the same time, loaf volume was significantly higher for the awnleted compared to awned wheat cultivars. Differences between awned and awnleted wheat were not obtained in flour ash, flour protein, mixing time or bread quality.

1.3.3.3. Genetics of awnedness

The genetic control of awns has been studied for almost a century. Early in the 20th century, it was demonstrated that awnless condition is dominant compared to the awned condition, with at least two genes controlling this trait (Stewart, 1928). However, Watkins and Ellerton (1940) suggested the presence of three major genes leading the production of the awn classes: 1) The gene B_1 controlling Tipped 1 (and presumably half-awned); 2) the gene B_2 related with Tipped 2 (and presumably half-awned); and 3) the gene Hd associated to hooded types (hooded bearded and hooded beardless). Awned wheat plants have the alleles hd , $b1$ and $b2$ in homozygous condition, while the awnless phenotypes carry dominant alleles. For instance, the awnless varieties “Chinese Spring” and “Federation” have the gene combination $Hd B2$ and $B1 B2$ respectively (Sourdille et al., 2002). Nevertheless, Watkins and Ellerton (1940) suggested that other genes could be associated with the phenotypes where the awns length is equal in all parts of the spikes.

Using SSR, Sourdille et al. (2002) mapped a Double Haploid population segregating for awnness. The parents of this population were the awnless line “Chinese Spring” and the awned lines “Courtlot”. Two major QTLs were detected on chromosome 4AS and 6BL. Subsequently, these authors used deletion lines to confirm that the QTL on chromosome 4A belongs to the Hd gene; while the QTL on chromosome 6B is the $B2$ gene.

1.3.4. Variations in glume pubescences

1.3.4.1. Natural variation for glume pubescences

The glumes are two opposite rigid boat-shaped bracts located at the base of the spikelet. Their size, shape, texture, form of keel, and terminal tooth are characters used in wheat classification (Percival, 1921). However, the notorious difference between absence and presence of hairs (pubescences) on glumes has been considered a major criterion of classification of wheat varieties (Briggle and Ritz, 1963). Most of the commercial wheat varieties are named glabrous because of lack of hairs on glumes. Instead, wheat varieties with hairs are denominated as pubescent. The glume hairs are unicellular structures that terminate in a fine point. The hairs vary in longitude; the long hairs ranged from 6 mm to 1.2 mm, while the shorter one measure from 2 mm to 4 mm (Percival, 1921).

In the past, the adaptive role of pubescent glumes has been discussed in few studies. Jain et al. (1975) were not able to find a geographic pattern of the glume pubescence in a world collection of durum wheat. In a similar study, but using a world collection of bread wheat, Börner et al. (2005) found that this trait is randomly distributed at a low frequency throughout the world. However, 20% of the hairy accession were originated from northern Europe (Finland, Sweden), Asia, (Afghanistan, India, Iran, Nepal, Pakistan, Turkey) and North Africa (Libya). Among these regions, the maximum frequencies of accessions with pubescences were found in Libya (65%) and Nepal (41%). Therefore, they concluded that their data provides some support for a positive effect of pubescence on dry and cold environments. On the other hand, Maes et al. (2001) reported a beneficial effect of pubescences at low temperatures under controlled condition in a growth chamber. Overall, they reported that floret temperature in pubescent varieties is high in relation to no-pubescent material. However, individual comparison between some lines indicated significant differences among few but not all the genotypes. Therefore, the authors concluded that in addition to the pubescences, other possible factors may be involved in frost tolerance. Maes et al. (2001) also reported that the presence of pubescences was also related to a delay in the time needed to reach damaging temperatures and pubescent plants produced more grains per spikelets compared with non-pubescent material.

1.3.4.2. Associations of glumes pubescences and other wheat traits

Studies reporting the associations of glumes pubescences with any other trait are scarce. Briggles and Sears (1966) reported association between the presence of pubescences on glumes and resistance to powdery mildew. Maes et al. (2001) found a positive relation between the number of kernels per spikelets and the presence of pubescences in the glumes under growth chamber conditions.

1.3.4.3. Genetic of glume pubescences

Genetically, the trait glume pubescence is designed by the symbol *Hg*. Percival (1921) described the pubescences on glumes as a characteristic with dominant inheritance and controlled by two alleles. However, Sheybani and Jenkins (1961) proposed an allelic series controlling this trait in durum; *Hg*, *hg*, and *hg*₁. According to these authors, when the pubescent varieties “reichenbachii” and “Golden Ball” are crossed with any glabrous variety, a ratio 3:1 of pubescent to glabrous (respectively) is obtained.

Nevertheless, when the pubescent variety “Khala” is crossed with a glabrous variety, a 3:1 ratio of glabrous to pubescent (respectively) is obtained. Therefore, the authors suggested that *Hg* was a dominant allele for pubescences, and *hg* is a recessive allele for glabrous, but dominant to *hg₁*. Thus, the genotypes “reichenbachii” and “Golden Ball” are *HgHg*, Khala is *hg₁hg₁*, and the glabrous germplasm is *hghg*.

The gene *Hg* has been located on the short arm of chromosome 1A tightly linked to the locus *Bg* (dominant gene that produces black color of glumes during maturation) and the gliadin locus (*Gli-A1*) (Khlestkina et al., 2000). In addition, *Hg* is located close to a number of major fungal resistance genes. Therefore, the absence/presence of pubescences could be used as a morphological marker for disease resistance selection (Khlestkina et al., 2006).

1.3.5. Genetics of several agronomic and quality traits in wheat

Since we mapped several genes affecting several agronomic and quality traits in this study, we consider that is important to describe previous QTL mapped for these traits. Kumar (2009) presented a summary of QTL mapping studies for some important traits in wheat. In Table 1-3 we are presenting a section of the summary reported by Kumar (2009) as well as an updated summary of most important QTL related to the agronomic and quality traits.

Table 1-3. Summary of information related to QTL governing agronomic and quality traits in common wheat

Trait	Authors	Chromosomal Location of QTLs	Population	Marker
<i>Agronomic Traits</i>				
Grain yield	Groos et al. (2003)	2B, 3BS, 4AL, 4BL, 5AL, 5BL, 7DL	RILs	RFLP, SSR
	Huang et al. (2003)	1AL, 1BL, 2AS, 2BL, 2DS, 2DL, 3BS, 4DS, 4DL, 5BS	BC ₂ F ₂	SSR
	Huang et al. (2004)	1AS, 3DS, 4DL, 5AS, 5AL, 5BL, 6BS, 6DS, 6DL	BC ₂ F ₂	SSR
	McCartney et al. (2005)	2AS, 2BS, 3DL, 4AL, 4DS	D-H	SSR, EST (SNP)
	Huang et al. (2006)	5AL, 7AS, 7BS	D-H	SSR
	Marza et al. (2006)	1AL, 1B, 2BL, 4AL, 4B, 5A, 5B, 6B, 7A, 7DL	RILs	AFLP, SSR
	Narasimhamoorthy et al. (2006)	2DS, 7DL	BC ₂ F _{2:4}	SSR
	Kirigwi et al. (2007)	4AL	RILs	SSR, EST
	Kuchel et al. (2007)	1BS, 2DL, 3DL, 4AL, 4DL, 5BL, 6AL, 6DL, 7B	D-H	SSR
	Kumar et al. (2007)	1AL, 2AS, 2DS, 2DL, 3BS, 4BL, 6AL, 6DL	RILs	RFLP, SSR
	Li et al. (2007)	2AS, 2DL, 3BS, 6AL	RILs	SSR, EST-SSR, ISSR
	Cuthbert et al. (2008)	1AS, 2DL, 3BS, 5AL	D-H	SSR
	Heidari et al (2011)	6A, 6D	D-H	AFLP, RAPD, SSR
	Bennet et al. (2012)	1A, 1B, 2A, 2B, 2D, 4D, 6D, 7A	D-H	DArT
	Mergoum et al (2013)	5AS, 6BS	D-H	DArT, SSR

(Continues)

Table 1-3. Summary of information related to QTL governing agronomic and quality traits in common wheat (continued)

Trait	Authors	Chromosomal Location of QTLs	Population	Marker
Days to heading	Keller et al (1999)	1A, 2A, 2B, 4A, 4B, 5A, 5B, 7B	RILs	RFLP, SSR
	Börner et al (2002)	2DS, 5DL, 5DS, 7DS	RILs	SSR
	Sourdille et al(2000a)	1AL, 2BS, 4DL, 5AL, 7BS	D-H	RFLP, SSR, AFLP
	Sourdille et al(2003)	2B, 5A, 7B, 7D	D-H	RFLP, AFLP
	Huang et al .(2004)	2DL, 3AL, 4AL, 7AL, 7DS	BC ₂ F ₂	SSR
	Huang et al. (2003)	2AS, 2DS, 3BS, 5AL, 5BL, 6AL, 7BS	BC ₂ F ₂	SSR
	Xu et al. (2005)	2D	RILs	AFLP, SSR
	Marza et al. (2006)	3BL, 5B, 6B	RILs	AFLP, SSR
	Narasimhamoorthy et al. (2006)	2D, 3D	BC ₂ F _{2:4}	SSR
Cultbert et al. (2008)	1BS, 2D, 3A, 5AL, 6BS, 7BS, 7DL	D-H	SSR	
Days To maturity	Li et al. (2002)	2AS, 2DS	RILs	RFLP, SSR
	McCartney et al. (2005)	3BS, 4AL, 4DS, 7DS	D-H	SSR
	Marza et al. (2006)	1B, 3AS, 6B	RILs	AFLP, SSR
	Huang et al. (2006)	2D, 5D, 7D	D-H	SSR
	Cuthbert et al. (2008)	1BS, 3B, 5AL,5B, 6BS, 7A, 7BS, 7DL	D-H	SSR

(Continues)

Table 1-3. Summary of information related to QTL governing agronomic and quality traits in common wheat (continued)

Trait	Authors	Chromosomal Location of QTLs	Population	Marker
Plant height	Cadalen et al. (1998)	3DL, 5AL, 5BL, 4BS, 4DL, 6DS, 7AL, 7BL	D-H	RFLP, SSR
	Araki et al. (1999)	4AS, 4AL	SbLi	RFLP
	Ahmed et al. (2000)	1AL, 1DL, 2BS, 2DS, 2DL 4BL	RILs	RFLP
	Börner et al (2002)	1AS, 1BL, 2BS, 2DS, 3AL, 3BS, 3DL, 4AL, 4BL, 4DL, 5DL, 6AS, 6BS	RILs	SSR
	Sourdille et al. (2003)	4BS, 4DL, 7AL, 7BL	D-H	RFLP
	Huang et al. (2004)	1AS, 1DL, 3AS, 3BL, 4AS, 4BL, 5AS, 5AL, 5BL, 6AL, 6DS, 7AS, 7AL, 7DS	BC ₂ F ₁	SSR
	McCartney et al. (2005)	2DS, 4BS, 4DS, 5BL, 7AL, 7BS	D-H	SSR, EST (SNP)
	Huang et al. (2006)	4BL, 4DS, 5DL, 7BS	D-H	SSR
	Marza et al. (2006)	2BL, 2BS, 2DL, 3BL, 4B, 6A	RILs	AFLP, SSR
	Spielmeier et al. (2004)	2DS, 6AS	RILs	SSR
	Chu et al. (2008)	4DS, 5AL	DH	SSR
	Wang et al. (2009)	1D, 2D, 3D, 4D	RILs	SSR
	Bennett et al. (2012)	1D, 2D, 3A, 5A	D-H	DArT
Lodging	Keller et al (1999)	1BS, 2AS, 2D, 3AS, 4AS, 5AL, 5BL, 6BL, 7BL	RILs	RFLP, SSR
	Börner et al (2002)	2D, 6AS	RILs	SSR
	McCartney et al. (2005)	3DL, 4BS, 4DS	D-H	SSR, EST (SNP)

(Continues)

Table 1-3. Summary of information related to QTL governing agronomic and quality traits in common wheat (continued)

Trait	Authors	Chromosomal Location of QTLs	Population	Marker
Lodging	Verma (2005)	1D, 2B, 4B, 4D, 6D, 7D	D-H	SSR
	Marza et al. (2006)	1B, 4AL, 5A	RILs	AFLP, SSR
	Huang et al. (2006)	4D, 5A, 6D	D-H	SSR
Kernels per spike	Börner et al (2002)	2DS, 4AL	RILs	RFLP, SSR
	Huang et al. (2004b)	1DL, 2AS, 3DS, 6AS, 6AL, 7AS, 7AL, 7DS	BC ₂ F ₁	SSR
	Marza et al. (2006)	1AL, 1B, 2BS, 2DL, 3BS, 4B, 6A, 7BS	RILs	AFLP, SSR
	Narasimhamoorthy et al. (2006)	3DS	BC ₂ F _{2:4}	SSR
	Kumar et al. (2007)	1AL, 1BL, 2AS, 2BS, 2DS, 2DL, 3BS, 3DL, 4BS, 7AS, 7AL	RILs	RFLP, SSR
	Li et al. (2007)	1DS, 2AS, 3BS, 6AL, 6BS	RILs	SSR, EST-SSR, ISSR, SRAP, TRAP
	Cuthbert et al. (2008)	1AS, 2DL, 3BS, 5AL, 7AL	D-H	SSR
	Wang et al.(2009)	1DL, 2AS, 2DS, 3A, 4D, 6DS	RILs	SSR
	Heidari et al (2011)	1A, 2B	D-H	AFLP, RAPD, SSR
	Bennett et al.(2012)	1A, 1B, 3A, 7 ^a	D-H	DArT
Mergoum et al. (2013)	5AS, 6BS	RILs	DArT, SSR	

(Continues)

Table 1-3. Summary of information related to QTL governing agronomic and quality traits in common wheat (continued)

Trait	Authors	Chromosomal Location of QTLs	Population	Marker
Spikes per m ⁻²	Huang et al (2003)	1BL, 2AL, 2DL, 3BS, 4DS, 5DL, 6DL, 7AS	BC ₂ F ₂	SSR
	Huang et al. (2004)	1BS, 7AS	BC ₂ F ₁	SSR
	Marza et al. (2006)	3BS	RILs	AFLP, SSR
	Kirigwi et al. (2007)	4AL	RILs	SSR, EST
	Li et al. (2007)	1AL, 1DS, 2AL, 2DL, 3BS, 3BL, 4BL, 7DL	RILs	SSR, ISSR,
	Cuthbert et al. (2008)	3BS, 5AS, 5AL, 5BS, 7DL	D-H	SSR
	Heidari et al (2011)	1A, 2D, 7 ^a	D-H	AFLP, RAPD, SSR
	Bennett et al (2012)	1DL, 2B, 2DS, 4BL, 6A	D-H	DArT
Quality Traits				
Thousand-Kernel-weight	Araki et al. (1999)	4AL-4AS	SbLi	RFLP
	Börner et al. (2002)	5A	RILs	RFLP, SSR
	Kato et al. (2000)	5AL	RILs	RFLP
	Groos et al. (2003)	1D, 2BS, 2DS, 3AL, 5BL, 6AL, 6DS, 7AS, 7DL	RILs	RFLP, SSR
	Huang et al. (2003)	2AS, 2DL, 4DS, 5BS, 7AS, 7BL, 7DS	BC ₂ F ₂	SSR
	Huang et al. (2004)	1BS, 1DL, 2AS, 2DL, 3AL, 3BS, 3BL, 3DS, 4BL, 6AS, 6AL, 7AS, 7AL, 7DS	BC ₂ F ₁	SSR

(Continues)

Table 1-3. Summary of information related to QTL governing agronomic and quality traits in common wheat (continued)

Trait	Authors	Chromosomal Location of QTLs	Population	Marker
Thousand-Kernel-weight	McCartney et al. (2005)	2AS, 3DL, 4AL, 4BL, 4DS, 6DL	D-H	SSR EST (SNP)
	Huang et al. (2006)	2BS, 2DS, 3BL, 4BL, 4DS, 6AL	D-H	SSR
	Kumar et al. (2006)	1AS, 2BS, 7AS	RILs	SSR, AFLP
	Li et al. (2007)	1DS, 3BL, 5DL, 6AL, 7DL	RILs	SSR, EST-SSR, ISSR, SRAP, TRAP
	Cuthbert et al. (2008)	2DL, 3BS, 5AS, 5AL, 7AS	D-H	SSR
	Wang et al. (2009)	1AS, 1BS, 2AS, 2DL, 3BS, 4AS, 4DS, 5AS, 6DL, 7DS	RIL	SSR
	Sun et al. (2009b)	1DS, 2AL, 5DS, 6AL	RILs	SSR, EST-SSR, ISSR, SRAP, TRAP
	Tsilo et al. (2010)	2AS, 5B, 6B, 7A	RILs	SSR, DArT
	Heidari et al (2011)	2B, 2D, 4B	D-H	AFLP, RAPD, SSR
	Simons et al. (2012)	1BS, 5BL, 6AS	RILs	SSR
	Bennett et al (2012)	1D, 2BS, 3D, 4A, 5B, 6A, 6B, 7AL, 7D	D-H	DArT
Grain protein content	Galande et al.(2001)	2B, 6B	RILs	ISSR
	Groos et al. (2003)	1A, 2AS, 3AL, 3BS, 4AS, 4DL, 5BL, 6AL, 7AS, 7DL	RILs	RFLP, SSR
	Prasad et al. (2003)	2AS, 2BL, 2DL, 3DS, 4AL, 6BS, 7AS, 7DS	RILs	SSR

(Continues)

Table 1-3. Summary of information related to QTL governing agronomic and quality traits in common wheat (continued)

Trait	Authors	Chromosomal Location of QTLs	Population	Marker
Grain protein content	Sourdille et al. (2003)	1BL, 6AS	D-H	RFLP, AFLP
	Kulwal et al. (2005)	1AS, 1BL, 1DL, 2AS, 2AL, 2BL, 2DS, 2DL, 3BS, 4AS, 5BL, 5DL, 6DL, 7AL, 7DS	RILs	RFLP, SSR
	Huang et al. (2006)	4DS, 7BL	D-H	SSR
	Kunert et al. (2007)	3AL, 4AL, 4BL, 5DL, 7BS, 7DS	BC ₂ F ₃	SSR
	Mann et al (2009)	1B, 3A	D-H	SSR
	Nelson et al (2006)	2A, 2D,	RILs	RFLP
	Raman et al. (2009)	4A	D-H	DArTs
	Sun et al. (2010)	3AS, 4B	RILs	SSR
	Tsilo et al. (2010)	2BS, 5A, 6D	RILs	SSR, DArT
	Zhao et al. (2010)	3A, 3B, 5D, 6DS	D-H	EST, ISSR, RFLP, SSR
	Conti et al (2011)	1BS, 2AL, 2BS, 3BS, 3BL, 4AL, 5AS, 5BL, 7AS, 7BL	RILs	SSR, SNP, RFLP, STS
	Li et al. (2012a)	1A, 1B, 2A, 2B, 2D, 3A, 4A, 4B, 4D, 5A, 5B, 5D, 6B, 7A, 7B, 7D	RILs	G-SSR, EST-SSR
	Li et al (2012b)	1AS, 2DL, 4BL, 5DL, 6AS, 6BL, 6D, 7B	BC ₅ , IL	SSR
Carter et al. (2012)	3BL	RILs	SSR, SNP	

(Continues)

Table 1-3. Summary of information related to QTL governing agronomic and quality traits in common wheat (continued)

Trait	Authors	Chromosomal Location of QTLs	Population	Marker
Kernel Volume Weight	Maphosa et al. (2013)	2B, 2D, 3A, 4A, 6A, 7A	D-H	DArT, SSR
	Campbell et al. (1999)	2B, 4A, 5A, 6A, 7A	RILs	RFLP
	McCartney et al. (2005)	1BS, 1DL, 2BL, 2DL, 3B, 3DL, 4DS, 5DL, 6BS, 7D	D-H	SSR EST(SNP)
	Narasimhamoorthy et al. (2006)	2DS	BC ₂ F _{2:4}	SSR
	Huang et al. (2006)	2DS, 4AL, 4DS, 5AL, 7AS	D-H	SSR
	Kunert et al. (2007)	3B, 4AL, 6BL, 7AS, 7BS	BC ₂ F ₃	SSR
	Sun et al. (2009)	2AL, 3BL, 4AL, 5DS, 6AL, 6BS, 7BL	RILs	SSR, EST-SSR, ISSR, SRAP, TRAP
	Sun et al. (2010)	1DL, 2DL, 4AS, 5AS, 5AL, 5BS, 6AS	RILs	SSR
Kernel Volume Weight	Bennett et al. (2012)	1B, 1D, 2A, 3A, 4A, 4D, 6A	D-H	DArT
	Simons et al. (2012)	1BS, 4BL, 5BL	RILs	SSR
	Carter et al. (2012)	5B	RILs	SSR, SNP
Flour Extraction	Campbell et al. (2001)	3S, 5AS, 5BS, 5DS	RILs	RFLP
	Kuchel et al. (2006)	1A, 2A, 6A	D-H	SSR, STS, Proteins
	Nelson et al. (2006)	4A	RILs	RFLP

(Continues)

Table 1-3. Summary of information related to QTL governing agronomic and quality traits in common wheat (continued)

Trait	Authors	Chromosomal Location of QTLs	Population	Marker
Flour Extraction	Raman et al. (2009)	7A	D-H	DArTs
	Carter et al. (2012)	3BL, 4DS, 6DL	RILs	SSR, SNP
	Simons et al. (2012)	1BL, 4BL	RILs	SSR
	Maphosa et al. (2013)	2D, 3A, 3D, 4A	D-H	DArT, SSR
Mixogram Pattern	Tsilo et al. (2011)	1B, 1D, 3B, 6D	RILs	SSR, DArT
Mixogram Midline peak time, min	Campbell et al. (2001)	1DL, 4AL, 7AS, 7DS	RILs	RFLP
	Tsilo et al. (2011)	1B1, 1D, 2A, 6D, 7D	RIL	SSR, DArT
	Li et al. (2012b)	2DL, 4A	BC5, IL	SSR
Mixogram Midline peak time, min	Simons et al. (2012)	1DL	RILs	SSR
	Mergoum et al. (2013)	2B, 7BS	RILs	DART, SSR
Mixogram Midline peak value, %	Tsilo et al. (2011)	1A, 1B, 1D, 6D	RILs	SSR, DArT
Mixogram Midline peak value, %	Li et al. (2012b)	1AL, 1BS, 1DS, 2B, 2DL, 3AL, 4BL, 5AS, 5B, 6AL, 7B	BC5, IL	SSR

(Continues)

Table 1-3. Summary of information related to QTL governing agronomic and quality traits in common wheat (continued)

Trait	Authors	Chromosomal Location of QTLs	Population	Marker
Mixogram Midline peak value, %	Simons et al. (2012)	1BS, 1DL, 5BL	RILs	SSR
Mixogram line peak width, %	Tsilo et al. (2011)	1A, 1B, 6D	RILs	SSR, DArT
	Li et al. (2012b)	1AS, 1BS, 1DS, 2B, 2DL, 3AL, 4BL, 5AS	BC ₅ , IL	SSR
	Simons et al. (2012)	1BS, 1DL	RILs	SSR
Mixogram line peak integral	Tsilo et al. (2011)	1B, 1D, 6D, 7D	RILs	SSR, DArT
	Simons et al. (2012)	1DL	RILs	SSR
Mixogram Mixing Tolerance	Campbell et al. 2001	1AL, 1BL	RILs	RFLP
	Li et al. (2012b)	1BS, 2DL, 4A, 5AS, 6AL	BC ₅ , IL	SSR
Mixogram Weakening slope	Li et al. (2012b)	4A, 6AL	BC ₅ , IL	SSR
Mixogram Mixing development time	Huang et al. (2006)	1B, 1DL, 3B	D-H	SSR
	McCartney et al. (2006)	1B, 4D	D-H	SSR

(Continues)

Table 1-3. Summary of information related to QTL governing agronomic and quality traits in common wheat (continued)

Trait	Authors	Chromosomal Location of QTLs	Population	Marker
Mixogram Peak Height	Huang et al. (2006)	1B, 1DL, 4DS	D-H	SSR
Mixogram Peak Height	McCartey et al. (2006)	4D	D-H	SSR
Mixogram Energy to peak	Huang et al. (2006)	1B, 1DL, 3B	D-H	SSR
Mixogram First minute slope	McCartney et al. (2006)	1B, 4D	D-H	SSR
	Huang et al. (2006)	1DL, 4DS	D-H	SSR
Mixogram Peak bandwidth	McCartey et al. (2006)	1B, 4D, 7B, 7D	D-H	SSR
	Huang et al. (2006)	1DL	D-H	SSR
Mixogram Slope after peak	McCartey et al. (2006)	2B, 4D, 7D	D-H	SSR
	Huang et al. (2006)	1DL, 4DS	D-H	SSR
Mixogram Total energy	McCartey et al. (2006)	1B, 4D	D-H	SSR
	Huang et al. (2006)	1B, 5DS	D-H	SSR
Mixogram Bandwidth energy	McCartey et al. (2006)	1B, 2B, 4D, 7D	D-H	SSR
	Huang et al. (2006)	1B, 5DS	D-H	SSR

(Continues)

Table 1-3. Summary of information related to QTL governing agronomic and quality traits in common wheat (continued)

Trait	Authors	Chromosomal Location of QTLs	Population	Marker
Mixogram Bandwidth energy	McCartey et al. (2006)	1B, 2A, 2B, 6A, 7D	D-H	SSR
Mixogram Midline time x=8 width	Li et al. (2012b)	1BS, 1DS, 2DL, 3AL, 4A, 4BL, 5AS, 5DL, 6AL	BC ₅ , IL	SSR
Midline time x=8 min value	Li et al. (2012b)	1AS, 1BS, 1DS, 2B, 3AL, 4A, 4BL, 5AS, 5B, 6AL	BC ₅ , IL	SSR
Mixogram Midline right of peak width	Li et al. (2012b)	1AL, 1BS, 2B, 2DL, 3AL, 4A, 4BL, 5AS, 6AL	BC ₅ , IL	SSR
Mixogram Midline right of peak value	Li et al. (2012b)	1AL, 1BS, 1DS, 2B, 2DL, 4BL, 5B, 6AL	BC ₅ , IL	SSR
Mixogram Midline left of peak width	Li et al. (2012b)	1AL, 1BS, 1DS, 2B, 3AL, 4BL, 5AS, 6AL	BC ₅ , IL	SSR
Mixogram Midline left of peak value	Li et al. (2012b)	1AL, 1BS, 1DS, 2B, 2DL, 4BL, 5AS, 6AL	BC ₅ , IL	SSR
Mixing Time	Mann et al. (2009)	1A, 1B, 1D	D-H	SSR

(Continues)

Table 1-3. Summary of information related to QTL governing agronomic and quality traits in common wheat (continued)

Trait	Authors	Chromosomal Location of QTLs	Population	Marker
Maximum band width	Mann et al. (2009)	1A, 1B, 4D, 5D, 7B	D-H	SSR
Resistance break down	Mann et al. (2009)	1D, 7B	D-H	SSR
Dough stability	Maphosa et al. (2013)	1B, 3B	D-H	DArT, SSR
Dough development time	Maphosa et al. (2013)	1B, 3B	D-H	DArT, SSR

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CHAPTER 2. GENOME-WIDE GENETIC DISSECTION OF SUPERNUMERARY SPIKELET AND RELATED TRAITS IN COMMON WHEAT (*TRITICUM AESTIVUM* L.)

2.1. Abstract

Branched spike or supernumerary spikelet (SS) is a naturally occurring variant in wheat and holds great potential for increasing the number of grains per spike, and ultimately, increasing wheat yield. However, detailed knowledge of the molecular basis of spike branching in common wheat is lacking. In the present study, a recombinant inbred line (RIL) population derived from the cross of an accession with SS and an elite line with non-SS was developed and evaluated over four to six environments for seven SS-related traits to identify the genetic basis of SS in wheat (*Triticum aestivum* L.). A framework linkage map was generated using 939 DArT markers. Composite interval mapping (CIM) identified a total of seven consistent QTL located on five chromosomes (2D, 5B, 6A, 6B and 7B), suggesting a polygenic inheritance of SS. The phenotypic variation explained (PVE) by individual QTL ranged from 3.3-37.3%. The QTL located on 2D (*QSS.ndsu-2D*) and 7B (*QSS.ndsu-7B.2*) have major effects (PVE>15%), while the remaining five QTL (*QSS.ndsu-5B*, *QSS.ndsu-6A*, *QSS.ndsu-6B.1*, *QSS.ndsu-6B.2*, *QSS.ndsu-7B.1*) have minor effects (PVE <15%). Comparison of the genomic locations of the QTL suggested that *QSS.ndsu-2D* was located in the same regions on 2DS where QTL for several traits have been reported. However, the remaining six QTL for SS are reported for the first time. Multiple interval mapping showed that all these six QTL are involved in epistatic interaction. The major genomic region controlling the SS related-traits may prove invaluable for wheat improvement and could also be the target for future studies aimed at cloning this gene.

2.2. Introduction

World food production is challenged due to loss of arable land, climate changes, volatile prices, biofuel demand, high meat demand, and poor commodity distribution (Godfray et al., 2010). An increase in food grain production is needed to feed the ever-increasing population, which is expected to reach 9 billion by 2050. Wheat (*Triticum aestivum* L.), one of the most important world food crops, will play a major role in ensuring world food security, (Rajaram, 2002). However, in the last few decades, only limited

breeding efforts were focused on enhancing the yield potential of this important crop (Reynolds et al., 2011).

Grain yield is a complex trait determined by multiple components such as spikes per unit area, grain weight, and grains per unit area (Hucl and Fowler, 1992; Ma et al., 2007). In turn, these components are impacted by the inflorescence architecture, spike meristem growth, and spike fertility (Sreenivasulu and Schnurbusch, 2012). Considering that conventional wheat cultivars bear one spikelet per rachis node and produce between 20 and 50 kernels per spike (Bonnett, 1966; Perkins, 1997), the implementation of exotic germplasm bearing more than one spikelet per rachis node has been suggested as a strategy to increase grain yield (Saluke and Asana, 1970; Rawson and Ruwali, 1972; Pennell and Halloran, 1984a, 1984b; Hucl and Fowler, 1992; Peng et al., 1998; Yang et al., 2005; Sun et al., 2009; Aliyeva and Aminov, 2011; Li et al., 2011; Sreenivasulu and Schnurbusch, 2012). These spike characteristics are collectively termed “branched spikes”, “Blé d’Osisris”, “Egyptian wheat”, “miracle spikes”, “mummy wheat”, “spike branching”, “wonder wheat”, or “Wunderweizen” (Percival, 1921; Sharman, 1944, 1967; Martinek and Bednár, 1998; Sreenivasulu and Schnurbusch, 2012). Branched spikes were originally identified in tetraploid wheat (*Triticum turgidum* L. var *durum* Desf.) (Percival, 1921; Sharman, 1944, 1967) and later in hexaploid wheat (Koric, 1973). Branched spikes have extra sessile spikelets at a rachis node, or additional spikelets extended on rachillas (Pennell and Halloran, 1983, 1984a, 1984b), and potentially could produce 150 kernels per spike (Percival, 1921). Pennell and Halloran (1983) later suggested the term supernumerary spikelets (SS) to include both types of spikelet organization.

Studies aimed at understanding the genetics of SS phenotype started almost a century ago (Percival, 1921). Earliest studies in tetraploid wheat observed that SS is controlled by a single recessive gene (Percival, 1921; Sharman, 1942, 1967). In hexaploid wheat, however, three genes designated as *Ramifera* (*Rm*), *Tetrastichon* (*Ts*) and *Normalizator* (*Nr*) were reported to control SS (Koric, 1973). The *Rm* and *Ts* genes work in a complementary fashion in the formation of the branched spike, while *Nr* is a dominant repressor of the branched phenotype. Therefore, the branched phenotype is possible when the inhibitor is silenced or absent. Other studies in hexaploid wheat also suggested that the branched spike phenotype was controlled by three genes (two genes working in a recessive or complementary fashion and a repressor) (Pennell and Halloran, 1983; Denčić, 1988). Pennell and Halloran (1983) also reported

the presence of at least one gene for SS in the conventional spike (or non-SS) variety “Phoenix”. This observation suggests that some of the high-yielding wheat cultivars in the world might have branched traits suppressed by one gene. However, a study in tetraploid wheat suggested that SS trait is inherited quantitatively with one major recessive gene and numerous minor genes (Klindworth et al., 1990a). Another recent study using line “51885” (a SS genotype derived from the cross of octoploid Triticale and common wheat) also found that the inheritance of SS trait is controlled by two dominant genes and probably a few minor genes (Sun et al., 2009). In contrast, another study in a vavilovii-branched line called “166-Schakheli” suggested that the branched spike trait is controlled by a single recessive gene (Aliyeva and Aminov, 2011).

Initial efforts to identify the chromosomal location of genes controlling SS trait were based on cytogenetic analyses (Millet, 1986, 1987; Klindworth et al., 1990b; Peng et al., 1998). In common wheat, Millet (1986, 1987) crossed the SS line “Noa” with the non-SS line “Mara” as well as with monosomic lines derived from “Mara.” The results showed that chromosome 2D of “Noa” carries a major recessive gene for the number of spikelets per spike, while chromosomes 5D and 7A have genes with minor effects for the spikelet number. Using a set of “Langdon” D-genome disomic substitution lines, Klindworth et al. (1990b) identified a major gene on chromosome 2A and one minor gene on chromosome 2B for SS in tetraploid wheat. Additional experiments with a “Langdon” 2A telosomic line located the major gene to the short arm of chromosome 2A. Interestingly, they observed that 2D monosomic addition lines had an inhibitory effect on the expression of SS (Klindworth et al., 1990b). Considering that D chromosomes come from “Chinese Spring” wheat, it was concluded that a strong inhibitor for SS is located on chromosome 2D in hexaploid wheat (Klindworth et al., 1990b). Later, Peng et al. (1998) reported the presence of major genes for SS on chromosomes 2D, 4A, 5A and a minor gene on 4B in the hexaploid wheat line “Yupi Branching” when it was crossed with monosomic lines derived from “Chinese Spring” wheat.

Although several progeny analysis and cytogenetic studies defined the inheritance of SS in wheat, only few studies reported genetic dissection of SS using molecular markers. Using multi row spikes (MRS) wheat (a type of branched spike phenotype), Dobrovolskaya et al. (2009) evaluated two F₂ populations and identified a major gene (*Mrs1*) on chromosome 2D linked to microsatellite locus *Xwmc453*. Subsequent analysis with chromosome deletion bin lines delimited the physical location of the

gene *Mrs1* to the distal half of chromosome 2DS (Dobrovolskaya et al., 2009). Another study using the Tibetan triple spikelet trait in wheat identified one QTL on the short arm of chromosome 2A associated with SSR markers, *Xgwm275* and *Xgwm122* (Li et al., 2011). The presence of a gene for SS trait on 2AS was further confirmed by another study which used three F₂ mapping populations of durum wheat to map a major recessive locus for the branched head (*bh*) phenotype on the short arm of chromosome arm 2A (Haque et al., 2012). This locus was associated with the SSR marker *Xgwm425*.

These molecular analyses of the SS phenotype focused on either a single chromosome or a single homeologous group. Yet, previous research also suggested that SS might be under polygenic control (Huang and Yen 1988; Klindworth et al., 1990a; Peng et al., 1998). Therefore, it becomes very important to dissect this trait at the whole genome level. The present study was aimed at identification of QTL influencing SS in common wheat at the whole genome level using a recombinant inbred line (RIL) population derived from an exotic line WCB617 and an elite line WCB414. The genotype WCB617 has spikes with SS phenotype which are similar to the spikes of *Triticum aestivum ramifera* (Koric, 1973) and “Miracle spikes” (Percival, 1921; Sharman, 1944, 1967); while, WCB414 is an elite white wheat (WW) line with non-SS phenotype. In the present study, the SS trait is segmented into seven component traits (or SS-related traits) in order to improve QTL detection power and to understand the role of the component traits in determining SS phenotype. To the best of our knowledge, this is the first detailed report on whole genome dissection of SS phenotype using molecular markers.

2.3. Material and Methods

2.3.1. Plant material

The present study was conducted using a population of 163 RILs derived from a cross between the hexaploid hard white wheat line WCB414 and the hexaploid hard red wheat line WCB617 (used as pollen donator). WCB414 was developed by the Hard White and Specialty Wheat breeding program at North Dakota State University (NDSU), Fargo, ND USA, and has conventional spikes with fusiform architecture, awned and glabrous glumes. WCB617 was identified and maintained by the NDSU wheat Germplasm Enhancement project as a source for the SS phenotype, and has awns, pubescence on the

glumes, and heterobranching behavior (variation in the penetrance of SS, in which a plant shows both branched and conventional spikes).

The RIL population was advanced to F₇ generation through single seed descent method. Later, the seeds of this population were increased and advanced to F₈ generation in greenhouse facilities. To ensure genetic purity, one spike derived from a main tiller of each RIL was collected in each season of field evaluations at Carrington (2009 and 2010) and sent to New Zealand for planting as spike-rows. The seeds harvested from each RIL in New Zealand were then planted in the next growing season in North Dakota, and phenotypic data was collected. The F_{7:9}, F_{10:11}, and F_{12:13} populations were assessed during the years 2009, 2010, and 2011.

Six hard red spring wheat (HRSW) and one white wheat (WW) cultivars were used as checks in this study. The HRSW cultivars were “Alsen” (PI 615543) (Frohberg et al., 2006), “Steel-ND” (PI 634981) (Mergoum et al., 2005), “Glenn” (PI 639273) (Mergoum et al., 2006), “Faller” (PI 648350) (Mergoum et al., 2008), “Barlow” (PI 658018) (Mergoum et al., 2011), and “Briggs” (PI 632970) (Devkota et al., 2007), while the WW cultivar was “Alpine” (Agripro® wheat variety, USA).

2.3.2. Field experiment

Field trials were conducted at two different locations (Prosper and Carrington) in North Dakota, USA over a period of three years (2009, 2010 and 2011). In total, the phenotypic data was recorded in six environments designated as I-VI (I=Prosper 2009, II= Carrington 2009, III= Prosper 2010, IV= Carrington 2010, V= Prosper 2011, and VI= Carrington 2011). Prosper is located in eastern North Dakota (46.9630°N, 97.0198° W) at an altitude 274 m and its soil are of the Bearden series. Carrington is located in central North Dakota (47.4500°N, 99.1239° W) at an altitude of 484 m and its soils are of the Heimdal-Emrick series. The average air temperatures during the growing seasons of 2009, 2010, and 2011 were 16.3°C, 18.7°C, 18.7°C respectively in Prosper and 15.7°C, 16.5°C, and 17.3°C, respectively in Carrington. While, the total rainfall during the growing seasons of 2009, 2010, and 2011 was 39.6 mm, 87.6 mm, and 106.3 mm, respectively in Prosper and 41 mm, 52.3 mm, and 113.1 mm, respectively in Carrington (NDAWN, 2012).

In each environment, the RILs, parents, and seven checks were planted in a 13 × 13 partially balanced square lattice design with two replicates. Each genotype was planted in plots comprised of

seven rows of 2.44 m length; with a row to row distance of 12.7 cm. Sowing rate for every genotype was 113 kg ha⁻¹.

2.3.3. Phenotypic data

In the field, a scale of 0 to 4 was used to determine the ratios of spikes with SS and conventional type (non-SS) in each experimental unit. This scale provides a measurement at the level of penetrance of SS (PSS) or heterobranching behavior. When all the spikes in the experimental unit had SS phenotype, a score of 4 was assigned. A ratio of 3:1 (SS: conventional spike type) corresponded to a score of 3, a ratio 1:1 was scored as 2, and a ratio 1:3 (SS: conventional spike type) was scored as 1. If all the spikes for any genotype were conventional type, a score of 0 was assigned.

Four spikes were taken randomly from the primary tillers from each experimental unit to measure other SS components (SS-related traits). The spikes of the prevalent phenotype were collected for the genotypes with PSS scores of 3 and 1. When two phenotypes (branched and non-branched) were present in equal proportions (ratio 1:1, score 2) in any particular replicate of any environment, the prevalent phenotype for that particular genotype was decided based on the phenotype of same genotype in other replicates and environments. The other SS-related traits for which data was recorded include i) number of spikelets per spike (Sk), in which immature spikelets at the spike base were excluded; ii) spike density (SD) (spikelets cm⁻¹) measured as the ratio between the number of spikelets and the spike length in centimeters, measured from the base of the rachis to the top of the uppermost spikelet, excluding awns; iii) number of spikelets per node (SkNd), in which conventional spikes were always given a value of 1, while in branched spikes the information for each spike was collected from the mean of five nodes randomly chosen; iv) number of nodes per spike with supernumerary spikelets (NdSS), in which conventional spikes were always given a value of 0; v) number of nodes with extended rachillas (NdR), in which conventional spikes were always given a value of 0; and vi) number of nodes per spike with non-SS (NdNonSS). In conventional spikes, NdNonSS was equivalent to the number of spikelets per spike excluding immature spikelets at the spike base, while in spikes with SS, NdNonSS was equivalent to the number of nodes bearing one spikelet excluding immature spikelets at the spike base. For each trait, the mean from the four spikes was calculated. In 2011, PSS assessment was performed in both locations, but it was not possible to collect other spike measurements because Carrington location was severely

affected by hail, and the Prosper location was affected by flood. Consequently, for all traits except PSS, data was recorded in four environments designated as I (Prosper 2009), II (Carrington 2009), III (Prosper 2010) and IV (Carrington 2010).

2.3.4. Statistical analysis

Data from all traits were subjected to analyses of variance (ANOVA) for a lattice design using the MIXED procedure of the Statistical Analysis System (SAS, 2004). The RILs, parental genotypes, and checks were considered fixed effects, whereas environments and blocks were considered as random effects. ANOVA was estimated for each environment separately and combined over locations to estimate genotype \times environment interaction. A F_{\max} ratio less than 10-fold were tested to combine analysis of variance (Tabachnik and Fidell, 2001). The mean separation tests were conducted using an F-protected least significant difference (LSD) value. *F*-tests were considered significant at $p < 0.05$.

The MIXED procedure of SAS/STAT was used to calculate broad-sense heritability for each trait on plot basis (Holland et al. 2003) excluding the means of parents and checks. All the sources of variation were used as random component. $h_B^2 = \sigma_g^2 / [\sigma_g^2 + (\sigma_{ge}^2) + (\sigma_e^2)]$, where σ_g^2 is the genotype variance, σ_{ge}^2 is the G \times E interaction variance and σ_e^2 is the error variance. All the variances were directly obtained from the output of covariance parameter estimate obtained from the MIXED procedure.

2.3.5. Molecular marker analysis, map construction, QTL identification, and epistasis analysis

Lyophilized young leaves were used to isolate genomic DNA for each genotype at F_{12:13} using DNeay® Plant Mini Kit (Quiagen, cat. no. 69106). From the 163 RILs considered in this study, DNA was isolated from 159 RILs, which were subsequently used for map construction and QTL mapping. DNA quality was assessed through visual observation on 0.8% agarose gel. DNA concentrations were measured with a NanoDrop 1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA), and dilutions from each genotype were prepared as per Triticarte Pty. Ltd (Camberra, Australia; <http://www.triticarte.com.au>) guidelines. The DNA belonging to 159 RILs and two parental genotypes was sent to Triticarte Pty. Ltd (Camberra, Australia; <http://www.triticarte.com.au>) to conduct Diversity Array Technology (DArT) analysis (Akbari et al. 2006). An expanded version of the WHEAT 2.6 DArT array with

increased genomic representation of the D-genome was used (Wenzl et al., 2010). Markers showing polymorphism between parental genotypes were used for construction of a genetic map.

The genetic map was constructed using a combination of MapMaker 3.0 (Lander et al., 1987) and CarthaGène V.1.2.3R (de Givry et al., 2005) software with a minimum LOD score of 3.0 and maximum recombination frequency of 50% for each program. Public genetic maps available at GrainGenes 2.0 (<http://wheat.pw.usda.gov/GG2/index.shtml>) were used in the selection of DArT anchor markers. MapMaker 3.0 was used to assign markers to linkage groups generated with anchor markers, while CarthaGène V.1.2.3R was used to order the markers in each linkage group separately. The robustness of the maps of each linkage group was tested with the commands Flips and Annealing of CarthaGène V.1.2.3R. Final genetic distances were obtained using the Kosambi's mapping function (Kosambi, 1944). The final maps were compared with the DArT consensus maps (Huang et al., 2012) using the program Autograph (Derrien et al., 2007; <http://autograph.genouest.org/>) to check the accuracy of the marker order.

Composite interval mapping (CIM) was conducted to identify main-effect QTL for each trait in each environment as well across environments (AE) using QTL Cartographer V2.5_011 (Wang et al., 2012). In QTL Cartographer, model 6 (standard model), forward regression, five control markers (cofactors), windows size of 10 cM, and walk speed of 1 cM was used. A minimum LOD score of 2.5 was used to declare a putative QTL. Permutation test with 500 permutations was used to determine critical LOD threshold (experimental wide significance of 0.05). QTL explaining at least 15% of phenotypic variation (PV) in one environment were declared as major QTL. Confidence intervals (CI) were calculated using ± 2 LOD (from the peak) method. QTL with overlapping CIs were considered as one QTL. When the same QTL was detected for more than one trait, the region overlapping across traits (ROAT) was determined. To calculate a ROAT, the genetic regions covered by the common QTL in each trait were aligned to determine shared (overlapping) regions. A ROAT was determined by the confidence intervals of the shared regions among traits. The QTL that were detected in at least three environments were called consistent QTL and were the only QTL considered in this study. However, when a QTL was detected for more than one trait, the QTL was reported in the present study even if it was consistent (present in minimum three environments) for at least one trait. Multiple interval mapping (MIM), using

default setting in Cartographer V2.5_011 (Wang et al., 2012), was also conducted to identify epistatic interaction between the QTL detected by CIM. Only those epistatic interactions which have $r^2 > 5\%$ were reported in for this study. Graphical genotypes were prepared using the software GGT (van Berloo 2008). The program MapChart 2.2 (Voorrips, 2002) was used to draw the linkage groups and QTL.

2.4. Results

2.4.1. Phenotypic analyses

The phenotypic data clearly showed segregation among the RILs for the presence/absence of spikes with SS. However, the prevalent spike type in the RIL population was conventional (non-SS). In branched RILs, SS were either observed as sessile spikelets at a rachis node or additional spikelets on extended rachillas or a combination of both (Fig. 2-1). The WCB617parent and the RILs with prevalence of the branched phenotype showed both classes of SS. The exception was the branched RIL 1021, which had only sessile SS (data not shown) in all the environments.

The heterobranching behavior (occurrence of branched spikes and conventional spikes on a plant), measured as PSS, was observed in most of the RIL with presence of spike bearing SS. Combined ANOVA analysis showed that a total of 20 RILs had an estimate mean for PSS equal or greater than 1 (data not shown). For four RILs, the PSS score ranged from 1 to 2, demonstrating the predominance of conventional spikes in these genotypes. While, for 16 RILs the PSS score ranged from 2.5 to 4, showing the prevalence of the branched phenotype in these genotypes. RIL 1070 was the only line with an average score of 4, indicating the absence of heterobranching behavior in all the environments. A total of 31 RILs had a PSS score between 0.01 and 0.99 indicating the presence of at least one replicate with some branched spikes. While a total of 112 RIL did not showed spikes with SS in any of the environments.

The estimated means and ranges of all traits for the RILs, parents, and checks in all environments are presented in Appendix Table A1. In all environments as well as in the combined analysis of the environments, the traits Sk, SD, and NdNoSS showed transgressive segregation in both directions of the parental means. While, in all the environments as well as in the combined analysis of the environments, PSS, SkNd, and NdR had transgressive segregation in the direction to the parent with SS

(WCB617). In all environments, NdSS had transgressive segregation in direction to the parent with SS, but did not show transgressive segregation in the combined analysis of the environments.

