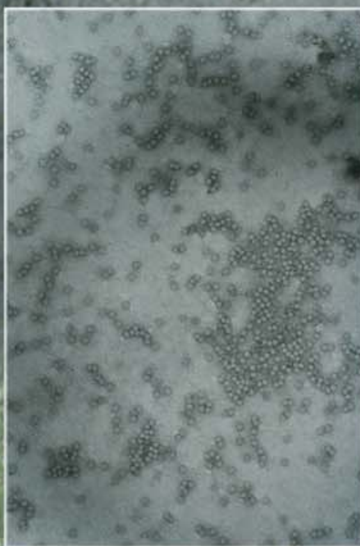
Barley Yellow Dwarf Disease: Recent Advances and Future Strategies

M. Henry and A. McNab, editors



CIMMYT MR

Barley Yellow Dwarf Disease: Recent Advances and Future Strategies

Proceedings of an International Symposium
Held at El Batán, Texcoco, Mexico
1-5 September 2002

M. Henry and A. McNab, editors

Sponsored by the CIMMYT Wheat Program, the United States
Department of Agriculture-ARS, and the Monsanto Company

CIMMYT® (www.cimmyt.org) is an internationally funded, nonprofit, scientific research and training organization. Headquartered in Mexico, CIMMYT works with agricultural research institutions worldwide to improve the productivity, profitability, and sustainability of maize and wheat systems for poor farmers in developing countries. It is one of 16 food and environmental organizations known as the Future Harvest Centers. Located around the world, the Future Harvest Centers conduct research in partnership with farmers, scientists, and policymakers to help alleviate poverty and increase food security while protecting natural resources. The centers are supported by the Consultative Group on International Agricultural Research (CGIAR) (www.cgiar.org), whose members include nearly 60 countries, private foundations, and regional and international organizations. Financial support for CIMMYT's research agenda also comes from many other sources, including foundations, development banks, and public and private agencies.

FUTURE℠ HARVEST Future Harvest® builds awareness and support for food and environmental research for a world with less poverty, a healthier human family, well-nourished children, and a better environment. It supports research, promotes partnerships, and sponsors projects that bring the results of research to rural communities, farmers, and families in Africa, Asia, and Latin America (www.futureharvest.org).

© International Maize and Wheat Improvement Center (CIMMYT) 2001. All rights reserved. The opinions expressed in this publication are the sole responsibility of the authors. The designations employed in the presentation of materials in this publication do not imply the expression of any opinion whatsoever on the part of CIMMYT or its contributory organizations concerning the legal status of any country, territory, city, or area, or of its authorities, or concerning the delimitation of its frontiers or boundaries. CIMMYT encourages fair use of this material. Proper citation is requested.

Correct citation: Henry, M., and A. McNab (eds.). 2002. *Barley Yellow Dwarf Disease: Recent Advances and Future Strategies*. Mexico, D.F.: CIMMYT.

AGROVOC Descriptors: Barley yellow dwarf virus; Plant diseases; Disease resistance; Pathogens; Feed cereals; Viruses; Transgenic plants; Hybridization; Genomes; Genetic resistance; Yields

AGRIS Category Codes: H20 Plant Diseases
F30 Plant Genetics and Breeding
Dewey Decimal Classification: 632.3
ISBN: 970-648-095-1

Design and layout: Marcelo Ortiz S. and Antonio Luna

Printed in Mexico.

Table of Contents

v **Preface**

Papers

1 **Structure, Function, and Variation of the *Barley Yellow Dwarf Virus* and *Cereal Yellow Dwarf Virus* Genomes**
W.A. Miller, R. Beckett, and S. Liu

9 **Assessment of Biological and Molecular Variability of Moroccan BYDV-PAV Isolates**
B. Bencharki, M. El Yamani, J. Mutterer, V. Ziegler-Graff, D. Zaoui, and G. Jonard

13 **Preliminary Study on the RNA-Binding Properties of BYDV-GAV and BYDV-GPV Movement Proteins**
Z. Xia, Y. Zhang, K. Liu, and D. Wang

16 **Progress in Characterizing the Chinese GAV Isolate of the BYDV Luteovirus**
X. Wang, LiLi, and G. Zhou

18 **Serial Passages of a BYDV-PAV Isolate Modify Its Biological Properties**
F. Chain, G. Riault, O. Valles, G. Mauvais, S. Tanguy, C. Kervarrec, C.-A. Dedryver, M. Trottet, and E. Jacquot

22 **A Single Copy of Virus-Derived, Transgene-Encoding Hairpin RNA Confers BYDV Immunity**
D. Abbott, M.-B. Wang, and P.M. Waterhouse

27 **Genetically Engineered Wheat for *Barley Yellow Dwarf Virus* Resistance**
P. Dupré, M. Henry, G. Posadas, A. Pellegrineschi, M. Trottet, and E. Jacquot

29 **Nucleotide Sequence Analysis of the BYDV-GPV Genome, and Transgenic Wheat Obtained via Pollen Tube Pathway**
Z. Cheng, M. Wu, X. He, L. Xu, G. Zhou, and P.M. Waterhouse

32 **Real-Time RT-PCR Quantification of Yellow Dwarf Virus Accumulation and Defense Gene Expression**
B. Balaji, D.B. Bucholtz, H.W. Ohm, and J.M. Anderson

34 **BYDV: The Heat Is On**
R. Harrington

40 **Vector Dynamics and BYD Disease Progress on Barley and Maize: Two Contrasting Cases and Consequences for Control**
L. Haack, F. Leclerq-Le Quillec, M. Henry, H. Albert, and C.-A. Dedryver

44 **Use of Aphids' Abundance in Autumn to Predict BYD Yield Losses**
F. Fabre, C.-A. Dedryver, J.L. Leterrier, and M. Plantegenest

45 **Incidence of *Barley Yellow Dwarf Viruses* in Symptom-Exhibiting Cereal Species**
E. Pocsai, I. Murányi, M. Papp, L. Szunics, A. Tomcsányi, and G. Vida

50 **Importance of BYDV and Its Vectors in Central Germany**
A. Habekuss and E. Schliephake

51 **Report on Barley Yellow Dwarf Disease Incidence in Latvia**
M. Bisnieks, A. Kvarnheden, and R. Sigvald