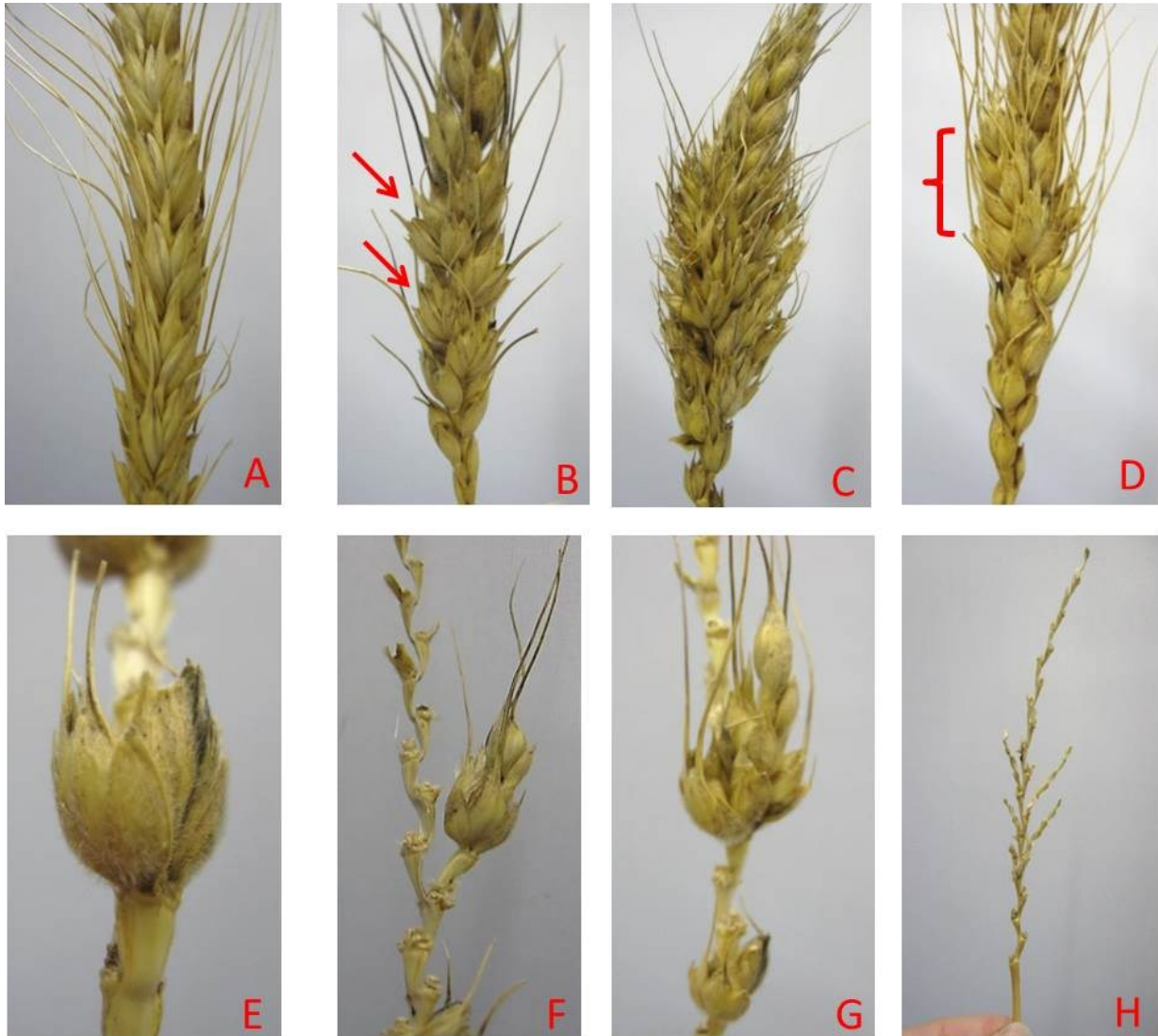


Fig. 2-1. Segregation in spike morphology in a spring wheat RIL population derived from the cross of an elite line (WCB414) with non-supernumerary spikelets (SS) and a PI with SS (WCB617). A. Conventional spike with non-SS, B. spike with sessile SS, C. spike with high expression of SS, D. spike with SS observed on an extended rachilla, E. two sessile spikelets attached to one node, F. spike with additional spikelets on extended rachilla (most of the spikelets were removed to get better detail), G. spike in whose node are observed sessile SS and SS extended on a rachilla, H. rachis and extended rachillas of a spike with high number of SS.

Coefficients of variation and lattice efficiency in each environment were calculated using the ANOVA results (Appendix Table A2). The error variances among the environments were homogenous for

all the traits except NdSS (Appendix Table A3). Combined ANOVA with six environments was conducted for PSS; with four environments for Sk, SD, SkNd, NdR, and NdNoSS; and with three environments for NdSS (Table 1). Combined ANOVA analysis showed that all traits had significant genotype × environment interaction. For all traits, the sum of squares calculated from the combined ANOVA showed that genotypes (RILs, parents and checks) are the main source of variation (data not shown). For all traits, broad sense heritability ranged from 0.69 to 0.86 (Table 2-1). Correlations between the SS-related traits are presented in Appendix Table A4.

Table 2-1. Mean squares, coefficient of variation and heritabilities for supernumerary-spikelets-related traits of parents, RILs and checks evaluated in a maximum of six environments

Trait [†]	NECAV [‡]	Mean Squares				CV(%) [¶]	H [#]	SE ^{††}
		Genotype	Environment	G x E	Error			
PSS	6	11.30**	0.75	0.24**	0.07	59.58	0.86	0.01
Sk	4	439.02**	288.25	21.56**	7.78	14.00	0.79	0.02
SD	4	3.79**	0.73	0.19**	0.08	13.41	0.79	0.02
SkNd	4	2.23**	0.22	0.12**	0.05	18.69	0.77	0.02
NdSS [§]	3	38.88**	4.23	0.98**	0.49	77.59	0.90	0.01
NdR	4	6.79**	1.83	0.53**	0.23	163.62	0.69	0.03
NdNoSS	4	60.05**	65.63	2.90**	1.38	7.09	0.78	0.02

* Significance at P < 0.05 probability level

** Significance at P < 0.01 probability level.

[†]PSS level of penetrance of supernumerary spikelets (scale from 0 to 4); Sk, number of spikelets (spikelets spike-1); SD spike density (Sk spike-length-1); SkNd spikelets per node (spikelet node-1); NdSS number of nodes with supernumerary spikelets per spike; NdR number of nodes with extended rachillas (extended rachillas spike-1); NdNoSS number of nodes with no supernumerary spikelets per spike.

[‡]Number Environments in combined analysis of variance.

[§]The four environments were not homogeneous in NdSS. Combined analysis of variance for NdSS was performed with the three more homogenous environments (Carr09, Carr10, and Pros10).

[¶]Coefficient of variation

[#]Broad sense heritability on plot-mean basis calculated in the RILs.

^{††}Standard error for heritability.

Comparison of top-ranked genotypes in each environment showed that few lines with SS were consistent in the level of expression of Sk, SD, SkNd, NdSS, and NdR (data not shown). For SK, RILs 1070, 1097, and 1099 were always ranked in the 10 genotypes with highest SK in each of the four environments tested. For SD, RILs 1017, 1053, 1068, 1070, 1097 and 1099 were consistently ranked in the top 10 genotypes in each of the four environments. For SkNd, RILs 1053, 1070, 1099 and 1151 were always ranked among the top 10 genotypes in each environment. For NdSS, RILs 1053, 1070, 1097, and

1134 were consistently ranked in the top 10 genotypes in each of the four environments. Finally, for NdR, RILs 1053, 1070, 1097, 1099, and 1151 were always ranked in the top 10 genotypes in each environment. The distribution of the RIL distribution across environments for the seven SS-related traits is showed in the Appendix Fig. A1.

2.4.2. Map construction

DArT analysis resulted in the identification of 1,004 markers polymorphic between parental genotypes. A total of 27 highly distorted markers were removed, and the remaining 977 markers were used for the construction of the framework genetic map. At a minimum LOD score of 3, a total 939 markers were assigned to 38 linkage groups. Thirty-eight markers could not be assigned to any linkage group. Comparison with a consensus map (Huang et al. 2012) showed that these 38 linkage groups represent 20 chromosomes of wheat. No linkage group was associated with chromosome 4D. Five chromosomes (1B, 3A, 3B, 7A, 7B) had three groups each; eight chromosomes (1A, 2B, 3D, 4B, 5A, 6A, 6B, 7D) showed 2 groups each, while seven chromosomes (1D, 2A, 2D, 4A, 5B, 5D, 6D) showed a single group. A total of 14 groups were assigned to the A-genome, 16 groups were assigned to the B-genome, while 8 groups were assigned to the D-genome.

The 939 markers mapped in the present study represented 671 unique loci; 268 markers co-segregated with other loci. The B-genome had the highest number of mapped loci (354 markers representing 285 loci), followed by A-genome (292 markers representing 222 loci) and D-genome (293 markers representing 164 loci). The number of markers on individual linkage groups ranged from three (3A.1, 5A.1 and 5A.2) to 97 (2D), while for individual chromosomes, it ranged from four (chromosome 5D) to 97 (chromosome 2D).

The total genetic distance covered by these 939 markers (671 loci) was 3,114.2 cM, and the average distance between any two marker loci was 4.6 cM. The B genome chromosomes covered a total length of 1,530.9 cM with an average distance of 5.4 cM between two loci. The A genome had a total map length of 1145.9 cM with an average distance of 5.2 cM between two loci. The D genome covered a total map length of 437.4 cM with an average density of 2.7 cM between two loci. Individually, chromosome 5B markers have the maximum coverage (309.1 cM), while chromosome 5D markers had minimum coverage

(3.7 cM). Marker order was generally consistent with the DArT consensus map (Huang et al., 2012) with only few exceptions.

2.4.3. QTL analysis

Composite interval mapping was used to identify QTL associated with supernumerary spikelet and related traits. The trait-associated QTL, as well as their flanking markers, confidence intervals and phenotypic variation (r^2) explained by each QTL are summarized in the Table 2. A total of 7 consistent QTL located on five chromosomes (2D, 5B, 6A, 6B and 7B) were identified. Chromosomes 2D (*QSS.ndsu-2D*), 5B (*QSS.ndsu-5B*) and 6A (*QSS.ndsu-6A*) had one QTL each, while chromosomes 6B (*QSS.ndsu-6B.1* and *QSS.ndsu-6B.2*) and 7B (*QSS.ndsu-7B.1* and *QSS.ndsu-7B.2*) carried two QTL each (Fig. 2).

Table 2-2.. QTL identified for supernumerary-spikelet-related traits in WCB414 × WCB617 RIL population of hexaploid wheat

QTL/Trait [†]	Environment [‡]	Flanking Markers	Pos. (cM)	Ci [§] (cM)	LOD	[¶] Thresh.	a [#]	R ² (%)
<i>QSS.ndsu-2D</i>								
PSS	I, II, III, IV, V, VI, AE	wPt-8134-wPt-667266	33.4-50.6	27.2-51.3	8.4-14.9	10.8-63.1	-0.4_-0.6	13.9-24.4
Sk	I, II, III, IV, AE	wPt-3677-wPt-667266	39.7-50.6	32.9-51.1	10.2-15.3	3.5-30.8	-3.7_-4.9	19.0-25.2
SD	I, II, III, IV, AE	wPt-741029-wPt-667266	39.6-50.6	29.5-51.5	8.6-12.9	3.3-30.1	-0.3_-0.4	13.8-21.8
NdR	I, II, III, IV, AE	wPt-3677-wPt-667266	39.7-50.6	28.4-51.3	6.2-9.7	3.4-46.9	-0.4_-0.5	10.9-18.6
SkNd	I, II, III, IV, AE	wPt-5574-wPt-667266	41.5-50.6	27.6-51.5	5.8-10.8	3.1-43.3	-0.2_-0.3	11.5-18.3
NdSS	I, II, III, IV, AE	wPt-5574-wPt-667785	41.5	27.2-51.3	7.2-9.4	71.6-93.5	-1.1_-1.3	13.2-15.5
				ROAT ^{††}	32.9-51.1			
<i>QSS.ndsu-5B</i>								
PSS	I, II, III, IV, VI, AE	wPt-1348-wPt-744851	278.9-292.6	265.6-308.3	2.9-4.5	10.8-63.1	-0.2_-0.4	4.1-9.1
Sk	III, AE	wPt-3995-wPt-3569	291.1	270.1-307.1	3.4-4.4	3.5-30.8	-1.7_-2.7	4.3-6.7
SD	III	wPt-3995-wPt-3569	291.1	275-307.9	4.9	30.1	-0.3	7.9
NdR	III	wPt-3995-wPt-3569	290.1	278.6-307.5	5.3	33.63	-0.4	9.4
SkNd	III, IV, AE	wPt-3995-wPt-744851	289.1-303.6	270.1-307.9	2.9-5.4	25.6-43.3	-0.1_-0.2	4.8-8.8
NdNoSS	II, III, IV, AE	wPt-3569-wPt-744851	292.6-307.6	265.4-307.6	2.6-3.9	3.0-3.3	0.7-0.9	5.4-9.2
NdSS	I, II, III, IV, AE	wPt-3995-wPt-744851	291.1-292.6	267.8-308.0	3.1-4.3	71.6-93.5	-0.6_-0.8	4.8-6.7
				ROAT ^{††}	278.6-307.1			
<i>QSS.ndsu-6A</i>								
PSS	I, II, III, IV, V, VI, AE	wPt-671561-wPt-733976	43.6-46.7	32.4-52.9	5.1-7.4	10.8-63.1	-0.3_-0.4	7.3-9.6
Sk	I, III, IV, AE	wPt-671561-wPt-0562	43.6	32.3-53.8	3.2-5.9	3.5-30.8	-1.9_-3.0	5.1-8.6
SD	I, III, IV, AE	wPt-671561-wPt-732760	43.6-61.8	34.5-75.5	3.7-5.1	3.3-30.1	-0.2_-0.3	5.5-7.7
NdR	I, III, IV, AE	wPt-671561-wPt-732760	43.6-61.8	33.3-77.9	3.0-4.8	33.6-46.9	-0.3_-0.4	5.4-8.0
SkNd	I, III, IV, AE	wPt-671561-wPt-9679	43.6-53.8	32.3-72.3	4.1-6.1	25.6-43.3	-0.2_-0.2	6.9-9.6
NdNoSS	I, III, IV, AE	wPt-9687-666574	20.8-60.1	7.9-69.8	4.5-6.5	3.1-3.3	1.1-1.3	10.1-13.5
NdSS	I, II, III, IV, AE	wPt-671561-wPt-0562	43.6	33.3-53.9	3.1-5.5	71.6-93.5	-0.6_-0.9	5.4-8.5
				ROAT ^{††}	34.5-52.9			
<i>QSS.ndsu-6B.1</i>								
PSS	II, III, V, AE	wPt-6116-wPt-6878	5.5-21.8	0-26.4	2.7-3.3	30.6-63.1	-0.2_-0.3	3.3-5.6
NdNoSS	IV	wPt-9256-wPt-0406	12.2	3.3-26.1	3.1	3.1	0.7	6.0
NdSS	III, IV, AE	wPt-1264-wPt-3207	18.8-27.0	0.4-31.7	2.6-3.0	71.6-89.0	-0.5_-0.6	3.6-4.5
				ROAT ^{††}	3.3-26.1			

(Continues)

Table 2-2. QTL identified for supernumerary-spikelet-related traits in WCB414 × WCB617 RIL population of hexaploid wheat (Continued)

QTL/Trait [†]	Environment [‡]	Flanking Markers	Position (cM)	CI [§] (cM)	LOD	¶Thresh	a [#]	R ² (%)
<i>QSS.ndsu-6B.2</i>								
PSS	I, II, III, IV, V, VI, AE	wPt-3284-wPt-3581	208.9-210.9	199.1-220.4	4.4-7.9	10.8-63.1	0.3-0.4	8.0-10.6
Sk	I, III, IV, AE	wPt-743099-wPt-3581	208.6-208.9	196.8-220.9	3.3-6.1	3.5-30.8	1.8-3.26	4.6-9.04
SD	I, III, IV, AE	wPt-743099-wPt-3581	207.6-208.9	195.8-220.1	2.9-4.8	3.3-30.1	0.2-0.27	5.0-8.1
NdR	I, III, AE	wPt-2564-wPt-3581	185.5-208.9	172.2-220.9	3.2-5.3	33.6-46.9	0.3-0.43	5.8-9.2
SkNd	I, III, IV, AE	wPt-743099-wPt-3581	207.6-208.9	198.1-219.3	4.5-6.6	25.6-43.3	0.2-0.24	7.9-11.1
NdNoSS	III, IV, AE	wPt-743099-wPt-3581	205.6-212.9	196.1-221.2	4.1-5.6	3.1-3.3	-1.0_-1.4	9.9-14.8
NdSS	I, II, III, IV, AE	wPt-743099-wPt-3284	206.6-208.6	195.6-221.2	3.1-6.2	71.6-93.5	0.7-1.07	6.5-11.4
				ROAT ^{††}	199.1-219.3			
<i>QSS.ndsu-7B.1</i>								
PSS	I, II, III, IV, V, VI, AE	wPt-0194-wPt-8615	97.7	84.6-111.5	2.8-4.6	10.8-63.1	0.2-0.3	3.9-5.8
SK	I, III, IV, AE	wPt-0194-wPt-8615	97.7	82.1-118.0	2.8-4.0	3.5-30.8	1.6-2.0	4.0-4.6
SD	I, III, IV, AE	wPt-0194-wPt-665428	97.7-115.0	93.4-123.7	2.6-5.5	3.3-30.1	0.2-0.2	4.8-8.1
NdR	IV, AE	wPt-0194-wPt-8615	97.7	84.9-120.9	2.7-3.2	42.1-46.9	0.2-0.2	4.4-4.8
SkNd	I, III, IV, AE	wPt-0194-wPt-8615	97.7	82.6-111.5	2.5-4.3	25.6-43.3	0.1-0.2	3.6-6.6
NdNoSS	III, IV, AE	wPt-0194-wPt-2407	97.7-101.3	81.2-115.4	2.6-2.9	3.1-3.3	-0.6_-0.8	4.6-5.9
NdSS	I, II, III, IV, AE	wPt-0194-wPt-8615	97.7	82.1-118.7	2.8-4.4	71.6-93.5	0.6-0.7	4.4-6.9
				ROAT ^{††}	93.4-111.5			
<i>QSS.ndsu-7B.2</i>								
PSS	III, IV, VI	wPt-3723-wPt-4258	72.6-73.6	59.8-78.9	2.9-4.7	30.6-41.3	-0.5_-1.3	10.4-25.3
SK	AE	wPt-3723-wPt-4258	74.6	64.4-79	3.2	30.8	-3.9	12.1
SD	IV	wPt-3723-wPt-4258	73.6	72.3-74.8	9.9	3.3	-1.1	33.5
SkNd	I, IV, AE	wPt-3723-wPt-4258	73.6-75.6	62.3-78.8	2.6-20.7	25.6-41.2	-0.3_-0.9	10.9-37.3
NdSS	III, AE	wPt-3723-wPt-4258	72.6	68.7-75.8	10.0-15.1	83.1-89.0	-4.0_-4.3	32.4-34.8
				ROAT ^{††}	72.3-74.8			

[†]Traits were defined in table 1[‡]PSS was studied in six environments; the other traits were studied in four environments[§]CI Confidence Interval[¶]Thresh calculated by permutation test.[#]Additive Effect^{††}ROAT region overlapping across traits

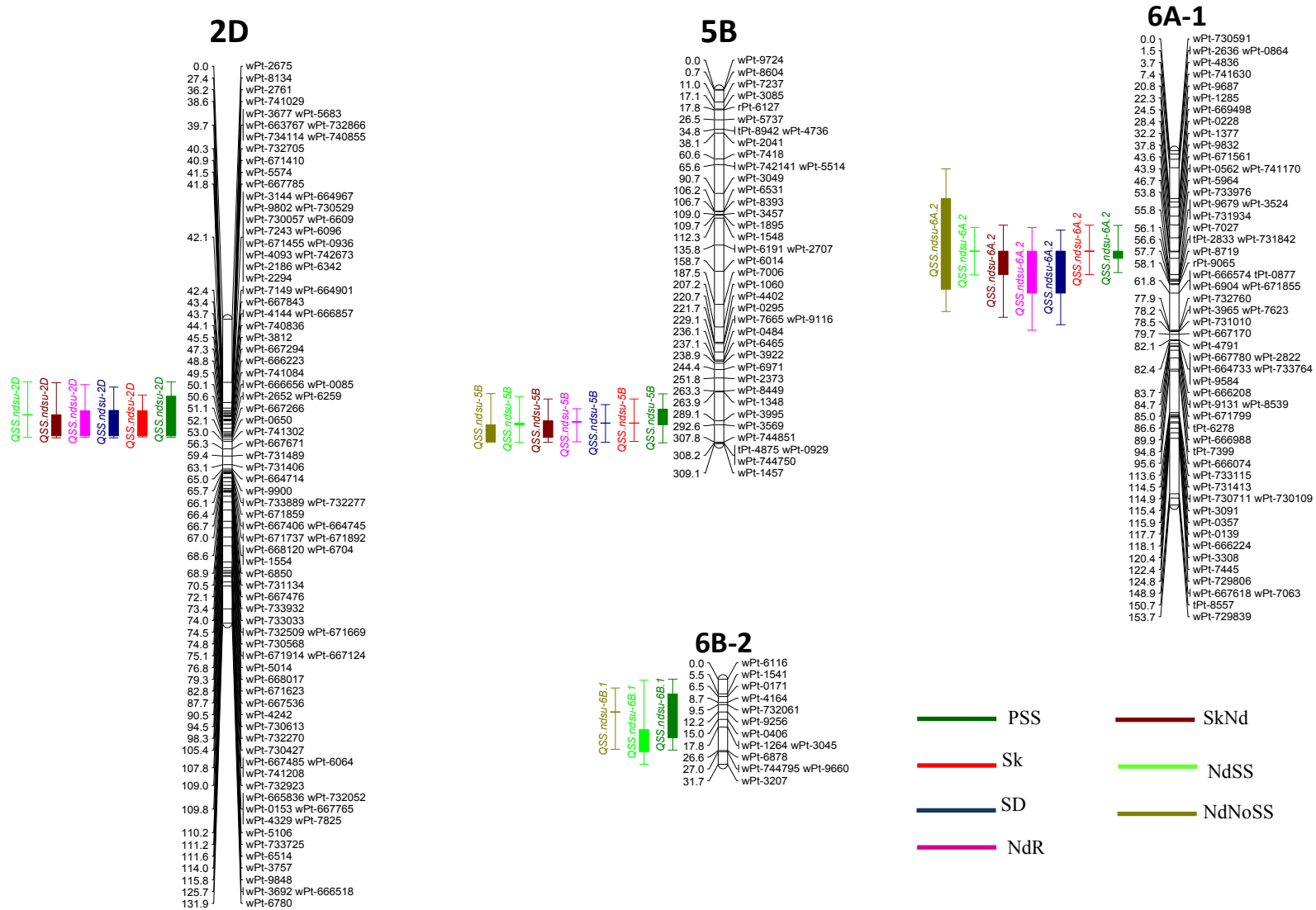


Fig. 2-2. Genetic linkage maps bearing stable QTL associated to seven supernumerary-spikelet-related traits in a RIL population of 159 individual derived from the cross between the elite line WCB414 and the PI with supernumerary spikelets (SS) WCB617

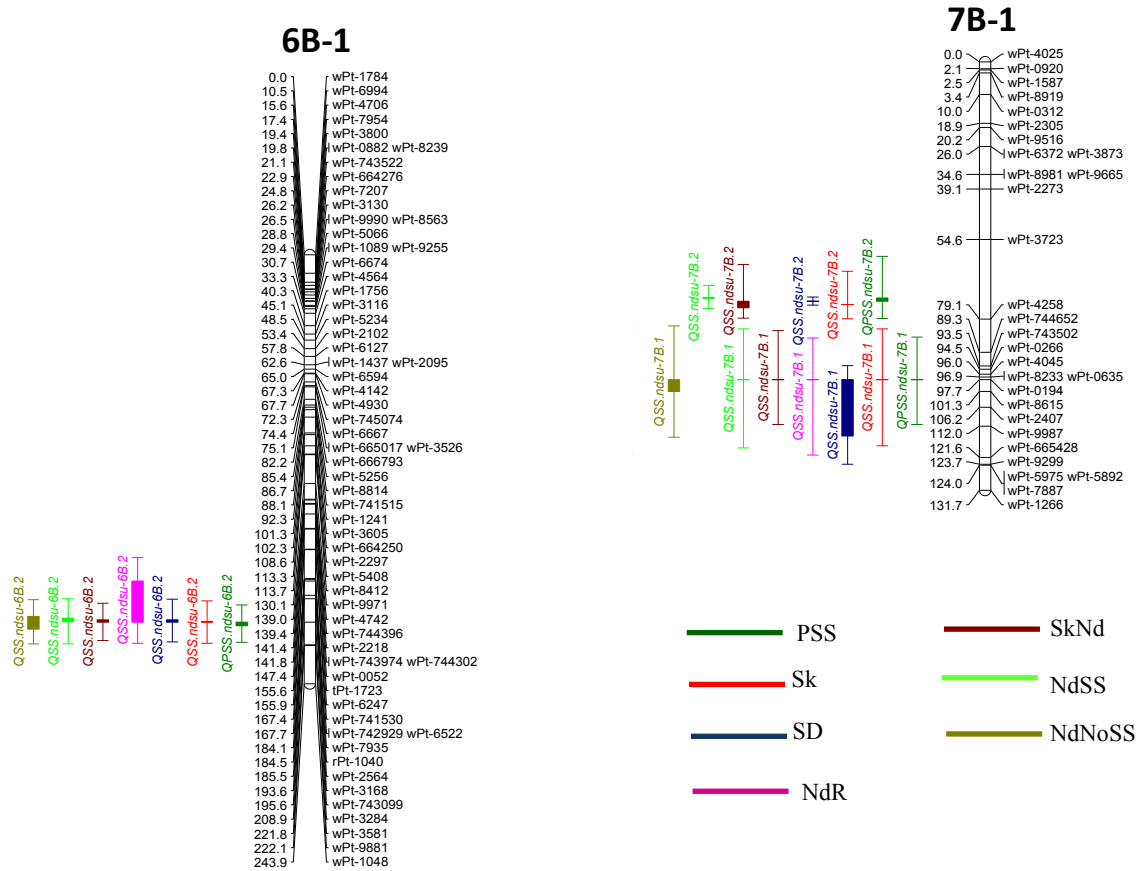


Fig. 2-2. Genetic linkage maps bearing stable QTL associated to seven supernumerary-spikelet-related traits in a RIL population of 159 individual derived from the cross between the elite line WCB414 and the PI with supernumerary spikelets (SS) WCB617 (Continued)

All seven QTL were associated with PSS and NdSS However, only six QTL were associated with Sk, SD and SkNd (except *QSS.ndsu-6B.1*), while five QTL each were associated with NdR (except *QSS.ndsu-6B.1* and *QSS.ndsu-7B.2*) and NdNoSS (except *QSS.ndsu-2D* and *QSS.ndsu-7B.2*). The phenotypic variation explained (PVE) by individual QTL in different environments ranged from 3.3-25.3% for PSS, 4.0-25.2% for Sk, 4.6-14.8% for NdNoSS, 4.4-18.6% for NdR, 3.6-34.8% for NdSS, 4.8-33.5% for SD and 3.6-37.3% for SkNd. The maximum PVE explained by all the QTL in individual environment was 77.39, 43.27, 37.53, 82.41, 59.69, 73.86 and 81.78 for PSS, NdNoSS, NdR, NdSS, NSk, SD, and SkNd, respectively.

Among the seven QTL, two QTL (*QSS.ndsu-2D* and *QSS.ndsu-7B.2*) have major effect (PVE> 15%), while five (*QSS.ndsu-5B*, *QSS.ndsu-6A*, *QSS.ndsu-6B.1*, *QSS.ndsu-6B.2*, *QSS.ndsu-7B.1*) have minor effect (PVE<15%) on phenotypic variation. The QTL on chromosome 2D (*QSS.ndsu-2D*) was

associated with all the traits except NdNoSS in all environments. This QTL explained up to 25.2% of the phenotypic variation. Another major QTL was identified on chromosome 7B (*QSS.ndsu-7B.2*) and was associated with five traits (PSS, Sk, SD, SkNd, NdSS). This QTL contributed up to 37.3% of the phenotypic variation for the associated traits.

Additive effects with a negative symbol indicate that alleles from the parent with SS (WCB617) are responsible for increased trait values, while the additive effects with a positive value indicate that the alleles from WCB414 are contributing towards increased trait values (Table 2-2). In the present study, both parents contributed alleles for increased trait values (Table 2-2). For traits with high phenotypic values in branched lines (PSS, Sk, SD, NdR, SkNd and NdSS), alleles from WCB617 contributed to increased trait values at *QSS.ndsu-2D*, *QSS.ndsu-5B*, *QSS.ndsu-6A*, *QSS.ndsu-6B.1*, and *QSS.ndsu-7B.2* loci, while alleles from WCB414 were responsible for increased trait values at *QSS.ndsu-6B.2* and *QSS.ndsu-7B.1* loci. For NdNoSS, a trait whose expression is higher in conventional spikes, alleles from WCB617 contributed to increased trait values at *QSS.ndsu-6B.2* and *QSS.ndsu-7B.1* loci, while alleles from WCB414 increase the trait values of NdNoSS at *QSS.ndsu-5B*, *QSS.ndsu-6A*, and *QSS.ndsu-6B.1*.

MIM showed that *QSS.ndsu-2D* had significant additive epistatic interactions with all other QTL (Table 2-3). In addition, epistatic interactions were also observed between *QSS.ndsu-5B* and *QSS.ndsu-6B.2*, *QSS.ndsu-6A* and *QSS.ndsu-6B.2*, *QSS.ndsu-6A* and *QSS.ndsu-7B.1*, and *QSS.ndsu-7B.1* and *QSS.ndsu-7B.2* for several SS related traits (for details, see Table 2-3). All of the epistatic interactions, except between *QSS.ndsu-5B* and *QSS.ndsu-6B.2*, and *QSS.ndsu-6A* and *QSS.ndsu-7B.1*, were detected for at least two SS-related multiple traits. These interactions also showed a differential phenotypic response (variation in R^2) in different environments (Table 2-3). The maximum value of PV explained (70.4%) by these interactions was observed for interaction between *QSS.ndsu-7B.2* and *QSS.ndsu-7B.1*. However, excluding that particular case, the remaining interaction explained up to 22.4% of the PV for different traits in different environments (Table 2-3).

Table 2-3. Additive Epistatic Interaction among QTL associated to SS-related traits

Epistatic Interaction	Trait[†]	Environment	Effect[‡]	Effect (%)
<i>QSS.ndsu-2D</i> × <i>QSS.ndsu-5B</i>	PSS	VI	0.80	15.2
	NdSS	I	0.97	8.2
<i>QSS.ndsu-2D</i> × <i>QSS.ndsu-6A</i>	PSS	IV, V, AE	0.27-0.58	5.4-13.8
	Sk	I, III, IV	2.23-2.95	5.4-8.2
	SD	III, AE	0.21-0.30	5.6-5.8
	SkNd	I, III	0.15-0.20	5.3-6.2
	NdR	AE	0.38	8.9
<i>QSS.ndsu-2D</i> × <i>QSS.ndsu-6B.1</i>	PSS	II	0.55	15.5
	NdSS	III, AE	2.16-2.31	14.5-18.9
<i>QSS.ndsu-2D</i> × <i>QSS.ndsu-6B.2</i>	Sk	I	-2.45	5.2
	SD	I, IV	-0.25_-0.53	5.6-18.1
	SkNd	IV	-0.22	8.7
	NdSS	II	-2.21	22.4
<i>QSS.ndsu-2D</i> × <i>QSS.ndsu-7B.1</i>	SD	IV	-0.48	17.2
	PSS	III	-0.31	6.3
	Sk	IV, AE	-2.38_-3.19	7.4-9.2
	SkNd	I	-0.35	13.3
	NdR	IV, AE	-0.29_-0.40	6.5-9.4
	NdSS	IV	-1.00	12.7
<i>QSS.ndsu-2D</i> × <i>QSS.ndsu-7B.2</i>	Sk	AE	3.08	8.1
	SD	IV	0.42	12.2
	SkNd	I	0.33	7.9
<i>QSS.ndsu-5B</i> × <i>QSS.ndsu-6B.2</i>	NdSS	I	-0.88	9.4
<i>QSS.ndsu-6A</i> × <i>QSS.ndsu-6B.2</i>	Sk	I	-1.77	6.4
	SD	AE	-0.13	4.5
	SkNd	IV	-0.20	9.2
	NdR	III	-0.36	6.4
	NdNoSS	III-AE	1.70-1.82	10.6-13.9
<i>QSS.ndsu-6A</i> × <i>QSS.ndsu-7B.1</i>	NdNoSS	III, IV, AE	0.49-0.75	5-5.7

(Continues)

Table 2-3. Additive Epistatic Interaction among QTL associated to SS-related traits (Continued)

Epistatic Interaction	Trait [†]	Environment	Effect [‡]	Effect (%)
<i>QSS.ndsu-7B.2</i> × <i>QSS.ndsu-7B.1</i>	PSS	IV	-0.37	12.1
	SD	IV	-0.14	6.7
	SkNd	AE	-0.74	70.4
	NdSS	III, AE	-1.78 -2.11	7.2-7.8

[†]Traits were defined in Table 1

[‡]additive by additive interaction between two loci, positive value indicate that epistatic interaction with parental two locus combination has increased trait values, negative value indicate that epistatic interaction with recombinant two locus combinations has increased trait values

In order to identify the highly significant marker from each QTL interval, single marker regression analysis was conducted using all the markers present in each ROAT/QTL confidence interval. For *QSS.ndsu-2D*, the closest linked marker with SS-related traits was wPt-667785, which was present at a distance of about 0.3 cM away from the QTL peak. The closest markers associated with SS related traits for *QSS.ndsu-5B*, *QSS.ndsu-6A*, *QSS.ndsu-6B.1*, *QSS.ndsu-6B.2*, *QSS.ndsu-7B.1* and *QSS.ndsu-7B.2* were wPt-3569, wPt-671561, wPt-0406, wPt-3284, wPt-0194 and wPt-4258, respectively. All these markers were present at distances ranging from 0.0 to 5.5 cM away from the respective QTL peaks (Table 2-2; Fig. 2-2).

2.5. Discussion

Previous studies have consistently shown that increased seeds per spike results in increased yield (McNeal, 1960; Hucl and Baker, 1987; Feil, 1992; Calderini et al., 1995; Wang et al., 2002; Green et al., 2012). Other studies have reported a positive association between seeds per spikelet and yield (McNeal, 1960; Siddique et al., 1989; Feil, 1992) and between kernel weight and yield (McNeal, 1960; McNeal et al., 1978; Hucl and Baker, 1987; Wang et al., 2002). Based on these studies, branched spikes with SS can affect directly the number of kernel per spike and therefore, it should be of prime interest for wheat breeders. Additionally, SS can affect the kernel size and shape which may affect kernel weight, another yield component, and kernel weight volume. Mapping SS and eventually developing markers for these QTL would potentially be of great help to improve kernel yield and quality.

Our study showed that about 29% of RILs expressed SS phenotype in at least one experimental replicate across the environments. The presence of a heterobranching behavior observed in most of the

RILs bearing SS is in agreement with previous studies (Percival, 1921; Denčić, 1988; Huang and Yen, 1988; Martinek and Bernard, 1998; Klindworth et al., 1990a) and it is a challenge in the phenotyping of the SS trait. Considering that this phenomenon could be confused with seed admixtures, in the present research, the seeds for each genotype were obtained from individual head-rows, thus avoiding any chances of admixture. The cause of heterobranching is still not fully understood; however, the influence of the environmental conditions has been suggested as a reason for this phenomenon (Denčić, 1988; Huang and Yen, 1988; Klindworth et al., 1990a). In case of genotypes showing heterobranching behavior, predominant spike types were collected for data recording to avoid mis-phenotyping. When PSS screening resulted in a score of 2 (ratio 1:1) both types of spikes were collected; but the data on SS-related traits was recorded for the dominant phenotype, determined through the replicates and environments, to control a possible bias produced by the visual screening of PSS.

High values of heritability observed for all SS traits (Table 2-1) suggest that the phenotypic variation observed among the RILs was mainly due to genetic factors. Indeed, most of the RIL were stable in the presence/absence of the SS trait. The presence of significant $G \times E$ interaction for all traits was most likely caused by differences in magnitude rather than the difference in rank. Prosper 2010 was the most conducive environment for Sk (Table Appendix A1). Interestingly, among the four environments in which this trait was studied, Prosper 2010 had the highest values for air temperature and rain fall (19⁰C and 80.8 mm respectively) (NDAWN, 2012). Contrarily, Pennell and Halloran (1984a, 1984b) suggest that low temperature, strong vernalization response, and short photoperiods are conducive for the expression of SS.

In the past, variable number of DArT markers have been mapped in different genetic populations in hexaploid wheat; 339 by Akbari et al. (2006), 189 by Semagn et al. (2006), 1,348 by Sorrells et al. (2011), 246 by Bennett et al. (2012). Mapping of a large number of DArT markers by Sorrells et al. (2011) compared to other studies was due to the fact that the DH population used in that study was developed from the divergent cross of Synthetic W7984 (Altar84/*Aegilops tauschii* (219) CIGM86.940) and Opata M85. Similar to the study of Sorrells et al. (2011), a much higher number of markers (939 DArT markers) were mapped in the present study relative to previous studies in wheat (Akbari et al., 2006; Semagn et al., 2006; Bennett et al., 2012). This is likely due to the reason that one of the parental genotype (WCB

617) used in the development of this population was an exotic line. This population, developed from an exotic and an elite wheat genotype, also resulted in a better coverage across the whole genome. This is evident from the fact that a large number of markers (almost similar to that of A and B-genome) were also mapped to the D-genome chromosomes which generally shows very low level of polymorphism in cultivated germplasm pool. This is because D-genome is a recent evolutionary addition to the hexaploid wheat genome (> 10,000 years old), and there has been limited gene flow from *Ae. tauschii* (Dubcovsky and Dvorak, 2007), thus, decreasing the rate of polymorphism. The only chromosome for which a framework genetic map could not be established due to lack of polymorphic markers, was chromosome 4D. Similar to the present study, several studies in the past have also reported a low level of polymorphism for wheat chromosome 4B, 4D, 5A, 5D (Akbari et al., 2006; Semagn et al., 2006; Bennett et al., 2012; Kumar et al., 2013). The length of the framework map (3,114.2 cM) developed in the present study is in agreement with the previous studies (Akbari et al., 2006; Semagn et al., 2006; Bennett et al., 2012; Kumar et al., 2013) and also suggests a good representation of the whole wheat genome. The average distance between any two markers was 4.6 cM which is high enough for any QTL mapping study.

A total of seven consistent QTL (detected in at least three environments for one trait) were identified for SS related traits. Except for *QSS.ndsu-6B.1*, all the other QTL reported in this study had a LOD larger than the empirical LOD threshold (calculated through permutation test) for at least one trait in one environment. The presence of QTL with LOD below of the empirical LOD threshold could be caused by the skewed distributions of the phenotypic data. Previous studies have reported that this type of distributions tend to produce large significance thresholds and increases Type II error (Manichaikul et al. 2007). Therefore, we believe our approach based on declaring the existence of a consistent putative QTL across environments is a valid approach to avoid ignoring real QTL as indicated by previous reports (Börner et al. 2002; Tsilo et al. 2010).

Four QTL (*QSS.ndsu-5B*, *QSS.ndsu-6A*, *QSS.ndsu-6B.2*, and *QSS.ndsu-7B.1*) were involved in the control of all SS-related traits. Of the other three QTL, *QSS.ndsu-2D* was associated with all traits except NdNoSS; *QSS.ndsu-6B.1* was associated only with PSS, NdNoSS, and NdSS; and *QSS.ndsu-7B.2* was associated with all traits except NdR and NdNoSS (Table 2-2). These results demonstrates that

wheat SS is regulated by several genes as suggested in earlier studies based on progeny and monosomic analysis (Huang and Yen, 1988; Klindworth et al., 1990a; Peng et al., 1998). At the same time, these results in terms of inheritance patterns are different from few other studies in hexaploid branched wheat which suggested that only two genes control the formation of branched spikes, while another gene suppresses the expression of branched spikes (Koric, 1973; Pennell and Halloran, 1983; Denčić, 1988). The differences in the number of genes for SS phenotype reported in present and previous studies could be the result of the different methodologies used for the genetic analysis (QTL mapping, monosomic analysis, progeny analysis) or even differences among the material used for these studies. Indeed, the different approaches used to classify branched spikes in *triticum* (Sharman, 1967; Koric, 1973; Pennell and Halloran, 1983, 1984a, 1984b; Martinek and Bednár, 1998; Aliyeva and Aminov, 2011; Yang et al., 2005) may also lead to different conclusions. However, it is worth mentioning that in contrast to this study, most of the previous studies did not use the recently developed genomic tools and/or were limited to only few chromosomes of the wheat genome.

The partitioning of the SS-trait into seven components and the use of RIL population contrasts with the methodologies used in previous studies to genetically dissect the SS phenotype in wheat. Earlier studies disregarded the heterobranching behavior and scored the SS trait as present/absent (qualitative evaluation) in individual plants belonging to F₁, and F₂ generations (Peng et al., 1998; Dobrovolskaya et al., 2009). In this study too, in addition to QTL analysis of the categorical data for SS, we also attempted to identify the genes using presence/absence data of SS in RIL population. The PSS scores across six environments were used to classify the RILs into two discrete categories, branched (score ≥ 0.5) and non-branched (score < 0.5). Mapping for these binary qualitative data resulted in the identification of only four of the seven QTL detected in this study (*QSS.ndsu-2D*, *QSS.ndsu-6A*, *QSS.ndsu-6B.1*, and *QSS.ndsu-6B.2*). Three QTL *QSS.ndsu-5B*, *QSS.ndsu-7B.1* and *QSS.ndsu-7B.2* could not be detected in this analysis (data not shown), suggesting that the scale (0-4) used in this study was more informative for QTL detection than a binary scale (present/absent). Therefore, we concluded that it is important to make use of all available variation for any trait and that it is not appropriate to arbitrarily classify quantitatively varying data into discrete categories.

The traits for which all the detected QTL explained higher percentage of phenotypic variation in a determined environment was NdSS (PVE = 82.41%) While, the traits for which QTL explained the lower percentage of phenotypic variation in a determined environment was NdNoSS (PVE = 6.28%).

Considering the high heritability observed for all the traits (Table 2-1), the differences in phenotypic variation explained by same QTL in different environments and for different traits could be due to environmental factors.

The QTL on 2D (*QSS.ndsu-2D*) was associated with all traits except NdNoSS in all the environments and explained the major proportion of the phenotypic variation for these traits. The graphical genotype of the RILs with SS and non-SS phenotype clearly shows the impact of this major gene in controlling the SS phenotype (Fig. 2-3). All the RILs except one with LPSS score ≥ 1 showed the presence of WCB617 (parental genotype with SS phenotype) allele at *QSS.ndsu-2D*, while the alleles of WCB414 (parental genotype with non-SS phenotype) were present in RILs showing non-SS phenotype with only very few exceptions which could be the result of double recombination occurring between the gene and the markers and also may be due to experimental errors (Fig. 2-3). Previous studies conducted using monosomic analysis also reported the presence of a major gene controlling SS on 2D (Millet 1986, 1987; Peng et al. 1998). Using molecular markers, Dobrovolskaya et al. (2009) also identified a gene, *Mrs1*, on 2D which was responsible for an SS-like trait (multirow spike) in hexaploid wheat. However, in contrast to other studies on SS, including the present, it was reported that MRS is controlled by only one recessive gene located on chromosome 2D. *Mrs1* co-segregate with the microsatellite locus *Xwmc453* and is located in a gene rich region (2S0.8) of the distal half of the short arm of chromosome 2D (Erayman, 2004; Dobrovolskaya et al., 2009). Comparative study shows that *Mrs1* was mapped at 73.4 cM (total 2D map length= 119.6 cM), whereas, *QSS.ndsu-2D* was mapped at 41.5 cM (total 2D map length= 131.6 cM). This suggests that *Mrs1* and *QSS.ndsu-2D* could be different genes involved in spike morphology. Moreover, multirow spike studied by Dobrovolskaya et al. (2009) and the phenotype evaluated in the present study have been classified into different groups (Martinek and Bednar, 1988). However, only future studies involving detailed characterization or cloning of these genes will be able to answer the question if *Mrs1* and *QSS.ndsu-2D* represent same gene or not.

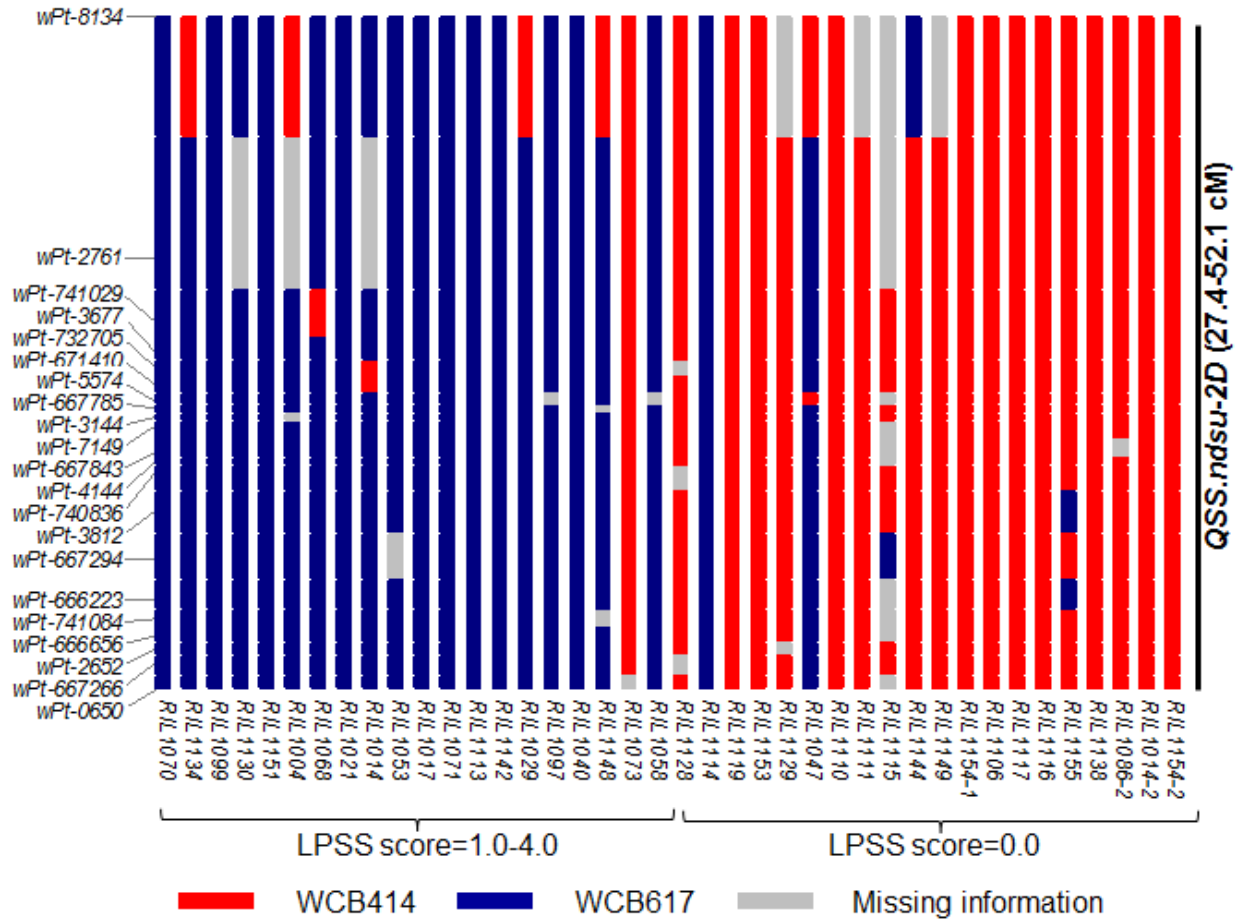


Fig. 2-3. Graphical genotypes of RILs with SS phenotype (All RILs with LPSS score ≥ 1) and non-SS phenotype (20 random RILs with LPSS score=0) for the 2DS region harboring major QTL (*QSS.ndsu-2D*)

In tetraploid wheat, it was reported that branched head is controlled by one major gene (*bh*) located on 2A and one minor gene located on 2B. However, these genes are inhibited when the chromosome 2D from “Chinese spring” (conventional spikes) is incorporated into 2D monosomic additional lines (Klindworth et al., 1990b). Considering that the D-genome is not shared between hexaploid and tetraploid wheat, it was suggested that *bh* could be an orthologue to *Mrs1* (Dobrovolskaya et al., 2009). Major role played by homoeologous chromosomes 2A, 2B and 2D in controlling spike related traits has also been reported in several studies in the past. For common wheat with no-SS, Li et al. (2002) and Kumar et al (2007) identified major QTL associated with spikelet number in a gene rich region of the short arm of chromosome 2D; while Shitsukawa et al. (2006) reported that the chromosomes 2A, 2B and 2D of “Chinese Spring” carry three homoeologous copies of the gene *WFL*

(Wheat FLORICULA/LEAFY) involved in the spikelet formation. In addition, chromosome 2DS has been reported to harbor QTL/genes for a number of other traits including yield, threshability-related traits, days to heading (*Ppd-1*), growth related traits (Sourdille et al., 2000, 2003; Börner et al., 2002; Groos et al., 2003; Jantasuriyarat et al., 2004; Hanocq et al., 2004; Kumar et al., 2007). It would be interesting to know if this region on 2DS represents a cluster of genes controlling different genes for different traits or a single gene having pleiotropic effect on several traits.

In addition to the major QTL on chromosome 2D, a QTL on chromosome 7B (*QSS.ndsu-7B.2*) also explained a high percentage of the phenotypic variation for some of the traits (Table 2-2). However, the detection of *QSS.ndsu-7B.2* in only some environments suggests that its expression is influenced by environmental conditions.

A gene on chromosome 7B controlling SS in hexaploid wheat has not been reported previously, suggesting that this novel QTL could be related to the specific material used in the present study. But several studies in the past have mapped genes for heading or ear emergence date and earliness per se on chromosome 7B of wheat (Kuchel et al., 2006; Maccaferri et al., 2008; Griffiths et al., 2009; Bennett et al., 2012) and 7H in barley (Laurie et al., 1995). However, all these studies mapped genes/QTL on the short arm of the chromosome 7B or 7H, while the two QTL detected in the present study were located on long arm of 7B chromosome, suggesting that *QSS.ndsu-7B.1* and *QSS.ndsu-7B.2* are most likely different than the already reported QTL/genes for heading date and earliness per se on 7B.

The remaining five QTL (*QSS.ndsu-5B*, *QSS.ndsu-6A*, *QSS.ndsu-6B.1*, *QSS.ndsu-6B.2* and *QSS.ndsu-7B.1*) explained minor portions of phenotypic variation for SS-related traits (Table 2-2), which suggests that chromosomes 5B, 6A, 6B and 7B possess minor genes for the SS phenotype. The location of these minor QTL differs with the findings of Peng et al. (1998), which suggested the presence of minor genes associated with branched spikes on chromosomes 4A, 4B and 5A through monosomic analysis. The identification of different minor genes in both studies could be attributed to the differences in germplasm as well as methodologies (monosomic analysis vs. genetic mapping) used in each investigation. Another reason for different results could be differences in genetic polymorphism in the parental genotypes of the populations.

The five minor QTL (*QSS.ndsu-5B*, *QSS.ndsu-6A*, *QSS.ndsu-6B.1*, *QSS.ndsu-6B.2* and *QSS.ndsu-7B.1*) were also associated with NdNoSS; while the two major QTL *QSS.ndsu-2D* and *QSS.ndsu-7B.2* were not associated to this trait. These results suggest that these minor genes could be related to the formation of the non-SS spikelets. Although, for SS phenotype, these five minor QTL are being reported for the first time, QTL for several other related traits have been reported in these regions in the past studies. For example, on 6AS where *QSS.ndsu-6A* was identified, several studies have reported QTL for spikelets per spike, spike compactness, grains per spike and days to heading (Kumar et al., 2007, Jantasuriyarat et al., 2004; Huang et al., 2003; Huang et al., 2004). Similarly, on long arm of 6B where *QSS.ndsu-6B.1* and *QSS.ndsu-6B.2* were detected, QTL have been reported for grains per spike and spike fertility (Li et al., 2007).

The QTL *QSS.ndsu-5B* was mapped in the telomeric region of long arm of chromosome 5B (Fig 2-2). The long arm of 5BL also harbors vernalization gene *Vrn-B1* which control the flowering time in wheat. This gene has been physically mapped in the bin 5BL18-0.66-0.79 (Timonova et al. 2013). However, comparison of the maps shows that marker wPt-1348 which is associated with *QSS.ndsu-5B* and is located proximal to the QTL, is present in the 5BL18-0.79-1.00 deletion bin (Francki et al. 2009) suggesting that this QTL is present in the telomeric bin of 5BL. This also means that *QSS.ndsu-5B* is most likely a different gene other than *Vrn-B1*.

The additive effects observed for *QSS.ndsu-6B.2* and *QSS.ndsu-7B.1* indicate that non-branched parent contributed the alleles that modify the phenotypic values of SS-related traits. The presence of an SS-related gene in commercial varieties was previously observed by Pennell and Halloran (1983). According to this study, a recessive inheritance pattern for SS was observed in the F₁ of crosses between the commercial variety “Phoenix” (non-SS) and the branched line “AUS15910”. However, a progeny with SS phenotype was observed in the first backcross using “Phoenix” as recurrent parent. Pennell and Halloran (1983) suggested the presence of a suppressor in “Phoenix” which inhibits the production of SS, but no further experiments were conducted to characterize the gene action. Therefore, the contribution of positive alleles at *QSS.ndsu-6B.2* and *QSS.ndsu-7B.1* by the non-branched parent constitute new evidence in this direction. Considering that these QTL also were associated with NdNonSS, it is possible that they have a positive effect on spikelet production in varieties with non-SS. Therefore, they could be

useful to breeding programs for increasing grain yield. Further genetic and molecular experiments will be necessary to characterize these QTL in hexaploid wheat.

Previous studies have suggested a role of epistatic interaction in the expression of the SS trait (Koric, 1973; Klindworth et al., 1990a; Denčić, 1988). In view of this, all the seven main effect QTL were tested for di-genic epistatic interactions (Table 2-3), using multiple interval mapping. The results reported in the present study are the first evidence at molecular level to demonstrate the role of epistatic interactions in the expression of SS phenotype. The previous studies using progeny analyses have suggested that a complementary action of two dominant genes control SS phenotype in wheat line 51885 (Sun et al., 2009). Other studies also suggested complementary action of the factors *Rm-ramifera* and *Ts-tetrastichon* in absence of an inhibitor gene called N (Koric, 1973, Denčić, 1988). The inhibitor was later located on chromosome 2D (Klindworth et al., 1990a). In the present study, the major and consistent QTL *QSS.ndsu-2D*, had additive epistatic interaction with all the other QTL, which explain up to 22.4% of the PV (Table 2-3). The identified interaction involve both parental two locus combinations (interactions with positive additive effect) as well as recombinant two locus combinations (interactions with positive additive effect), suggesting that specific allele combinations among *QSS.ndsu-2D* and the other loci can increase or decrease the expression of the SS trait in a particular genotype. Indeed, a few of RILs had the *QSS.ndsu-2D* allele derived from the branched parent but never expressed the branched phenotype (data not shown). Nevertheless, epistatic interactions were not limited to locus *QSS.ndsu-2D*. All other identified QTL were also involved in epistatic interactions (Table 2-3), including the interaction between *QSS.ndsu-7B2* and *QSS.ndsu-7B1* which explained up to 70.4 of PV.

It may also be noted that most of the identified QTL for SS were involved in two or more digenic interactions (Table 2-3) suggesting a network which may represent higher order interactions. It has been suggested that the metabolic pathways that presumably underlie quantitative traits involve multiple interacting gene products and regulatory loci that could generate higher-order epistatic interactions (McMullen et al., 1998). However, most of the software dealing with epistasis includes only two-locus interaction. This is partly because including higher order interactions requires too many parameters in the genetic model, which would be difficult to estimate properly except in extremely large populations. For example, a three-locus model may need a population size of over 1000 lines to enable reasonably

reliable estimations for all parameters. As a result, it would be very difficult to work with an epistatic model involving more than three loci (Jannink and Jansen, 2001; Xu and Crouch, 2008). However, as this is the first study which used an RIL population to uncover the genetic basis of SS phenotype, future studies using larger populations, and suitable statistical methods may be able to identify the role of higher order interactions in the genetic control of SS.

Unlike wheat, in other grass species, the molecular basis behind branched phenotype is better understood (for a review, see Sreenivasulu and Schnurbusch, 2012). In maize (*Zea mays*), it is known that mutations in the genes *RAMOSA1 (RM1)*, *RM2*, *RM3* and *RAMOSA1 ENHANCER* produces branched phenotypes in the tassel and the ear (Vollbrecht et al., 2005; Bortiri et al., 2006; Satoh-Nagasawa et al., 2006; Gavalloti et al., 2010). These genes encode transcription factors (EAR-containing zinc-finger transcription factor and the REL2 transcriptional co-repressor) which works as repressors of the indeterminate fate of spikelet-pair meristem (Gavalloti et al., 2010). Although ramosa genes are mainly observed in the tribe Andropogoneae which includes maize, sugar cane (*Saccharum officinarum*) and sorghum (*Sorghum bicolor*) (Kellogg, 2007), similar genes playing an important role in inflorescence development have also been identified in other grasses. For instances, a sister gene of *RA3* called *SISTER OF RAMOSA (SRA)* was found in rice (*Oryza sativa*) (Satoh-Nagasawa et al., 2006); while a gene called *six-rowed spike4 (Vrs4)*, an ortholog of *RAMOSA2* of maize, was recently isolated in barley (*Hordeum vulgare* L.) (Koppolu et al., 2013). It would be interesting to find out, if genes controlling SS phenotype in wheat also belong to the same gene families as reported in maize, rice and barley. An initial step in this direction would be to design markers from the available wheat sequences orthologous to the above mentioned genes in maize, rice and barley and to map them in wheat. The new information reported in this paper provides an excellent basis for future studies directed at gaining additional functional and evolutionary knowledge about this important phenotype.

2.6. Conclusions

In this study, a whole genome genetic map of an RIL population derived from a cross between a branched spike genotype and conventional spike genotype in hexaploid wheat was reported and used for identification of QTL associated with branched spike phenotype. Whole genome QTL analysis for

branched spikes and related traits resulted in the identification of seven QTL. Consistent to the previous finding, a major QTL for branched spike phenotype was identified on chromosome 2D (*QSS.ndsu-2D*). However, another major QTL on 7B (*QSS.ndsu-7B.2*), several other novel minor QTL, and epistatic interactions involved in the genetic control of branched spike phenotype were also identified in the present study. The major QTL on chromosome 2D could be the initial target for future studies aimed at fine mapping and ultimately cloning the underlying gene. Finally, as spike-related traits are the major components of final yield of wheat crop, the identified gene network for SS phenotype can play an important role in increasing wheat yield since it allows the formation of more spikelets in the spike.

2.7. References

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CHAPTER 3. GENOME-WIDE MAPPING OF SPIKE-RELATED AND AGRONOMIC TRAITS IN A COMMON WHEAT POPULATION DERIVED FROM A SUPERNUMERARY PARENT AND AN ELITE PARENT

3.1. Abstract

Commonly grown wheat (*Triticum aestivum* L) cultivars are characterized by spikes with fusiform shape, and spikelets evenly distributed. However, exotic genotypes express a broad range of spike-related traits and could be used as a source of new genes to enrich the germplasm of wheat breeding programs. In the present study, a population of 163 recombinant inbred lines derived from an cross between an elite line (WCB414) and an exotic line with supernumerary spikelet (SS) (WCB617) was evaluated over four to six environments and used to identify QTL associated with ten spike-related, and ten agronomic traits. Composite interval mapping identified a total of 145 QTL which were located on 17 wheat chromosomes and included 37 consistent QTL. The QTL identified for individual traits ranged from two for apical awnleted expression to 14 in number of spikes per m². The phenotype variation explained (PVE) by individual QTL ranged from 0.61% to 91.8%. The major QTL for glume pubescences was located in a 1AS QTL cluster and was associated with other traits such as kernels per spike and spike length. Likewise, the major QTL for SS was co-located in a cluster of QTL on 2DS with a yield-related QTL with 40.3% of PVE and other QTL for agronomic and spike-related traits. Consistent and major QTL may be used in marker assisted breeding programs to transfer the desirable alleles into other germplasm. Desirable QTL alleles were also contributed by the exotic line, suggesting the possibility of enriching the breeding germplasm with alleles from exotic genotypes.

3.2. Introduction

Spikes (also named ears) are the most symbolic benchmark of common wheat (*Triticum aestivum* L.) and are the host of the main crop product: the grain. They are a photosynthetic site which provides carbohydrates accumulated in the mature grain impacting yield components (Grundbacher, 1963; Li et al., 2006). Spike-related traits define the spike architecture and determine the phenotype of the wheat subspecies. For instance, the spikes of spelt wheat (*T. aestivum* ssp. *spelta*) have a fragile and log

rachis, lax density with spikelets well separated from each other on the rachis, and are not free threshing (Percival, 1921; Winzeler et al., 1994; Bertin et al., 2001; Jantasuriyarat et al., 2004). The spikes of club wheat (*T. aestivum* ssp. *compactu*), however have a tough rachis, short length, laterally compressed with spikelets closely packed and free-threshing (Percival, 1921; Zwer et al., 1995; Jantasuriyarat et al., 2004). The cultivars of common wheat (*T. aestivum* ssp. *aestivum*) are characterized as having spikelets evenly distributed, tough rachis and free-threshing (Percival, 1921; Jantasuriyarat et al., 2004).

Variations in spike length, number of spikelets per spike, and density are also observed. Spikes with fusiform, oblong, and clavate shape are the result of changes in these traits (Briggle and Ritz, 1963). The impact of spike-dimension traits on grain yield has prompted the study of the genetic basis of spike architecture. Three major complexes of genes, Q, C, and S1, play an important role in the genetic control of the spike dimensions of most of the wheat subspecies (Miller, 1987; Jantasuriyarat et al., 2004). In addition, the evidence collected during the last couple of decades suggests that variation in spike-dimensions traits in common wheat is caused by multiple QTL distributed throughout the wheat genome (Sourdille et al., 2000b; Li et al., 2002; Börner et al., 2002; Jantasuriyarat et al., 2004; Marza et al., 2006; Kumar et al., 2007; Ma et al., 2007; Chu et al., 2008; Cui et al., 2012).

QTL mapping studies typically using elite × elite populations discovered QTL and associated molecular markers suitable for breeding programs (Würschum, 2012). However, elite × elite populations often show low polymorphism, which results in non-saturated maps and the identification of fewer QTL. Moreover, the QTL identified in such studies are mostly the ones which are already fixed in the cultivated germplasm. The use of populations derived from exotic × elite crosses could be a way forward to identify new desirable QTL and/or alleles. In the past, synthetic lines derived from the cross of tetraploid *T. turgidum* ssp. and diploid *T. taushii* have been used to map QTL for agronomic (Börner et al., 2002; Kumar et al., 2007), morphologic (Li et al., 2002; Jantasuriyarat et al., 2006) and quality traits (Nelson et al., 2006).

Exotic wheat germplasm exhibits broad spike diversity for spike-related traits (Sharman, 1967; Martinek and Bednár, 1998). Branched spikes, also known as supernumerary spikelets (SS), are one of the most well-known exotic variations in spike architecture (Sharman, 1944; Sharman, 1967; Rawson and Ruwali, 1972; Koric, 1973; Klindworth et al., 1990a, 1990b; Pennel and Halloran, 1983; Yang et al., 2005;

Dobrovolskaya et al., 2009; Sun et al., 2009; Haque et al., 2012; Sreenivasulu and Schnurbusch, 2012; Zhang et al., 2012). These spikes have more than one spikelet per rachis node and have been suggested as a means to increase the yield of wheat through increasing the number of fertile florets where the grains are developed (Pennell and Halloran, 1983, 1984a, 1984b; Hucl and Fowler, 1992; Peng et al., 1998). Several studies suggest that the SS trait is under control of at least three genes (Koric, 1973; Pennell and Halloran, 1983; Klindworth et al., 1990a; Peng et al., 1998) (Chapter 2) with a major gene located on chromosome 2D (Klindworth et al., 1990b; Peng et al., 1998; Dobrovolskaya et al., 2009) (Chapter 2). Apparently this gene is a suppressor of the expression of SS gene in commercial wheat varieties (Pennell and Halloran, 1983) (Chapter 2).

The impact of SS on other spike-related traits is not well known; but in terms of SS impact on agronomic traits, it is known that branched genotypes are mostly associated with reduced number of tillers, fewer grains per spike, low kernel weight, sterility in several florets, delayed days to heading and maturity, and low grain yield (Percival, 1921; Saluke and Asana, 1971; Rawson and Ruwali, 1972; Koric, 1973; Millet, 1986, 1987; Hucl and Fowler, 1992; Zhang et al., 2012). However, considering the broad genetic diversity of branched spikes in *Triticum* (Sharman, 1967; Martinek and Bednár, 1998), some branched genotypes break this trend. For instance, some lines in Tibetan Triple-Spikelet wheat have excellent spike grain production (>120 seeds per spike) with adequate thousand kernel weight (Yang et al., 2005). Moreover, it has also been suggested that the grain yield of branched genotypes can be increased if the fertility of the florets is improved (Rawson and Ruwali, 1972; Pennell and Halloran, 1984a, 1984b).

Past studies have predominantly investigated the association of agronomical important traits in branched genotypes using traditional methods. Few studies have, however, used molecular markers to identify genetic regions controlling the SS trait (Dobrovolskaya et al., 2009; Li et al., 2011; Haque et al., 2012) (Chapter 2). However, those studies did not investigate the association of these loci with other agronomical important traits. In the present study, a recombinant inbred line (RIL) population derived from a cross between elite white wheat line (WCB414) and a wheat line with SS and pubescences on glumes (WCB617) was developed to 1) detect QTL for 10 spike-related traits and 10 important agronomical traits of spring wheat, 2) identify if the QTL/genes for SS-related and other spike-related traits share common

genomic region with important agronomic loci and 3) identify novel genes and/or alleles with potential use in breeding programs.

3.3. Material and Methods

3.3.1. Plant material

A population of 163 RIL developed from a cross between the hexaploid hard white wheat (HWW) elite line WCB414 with the exotic hexaploid hard red wheat (HRSW) line WCB617 was evaluated in this study. The RIL population was assessed at $F_{8:9}$, $F_{10:11}$ and $F_{12:13}$ during the years 2009, 2010 and 2011, respectively. At the end of the growing seasons of 2009 and 2010 one spike from each experimental unit was collected and sent to New Zealand to grown as head-row. The bulked seed from each head row were planted in the next season. More details about the mapping population have been described elsewhere in Chapter 2. WCB414 is an elite line with fusiform architecture, awned, and glabrous glumes, while WCB617 is an exotic line with heterobranching behavior, awns, and pubescence on the glumes. Seven checks, “Alsen” (PI 615543) (Frohberg et al., 2006), “Steele-ND” (PI 634981) (Mergoum et al., 2005), “Glenn” (PI 639273) (Mergoum et al., 2006), “Faller” (PI 648350) (Mergoum et al., 2008), “Barlow” (PI 658018) (Mergoum et al., 2011), and “Briggs” (PI 632970) (Devkota et al., 2007), and “Alpine” (Agripro® wheat variety, USA) were also included. All the checks are HRSW except “Alpine” which is a HWW.

3.3.2. Field experiment

The parents, RILs and checks were planted in a 13 × 13 partially balanced square lattice design with two replicates at Prosper and Carrington, North Dakota, USA, during the years 2009, 2010 and 2011. The environments were designated as I-VI (I=Prosper 2009, II= Carrington 2009, III= Prosper 2010, IV= Carrington 2010, V= Prosper 2011, and VI= Carrington 2011). Each genotype was planted in plots comprised of seven rows 2.44 m length, and 12.7 cm apart with a seeding rate of 113 kg ha⁻¹. Due to a limited number of seeds in 2009, the parent WCB617 was planted in two rows plot in each environment. The remaining five rows were planted to the commercial variety “Glenn”. The two rows of WCB617 were harvested by hand in 2009.

3.3.3. Spike-related traits

Penetrance of pubescence (PP) measuring glabrous spikes and penetrance of clavate architecture (PC) measuring fusiform architecture were assessed using a scale of 0 to 4, where 4 indicate that all spikes in the experimental unit were pubescent or clavate, and 0 indicates that all spikes were glabrous or fusiform. An experimental unit with a ratio of 3:1 (Pubescent: glabrous spike type; or, clavate: fusiform spike type) corresponded to a score of 3, a ratio 1:1 was scored as 2, and a ratio 1:3 was scored as 1. Experimental units with apical awnleted expression (Aless) were scored as 1, and awned experimental units were scored as 0.

Four spikes were taken randomly from the primary tillers from each experimental unit to measure spike-related traits. The spikes of prevalent phenotype were collected in plots with penetrance of supernumerary spikelets (PSS) (Chapter 2), PP and PC scores of 3 and 1 following methodology used in Chapter 2. When two phenotypes were present in equal proportions (ratio of 1:1; score 2) in any particular replicate of any environment, the prevalent phenotype for that particular genotype was decided based on the phenotype of other replicates and environments. The other spike-related traits for which data were recorded include spike length (SL) in centimeters, measured from the base of the rachis to the top of the uppermost spikelet, excluding awns; the number of nodes per spike (Nd); nodes density (NdD) (nodes cm^{-1}) calculated as Nd/SL ; number of nodes with immature spikelets at the spike base (NNdISk); awn length at the bottom of spike (ALB), determined from the mean length of three awns collected randomly in the first three fully developed spikelets located at the spike base; awn length at the top of spike (ALT), calculated from the mean length of three awns collected in the last four fully developed spikelets at the spike top; awn length at middle of the spike (ALM), obtained from the average length of three awns collected in the spikelets located between the first three fully developed spikelets at the spike base and the last four full-developed spikelets at the spike top; and awn length total averaged (AAL), calculated as the mean of all the awn lengths measured per spike. In all the measurements of awns, the awns were chosen randomly from all the lemmas in specific spikelets; awns with evidence of damage were discarded. For each trait, the mean from the four spikes was calculated.

In 2011, PSS, PP, and PC assessments were performed in both locations, but other spike measurements could not be collected because Carrington was severely affected by hail and Prosper by

flood. Consequently, for all traits except PSS, PP and PC, data were recorded in four environments. The evaluations of PSS reported in Chapter 2 were correlated with the spike-related and agronomic traits to identify effect of SS on other traits.

3.3.4. Agronomic traits

The agronomic traits assessed in this study were days to heading (DH), determined as the number of days from planting to heading; plant height (PH), measured (cm) at maturity from the soil surface to the tip of the spike, excluding the awns; days to maturity (DM) determined as the number of days from planting to maturity (appearance of a yellow peduncle at the base of the spikes); number of spikes (NS) (spikes m⁻²), determined by counting the number of stems in two 61-cm-long rows per plot and subsequently converted to stems per square meter; percentage of lodging (Ld), visually estimated by the degree (angle) and number of plants that lodged; kernels per spike (KS), determined from ten random spikes per plot collected before the harvesting of each experimental unit; kernels per spikelet (KSk) calculated from the means of five fully-developed spikelets per spike randomly selected; and kernels per node (KNd) obtained from the mean of five randomly selected nodes per spikes with fully-developed spikelets. In the case of spike with non-SS, the mean obtained for KSk was used for KNd. The four spikes used in the spike-related measurements were used to collect information of KSk and KNd. Therefore, a total of 20 measurements of KSk and KNd per genotype were collected and then averaged. After harvesting, the grain samples were cleaned using a clipper grain cleaner before grain yield (GY) was measured. The GY (kg ha⁻¹) of each genotype was determined by the weight of the total seed harvested from each plot. Considering that the parent WCB617 was only planted in two rows in 2009, an estimated GY for the entire plot was determined based on grain yield from the two rows.

Plant height was scored in all six environments, and NS was also collected in all the environments except Prosper 2011. Similarly, DH was collected in all environments except Carrington 2011 and DM was collected in all environments except Carrington 2009. Lodging was scored in the environments Prosper 2009, 2010, and 2011; and Carrington 2011. Data on the traits GY and KS were collected at Carrington and Prosper in 2009 and 2010.

3.3.5. Statistical analysis

Analysis of variance (ANOVA) for a lattice design was carried out for every trait using the single environment data. The MIXED procedure of the Statistical Analysis System (SAS, 2004) was used for these analyses, where the RILs, parental genotypes, and checks were considered as fixed effects, and the environments and blocks were considered as random effects. The *F*-tests were considered significant at $P \leq 0.05$. Combined ANOVA analysis over environments was performed to estimate genotype \times environment interaction, if the ratio between the largest and smallest experimental error of each environment was less than 10-fold (F_{\max} ratio) (Tabachnik and Fidell, 2001). The mean separation tests were conducted using an F-protected least significant difference (LSD) value. Pearson's correlation between traits was calculated in each environment. Correlations coefficients were considered significant at $P \leq 0.05$. Significant correlation coefficients obtained in each environment were assessed for homogeneity across environments at $P \leq 0.005$, following the steps described by Gomez and Gomez (1984). Pooled correlations were calculated among homogenous correlations and considered as significant at $P \leq 0.05$.

Broad sense heritability was estimated on a plot basis (Holland et al., 2003), excluding the parent and check means. $h_B^2 = \sigma_g^2 / [\sigma_g^2 + (\sigma_{ge}^2) + (\sigma_e^2)]$, where σ_g^2 is the genotype variance, σ_{ge}^2 is the G \times E interaction variance, and σ_e^2 is the error variance.

3.3.6. Molecular marker analysis, map construction, QTL identification, and genotypic analysis

Molecular map construction for this population was described in Chapter 2. The software, QTL Cartographer V2.5_011 (Wang et al., 2012), was used to conduct composite interval mapping (CIM) using forward regression method and default conditions. The QTL analysis was conducted for each trait in each environment as well across environments (AE). A minimum LOD score of 2.5 was used to declare putative QTL. Permutation test with 500 permutations was used to determine critical LOD threshold ($\alpha=0.05$). Putative QTL below this critical threshold and identified in only one environment were not considered. In this study, to avoid obvious Type II error, if a putative QTL was identified in more than one environment (including AE), the QTL was reported regardless their significance in the permutation test. A QTL was declared consistent when it was detected in at least 50% of the environments in which it was studied. Confidence intervals (CI) were calculated using ± 2 LOD (from the peak) method (Lander and

Bostein, 1989). QTL with overlapping CIs were considered as one QTL. A QTL was declared as major when explained at least 15% of phenotypic variation (PV) in one environment. The program MapChart 2.2 (Voorrips, 2002) was used to draw the linkage groups and QTL.

3.4. Results

3.4.1. Phenotypic variation

The RIL population segregated for presence/absence of SS, pubescences, clavate/fusiform architecture, and awned/apical-awnleted spikes (Fig. 3-1). However, the most prevalent spike phenotype in the RIL population was non-branched, glabrous, fusiform, and awned. For some RIL with presence of pubescences, glabrous spikes were also observed. This phenomenon was called hetero-pubescent and was assessed through the PP trait. Similarly, for RILs with presence of clavate architecture, fusiform spikes were also observed. This phenomenon was referred as hetero-clavate and was assessed by PC trait. Appendix Fig. B1 and Appendix Fig. B2 show the distribution of the RIL across the environments for spike-related and agronomic traits, respectively. The estimated means and ranges of all traits for the RILs, parents, and checks in all environments as well in the combined analysis are presented in Appendix Tables B1 and B2.

The traits had significant genotype × environment interaction (Table 3-1). For spike-related traits, broad sense heritability ranged from 0.43 for ALT to 0.89 for PP (Table 3-1). For agronomic traits, broad sense heritability ranged from 0.13 for KS to 0.67 for NNdISk (Table 3-1). The significant correlations among all traits including PSS are summarized in Table 3-2, Table 3-3 and Table 3-4. Coefficients of variation and lattice efficiency for each environment are presented in Appendix Table B3; while Appendix Table B4 shows that error variances among the environments were homogenous for the traits.

In most of the environments the parental line WCB617 was pubescent. The exception was observed in Carrington 2009, where WCB617 had a hetero-pubescent phenotype. Through combined ANOVA analysis, we observed that 86 RIL were classified as glabrous ($PP=0$), 29 RIL were hetero-pubescent with prevalence of glabrous type ($0.1 \leq PP \leq 1.4$), 24 RIL were hetero-pubescent with prevalence of pubescent type ($1.4 \leq PP \leq 3.9$), and 24 RILs had a PP mean of four. Non-transgressive segregation was

observed for PP in all the environments except Carrington 2009 where we observed transgressive segregation toward the mean of the exotic parent (WBC617) (Appendix Table B1).

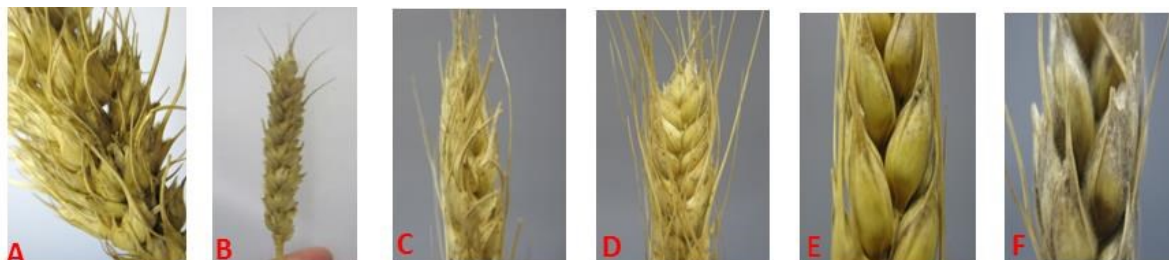


Fig. 3-1. Types of spikes identified in the RIL population derived from the cross of the elite line WCB414 and the exotic line with supernumerary spikelets WBC617. A. Spike with supernumerary spikelets. B. Spike apically awnleted. C. Spike with fusiform architecture at the top of the spike. D. Spike with clavate architecture at the bottom of the spike. E. Spike with glabrous glumes. F. Spike with pubescences on the glumes.

In all the environments, both parents were fusiform at the apical region of the spike ($PC=0$) (Fig. 3-1). Through combined ANOVA we observed that a total of 136 RIL were fusiform ($PC=0$), 21 RIL were hetero-clavate with prevalence of fusiform spikes ($0.1 \leq PC \leq 1.9$), and 6 RIL were hetero-clavate with prevalence of clavate spikes ($2.3 \leq PC \leq 3.8$) (Fig 3-1).

The RIL 1069, 1087, 1131 and 1134 were apically awnleted (Fig 3-1) across all replicates and environments. For other spike-related traits, SL, NdD, ALB, ALT, ALM, and AAL showed transgressive segregation in both directions of the parental means in all environments (Appendix Table B1 and Appendix Fig. S1). Nd had transgressive segregation similarly in both directions of the parental means, but when the data was pooled across environments transgressive segregation only was observed toward the mean of the elite parent (WCB414) (Appendix Table B1 and Appendix Table B2). In Carrington 2010, Prosper 2009 and 2010, and AE, NNdISK had transgressive segregation in both directions of the parental means, however in Carrington 2009, it showed transgressive segregation in direction of the mean of the elite parent (WCB414) only (Appendix Table B1).

Table 3-1. Mean squares, coefficient of variation and heritabilities for spike-related and agronomic traits

Trait [†]	NECAV [‡]	Mean squares				CV(%) [§]	H	SE [#]
		Genotype	Environment	G x E	Error			
Spike-related Traits								
PP	6	29.63**	0.22	0.53**	0.06	22.3	0.89	0.01
PC	6	1.96**	3.54	0.38**	0.1	185.4	0.57	0.03
SL	4	4.67**	19.10	0.42**	0.22	4.9	0.62	0.03
Nd	4	31.50**	239.61	2.46**	0.71	4.4	0.69	0.03
NdD	4	0.23**	0.77	0.02**	0.01	4.9	0.63	0.03
ALB	4	3.13**	93.83	0.44**	0.26	17.5	0.52	0.03
ALT	4	3.29**	143.89	0.60**	0.31	17.3	0.43	0.04
ALM	4	10.67**	19.39	0.56*	0.46	10.9	0.73	0.02
AAL	4	4.42**	61.09	0.29**	0.17	9.9	0.72	0.03
Agronomic Traits								
DH	5	78.61**	15683	8.55**	1.28	2.0	0.60	0.03
PH	6	761.75**	14462	51.64**	25.37	5.8	0.63	0.03
DM	5	54.17**	19931	10.05**	2.22	1.7	0.44	0.04
NS	6	13587**	319930	2204.96**	1623.44	16.0	0.26	0.03
Ld	4	1640.19**	286472	885.09**	209.99	33.7	0.14	0.04
KS	4	81.99**	13657	43.57**	18.06	12.7	0.13	0.03
KSk	4	14.66**	1.08	0.12**	0.07	10.3	0.59	0.03
KNd	4	0.5**	17.07	0.16**	0.08	10.8	0.26	0.04
NNdISk	4	6.03**	75.18	0.44**	0.3	37.0	0.67	0.03
GY	4	2086113**	74853141	391215**	99222	11.72	0.40	0.04

*, ** Significance at P < 0.05, 0.01, respectively; ns not significant at P < 0.05

[†]PP, penetrance of pubescences; PC, penetrance of clavate architecture; SL, spike length; Nd, number of nodes; NdD, node density; ALB, awns length at the bottom of spike; ALT, awns length at the top of spike; ALM, awns length at middle of the spike; AAL, awns length total averaged, DH, days to heading; PH, plant height; DM, days to maturity; NS, number of spikes; Ld, lodging susceptibility; KS, kernel spike; KSk, kernels per spikelet; KNd, kernels per node; NNdISk, number of nodes with immature spikelets at the spike base; GY, grain yield.

[‡]Number Environments in combined analysis of variance.

[§]Coefficient of variation.

^{||}Broad sense heritability on plot basis calculated for the RILs.

[#]Standard error for heritability.

Table 3-2. Correlations coefficients between spike-related traits

Traits [†]	PP	PC	SL	Nd	NdD	ALB	ALT	ALM	AAL	Aless	PSS
PP	1	Ns	0.39**††	0.47**††	0.24**#	-0.16*§	-0.33**#	-0.19**¶	-0.23**¶	ns	0.38**‡‡
PC		1	-0.22**††	0.22**§	0.38**††	0.15**§	-0.17**§	ns	ns	ns	ns
SL			1	0.64**# 0.35**§	-0.31**#	Ns	-0.33**¶	ns	ns	-0.19**¶	0.29**††
Nd				1	0.64**††	Ns	-0.49**#	ns	-0.23**¶	-0.19*§	0.52**††
NdD					1	Ns	-0.33**#	-0.19**§	-0.21**¶	-0.16*§	0.32**††
ALB [‡]						1	0.61**††	0.76**††	0.87**# 0.91**§	-0.54**††	-0.33**††
ALT							1	0.60**††	0.83**# 0.70**§	-0.35**††	-0.45**††
ALM								1	0.94**††	-0.65**††	-0.42**††
ALTA									1	-0.61**††	-0.45**††
Aless										1	ns

*, ** Significance at $P < 0.05$, 0.01 , respectively; ns not significant at $P < 0.05$

[†]Traits defined in Table 3.1

[‡]Alternative pooled correlations were observed between the traits ALB-AAL. The lowest pooled r value is presented.

[§]r from one environment; [¶]r pooled from two environments; [#]r pooled from three environments; ^{††}r pooled from four environments; ^{‡‡}r pooled from six environments.

Table 3-3. Correlations coefficients between agronomic traits

Trait [†]	DH	PH	DM	NS	Ld	KSk	KNd	KS	NNdISk	GY
DH [‡]	1	0.23** [¶]	0.73** ^{††}	-0.27** ^{‡‡}	0.55** [¶]	-0.29** ^{††}	0.17** [¶]	0.30** ^{††}	0.23** [#]	-0.32** ^{††}
		-0.24** [¶]	0.51** [¶]	-0.50** [¶]	-0.50** [¶]		-0.33** [¶]	-0.40** [¶]		-0.78** [¶]
PH [§]		1	0.26** ^{††}	-0.24** ^{††}	0.46** ^{††}	ns	0.16* [¶]	0.25** ^{††}	ns	0.21** [¶]
				-0.17** ^{††}	0.17** [¶]					
DM			1	-0.17** ^{††}	-0.33** [¶]	-0.18** [#]	-0.26** [#]	0.23** [#]	0.17* [¶]	-0.35** [#]
				0.22** [¶]	0.24** [¶]	0.15* [¶]				
NS				1	-0.39** [#]	0.20** ^{††}	0.20* [¶]	-0.20** ^{††}	-0.22** [#]	0.53** ^{‡‡}
								0.28** [¶]		
Ld					1	-0.24** [#]	-0.20** [¶]	-0.51* [¶]	0.21** [#]	0.16* [¶]
										-0.69** [¶]
KSk						1	0.33** ^{††}	0.28** [#]	-0.76** ^{‡‡}	0.33** ^{‡‡}
							0.70** [¶]			
KNd							1	0.51** ^{††}	-0.23** [¶]	0.26** [#]
								0.71** [¶]		
KS								1	-0.19* [¶]	0.58** [#]
NNdISk									1	-0.27** ^{‡‡}

*, ** Significance at P < 0.05, 0.01, respectively; ns not significant at P < 0.05

[†] Traits defined in Table 3.1

[‡] Alternative pooled correlations were observed between the traits DH-NS. The lowest pooled r value is presented.

[§] Alternative pooled correlations were observed between the traits PH-LD. The lowest pooled r value is presented.

[¶]r from one environment; [#]r pooled from two environments; ^{††}r pooled from three environments; ^{‡‡}r pooled from four environments.

Table 3-4. Correlations coefficients between spike-related traits and agronomic traits

Spike-related traits	Traits [†]	Agronomic traits									
		DH	PH	DM	NS	LD	KSk	KNd	KS	NNdISk	GY
	PP	0.43**§§	-0.26**¶¶	0.28**††	-0.23**‡‡	0.25**¶¶ -0.27**¶¶	-0.31**‡‡	-0.21**¶¶ 0.23**¶¶	0.25**†† -0.18**¶¶	0.31**‡‡	-0.39**‡‡
	PC	0.29**‡‡	ns	0.24**#	-0.18**#	0.20*¶¶	ns	-0.18**¶¶	0.16*#	ns	-0.15**¶¶
	SL [‡]	0.41**‡‡	0.30**‡‡	0.37**#	-0.38**‡‡	0.42**¶¶	-0.22**#	-0.28**#	0.46**††	0.30**††	-0.33**†† -0.55**¶¶
	Nd	0.62**‡‡	ns	0.48**#	-0.39**‡‡	0.49**¶¶	-0.57**‡‡	-0.31**#	0.29**†† -0.34**¶¶	0.58**‡‡	-0.45**†† -0.73**¶¶
	NdD [§]	0.36**‡‡	-0.28**††	0.27**#	-0.21**#	0.22**¶¶	-0.53**‡‡	-0.27**†† -0.53**¶¶	-0.37**¶¶	0.42**‡‡	-0.27**‡‡
	ALB	0.21**#	-0.20**#	0.23**#	0.23**#	ns	0.33**††	-0.29**¶¶	ns	-0.30**#	0.16*#
	ALT	-0.25**††	-0.23**††	-0.18*¶¶	0.36**‡‡	ns	0.41**‡‡	0.16*¶¶ -0.18*¶¶	-0.27**¶¶	-0.34**‡‡	0.29**‡‡
	ALM	0.16*¶¶	-0.19**#	0.18*¶¶	ns	ns	0.43**‡‡	ns	0.15*¶¶	-0.34**‡‡	ns
	AAL	-0.18*¶¶	-0.21**††	0.18**#	0.22**‡‡	-0.15*¶¶	0.43**‡‡	ns	ns	-0.35**‡‡	0.19**††
	Aless	-0.16*¶¶	ns	-0.19**#	ns	ns	ns	-0.22**¶¶	-0.22**††	-0.18*¶¶	ns
	PSS	0.24**§§	ns	ns	-0.21**‡‡	0.22**¶¶ -0.17**¶¶	-0.70**‡‡	0.25**††	0.29**# -0.17**¶¶	0.75**‡‡	-0.32**‡‡

*, ** Significance at P < 0.05, 0.01, respectively; ns not significant at P < 0.05

[†]Traits defined in Table 3.1.

[‡]Alternative pooled correlations were observed between the traits SL-GY. The lowest pooled r value is presented.

[§]Alternative pooled correlations were observed between the traits NdD-KNd. The lowest pooled r value is presented.

¶r from one environment; #r pooled from two environments; ††r pooled from three environments; ‡‡r pooled from four environments; §§ r pooled from five environments.

The agronomic traits NS, PH, and DH had transgressive segregation in both directions of the parental means in all environments as well as when the data was pooled across environments (Appendix Table B2 and Appendix Fig. B2). In Carrington 2009, Prosper 2009, and AE, the trait GY showed transgressive segregation toward the elite parent (WCB414), while in Carrington 2010 and Prosper 2010, GY showed transgressive segregation in both directions of the parental means (Appendix Table B2). In Carrington 2009 and 2010, Prosper 2010, and AE the traits DM, KS and KNd had transgressive segregation in both directions of the parental means (Appendix Table B2 and Appendix Fig. B2). However, in Prosper 2009 these traits only showed transgressive segregation in direction of the elite line (WBCB414) (Appendix Table B2). In Carrington 2011, Prosper 2010, and AE the trait Ld had transgressive segregation in both directions of the parental means (Appendix Table B2 and Appendix Fig. B2); however, in Prosper 2009 the trait Ld only showed transgressive segregation in direction of the PI (WBCB617), whereas in Prosper 2011 only showed transgressive segregation in direction of the elite line (WBCB414) (Appendix Table B2). The trait KSk segregated transgressively in both directions of the parental means in Carrington 2010 and Prosper 2009 (Appendix Table B2); however, in Carrington 2009, Prosper 2010 and AE, the trait KSk only showed transgressive segregation in direction of the elite line (WBCB414) (Appendix Table B2 and Appendix Fig. B2).

3.4.2. QTL for spike size and pubescences

Composite interval mapping for SL resulted in the identification of three consistent QTL (*QSL.ndsu.1A.1*, *QSL.ndsu.2D* and *QSL.ndsu.4B*) and three putative QTL (*QSL.ndsu.1A.2*, *QSL.ndsu.2B.2*, and *QSL.ndsu.4A*) (Table 3-5, Fig 3-2). The QTL *QSL.ndsu.4A* explained more than 15% PV and was considered as major QTL; while *QSL.ndsu.1A.1* and *QSL.ndsu.4B* explained up to 14.90% of PV, a close value to 15%, the threshold used for major QTL. The QTL *QSL.ndsu.1A.2*, *QSL.ndsu.2B*, and *QSL.ndsu.2D* are minor QTL which explained between 5.1% to 9.8% of PV of SL. Depending of the environments, these QTL together explained 19.93 to 27.46 of PV. Alleles from the branched parent (WCB617) were the only responsibly to increase the length of the spike in the population

The QTL analysis for Nd data resulted in the identification of three consistent QTL (*QNd.ndsu.1A.1*; *QNd.ndsu.1D*; and *QNd.ndsu.2D*) and four putative QTL (*QNd.ndsu.1A.2*; *QNd.ndsu.2A*, *QNd.ndsu.4A*, and *QNd.ndsu.4B.2*) (Table 3-5, Fig 3-2). *QNd.ndsu.2D* had major effect

and explained up to 55.9% of PV for Nd. This QTL was observed in all the environments as well as AE. The other six QTL associated to Nd (*QNd.ndsu.1A.1*, *QNd.ndsu.1A.2*, *QNd.ndsu.1D*, and *QNd.ndsu.4B.2*) are minor QTL which explaining between 3.63- 6.65% of PV. These QTL explained 16.77-67.42% of PV for Nd in different environments. Additives effects observed in all the QTL associated to Nd demonstrated that WCB617 has alleles that increase the phenotypic values of this trait.

For NdD a total of five QTL including one consistent QTL (*QNdD.ndsu.2D*) were identified (Table 3-5, Fig 3-2) and together explained 20.5-47.0% of PV depending of the environments. The consistent QTL *QNdD.ndsu.2D* had major effect and explained up to 35.1% of PV, while the remaining QTL had minor effects, with PVE ranging from 5.3 to 8.6% for NdD. Alleles from WCB617 were responsible for increased values of NdD at *QNdD.ndsu.2D*, *QNdD.ndsu.5B*, and *QNdD.ndsu.7A.2*; while alleles from WCB614 at *QNdD.ndsu.7A.1* and *QNdD.ndsu.7B* contributed to increased values of NdD.

Data analysis revealed a total of seven QTL located on six chromosomes for PC. These seven QTL include one consistent QTL (*QLPC.ndsu.4A*) and six putative QTL (*QLPC.ndsu.1B*, *QLPC.ndsu.3B.1*, *QLPC.ndsu.3B.2*, *QLPC.ndsu.5B.1*, *QLPC.ndsu.5B.2* and *QLPC.ndsu.6B.1*) (Table 3-5, Fig 3-2). None of the QTL explained more than 15% of PV, therefore these genetic regions were considered to harbor minor QTL. However, the QTL located on chromosome 4A (*QLPC.ndsu.4A*) was identified in five environments as well as AE and was the only QTL for PC where alleles from the elite parent (WCB414) contributed to the increase of expression of this trait. Together, the QTL associated to PC explained 13.4-27.5% of PV in different environments.

A total of seven QTL located on four chromosomes (1A, 4A, 5B, and 6B) (Table 3-5, Fig. 3-2) were identified for variation in PP and together explained 60.4-95.0% of PV in different environments. The QTL *QPP.ndsu.1A.1* showed major effect and explained 56.8- 91.8% of PV for PP. The PV explained by other six minor QTL ranged from 0.6% to 5.3%. Two of the QTL (*QLPP.ndsu.5B*, and *QPP.ndsu.6B.1*) were consistent QTL (Table 3-5). Alleles that increased PP at *QPP.ndsu.1A.1*, *QPP.ndsu.1A.3*, *QPP.ndsu.4A*, *QLPP.ndsu.5B*, and *QPP.ndsu.6B.2* loci were derived from the branched and pubescent parent WCB617; while at *QLPP.ndsu.1A.2*, and *QLPP.ndsu.6B.1*, alleles from the glabrous parent WCB414 increased PP.

3.4.3. Awn-related QTL

QTL analysis for ALB resulted in the detection of two consistent QTL (*QALB.ndsu.1B* and *QALB.ndsu.7B*) and five putative QTL (*QALB.ndsu.1D*, *QALB.ndsu.2A*, *QALB.ndsu.3A*, *QALB.ndsu.4A*, and *QALB.ndsu.6A*) (Table 3-5, Fig 3-2). *QALB.ndsu.2A* and *QALB.ndsu.4A* were major QTL and explained more than 15% of PV in some environments. While the remaining QTL, each contributed less than 15% of PV for ALB. Together these QTL explained 31.5-38.3% of PV in the different environments studied. Alleles from the branched parent (WCB617) increased ALB values at *QALB.ndsu.1B*, *QALB.ndsu.1D*, *QALB.ndsu.2A*, and *QALB.ndsu.4A*; while alleles from the elite parent (WCB414) contributed to increase ALB values at *QALB.ndsu.3A*, *QALB.ndsu.6A*, and *QALB.ndsu.7B* loci.

A total of three consistent QTL (*QALT.ndsu.1A*, *QALT.ndsu.2D*, and *QALT.ndsu.5B*) and four putative QTL (*QALT.ndsu.2A*, *QALT.ndsu.5A*, *QALT.ndsu.6A*, and *QALT.ndsu.7B*) were associated to ALT (Table 3-5, Fig 3-2). The PV explained by all these QTL varied from 15.8% to 61.4% in different environments. The QTL *QALT.ndsu.1A*, *QALT.ndsu.2A* and *QALT.ndsu.2D* had major effect and explained up to 17.1%, 15.5% and 20% of phenotypic variation, respectively. The PVE by other minor QTL ranged from 6.5% to 12.3%. Only the elite parent (WCB14) contributed alleles for increased ALT values at each of these QTL

The QTL analysis for ALM in the RIL population studied resulted in the detection of one consistent QTL (*QALM.ndsu.5B*) and five putative QTL (*QALM.ndsu.4B*, *QALM.ndsu.5A*, *QALM.ndsu.6A*, *QALM.ndsu.6B.1*, *QALM.ndsu.6B.2*, *QALM.ndsu.7B.1* and *QALM.ndsu.7B.2*) (Table 3-5, Fig 3-2). Only *QALM.ndsu.5B* explained more than 15% of PV in one of the environment and can be considered as major QTL. The PVE by other QTL ranged from 6.1% to 10.6%. Together, these QTL explained 6.1-40.9% of PV in different environments. The branched parent (WCB617) contributed the alleles that increase the phenotypic values of ALM at *QALM.ndsu.4B* and *QALM.ndsu.6B.2*, while WCB414 contributed towards increased phenotypic values of this trait at *QALM.ndsu.5A*, *QALM.ndsu.5B*, *QALM.ndsu.6A*, *QALM.ndsu.6B.1*, *QALM.ndsu.7B.1* and *QALM.ndsu.7B.2*.

The genetic analysis for AAL resulted in the detection of two consistent QTL (*QAAL.ndsu.5B1* and *QAAL.ndsu.7B.3*) and two putative QTL (*QAAL.ndsu.5B.2* and *QAAL.ndsu.6A*,) (Table 3-5, Fig 3-2). The PVE explained for these QTL ranged from 6.1% to 13.3%. However, these QTL explained together

8.0-20.2% of PV in different environments and the elite parent (WCB414) contributed positive alleles for increased phenotypic values at all AAL loci.

Composite interval mapping of data for Aless revealed two QTL, both located on chromosome 2A (*QAless.ndsu.2A.1* and *QAless.ndsu.2A.2*) (Table 3-5, Fig 3-2). The PVE by these QTL ranged from 22.4% to 33.4% and together explained 55.6-57.0% of PV in different environments. Additive effects of these QTL showed that the alleles that produced the apical awnleted phenotype were derived from the elite parent (WCB414).

3.4.4 .QTL for yield components

The CIM analysis for KSk resulted in the detection of three consistent (*QKSk.ndsu.2B*, *QKSk.ndsu.2D*, and *QKSk.ndsu.6B*) and two putative QTL (*QKSk.ndsu.1B* and *QKSk.ndsu.7B*) (Table 3-6, Fig. 3-2). Only *QKSk.ndsu.2D* is a major QTL that explain between 21.5% and 38.8% of PV. The other QTL had minor effects and explain 3.14% to 13.48% of PV. However, these QTL explained together 34.7-48.6% of PV in different environments. The elite parent (WCB414) contributed the positive alleles to increase KSk at all the loci except *QKSk.ndsu.7B*.

A total of eight QTL were detected for the trait KNd, one of which was consistent (*QKNd.ndsu.1A*) and seven were putative (*QKNd.ndsu.1D*, *QKNd.ndsu.2B*, *QKNd.ndsu.3A*, *QKNd.ndsu.4A*, *QKNd.ndsu.5B*, *QKNd.ndsu.6B*, and *QKNd.ndsu.7A*) (Table 3-6, Figure 3-2). All these QTL were minor QTL explaining between 6.93% and 13.09% of PV. Depending of the environment, these QTL together explained 7.1-20.2 of PV of KNd. Additive effects showed that the branched parent (WCB617) alleles contributes the positively to increase KNd at *QKNd.ndsu.1A*, *QKNd.ndsu.4A*, and *QKNd.ndsu.6B*, while the elite parent (WCB414) contributed the positive alleles for increased trait values at *QKNd.ndsu.1D*, *QKNd.ndsu.2B*, *QKNd.ndsu.3A*, *QKNd.ndsu.5B*, and *QKNd.ndsu.7A* loci.