53 ***Barley Yellow Dwarf Virus* in Argentina**
G. Truol

55 **Estimating Yield Losses in Cereals Infected with *Barley Yellow Dwarf Virus***
K.M. Makkouk and W. Ghulam

58 **Spring Oat and Soft Winter Wheat Lines with BYDV Resistance**
H. Ohm, J. Anderson, H. Sharma, L. Ayala-Navarrete, and D. Bucholtz

60 **Utilizing *Bdv*2, the *Thinopyrum intermedium* Source of BYDV Resistance, to Develop Wheat Cultivars**
P.J. Larkin, S. Kleven, and P.M. Banks

64 **Evaluating Resistance to BYDV-PAV, BYDV-MAV, and CYDV-RPV in *Thinopyrum intermedium*-Derived Wheat Lines**
M. Henry, G. Posadas, J. Segura, and S. Rajaram

67 **Advances in Breeding Wheat for BYDV Resistance Using Biotechnology**
Z.Y. Xin, Z.Y. Zhang, Z.S. Lin, X. Chen, H.J. Xu, Y.Z. Ma, D.L. Wu, Q. F. Xu, L.P. Du, P.M. Banks, and P.J. Larkin

70 **Development and Application of PCR Markers for Breeding Wheat for BYDV Resistance**
Z. Zhang, J. Xu, Q. Xu, L. Du, Z. Lin, and Z. Xin

75 **A Map-based Approach towards Cloning the *Yd*2 Resistance Gene in Barley**
B. King, N. Paltridge, N. Collins, C. Ford, R. Li, R. Symons, and P. Landridge

78 **Exploitation of Detected BYDV Resistance Genes in Barley Breeding**
J. Ovesna, V. Sip, J. Chrpova, J. Vacke, M. Skorpik, K. Polakova, and L. Kucera

82 **Breeding for BYDV Tolerance in Wheat as a Basis for a Multiple Stress Tolerance Strategy**
A. Comeau and S. Haber

93 **Breeding for BYDV Tolerance/Resistance in CIMMYT Bread Wheats Targeted to Developing Countries**
M. van Ginkel and M. Henry

Posters

97 **Coat Protein Sequence of an Egyptian BYDV-PAV Isolate**
H.M Waziri, M. Abd El Gaffar, E.K. Allam, and A.S. Gamal El Din

100 **Diagnosis of *Barley Yellow Dwarf Virus* and *Cereal Yellow Dwarf Virus* Isolates in Infected Plants and Vectors by Group PCR and ELISA**
B. Kulemeka, W. Bliss, A. Harness, C. Sutula, M. Bandla, and S.M. Gray

101 **Preparing Monoclonal Antibodies against BYDV Using Viruses Purified from Naturally Infected Plants**
T.N. Erokhina, T.B. Kastalieva, and K.A. Mozhaeva

104 **Improvement of BYDV-PAV Detection in Single Aphid by Using Real-Time RT-PCR Fluorescent Method**
F. Fabre, C. Kervarrec, L. Mieuzet, A. Vialatte, and E. Jacquot

106 **Risk Index for *Barley Yellow Dwarf Virus* and *Cereal Yellow Dwarf Virus* of Wheat in Alabama**
K.L. Brown, K.L. Flanders, P. Mask, and J.F. Murphy

108 **Identification of Cereal Viruses in Uzbekistan**
Z.N. Kadirova, A.A. Zueva, and A.A. Abdukarimov

110 **Distribution of *Barley Yellow Dwarf Virus* Vic-PAV Strain in South Korea**
J.-C. Park, J.-H. Seo, J.-S. Choi, S.-L. Park, D.-Y. Suh, and M.-H. Lee

112 **Host Specialization in BYDV Vectors and Its Impact on Virus Spread**
A. Vialatte, F. Fabre, E. Jacquot, J.-C. Simon, and M. Plantegenest

115 **Barley Yellow Dwarf Virus in Egypt: Current Situation and Prospects**
A.E. Aboulata

117 **Breeding BYDV Resistant Barley with an Agronomically Improved Background**
F. Capettini, M. Henry, and H. Vivar

119 **Problems and Solutions in the Development of New Sources of BYDV Tolerance for Bread Wheat**
A. Comeau and S. Haber

120 **Barley Yellow Dwarf in Russia**
K.A. Mozhaeva and T.B. Kastalyeva

123 **Use of PCR Markers to Select *Barley Yellow Dwarf Virus* Resistant Plants**
K.M. Makkouk, W. Ghulam, M. Baum, S. Ceccarelli, and S. Grando

127 **Resistance to BYDV-PAV and CYDV-RPV in a Bread Wheat x *Agrotricum* Cross**
G. Posadas and M. Henry

130 **Resistance and Tolerance to *Barley Yellow Dwarf Virus* in Wheat Wild Relative *Aegilops geniculata***
M. Zaharieva, J. Segura, and M. Henry

133 **Development of 'Thelin' Wheat Lines with Genes *Lr*19 and *Bdv*2 from *Thinopyrum elongatum* and *Th. intermedium***
R.P. Singh, M. Henry, J. Huerta-Espino, A. Mujeeb-Kazi, and R.J. Peña

137 **List of Participants**

Preface

Given the complex nature of the disease known as barley yellow dwarf (BYD), it is essential that researchers working to combat it come together periodically to share what they have learned about the virus pathogen and its vectors, report on the use of new methodologies, and present the latest findings in different regions of the world. The latter is especially important given that individual strains of the pathogens classified as *Barley yellow dwarf virus* (BYDV) and *Cereal yellow dwarf virus* behave differently in different regions of the world, a fact that must be taken into account when breeding crop varieties that will provide farmers with effective BYD control. Since the last time involved researchers came together was at a meeting held at the International Center for Agricultural Research in the Dry Areas (ICARDA) in Aleppo, Syria, in 1989, a BYD symposium was long overdue.

The incidence of BYD in any given year is very unpredictable. It depends not only on host-pathogen dynamics and environmental conditions that favor disease development, but also on fluctuations in the aphid populations that vector the disease. This unpredictability makes it very difficult to know when it makes sense to apply control measures—for example, insecticide applications to reduce aphid populations. Expensive and not always effective, insecticides should almost never be applied except in places where it is fairly certain that both vector and virus will be endemic each year. Moreover, in wheat-producing areas of the developing world where BYD is a problem (parts of North Africa, the Eastern African Highlands, several Asian countries, China included, and parts of Latin America), most farmers cannot afford chemical control methods. For all these reasons, durable genetic resistance and/or tolerance to BYDV is the best and most cost-effective option for bringing this unpredictable disease under control.