Table 3-5. QTL identified for spike-related traits in a RIL population of hexaploid wheat derived from the cross of WCB414 and WCB617

QTL	Environment [†]	Flanking Markers	Pos [‡] . (cM)	CI [§] (cM)	LOD	Thresh. [¶]	a [#]	R ² (%)
<i>Spike Length</i>								
QSL.ndsu.1A.1	I, II, IV, AE	wPt-664586-wPt-667180	14.0-26.6	7.5-33.2	4.0-7.9	3.1-3.3	-0.3_-0.3	7.2-14.9
QSL.ndsu.1A.2	IV	wPt-733588-wPt-665613	95.9	85.7-109.9	4.7	3.2	-0.3	8.0
QSL.ndsu.2B	IV	wPt-7859-wPt-1133	2.0	0-12.2	5.4	3.2	-0.3	9.8
QSL.ndsu.2D	I, III	wPt-730568-wPt-667536	74.8-82.8	62.6-93.9	2.7-2.8	3.1	-0.2_-0.3	5.1-5.5
QSL.ndsu.4A	III	wPt-4645-wPt-0798	83.7	72.7-94.4	5.1	3.1	-0.4	17.8
QSL.ndsu.4B	I, II, AE	wPt-74434-wPt-1101	31.0	23.9-37.2	3.4-7.3	3.1-3.3	-0.2_-0.3	15.0
							^{††} RTPVE	19.9-27.5
<i>Number of Nodes</i>								
QNd.ndsu.1A.1	I, III	wPt-664586-wPt-664772	14.0-39.0	9.5-54.5	2.6-4.3	3.32-3.40	-0.6_-0.69	4.6-6.7
QNd.ndsu.1A.2	IV, AE	wPt-8172-wPt-9429	106.9-113.9	79.4-127.3	4.4-4.6	3.28-3.36	-0.5_-0.51	5.8-6.1
QNd.ndsu.1D	I, III, AE	wPt-730475-wPt-665360	131.9	105.4-146.2	2.8-3.9	3.28-3.40	-0.4_-0.62	3.0-5.1
QNd.ndsu.2A	II	wPt-2372-wPt-2850	48.2	39.8-60.6	3.5	3.20	-0.6	5.2
QNd.ndsu.2D	I, II, III, IV, AE	wPt-671859-wPt-671914	66.4-74.8	66.0-78.0	6.5-37.5	3.20-3.40	-1.2_-1.7	8.6-56.0
QNd.ndsu.4A	IV	wPt-2946-tPt-9400	6.9	0-18	4.3	3.36	-0.4	4.1
QNd.ndsu.4B	III, AE	wPt-8892_wPt-1101	29.6-31.0	11.8-37.6	2.9-3.9	3.28-3.32	-0.4_-0.6	3.6-4.5
							^{††} RTPVE	16.8-67.4
<i>Nodes density</i>								
QNdD.ndsu.2D	I, II, III, IV, AE	wPt-2675-wPt-666223	21.0-48.3	10.1-51.0	6.6-16.6	3.14-3.4	-0.1_-0.1	20.5-35.1
QNdD.ndsu.5B	I	wPt-1548-wPt-6191	133.3	117.0-152.7	3.8	3.23	-0.1	7.9
QNdD.ndsu.7A1	II	wPt-1706-wPt-5524	40.9	35.3-41.2	4.0	3.28	0.1	6.6
QNdD.ndsu.7A2	II	wPt-0971-wPt-2083	68.2	64.7-80.2	5.0	3.28	-0.1	8.6
QNdD.ndsu.7B	III, AE	wPt-6665428-wPt-5975	123.6-123.7	112.0-131.7	3.3-3.7	3.14	0.0-0.1	5.3-6.6
							^{††} RTPVE	20.5-47.0

(Continues)

Table 3-5. QTL identified for spike-related traits in a RIL population of hexaploid wheat derived from the cross of WCB414 and WCB617 (continued)

QTL	Environment [†]	Flanking Markers	Pos. [‡] (cM)	CI [§] (cM)	LOD	Thresh. [¶]	a [#]	R ² (%)
<i>Penetrance of clavate architecture</i>								
QPC.ndsu.1B	III, VI	wPt-0308-wPt-8240	22.5	16.3-32.2	3.4-5.4	2.8-3.4	-0.2_-0.3	7.6-11.7
QPC.ndsu.3B.1	V	wPt-6945-wPt-1159	108.9	99.1-124.0	3.0	2.4	-0.1	6.9
QPC.ndsu.3B.2	I, II, AE	wPt-4412-wPt-1311	77.2	66.3-80.6	3.0-5.4	3.1-54.3	-0.2_-0.3	6.4-11.5
QPC.ndsu.4A	I, II, III, IV, VI, AE	wPt-0798-wPt-3250	90.0-124.2	79.7-127.1	3.5-4.3	2.8-54.3	0.2-0.3	7.0-9.6
QPC.ndsu.5B.1	IV	wPt-6191-wPt-6014	155.8	135.7-182.4	3.4	3.4	-0.2	10.3
QPC.ndsu.5B.2	V	wPt-0295-wPt-7665	221.7	212.0-227.9	4.4	2.4	-0.2	10.1
QPC.ndsu.6B.1	IV, V	wPt-6116-wPt-1541	0.0-1.0	0-17.2	3.4-3.8	2.4-3.4	-0.1_-0.2	7.6-9.1
							RTPVE ^{††}	13.4-27.5
<i>Penetrance of pubescences</i>								
QPP.ndsu.1A.1	I, II, III, IV, V, VI, AE	wP1924-wPt-667180	18.4-23.6	17.0-25.4	51.6-134.4	4.0-84.8	-1.6_-1.9	56.8-91.8
QPP.ndsu.1A.2	V, VI	wPt-3347-wPt-666424	2.6	0-3.5	2.6-3.7	4.3-84.8	0.3-0.4	0.8-1.2
QPP.ndsu.1A.3	V, VI	wPt-665784-wPt-9429	98.0-108.9	95.2-119.2	3.2-4.0	4.3-84.8	-0.2	1.0-1.1
QPP.ndsu.4A	IV	wPt-7926-wPt-9833	62.1	48.3-81.2	6.2	3.7	-0.3	2.4
QPP.ndsu.5B	I, V, VI	rPt6127-tPt8942	22.8-27.5	16.7-38.2	2.5-4.1	4.3-84.8	-0.2_-0.4	0.7-5.3
QPP.ndsu.6B.1	III, IV, V	wPt-742929-wPt-7935	167.7-178.7	157.0-189.1	2.6-4.2	3.7-84.8	0.2-0.4	0.6-3.9
QPP.ndsu.6B.2	VI, AE	wPt-1784-wPt-4706	5.0-13.5	0-17.6	2.6-5.1	4.1-4.3	-0.2_-0.4	0.7-2.3
							RTPVE ^{††}	60.4-95.0
<i>Awn length at the bottom of spike</i>								
QALB.ndsu.1B	II, III, IV, AE	wPt-671415-wPt-665480	1.5-10.9	0-14.6	2.9-4.1	3.1-3.2	-0.1_-0.3	5.7-9.3
QALB.ndsu.1D	IV	wPt-672077-wPt-730172	100.0	93.7-109.2	4.2	3.2	-0.3	8.6
QALB.ndsu.2A	IV, AE	wPt-798339-wPt-2372	41.0-43.0	34.1-52.6	2.7-4.5	3.1-3.2	-0.4_-0.4	7.9-16.8
QALB.ndsu.3A	III	wPt-667640-tPt-1079	35.9	22.0-43.4	3.9	3.1	0.2	9.7
QALB.ndsu.4A	II	wPt-4645-wPt-0798	80.7	66.6-96.2	4.3	3.1	-0.3	17.6
QALB.ndsu.6A	IV, AE	rPt9065-wPt-666574	58.1	54.3-71.1	3.1-4.2	3.1-3.2	0.2-0.3	6.5-8.6
QALB.ndsu.7B	II, III, AE	wPt-2305-wPt-6372	18.9-20.2	6.6-30.1	3.5-6.5	3.1-3.1	0.2-0.2	7.9-14.9
							RTPVE ^{††}	31.5-38.3

(Continues)

Table 3-5. QTL identified for spike-related traits in a RIL population of hexaploid wheat derived from the cross of WCB414 and WCB617 (continued)

QTL	Environment [†]	Flanking Markers	Pos [‡] .(cM)	CI [§] cM)	LOD	Thersh. [¶]	a [#]	R ² (%)
<i>Awn length at the top of spike</i>								
QALT.ndsu.1A	I, II, III	wPt-6654-wPt-3272	125.0-131.01	112.0-142.6	3.5-4.5	3.1-3.3	0.1-0.4	7.5-17.1
QALT.ndsu.2A	I	wPt-0003-wPt-4201	131.4	113.0-144.9	3.6	3.3	0.4	15.50
QALT.ndsu.2D	I, II, III, IV, AE	wPt-6850-wPt-667476	68.9-70.51	62.1-82.0	3.9-9.9	3.1-3.4	0.2-0.4	8.3-20.0
QALT.ndsu.5A	IV, AE	wPt-1038-wPt-1903	0.0	0-8.5	3.4-3.8	3.3-3.4	0.2-0.3	6.9
QALT.ndsu.5B	I, II	wPt-742141-wPt-3049	65.6	60.9-82.5	3.1-5.9	3.2-3.3	0.2-0.4	6.4-12.3
QALT.ndsu.6A	IV	wPt-666208-wPt-9131	83.7	65.2-94.3	3.6	3.3	0.3	7.1
QALT.ndsu.7B	AE	wPt-2305-wPt-9516	18.9	12.8-25.6	3.7	3.4	0.2	6.5
							RTPVE ^{††¶}	15.8-61.4
<i>Awn length at middle of the spike</i>								
QALM.ndsu.4B	IV, AE	wPt-7412-wPt-5303	103.51	91.2-111.6	4.2-4.8	3.0-3.1	-0.4_-0.5	8.6-10.4
QALM.ndsu.5A	IV	wPt-1038-wPt-1903	0.01	0-5	5.0	3.0	0.5	10.6
QALM.ndsu.5B	I, II, AE	wPt-2041-wPt-3049	49.11-65.61	38.2-91.4	2.6-4.6	3.0	0.4-0.7	9.7-21.0
QALM.ndsu.6A	IV	wPt-2636-wPt-4836	1.51	0-6.7	3.3	3.0	0.3	6.6
QALM.ndsu.6B.1	IV	wPt-0171-wPt-4164	7.51	5.6-11.9	3.5	3.0	0.4	7.8
QALM.ndsu.6B.2	II	wPt-9990-wPt-5066	26.51	17.6-32.7	3.8	3.0	-0.4	8.5
QALM.ndsu.7B.1	III, AE	wPt-2305-wPt-9516	18.91	9.7-33.4	2.9-3.0	3.1-3.2	0.3-0.4	6.1-6.3
QALM.ndsu.7B.2	IV	wPt-2273-wPt-3723	52.11	38.9-73.7	3.2	3.0	0.4	7.3
							RTPVE ^{††¶}	6.1-40.9
<i>Awn length total averaged</i>								
QAAL.ndsu.5B.1	I, II, AE	wPt-742141-wPt-3049	65.61	61.7-89.4	2.9-5.1	3.0-3.3	0.2-0.4	6.2-11.9
QAAL.ndsu.5B.2	III	wPt-1348-wPt-3995	280.91	267.7-299.8	3.6	3.2	0.3	13.3
QAAL.ndsu.6A	IV, AE	rPt9065-wPt-666574	58.11	46.7-72.5	2.8-3.7	3.2-3.3	0.2-0.3	6.1-8.0
QAAL.ndsu.7B	II, III, AE	wPt-2305-wPt-6372	18.91-20.21	9.9-32.5	3.2-3.8	3.0-3.3	0.2-0.2	6.9-8.3
							RTPVE ^{††¶}	8.0-20.2

(Continues)

Table 3-5. QTL identified for spike-related traits in a RIL population of hexaploid wheat derived from the cross of WCB414 and WCB617 (continued)

QTL	Environment [†]	Flanking Markers	Pos [‡] .(cM)	CI [§] (cM)	LOD	Thersh. [¶]	a [#]	R ² (%)
<i>Apical awnleted expression</i>								
<i>QAless.ndsu.2A.1</i>	I, II, III, IV, V, VI, AE	wPt-798339-wPt-2372	39.0	35.8-44.9	9.8	2.3-2.5	0.1	33.2-33.4
<i>QAless.ndsu.2A.2</i>	I, II, III, IV, V, VI, AE	wPt-668027-wPt-798459	30.5	26.4-32.7	2.8-3.3	2.3-2.5	0.1	22.4-23.6
							RTPVE ^{††¶}	55.6-57.0

[†]I, Prosper 2009; II, Carrington 2009; III, Prosper 2010; IV, Carrington 2010; V, Prosper 2011; VI, Carrington 2011.

[‡]Position

[§]Confidence Interval

[¶]Threshold calculated by permutation test.

[#]Additive effects

^{††}rank of phenotypic variation explained per environment

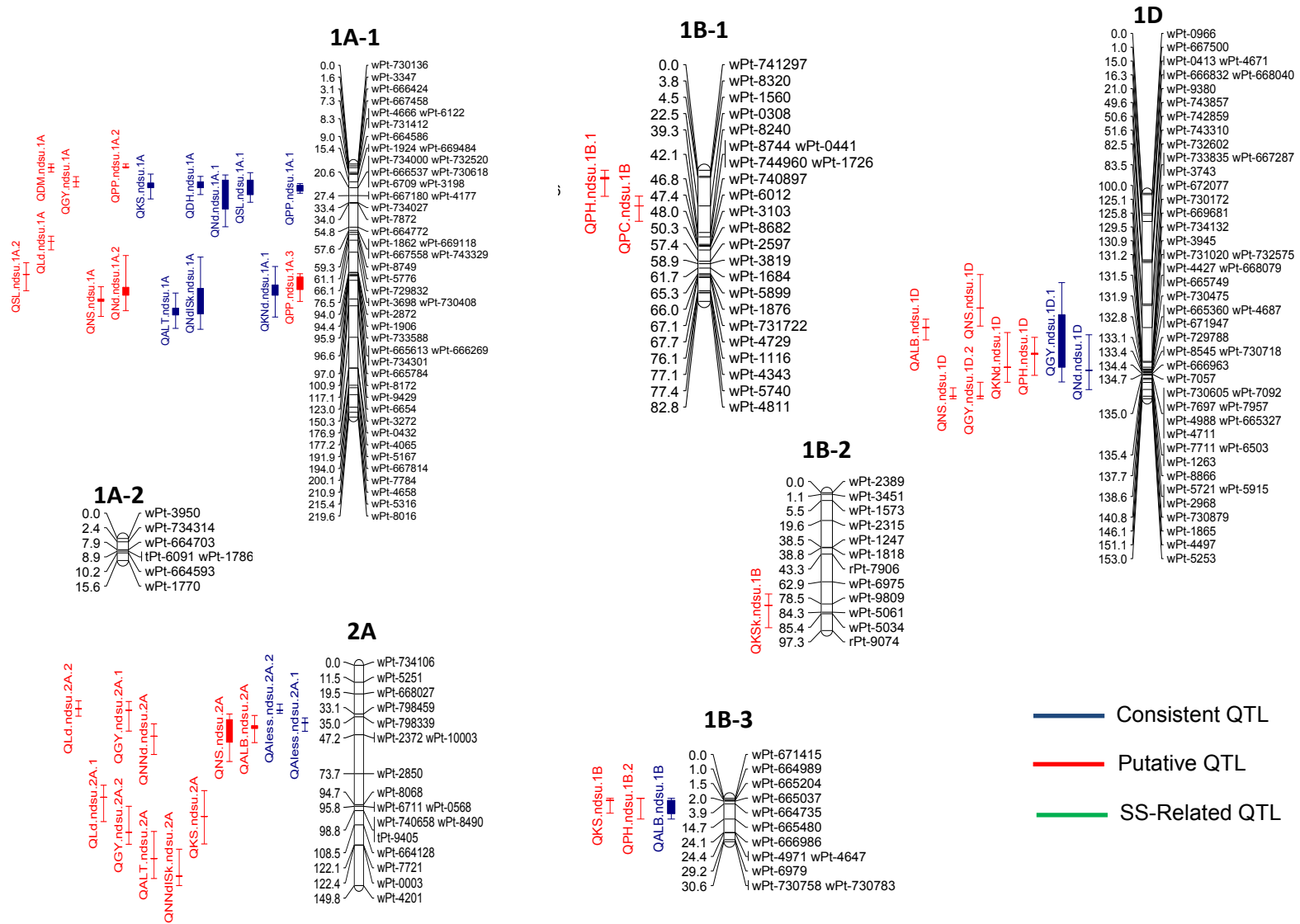


Fig. 3-2. Genetic map and QTL for 10 spike-related, and 10 agronomic traits of a RIL population derived from the cross of the elite line WCB414 and the exotic line WCB617 with SS trait, over four to six locations in ND, USA during 2009, 2010, and 2011

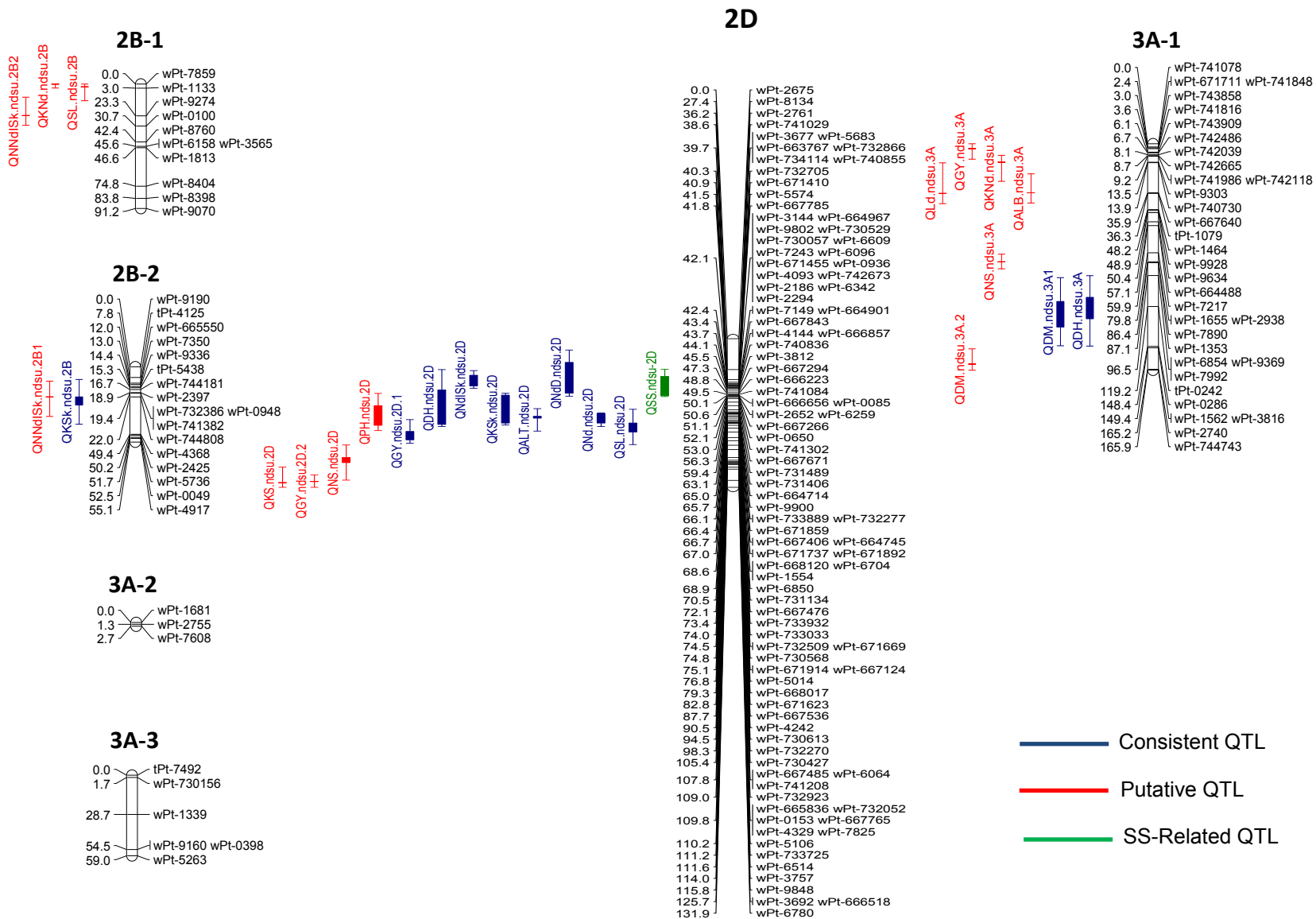


Fig. 3-2. Genetic map and QTL for 10 spike-related, and 10 agronomic traits of a RIL population derived from the cross of the elite line WCB414 and the exotic line WCB617 with SS trait, over four to six locations in ND, USA during 2009, 2010, and 2011 (Continued)

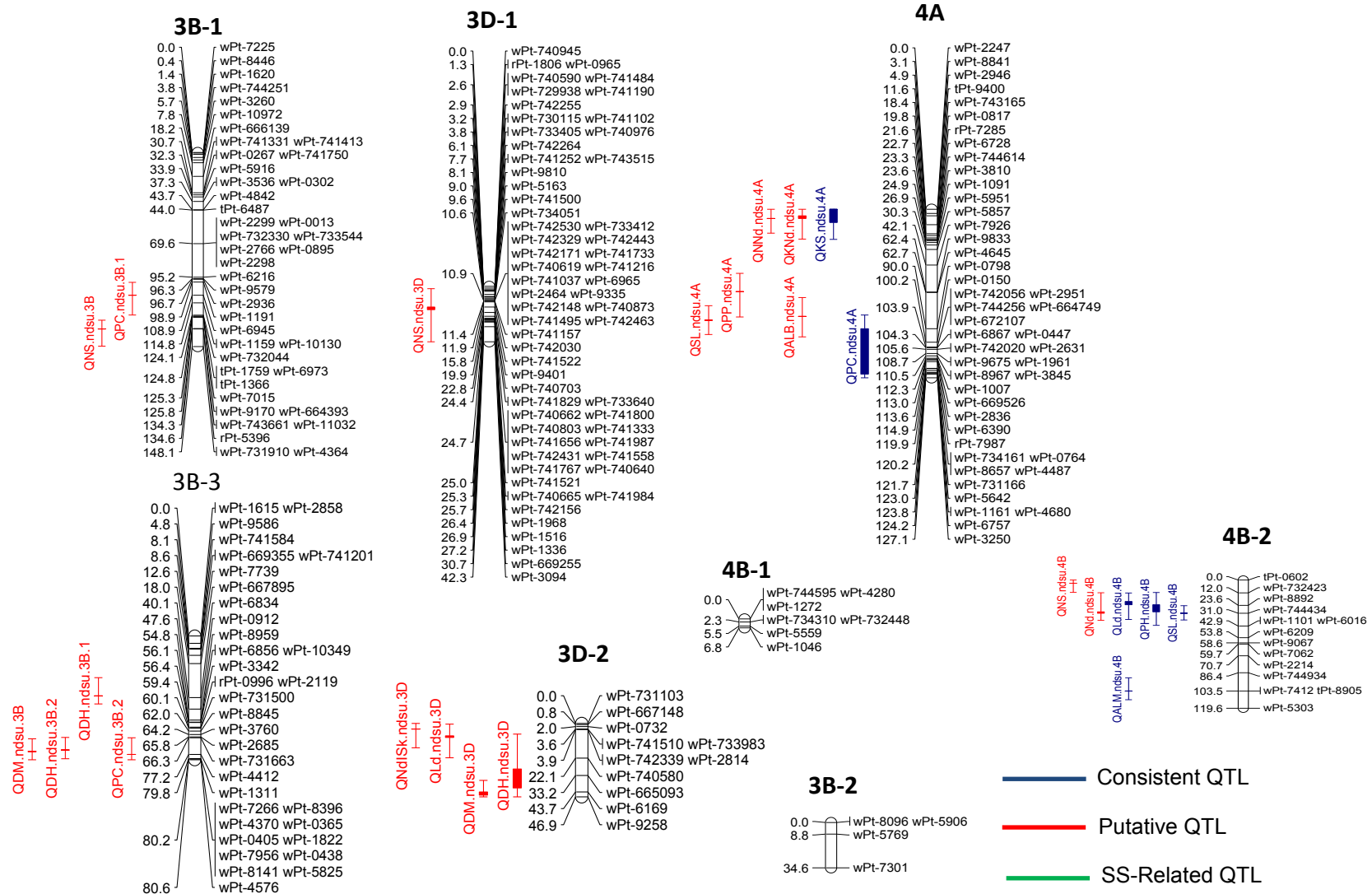


Fig. 3-2. Genetic map and QTL for 10 spike-related, and 10 agronomic traits of a RIL population derived from the cross of the elite line WCB414 and the exotic line WCB617 with SS trait, over four to six locations in ND, USA during 2009, 2010, and 2011 (Continued)

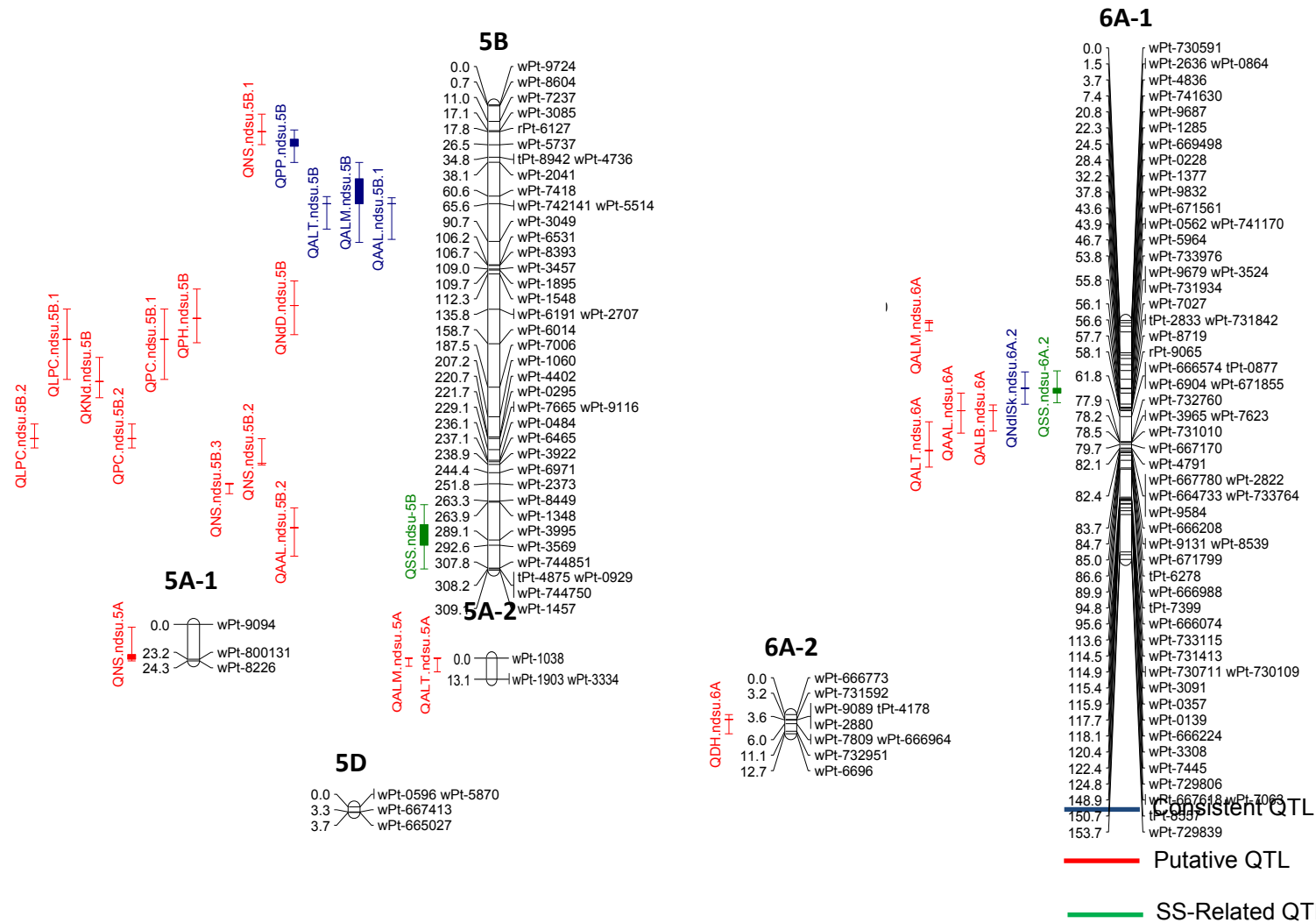


Fig. 3-2. Genetic map and QTL for 10 spike-related, and 10 agronomic traits of a RIL population derived from the cross of the elite line WCB414 and the exotic line WCB617 with SS trait, over four to six locations in ND, USA during 2009, 2010, and 2011 (Continued)

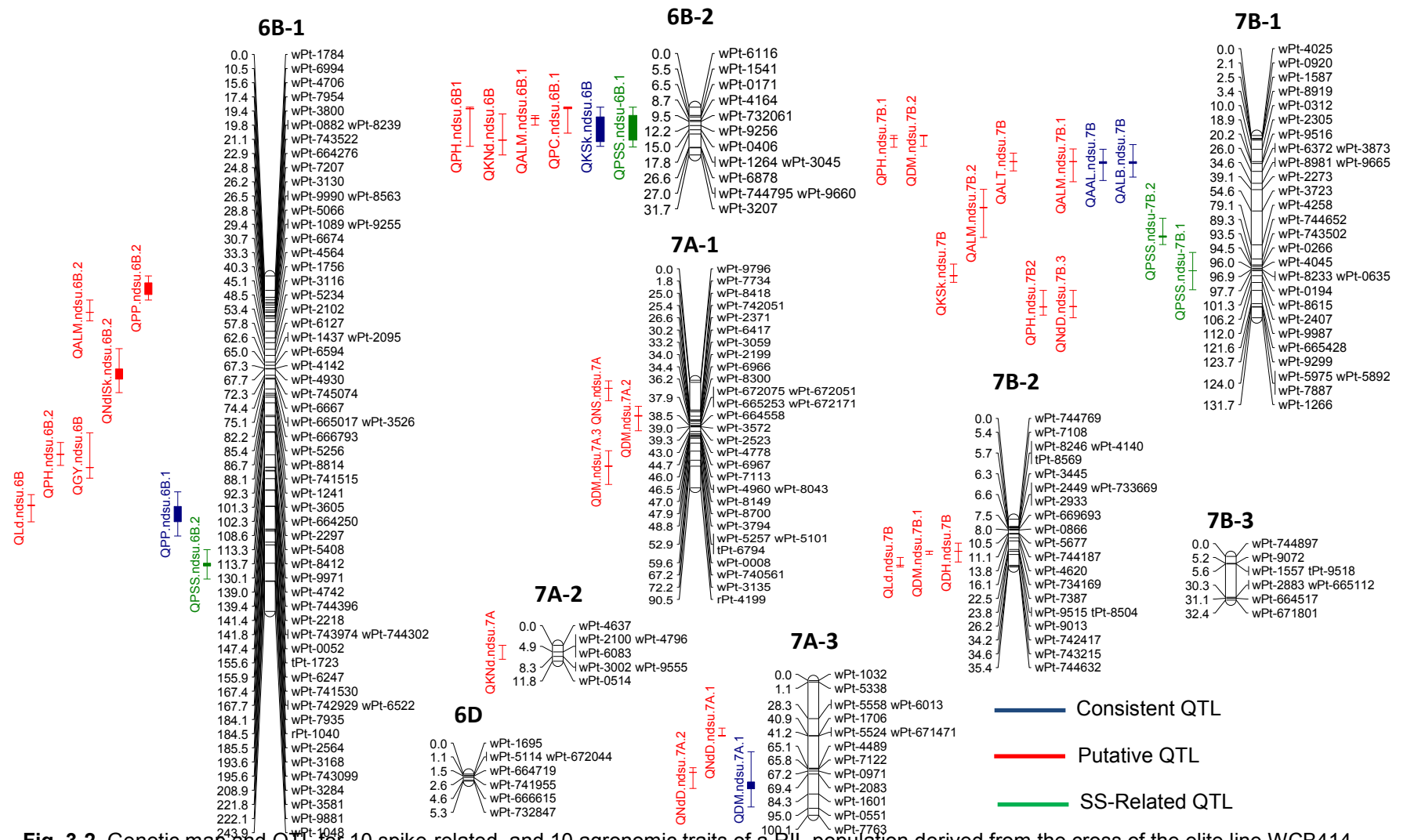


Fig. 3-2. Genetic map and QTL for 10 spike-related, and 10 agronomic traits of a RIL population derived from the cross of the elite line WCB414 and the exotic line WCB617 with SS trait, over four to six locations in ND, USA during 2009, 2010, and 2011 (Continued)

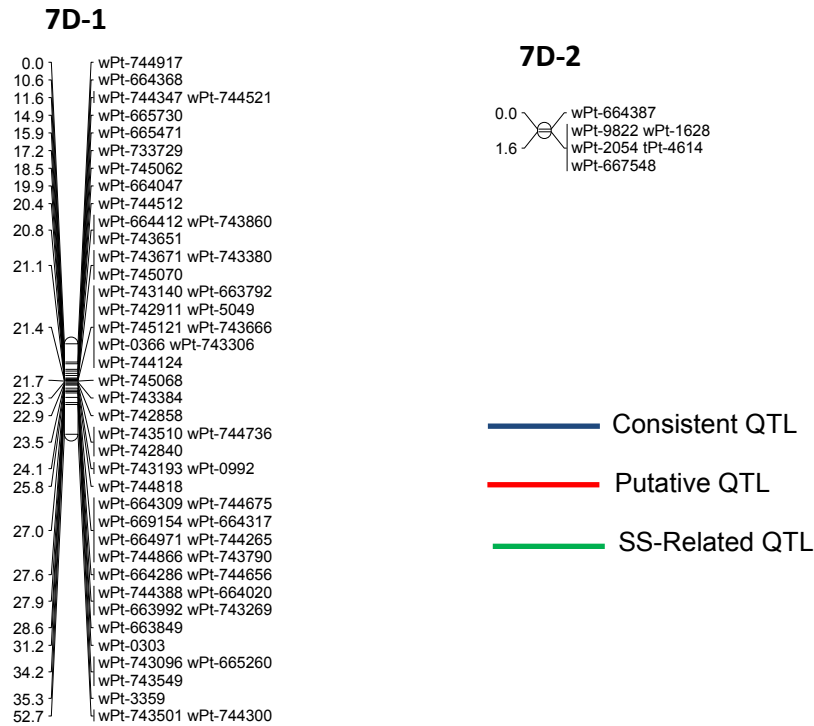


Fig. 3-2. Genetic map and QTL for 10 spike-related, and 10 agronomic traits of a RIL population derived from the cross of the elite line WCB414 and the exotic line WCB617 with SS trait, over four to six locations in ND, USA during 2009, 2010, and 2011 (Continued)

The genetic analysis of the RIL population resulted in the identification of two consistent QTL (*QKS.ndsu.1A1* and *QKS.ndsu.4A*) and three putative QTL for KS (*QKS.ndsu.1B*, *QKS.ndsu.2A*, and *QKS.ndsu.2D*) (Table 3-6, Figure 3-2). These QTL had minor effects and explain PV ranging from 6.22% to 12.53%. Together these QTL explained 7.1-20.2 of PV in different environments. Additive effects showed that the branched parent (WCB617) contributed the alleles for increased KS at *QKS.ndsu.1A1* and *QKS.ndsu.4A* loci, while the alleles for increase KS values at *QKS.ndsu.1B*, *QKS.ndsu.2A*, and *QKS.ndsu.2D* loci were contributed by WCB414.

A total of eight QTL were associated to NNdISk, three of which were consistent (*QNNdISk.ndsu.1A.1*, *QNNdISk.ndsu.2D*, and *QNNdISk.ndsu.6A.2*) and five were putative (*QNNdISk.ndsu.2A*, *QNNdISk.ndsu.2B.1*, *QNNdISk.ndsu.2B.2*, *QNNdISk.ndsu.3D*, and *QNNdISk.ndsu.6B.2*) (Table 4 Fig.2) and explained 34.2-43.6% of PV in different environments. Only *QNNdISk.ndsu.2D* was a major QTL which explained 21.32% to 34.22% of the PV for NNdISk in different environments. The other QTL had minor effects and explain 3.72% to 12.63% of PV of this trait. Additive

effects showed that most of the alleles that increased the values of NNdISk were contributed by WCB617. The exceptions were observed for QTL *QNNdISk.ndsu.3D* and *QNNdISk.ndsu.6B.2* where the elite parent WCB414 contributed the positive alleles to increase NNdISk (Table 3-6).

In this population, our results showed that NS was controlled by 14 QTL. This trait was studied in five environments and none QTL was detected in at least 50% of them (Table 3-6). Therefore, *QNS.ndsu.1A*, *QNS.ndsu.1D.1*, *QNS.ndsu.1D.2*, *QNS.ndsu.2A*, *QNS.ndsu.2D*, *QNS.ndsu.3A*, *QNS.ndsu.3B*, *QNS.ndsu.3D*, *QNS.ndsu.4B*, *QNS.ndsu.5A*, *QNS.ndsu.5B.1*, *QNS.ndsu.5B.2*, *QNS.ndsu.5B.3*, and *QNS.ndsu.7A* are putative QTL for NS. Among these, three of those QTL (*QNS.ndsu.2A*, *QNS.ndsu.5A.1* and *QNS.ndsu.7A3*) were major QTL explaining 15.9% to 20.6% of PV. The other QTL were minor explaining 4.84% to 11.63% of the PV. The PVE explained by these QTL in the environments ranked from 11.6% to 54.5%. The alleles for increased trait values were contributed by WCB414 at all the loci, except *QNS.ndsu.3D* and *QNS.ndsu.5B3*.

Composite interval mapping for GY identified two consistent (*QGY.ndsu.1D* and *QGY.ndsu.2D.1*) and seven putative QTL (*QGY.ndsu.1A*, *QGY.ndsu.2A.1*, *QGY.ndsu.2A.2*, *QGY.ndsu.2D.2*, *QGY.ndsu.3A* and *QGY.ndsu.6B*) (Table 3-6, Figure 3-2) which explained 8.8-58.6% of PV in different environments. *QGY.ndsu.2D.1* explained up to 40.34% of PV and can be considered a major QTL. The PVE by other QTL ranged from 4.6% to 9.76% and they were considered as minor QTL. The elite parent (WCB414) contributed the alleles for increased GY at all the loci except *QGY.ndsu.3A* and *QGY.ndsu.6B*.

3.4.5. QTL for important agronomical traits

In this population, data analysis showed that DH is controlled by two consistent (*QDH.ndsu.2D* and *QDH.ndsu.3A*) and six putative QTL (*QDH.ndsu.1A.1*, *QDH.ndsu.3B.1*, *QDH.ndsu.3B.2*, *QDH.ndsu.3D*, *QDH.ndsu.6A*, and *QDH.ndsu.7B*) (Table 3-6, Figure 3-2), which together explained 26.4-43.2% of PV in different environments. One QTL on chromosome 2D (*QDH.ndsu.2D*) and another on chromosome 3A (*QDH.ndsu.3A*) have major effect, explaining up to 21.12% and 15.75% of PV, respectively. The other QTL are minor and explained between 6.73% and 11.09% of PV for DH. The branched parent (WCB617) provided the allele that increased the phenotypic value of DH at most of the loci, except at *QDH.ndsu.3A1* and *QDH.ndsu.6A*.

Table 3-6. QTL identified for agronomic traits in a RIL population of hexaploid wheat derived from the cross of WCB414 and WCB617

QTL	Environment [†]	Flanking markers	Pos. [‡] (cM)	CI [§] (cM)	LOD	Thresh	a [#]	R ² (%)
Kernels per spikelet								
<i>QKSk.ndsu.1B</i>	II	wPt-9809-wPt-5061	79.5	71.5-95.3	4.7	3.2	0.1	9.8
<i>QKSk.ndsu.2B</i>	I, IV, AE	wPt-744808-wPt-4368	22.0-28.0	9.3-42.0	3.1-6.8	3.1-3.4	0.1-0.2	3.9-13.5
<i>QKSk.ndsu.2D</i>	I, II, III, IV, AE	wPt-666656-wPt-671914	50.1-74.8	48.4-76.6	14.2-21.0	3.1-3.4	0.2-0.3	21.5-38.8
<i>QKSk.ndsu.6B</i>	I, III, IV, AE	wPt-0171-wPt-6878	6.5-22.8	0-26.0	2.8-4.0	3.1-3.4	0.1-0.1	3.1-7.1
<i>QKSk.ndsu.7B</i>	IV	wPt-8615-wPt-2407	101.3	92.9-106.1	4.3	3.3	-0.1	5.2
							RTPVE ^{††}	34.7-48.6
Kernels per node								
<i>QKNd.ndsu.1A</i>	II, IV, AE	wPt-8172-wPt-9429	104.9-113.9	88.9-132.7	2.9-4.4	3.1-3.3	-0.1_-0.1	8.3-11.3
<i>QKNd.ndsu.1D</i>	III	wPt-734132-wPt-3945	129.5	103.7-140.7	4.3	3.2	0.1	8.7
<i>QKNd.ndsu.2B</i>	III	wPt-7859-wPt-1133	0.0	0-2.9	6.3	3.2	0.1	13.1
<i>QKNd.ndsu.3A</i>	IV	wPt-9303-wPt-740730	13.5	8.5-27.5	3.3	3.05	0.1	6.9
<i>QKNd.ndsu.4A</i>	III, AE	wPt-2946-tPt9400	4.9-6.9	0-22.5	2.7-3.2	3.2-3.3	-0.1_-0.1	5.3-7.6
<i>QKNd.ndsu.5B</i>	I	wPt-6014-wPt-7006	183.7	167.8-194.6	4.4	3.2	0.1	12.9
<i>QKNd.ndsu.6B</i>	III	wPt-1264-wPt-6878	21.8	14.5-31.7	3.1	3.1	-0.1	8.9
<i>QKNd.ndsu.7A</i>	I	wPt-4637-wPt-2100	0.0	0-10.4	4.2	3.2	0.1	9.2
							RTPVE ^{††}	15.9-27.1
Kernels per spike								
<i>QKS.ndsu.1A</i>	I, IV, AE	wPt-1924-wPt-667180	16.4-20.6	8.7-30.3	2.9-4.0	3.2-3.4	-0.9_-2.0	6.2-8.9
<i>QKS.ndsu.1B</i>	II	wPt-665204-wPt-665037	1.5	0-10.5	4.8	3.3	1.8	11.0
<i>QKS.ndsu.2A</i>	IV	wPt-740658-wPt-664128	102.8	85.2-121.5	4.5	3.2	2.0	12.3
<i>QKS.ndsu.2D</i>	IV	wPt-3692-wPt-6780	127.7	113.9-131.9	3.3	3.2	1.5	7.9
<i>QKS.ndsu.4A</i>	II, IV, AE	wPt-2247-wPt-9400	0.0-9.9	0-22.7	3.1-5.4	3.2-3.4	-1.2_-1.7	7.3-12.5
							RTPVE ^{††}	7.1-20.2

(Continues)

Table 3-6. QTL identified for agronomic traits in a RIL population of hexaploid wheat derived from the cross of WCB414 and WCB617 (Continued)

QTL	Environment [†]	Flanking markers	Pos. [‡] (cM)	CI [§] (cM)	LOD	Thresh [¶]	a [#]	R ² (%)
<i>Number of nodes with immature spikelets at the spike base</i>								
QNNdISk.ndsu.1A.1	II, IV, AE	wPt-8172-wPt-3272	107.9-130.0	80.8-143.4	2.6-5.4	3.1-3.5	-0.2_-0.3	4.0-12.6
QNNdISk.ndsu.2A	III	wPt-0003-wPt-4201	143.4	125-149.8	3.9	3.4	-0.4	10.4
QNNdISk.ndsu.2B.1	II	wPt-744808-wPt-4368	22.0	10.5-36.4	4.0	3.1	-0.2	6.2
QNNdISk.ndsu.2B.2	AE	wPt-1133-wPt-9274	23.0	9.6-30.2	3.5	3.5	-0.2	5.1
QNNdISk.ndsu.2D	I, II, III, IV, AE	wPt-8134-wPt-3144	32.4-41.8	28.3-43.9	10.0-17.7	3.1-3.5	-0.5_-0.7	21.3-34.2
QNNdISk.ndsu.3D	IV	wPt-741510-wPt-742339	3.6	0-15.6	4.3	3.2	0.3	5.9
QNNdISk.ndsu.6A.2	II, IV	wPt-671561-wPt-0562	43.6	33.1-53.9	3.3-4.1	3.1-3.2	-0.2_-0.2	5.1-5.6
QNNdISk.ndsu.6B.2	III, AE	wPt-4930-wPt-666793	67.7-75.1	52.9-84.9	2.7-3.5	3.4-3.5	0.2-0.3	3.7-5.7
							RTPVE ^{†††}	34.2-43.6
<i>Number spikes/area</i>								
QNS.ndsu.1A	II, AE	wPt-9429-wPt-6654	117.1-119.1	106.5-132.2	3.8-5.2	3.3	8.3-11.0	6.6-11.6
QNS.ndsu.1D.1	IV	wPt-733835-wPt-672077	85.5	60.5-98.9	3.5	3.1	11.2	8.2
QNS.ndsu.1D.2	AE	wPt-4497-wPt-5253	151.1	144.8-153	6.1	3.3	10.2	10.6
QNS.ndsu.2A	V, VI, AE	wPt-798339-wPt-2850	37.0-52.2	33.1-65.4	4.1-4.8	3.2-3.3	12.9-23.5	11.7-15.9
QNS.ndsu.2D	III, VI, AE	wPt-730427-wPt-665836	105.4-109.0	94.3-125.5	2.7-5.8	3.2-3.3	9.9-11.8	4.8-10.0
QNS.ndsu.3A	IV	wPt-7890-wPt-1353	86.4	80.9-91.6	5.3	3.1	12.7	10.9
QNS.ndsu.3B	VI	rPt-5396-wPt-731910	134.6	127.9-147.7	3.7	3.2	12.2	6.8
QNS.ndsu.3D	IV, VI	wPt-741522-wPt-9401	15.8-17.8	1.8-42.3	2.7-2.7	3.1-3.2	-9.4_-10.6	4.8-5.5
QNS.ndsu.4B	IV	tPt-0602-wPt-732423	3.0	0-11.5	3.4	3.1	12.0	9.0
QNS.ndsu.5A	I, III, AE	wPt-9094-wPt-8226	20.0-23.2	1.9-24.3	2.8-8.1	3.2-3.3	12.2-13.3	7.5-15.6
QNS.ndsu.5B.1	I	rPt-6127-wPt-5737	17.8	6.1-26.4	4.4	3.2	15.3	9.5
QNS.ndsu.5B.2	III	wPt-6465-wPt-3922	238.1	221.8-239.2	3.7	3.3	18.1	8.2
QNS.ndsu.5B.3	III	wPt-2373-wPt-8449	251.8	251.4-258.3	3.6	3.3	-17.2	7.8
QNS.ndsu.7A	IV	wPt-7734-wPt-8418	6.8	0.6-17.2	6.7	3.1	17.7	20.59
							RTPVE ^{†††}	11.6-54.5

(Continues)

Table 3-6. QTL identified for agronomic traits in a RIL population of hexaploid wheat derived from the cross of WCB414 and WCB617 (Continued)

QTL	Environment [†]	Flanking markers	Pos. [‡] (cM)	CI [§] (cM)	LOD	Thresh [¶] .	a [#]	R ² (%)
Grain yield								
QGY.ndsu.1A	III	wPt-1924-wPt-734000	15.4	11.0-19.9	4.6	3.3	211.9	8.0
QGY.ndsu.1D	III, IV, AE	wPt-733835-wPt-3945	90.5-129.5	66.5-140.5	2.9-3.6	3.3-3.4	142.2-176.6	5.0-8.5
QGY.ndsu.1D	IV	wPt-4497-wPt-5253	151.1	140.7-153	3.8	3.3	169.6	5.7
QGY.ndsu.2A1	AE	wPt-668027-wPt-798459	30.51	24.6-44.7	4.9	3.4	215.7	9.8
QGY.ndsu.2A2	AE	wPt-664128-wPt-7721	113.5	95.9-121.7	3.6	3.4	133.2	6.9
QGY.ndsu.2D1	I, III, IV, AE	wPt-668017-wPt-4242	82.3-89.7	71.8-92.7	4.7-20.0	3.3-3.4	198.6-439.6	10.6-40.3
QGY.ndsu.2D2	III	wPt-3692-wPt-6780	126.7	120.8-131.9	4.9	3.3	218.8	8.7
QGY.ndsu.3A	AE	wPt-741816-wPt-743909	3.6	0-11.3	3.5	3.4	-112.1	4.6
QGY.ndsu.6B	IV	wPt-744396-wPt-2218	139.4	114.1-147.1	4.0	3.3	-168.4	6.1
							RTPVE ^{††}	8.8-58.6
Plant height								
QPH.ndsu.1B.1	II, VI	wPr1560-wPt-0308	4.5-5.5	0-16.4	3.8-6.6	3.3-3.4	3.3-3.3	7.8-14.7
QPH.ndsu.1B.2	IV, V, AE	wPt-671415-wPt-664989	0.0	0-14.6	3.2-3.3	3.3-3.4	2.0-2.6	5.9-6.3
QPH.ndsu.1D	III, IV, AE	wPt-672077-wPt-730172	119.0-120.0	107.2-135.5	2.8-4.7	3.1-3.4	-2.7_-4.1	9.0-15.9
QPH.ndsu.2D	V, AE	wPt-5014-wPt-731406	59.4-76.8	48.5-81.5	2.9-9.6	3.3-3.4	2.0-3.9	5.3-19.1
QPH.ndsu.4B	III, IV, V, VI, AE	wPt-732423-wPt-744434	23.0-29.6	11.6-42.3	2.81-7.3	3.1-3.4	-2.0_-3.5	5.3-13.8
QPH.ndsu.5B	V	wPt-6191-wPt-6014	141.8	122.3-158.0	3.4	3.3	2.4	8.5
QPH.ndsu.6B.1	VI	wPt-6116-wPt-1541	1.0	0-26	3.4	3.3	-2.4	6.9
QPH.ndsu.6B.2	II	wPt-8412-wPt-9971	129.7	121.1-137.8	6.3	3.4	4.5	13.9
QPH.ndsu.7B.1	II	wPt-0920-wPt-1587	2.1	0-8.5	5.2	3.4	4.0	10.8
QPH.ndsu.7B.2	III, IV, AE	wPt-9299-wPt-1266	123.7-124.0	112.0-129.8	4.2-5.4	3.1-3.4	-2.6_-3.4	8.4-10.8
							RTPVE ^{††}	26.9-47.3
Lodging								
QLd.ndsu.1A	VI	wPt-729832-wPt-3698	67.1	62.6-74.5	5.6	3.4	-6.5	12.4
QLd.ndsu.2A.1	III	wPt-2850-wPt-8068	89.7	81.5-106.3	5.6	3.4	-11.8	18.3
QLd.ndsu.2A.2	V	wPt-668027-wPt-798459	29.5	24.2-34.6	5.0	3.1	6.6	22.8

(Continues)

Table 3-6. QTL identified for agronomic traits in a RIL population of hexaploid wheat derived from the cross of WCB414 and WCB617 (Continued)

QTL	Environment [†]	Flanking markers	Pos. [‡] (cM)	CI [§] (cM)	LOD	Thresh	a [#]	R ² (%)
Lodging								
<i>QLd.ndsu.3A</i>	III	tPt-1079-wPt-1464	36.3	14.0-43.8	4.6	3.4	7.9	9.4
<i>QLd.ndsu.3D</i>	I, AE	wPt-742339-wPt-740580	7.9-8.9	0.4-21.9	3.5-7.3	3.3	6.8-12.6	11.9-20.3
<i>QLd.ndsu.4B</i>	I, VI, AE	wPt-732423-wPt-8892	20.0-23.0	12.1-36.8	3.7-7.6	3.3	-6.5_-10.6	8.2-18.3
<i>QLd.ndsu.6B</i>	AE	wPt-6247-wPt-741530	166.9	159.0-178.8	5.2	3.3	5.1	10.5
<i>QLd.ndsu.7B</i>	AE	wPt-742417-wPt-743215	34.2	28.4-35.4	3.8	3.3	-4.5	7.4
							RTPVE ^{††}	20.1-56.5
Days to heading								
<i>QDH.ndsu.1A.1</i>	III, IV, AE	wPt-1924-wPt-667180	15.4-20.6	10.7-26.5	5.6-6.3	3.2	-1.0_-1.4	9.2-10.9
<i>QDH.ndsu.2D</i>	I, II, III, IV, V, AE	wPt-3812-wPt-5014	45.5-76.1	27.4-78.0	3.7-10.9	3.2-3.4	-0.7_-1.3	6.0-21.1
<i>QDH.ndsu.3A</i>	I, III, V, AE	wPt-6854-wPt-0286	112.5-128.2	96.6-148.2	2.7-6.6	3.2-3.4	1.2-1.9	8.4-15.8
<i>QDH.ndsu.3B.1</i>	IV	wPt-667895-wPt-6834	39.0	27.2-44.3	5.4	3.2	-0.9	10.3
<i>QDH.ndsu.3B.2</i>	V	wPt-731663-wPt-4412	74.3	66.1-80.0	3.5	3.3	-1.2	6.9
<i>QDH.ndsu.3D</i>	I, AE	wPt-740580-wPt-6169	29.1-41.2	6.8-46.9	2.9-4.7	3.2-3.3	-0.8_-1.4	5.8-11.1
<i>QDH.ndsu.6A</i>	II	wPt-731592-wPt-9089	3.2	0-12.7	3.8	3.4	0.6	6.7
<i>QDH.ndsu.7B</i>	V	wPt-9515-wPt-9013	23.8	17.8-31.8	5.3	3.3	-1.4	8.9
							RTPVE ^{††}	26.4-43.2
Days to maturity								
<i>QDM.ndsu.1A</i>	III	wPt-666424-wPt-667458	3.1	0-6.9	3.8	3.2	-0.7	7.7
<i>QDM.ndsu.3A.1</i>	I, III, IV, V, VI AE	wPt-6854-wPt-0286	111.5-134.2	98.1-148.0	2.9-7.6	3.2-3.3	0.6-2.1	8.2-25.0
<i>QDM.ndsu.3A.2</i>	III	wPt-1562-wPt-2740	161.4	150.2-165.9	4.6	3.2	0.9	12.9
<i>QDM.ndsu.3B</i>	V	wPt-731663-wPt-4412	75.3	67.0-80.6	3.8	3.2	-1.2	9.2
<i>QDM.ndsu.3D</i>	I, AE	wPt-6169-wPt-9258	43.7-45.7	36.4-46.9	5.0-5.4	3.3	-0.8_-1.4	9.2-11.7
<i>QDM.ndsu.7A.1</i>	IV, VI, AE	wPt-2083-wPt-1601	75.4-80.4	52.9-93.9	4.4-5.5	3.2-3.3	0.8-1.2	10.9-15.4
<i>QDM.ndsu.7A.2</i>	VI, AE	wPt-2371-wPt-3059	29.6-30.2	21.9-42.5	4.1-4.6	3.3	0.7-1.1	7.5-9.3
<i>QDM.ndsu.7A.3</i>	I	wPt-3135-rPt-4199	72.2	59.3-87.6	3.8	3.3	1.0	7.5

(Continues)

Table 3-6. QTL identified for agronomic traits in a RIL population of hexaploid wheat derived from the cross of WCB414 and WCB617 (Continued)

QTL	Environment [†]	Flanking markers	Pos. [‡] (cM)	CI [§] (cM)	LOD	Thresh [¶] .	a [#]	R ² (%)
Days to maturity								
<i>QDM.ndsu.7B.1</i>	V	wPt-9515-wPt-9013	23.8	23.7-26.1	5.5	3.2	-1.4	11.0
<i>QDM.ndsu.7B.2</i>	IV	wPt-4025-wPt-0920	0.0	0-7.8	4.7	3.2	0.6	10.1
							RTPVE ^{††}	28.7-52.7

[†]I, Prosper 2009; II, Carrington 2009; III, Prosper 2010; IV, Carrington 2010; V, Prosper 2011; VI, Carrington 2011.

[‡]Position

[§]Confidence Interval

[¶]Threshold calculated by permutation test.

[#]Additive effects

^{††}rank of phenotypic variation explained per environment

Composite interval mapping analysis for DM resulted in the identification of one consistent QTL (*QDM.ndsu.3A1*) and seven putative QTL (*QDM.ndsu.1A*, *QDM.ndsu.3A.2*, *QDM.ndsu.3B*, *QDM.ndsu.3D*, *QDM.ndsu.7A.1*, *QDM.ndsu.7A.2*, *QDM.ndsu.7A.3*, *QDM.ndsu.7B.1* and *QDM.ndsu.7B.2*) (Table 3-6, Fig.3-2). The QTL *QDM.ndsu.3A.1* and *QDM.ndsu.7A.1* were major and explained up to 24.97% and 15.41% of PV, respectively. Together, the QTL associated with DM explained 28.7-52.7% of PV in different environments. The elite parent WCB414 provided alleles that increased the phenotypic values of DM at *QDM.ndsu.3A1*, *QDM.ndsu.3A.2*, *QDM.ndsu.7A.1*, *QDM.ndsu.7A.2*, *QDM.ndsu.7A.3*, and *QDM.ndsu.7B.2*; while the branched parent WCB617 contributed with alleles that increased DM at *QDM.ndsu.1A*, *QDM.ndsu.3B*, *QDM.ndsu.3D*, and *QDM.ndsu.7B.1*.

In this RIL population, the segregation for PH was controlled by one consistent QTL (*QPH.ndsu.4B*) and nine putative QTL (*QNS.ndsu.1B.1*, *QPH.ndsu.1B.2*, *QPH.ndsu.1D*, *QPH.ndsu.2D*, *QPH.ndsu.5B*, *QPH.ndsu.6B.1*, *QPH.ndsu.6B.2*, *QPH.ndsu.7B.1* and *QPH.ndsu.7B.2*) (Table 3-6, Figure 3-2). Two major QTL were identified for PH, one each on chromosomes 1D and 2D. The QTL on chromosome 1D (*QPH.ndsu.1D*) explained up to 15.90% of PV, while the QTL on chromosome 2D explained up to 19.12% of PV for PH. The minor QTL explained between 5.28% and 14.70% of PV for PH. Together these QTL explained 26.9-47.3% of PV. The alleles for increased PH at *QPH.ndsu.1B1*, *QPH.ndsu.1B2*, *QPH.ndsu.2D*, *QPH.ndsu.5B*, *QPH.ndsu.6B2*, and *QPH.ndsu.7B1* loci were contributed by WCB414; while WCB617 provided alleles for increased PH at *QPH.ndsu.1D*, *QPH.ndsu.4B*, *QPH.ndsu.6B1* and *QPH.ndsu.7B2* loci.

Composite interval mapping for Ld resulted in the detection of one consistent (*QLd.ndsu.4B*) and seven putative QTL (*QLd.ndsu.1A*, *QLd.ndsu.2A.1*, *QLd.ndsu.2A.2*, *QLd.ndsu.3A*, *QLd.ndsu.3D*, *QLd.ndsu.6B* and *QLd.ndsu.7B*) (Table 3-6, Figure 3-2) and explained 20.1-56.5% of PV depending of environments. Among these QTL, *QLd.ndsu.2A.1*, *QLd.ndsu.2A.2*, *QLd.ndsu.3D* and *QLD.ndsu.4B* have major effect and explained up to 18.3%, 22.8%, 20.3% and 18.3% of PV, respectively. The elite parent (WCB414) contributed with alleles for increased Ld at *QLd.ndsu.2A.2*, *QLd.ndsu.3A*, *QLd.ndsu.3D*, and *QLd.ndsu.6B* loci, while WCB617 provided alleles that increased Ld at *QLd.ndsu.1A*, *QLd.ndsu.2A1*, *QLd.ndsu.4B*, and *QLd.ndsu.7B* loci.

3.4.6. Distribution of QTL at whole genome level and important QTL clusters

A total of 145 QTL on 17 chromosomes were identified in this study. Out of these, 40 were consistent QTL and 105 were putative QTL; meanwhile a total of 28 QTL were major (explained more than 15% of PV) and 117 QTL were minor QTL. A maximum of 16 QTL were identified on chromosome 1A in this study, followed by chromosomes 5B and 7B with 13 QTL each. Chromosomes 2A, 2D, and 6B had each 12 QTL; chromosomes 3A and 1D had 8 QTL each; chromosomes 4A, and 7A had 7 QTL each; chromosomes 1B, 3B, 4B and 6A had each 6 QTL; chromosomes 2B and 3D had 5 QTL each; and chromosome 5A had 3 QTL. No QTL were observed in the linkage groups belonging to chromosomes 5D, 6D and 7D. The B-genome had the highest number of QTL (61), followed by A-genome (59), and D-genome (25). The largest clusters of consistent QTL were located on chromosomes 1A and 2D. On chromosome 1A eight QTL were detected on the short arm (*QLPP.ndsu.1A1*, *QLPP.ndsu.1A2*, *QSL.ndsu.1A1*, *QNd.ndsu.1A.1*, *QDH.ndsu.1A.1*, *QDM.ndsu.1A*, *QGY.ndsu.1A*, and *QKS.ndsu.1A*) and eight QTL on long arm. In chromosome 2DS eight QTL (*QKSk.ndsu.2D*, *QNNdISk.ndsu.2D*, *QGY.ndsu.2D1*, *QDH.ndsu.2D*, *QSL.ndsu.2D*, *QNd.ndsu.2D*, *QNdD.ndsu.2D*, *QALT.ndsu.2D*) were grouped close to the major QTL for SS (*QSS.ndsu.2D*).

Other clusters of at least three consistent QTL were observed on 1DL (*QGY.ndsu.1D*, *QPH.ndsu.1D*, and *QNd.ndsu.1D*), 2A (*QNS.ndsu.2A*, *QAless.ndsu.2A.1* and *QAless.ndsu.2A.2*), 4BL (*QPH.ndsu.4*, *QLd.ndsu.4B*, and *QSL.ndsu.4B*), and 5BS (*QALM.ndsu.5B*, *QALT.ndsu.5B*, *QAAL.ndsu.5B* and *QPP.ndsu.5B*).

3.5. Discussion

3.5.1. Phenotypic variation

The cross between a branched and pubescent exotic line (WCB617) with a glabrous and conventional-spike-type elite line (WCB414) resulted in the segregation of a large number of spike-related traits. Most of these traits had transgressive segregation, and in the cases of PC and Aless traits their expression was absent in the parents. This situation reveals the presence of new allelic combinations which are usually absent in the elite × elite cross where most of the alleles are fixed.

Genotypes with the clavate trait also expressed fusiform spikes, while some RIL with glume pubescences had plants with glabrous spikes. A similar situation known as heterobranching was described for the SS trait in this population (Chapter 2). The presence of seed admixture as a cause for these phenomena was discarded considering that the seeds from each genotype were derived from individual head-rows. To the best of our knowledge, this was not reported previously (for the presence of clavate architecture and glume pubescences). Therefore, we named these novel phenotype as hetero-clavate and hetero-pubescent phenotypes and the causes of their expression need to be clarified.

This was the first time that a RIL population segregating for SS was used to study spike-related and agronomical important traits. With some exceptions, PSS and PP had the same type of correlations with other traits (Table 3-2, and Table 3-4). Both traits are positively correlated with SL, Nd, NdD, NNdISk, and DH; but negatively associated to awn lengths, NS, KSk, TKW and GY. These results demonstrate a poor agronomic performance of branched lines and pubescent lines. Previous studies also indicated a poor agronomic performance of genotypes with SS (Percival, 1921; Koric, 1973; Millet, 1986, 1987; Hucl and Fowler, 1992; Zhang et al., 2012).

The genetics of Nd have been studied previously by counting the number of spikelets per spike (Sk) (Araki et al., 1999; Kato et al., 2000; Sourdille et al., 2000a; Li et al., 2002; Sourdille et al., 2003; Jantasuriyarat et al., 2004; Kumar et al., 2007; Li et al., 2007; Chu et al., 2008; Cui et al., 2012). In these studies, the spikes had one spikelet per rachis node making Nd and Sk equivalent. In our study, however, RILs with SS have different value for Nd and Sk. In fact, we studied the genetics of Sk as a component of SS in a previous investigation (Chapter 2). Likewise, the trait NdD is comparable to the trait spike density (SD) or compactness reported in previously (Sourdille et al., 2000b; Sourdille et al., 2003; Jantasuriyarat et al., 2004; Marza et al., 2006; Ma et al., 2007; Chu et al., 2008; Cui et al., 2012). Spike density is determined as the ratio between Sk and SL. However, based on our previous work (Chapter 2), where it was considered SS and SD as the same trait, we determined NdD as the ratio between Nd and SL.

3.5.2. QTL for spike-related traits

In the present study, the identification of relatively few major and several minor genes for all spike-related traits shows that these traits have a polygenic control. The detection of some consistent and a number of environment specific QTL for all those traits suggests that the inheritance of these traits are

also influenced by the environmental factors. Quantitative genetic control and/or influence of environment on these traits is in agreement with previous studies (Araki et al., 1999; Kato et al., 2000; Sourdille et al., 2000a; Börner et al., 2002; Li et al., 2002; Sourdille et al., 2003; Jantasuriyarat et al., 2004; Verma et al., 2005; Marza et al., 2006; Kumar et al., 2007; Ma et al., 2007; Chu et al., 2008; Heidari et al., 2011; Cui et al., 2012).

Overlapping between positions and/or confidence intervals of QTL for SL and Nd were observed in this population (Table 3-5; Fig. 3-2) demonstrating a close linkage between genes and/or pleiotropic effects of a gene on these spike-dimension-related traits. These situations were observed at *QSL.ndsu.1A.1* and *QNd.ndsu.1A.1*; *QSL.ndsu.1A.2* and *QNd.ndsu.1A.2*; *QSL.ndsu.2D* and *QNd.ndsu.2D*; and *QSL.ndsu.4B* and *QNd.ndsu.4B*. This confirms the positive correlations observed between SL and Nd (Table 3-2). *QSL.ndsu.1A1* and *QNd.ndsu.1A1* were consistent QTL for SL and Nd identified on 1AS (Table 3-5; Fig 3-2). Interestingly, chromosome 1A has been recognized in most of the previous (Sourdille et al., 2000; Li et al., 2002; Marza et al., 2006; Kumar et al., 2007; Ma et al., 2007; Heidari et al., 2011) as a carrier of SL genes; whereas the presence of genes associated with Nd on chromosome 1A only was reported in two previous studies (Heidari et al., 2011; Cui et al., 2012). Although the QTL, *QSL.ndsu.2D* and *QNd.ndsu.2D* were consistent for these spike-dimension traits, *QNd.ndsu.2D* was a major QTL while *QSL.ndsu.2D* was minor QTL. The position of these QTL on 2DS chromosome coincides with previous QTL found for SL and Nd (Sourdille et al., 2000b; Li et al., 2002; Verma et al., 2005; Kumar et al., 2007). Certainly, these QTL are located on a highly rich gene region of the wheat genome (Erayman, 2004) in which the major gene for SS was also located in this population (Chapter 2). On the other hand, although *QSL.ndsu.4B* and *QNd.ndsu.4B* were also detected in the same position on 4BS, it is important to note that *QSL.ndsu.4B* was a consistently detected in two environments and AE; while *QNd.ndsu.4B* was detected in Prosper 2010 and AE only (Table 3-5; Fig 3-2). We believe this is the first time that QTL for SL and Nd are detected on chromosome 4B.

Unique SL and Nd QTL were observed on 2BS (*QSL.ndsu.2B*), 4AL (*QSL.ndsu.4A*, *QNNd.ndsu.4A*), 1DL (*QNd.ndsu.1D*), and 2A (*QNNd.ndsu.2A*). Among these QTL, it is worth to focus on *QSL.ndsu.4A* and *QNd.ndsu.1D*. The putative QTL for SL located on 4AL, *QSL.ndsu.4A*, was a major QTL that explained up to 17.8 % of PV. Most of the previous QTL-mapping studies for SL have also

detected a QTL for this trait on 4AL (Börner et al., 2002; Li et al., 2002; Jantasuriyarat et al., 2004; Kumar et al., 2007; Chu et al., 2008). Meanwhile, *QNd.ndsu.1D* was a minor QTL, but it was consistently detected in Prosper 2009, 2010 and AE. This QTL was located in a cluster of genes which will be discussed later. At the QTL associated to SL and Nd, the exotic parent WCB617 contributed the alleles that increased phenotypic values of these traits. This is consistent with the differences for SL and Nd between the parents, which resulted in favor of WCB617 in all the environments (Appendix Table B1).

The identification of one QTL associated to NdD on chromosome 2D (*QNdD.ndsu.2D*) is in agreement with previous reports by Sourdille et al. (2000b, 2003), Ma et al. (2007), Heidari et al. (2010), and Cui et al. (2012). However, the PV explained by *QNdD.ndsu.2D* is higher compared to the previous studies. *QNdD.ndsu.2D* explained up to 35.1% of PV (Table 3-5), while the maximum PV reported by Sourdille et al. (2000b), Ma et al. (2007), Heidari et al. (2011), Cui et al. (2012) were 11.2%, 23.2%, 26.1%, 13.54%, respectively. The QTL on chromosome 2D was located in the same region where *QSS.ndsu.2D*, a major QTL for SS was detected (Chapter 2). Interestingly, although NdD is derived from the ratio of Nd and SL, none QTL detected for NdD shared the same positions as QTL for Nd and SL. Instead, NdD and PC shared a minor a putative QTL on 5BL (*QNdD.ndsu.5B* and *QPC.ndsu.5B.1*), which is in agreement with the fact that clavate spikes are produced by changes in spike density. The identification of *QNdD.ndsu.5B* on 5BL was confirmed by previous results reported by Ma et al. (2007) and Chu et al. (2008) for SD; while the discovery of QTL on chromosome 7B was also confirmed for SD by Marza et al. (2006). To our best knowledge, this is the first time that QTL for NdD and indirectly for SD (for non-SS genotypes) are reported on chromosome 7A (*QNdD.ndsu.7A1*, *QNdD.ndsu.7A2*).

The PC was expressed in a total of 27 RIL but was not expressed by either parent. Therefore we assumed that this trait is the result of recombination process between WCB414 and WCB617 and epistatic interactions. The identification of seven QTL associated with PC (*QPC.ndsu.1B*, *QPC.ndsu.4A*, *QPC.ndsu.3B1*, *QLPC.ndsu.3B2*, *QPC.ndsu.5B1*, *QPC.ndsu.5B2* and *QPC.ndsu.6B1*) suggests that this trait is quantitatively inherited. To the best of our knowledge, this is also the first time that clavate spike architecture was studied and genetically dissected in common wheat. The most similar studies on clavate-like spike have been conducted on club wheat sub-species. In club wheat, the spikes are uniformly dense, short, stiff, compacted, with a spike length of 3.5 to 6 cm with 17 to 25 closely packed

spikelets (Percival, 1921). The spike architecture of club wheat is determined by the C locus which has been located close to the centromere of chromosome 2D. Apparently in common wheat, the C locus is monomorphic because no studies located QTL associated with spike morphology in the same location as C locus (Johnson et al., 2008). The absence of QTL for PC in chromosome 2D indicate that the clavate architecture is not controlled by the C locus. Indeed, the identification of seven QTL associated with PC is in agreement with results reported in many papers that indicate that spike architecture in common wheat is controlled by multiples QTL scattered through the genome. Although none of the QTL associated with PC explained more than 15% of PV, it is worth to highlight the consistency observed for *QPC.ndsu.4A* across the environments. Most likely, *QPC.ndsu.4A* is the most important QTL for PC, but the estimation of the effects of this QTL could be influenced by experimental error or environmental conditions that limited the expression of this trait in other RILs.

Despite the fact that the genetics of pubescences on glumes in wheat has been studied for almost one century, few studies have dissected this trait using molecular markers. In the present study, although one major QTL and seven minor QTL associated to PP were identified, PVE by these QTL suggests that this trait is mainly controlled by one gene located on the short arm of chromosome 1A (*QPP.ndsu.1A1*) (Table 3-5). Previous studies have also shown that hairy glumes are controlled by one gene (*Hg*) located on 1AS in *Triticum* (Briggle and Sears, 1966; McIntosh and Bennett, 1978; Blanco et al., 1998; Khlestkina et al., 2000; Spielmeyer and Richards, 2004; Khlestkina et al., 2006). Additionally, interactions of the minor QTL located on 1AS, 4AL, 5BS, and 6BS with *QPP.ndsu.1A1* could explain the hetero-pubescent behavior observed in some RIL.

The phenotypic dissection of awn length into three spikes areas (ALB, ALM, ALT) detected more QTL than a simple average of all the awn measurements (AAL). The traits ALB, ALT, and ALM resulted in the detection of 7, 7, and 8 QTL, respectively; while for AAL, only 4 QTL were detected (Table 3-5; Fig 3-2). Common QTL were observed among the different awn measurements. However, only one QTL located on chromosome 7B was common across the different awn measurements. This finding suggests that chromosome 7B carries a gene controlling awn length across the entire spike. The traits ALT and ALM shared QTL at chromosomes 5A (*QALT.ndsu.5A* and *QALM.ndsu.5A*) and 5B (*QALT.ndsu.5B* and *QALM.ndsu.5B*). The QTL on chromosome 5B was also detected for AAL trait suggesting its importance

in the determinations of the awn length especially at the top and middle of the spike. Similarly, ALB and AAL had a common QTL on chromosome 6A (*QALB.ndsu.6A* and *QAAL.ndsu.6A*). Among the QTL detected for only one awn-related trait, *QALB.ndsu.2D* detected for ALT, seems important as it was detected in all the environments and explained up to 20% of PV.

The presence of four apically awnleted RIL in this experiment was not expected considering the awned phenotype of both parents. Composite interval mapping determined that two QTL on chromosome 2A (*QAless.ndsu.2A1* and *QAless.ndsu.2A2*) were associated to this trait. The position of *QAless.ndsu.2A1* coincides with the position of the exclusive QTL for ALB named *QALB.ndsu.2A*. The elite parent (WCB414) provided the alleles for the awn inhibition in the apically awnleted phenotype as well as the alleles for a reduction in the length of the awns at the bottom of the spike (ALB). Given that this parent is awned, we believe that an undetermined genetic process should be responsible of the increased expression of this gene in the four apically awnleted RIL.

In wheat, the genes *Hd*, *B1*, and *B2* located on chromosomes 4AS, 5AL, and 6BL respectively, have been previously associated with awnness (Rao 1981). Moreover, the presence of three QTL on chromosomes 2DS, 4AS and 6BL associated with ALB, ALM, ALT and AAL have also been reported (Sourdille et al., 2002; Sourdille et al., 2003). QTL located on chromosome 4AS and 6BL have been found to co-segregate with the genes *Hd* and *B2*, respectively (Sourdille et al., 2002). The present study also identified QTL associated with awns on 2DS, 5AL and 6BL (*QALT.ndsu.2D*, *QALT.ndsu.5A*, *QALM.ndsu.5A*, and *QALM.ndsu.6B.1*). However, contrary to previous studies, the present research identified QTL on 1AL, 1B, 2AL, 3A, 4AL, 4BL, 5BS, 6AS, 6BS and 7BL demonstrating the polygenic control of awn length in wheat. Nevertheless, it is worth to point out that this is the first time that a RIL population derived from a branched and awned parent and an awned elite line was used to study the genetics of awn length and awn inhibition. Therefore, differences between our results and previous studies may be explained by differences in the germplasm studied.

3.5.3. QTL for yield and yield components

The identification of multiple QTL for GY and its components KSk, KNd, KS, NNdISk, and NS (Table 3-6, Fig 3-2) is in accordance with previous studies that identified a polygenic control of these traits

and/or high effects of the environment on their expression (Kato et al., 2000; Börner et al., 2002; Gross et al., 2003; Huang et al., 2003; Huang et al., 2004; McCartney et al., 2005; Verma et al., 2005; Marza et al., 2006; Kuchel et al., 2007; Kumar et al., 2007; Li et al., 2007; Ma et al., 2007; Cuthbert et al., 2008; Wang et al., 2009; Heidari et al., 2011; Bennet et al., 2012a; Cui et al., 2012; Mergoum et al., 2013). The number of kernels per spikelet is an indirect measurement of floret fertility. The genetics of kernels per spikelet has been traditionally studied through progeny analysis (Keteta et al., 1976; Sidwell et al., 1976; Sayed., 1978; Ibrahim et al., 1983), but there are limited studies reporting the genetic dissection of this trait using molecular markers (Bennet et al., 2012a). Although an earlier study for KSk conducted under water-limited environments reported six QTL, none of them were located on chromosomes 2BL, 2DS, 6BL and 7BL where QTL were identified in the present study. This could be due to either different genetic material used in these studies or to differences in environmental conditions. Most of the alleles that contributed to increase KSk were derived from the elite parent WCB414. This was expected considering that WCB414 had always superior values of KSk compared to WCB617 (Appendix Table B2) and for which PSS and KSk were inversely correlated (Table 3-2).

Considering that branched spikes have several spikelets per rachis node, we analyzed the number of kernels per node. Considering that conventional spikes (non-SS) have the same phenotypic value for KSk and KNd, it was expected to discover common QTL between these traits. However, only *QKSk.ndsu.6B* and *QKNd.ndsu.6B* (detected in only one environment) shared the same position. This result suggests an independent genetic control of these traits. Among the QTL associated with KNd, only *QKNd.ndsu.1A* was consistently detected in 50% of the environment studied and had the largest percentage of PVE (up to 11.31%). This QTL was located in a QTL cluster on 1AL together with QTL for spike-related traits.

Kernels per spike (KS) is an important yield component and was studied widely in the past. For this trait, the identification of one consistent QTL on 1AS (*QKS.ndsu.1A*) and 4AL (*QKS.ndsu.4A*) as well as two putative QTL on 1B (*QKS.ndsu.1B*) and 2DS (*QKS.ndsu.2D*) coincides with the findings of previous studies (Börner et al., 2002; Marza et al., 2006; Cuthbert et al., 2008; Wang et al., 2008; Bennett et al., 2012a). *QKS.ndsu.4A* is co-located with *QKNd.ndsu.4A* and *QNd.ndsu.4A*, two putative QTL for KNd and Nd (Fig. 3-2), suggesting pleiotropic effect of this genetic region on these traits. Interestingly, we

found that the branched parent (WCB617) is the source of the alleles that increased KS at *QKS.ndsu.1A* and *QKS.ndsu.4A*. Considering the consistency of these QTL, these alleles could be of great interest for breeding programs aimed to increase KS.

The spikelets in the middle of the wheat spikes are developed before the other spikelets at the bottom or top (Sharman, 1967). Hence, it is common to observe immature spikelets at the spike base. The genetics of immature spikelets have received the attention of some studies considering their impact on the number of grain per spikes and ultimately on grain yield (Ma et al., 2007; Li et al., 2007). The trait NNdISk assessed in this study is comparable to the basal sterile spikelet number studied in conventional spikes (Ma et al., 2007; Cui et al., 2012). In the branched spikes studied in this population, the presence of a large number of immature spikelets at the bottom of the spike is obvious, but it is often not possible to distinguish immature spikelets from others at each rachis node. Therefore, it was decided to count the nodes with immature spikelets instead of the number of spikelets. The location of consistent QTL for NNdISk on 1AL (*QNNdISk.ndsu.1A*) 2DS (*QNNdISk.ndsu.2D*) is in agreement with a previous study by Cui et al. (2012).

Fourteen QTL related to NS were identified, the largest number of QTL found for a trait in this study. However, none of these QTL were consistently detected in 50% of the 6 environments of the study. Only four QTL were consistent in more than one environment, which suggests a high impact of the environment on the expression of the genes associated with NS. Among these putative QTL for NS, three of them explained more than 15% of PV in some environments. This was the cases of *QNS.ndsu.2A*, *QNS.ndsu.5A*, and *QNS.ndsu.7A* which explained 15.9% 15.6% and 20.6% of PV, respectively. The location of these chromosomes on chromosomes 2A, 5A and 7A confirms previous findings (Huang et al. 2003; Huang et al. 2004; Li et al., 2007; Cuthbert et al., 2008; Heidari et al., 2011). In general, the alleles which increased NS were derived from the elite parent (WCB414). This result suggest that the alleles from the branched parent (WCB 617) are associated with poor tiller capacity, which confirm the negative correlation observed between PSS and NS (Table 3-4) observed previously (Percival, 1921; Hucl and Fowler, 1992).

The location of a consistent QTL associated with GY on 1DL (*QGY.ndsu.1D*) has been not reported previously. This minor QTL overlapped with QTL for PH (*QPH.ndsu.1D*) and Nd (*QNd.ndsu.1D*).

On the other hand, the location of a QTL for GY on 2DS (*QGY.ndsu.2D1*) confirms previous genetic analyses (Huang et al. 2003; Narasimhamoorthy et al. 2006; Kumar et al., 2007). Indeed, this QTL is very close to the major gene for SS (*QSS.ndsu.2D*) and explained up to 40.3% PV. In fact, only one study (Kumar et al., 2007) had reported a QTL on chromosome 7A explaining more than 40% of PV of GY in common wheat.

3.5.4. QTL for other important agronomic traits

In addition to yield and its components, other important agronomic traits considered by breeders are PH, Ld, DH and DM. These traits play an important role in cultivar adaptation and ultimately in grain yield. During the last 15 years, a large number of QTL studies have shown that PH, Ld, DH and DM are controlled by several loci located throughout the wheat genome, whose expression is influenced by environmental conditions (Cadalen et al., 1998; Araki et al., 1999; Keller et al., 1999; Kato et al., 1999; Ahmed et al., 2000; Börner et al., 2002; Sourdille et al., 2000a ; Sourdille et al., 2003; Huang et al., 2003; Huang et al., 2004; McCartney et al., 2005; Verma et al., 2005; Huang et al., 2006; Marza et al., 2006; Narasimhamoorthy et al., 2006; Chu et al., 2008; Cuthbert et al., 2008; Wang et al., 2009; Bennet et al., 2012a). The positive correlations observed between PH and Ld (Table 3-3) can be expected, since tall plants are usually more susceptible to lodging. This result is in agreement with the overlapping position of *QPH.ndsu.4B* and *QLd.ndsu.4B* on 4BS. These consistent QTL explained up to 13.8% of PV of PH and up to 18.3% of PV of Ld. The position of *QPH.ndsu.4B* and *QLd.ndsu.4B* coincides with the position of the major gibberellic acid insensitive semi dwarf gene *Rht-B1b (Rht1)* (Cadalen et al., 1998, McCartney et al., 2005a; Cuthbert et al., 2008). Interestingly, *QPH.ndsu.4B* and *QLd.ndsu.4B* are also overlapping with a consistent QTL for SL (*QSL.ndsu.4B*) and a putative QTL for Nd (*QSL.ndsu.4B*). This result suggests that selection for semi dwarf alleles may also be coupled with reduced spike dimensions. The reduced spike size observed in the checks (commercial varieties) as well as in the elite parent is probably caused by this gibberellic acid insensitivity gene. However, the inability to detect any QTL for plant height on chromosome arm 4DS where another major gene for plant height *Rht-D1b (Rht2)* is located (Sourdille et al., 1998, McCartney et al., 2005a), suggest that either there was no polymorphism for that locus in the population or *Rht-D1b (Rht2)* has no role in the genetic control of PH in this populations. On the other

hand, the detection of other QTL not shared between PH and Ld suggests that other biological process may be involved in the regulation of these traits. For instance a new QTL was detected on chromosome 1A for Ld, which was not detected for PH.

Wheat genotypes with earlier DH are expected to mature early in general; therefore the positive associations observed between DH and DM were expected (Table 3-3). We observed that positions and/or CI for QTL associated to DH and DM overlapped on 3A, 3BL, 3D, and 7BL. *QDH.ndsu.3A* and *QDM.ndsu.3A.1* were consistent and major QTL that explained up to 15.8% and 25.0% of PV of DH and DM, respectively. Previous studies (Huang et al., 2004) have also reported QTL associated with DH on 3A. Most likely, the 3A region corresponds to the gene associated with earliness per se (*Eps*) identified on this chromosome (Shah et al., 1999). Another important QTL for DH identified in our population was *QDH.ndsu.2D* which was located on 2DS and was consistently identified in five environments and AE. This is major QTL that explained up to 21.1% of PV of DH (Table 3-6). It is well known that chromosome 2D carries the photoperiod response gene (*Ppd1*) on 2DS (Scarath and Law, 1984; Millet, 1986, 1987; Xu et al., 2005), and several studies have reported QTL on 2DS associated with DH using SSR markers (Börner et al., 2002; Huang et al., 2003; Narasimhamoorthy et al., 2006) and DArT markers (Sorrells et al., 2011; Bennet et al., 2012b). The flanking markers of *QDH.ndsu.2D* (*wPt-3812* and *wPt-5014*) were also located on chromosome 2D at \approx 17cM apart from *Ppd1* by Sorrells et al. (2011). These results suggest that *QDH.ndsu.2D* may represent *Ppd1* gene.

3.5.5. Genetic associations of spike pubescences and SS with other traits

On 1AS, a cluster of five consistent QTL for PP, SL, Nd, DH and KS (*QPP.ndsu.1A1*, *QSL.ndsu.1A1*, *QNd.ndsu.1A.1*, *QDH.ndsu.1A.1*, and *QKS.ndsu.1A*) were detected suggesting a pleiotropic effect of this chromosome region on these traits. Considering that *QPP.ndsu.1A1* was the major gene that explained up to 91.82% of PV of PP, it is possible that the co-location of these QTL corresponds to an effect on glume pubescences on SL, Nd, DH and KS. Certainly, PP was positively correlated with these traits (Table 3-2; and Table 3-4). The effect of glume pubescences on other wheat traits has scarcely been studied. Among these few studies, it was reported under growth chamber conditions that glume pubescences are associated with more kernels per spikelets (Maes et al., 2001). Interestingly, we found that at *QKS.ndsu.1A* the alleles that increased the number of KS were derived

from the pubescent parent. On the other hand, the positive association of glume pubescences with SL, Nd, and DH should be considered in further studies.

In the long arm of chromosome 1A (1AL) we observed another cluster of consistent QTL for KNd (*QKNd.ndsu.1A*), NNdISk (*QNNdISk.ndsu.1A*), and ALT (*QALT.ndsu.1A*). The co-location on 1AL of QTL for NNdISk and ALT is in agreement with the negative association between these traits (Table 3-2), suggesting a significant role of the awns at the top of the spikes on the number of immature spikelets at the spike bottom.

Using the same population, we have shown previously, (Chapter 2) that SS-related traits are mainly controlled by a major and consistent QTL located on 2DS (*QSS.ndsu-2D*), a major and putative QTL located on 7B (*QSS.ndsu-7B.2*) and six minor QTL located on chromosome 5B, 6A, 6B and 7B (*QSS.ndsu-5B*, *QSS.ndsu-6A.2*, *QSS.ndsu-6B.1*, *QSS.ndsu-6B.2*, and *QSS.ndsu-7B.1*). The position and/or CI of *QSS.ndsu-2D* are overlapping with the consistent QTL for NdD (*QNdD.ndsu.2D*), NNdISk (*QNNdISk.ndsu.2D*), DH (*QDH.ndsu.2D*), and Ksk (*QKSk.ndsu.2D*) (Fig. 3-2). This finding suggests either a pleiotropic effect of the region associated with SS on these traits or a closely linked QTL; and it is in agreement with the correlations observed between PSS and these traits (Table 3-2 and Table 3-4). Interestingly, previous studies reported that chromosome 2D of the multi-spikelet line “Noa” had in addition to the gene associated to a large number of spikelets, a gene for late heading date which is independent of the day-length sensitive gene *ppd* (Millet, 1986, 1987). Other consistent QTL were also shown to be closely linked to the major QTL associated with SS (*QSS.ndsu-2D*), demonstrating the impact of SS on other spike-related and agronomic traits of wheat. This was the case of QTL for SL (*QSL.ndsu.2D*), Nd (*QNd.ndsu.2D*), ALT (*QALT.ndsu.2D*), and GY (*QGY.ndsu.2D*) which were clustered on 2DS. In addition, PSS was also correlated with these traits demonstrating that branched spikes tend to be long, with a high number of nodes, reduced awn length at the top of the spike, and poor grain yield (Table 3-2 and Table 3-4). Among these associations, the association between PSS and GY is of interest, considering that *QGY.ndsu.2D* explained up to 40.3% of PV of GY. It could be possible that the branched genotypes improve their agronomic performance to break the linkage between negative alleles for GY at *QGY.ndsu.2D* and alleles for SS at *QPSS.ndsu.2D*.

3.6. Conclusion

A genetic study using a RIL population derived from a cross between an elite parent (WCB414) and an exotic non-adapted line with SS and pubescences (WCB617) resulted in the identification of 60 spike-related QTL and 85 agronomic QTL. This large number of QTL identified demonstrates the richness of using elite x exotic cross for the genetic analysis of traits in common wheat. The major QTL associated to SS located on 2DS identified in this study has either pleiotropic effect or it is closely linked to consistent QTL for SL, Nd, NdD, ALT, NNdISk, DH, KSK, and GY which demonstrate a remarkable impact of the SS on spike morphology and ultimately on GY. Similarly, the influence of glume pubescences (PP) on SL, Nd, DH and KS was supported by locating a cluster of consistent QTL for these traits on 1AS. Considering the consistency across environments of several of the QTL reported in this study, the development of molecular markers from these loci could be valuable to increase genetic diversity of wheat breeding programs.

3.7. References

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CHAPTER 4. GENETIC DISSECTION OF QUALITY TRAITS IN WHEAT USING AN ELITE× EXOTIC RIL POPULATION

4.1. Abstract

Recognizing new QTL and alleles in exotic germplasm is paramount for the future of wheat traits and particularly quality improvement. In the present study, a RIL population developed from a cross of an elite wheat elite line (WCB414) and an exotic genotype with supernumerary spikelets (SS) and pubescence (WCB617), was used to identify QTL and new alleles for eight quality traits. Composite interval mapping for thousand kernels weight (TKW), kernel volume weight (KVV), grain protein content (GPC), percent of flour extraction (FE) and four mixograph-related traits identified a total of 69 QTL including 19 consistent QTL. These QTL were located on 18 different chromosomes. Thirteen of these QTL explained more than 15% of phenotypic variation (PV) and were considered as major QTL. The exotic parent contributed positive alleles that increased PV of the traits at 56% of loci across the genome. In this study we identified 12 QTL for TKW ($R^2 = 7.2\%-17.1\%$); 10 QTL for KVV ($R^2 = 6.7\%-22.5\%$); 11 QTL for GPC ($R^2 = 4.7\%-16.9\%$); six QTL for FE ($R^2 = 4.8\%-19\%$) and 31 QTL for mixogram-related traits ($R^2 = 3.2\%-41.2\%$). The co-localization of QTL for SS and quality-related QTL suggests a pleiotropic effects among these genetic regions. A cluster of QTL for quality traits was observed on 6BS. Closely linked QTL suggest pleiotropic effects between some quality traits.

4.2. Introduction

Common wheat (*Triticum aestivum* L.) was in 2012 the cereal with most area harvested (about 216 million Ha), and the third cereal with highest production worldwide (about 720 million tons), surpassed only by maize and rice. In 2009, wheat was the main food supply crop in the world (66 kg/capita/year) and the most important source of proteins (16.2 g/capital/day) worldwide (FAO-FAOSTAT, 2014). These statistics make wheat breeding a paramount activity for food security in the world and for the dynamics of the global markets. Breeding programs of wheat have the objective to supply varieties for a diverse market and end-users including growers, millers, bakers, and consumers. This is a daunting challenge since selection for optimal characteristics for one of these customers could be detrimental for

others (Carena, 2009). Wheat breeders select the best genotypes through the assessment of a large number of agronomic and quality traits. Plant height, grain yield, disease resistance, and days to heading are examples of agronomic characters tested; while grain protein content, flour extraction, dough resistance and baking performance are quality traits considered in the selection of superior cultivars. The assessment of quality traits, at difference of agronomic traits, requires large samples, time, and specialized personal to conduct complex procedures. In addition, most of the quality traits have a quantitative inheritance and their expression is affected by environmental conditions (Campbell et al., 2001; Nelson et al., 2006; Tsilo et al., 2010; Tsilo et al., 2011; Simons et al., 2012) limiting the genetic gain through breeding cycles.

Molecular-assisted selection (MAS) has been presented as a shortcut for the development of premium cultivars (Nelson et al., 2006; Raman et al., 2009; Simons et al., 2012). This approach offers the opportunity for wheat breeders to select genotypes bypassing the assessment of quality traits. Therefore, in the last 15 years a broad number of genetic studies in wheat have been conducted to map QTL/genes associated with quality traits in wheat and identify suitable markers to use in MAS (Campbell et al., 2001; Gross et al., 2003; Huang et al., 2006; Kuchel et al., 2006; McCartney et al., 2006; Nelson et al., 2006; Kunert et al., 2007; Mann et al., 2009; Raman et al., 2009; Sun et al., 2010; Zhao et al., 2010; Tsilo et al., 2011; Carter et al., 2012; Li et al., 2012a; Li et al., 2012b; Simons et al., 2012; Maphosa et al., 2013). Recently, wheat breeding programs have started to take advantage of mass genotyping strategies such as Diversity array Technology (DArT) and Illumina Infinium analysis, which permit the development of (saturated) genetic maps identifying suitable QTL and alleles for quality traits (Raman et al., 2009; Tsilo et al., 2011; Simons et al., 2012; Kumar et al., 2013; Maphosa et al., 2013).

Among the quality traits, GPC has received special attention because it is an indicator of the performance of wheat derived products (Zhao et al., 2010). In addition, wheat markets are determined by the percentage of protein in the grain (Regional Quality Report, 2011). Several studies have genetically dissected this trait and reported the existence of genes associated with this trait on all wheat chromosomes (Galante et al., 2001; Gross et al., 2003; Prasad et al., 2003; Sourdille et al., 2003; Huang et al., 2006; Kunert et al., 2007; Mann et al., 2009; Nelson et al., 2006; Raman et al., 2009; Sun et al., 2010; Tsilo et al., 2010; Zhao et al., 2010; Conti et al., 2011; Li et al., 2012a; Li et al., 2012b; Carter et al.,

2012; Maphosa et al., 2013). In several of these studies, molecular markers associated with genes regulating gluten proteins have been reported. Gluten is the coherent mass formed by the binding of glutenin and gliadin (storage proteins) after water is added to flour (Stone and Savin, 1999). Glutenins are responsible for dough strength and are conformed by subunits of high molecular weight (HMW) and subunits of low weight (LMW). The major genes controlling HMW (Glu-1, Glu-A1, Glu-B1 and Glu-D1) are located on the long arms of the homeologous group 1; while the major genes controlling LMW (Glu-A3, Glu-B3, and Glu-D3) are also in the same homeologous group but in the short arm of these chromosomes. Gliadins, responsible for dough viscosity, are controlled mainly by genes located on the short arm of homeologous groups 1 (ω -gliadins and γ -gliadins) and 6 (α -gliadins, β -gliadins) (Payne 1987).

Wheat varieties with good grading standards such as thousand kernel weight (TKW) and kernel volume weight (KVV) usually has more flour extraction (FE) with high quality (Gwirtz et al., 2006). These important traits for millers have been also dissected genetically. Loci controlling TKW have been identified on all wheat chromosomes (Araki et al., 2001; Börner et al., 2002; Kato et al., 2000; Gross et al., 2003; Huang et al., 2003; Huang et al., 2004; McCartney et al., 2005; Huang et al., 2006; Kumar et al., 2006; Li et al., 2007; Cuthbert et al., 2008; Wang et al., 2009; Sun et al., 2009; Tsilo et al., 2010; Heidari et al., 2011; Simons et al., 2012; Bennett et al., 2012). For KVV also, QTL have been reported on all chromosomes except 1A and 6D (Campbell et al., 1999; McCartney et al., 2005; Narasimhamoorthy et al., 2006; Kunert et al., 2007; Huang et al., 2006; Sun et al., 2009; Sun et al., 2010; Bennett et al., 2012; Simons et al., 2012; Carter et al., 2012). In the case of FE, QTL have been detected on chromosomes 1A, 1B, 2A, 3B, 4A, 4B, 4D, 5A, 5B, 5D, 6A, 6D and 7A (Campbell et al., 2001; Kuchel et al., 2006; Nelson et al., 2006; Raman et al., 2009; Carter et al., 2012; Simons et al., 2012; Maphosa et al., 2013).

Rheological properties govern the performance of wheat flour dough during mechanical treatment (Alamri 2009a, 2009b). Mixograph, Farinograph, and Alveograph are used to assess these rheological characteristics giving an insight about baking performance (Gwirtz et al., 2006). Several studies have also reported QTL for rheological traits derived from Mixograph, Farinograph and/or Alveograph assessments (Campbell et al., 2006; Huang et al., 2006; Mann et al., 2009; Tsilo et al., 2011; Li et al., 2012b; Simons et al., 2012; Mergoum et al. 2013, Maphosa et al. 2013). In the case of Mixograph, QTL have been found

in all chromosomes except chromosomes 3D and 6B (Campbell et al., 2006; Huang et al., 2006; Mann et al., 2009; Tsilo et al., 2011; Li et al., 2012b; Simons et al., 2012; Mergoum et al., 2013, Maphosa et al., 2013).