However, in affected countries there has been limited progress in developing crop varieties that are BYD resistant/tolerant and also agronomically appealing to the farmer. As yet, no progress has been made on developing methods to combat the virus directly. It is obvious that large advances remain to be made in combating BYD.

Progress in controlling the disease is more likely to accelerate if BYD researchers from all over the world pool the latest data and insights they have gleaned. CIMMYT and, more specifically, the Wheat Program, is happy to organize and host an occasion for doing just that, and hopes that it will also serve to make funding sources aware that there are still wide gaps in the knowledge about this complex disease. We expect these proceedings will allow researchers who did not attend the event to access the wealth of information that we were privileged to share during the symposium.

Sanjaya Rajaram
Director
CIMMYT Wheat Program

Structure, Function, and Variation of the *Barley Yellow Dwarf Virus* and *Cereal Yellow Dwarf Virus* Genomes

W.A. Miller, R. Beckett, and S. Liu

Plant Pathology Dept., Iowa State University, Ames, Iowa, USA

In 1988, the nucleotide sequence of the entire barley yellow dwarf virus, PAV serotype (BYDV-PAV), genome was completed (Miller *et al*., 1988). This first sequence of a luteovirus was the starting point for years of discovery on the fascinating workings of this small, 5,677 nucleotide stretch of RNA, one-millionth the size of the genomes of its hosts, in which it causes widespread disease and yield loss. As a subject of research, BYDV has provided new insight about the inner workings of the cellular protein synthesis machinery and virus replication strategies. Here we describe some ways in which the sequence information has been put to use, including engineered resistance, the discovery of elaborate translation mechanisms, and an awareness of the remarkable diversity of BYDV isolates.

Current Classification

BYDV is the sole member of genus *Luteovirus* and the type member of the *Luteoviridae* family (formerly luteovirus group) (D'Arcy *et al*., 2000). BYDV serotypes were divided into two subgroups, which were subsequently reclassified as separate species. Currently, only BYDV-MAV (transmitted primarily by *Sitobion avenae*) and BYDV-PAV (transmitted efficiently by *S. avenae* and *Rhopalosiphum padi*) are barley yellow dwarf viruses. Former BYDV serotype RPV (transmitted primarily by *R. padi*) was given a new name, *Cereal yellow dwarf virus*-RPV (CYDV-RPV) and placed in genus *Polerovirus* along with four non-BYDV viruses in the *Luteoviridae*. A third genus, *Enamovirus*, consists only of RNA-1 of the bipartite *Pea enation mosaic virus* (PEMV). Its organization resembles poleroviruses, but lacks open reading frame (ORF) 4. For comprehensive reports on all aspects of the *Luteoviridae*, see the book *The* Luteoviridae (Smith *et al*., 1999).

After publication of the BYDV sequence, the sequences of several other luteoviruses were determined in rapid succession. These revealed a taxonomic dilemma that has continued to this day. Essentially the replication machinery of the *Luteoviridae* has two different evolutionary histories, whereas the proteins that form the virus particles and interact with the aphid vectors clearly have a common origin. Functional and comparative genomic analyses of BYDV and related viruses indicate that, from a molecular virological point of view, BYDV belongs in the *Tombusviridae* family (Miller *et al*., 2002). The replication proteins and the RNA sequences that control replication and translation most closely resemble those of viruses in the *Tombusviridae* family (Figure 1). Yet the coat protein, movement and aphid transmission proteins clearly

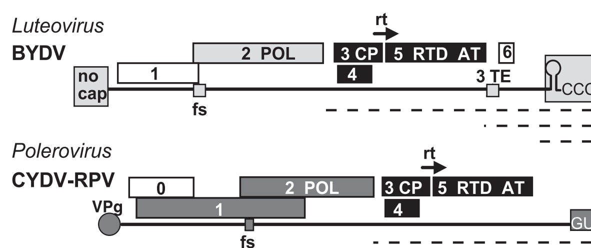


Figure 1. Genome organization of BYDV and CYDV-RPV.
Notes: Bold black line indicates genomic RNA; bold dashed line, subgenomic RNA. Open reading frames are numbered and functions indicated where known. POL, RNA-dependent RNA polymerase; CP, major coat protein; RTD, readthrough domain required for aphid transmission (AT); 3'TE, 3' cap-independent translation element; fs, frameshift signal; rt, readthrough site; VPg, viral genome-linked protein. Stem-loops and terminal bases are shown at 3' ends of genomes. Light gray shading, features in common with at least one genus of the *Tombusviridae* and not with genus *Polerovirus*. Dark gray shading, shared among *Polerovirus* and *Enamovirus* genera and not genus *Luteovirus*. Black boxes, ORFs common to all *Luteoviridae* and absent in other families. White boxes, ORFs unique to the genus.

resemble those of the other *Luteoviridae*, including genus *Polerovirus*. Like the *Tombusviridae*, BYDV RNA lacks a 5′ cap or any other modification (Allen *et al*., 1999), and terminates at the 3′ end with the sequence CCC, preceded by a conserved stem-loop (Koev *et al.*, 2002). In contrast, the poleroviruses, including CYDV-RPV have a protein (VPg) linked to the 5′ end and terminate in GU. In poleroviruses ORF 0 codes for a suppressor of the post-transcriptional gene silencing defense response (Pfeffer *et al*., 2002). This ORF is absent in BYDV. ORF 1 of poleroviruses encodes a proteinase and the VPg which also are lacking in BYDV. All of the luteovirus-like genes of BYDV can be deleted and the remaining RNA can still replicate in protoplasts (discussed below). Thus, the core of the virus, i.e. the gene expression and replication framework is more closely related to the *Tombusviridae* family than to other members of the *Luteoviridae* (Miller *et al.*, 2002).

Yet with regard to symptomatology and practical plant pathology, BYDV is clearly a member of the *Luteoviridae*, defined as those viruses that: 1) are transmitted only by aphids in a persistent manner and not mechanically; 2) circulate but do not replicate in the aphid; 3) are confined to the phloem in the plant; and 4) have 25 nm icosahedral particles consisting of a major ~22 kDa coat protein and a minor component. None of these properties apply to the *Tombusviridae*.

BYDV Gene Function and Expression

BYDV has a positive sense, 5.7 kb genomic RNA that encodes six open reading frames (ORFs) and produces three subgenomic RNAs (Kelly *et al.*, 1994) that serve as mRNAs for downstream genes (Figures 1 and 2). Only ORFs 1 and 2 are translated from genomic RNA. ORF 2 is translated only as a fusion with ORF 1 via –1 ribosomal frameshifting (Di *et al.*, 1993). ORF 2 encodes the active site of the viral RNA-dependent RNA polymerase (RdRp); the role of the ORF 1 product alone is unknown. These are the only two ORFs that are essential for RNA replication in plant cells (protoplasts) (Mohan *et al.*, 1995). ORF 3 codes for the major coat protein (CP). ORF 4, which is imbedded in the sequence that codes for ORF 3, but in a different reading frame, codes for a protein required for systemic infection of plants (Chay *et al*., 1996a). ORF 5 codes for an extension to the CP and is required for aphid transmission (Chay *et al*., 1996a).

ORFs 3, 4, and 5 all are translated only from subgenomic RNA1 (sgRNA1). ORF 5 is translated as a fusion with CP via in-frame readthrough of the CP stop codon (Brown *et al*., 1996). ORF 4 is translated via leaky ribosomal scanning (Dinesh-Kumar *et al*., 1993). ORF 6 is translatable only from sgRNA2 *in vitro* (Wang *et al.*, 1999), via the TE in its 5' UTR. Its role, if any, is unknown. sgRNA3, consists of the 3′-terminal 300 nucleotides of the BYDV genome. It is scarce in protoplast infections after 48 h, but accumulates to very high levels in plants, weeks after inoculation (Kelly *et al*., 1994; Koev *et al.*, 1998). sgRNA3 has no ORFs and no known function.

Translational Control

As indicated above, BYDV RNA undergoes a host of unusual events during translation (protein synthesis) (Figure 2). It lacks the 5′ cap and poly(A) tail that are necessary for translation of normal host mRNAs. Normally eukaryotic mRNAs must circularize via proteins that bind the 5′ cap, the poly(A) tail, and each other, prior to binding of the ribosome (Sachs *et al.*, 1997). Instead, BYDV RNA harbors a sequence in the 3′ untranslated region (UTR) called the 3′ TE that brings about efficient translation initiation at the first AUG at the 5′ end of the genome and subgenomic RNA1 (Wang *et al.*, 1997). The 3′ TE base pairs with a sequence in the 5′ UTR to circularize the mRNA and facilitate translation initiation (Guo *et al*., 2001). This is the first known case of a functional mRNA formed by base pairing between the UTRs rather than by protein-mediated circularization. This provides an example of how BYDV research has revealed fundamental new insight on the workings of the eukaryotic translation machinery. A similar structure and interaction exists in genus *Necrovirus* of the *Tombusviridae*, but not in genus *Polerovirus* of the *Luteoviridae*. The translation signals of poleroviruses, which also lack a cap and a poly(A) tail, have not been well-characterized, but they bear no resemblance to those of BYDV.

The BYDV polymerase is expressed via –1 ribosomal frameshifting, i.e. in the region of overlap between ORF 1 and ORF 2, a small number of ribosomes translating ORF 1 shift back one nucleotide relative to the mRNA and resume translation in the new ORF (ORF 2). This event, which is common among retroviruses such as HIV (Dinman *et al*., 2002) and various other RNA viruses, is controlled by the mRNA sequence in and around the frameshift site. The exact mechanism of frameshifting is unknown. Because host

genes are not known to use frameshifting, disruption of frameshifting is a promising target for antiviral research (Dinman *et al*., 2002). BYDV provides new insight into the mechanisms of ribosomal reading frame maintenance because its RNA has a novel structure for bringing about the frameshift (Figure 2). Unlike any other known RNA, base pairing between a region four kilobases downstream in the 3′ UTR, and the frameshift site is necessary for frameshifting (Barry and Miller, 2002). In contrast, for the poleroviruses, a small pseudoknotted RNA structure is sufficient to bring about frameshifting (Kim *et al*., 1999). Viruses in genus *Dianthovirus* (*Tombusviridae*) have a frameshift structure very similar to BYDV (Kim and Lommel, 1998), although it is not known if long-distance base pairing to a downstream sequence modulates dianthoviral frameshifting. However, it emphasizes yet again how BYDV in many ways is more like the *Tombusviridae* than other *Luteoviridae*.

Figure 2. Control of BYDV gene expression.
Notes: Genomic (gRNA) and subgenomic (sgRNA) RNAs are bold black lines. ORFs are shown above RNAs. White ORFs are translatable from the indicated RNA, black ORFs are not. Boxes on the bold lines (RNAs) control cap-independent translation [white (in vitro and in vivo), and light gray (*in vivo* only)], ribosomal frameshifting (dark gray), in-frame readthrough of CP stop codon (black). Secondary structures of translational control regions, 3'-terminus (replication origin), and subgenomic promoters (minus strand) are indicated; thick, gray loops base-pair to distant regions as shown by dotted lines with double arrowheads.
Source: Miller *et al*., 2002.

A Molecular Traffic Signal?

The control of the above translational events in the 5′ end of the genome by sequences in the 3′ UTR, led us to propose a new model for regulating the switch from translation to replication. This is a key step in the virus life cycle because viral replicase cannot copy RNA that is being translated (Gamarnik and Andino, 1998). We proposed a "molecular traffic signal" model as follows. (See Barry and Miller, 2002, for details.) When BYDV RNA is released from the virion upon cell entry, long-distance base-pairing allows cap-independent translation initiation at the 5′-proximal AUG, and ribosomal frameshifting (Figure 2). This allows translation of the replicase which accumulates and then initiates negative strand synthesis at the 3′ end of the BYDV genome (Koev *et al*., 2002). As it proceeds upstream on the template, the replicase would disrupt the short base-paired regions in the 3′ UTR necessary for frameshifting and for cap-independent translation. This would clear the upstream portion of the RNA (ORFs 1 and 2) of ribosomes and allow the replicase to proceed all the way to the 5′ end, unimpeded by ribosomes. After several rounds of replication, enough (+) strand would accumulate to outnumber the replicase molecules and thus be capable of forming the long-distance base pairing necessary for translation and the cycle would start over. Similar control could be envisioned for viruses that lack base-pairing but use protein-mediated ways of interacting with the 5′ UTR. Thus, as a model for RNA virus replication, BYDV has contributed to understanding of regulation of replication that could apply to many viruses.