Although the recent information derived from QTL analysis suggests that the QTL/genes controlling wheat quality are contributed by whole-wheat genome, previous studies have suggested that D-genome plays a pivotal role in bread wheat attributes (Kerber and Tipples, 1969; Nelson et al., 2006). Due to the relatively recent addition of the D-genome into hexaploid wheat, the D-genome is the less diverse than other wheat genomes (Akunov et al., 2010). Consequently, wheat varieties show low polymorphism for D-genome. This could jeopardize future improvement of wheat quality traits, particularly for those loci located on D-genome. The use of the genetic diversity present in landraces and exotic wheat has been suggested as a mechanism to enrich wheat genetic pools (Raman et al., 2010). In the past, a combination of molecular markers and breeding approaches has allowed the identification and introgression of novel QTL present in exotic wheat lines and landraces (Nelson et al., 2006, Huang et al., 2003; Huang et al., 2004; Narasimhamoorthy et al., 2006; Kunert et al., 2007; Naz et al., 2008). Wheat genotypes with supernumerary spikelets (SS) is an unexplored exotic germplasm for quality traits. Usually wheat bears one spikelet per rachis node, however, in some landraces; it is possible to find genotypes in which a rachis node has more than one spikelet (Sharman 1967; Martinek and Bednár 1998). This trait was reported to be controlled by a major gene located on chromosome 2D and some minor genes located on other chromosomes (Koric, 1973; Peng et al., 1998; Klindworth et al., 1990; Dobrovolskaya et al., 2009)(Chapter 2). Considering the high number of spikelets in which grains could be developed, SS have been suggested as a means to increase grain yield (Pennell and Halloran, 1983, 1984, Hucl and Fowler. 1992). The quality performance of genotypes with SS is mostly unknown. In the present paper, a RIL population derived from the cross of a white wheat (WW) genotype and an exotic line with SS, was used to genetically dissect eight quality traits. White wheat (WW) is a commercial alternative to hard red spring wheat (HRSW) in the northern plains of USA (Ransom et al., 2006) and is characterized by good-quality performance. The impact of genetic regions controlling SS on quality traits was studied.

4.3. Material and Methods

4.3.1. Plant material

A RIL population derived from a cross between an elite genotype WCB414 and an exotic genotype WCB617 was used in this study. WCB414 is a white wheat (WW) genotype developed by the Hard White and Specialty Wheat breeding program at North Dakota State University (NDSU), Fargo, ND USA. This line was chosen for its adaptation and good-quality performance and has a conventional spike morphology. The genotype WCB617 is an exotic line with SS phenotype and glume pubescence, maintained by the NDSU wheat Germplasm Enhancement project as a source to enrich genetic diversity in wheat breeding programs at NDSU. Single seed descent method was used to advance the RIL population to F7 generation. Afterward, plants were grown in greenhouse facilities at NDSU to increase seeds and advance the population to F8 generation. In the growing seasons of 2009, spikes from each plot were collected and grown in New Zealand winter nursery as head rows in order to ensure genetic purity of each RIL. Thus, in the years 2009 and 2010, the generations F7:9, and F10:11 were included in this study. The HRSW cultivars “Alsen” (PI 615543) (Frohberg et al. 2006), “Steele-ND” (PI 634981) (Mergoum et al. 2005), “Glenn” (PI 639273) (Mergoum et al. 2006), “Faller” (PI 648350) (Mergoum et al. 2008), “Barlow” (PI 658018) (Mergoum et al. 2011), “Briggs” (PI 632970) (Devkota et al. 2007) and WW cultivar “Alpine” (Agripro® wheat variety, USA) were included as checks in this study.

4.3.2. Field experiment

During the years of 2009 and 2010, the parents, 163 RILs and seven checks were planted in a 13 × 13 partially balanced square lattice design with two replicates, at two different location, Prosper (46.96300N, 97.01980 W, altitude 274 m, Bearden series soils) and Carrington (47.45000N, 99.12390 W, 484 m of altitude, Heimdal-Emrick series soils) in North Dakota (ND), USA. Planting and environmental conditions were previously described in Chapters 2 and 3. The environments were designated as: I=Prosper 2009, II= Carrington 2009, III= Prosper 2010, IV= Carrington 2010.

4.3.3. Phenotypic data collection

The grain samples collected from the field experiments were cleaned by using a clipper grain cleaner before recording phenotypic data. The quality traits analyzed in this study were TKW, KVV, GPC,

FE and mixograph related trait which include mixograph envelope peak time (MEPT), mixograph MID line peak time (MMLPT), mixograph MID peak integral (MMLPI) and general mixograph pattern (Mx). TKW (g) was obtained from the number of seeds in 10 g sample, counted using a seed counter (Seedburo Equipment Co., Chicago, IL). KVW (kg m⁻³) was measured according to the American Association of Cereal Chemist International (AACCI) method 55-10.01 (AACCI, 2008). Whole-grain GPC (%) in on a 12 percent basis was measured using Near-Infrared Reflectance following the AACCI standard method 39.25.01 (AACC International, 2008). FE was determined using 100 or 150 g grain sample tempered to 16.0% moisture. Brabender Quadrumat Junior Mill was used to mill the grain sample and bran was discarded from the flour. Flour extraction was reported on clean dry wheat basis (Bass, 1988). Mixograph measurements were obtained from 35 g of flour in a National Manufacturing Mixograph (National Manufacturing, TMCO Division, Lincoln NE) following the AACCI 54-40.02 (AACC International 2008). The Mixmart software was used to collect information of MEPT (min), MMLPT (min) and MMLPI (% torque x min). General Mixograph pattern (Mx) was based on a 0-9 scale of (0 = weakest; and 9, = strongest). The traits MEPT, MMLPT, MMLPI and Mx are referred as Mixograph-related traits in this study.

Due to small seed sample of WCB617 in Prosper 2009 and Carrington 2009, different procedures were used for estimating KVW and GPC. A miniature test weight device was used for KVW. The GPC was calculated from flour following the combustion method 46-30.01 of AACCI (AACC International 2008) using a Leco FP 528 (Leco Corporation 3000 Lakeview Avenue, St. Joseph MI). The information of flour protein content of these two samples was used as equivalent of GPC considering the close correlation between both traits. Data of FE and mixograph-related traits for WCB617 in Prosper 2009 could not be obtained due to the small seed sample.

4.3.4. Data analysis

Data from Prosper 2009 for FE and mixograph related traits were subjected to analyses of variance (ANOVA) for a random complete block design using the MIXED procedure of the Statistical Analysis System (SAS) (SAS 2004). The data for FE and mixograph related traits from Carrington 2009, Prosper 2010, and Carrington 2010, as well as data for TKW, TW and GPC from all the environments were subjected to ANOVA for a lattice design using the MIXED procedure of SAS (SAS 2004).

Differences in the statistical analysis methodologies used in this study were due to missing data. ANOVA was estimated for each environment separately. To estimate genotype × environment interaction, combined ANOVA over location was performed. The Fmax test (Tabachnik and Fidell, 2001), considering a ratio of less than 10-fold, was conducted to verify homogeneity of variance before conducting combined ANOVA analysis. In ANOVA analyses, the RILs, parents, and checks were considered as fixed effects; while environments and blocks were considered as random effects. F-tests were considered significant at $p < 0.05$. Significant differences between genotypes were assessed using an F-protected least significant difference (LSD) value at $p < 0.05$. Correlations between two traits were assessed using significant Pearson coefficients at $P < 0.05$. Procedures described by Gomez and Gomez (1984) were followed to test homogeneity among correlation coefficients of different environments at $P < 0.005$. Pooled homogeneous correlation coefficients were considered significant at $P \leq 0.05$. Broad-sense heritability for each trait on plot-basis (Holland et al. 2003) was calculated excluding the means of parents and checks. The mixed procedure of SAS (SAS 2004) was used to conduct this analysis considering all sources of variation as random component. The outputs of the covariance parameter estimates were used in the equation $h_B^2 = \sigma_g^2 / [\sigma_g^2 + (\sigma_{ge}^2 / e) + (\sigma_e^2 / r)]$, where σ_g^2 is the genotype variance, σ_{ge}^2 is the G × E, e is the number of environments and r is the number of replicates.

The evaluations of penetrance of supernumerary spikelets (PSS), penetrance of pubescences (PP), penetrance of clavate architecture (PC) and apical awnleted expression (Aless) reported in Chapter 2 and Chapter 3 were correlated with the traits investigated in this study and co-located in the genetic map to identify the influence of these traits on quality traits. Other spike-related traits described in chapter 3 were co-located in the genetic map.

4.3.5. QTL analysis

The molecular map developed in chapter 2 was used in the present study. The genetic map consisted of 939 DArT markers located on 671 unique loci in 38 linkage groups. The total genetic distance of the genetic map was 3,114.2 cM with a average distance between any two markers of 4.6 cM. The map represented 20 of the 21 wheat chromosomes. The program QTL Cartographer V2.5_011 (Wang et al. 2012) was used to conduct confidence interval mapping (CIM) following the steps described in chapter 3. QTL discrimination of putative QTL was conducted following the parameters described in

chapter 3. In summary, a putative QTL was declared at 2.5 LOD. Five hundred permutations were performed to determine critical LOD threshold. QTL in only one environment with a LOD lower than the critical threshold were discarded. Putative QTL detected in at least two environments (including AE) were reported regardless the critical LOD threshold. A QTL was considered as consistent if was detected in 50% of the environments studied. A QTL was called as major when explained 15% or more of the phenotypic variance (PV). Linkage groups and QTL were identified using MapChart 2.2 program (Voorrips, 2002).

4.4. Results

4.4.1. Phenotypic data

The elite parent (WCB 414) had better performance than the exotic parent (WCB 617) in all the environments for the traits TKW, KVV, and FE (Appendix Table C1). However, the exotic parent (WCB 617) had higher GPC than the elite parent (WCB 414) (Appendix Table C1). For different mixograph measurements (MX, MEPT, MMLPT, MMLPI), although WCB 414 had better performance than WCB 617 in most of the environments, these differences were not significant at $P < 0.05$ (Appendix Table C1). Transgressive segregation in direction of both parents was observed for KVV and GPC (Fig. 4-1; Appendix Table C1) in all the environment. A similar segregation was observed for MX, MEPT, MMLPT, and MMLPI in all environments, except at Prosper 2009. In this case, phenotypic values of the exotic parent were missing. Nevertheless, in this environment, the mixograph-related traits showed transgressive segregation in direction of WCB 414 parent (Appendix Table C1). For TKW, transgressive segregation in both parent directions was observed in all the environments except in Carrington 2009, where the segregation was in direction of the elite parent only (Appendix Table C1). For the trait FE, transgressive segregation in both directions was observed at Carrington 2010 and Prosper 2010, while at Carrington 2009, it was observed in the direction of the exotic parent (Fig. 4-1; Appendix Table C1). At Prosper 2009, where the phenotypic values of FE for the exotic parent could not be estimated, no transgressive segregation in the direction of the parent WCB 414 was observed (Appendix Table C1).

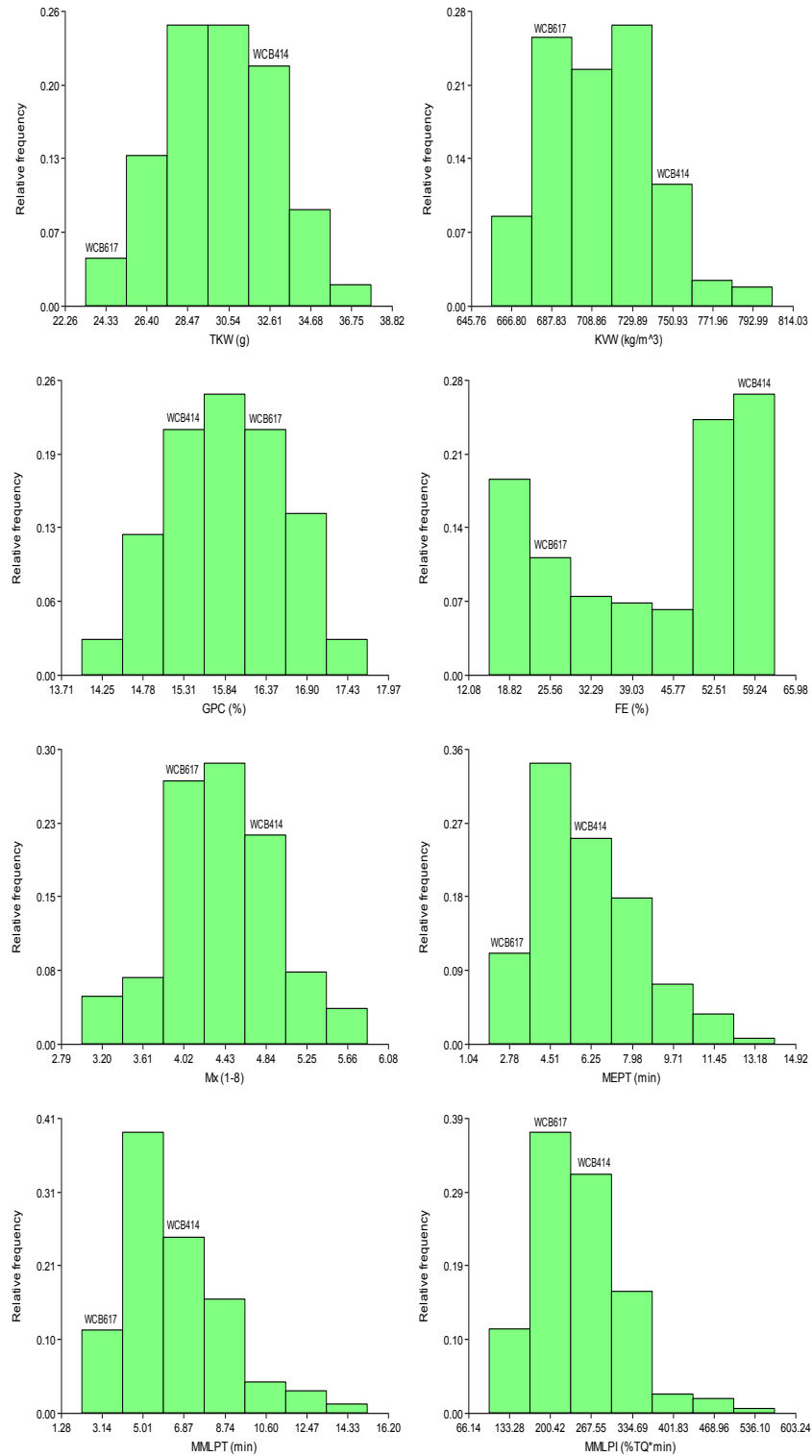


Fig. 4-1. Frequency distribution of 163 RIL for mean of eight quality traits over four six environments

Table 4-1. Mean squares, coefficient of variation and heritabilities for quality traits of the parents, RILs and checks evaluated in four environments in North Dakota, USA, during 2009 and 2010.

Trait [†]	Mean squares				CV(%) [‡]	H [§]	SE [¶]
	Genotype	Environment	G x E	Error			
TKW	52.8**	3294.73	9.67**	2	4.6	0.50	0.04
KVW	6583.2**	593271.0	1169.6**	130.4	1.6	0.47	0.04
FE	1848.8**	4591.3	67.8**	12.4	8.2	0.84	0.02
GPC	4.0**	224.9	1.1**	0.2	2.9	0.39	0.04
MX	2.3**	25.31	0.7**	0.6	17.1	0.24	0.03
MEPT	34.1**	638.0	6.2**	2.4	26.0	0.48	0.04
MMLPT	36.3**	531.2	6.3**	2.6	25.3	0.49	0.04
MLPI	38855.0**	354470.0	47663.1**	1824.3	17.2	0.60	0.03

** Significance at P < 0.01, respectively; ns, not significant at P < 0.05

[†]TKW, thousand Kernel weight; KVW, kernel volume weight; FE, flour extraction; GPC, grain protein content; Mx, mixogram score; MEPT, mixogram envelope peak time; MMLPT, mixogram MID line peak time; MLPI, mixogram MID peak Integral.

[‡]Coefficient of variation.

[§]Broad sense heritability on plot-means basis calculated in the RILs.

[¶]Standard error of heritability.

Error variances among the environments were homogenous (Appendix Table C2) allowing a combined ANOVA to be performed. The eight quality traits had significant G×E interactions (Table 4-1). Broad sense heritability on plot means basis ranged from 0.24 for Mx to 0.84 for FE (Table 4-1). Several inconsistent correlations (positive and negative correlations for the same pair of traits in different environments) were observed among several quality traits (Table 4-2). Flour extraction was positively and significantly associated with TKW and KVW, but negatively associated with mixograph-related traits; while these traits had strong positive correlations among them (Table 4-2). The presence of spikes with SS (PSS) was positively associated with GPC and mixograph-related traits, but negatively associated with TKW. Meanwhile spikes with PP trait were positively correlated with GPC, MEPT, MMLPT and MMLPI; but negatively associated to TKW, KVW and FE. The traits PC and Aless only showed weakly associations with GPC (Appendix Table C3).

Table 4-2. Pearson's correlation coefficients among traits and their significance for the RIL, their parents and checks grown at Prosper and Carrington, ND, in 2009 and 2010.

	TKW	KVW	GPC	FE	MX	MEPT	MMLPT	MMLPI
TKW	1	0.26** [§] 0.67** [‡]	-0.40** [‡] 0.22** [†]	0.28** [¶]	Ns	-0.31** [‡]	-0.31** [‡]	-0.29** [‡]
KVW		1	0.37** [‡] -0.18* -0.59**	0.41** [¶]	0.22** [†]	0.27** [‡] -0.26** [†]	0.25** [‡] -0.25** [†]	0.24** [‡] -0.15* [†]
GPC			1	ns	0.33** [¶]	-0.31** [§]	-0.30** [§]	-0.27** [†]
FE				1	-0.16* [†]	-0.25** [†]	-0.26** [†]	-0.20** [†]
MX					1	Ns	ns	0.26** [§]
MEPT [#]						1	0.98** [‡] 0.99** [‡]	0.88** [‡] 0.92** [‡]
MMLPT ^d							1	0.93** [‡] 0.92** [‡]

*, ** Significance at P < 0.05, 0.01, respectively; ns not significant at P < 0.05

TKW, thousand Kernel weight; KVW, kernel volume weight; FE, flour extraction; GPC, grain protein content; Mx, mixograph score; MEPT, mixograph envelope peak time; MMLPT, mixograph MID line peak time; MMLPI, mixograph MID peak Integral

[†]r from one environment, [‡]r pooled from two environments, [§]r pooled from three environments, [¶]r pooled from four environments

[#]Alternative pooled correlations were observed between the traits MEPT and MMLPI. The lowest pooled r value is presented.

^{††}Alternative pooled correlations were observed between the traits MMLPT and MMLPI. The lowest pooled r value is presented.

4.4.2. Identification of genomic regions (QTL) associated with eight quality traits

A total of 69 QTL were detected for eight quality traits investigated in the present study (Table 4-3). These QTL were located on eighteen different chromosomes (All accept chromosome 4D, 5D and 6D). A total of nine QTL were detected on chromosome 6B; seven QTL on each chromosomes 1B and 2D; five QTL on each chromosomes 2B and 3A; four QTL each on chromosomes 1A, 5B, 6A, and 7B; three QTL on each chromosomes 1D, 2A, 3B, 3D and 4A; two QTL on each chromosomes 4B, and 7D; one QTL on each chromosomes 5A, and 7A. In terms of genome wide distribution of QTL, a total of 34, 21 and 15 quality-related QTL were detected on B, A and D-genome, respectively. Among all the 70 QTL, a total of 18 QTL were consistent (identified in at least 50% of the environments) (Table 4-3). A total of 13 QTL explained more than 15% of PV and were considered as major QTL, while the remaining 57 QTL explained less than 15% of PV and were considered as minor QTL. The alleles for increased phenotypic

values at 31 loci were contributed by WCB414, while exotic parent WCB617 contributed alleles for 39 loci that increased the effect of quality traits.

4.4.3. QTL for TKW, KVV, and FE

A total of 11 QTL were associated with TKW, including one consistent QTL (*QTKW.ndsu.4A*) (Table 4-3, Fig.4-2). Only the consistent QTL *QTKW.ndsu.4A* could be considered as major QTL as it explained up to 17.1% of PV of TKW. The other QTL explained between 6.2% and 13.9% of PV for TKW. In individual environments, the number of QTL identified for TKW ranged from three to five and PV explained by all the QTL identified in individual environments ranged from 24 to 47.3% (Table 4). Both parents contributed with alleles that increased the phenotypic values of TKW. For the consistent QTL *QTKW.ndsu.4A*, elite parent WCB414 contributed the alleles for increased TKW.

For KVV, a total of 10 QTL including three consistent QTL (*QKVV.ndsu.1A.1*, *QKVV.ndsu.2A.1* and *QKVV.ndsu.6A.1*) were detected (Table 4-3; Fig. 4-2). The number of QTL identified for TKW in individual environments ranged from three to four. The PV explained by all the QTL in individual environments ranged from 40.8 to 51.2%. Four QTL (*QKVV.ndsu.1B.1*, *QKVV.ndsu.2A.1*, *QKVV.ndsu.2A.2*, and *QKVV.ndsu.6A.1*) including two consistent QTL, had major effect (PV>15%) on KVV, at least in some of the environments. Additive effects indicated that the elite parent WCB414 contributed positive alleles for increasing KVV at most of the loci, including the three consistent QTL.

A total of six QTL including four consistent QTL (*QFE.ndsu.1A*, *QFE.ndsu.1B*, *QFE.ndsu.3D*, and *QFE.ndsu.6A*) were associated with FE in this RIL population (Table 4-3; Fig. 2). The number of QTL identified for FE in individual environments ranged from two (Prosper 2009) to six (Prosper 2010) and the PV explained by all the QTL in individual environments ranged between 15.3% and 51.5%. The PV explained by individual QTL ranged from 4.8% to 19.0%. Only one QTL (*QFE.ndsu.2B*), which was identified in a single environment, explained more than 15% PV of FE. The elite parent WCB 414 provided the alleles that increased phenotypic values of FE at all loci except *QFE.ndsu.6A*.

4.4.4. QTL for GPC

GPC was controlled by 11 different QTL in this population (Table 4-3; Fig. 2). These include three consistent QTL (*QGPC.ndsu.5B*, *QGPC.ndsu.6B.1* and *QGPC.ndsu.7B*) located on chromosome 5B, 6B and 7B (Table 4-3; Fig. 4-2). The number of QTL identified in individual environments ranged from three (Prosper 2009) to five (Carrington 2010). The PV explained by individual QTL ranged from 4.7% to 16.9% and the PVE explained by all the QTL in individual environments ranged from 38.5%- 40.3%. Two QTL had major effect and explained up to 16.5% (*QGPC.ndsu.1A.1*) and 16.9% (*QGPC.ndsu.6B.1*) of PV for GPC. The other nine QTL were minor and explained between 4.7% and 13.7 % of PV. The elite parent contributed alleles for increased GPC at five loci (*QGPC.ndsu.1B*, *QGPC.ndsu.4B*, *QGPC.ndsu.6B.1*, *QGPC.ndsu.6B.2*, and *QGPC.ndsu.7B*), while the exotic parent contributed alleles for increased GPC at six loci (*QGPC.ndsu.1A.1*, *QGPC.ndsu.1A.2*, *QGPC.ndsu.2B*, *QGPC.ndsu.2D*, *QGPC.ndsu.3D* and *QGPC.ndsu.5B*).

4.4.5. QTL for mixograph-related traits

The genetic dissection of MEPT resulted in the detection of two consistent QTL (*QMEPT.ndsu.1D*, and *QMEPT.ndsu.5B*) and five putative QTL (*QMEPT.ndsu.1B*, *QMEPT.ndsu.2B*, *QMEPT.ndsu.2D.1*, *QMEPT.ndsu.2D.2*, and *QMEPT.ndsu.4A*) (Table 4-3; Fig. 4-2). In individual environments, the number of identified QTL ranged from one (Prosper 2010) to six (Carrington 2010). The PV explained by all QTL identified in individual environments ranged from 25.9%-64.5%. The QTL located on 1DL (*QMEPT.ndsu.1D*) seems to be the most important locus in controlling MEPT as it was detected in all environments and explained up to 41.2% of PV. The remaining six QTL were minor and explained between 3.2% and 10.1% of PV (Table 4-3). Both parents contributed QTL alleles to increase MEPT, however, for both consistent QTL (*QMEPT.ndsu.1D*, and *QMEPT.ndsu.5B*), the alleles for increased values of MEPT were contribute by elite parent (Table 4-3).

Table 4-3. Summary of information for QTL identified in a RIL population derived from the cross of an elite line (WCB414) and exotic line (WCB617) with SS at four locations in ND, USA during 2009 and 2010.

QTL	Environment [†]	Flanking markers	Pos [‡] .(cM)	CI [§] (cM)	LOD	Thresh.	a [#]	R ² (%)
<i>Thousand kernel weight</i>								
QTKW.ndsu.2A	II	wPt-2372-wPt-2850	70.2	58.5-84.4	4.8	3.3	-1.3	13.5
QTKW.ndsu.2B	IV	wPt-8760-wPt-6158	43.4	32.1-60.8	4.1	3.2	1.1	8.8
QTKW.ndsu.2D	II	wPt-5014-wPt-668017	76.8	66.6-86.8	4.1	3.3	1.0	7.8
QTKW.ndsu.3A1	IV, AE	wPt-741078-wPt-743858	2.0-2.4	0-12.9	5.0-8.0	3.2-3.4	-0.9_-1.6	9.2-13.9
QTKW.ndsu.3A2	III	wPt-6854-tPt-0242	97.5	89.6-115.1	3.8	3.2	-1.2	7.9
QTKW.ndsu.3B1	I	wPt-9170-wPt-743661	128.8	116.8-147.3	3.4	3.3	-0.8	7.8
QTKW.ndsu.3B2	IV	tPt-6487-wPt-2299	44.0	38.3-57.6	4.4	3.2	-1.1	7.3
QTKW.ndsu.3B3	I	wPt-9586-wPt-741584	4.8	0-17.7	3.6	3.3	-0.8	7.2
QTKW.ndsu.4A	I, II, III, IV, AE	wPt-2247-wPt-744614	1.0-22.7	0-38.1	3.6-9.4	3.2-3.4	1.0-1.7	7.3-17.1
QTKW.ndsu.5A	AE	wPt-9094-wPt-800131	12.0	0-24.3	4.2	3.4	-1.0	12.9
QTKW.ndsu.6B	IV	wPt-664250-wPt-2297	102.3	96.5-106.4	5.5	3.2	1.4	9.2
							^{††} RTPVE	24-47.3
<i>Kernel volume weight</i>								
QKVW.ndsu.1A	I, IV, AE	wPt-4065-wPt-5167	179.2-180.2	161.0-191.6	4.5-6.0	3.2-3.5	8.1-10.0	9.5-12.7
QKVW.ndsu.1B	I, AE	wPt-0308-wPt-8240	22.5	10.1-29.8	5.1-6.6	3.4-3.5	9.5-10.3	9.3-12.2
QKVW.ndsu.2A1	II, IV, AE	wPt-798339-wPt-2372	38.0-40.0	30.5-45.7	4.1-8.3	3.2-3.5	9.7-17.0	10.3-22.5
QKVW.ndsu.2A2	III	wPt-2850-wPt-8068	88.7	79.7-106.5	5.8	3.4	25.0	17.7
QKVW.ndsu.4B	I	wPt-5559-wPt-1046	5.5	0.4-6.8	4.2	3.4	7.5	7.6
QKVW.ndsu.5B	III	wPt-2373-wPt-8449	252.8	247.8-260.4	5.5	3.4	-21.1	11.9
QKVW.ndsu.6A.1	I, II, III, IV, AE	wPt-733115-wPt-667618	113.6-135.8	98.8-149.3	2.7-8.9	3.2-3.5	7.4-17.0	7.2-21.0
QKVW.ndsu.6A.2	II	wPt-667170-wPt-4791	79.7	61.8-85.2	4.4	3.2	-9.2	7.8
QKVW.ndsu.6B	III	wPt-5408-wPt-8412	113.3	102.2-122.1	3.5	3.4	14.7	6.7
QKVW.ndsu.7B	II	wPt-665428-wPt-9299	121.6	96.7-134.3	4.5	3.3	-6.8	8.5
							^{††} RTPVE	40.8-51.2

(Continues)

Table 4-3. Summary of information for QTL identified in a RIL population derived from the cross of an elite line (WCB414) and exotic line (WCB617) with SS at four locations in ND, USA during 2009 and 2010. (Continued)

QTL	Environment [†]	Flanking markers	Pos [‡] . (cM)	CI [§] (cM)	LOD	Thresh. [¶]	a [#]	R ² (%)
<i>Flour extraction</i>								
QFE.ndsu.1A	I, II, III, IV, AE	wPt-3698-wPt-1906	93.5-94.0	81.1-110.8	2.6-5.6	3.3-3.7	3.7-6.0	4.9-11
QFE.ndsu.1B	III, IV, AE	wPt-2389-wPt-3451	0.0	0-15.1	2.9-4.5	3.3-3.6	3.8-5.2	5.0-8.2
QFE.ndsu.2B	III	wPt-1813-wPt-8404	57.6	42.9-68.8	4.1	3.3	7.3	19.0
QFE.ndsu.3D	II, III, IV	wPt-665093-wPt-6169	39.2-42.2	33.1-46.9	2.7-3.3	3.3-3.7	4.6-4.6	5.8-7.7
QFE.ndsu.4A	III, AE	wPt-0817-wPt-7926	20.8-30.3	12.5-39.1	2.6-3.4	3.3-3.6	3.5-4.3	4.8-6.5
QFE.ndsu.6A	I, II, III, IV, AE	wPt-666773-wPt-731592	1.0-2.0	0-12.7	3.2-4.5	3.3-3.7	-4.4_-4.9 ††RTPVE	6.0-9.1 15.3-51.5
<i>Grain protein content</i>								
QGPC.ndsu.1A.1	III, AE	wPt-1924-wPt-7872	15.4-33.4	12.4-44.4	4.9-8.7	3.2-3.4	-0.2_-0.6	8.1-16.5
QGPC.ndsu.1A.2	I	wPt-8172-wPt-9429	101.9	85.4-113.4	4.1	3.2	-0.3	8.2
QGPC.ndsu.1B	II, AE	wPt-1684-wPt-5899	61.7	51.7-75.7	2.8-3.4	3.3-3.4	0.2-0.2	4.7-5.3
QGPC.ndsu.2B	IV	wPt-744808-wPt-4368	30.0	14.4-48.1	3.4	3.4	-0.3	10.5
QGPC.ndsu.2D	III	wPt-8134-wPt-2761	30.4	13.9-38.5	3.7	3.2	-0.4	7.8
QGPC.ndsu.3D	III	wPt-740945-rPt-1806	0.0	0-9.8	5.5	3.2	-0.4	9.9
QGPC.ndsu.4B	II	wPt-732423-wPt-8892	12.0	3.6-17.8	5.5	3.3	0.3	8.8
QGPC.ndsu.5B	I, II, IV, AE	wPt-1895-wPt-6191	111.7-113.3	101.1-127.5	3.6-6.6	3.2-3.4	-0.2_-0.3	5.3-13.7
QGPC.ndsu.6B.1	I, II, IV, AE	wPt-5234-wPt-1437	52.5-57.8	41.0-71.5	2.8-9.2	3.2-3.4	0.3-0.4	5.5-16.9
QGPC.ndsu.6B.2	IV	wPt-1241-wPt-3605	95.3	88.1-102.2	3.6	3.4	0.3	7.7
QGPC.ndsu.7B	III, IV, AE	wPt-0266-wPt-9299	95.5-121.6	90.2-131.7	2.7-6.0	3.2-3.4	0.3 ††RTPVE	4.7-10.4 38.5-40.3
<i>Mixogram envelope peak time</i>								
QMEPT.ndsu.1B	IV, AE	wPt-8320-wPt-1560	3.8	0-19.3	2.5-4.6	3.3-3.3	-0.6_-0.6	3.2-6.0
QMEPT.ndsu.1D	I, II, III, IV, AE	wPt-7333835-wPt-672077	83.5-85.5	68.8-93.1	8.0-23.6	3.2-3.3	0.8-1.9	18.7-41.2
QMEPT.ndsu.2B	IV	wPt-744808-wPt-4368	22.0	11.1-35.6	4.1	3.3	0.8	5.6
QMEPT.ndsu.2D.1	II	wPt-730568-wPt-671914	74.8	64.8-86.7	3.9	3.3	-0.5	7.2
QMEPT.ndsu.2D.2	IV	wPt-741084-wPt-666656	49.5	46.6-55.8	5.6	3.3	-1.0	7.9
QMEPT.ndsu.4A	IV	rPt-7285-wPt-6728	21.6	16.4-33.6	3.5	3.3	-0.8	4.8
QMEPT.ndsu.5B	I, IV, AE	wPt-1895-wPt-6191	110.7-118.3	94.6-135.3	3.0-5.5	3.3-3.3	0.5-1.1 ††RTPVE	5.5-10.1 25.9-64.5

(Continues)

Table 4-3. Summary of information for QTL identified in a RIL population derived from the cross of an elite line (WCB414) and exotic line (WCB617) with SS at four locations in ND, USA during 2009 and 2010. (Continued)

QTL	Environment [†]	Flanking markers	Pos [‡] . (cM)	CI [§] (cM)	LOD	Thresh. [¶]	a [#]	R ² (%)
<i>Mixogram MID line peak time</i>								
QMMLPT.ndsu.1B	IV, AE	wPt-8320-wPt-1560	3.8	0-20	2.9-4.8	3.1-3.2	-0.6_-0.7	4.1-6.5
QMMLPT.ndsu.1D	I, II, III, IV, AE	wPt-743310-wPt-672077	78.6-84.5	67.4-93.3	7.1-22.6	3.0-3.2	0.8-2.3	15.7-40.3
QMMLPT.ndsu.2D	II	wPt-730568-wPt-671914	74.8	64.8-86.8	4.0	3.0	-0.6	7.6
QMMLPT.ndsu.3A	AE	tPt-1079-wPt-1464	47.3	36.0-59.5	2.6	3.2	0.4	3.5
QMMLPT.ndsu.5B	I, III, IV, AE	wPt-1895-wPt-6191	110.7-127.3	101.1-157.2	2.9-4.1	3.1-3.2	0.6-1.0	6.0-7.9
QMMLPT.ndsu.6B	IV	wPt-0052-tPt-1723	150.4	142.0-164.1	3.3	3.1	0.8	5.6
							^{††} RTPVE	23.3-56.2
<i>Mixogram MID peak integral %TQ*MIN</i>								
QMMLPI.ndsu.1B	I, III	wPt-8320-wPt-1560	3.8	0-17.8	2.8-3.4	3.2-3.3	-17.5_-23.1	4.7-6.1
QMMLPI.ndsu.1D	I, II, III, IV, AE	wPt-743310-wPt-672077	79.6-83.5	68.8-94.1	7.0-22.2	3.1-3.3	28.9-60.5	16.4-37.1
QMMLPI.ndsu.2D.1	II	wPt-2675-wPt-8134	27.0	18.9-32.2	8.6	3.1	-36.7	17.7
QMMLPI.ndsu.2D.2	IV, AE	wPt-741084-wPt-666656	49.5	38.5-62.7	3.4-3.6	3.1-3.2	-18.2_-23.4	4.2-4.8
QMMLPI.ndsu.3D	III	wPt-669255-wPt-3094	33.7	20.0-42.3	3.4	3.3	-26.1	6.3
QMMLPI.ndsu.6B.1	I, AE	wPt-745074-wPt-6667	72.3	66.7-85.3	3.5-3.7	3.2-3.2	17.2-18.6	4.7-6.7
QMMLPI.ndsu.6B.2	IV	wPt-0052-tPt-1723	148.4	141.3-154.5	7.4	3.1	40.3	10.8
QMMLPI.ndsu.7D	II, AE	wPt-744917-wPt-664368	0.0	0-10.4	2.8-2.8	3.1-3.2	-15.5_-16.2	3.8-4.9
							^{††} RTPVE	32.4-52.1
<i>Mixogram</i>								
QMx.ndsu.2B.2	AE	wPt-8404-wPt-8398	76.8	63.8-86.4	5.3	3.3	-0.2	10.1
QMx.ndsu.3A.1	II	tPt-0242-wPt-0286	122.2	108.3-134.1	6.8	3.3	-0.4	15.6
QMx.ndsu.3A.2	II	wPt-1562-wPt-2740	163.4	153.7-165.9	5.6	3.3	0.3	11.0
QMx.ndsu.6A	III	wPt-666074-wPt-733115	109.6	99.0-141.0	3.9	3.3	0.2	12.6
QMx.ndsu.6B.1	II	wPt-0171-wPt-4164	8.5	0-14.6	3.8	3.3	-0.3	6.5
QMx.ndsu.6B.2	I, II, IV, AE	wPt-1756-wPt-5234	44.3-45.1	38.5-48.1	5.2-10.5	3.2-3.3	0.3-0.4	11.3-19.9
QMx.ndsu.7A	IV	wPt-3135-rPt-4199	72.2	63.9-85.3	2.8	3.2	-0.2	5.8

(Continues)

Table 4-3. Summary of information for QTL identified in a RIL population derived from the cross of an elite line (WCB414) and exotic line (WCB617) with SS at four locations in ND, USA during 2009 and 2010. (Continued)

QTL	Environment [†]	Flanking markers	Pos [‡] . (cM)	CI [§] (cM)	LOD	Thresh. [¶]	a [#]	R2(%)
<i>Mixogram MID line peak time</i>								
<i>QMx.ndsu.7B.1</i>	IV	wPt-8246-wPt-3445	5.7	0-20.3	2.7	3.2	-0.2	5.3
<i>QMx.ndsu.7B.2</i>	AE	wPt-744769-wPt-7108	1.0	0-7.4	3.8	3.3	-0.2	6.9
<i>QMx.ndsu.7D</i>	I, IV, AE	wPt-744917-wPt-664368	0.0	0-9.4	3.1-4.3	3.2-3.3	-0.2_-0.2	6.6-7.1
							^{††} RTPVE	29.0-48.8

[†]I, Prosper 2009; II, Carrington 2009; III, Prosper 2010; IV, Carrington 2010; V, Prosper 2011; VI, Carrington 2011.

[‡]Position

[§]Confidence Interval

[¶]Threshold calculated by permutation test.

[#]Additive effects

^{††}rank of phenotypic variation explained per environment

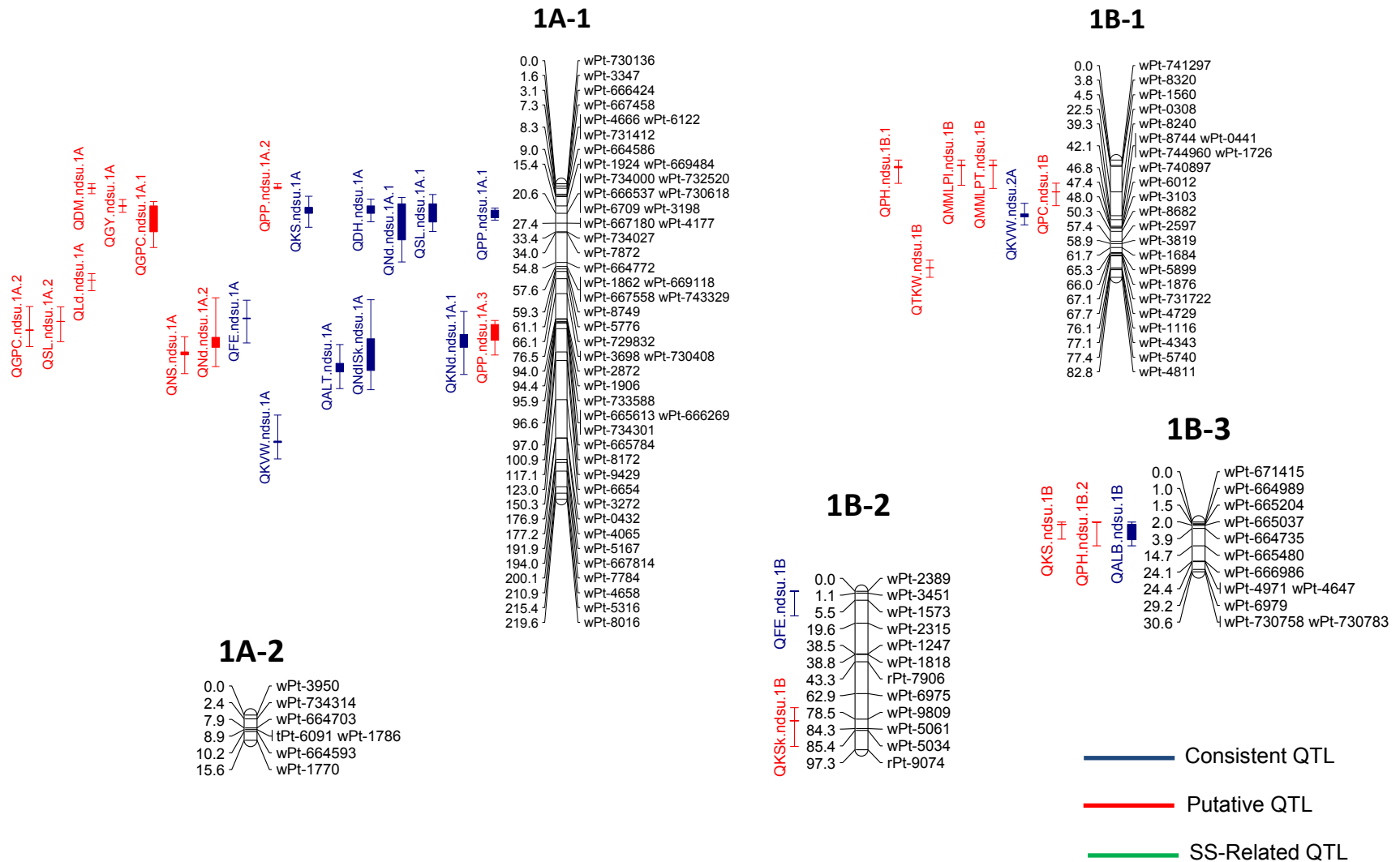


Fig. 4-2. Genetic map and QTL for 35 spike-related, agronomic, and quality traits of a RIL population derived from the cross of the elite line WCB414 and the exotic line WCB617 with SS trait, over four to six locations in ND, USA during 2009, 2010, and 2011

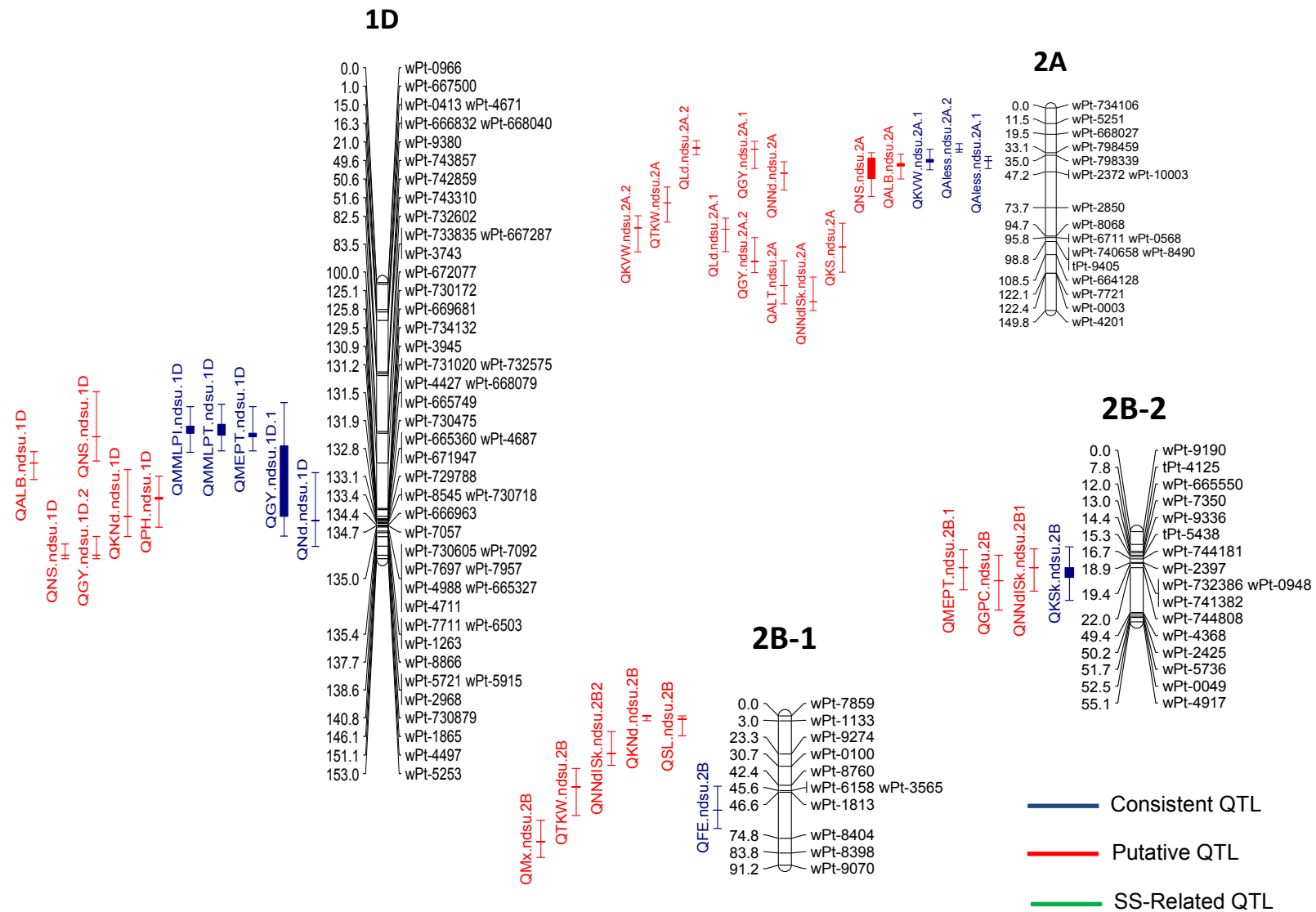


Fig. 4-2. Genetic map and QTL for 35 spike-related, agronomic, and quality traits of a RIL population derived from the cross of the elite line WCB414 and the exotic line WCB617 with SS trait, over four to six locations in ND, USA during 2009, 2010, and 2011 (Continued)

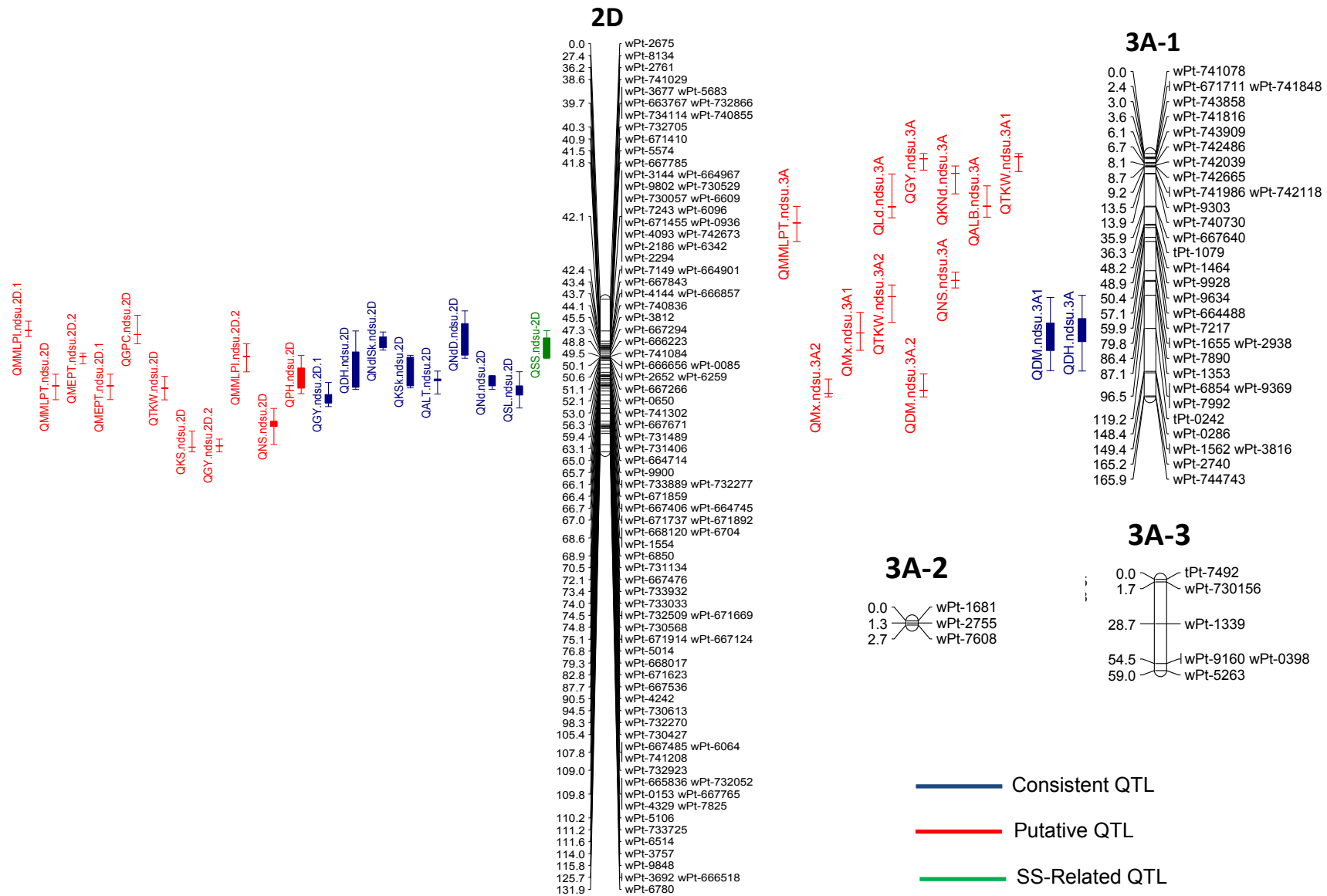


Fig. 4-2. Genetic map and QTL for 35 spike-related, agronomic, and quality traits of a RIL population derived from the cross of the elite line WCB414 and the exotic line WCB617 with SS trait, over four to six locations in ND, USA during 2009, 2010, and 2011 (Continued)