Transgenic Resistance

The most cost-effective and environmentally desirable form of disease control is genetic resistance. However, natural resistance genes to BYDV are few (Burnett *et al*., 1995; Jin *et al*., 1998). Yet, because of the threat of BYDV, breeders are limited to lines that have significant natural BYDV resistance or tolerance. To allow breeders to expand beyond these limitations, we, with collaborator David Somers at the University of Minnesota, developed transgenic oats tolerant to

BYDV (Koev *et al.*, 1998). Oats were transformed with a gene designed to express the 5’ half of the BYDV genome driven by a CaMV 35S promoter. The most resistant line of transgenic oats initially showed mild symptoms but then recovered and grew to maturity. In laboratory growth conditions, yield was slightly reduced compared to uninoculated controls and virus was sometimes detectable, but the yield was infinitely greater than in the inoculated non-transgenic controls which were actually killed by virus infection long before flowering. The transgene was stable inherited in a Mendelian fashion.

Field trials were less promising, mainly because the only line of oats that could be transformed (genetically engineered) at the time was not agronomically useful (Somers *et al.*, 1992). It was derived by artificial hybridization of *Avena fatua* with the Park cultivar of oat (*A. sativa*), followed by back crosses to oat. These plants (with or without a transgene) were smaller and less robust than agronomic cultivars, in the presence or absence of virus infection. Fortunately, recent improvements in technology now permit transformation of such agronomic lines as Bell.

Peter Waterhouse and colleagues greatly improved design of transgenes to engineer resistant plants with high efficiency. Barley plants transformed with inverted sequences of BYDV genes, causing the transcripts to form long, double-stranded hairpin RNAs were immune to BYDV infection (Wang *et al.* 2000). Presumably the double-stranded RNA induces the host’s post-transcriptional gene silencing system (Waterhouse *et al.*, 2001).

To the best of our knowledge, no plants engineered for BYDV resistance have been released for use by growers. Unfortunately, due to the low value of oats as a profit-making enterprise, and perhaps due to concern about consumer acceptance of food derived from GMO crops, corporate interest in funding transgenic oat research has waned. Perceived risks imposed by transgenic BYDV-resistant oats drew attention in a *Science* magazine article about an unpublished poster presentation at a scientific conference (Kaiser, 2001). The experiments alleging that pollen escape from transgenic BYDV-resistant oats could lead to “superweeds” were confined to the greenhouse and used no transgenic plants. Yet the benefits of new resistance genes, such as reduced pesticide inputs and increased yields, are clear (Miller *et al.*, 1997). Application of insecticides on wheat in the United Kingdom and Australia to control the aphid vectors of BYDV often results in substantial yield increases (Plumb and Johnstone, 1995) that are attributable to the absence of BYDV infection. For more details on the economic costs and worldwide occurrence of BYDV diseases, and the economic and environmental costs of controlling them, we refer the reader to other reports in these proceedings, and to the book *BYDV: Forty Years of Progress* (D’Arcy and Burnett, 1995).

One legitimate concern with regard to the applicability (but not safety) of virus-derived transgenes for resistance to BYDV is the wide range of sequence variation among isolates. BYDV isolates that lack high homology to the transgene would not be hindered by transgene-induced post-transcriptional gene silencing (Miller *et al.*, 1997; Wang *et al*., 2000; Waterhouse *et al.*, 2001). Thus, virus-derived transgenes may confer resistance to only a subset of BYDV isolates in the field. The diversity of BYDV isolates is discussed below.

Relationships among BYDV Isolates

Sequences of coat proteins of many isolates of BYDV have been determined, revealing much variation within serotypes. Based on these sequences, the most common serotype, PAV, has been unofficially subdivided into two subgroups, A and B, which have about 90% amino acid sequence homology in the CP gene (Mastari *et al*., 1998). Coat proteins of PAV isolates within subgroup A have at least 96% amino acid sequence identity to each other (Table 1). The complete genomes of few isolates have been determined. We sequenced the completed genome of a severe isolate called PAV-129 by its discoverer Stewart Gray and colleagues (Chay *et al*., 1996b).

This isolate is quite divergent from other completely sequenced PAV isolates (Table 1). In the replicase ORFs 1 and 2, PAV-129 has only 80 and 88% sequence homology, respectively, to ORFs 1 and 2 of all other PAV isolates and MAV, which are all around 97% identical to each other. The 3’ terminus also differs significantly in sequence, but retains a similar secondary structure to the other PAV isolates (Koev *et al.*, 2002). The coat protein sequence of PAV-129, with 86% identity to subgroup A PAV isolates, falls into subgroup B (Miller *et al*., 2002), and PAV-129 is the first subgroup B isolate for which the complete genome has been determined.

If other members of subgroup B prove to be as different from subgroup A members throughout their genomes as PAV-129, then there is more taxonomic confusion. For example, BYDV-MAV has the most divergent coat and readthrough proteins (76% and 60% homology, respectively, to all PAVs), but the remainder of its genome is more closely related to PAV subgroup A than subgroup A is to PAV-129 (Table 1).

To map the severe symptom determinants of PAV-129, we made chimeric isolates of our infectious clone PAV6 (originally derived from the PAV-Ill and PAV-Aus isolates) (Di *et al.*, 1993; Mohan *et al.*, 1995) and PAV-129. A genome with the 5' half (replication genes) derived from PAV6 and the 3' half (structural genes) derived from PAV-129, replicated only about 10% as efficiently as full-length PAV6 (Koev *et al.*, 2002). Given the divergence of the replication genes and *cis*-acting signals recognized by them, it is not surprising that replication was reduced. Despite the reduced virus accumulation, the chimera still caused more severe symptoms than PAV6. The chimera also was more efficiently aphid transmitted (32%) than full-length PAV6 (13%). Thus the 3' half of the PAV-129 genome, which includes the CP, aphid transmission, and system movement genes, contains symptom determinants and (not surprisingly) aphid transmission efficiency determinants.