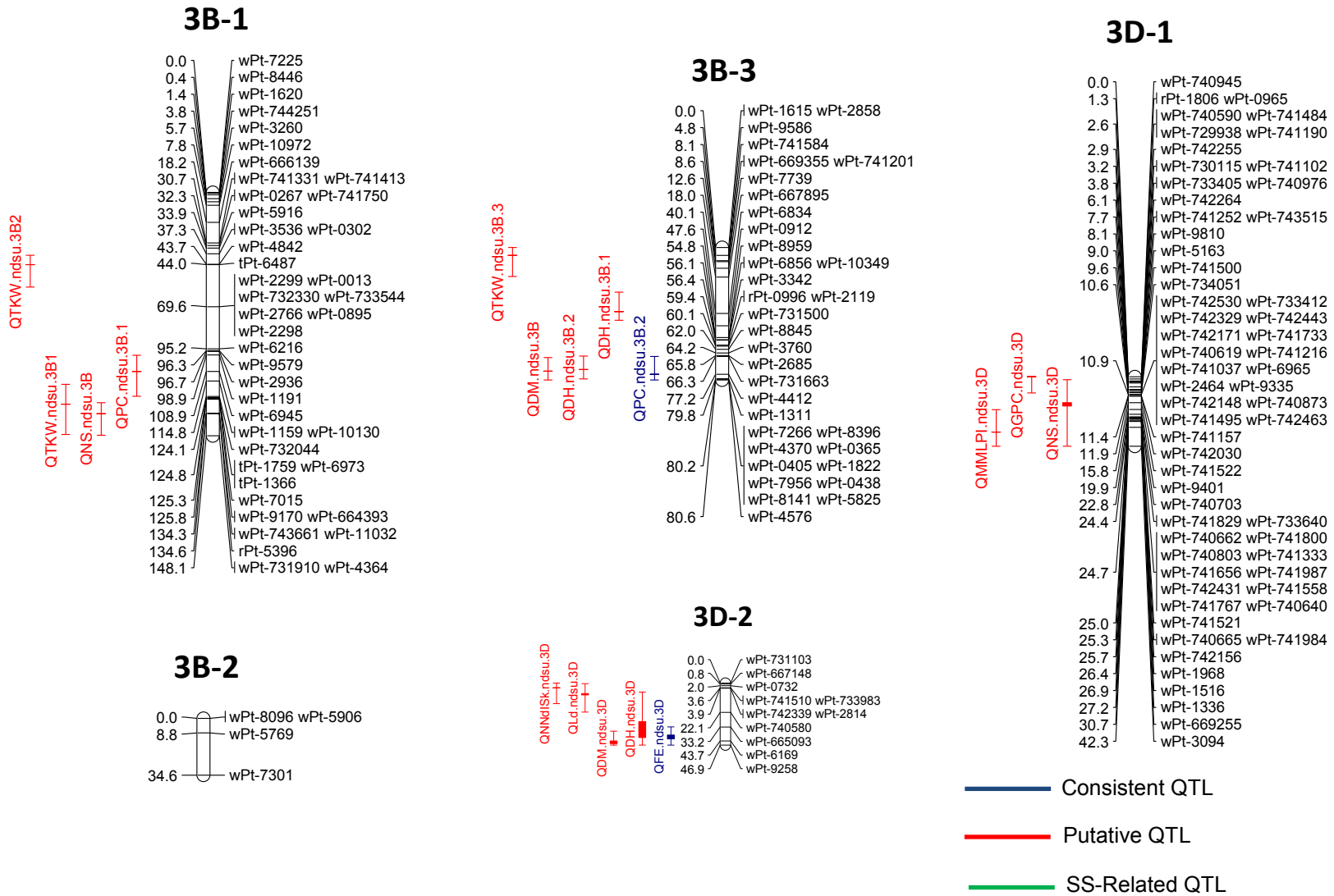


Fig. 4-2. Genetic map and QTL for 35 spike-related, agronomic, and quality traits of a RIL population derived from the cross of the elite line WCB414 and the exotic line WCB617 with SS trait, over four to six locations in ND, USA during 2009, 2010, and 2011 (Continued)

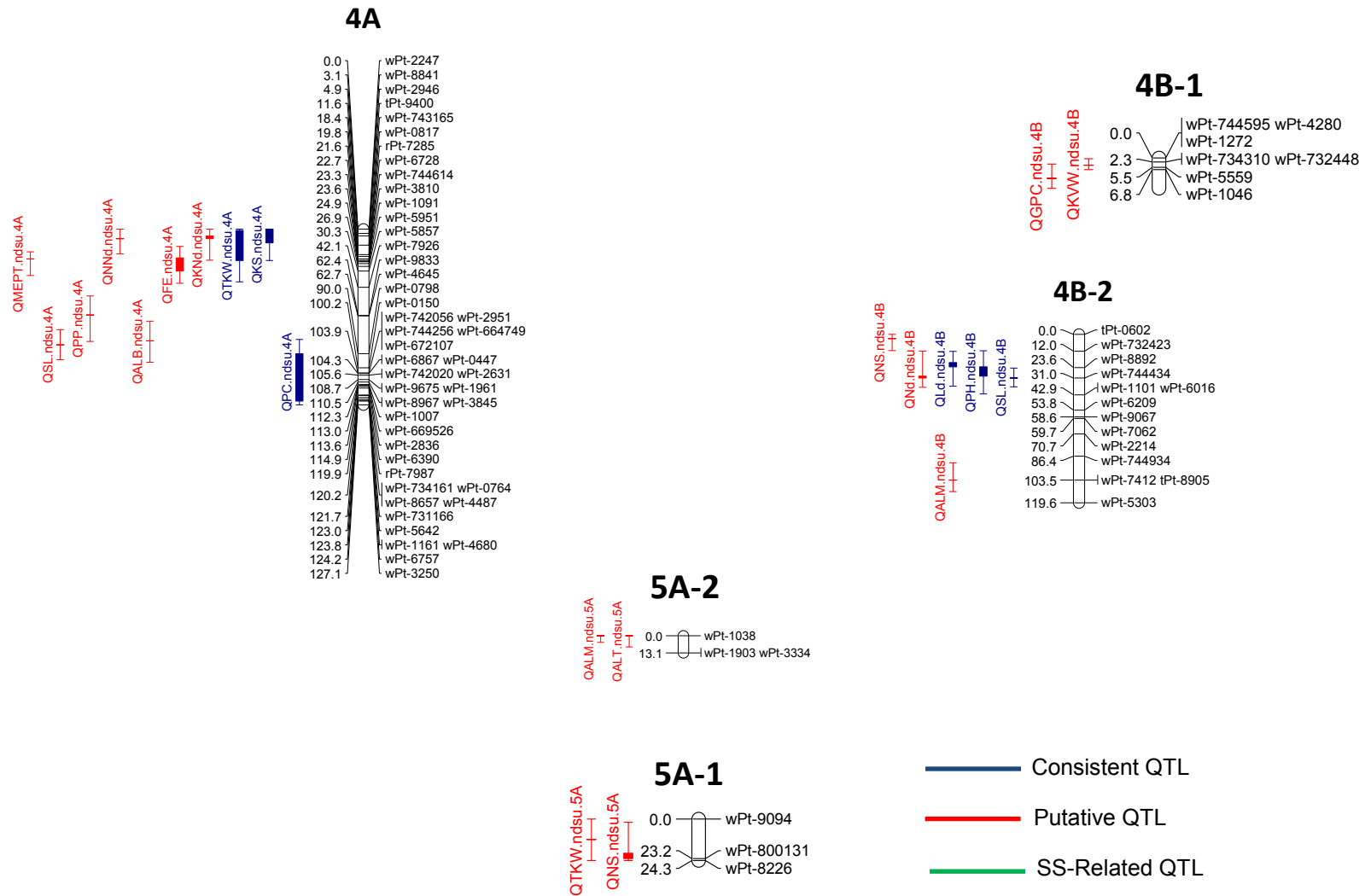


Fig. 4-2. Genetic map and QTL for 35 spike-related, agronomic, and quality traits of a RIL population derived from the cross of the elite line WCB414 and the exotic line WCB617 with SS trait, over four to six locations in ND, USA during 2009, 2010, and 2011 (Continued)

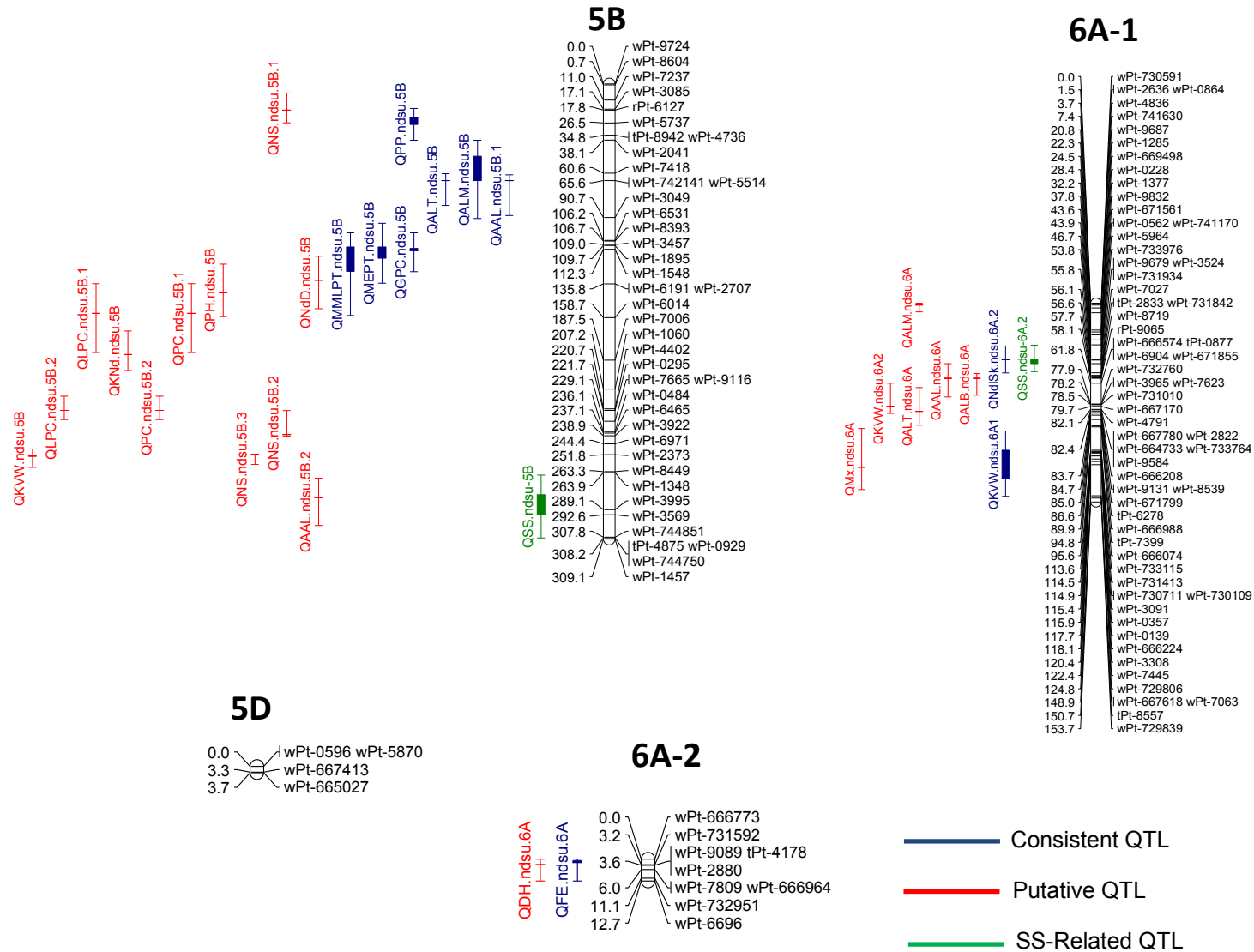


Fig. 4-2. Genetic map and QTL for 35 spike-related, agronomic, and quality traits of a RIL population derived from the cross of the elite line WCB414 and the exotic line WCB617 with SS trait, over four to six locations in ND, USA during 2009, 2010, and 2011 (Continued)

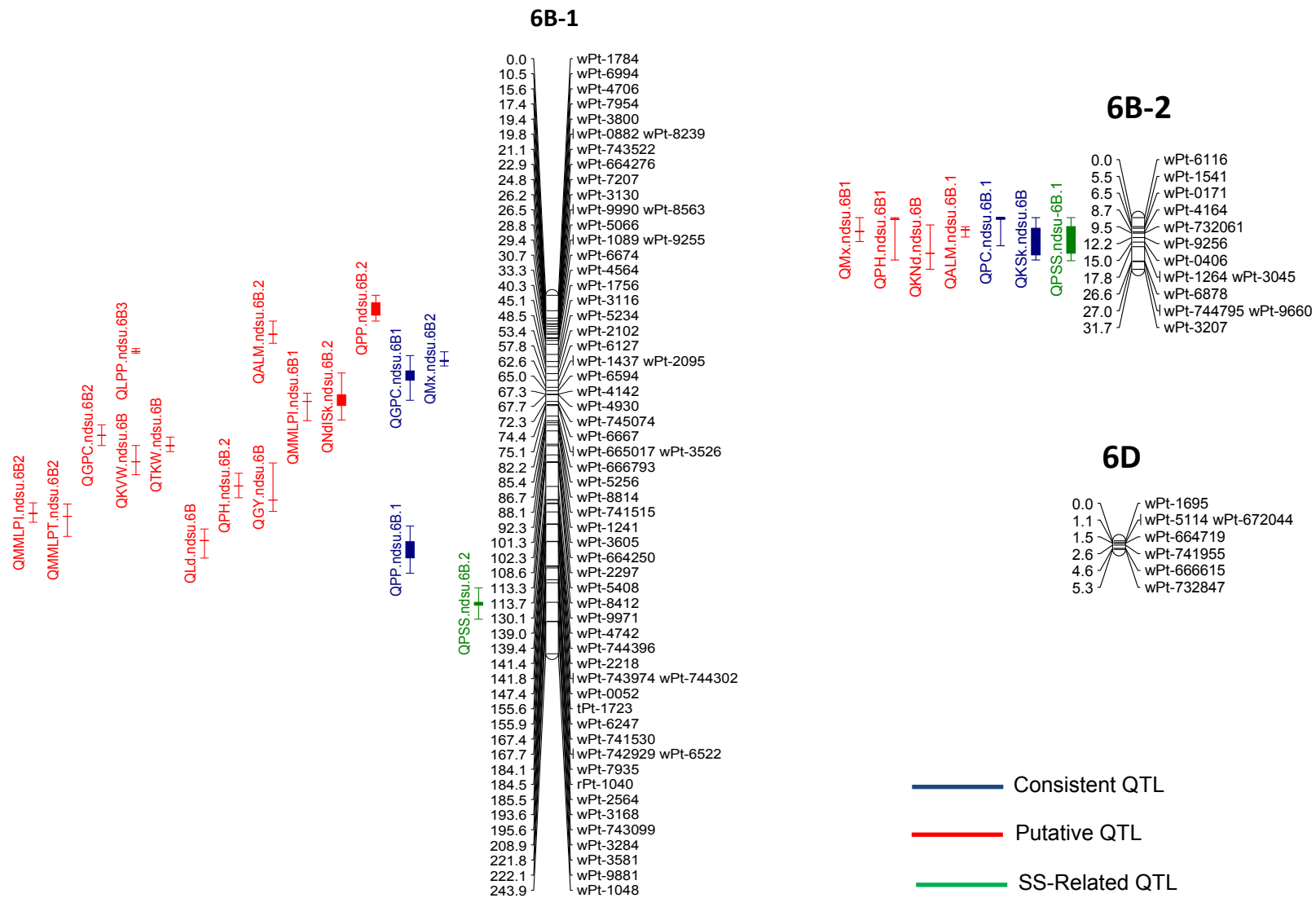


Fig. 4-2. Genetic map and QTL for 35 spike-related, agronomic, and quality traits of a RIL population derived from the cross of the elite line WCB414 and the exotic line WCB617 with SS trait, over four to six locations in ND, USA during 2009, 2010, and 2011 (Continued)

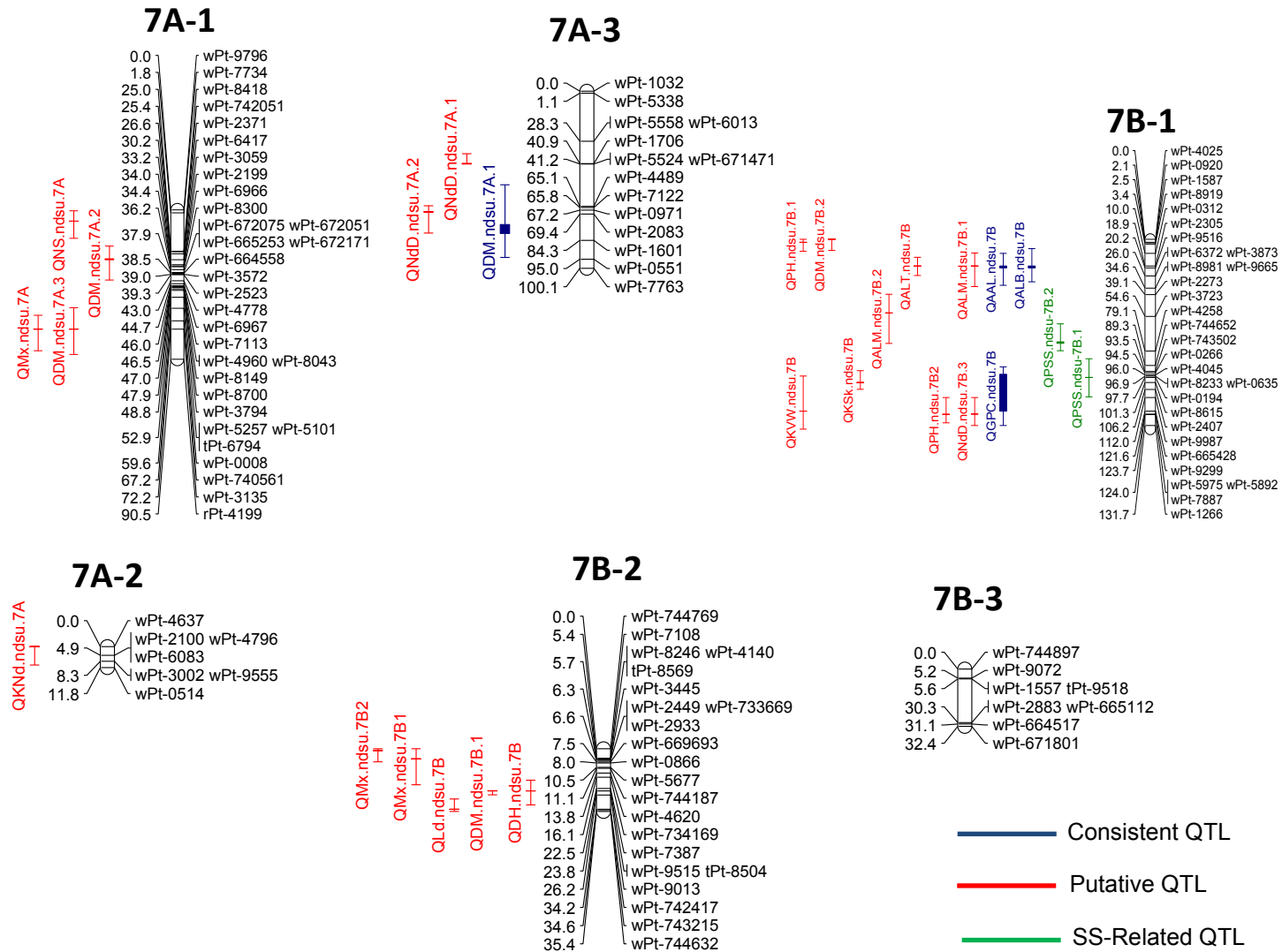


Fig. 4-2. Genetic map and QTL for 35 spike-related, agronomic, and quality traits of a RIL population derived from the cross of the elite line WCB414 and the exotic line WCB617 with SS trait, over four to six locations in ND, USA during 2009, 2010, and 2011 (Continued)

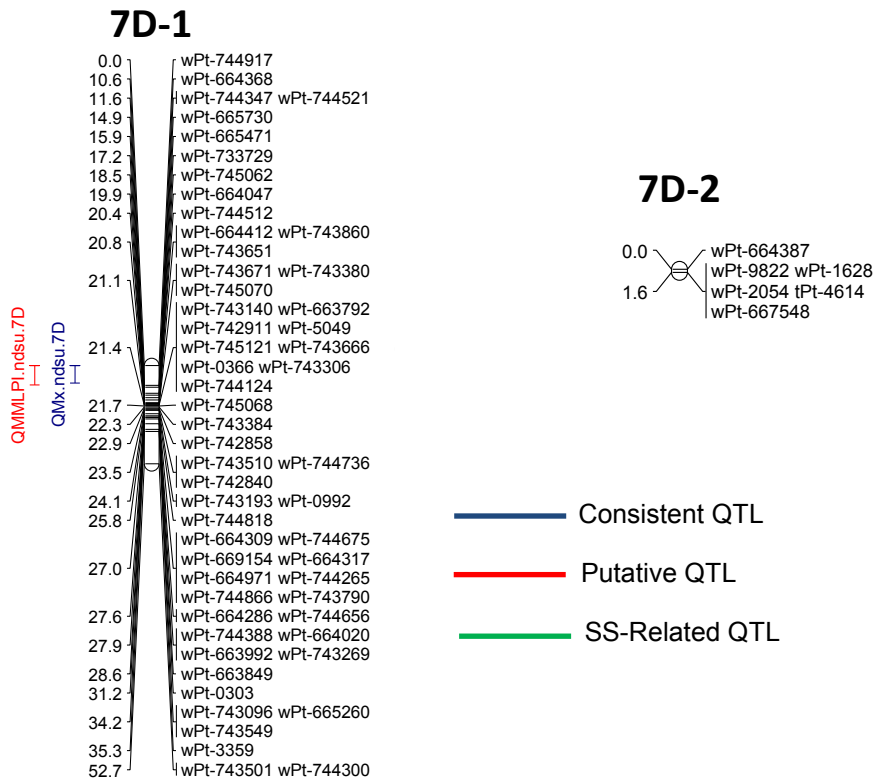


Fig.4-2. Genetic map and QTL for 35 spike-related, agronomic, and quality traits of a RIL population derived from the cross of the elite line WCB414 and the exotic line WCB617 with SS trait, over four to six locations in ND, USA during 2009, 2010, and 2011 (Continued)

The QTL mapping of MMLPT resulted in the identification of six QTL including two consistent QTL (*QMMLPT.ndsu.1D*, and *QMMLPT.ndsu.5B*) (Table 4-3). The number of QTL identified in individual environments for MMLPT ranged from two (Prosper 2009; Carrington 2009; and Prosper 2010) to four (Carrington 2010). The PV explained by all the QTL identified in individual environments ranged from 23.3%-56.2%, while the PV explained by individual QTL ranged from 3.5% to 40.3%. Among all these QTL, *QMMLPT.ndsu.1D* had a major effect on MMLPT as it explained up to 40.3% of PV and was consistently detected in all the environments. The other five minor QTL explained between 3.5% and 7.9% of PV (Table 4-3). The elite parent WCB414 contributed alleles for increased values of MMLPT at four loci, including both the major (*QMMLPT.ndsu.1D*) and minor consistent QTL (*QMMLPT.ndsu.5B*) (Table 4-3).

The trait MMLPI was associated with two consistent QTL (*QMMLPI.ndsu.1B*, and *QMMLPI.ndsu.1D*) and six putative QTL in this population (Table 4-3). The PV explained by individual

QTL ranged from 3.8% to 37.1% and the PV explained by all QTL in individual ranged 32.4% to 52.1%. A major QTL (*QMMLPI.ndsu.1D*) explaining up to 37.1% of PV for MLPI, located on 1DL was consistently detected in all the environments. Another major QTL which explained up to 17.7% PV for MMLPI was located on 2DS (*QMMLPI.ndsu.2D.1*), but was identified in only one environment. The remaining minor QTL explained 3.8% to 10.8% of PV (Table 4-3). The elite parent WCB414 contributed the alleles for increased values of MMLPI at *QMMLPI.ndsu.1D*, *QMMLPI.ndsu.6B.1*, and *QMMLPI.ndsu.6B.2*); while the exotic parent WCB617 provided the alleles that increased the values of this trait at the remaining loci (Table 4-3).

CIM for Mx resulted in the detection of a total of 10 QTL which include two consistent QTL (*QMx.ndsu.6B.2* and *QMx.ndsu.7D*). The PV explained by individual QTL for Mx ranged from 5.3% to 19.9%, while PV explained by all QTL in individual environments ranged from 29.0% to 48.8%. Among all the consistent QTL located on 6BS (*QMx.ndsu.6B.2*) explained the highest PV by 19.9% for Mx. Another major QTL of Mx, explained up to 15.6% PV and was located on 3A (*QMx.ndsu.3A.1*), but could be detected in single environment only. The remaining eight QTL explained a minor portion of the PV, ranging from 5.3% to 11.0%. WCB 414 alleles increased the trait values at three loci (*QMx.ndsu.3A.2*, *QMx.ndsu.6A*, and *QMx.ndsu.6B.2*), while WCB 617 alleles contributed to increase phenotypic values of Mx at remaining seven loci (*QMx.ndsu.2B.2*, *QMx.ndsu.3A.1*, *QMx.ndsu.6B.1*, *QMx.ndsu.7A*, *QMx.ndsu.7B.1*, *QMx.ndsu.7B.2*, and *QMx.ndsu.7D*).

4.5. Discussion

4.5.1. Phenotypic variations

A better performance of WCB414 for TKW, KVV, FE, and mixograph-related traits was expected, considering that this genotype is an elite line. In most of the environments, the RIL population showed transgressive segregation (Fig. 4-1; Supplementary Table 4-1) for all the traits, demonstrating the allelic contribution of both parents and the formation of new recombinant lines. As was reported in previous studies, the quality traits had genotype × environment interaction (Lukow and McVetty, 1991; Peterson et al., 1992; Gross et al., 2003; Huang et al., 2006; Raman et al., 2009; Sun et al., 2009; Wang et al., 2009; Tsilo et al., 2010; Heidari et al., 2011; Tsilo et al., 2011; Simons et al., 2012) (Table 4-1). The influence of

the environment on the expression of the quality traits was also confirmed by the inconsistent correlations between some traits. For instance, TKW and GPC were negatively associated in two environments, but positively associated in one environment. Likewise, the low values of heritability observed in most of these traits (except FE) indicate a strong influence of the environment on the phenotypic the values.

The positive significant associations observed between FE and TKW, as well as between FE and KVV are expected considering the impact of grain characteristics on milling characteristics. Similarly, the associations between GPC and mixograph related traits were expected considering that dough mixing properties are influenced by the quality and quantity of proteins (Finney, 1997). The positive and strong associations among several of the mixograph-related traits are suggesting the presence of common genes controlling these traits.

4.5.2. QTL for TKW, KVV and FE

The detection several QTL associated to TKW, KVV, and FE is in agreement with the quantitative genetic control and high environment influence on these traits, as demonstrated in previous studies (Campbell et al., 1999; Campbell et al., 2001; Börner et al., 2002; Gross et al., 2003; Huang et al., 2003; Huang et al., 2004; Huang et al., 2006; McCartney et al., 2005; Kuchel et al., 2006; Kunert et al., 2007; Li et al., 2007; Cuthbert et al., 2008; Sun et al., 2009; Sun et al., 2010; Raman et al., 2009; Wang et al., 2009; Tsilo et al., 2010; Heidari et al., 2011; Bennet et al., 2012; Simons et al., 2012; Maphosa et al., 2013). Breeders and millers use both TKW and KVV to determine the potential phenotypic values of FE in their genotypes (Wheat Marketing Center, 2008). Indeed, in this study, these three traits were positively correlated (Table 4-3). Consequently, it is reasonable to expect QTL with pleiotropic effects or close linkage, for these traits. CIM indicated that 1B is the only chromosome bearing QTL for TKW (*QTKW.ndsu.1B*), KVV (*QKVV.ndsu.1B*), and Fe (*QFE.ndsu.1B*). However, these QTL were located at different positions and could be considered different loci. Nevertheless, other chromosomes also harbored QTL for two of these traits as well. For example, chromosomes 2A and 6B had QTL for TKW (*QTKW.ndsu.2A*) and KVV (*QKVV.ndsu.2A.1* and *QKVV.ndsu.2A.2*); 1A and 6A had QTL for KVV (*QKVV.ndsu.1A*) and FE (*QFE.ndsu.1A*); and 2B had QTL for TKW (*QTKW.ndsu.2B*) and FE (*QFE.ndsu.2B*). However, in all of these cases, the QTL for different traits were located at different

positions suggesting absence of linked genes or common genes with pleiotropic effects. Exception to this trend was observed on chromosome 4A, where the positions of *QTKW.ndsu.4A* and *QFE.ndsu.4A* overlapped suggesting a pleiotropic effect of this genetic region on TKW and FE (Table 4-3; Fig. 4-2).

QTKW.ndsu.4A was a major QTL for TKW located on 4AL (Table 4-3, Fig. 4-2). This could be an excellent target for the selection of TKW through molecular markers, considering the fact that this QTL was consistently detected in all the environments. Previous study also reported the presence of a QTL controlling TKW on 4AL (Araki et al., 1999; McCartney et al., 2005). In addition to the co-localization with *QFE.ndsu.4A*; *QTKW.ndsu.4A* was also co-located with putative QTL associated to KNd (*QKNd.ndsu.4A*), Nd (*QNNd.ndsu.4A*) and KS (*QKS.ndsu.4A*) identified earlier in the same population (Chapter 3) (Fig. 4-2). This cluster of QTL suggests a pleiotropic effect of this genetic region on spike and kernel development with impact on grain-quality traits. Chromosome 4A is affected by a pericentrical inversion that moved several of the ancestral genes located on 4AS to 4AL and vice versa (Hernandez et al., 2012). QTL for KVV (Huang et al., 2006; Sun et al., 2009), GY (Gross et al., 2003; McCartney et al., 2005; Marza et al., 2006), and Ld (Marza et al 2006) have been also located on 4AL.

Kernel volume weight (also called test weight) is a grade-determining factor of wheat quality defined as the weight of grain per unit of volume (Steve et al., 2004; Wheat Marketing Center, 2008). A major QTL *QKVV.ndsu.6A.1* for KVV, detected consistently in all the environments, was located on the distal region of 6AL. The QTL allele from WCB414 increased KVV and could be considered for MAS in wheat breeding programs aimed at increasing KVV. In this population, *QKVV.ndsu.6A.1* was also co-located with a minor and putative QTL for Mx detected in Carrington 2010. Indeed, a positive correlation was also observed between KVV and Mx (Table 4-2) in this environment. A previous study also detected a QTL associated to KVV on the distal region of 6AL in Chinese winter wheat varieties (Sun et al., 2009). Another QTL associated to KVV under a water-limited environment was also detected on 6AL but in a different position (Bennett et al., 2012). The distal region of 6AL is also known to harbor QTL for grain weight per ear and leaf erectness (Börner et al., 2002).

Another major and consistent QTL for KVV was located 2A (*QKVV.ndsu.2A.1*), which explained up to 22.5% of PV for this trait. In this population, this genomic region has also been found to be associated with QTL for Aless and ALB (Chapter 3; Fig 4-2). Although phenotypically there was no

evidence of association between Aless and KVV, there is possibility that either there are tightly linked different genes controlling different traits a common locus having pleiotropic effect on apical awnleted expression and KVV. Another novel and consistent QTL associated to KVV was located on the distal region of chromosome 1AL (*QKVV.ndsu.1A*). Although, this region was associated with only KVV in this population, the distal region of 1AL has been shown to harbor QTL associated to Fusarium head blight (FHB) and deoxynivalenol (DON) toxin produced by FHB (Semagn et al., 2007).

The trait FE has the highest proportion of consistent QTL among all the eight quality traits studied in this population, which makes them suitable for MAS. All the chromosomes except 2B, where QTL for FE were identified in this study, have been reported to carry QTL for FE (Nelson et al., 2006; Kuchel et al., 2006; Simons et al., 2012). The novel QTL located on 2BS (*QFE.ndsu.2B*), although had major effect, it was detected in one environment only and needs further validation before it could be used in MAS. *QFE.ndsu.1A* was detected in all the four environments studied as well as across environments (Table 4-3). A previous study (Kuchel et al. 2006) has also reported a QTL on 1A associated with FE in a double haploid population derived from two commercial wheat varieties. However this QTL was detected in one environment only. *QFE.ndsu.1A* was located on the long arm of chromosome 1A and its confidence interval (CI) overlapped a QTL rich region associated with ALT (*QALT.ndsu.1A*), NS (*QNS.ndsu.1A*), KNd (*QKNd.ndsu.1A*), NNdISk (*QNNdISk.ndsu.1A*), Nd (*QNd.ndsu.1A*), and PP (*QPP.ndsu.1A.3*) (Chapter 2; Fig 4-2).

Another consistent QTL *QFE.ndsu.1B* for FE was located on 1B and was detected in both locations (Prosper, Carrington) in the year 2010 as well as AE. Previous study also reported a QTL for FE in a population derived from the cross of a soft red spring wheat and a HRSW (Simons et al. 2012). The chromosome 3D also harbor a consistent QTL (*QFE.ndsu.3D*) for FE. Recently, a QTL for FE also was detected on the distal region of 3DL under water-limited conditions (Maphosa et al., 2013). QTL for other traits like DH (*QDH.ndsu.3D*) and DM (*QDM.ndsu.3D*) have also been identified in this population in the same region where *QFE.ndsu.3D* was located (Chapter 3; Fig.4-2). The QTL alleles for higher FE and early heading and maturity at those loci were contributed by WCB414 suggesting that the selection of QTL alleles for higher FE could also result in early heading and maturity. This was also supported by negatives correlation observed between FE and DH, as well as between FE and DM (data not shown).

QFE.ndsu.6A was the only QTL associated with FE, for which the alleles from the branched parent WCB617 were responsible for its increase. Considering the high stability in detection of *QFE.ndsu.6A* (Table 4-3), alleles of the exotic parent at this QTL are excellent candidate to be introduced into the wheat breeding programs. *QFE.ndsu.6A* was located on the long arm of chromosome 6AL, where QTL for DH was reported earlier in the same population (*QDH.ndsu.6A*). QTL for FE has also been reported on 6A in a previous study using a population derived from two commercial wheat varieties (Kuchel et al., 2006).

4.5.3. QTL for GPC and mixograph-related traits

GPC affects mixing properties of wheat (Finney, 1997). Therefore, it is important for breeding purpose to recognize genetic regions controlling GPC and dough strength. For this purpose, this study detected a consistent QTL on 5BL with pleiotropic effect on GPC (*QGPC.ndsu.5B*), MEPT (*QMEPT.ndsu.5B*), and MMLPT (*QMMLPT.ndsu.5B*) (Table 4-3; Fig. 4-2). For this QTL, alleles from the exotic parent increased GPC but decreased the mixograph-related values. These results are in agreement with the negative correlations observed between GPC among mixograph-related traits in the present study (Table 4-2) as well as with some previous studies that have showed negative associations among these traits (Huang et al., 2006; Simons et al., 2012). The QTL for GPC on 5BL by itself has been reported in several other studies (Gross et al., 2003; Kulwal et al., 2005; Conti et al., 2011; Bordes et al., 2011, 2013). However, similar to our finding, a recent study also reported a common QTL for GPC and MMLPT on 5BL (Simons et al., 2012). In both studies, the QTL were located in the distal region of 5BL. This QTL represents probably the gene earlier identified on 5BL (Cane et al., 2008; Maphosa et al., 2013) that encode for the protein Serpin (Serine proteinase inhibitor) (Rasmussen et al., 1996). This protein represents up to 4% total protein in the grains of monocot cereals (Østergaard et al. 2000) and has been shown to affect quality traits such as FE (Cane et al., 2008).

Another closely linked QTL for GPC and mixograph-related traits also were observed on 6BS. The QTL for GPC (*QGPC.ndsu.6B*) and Mx (*QMx.ndsu.6B*) had consistent and major effect, while another QTL found in this region for MMLPI (*QMLPI.ndsu.6B*) had minor effect. At these loci on 6BS, the elite parent WCB414 contributed alleles that increased phenotypic values of these traits. Probably the

highly consistent QTL, *QGPC.ndsu.6B.1*, is associated to gliadins genes located on 6BS (Payne et al., 1987).

Past studies have also studied a large number of mixograph-related traits. Examples of these traits are mixograph midline peak value, mixograph line peak integral, mixograph weakening slope, and mixograph total energy. However, to the date, there is no consensus on the merits of each of these mixograph-related traits in the selection of the best dough properties (Ingelin, 1997). Moreover, often time, the information generated from different studies is not interchangeable since it is derived from devices designed by different manufactures (Ingelin, 1997). In this study, to the best of our knowledge, this is the first time that QTL for MEPT are reported. However, QTL for MMLPT and MMLPI have been identified in few earlier studies (Campbell et al., 2001; Huang et al., 2006; Tsilo et al., 2011; Li et al., 2012b; Simons et al., 2012; Mergoum et al., 2013). QTL controlling MEPT (*QMEPT.ndsu.1B*), MMLPT (*QMMLPT.ndsu.1B*) and MMLPI (*QMMLPI.ndsu.1B*) were detected on chromosome arm 1BS, which coincides with the location of *Glu-B3* gene (McCartney et al., 2006, Mann et al., 2009; Maphosa et al., 2013), a gene that encode for a LMW-GS (Simons et al., 2012). The positive alleles responsible for increasing MEPT, MMLPT, and MMLPI for loci located on 1BS were contributed by the exotic parent, demonstrating the potential use of WCB617 in improving wheat quality traits. This locus on 1BS controlling mixograph-related traits, also overlaps with a cluster of other QTL associated with GPC (*QGPC.ndsu.1B*), and KVV (*QKVV.ndsu.1B*) as shown in the present study (Fig. 4-2) and PC (*QPC.ndsu.1B*) identified previously in the same RIL population (chapter 3). The chromosome 1B is well documented to play an important role in wheat quality as it harbors several genes for gluten strength and other quality traits (Campbell et al., 2001; Huang et al., 2006; Mann et al., 2009; McCartney et al., 2006; Tsilo et al., 2010; Kumar et al., 2013; Maphosa et al., 2013). Comparison of map position, however, suggests that the QTL identified in the present study on 1BS are probably different than *Glu-B1*. However further investigation may needed to clarify this result.

The most consistent genomic region associated with mixograph-related traits was identified on chromosome 1D, where major QTL for MEPT (*QMEPT.ndsu.1D*), MMLPT (*QMMLPT.ndsu.1D*), MMLPI (*QMMLPI.ndsu.1D*) were identified. The position of these QTL coincides with the position of *Glu-D1* gene at 73.0 cM (Mann et al., 2009) that encode for an *HMW-GS*. The high PV explained by these QTL

(15.4%-41.2%) and their stability across environments confirms previous studies results which identified major QTL/QTL-clusters associated with mixograph properties as well as with other quality traits, in the region near by *Glu-D1* (Campbell et al., 2001; Huang et al., 2006; Nelson et al., 2006; Mann et al., 2009; Tsilo et al., 2010; Simons et al., 2012).

As there were high correlations between MEPT, MMLPT and MMLPI, it was expected to identify common loci controlling these traits. However, some of the QTL specific to only one mixograph-related traits were also identified. For example, two QTL located on 2BL (*QMEPT.ndsu.2B*) and 4AL (*QMEPT.ndsu.4A*) were specific to MEPT; one QTL located on chromosome 3A (*QMMLPT.ndsu.3A*) was specific to MMLPT and three QTL located on 3DS (*QMMLPI.ndsu.3D*), 6BS (*QMMLPI.ndsu.6B.1*), and 7DS (*QMMLPI.ndsu.7D*) were specific to MMLPI. Most of these QTL, although reported first time, have minor effects and were unstable across environments. This suggests a need to further study these QTL, before any recommendations could be made for their use in improving wheat quality.

Interestingly, the trait Mx shared only one common QTL with MMLPI, which was located on chromosome 7DS at 0.01 cM (Table 4-3; Fig. 4-2). The lack of additional common QTL among Mx and mixograph-related traits could be due to difference in the methodologies conducted to assess each trait. Mx is assessed through a visual scale; while the information of MEPT, MMLPT, and MMLPI is collected from the Mixmart software. Although, Mx is broadly used by the breeders to select germplasm with optimal dough strength, the genetic dissection of this trait have not received much attention in the past. In one study, the presence of four QTL located on chromosomes 1B, 1D, 3B, 6D controlling Mx was reported (Tsilo et al., 2011). However, all those QTL differ from the QTL described in our study, which were located on 2BS, 3A, 6AL, 6BS, 6BL, 7AS, 7BL, and 7DS. Two consistent QTL associated with Mx were identified; one on chromosome 6B (*QMx.ndsu.6B.2*) and the other on 7D (*QMx.ndsu.7D*). Once again, the exotic parent WCB617 played an important role in providing alleles that increased the values of Mx at seven QTL, including the consistent QTL located on 7D (Table 4-3). This shows the potential use of WCB617 as a source for improving some important wheat quality traits.

4.5.4. Influence of SS loci on quality traits

The exotic parent (WCB617) used in the present study has SS phenotype. For this reason, it was used to compare the genomic locations of QTL identified for SS (Chapter 2) with QTL for quality traits identified in this study. This study identified QTL for TKW (*QTKW.ndsu.2D*), GPC (*QGPC.ndsu.2D*), MEPT (*QMEPT.ndsu.2D.1*, *QMEPT.ndsu.2D.2*), MMLPT (*QMMLPT.ndsu.2D*), and MMLPI (*QMMLPI.ndsu.2D.1*, *QMMLPI.ndsu.2D.2*) on chromosome 2D. The map location of these QTL have overlapping CI with a major QTL for the SS-phenotype and some other spike-related and agronomic traits mapped in the same region and/or they were mapped very close to each other on 2D (chapter 2 and chapter 3) (Fig. 4-2). This 2D region has been identified as a rich gene region previously (Erayman et al. 2004). Other genomic regions where minor quality QTL (KVW, Mx and GPC) were co-located/linked with QTL for SS were on chromosome 6B and 7B. Interestingly, the co-localization of QTL for SS and quality related traits was also supported by significant correlations observed between SS and quality related traits (TKW, GPC, mixograph-related traits) (Appendix Table C3).

Except for TKW QTL on 2D genomic region, the positive QTL alleles for SS and quality traits were contributed by the exotic parent WCB617. Supporting our findings a study identified a cluster of QTL for mixograph and baking traits on 2D and also reported that the favorable QTL alleles were derived from wild wheat specie at those loci (Li et al., 2012). This clearly suggests the potential usefulness of exotic germplasm for quality traits improvement in wheat.

4.6. Conclusion

The use of a RIL population generated from an exotic germplasm with SS and a white elite wheat line in our study resulted in the identification of a large number of QTL for eight important wheat quality traits. A total of 69 QTL were detected for these traits, in which the exotic parent provided alleles with increasing effect in 51% of these QTL. These results suggest that germplasm with SS is a valuable resource to improve quality traits in wheat. Therefore, identifying molecular markers associated with consistent and/or major QTL detected in this study could be of great interest for wheat breeding programs.

4.7. References

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CHAPTER 5. GENERAL CONCLUSIONS

In this study, for the first time, a RIL population derived from an elite wheat line and an exotic wheat genotype with SS and glume pubescences was used to map QTL of 35 wheat traits. A total of 221 QTL were detected (Fig 5.1), out of them, 30% were spike-related QTL (including QTL for SS), 39% agronomic-related QTL, and 31% quality-related QTL. These QTL were distributed across the entire wheat genome, with 44% of the QTL on the B-genome, 37% of the QTL on A-genome, and only 19% of the QTL on the D-genome. Chromosome 6B had the highest number of QTL (23 QTL); meanwhile none QTL were reported for chromosomes 4D, 5D and 6D. A polygenic control was found for most of the traits. Most (81%) of individual QTL explained less than 15% of PV associated with the traits (minor QTL), and 19% of individual QTL explained more than 15% of PV (major QTL). Most of the QTL were putative (71%) demonstrating a high influence of the environment on their expression. In general, both parental genotypes contributed equally with favorable alleles that increased the effects of phenotypic values of the traits. Precisely, the elite parent (WCB414) provided 52% of these alleles; meanwhile the exotic parent provided 48%. Therefore, these results demonstrate the suitability of the exotic germplasm to discover new genes and alleles with potential use into the wheat breeding programs.

Whole genome QTL mapping of seven SS-related traits (PSS, Sk, SD, NdSS, SkNd, NdR, and NdNonSS) resulted in the identification of seven stable QTL and showed a polygenic control of these traits. A major QTL was identified on 2DS (*QSS.ndsu-2D*) explained up to 25% of PV of SS trait. This QTL was also reported on previous studies. The presence of another major QTL for SS on 7BL (*QSS.ndsu-7B.2*), as well as the detection of five minor QTL located on 5BL (*QSS.ndsu-5B*), 6A (*QSS.ndsu-6A*), 6BL (*QSS.ndsu-6B.1* and *QSS.ndsu-6B.2*) and 7BL (*QSS.ndsu-7B.1*) were identified for the first time. Together these QTL explained up to 82% of PV of some traits such as NdSS). Digenic epistatic interaction observed between some QTL can be responsible for increase or decrease of SS expression in some genotypes. Further experiments will be required to physically detect and to clone *QSS.ndsu-2D*, as well as to assess the high level interactions between the seven QTL detected in order to understand the nature of the SS inheritance in hexaploid wheat.

Considering the impact of the SS trait on spike morphology, the genetic dissection of 10 spike-related traits (PP, PC, SL, Nd, NdD, ALT, ALM, ALB, AAL, Aless), was conducted. A total of 60 QTL were associated to these spike-related traits. Among these, 35% were consistent QTL and 65% putative QTL. Chromosome 1A and 5B had the highest number of QTL associated to spike-related traits (8 QTL each). Indeed, it was on 1AS where we detected *QPP.ndsu.1A1*, a QTL for glume pubescences that explained the maximum PV (92%) explained by a single QTL. For the spike-related traits, 55% of the alleles that increased phenotypic values were derived from the exotic parent (WCB 617).

To identify new QTL and/or alleles with agronomic potential value, ten agronomic traits (KS, KSk, KNd, NdImmSk, GY, NS, PH, Ld, DH and DM) were investigated in this study. A total of 85 agronomic-related QTL were identified of which 81% were putative QTL and 81% were minor QTL. Despite of this large number of putative and minor QTL, some agronomic-related QTL were exceptionally consistent and explained a high percentage of the PV. For instances, *QKSk.ndsu.2D* was detected in all the environments studied and explained up to 39% of PV of KSk; and *QGY.ndsu.2D1*, detected in three environments as well as AE, explained up to 40% of PV of GY (the highest PV explained so far for GY). Chromosomes 1A and 2D had the highest number of QTL (8 QTL each) associated with agronomic traits. The elite parent WCB617 provided 60% of the alleles that increased phenotypic values of agronomic traits.

The results obtained for quality traits assessed (TKW, KVV, GPC, FE, Mx, MEPT, MMLPT, and MMLPI) showed that the exotic parent has valuable genes which could be considered by breeders for the wheat improvement of traits such as GPC and gluten strength. The improvement of quality traits in wheat is a daunting challenge because most of these traits are controlled by several genes located in the D-genome which is the least diverse genome in wheat. A total of 69 quality-related QTL were identified in this study of which 72% were putative QTL. The parent with SS provided 52% of the alleles that increased phenotypic values of these quality traits. Several quality-related QTL were major and highly consistent QTL. For instance *QTKW.ndsu.4A* was detected in all the environments studied and explained up to 17% of the PV of TKW. Chromosome 6B had the highest number of QTL (9 QTL) for quality traits.

The impact of *QPP.ndsu.1A1* and *QSS.ndsu.2D*, two major QTL for PP and SS, on other traits was evidenced in the clusters of QTL where these QTL were located. *QPP.ndsu.1A1* was clustered on

1AS with loci for SL, Nd, DH, KS, GPC, GY and DM; meanwhile *QSS.ndsu.2D* was clustered on 2DS with loci for SL, Nd, NdD, ALT, KSk, NNdISk, DH, KS, MMLPI, PH, GY,TKW, GPC, MEPT, MMLPT, NS. These groups of QTL suggest either close linkage or pleiotropic effects of the major loci for glume pubescences and SS on different spike-related, agronomic, and quality traits. This could be advantage for breeder, who could take advance of these genetic linkage/associations. For instance, breeders could consider the selection of pubescent spikes considering that *QPP.ndsu.1A1* has a positive pleiotropic effect on number of kernels per spike. Likewise, breeders could attempt to break the repulsion phase linkage between *QSS.ndsu.2D* and *QGY.ndsu.2D*, to improve GY in genotyped with SS.