Table 1. Amino acid (shaded, upper right set) and nucleotide identity (lower left set) between major ORFs of five BYDV PAV-like isolates and BYDV MAV.

		PAV-129	PAV-Aus	PAV-Pur	PAV-Jpn	PAV-Ill	MAV-PS1
ORF1	PAV-129		79.4	79.7	79.0	79.7	79.1
	PAV-Aus	76.7		97.9	97.3	98.2	97.6
	PAV-Pur	76.6	93.2		97.6	98.5	97.9
	PAV-Jpn	76.0	92.9	95.6		97.9	97.3
	PAV-Ill	77.3	93.2	95.8	96.1		98.8
	MAV-PS1	75.5	92.9	95.6	95.6	96.1	
ORF2	PAV-129		88.5	88.3	88.7	88.9	87.9
	PAV-Aus	82.2		99.1	99.4	98.7	97.5
	PAV-Pur	82.5	97.1		99.2	98.9	97.7
	PAV-Jpn	82.6	97.7	97.2		98.9	97.7
	PAV-Ill	82.7	97.0	98.4	97.3		97.3
	MAV-PS1	81.8	94.6	96.1	94.8	95.6	
ORF3	PAV-129		86.5	86.0	85.6	86.1	70.7
	PAV-Aus	89.3		97.0	96.5	96.0	72.7
	PAV-Pur	89.9	95.0		97.5	98.0	72.2
	PAV-Jpn	89.3	94.5	98.5		97.5	70.3
	PAV-Ill	89.2	94.0	98.3	98.2		71.2
	MAV-PS1	75.5	76.0	76.5	76.2	75.5	
ORF4	PAV-129		89.0	90.3	89.0	87.7	73.4
	PAV-Aus	90.9		88.3	87.0	84.4	70.8
	PAV-Pur	91.3	94.8		96.7	96.1	72.1
	PAV-Jpn	91.3	94.4	98.7		97.5	69.4
	PAV-Ill	90.7	93.3	98.0	98.0		68.1
	MAV-PS1	78.2	79.2	80.3	79.6	78.6	
ORF5	PAV-129		86.9	88.0	88.0	88.2	59.2
	PAV-Aus	80.6		92.0	92.2	92.7	58.5
	PAV-Pur	81.8	87.6		96.4	97.3	59.2
	PAV-Jpn	82.1	87.6	97.0		96.7	59.2
	PAV-Ill	81.6	87.3	96.9	96.2		59.4
	MAV-PS1	60.5	60.1	60.2	59.9	61.1	

Notes: All data represent the percent identity between sequences as determined using the GAP program (GCG package). PAV-129: PAV isolate 129. PAV-Aus: PAV Australian isolate. PAV-Pur: PAV Purdue isolate. PAV-Jpn: PAV Japan isolate. PAV-Ill: PAV Illinois isolate. MAV-PS1: MAV PS1 isolate.

We discovered even greater intraserotype divergence among CYDV-RPV isolates. We sequenced a severe isolate from wheat, which was identified serologically as an RPV serotype by researchers at CIMMYT. This isolate, RPV-Mex1, causes corkscrewing and leaf notching in wheat, symptoms that are not commonly caused by milder isolates such as the New York isolate (RPV-NY). RPV-Mex1 has proved to be so different from RPV-NY that it may be reclassified as a different virus. ORFs 0 and 1 of RPV-Mex1 and RPV-NY have only 41% and 55% identity, respectively (Table 2). In contrast, the coat protein genes (ORF 3) are 92% identical, which explains why both isolates are serologically detected as RPV. Partial sequence of a severe RPV-like isolate from California (from Bryce Falk, University of California, Davis) revealed high homology to RPV-Mex1 (data not shown), indicating that the RPV-Mex1 virus is also present in the United States.

Table 2. Amino acid (shaded upper right set) and nucleotide identity (lower left set) of *Polerovirus* ORFs.

		RPV-NY	RPV-Mex	PLRV	BWYV	BMYV	CABYV
ORF0	**RPV-NY**		**40.6**	25.8	26.9	29.4	26.3
	RPV-Mex	**52.8**		29.3	21.7	26.2	28.9
	PLRV	43.6	44.0		22.3	28.6	26.4
	BWYV	45.9	40.6	42.1		28.4	28.7
	BMYV	42.5	43.5	43.6	44.0		42.3
	CABYV	42.9	42.3	41.6	47.2	55.9	
ORF1	**RPV-NY**		**54.7**	37.4	37.1	34.2	37.2
	RPV-Mex	**63.9**		35.3	36.1	30.9	34.5
	PLRV	51.0	48.7		40.3	32.0	32.2
	BWYV	51.5	50.2	50.4		38.6	39.2
	BMYV	48.2	46.9	49.0	51.2		51.7
	CABYV	48.8	47.7	46.4	52.4	59.5	
ORF2	**RPV-NY**		**81.4**	58.6	61.4	57.9	58.0
	RPV-Mex	**80.8**		58.0	58.4	58.0	56.8
	PLRV	64.0	61.8		61.0	59.5	58.4
	BWYV	61.3	60.6	63.4		64.1	64.9
	BMYV	59.9	59.0	61.0	63.3		68.9
	CABYV	61.5	58.7	59.8	64.6	68.5	
ORF3	**RPV-NY**		**91.7**	67.7	68.0	64.7	65.8
	RPV-Mex	**91.7**		69.0	66.2	64.8	66.3
	PLRV	69.4	69.4		65.7	65.2	62.3
	BWYV	65.3	65.5	69.1		91.6	69.7
	BMYV	65.0	65.3	69.1	93.9		67.3
	CABYV	65.8	65.3	68.7	69.7	69.3	
ORF4	**RPV-NY**		**89.6**	57.6	51.0	50.3	48.6
	RPV-Mex	**92.8**		58.9	53.0	52.3	49.3
	PLRV	69.9	71.6		50.3	51.7	57.8
	BWYV	65.1	65.3	68.9		91.4	47.7
	BMYV	65.1	65.5	70.0	94.5		50.9
	CABYV	66.2	65.1	68.9	68.5	68.2	
ORF5	**RPV-NY**		**86.6**	45.0	52.8	53.8	31.8
	RPV-Mex	**83.0**		43.8	51.5	52.2	29.7
	PLRV	53.4	53.0		46.7	43.9	34.4
	BWYV	57.8	55.4	51.4		87.1	42.6
	BMYV	56.5	56.4	48.8	86.6		41.2
	CABYV	43.7	44.0	48.4	49.3	46.0	

Notes: Isolates and Genbank accession numbers (in parentheses): RPV-NY = CYDV-RPV-NY (L25299); RPV-Mex = CYDV-RPV-Mex1 (AF235168); PLRV = PLRV-Pol (X74789); BWYV = BWYV-FL1 (X13063); BMYV = BMYV-2itb (X83110); CAYV = CABYV (X76931). Pairwise comparisons between RPV-NY and RPV-Mex1 are in bold. All data represent the percent identity between sequences as determined by the GAP program (GCG package). Gap penalty of 8, extension penalty of 2, Ends weighted.