APPENDIX A. SUPPLEMENTARY TABLES AND FIGURE FOR CHAPTER 2

Table A1. Mean values and RIL ranges of supernumerary-spikelet-related in each environment

Trait [†]	Experiment Categories	Environments [‡]						
		All	Carrington 2009	Carrington 2010	Carrington 2011	Prosper 2009	Prosper 2010	Prosper 2011
PSS	Experiment	0.4	0.4	0.4	0.5	0.5	0.4	0.4
	Parent WCB414	0.0	0.0	0.0	0.0	0.1	0.0	0.0
	Parent WCB 617	3.2	3.0	3.0	4.0	3.1	2.5	3.5
	RILs	0.4	0.4	0.4	0.5	0.5	0.4	0.4
	Minimum RILs	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Maximum RILs	4.0	4.0	4.0	4.0	4.1	4.0	4.0
	Checks	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	LSD 0.05	0.4	0.6	0.6	0.5	0.4	0.5	0.4
Sk	Experiment	19.9	19.3	19.7	-	19.6	21.3	-
	Parent WCB414	15.1	16.1	15.3	-	13.9	14.2	-
	Parent WCB 617	44.2	39.5	50.4	-	31.0	52.0	-
	RILs	20.0	19.3	19.7	-	19.8	21.4	-
	Minimum RILs	13.5	13.5	13.2	-	13.4	13.4	-
	Maximum RILs	51.0	60.3	51.5	-	52.9	63.8	-
	Checks	15.1	15.1	15.2	-	14.4	15.7	-
	LSD 0.05	6.6	3.5	7.2	-	2.9	6.7	-
SD	Experiment	2.1	2.1	2.1	-	2.1	2.2	-
	Parent WCB414	1.7	1.7	1.6	-	1.7	1.6	-
	Parent WCB 617	4.3	4.0	4.9	-	2.7	5.1	-
	RILs	2.1	2.1	2.1	-	2.1	2.2	-
	Minimum RILs	1.5	1.4	1.5	-	1.5	1.5	-
	Maximum RILs	5.1	6.0	5.5	-	5.0	6.2	-
	Checks	1.8	1.8	1.9	-	1.8	1.8	-
	LSD 0.05	0.4	0.4	0.7	-	0.3	0.7	-

(Continues)

Table A1. Mean values and RIL ranges of supernumerary-spikelet-related in each environment (continued)

Trait [†]	Experiment Categories	Environments [‡]						
		All	Carrington 2009	Carrington 2010	Carrington 2011	Prosper 2009	Prosper 2010	Prosper 2011
SkNd	Experiment	1.2	1.2	1.2	-	1.2	1.2	-
	Parent WCB414	1.0	1.0	1.0	-	1.0	1.0	-
	Parent WCB 617	2.6	3.1	2.5	-	1.8	3.0	-
	RILs	1.2	1.2	1.2	-	1.2	1.2	-
	Minimum RILs	1.0	1.0	1.0	-	1.0	1.0	-
	Maximum RILs	3.6	4.6	4.0	-	4.1	4.2	-
	Checks	1.0	1.0	1.0	-	1.0	1.0	-
	LSD 0.05	0.3	0.3	0.5	-	0.3	0.6	-
NdSS [§]	Experiment	0.9	0.8	0.9	-	1.0	1.0	-
	Parent WCB414	0.0	0.0	0.1	-	0.0	0.0	-
	Parent WCB 617	10.0	8.8	9.9	-	9.3	11.0	-
	RILs	0.9	0.8	0.9	-	1.0	1.0	-
	Minimum RILs	0.0	0.0	0.0	-	0.0	0.0	-
	Maximum RILs	10.0	10.8	10.2	-	11.5	12.9	-
	Checks	0.0	0.0	0.0	-	0.0	0.0	-
	LSD 0.05	1.2	1.2	1.8	-	0.5	1.0	-
NdR	Experiment	0.3	0.2	0.3	-	0.3	0.4	-
	Parent WCB414	0.0	0.0	0.0	-	0.0	0.0	-
	Parent WCB 617	3.2	3.5	4.4	-	0.5	3.6	-
	RILs	0.3	0.2	0.3	-	0.3	0.4	-
	Minimum RILs	0.0	0.0	0.0	-	0.0	0.0	-
	Maximum RILs	5.0	5.8	5.7	-	5.1	6.9	-
	Checks	0.0	0.0	0.0	-	0.0	0.0	-
	LSD 0.05	0.7	0.6	1.1	-	0.5	1.3	-

(Continues)

Table A1. Mean values and RIL ranges of supernumerary-spikelet-related in each environment (continued)

Trait [†]	Experiment Categories	Environments [‡]						
		All	Carrington 2009	Carrington 2010	Carrington 2011	Prosper 2009	Prosper 2010	Prosper 2011
NdNoSS	Experiment	16.6	16.5	16.2	-	16.4	17.2	-
	Parent WCB414	15.1	16.0	15.6	-	13.9	14.6	-
	Parent WCB 617	9.5	7.5	9.6	-	11.6	9.5	-
	RILs	16.6	16.6	16.3	-	16.6	17.3	-
	Minimum RILs	7.8	8.0	7.2	-	7.0	7.7	-
	Maximum RILs	22.1	21.6	20.8	-	23.1	25.4	-
	Checks	15.1	15.1	15.2	-	14.4	13.3	-
	LSD 0.05	1.7	2.3	2.7	-	2.0	2.4	-

[†]PSS level of penetrance of supernumerary spikelets (scale from 0 to 4); Sk, number of spikelets (spikelets spike⁻¹); SD spike density (Sk spike-longitude⁻¹); SkNd spikelets per node (spikelet node⁻¹); NdSS number of nodes with supernumerary spikelets per spike; NNdR number of nodes with extended rachillas (extended rachillas spike⁻¹); NdNoSS number of nodes with no supernumerary spikelets per spike

[‡]In Carrington 2011 and Prosper 2011 only were conducted measurements of PSS

[§]The four environments were not homogeneous in NdSS. Combined analysis for NdSS was performed with the three more homogeneous environments (Carrington 2009, Carrington 2010, and Prosper 2010)

Table A2. Coefficient of variance (CV%), and lattice efficiency (EF%) of supernumerary-spikelets-related traits

Trait [†]	Carrington 2009		Carrington 2010		Carrington 2011		Prosper 2009		Prosper 2010		Prosper 2011	
	CV%	EF%	CV%	EF%	CV%	EF%	CV%	EF%	CV%	EF%	CV%	EF%
PSS	78.00	100.80	76.01	99.44	48.50	99.18	51.43	99.80	58.10	102.18	41.01	99.03
Sk	9.08	101.35	18.84	105.03	-	-	7.52	101.73	16.29	98.95	-	-
SD	9.19	99.34	16.10	103.00	-	-	8.07	99.19	17.10	99.91	-	-
SkNd	10.94	100.36	22.50	101.89	-	-	10.96	100.06	24.79	99.47	-	-
NdSS	78.62	99.67	96.27	105.75	-	-	27.12	99.12	51.54	99.46	-	-
NNdR	143.82	99.84	164.92	108.05	-	-	95.40	102.44	177.97	99.09	-	-
NdNoSS	6.90	101.21	8.41	100.05	-	-	5.97	106.09	6.87	105.28	-	-

[†] PSS level of penetrance of supernumerary spikelets (scale from 0 to 4); Sk, number of spikelets (spikelets spike-1); SD spike density (Sk spike-longitude-1); SkNd spikelets per node (spikelet node-1); NdSS number of nodes with supernumerary spikelets per spike; NNdR number of nodes with extended rachillas (extended rachillas spike-1);NdNoSS number of nodes with no superpernumerary spikelets per spike
- non information collected

Table A3. Error mean square (EMS) and Fmax for supernumerary-spikelet-related traits

Trait †	Carrington 2009	Carrington 2010	Carrington 2011	Prosper 2009	Prosper 2010	Prosper 2011	Fmax ratio ^a
	EMS	EMS	EMS	EMS	EMS	EMS	
PSS	0.082	0.099	0.061	0.054	0.061	0.034	2.91
Sk	3.054	13.752	-	2.183	12.089	-	6.30
SD	0.036	0.113	-	0.028	0.135	-	4.82
SkNd	0.016	0.071	-	0.017	0.092	-	5.75
NdSS ^b	0.400	0.786	-	0.070	0.279	-	2.81
NNdR	0.1005	0.270	-	0.061	0.481	-	7.89
NdNoSS	1.290	1.852	-	0.962	1.397	-	1.93

† PSS level of penetrance of supernumerary spikelets (scale from 0 to 4); Sk, number of spikelets (spikelets spike-1); SD spike density (Sk spike-longitude-1); SkNd spikelets per node (spikelet node-1); NdSS number of nodes with supernumerary spikelets per spike; NNdR number of nodes with extended rachillas (extended rachillas spike-1);NdNoSS number of nodes with non- supernumerary spikelets per spike
- non information collected

a Ratio: test of homogeneity (greatest EMS / smallest EMS) should be smaller than 10-fold

b The four environments were not homogeneous in NdSS. Combined analysis of variance for NdSS was performed with the three more homogeneous environments (Carr09, Carr10, and Pros10).

Table A4. Correlation coefficients (r) among SS-related traits

Traits ^a	PSS	Sk	SD	SkNd	NdSS	NdR	NdNoSS
PSS ^b	1	0.85 ^{**§} 0.89 ^{**†}	0.80 ^{**§} 0.89 ^{**†}	0.88 ^{**§} 0.78 ^{**†}	0.91 ^{**¶}	0.74 ^{**§} 0.83 ^{**†}	-0.71 ^{**¶}
Sk		1	0.98 ^{**¶}	0.97 ^{**§} 0.94 ^{**†}	0.92 ^{**¶}	0.94 ^{**§} 0.90 ^{**†}	-0.62 ^{**¶}
SD			1	0.96 ^{**¶}	0.91 ^{**¶}	0.94 ^{**¶}	-0.66 ^{**¶}
SkNd				1	0.91 ^{**¶}	0.96 ^{**¶}	-0.76 ^{**¶}
NdSS					1	0.82 ^{**¶}	-0.80 ^{**¶}
NdR						1	-0.69 ^{**¶}

** Significant at $P < 0.01$; †r from one environment; §, ¶ r pooled from three and four environments, respectively

b Alternative pooled correlations were observed between the traits PSS-Sk, PSS-SD, and PSS-NdR. The lowest pooled r value is presented.

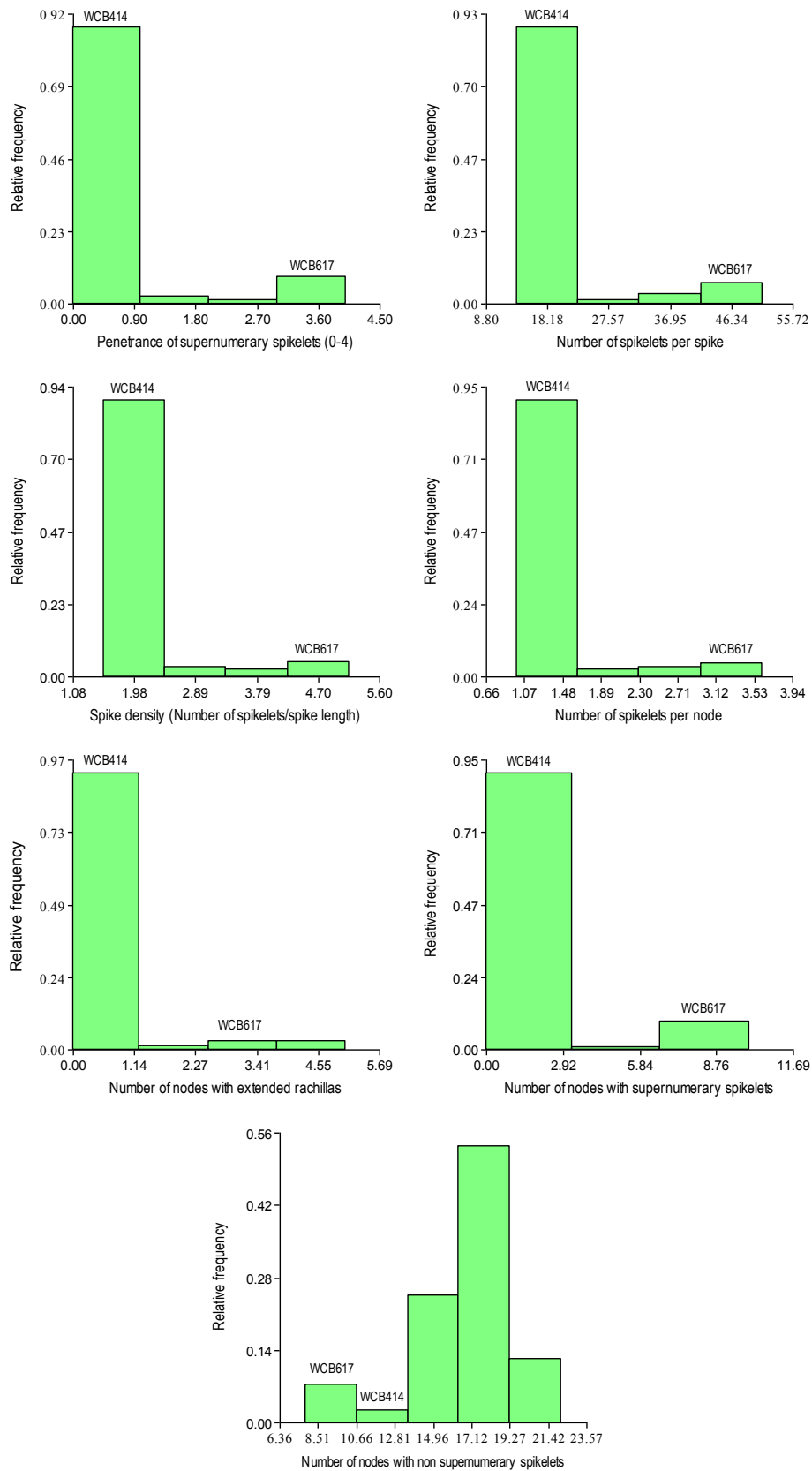


Fig. A1. Frequency distribution of 163 RIL for SS-related traits over four to six environments

APPENDIX B. SUPPLEMENTARY TABLES AND FIGURES FOR CHAPTER 3

Table B1. Mean values and RIL ranges of supernumerary-spikelet-related traits in each environment

Trait [†]	Experiment Categories	Environments						
		All	Carrington 2009	Carrington 2010	Carrington 2011	Prosper 2009	Prosper 2010	Prosper 2011
PP (0-4)	All Experiment	1.1	1.1	1.1	1.1	1.1	1.1	1.1
	Parent WCB414	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Parent WCB 617	4.0	3.9	4.0	4.0	4.0	4.0	4.0
	RILs	1.2	1.1	1.2	1.1	1.2	1.1	1.1
	Minimum RILs	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Maximum RILs	4.0	4.0	4.0	4.0	4.0	4.0	4.0
	Checks	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	LSD 0.05	0.6	0.7	0.3	0.5	0.6	0.3	0.4
PC (0-4)	All Experiment	0.2	0.3	0.2	0.1	0.3	0.2	0.1
	Parent WCB414	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Parent WCB 617	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	RILs	0.2	0.3	0.2	0.1	0.3	0.2	0.1
	Minimum RILs	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Maximum RILs	3.8	4.0	4.0	4.0	4.0	4.0	4.0
	Checks	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	LSD 0.05	0.5	0.8	0.8	0.3	0.6	0.6	0.5
SL (cm)	All Experiment	9.5	9.4	9.4	-	9.5	9.9	-
	Parent WCB414	9.1	9.3	9.5	-	8.2	9.2	-
	Parent WCB 617	10.3	9.8	10.3	-	11.1	10.1	-
	RILs	9.6	9.4	9.4	-	9.5	9.9	-
	Minimum RILs	7.9	7.3	7.4	-	7.8	7.8	-
	Maximum RILs	11.6	11.6	11.6	-	11.8	12.6	-
	Checks	8.3	8.3	8.1	-	8.1	8.7	-
	LSD 0.05	0.7	1.0	0.9	-	0.9	0.9	-

(Continues)

Table B1. Mean values and RIL ranges of supernumerary-spikelet-related traits in each environment (continued)

Trait [†]	Experiment Categories	Environments						
		All	Carrington 2009	Carrington 2010	Carrington 2011	Prosper 2009	Prosper 2010	Prosper 2011
Nd (nodes spike ⁻¹)	All Experiment	19.0	18.2	18.4	-	19.4	20.0	-
	Parent WCB414	16.1	16.4	16.7	-	15.2	16.2	-
	Parent WCB 617	24.0	22.2	22.6	-	26.3	25.1	-
	RILs	19.1	18.3	18.5	-	19.5	20.1	-
	Minimum RILs	15.2	14.4	14.9	-	14.8	14.5	-
	Maximum RILs	23.9	22.4	22.8	-	26.7	26.0	-
	Checks	16.4	16.0	16.4	-	16.1	17.3	-
	LSD 0.05	1.6	1.7	1.5		1.8	1.8	
NdD (nodes cm ⁻¹)	All Experiment	2.0	1.95	2.0	-	2.0	2.0	-
	Parent WCB414	1.8	1.8	1.7	-	1.8	1.8	-
	Parent WCB 617	2.3	2.2	2.2	-	2.4	2.5	-
	RILs	2.0	1.9	2.0	-	2.0	2.0	-
	Minimum RILs	1.5	1.4	1.6	-	1.5	1.6	-
	Maximum RILs	2.5	2.6	2.4	-	2.6	2.7	-
	Checks	2.0	1.9	2.0	-	2.0	2.0	-
	LSD 0.05	0.2	0.2	0.2		0.2	0.2	
ALB (cm)	All Experiment	2.9	2.7	3.3	-	3.4	2.3	-
	Parent WCB414	3.2	3.4	2.5	-	4.0	3.1	-
	Parent WCB 617	1.9	2.0	1.8	-	1.8	1.8	-
	RILs	2.9	2.7	3.3	-	3.4	2.3	-
	Minimum RILs	0.2	0.2	0.1	-	0.0	0.3	-
	Maximum RILs	4.3	4.6	6.0	-	5.1	3.8	-
	Checks	3.4	3.0	3.5	-	4.2	2.7	-
	LSD 0.05	0.7	1.0	1.1		1.2	0.9	

Table B1. Mean values and RIL ranges of supernumerary-spikelet-related traits in each environment (continued)

Trait [†]	Experiment Categories	Environments						
		All	Carrington 2009	Carrington 2010	Carrington 2011	Prosper 2009	Prosper 2010	Prosper 2011
ALT (cm)	All Experiment	3.2	3.2	3.7	-	3.7	2.3	-
	Parent WCB414	3.5	3.7	2.5	-	4.8	2.6	-
	Parent WCB 617	2.0	1.8	2.4	-	1.8	1.8	-
	RILs	3.2	3.2	3.7	-	3.6	2.3	-
	Minimum RILs	1.1	0.7	1.3	-	1.1	0.8	-
	Maximum RILs	4.8	5.0	6.3	-	5.9	4.2	-
	Checks	4.3	4.4	4.7	-	5.1	2.9	-
	LSD 0.05	0.8	1.0	1.2	-	1.2	1.0	-
ALM (cm)	All Experiment	6.3	6.0	6.6	-	6.2	6.2	-
	Parent WCB414	6.9	6.2	7.2	-	7.0	7.4	-
	Parent WCB 617	3.8	3.6	3.9	-	4.1	3.5	-
	RILs	6.3	6.0	6.6	-	6.2	6.2	-
	Minimum RILs	0.6	0.4	0.2	-	0.4	0.6	-
	Maximum RILs	8.1	8.3	9.4	-	8.2	9.3	-
	Checks	6.5	6.1	6.6	-	6.5	6.8	-
	LSD 0.05	0.8	1.3	1.2	-	1.2	1.6	-
ALTA (cm)	All Experiment	4.1	4.0	4.5	-	4.4	3.6	-
	Parent WCB414	4.5	4.4	4.1	-	5.3	4.4	-
	Parent WCB 617	2.6	2.5	2.7	-	2.7	2.4	-
	RILs	4.1	4.0	4.5	-	4.4	3.6	-
	Minimum RILs	0.7	0.4	0.6	-	0.5	0.6	-
	Maximum RILs	5.3	5.2	6.8	-	6.2	4.8	-
	Checks	4.7	4.5	4.9	-	5.3	4.1	-
	LSD 0.05	0.55	0.72	0.89	-	0.87	0.78	-

- non information collected

[†]PP, penetrance of pubescences; PC, penetrance of clavate architecture; SL, spike length; Nd, number of nodes; NdD, node density; ALB, awns length at the bottom of spike; ALT, awns length at the top of spike; ALM, awns length at middle of the spike; ALTA, awns length total averaged.

Table B2. Mean values and RIL ranges of agronomic traits in each environment

Trait [†]	Experiment Categories	Environments						
		All	Carrington 2009	Carrington 2010	Carrington 2011	Prosper 2009	Prosper 2010	Prosper 2011
DH (days)	All Experiment	55.8	58.9	66.3	-	51.6	50.0	52.1
	Parent WCB414	53.3	57.9	65.5	-	48.6	45.5	49.4
	Parent WCB 617	57.3	53.9	66.1	-	58.8	50.6	56.7
	RILs	55.9	59.0	66.4	-	51.7	50.2	52.2
	Minimum RILs	49.8	53.8	61.5	-	46.6	43.0	43.0
	Maximum RILs	65.0	64.0	74.0	-	61.7	61.2	66.5
	Checks	52.8	57.2	64.2	-	48.8	45.9	48.0
	LSD 0.05	2.7	1.5	2.1	-	1.8	2.5	3.1
PH (cm)	All Experiment	87.4	84.9	93.2	91.9	91.6	87.9	75.7
	Parent WCB414	86.7	86.6	92.4	89.9	83.1	90.2	80.5
	Parent WCB 617	73.5	81.3	77.3	76.3	70.7	72.4	64.1
	RILs	87.6	85.2	93.6	92.4	92.0	88.2	75.9
	Minimum RILs	60.2	60.4	69.9	69.5	62.0	62.3	53.2
	Maximum RILs	103.6	111.8	110.9	108.7	115.5	111.8	97.6
	Checks	81.8	79.4	86.1	83.4	86.4	82.5	72.8
	LSD 0.05	6.1	11.0	7.8	7.9	12.5	9.9	11.0
DM (Days)	All Experiment	89.0	-	99.6	93.7	87.8	82.2	81.6
	Parent WCB414	86.6	-	98.9	93.4	82.4	81.6	76.7
	Parent WCB 617	91.1	-	97.0	94.3	100.2	80.7	85.1
	RILs	89.0	-	99.6	93.6	87.8	82.2	81.6
	Minimum RILs	81.6	-	94.6	84.1	80.0	78.1	74.3
	Maximum RILs	98.0	-	107.1	103.2	96.5	90.8	93.3
	Checks	89.1	-	99.5	96.0	80.0	82.3	80.0
	LSD 0.05	3.0	-	2.5	2.5	3.4	2.2	4.1

(Continues)

Table B2. Mean values and RIL ranges of agronomic traits in each environment (continued)

Trait [†]	Experiment Categories	Environments						
		All	Carrington 2009	Carrington 2010	Carrington 2011	Prosper 2009	Prosper 2010	Prosper 2011
NS (spikes m ⁻²)	All Experiment	254.9	199.2	255.6	264.5	280.9	276.7	-
	Parent WCB414	337.0	280.9	335.3	349.6	363.1	352.6	-
	Parent WCB 617	224.8	242.3	230.1	237.9	199.8	235.7	-
	RILs	250.7	195.5	251.3	260.1	277.0	271.5	-
	Minimum RILs	159.2	95.0	172.6	164.5	155.0	148.4	-
	Maximum RILs	345.3	294.3	343.4	410.3	440.3	430.5	-
	Checks	345.2	264.4	345.7	356.7	368.9	391.3	-
	LSD 0.05	40.6	70.2	70.7	72.8	84.5	78.0	-
Ld %	All Experiment	43.0	0.0	0.0	72.9	32.6	60.7	7.7
	Parent WCB414	28.5	0.0	0.0	67.9	0.0	35.3	12.0
	Parent WCB 617	36.5	0.0	0.0	46.6	6.0	90.3	0.0
	RILs	43.8	0.0	0.0	74.7	32.5	62.4	7.7
	Minimum RILs	6.2	0.0	0.0	13.67	0.0	6.0	0.0
	Maximum RILs	72.8	0.0	0.0	102.16	98.7	101.8	55.0
	Checks	26.0	0.0	0.0	35.80	41.6	19.2	7.4
	LSD 0.05	31.0	0.0	0.0	22.75	38.3	33.1	19.4
KSk (Kernels spikelet ⁻¹)	All Experiment	2.5	2.6	2.7	-	2.4	2.3	-
	Parent WCB414	2.6	2.8	2.9	-	2.5	2.5	-
	Parent WCB 617	1.2	1.0	1.8	-	1.0	0.8	-
	RILs	2.5	2.6	2.7	-	2.4	2.3	-
	Minimum RILs	1.3	1.4	1.4	-	0.9	1.0	-
	Maximum RILs	3.0	3.7	4.0	-	3.2	3.0	-
	Checks	2.6	2.8	2.7	-	2.4	2.4	-
	LSD 0.05	0.4	0.5	0.5	-	0.5	0.5	-

(Continues)

Table B2. Mean values and RIL ranges of agronomic traits in each environment (continued)

Trait [†]	Experiment Categories	Environments						
		All	Carrington 2009	Carrington 2010	Carrington 2011	Prosper 2009	Prosper 2010	Prosper 2011
KNd (Kernels Node ⁻¹)	All Experiment	2.6	2.8	2.8	-	2.5	2.4	-
	Parent WCB414	2.7	2.8	2.9	-	2.5	2.5	-
	Parent WCB 617	2.1	2.2	2.8	-	1.5	1.7	-
	RILs	2.6	2.8	2.8	-	2.5	2.4	-
	Minimum RILs	2.0	1.8	2.0	-	1.6	1.3	-
	Maximum RILs	3.6	4.9	4.0	-	4.2	3.4	-
	Checks	2.6	2.8	2.7	-	2.4	2.4	-
	LSD 0.05	0.4	0.5	0.6	-	0.6	0.6	-
NNdISk (nodes with Immature spike ⁻¹)	All Experiment	1.5	0.9	1.3	-	1.9	1.8	-
	Parent WCB414	1.1	0.5	1.0	-	1.2	1.7	-
	Parent WCB 617	4.6	6.1	3.3	-	5.4	4.7	-
	RILs	1.5	0.9	1.3	-	1.9	1.8	-
	Minimum RILs	0.1	0.0	0.0	-	0.2	0.0	-
	Maximum RILs	4.9	4.2	4.8	-	6.1	5.8	-
	Checks	1.3	0.8	1.2	-	1.7	1.6	-
	LSD 0.05	0.7	0.9	1.0	-	1.1	1.2	-
KS (Kernels spike ⁻¹)	All Experiment	33.6	37.5	38.7	-	33.6	24.6	-
	Parent WCB414	30.8	34.2	34.7	-	30.3	23.9	-
	Parent WCB 617	25.9	27.5	42.1	-	14.2	14.7	-
	RILs	33.7	37.7	38.8	-	33.9	24.4	-
	Minimum RILs	25.1	23.2	23.8	-	21.4	10.3	-
	Maximum RILs	43.6	52.9	62.6	-	45.7	35.4	-
	Checks	32.0	34.2	35.4	-	29.3	29.0	-
	LSD 0.05	6.8	7.9	7.7	-	10.0	8.3	-

(Continues)

Table B2. Mean values and RIL ranges of agronomic traits in each environment (continued)

Trait [†]	Experiment Categories	Environments						
		All	Carrington 2009	Carrington 2010	Carrington 2011	Prosper 2009	Prosper 2010	Prosper 2011
GY (Kg ha ⁻¹)	All Experiment	2688.8	3119.0	2952.1	-	2632.0	2051.9	-
	Parent WCB414	3320.4	3954.1	3116.5	-	2943.1	3279.1	-
	Parent WCB 617	1359.0	972.5	2384.1	-	545.2	1226.9	-
	RILs	2641.3	3080.1	2904.4	-	2596.5	1981.9	-
	Minimum RILs	1561.8	1375.4	1317	-	1275.6	450.9	-
	Maximum RILs	4086.7	4951.5	4972.5	-	3787.7	4331.4	-
	Checks	3894.2	4195.7	4098.1	-	3696.1	3593.9	-
	LSD 0.05	648.0	592.7	566.2	-	854.0	514.5	-

- non information collected

[†] DH, days to heading; PH, plant height; DM, days to maturity; NS, number of spikes; Ld, lodging; KSk, kernels per spikelet; KNd, kernels per node; KS, kernel spike; NNdISk, number of nodes with immature spikelets at the spike base; GY, grain yield.

Table B3. Coefficient of variance (CV%), and lattice efficiency (EF%) of spike-related and agronomic traits

Trait [†]	Carrington 2009		Carrington 2010		Carrington 2011		Prosper 2009		Prosper 2010		Prosper 2011	
	CV%	EF%	CV%	EF%	CV%	EF%	CV%	EF%	CV%	EF%	CV%	EF%
Spike-related Traits												
PP	31.81	101.66	14.42	99.87	21.15	102.73	29.04	99.36	12.51	100.6	20.24	100.38
PC	151.41	98.95	275.91	99.15	107.1	100.21	111.25	101.49	181.78	99.36	339.22	99.59
SL	5.44	101.66	4.86	103.47	-	-	4.88	102.69	4.38	131.57	-	-
Nd	4.47	109.36	4.09	102.37	-	-	4.66	105.8	4.47	104.44	-	-
NdD	5.08	102.9	4.25	98.96	-	-	5.26	101.1	4.89	109.32	-	-
ALB	17.38	109.27	15.92	104.29	-	-	17.84	108.73	18.58	110.55	-	-
ALT	15.93	103.94	15.91	108.47	-	-	16.91	101.08	21.82	109.12	-	-
ALM	11.06	100.17	9.47	99.18	-	-	9.76	104.29	13	100.39	-	-
ALTA	9.22	99.47	9.78	104.08	-	-	9.91	101.13	10.65	111.04	-	-
Agronomic Traits												
DH	1.25	110.03	1.6	102.09	-	-	1.67	114.49	2.12	106.44	3	101.62
PH	6.48	103.38	4.12	109.35	4.22	117.6	6.76	107.09	5.73	99.51	7.05	136.38
DM	nd	nd	1.22	110.03	1.32	115.15	1.89	105.46	1.34	106.22	2.49	113.48
NS	17.76	101.25	13.53	117.86	13.74	103.78	15.01	103.36	13.9	107.91	-	-
Ld	-	-	-	-	15.3	111.6	58.2	106.5	26.76	112.58	126.01	105.99
KSk	10	101.61	2.69	111.66	-	-	10.93	101.33	11.07	103.5	-	-
KNd	9.52	103.81	9.67	119.74	-	-	11.37	100.1	12.83	99.49	-	-
KS	10.78	99.73	9.83	106.59	-	-	14.65	107.89	16.9	103.03	-	-
NNdISk	50.57	99.13	40.75	99.98	-	-	28.85	104.55	35.07	99.05	-	-
GY	9.24	121.92	9.39	116.03	-	-	15.82	117.96	12.3	111.38	-	-

- non information collected

[†] PP, penetrance of pubescences; PC, penetrance of clavate architecture; SL, spike length; Nd, number of nodes; NdD, node density; ALB, awns length at the bottom of spike; ALT, awns length at the top of spike; ALM, awns length at middle of the spike; ALTA, awns length total averaged, DH, days to heading; PH, plant height; DM, days to maturity; NS, number of stems; Ld, lodging susceptibility; KSk, kernels per spikelet; KNd, Kernels per node; KS, kernel per spike; NNdISk, number of nodes with immature spikelets at the spike base; GY, grain yield.

Table B4. Error mean square (EMS) and Fmax ratio for spike-related and agronomic traits

Trait [†]	Carrington 2009	Carrington 2010	Carrington 2011	Prosper 2009	Prosper 2010	Prosper 2011	Fmax ratio ^a
	EMS	EMS	EMS	EMS	EMS	EMS	
<i>Spike-related Traits</i>							
PP	0.12	0.03	0.05	0.11	0.02	0.05	6.61
PC	0.17	0.17	0.02	0.08	0.1	0.06	9.61
SL	0.26	0.21	-	0.21	0.19	-	1.4
Nd	0.66	0.57	-	0.81	0.8	-	1.43
NdD	0.01	0.01	-	0.01	0.01	-	1.57
ALB	0.22	0.28	-	0.37	0.18	-	2.03
ALT	0.26	0.35	-	0.39	0.25	-	1.53
ALM	0.45	0.39	-	0.36	0.66	-	1.81
ALTA	0.14	0.2	-	0.19	0.15	-	1.47
<i>Agonomic Traits</i>							
DH	0.54	1.13	-	0.74	1.53	2.44	4.54
PH	30.29	14.72	15.06	38.39	25.36	28.5	2.61
DM	-	1.48	1.53	2.77	1.2	4.11	3.41
NS	1251.06	1195.96	1321.18	1778.24	1479.01	-	1.49
LD	-	-	125.25	359.63	263.43	93.05	3.86
KSk	0.07	0.06	-	0.07	0.06	-	1.11
KNd	0.07	0.08	-	0.08	0.09	-	1.32
KS	16.37	14.45	-	24.21	17.22	-	1.68
NNdISk	0.21	0.27	-	0.32	0.39	-	1.85
GY	83068	76883	-	173370	63723	-	2.72

- non information collected

[†] PP, penetrance of pubescences; PC, penetrance of clavate architecture; SL, spike length; Nd, number of nodes; NdD, node density; ALB, awns length at the bottom of spike; ALT, awns length at the top of spike; ALM, awns length at middle of the spike; ALTA, awns length total averaged, NS, number of stems; PH, plant height; LD, lodging susceptibility; DH, days to heading; DM, days to maturity; KSk, kernels per spikelet; KNd, Kernels per node; KS, kernel spike; NNdISk, number of nodes with immature spikelets at the spike base; GY, grain yield

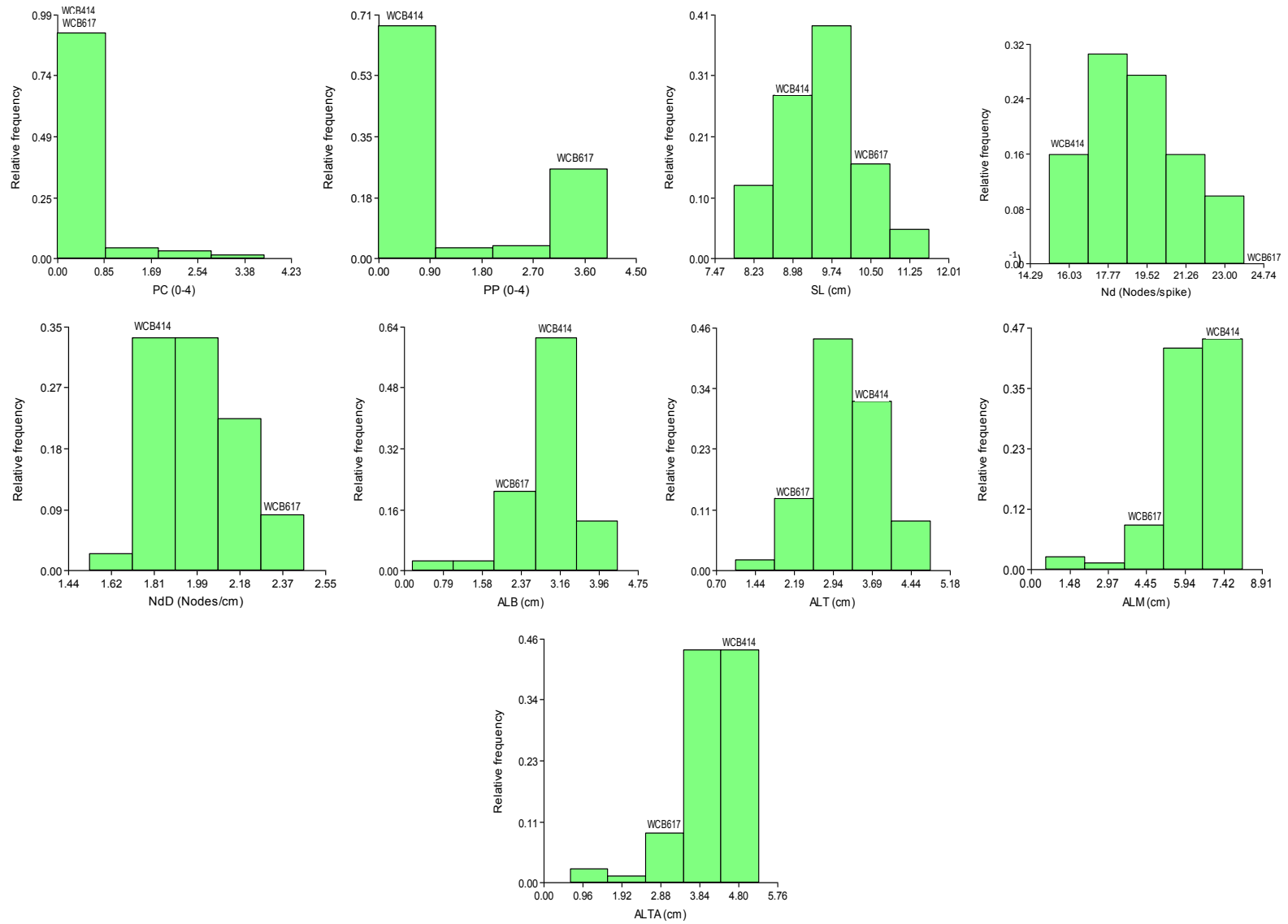


Fig. B1. Frequency distribution of 163 RIL for mean of spike-related traits over four to six environments

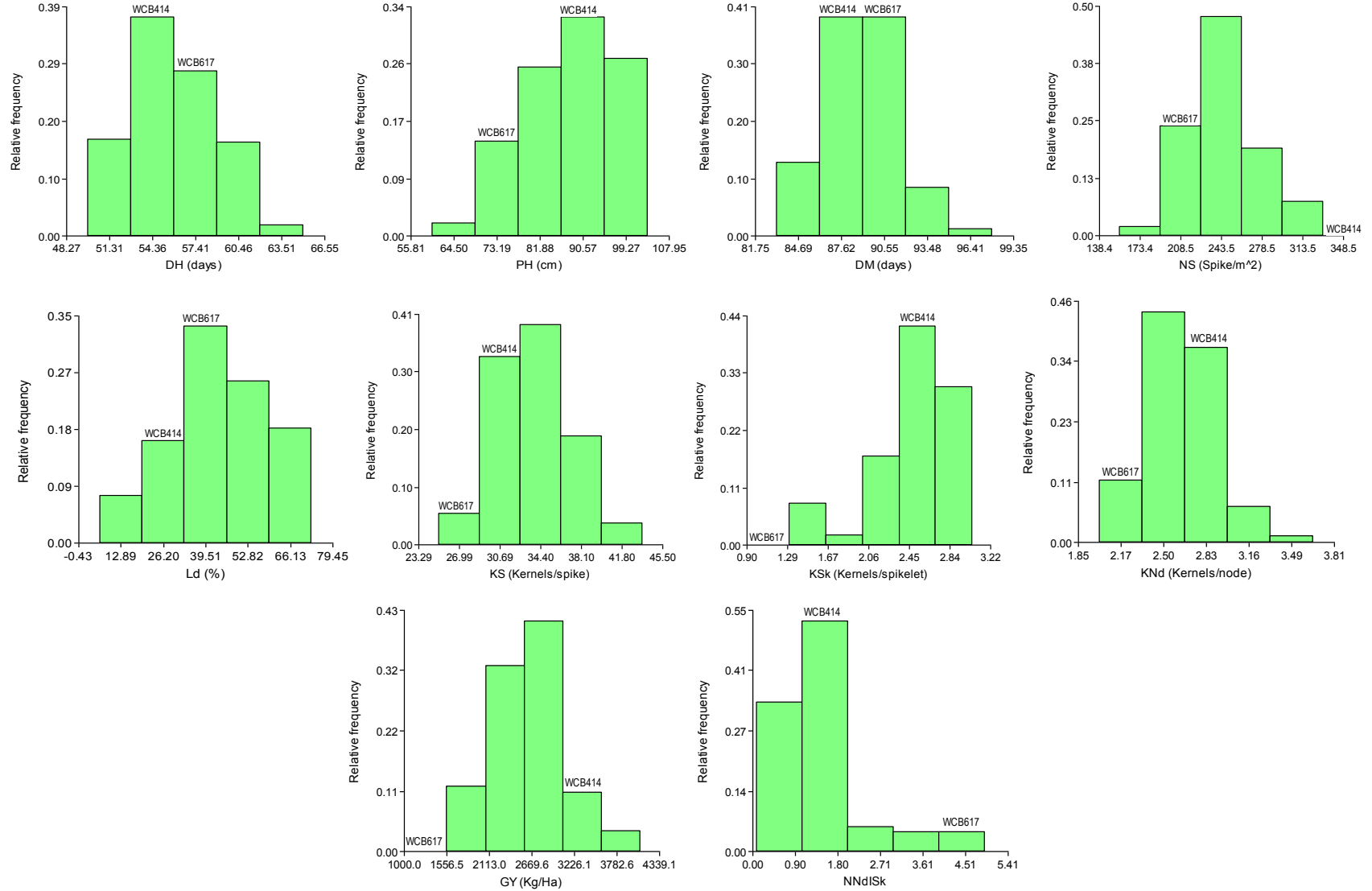


Fig. B2. Frequency distribution of 163 RIL for mean of agronomic traits over four to six environments

APPENDIX C. SUPPLEMENTARY TABLES FOR CHAPTER 4

Table C1. Estimated means of eight quality traits in a RIL population, their parents and seven checks evaluated in four environments

Trait [†]	Experiment Categories	Environments				
		All	Carrington 2009	Carrington 2010	Prosper 2009	Prosper 2010
TKW (g)	All Experiment	30.1	32.4	32.8	29.1	26.1
	Parent WCB414	32.9	34.4	34.1	33.3	30.0
	Parent WCB 617	24.9	20.5	29.0	26.2	22.9
	RILs	30.1	32.4	32.8	29.1	26.0
	Minimum RILs	23.3	24.0	23.8	22.6	15.2
	Maximum RILs	37.8	39.7	42.6	35.7	34.7
	Checks	30.6	32.8	32.7	28.9	28.1
	LSD 0.05	3.2	2.3	2.6	3.1	3.3
KVV (kg m-3)	All Experiment	716.6	756.7	736.3	715.0	659.4
	Parent WCB414	761.1	789.3	767.7	756.4	731.6
	Parent WCB 617	692.0	710.9	713.9	723.1	620.5
	All RILs	714.0	755.0	734.4	712.6	654.9
	Minimum RIL	656.3	702.3	675.3	637.9	543.6
	Maximum RIL	803.5	824.9	811.9	798.7	787.9
	Checks	774.6	797.6	778.6	763.1	759.1
	LSD 0.05	35.4	14.5	15.0	22.3	37.3
FE %	All Experiment	43.2	47.8	44.5	40.8	39.7
	Parent WCB414	63.0	66.9	59.9	61.9	61.7
	Parent WCB 617	26.0	38.7	20.7	-	22.2
	All RILs	42.4	47.1	43.8	40.0	38.8
	Minimum RIL	15.5	18.4	11.2	7.3	11.2
	Maximum RIL	62.6	64.5	68.5	61.6	68.2
	Checks	60.0	62.2	61.3	58.3	59.0
	LSD 0.05	8.5	5.2	8.6	4.8	9.3

(Continues)

Table C1. Estimated means of eight quality traits in a RIL population, their parents and seven checks evaluated in four environments (Continued)

Trait [†]	Experiment Categories	Environments				
		All	Carrington 2009	Carrington 2010	Prosper 2009	Prosper 2010
GPC %	All Experiment	15.8	15.2	15.4	15.7	17.0
	Parent WCB414	15.1	14.6	15.5	14.2	16.0
	Parent WCB 617	16.5	16.8	15.1	16.8	17.5
	All RILs	15.9	15.1	15.4	15.8	17.1
	Minimum RIL	14.0	12.4	12.9	13.6	13.8
	Maximum RIL	17.7	17.2	18.2	17.6	20.2
	Checks	15.5	15.7	15.7	15.1	15.7
	LSD 0.05	1.1	0.7	0.8	0.9	1.3
MX (1-8)	All Experiment	4.4	4.4	4.3	4.8	4.1
	Parent WCB414	4.8	5.0	4.8	4.5	5.0
	Parent WCB 617	4.2	4.0	4.5	-	3.5
	All RILs	4.4	4.3	4.3	4.8	4.1
	Minimum RIL	3.0	1.9	2.7	2.5	2.5
	Maximum RIL	5.9	6.9	6.1	7.0	5.5
	Checks	4.4	4.6	4.4	4.4	4.4
	LSD 0.05	0.9	1.5	2.0	1.3	1.2
MEPT min	All Experiment	6.0	4.4	7.0	5.1	7.3
	Parent WCB414	6.3	4.8	6.9	5.9	7.9
	Parent WCB 617	3.5	2.6	5.0	-	3.6
	All RILs	6.0	4.5	7.1	5.1	7.2
	Minimum RIL	1.9	1.6	1.6	1.2	2.2
	Maximum RIL	14.0	11.9	20.0	11.3	18.3
	Checks	6.2	4.0	6.2	6.0	8.7
	LSD 0.05	2.6	2.8	3.3	3.0	3.3

(Continues)

Table C1. Estimated means of eight quality traits in a RIL population, their parents and seven checks evaluated in four environments (Continued)

Trait [†]	Experiment Categories	Environments				
		All	Carrington 2009	Carrington 2010	Prosper 2009	Prosper 2010
MMLPT min	All Experiment	6.4	5.0	7.3	5.6	7.5
	Parent WCB414	6.4	4.9	7.1	6.0	7.8
	Parent WCB 617	3.9	2.4	5.6	-	3.9
	All RILs	6.4	5.0	7.4	5.5	7.5
	Minimum RIL	2.2	1.9	1.8	1.7	2.3
	Maximum RIL	15.3	13.7	20.1	11.9	18.4
	Checks	6.7	4.6	6.6	6.6	9.1
	LSD 0.05	2.6	2.9	3.4	3.1	3.5
%TQ*min.	All Experiment	249.0	201.0	268.0	250.6	273.5
	Parent WCB414	271.5	201.8	289.1	277.7	318.6
	Parent WCB 617	176.9	158.6	206.5	-	176.4
	All RILs	248.5	201.3	268.8	248.4	271.7
	Minimum RIL	99.7	84.3	82.8	101.3	99.0
	Maximum RIL	569.7	612.5	658.2	567.1	549.4
	Checks	267.7	198.1	256.0	296.9	321.5
	LSD 0.05	70.8	75.1	84.1	88.6	93.7

- non-data collected.

[†]TKW, Thousand kernel weight; KVW, grain-volume weight; GPC, grain protein content; FE, flour extraction; MX, mixogram score; MEPT, mixogram envelope peak time; MMLPT, mixogram mid line peak time; MMLPI, mixogram mid line peak integral.

Table C2. Coefficient of variance, lattice efficiency, error mean square, and FMax ratio for eight for quality traits

Trait [†]	Carrington 2009			Carrington 2010			Prosper 2009			Prosper 2010			Fmax ratio
	CV% [‡]	EF% [§]	EMS [¶]	CV% [‡]	EF% [§]	EMS [¶]	CV% [‡]	EF% [§]	EMS [¶]	CV% [‡]	EF% [§]	EMS [¶]	
TKW	0.8	116.5	40.4	1.0	120.6	53.1	1.4	106.7	98.5	2.8	120.5	329.9	8.2
GVW	2.3	118.0	0.1	2.4	126.0	0.1	2.6	112.8	0.2	3.7	111.6	0.4	3.3
GPC	5.4	107.7	6.6	9.4	158.6	17.4	6.0	-	6.0	11.3	146.2	20.2	3.4
FE	15.4	105.2	0.5	23.3	105.3	1.0	13.8	-	0.4	15.1	100.5	0.4	2.6
MX	32.0	99.3	2.0	23.1	106.2	2.6	30.0	-	2.4	22.9	99.9	2.8	1.4
MEPT	28.7	101.1	2.0	22.8	107.0	2.8	28.7	-	2.6	23.4	98.8	3.1	1.5
MMLPT	16.8	102.7	1146.5	15.8	101.8	1788.9	18.0	-	2045.5	17.7	98.6	2345.3	2.0

- non-data collected.

[†]TKW, Thousand kernel weight; KVW, grain-volume weight; GPC, grain protein content; FE, flour extraction; MX, mixogram score; MEPT, mixogram envelope peak time; MMLPT, mixogram mid line peak time; MMLPI, mixogram mid line peak integral.

[‡]Coefficient of variance

[§]Lattice efficiency

[¶]Error mean square

Table C3. Significant Pearson's correlation coefficients among four spike-related traits and eight quality traits in wheat

	Quality Traits							
	TKW	KVW	GPC	FE	MX	MEPT	MMLPT	MMLPI
<i>Spikes-Related Traits^a</i>								
PSS	-0.23**§	ns	0.31**§	ns	0.25**‡	0.19**‡	0.26**†	0.25**‡
PP	-0.26**§	-0.22**‡	0.36**†	-0.18**§	ns	0.17*†	0.19*†	0.21**†
PC	ns	-0.19**‡	0.26**†	ns	ns	ns	ns	ns
Aless	ns	ns	-0.16*†	ns	ns	ns	ns	ns