This sequence information is important for designing resistance transgenes. A single coat protein gene construct might confer resistance to both viruses, whereas sequence from the 5′ end of the genome clearly would not. This tendency in both BYDV and CYDV for greatest divergence toward the 5′ end of the genome has been observed in *Beet mild yellowing* and *Beet western yellows poleroviruses* as well (Guilley *et al*., 1995) (Table 2). It is interesting that the most variable gene is ORF 0, which encodes the suppressor of gene silencing. This ORF is a strong candidate for symptom determination, as this class of gene determines the ability of viruses to evade host defenses (Kasschau and Carrington, 1998). Indeed, the *Potato leaf roll polerovirus* ORF 0 induced characteristic leaf rolling symptoms when expressed alone as a transgene (van der Wilk *et al*., 1997).

Conclusions

With the growing number of sequenced or partially sequenced isolates of BYDV and CYDV around the world, we are in a better position 1) to develop rapid means of nucleic acid-based detection (e.g., PCR), 2) to understand the epidemiology of BYDV/CYDV, and 3) to develop transgenic and other means of disease control. The better understanding of BYDV molecular mechanisms that we have gained may ultimately lead to new means of controlling or mitigating the effects of the disease, and it sheds light on processes relevant to medically important viruses. However, the number of fully sequenced BYDV and CYDV isolates is far too low, given the immense variation of which we have only caught a glimpse. Although recent work on translation in particular has provided insight into viral mechanisms, the study of BYDV and CYDV lags behind other viruses in our understanding of host-virus interactions and host resistance mechanisms. If funding sources can be made aware of these gaping holes in knowledge about this important disease agent, clearly an exciting future of discovery lies ahead in diverse areas of BYDV and CYDV biology that ultimately will benefit the farmer.

Acknowledgments

The authors thank the USDA National Research Initiative (grant no. 2001-35319-10011), the National Science Foundation (grant no. MCB-9974590), and Quaker Oats for research funding.

References

Allen, E., S. Wang, and W.A. Miller. 1999. Barley yellow dwarf virus RNA requires a cap-independent translation sequence because it lacks a 5' cap. *Virology* 253:139-144.

Barry, J. K., and W.A. Miller. 2002. A programmed -1 ribosomal frameshift that requires base-pairing across four kilobases suggests a novel mechanism for controlling ribosome and replicase traffic on a viral RNA. *Proc Natl Acad Sci USA* 99: (in press).

Brown, C.M., S.P. Dinesh-Kumar, and W.A. Miller. 1996. Local and distant sequences are required for efficient read-through of the barley yellow dwarf virus-PAV coat protein gene stop codon. *J. Virol.* 70:5884-5892.

Burnett, P.A., A. Comeau, and C.O. Qualset. 1995. Host plant tolerance or resistance for control of barley yellow dwarf. In: C.J. D’Arcy and P.A. Burnett (eds.). *Barley Yellow Dwarf: 40 Years of Progress.* APS Press, St. Paul. pp. 321-343.

Chay, C.A., U.B. Gunasinge, S.P. Dinesh-Kumar, W.A. Miller, and S.M. Gray. 1996a. Aphid transmission and systemic plant infection determinants of barley yellow dwarf luteovirus-PAV are contained in the coat protein readthrough domain and 17-kDa protein, respectively. *Virology* 219:57-65.

Chay, C., D.M. Smith, R. Vaughan, and S.M. Gray. 1996b. Diversity among isolates within the PAV serotype of barley yellow dwarf virus. *Phytopathology* 86:370-377.

D’Arcy, C.J., and P.A. Burnett (eds.). 1995. *Barley Yellow Dwarf: 40 Years of Progress.* APS Press, St. Paul.

D’Arcy, C.J., L.L. Domier, and M.A. Mayo. 2000. Family *Luteoviridae.* In: M.H.V. van Regenmortel, C.M. Fauquest, D.H.L. Bishop, E.B. Carstens, M.K. Estes, S.M. Lemon, J. Maniloff, M.A. Mayo, D.J. McGeoch, C.R. Pringle, and R.B. Wickner (eds.). *Virus Taxonomy: Seventh Report of the International Committee on the Taxonomy of Viruses.* Academic Press, San Diego. pp. 775-784.

Di, R., S.P. Dinesh-Kumar, and W.A. Miller. 1993. Translational frameshifting by barley yellow dwarf virus RNA (PAV serotype) in *Escherichia coli* and in eukaryotic cell-free extracts. *Molec. Plant-Microbe Interact.* 6:444-452.

Dinesh-Kumar, S.P., and W.A. Miller. 1993. Control of start codon choice on a plant viral RNA encoding overlapping genes. *Plant Cell* 5:679-692.

Dinman, J.D., S. Richter, E.P. Plant, R.C. Taylor, A.B. Hammell, and T.M. Rana. 2002. The frameshift signal of HIV-1 involves a potential intramolecular triplex RNA structure. *Proc Natl Acad Sci USA* 99:5331-5336.

Gamarnik, A.V., and R. Andino. 1998. Switch from translation to RNA replication in a positive-stranded RNA virus. *Genes Dev* 12:2293-2304.

Guilley, H., K.E. Richards, and G. Jonard. 1995. Nucleotide sequence of beet mild yellowing virus RNA. *Arch. Virol.* 140:1109-1118.

Guo, L., E. Allen, and W.A. Miller. 2001. Base-pairing between untranslated regions facilitates translation of uncapped, nonpolyadenylated viral RNA. *Mol. Cell* 7:1103-1109.

Jin, H., L.L. Domier, F.L. Kolb, and C.M. Brown. 1998. Identification of quantitative loci for tolerance to barley yellow dwarf virus in oat. *Phytopathology* 88:410-415.

Kaiser, J. 2001. Breeding a hardier weed. *Science* 293:1425-1427.

Kasschau, K.D., and J.C. Carrington. 1998. A counterdefensive strategy of plant viruses: suppression of posttranscriptional gene silencing. *Cell* 95:461-470.

Kelly, L., W.L. Gerlach, and P.M. Waterhouse. 1994. Characterisation of the subgenomic RNAs of an Australian isolate of barley yellow dwarf luteovirus. *Virology* 202:565-573.

Kim, K.H., and S.A. Lommel. 1998. Sequence element required for efficient -1 ribosomal frameshifting in red clover necrotic mosaic dianthovirus. *Virology* 250:50-59.

Kim, Y.G., L. Su, S. Maas, A. O’Neill, and A. Rich. 1999. Specific mutations in a viral RNA pseudoknot drastically change ribosomal frameshifting efficiency. *Proc Natl Acad Sci USA* 96:14234-14239.

Koev, G., S. Liu, R. Beckett, and W.A. Miller. 2002. The 3'-terminal structure required for replication of barley yellow dwarf virus RNA contains an embedded 3' end. *Virology* 292:114-126.

Koev, G., B.R. Mohan, S.P. Dinesh-Kumar, K.A. Torbert, D.A. Somers, and W.A. Miller. 1998. Extreme reduction of disease in oats tranformed with the 5' half of the barley yellow dwarf virus-PAV genome. *Phytopathology* 88:1013-1019.

Mastari, J., H. Lapierre, and J.T. Dessens. 1998. Assymetrical distribution of barley yellow dwarf virus PAV variants between host plant species. *Phytopathology* 88:818-821.

Miller, W.A., G. Koev, and B.R. Mohan. 1997. Are there risks associated with transgenic resistance to luteoviruses? *Plant Disease* 81:700-710.

Miller, W.A., S. Liu, and R. Beckett. 2002. Barley yellow dwarf virus: *Luteoviridae* or *Tombusviridae*? *Mol. Plant Pathol.* 3:(in press).

Miller, W.A., P.M. Waterhouse, and W.L. Gerlach. 1988. Sequence and organisation of barley yellow dwarf virus genomic RNA. *Nucl. Acids Res.* 16:6097-6111.

Mohan, B.R., S.P. Dinesh-Kumar, and W.A. Miller. 1995. Genes and *cis*-acting sequences involved in replication of barley yellow dwarf virus-PAV RNA. *Virology* 212:186-195.

Pfeffer, S., P. Dunoyer, F. Heim, K.E. Richards, G. Jonard, and V. Ziegler-Graff. 2002. P0 of *Beet western yellows virus* is a suppressor of posttranscriptional gene silencing. *J. Virol.* 76:6815-6824.

Plumb, R.T., and G.R. Johnstone. 1995. Cultural, chemical, and biological methods for the control of barley yellow dwarf. In: C.J. D'Arcy and P.A. Burnett (eds.). *Barley Yellow Dwarf: 40 Years of Progress.* APS Press, St. Paul. pp. 307-319.

Sachs, A.B., P. Sarnow, and M.W. Hentze. 1997. Starting at the beginning, middle, and end: translation initiation in eukaryotes. *Cell* 89:831-838.

Smith, H.G., and H. Barker. 1999. *The* Luteoviridae. CABI Publishing, Wallingford, Oxon.

Somers, D.A., H.W. Rines, W. Gu, H.F. Kaeppler, and W.R. Bushnell. 1992. Fertile, transgenic oat plants. *Bio/Technology* 10:1589-1594.

van der Wilk, F., P. Houterman, J. Molthoff, F. Hans, B. Dekker, J.F. van den Heuvel, H. Huttinga, and R.W. Goldbach. 1997. Expression of the potato leafroll virus ORF0 induces viral-disease-like symptoms in transgenic potato plants. *Mol Plant Microbe Interact* 10:153-159.

Wang, M.-B., D.C. Abbot, and P.M. Waterhouse. 2000. A single copy of a virus-derived transgene encoding hairpin RNA gives immunity to barley yellow dwarf virus. *Mol Plant Pathol* 1:347-356.

Wang, S., K.S. Browning, and W.A. Miller. 1997. A viral sequence in the 3'-untranslated region mimics a 5' cap in facilitating translation of uncapped mRNA. *EMBO J* 16:4107-4116.

Wang, S., L. Guo, E. Allen, and W.A. Miller. 1999. A potential mechanism for selective control of cap-independent translation by a viral RNA sequence in *cis* and in *trans. RNA* 5:728-738.

Waterhouse, P.M., M.-B. Wang, and T. Lough. 2001. Gene silencing as an adaptive defence against viruses. *Nature* 411:834-842.

Assessment of Biological and Molecular Variability of Moroccan BYDV-PAV Isolates

B. Bencharki,[1] M. El Yamani,[2] J. Mutterer,[3] V. Ziegler-Graff,[3] D. Zaoui,[4] and G. Jonard[3]

[1] Université Hassan 1er, Faculté des Sciences et Techniques, Settat Morocco
[2] Centre Régional de la Recherche Agronomique de Settat, Settat, Morocco
[3] Institut de Biologie Moléculaire des Plantes du CNRS, Strasbourg, France
[4] Université Chouaib Doukkali, Faculté des Sciences d'El Jadida, Morocco

Barley yellow dwarf is an important viral disease. The virus (BYDV) has a wide host range including all major cereal crops and many annual and perennial weeds, pasture and range grasses (Lister and Ranieri, 1995). BYDVs are vectored by several aphid species in a persistent circulative manner and mainly limited to the phloem tissue of an infected plant (Gray, 1996). The virus occurs as a diverse complex of at least five species that vary greatly in aphid transmissibility and severity in cereal hosts (Rochow, 1969). The species CYDV-RPV, BYDV-RMV, BYDV-MAV and BYDV-SGV are readily transmitted by *Rhopalosiphum padi* (L.), *R. maïdis* (Fitch), *Sitobion avenae* (Fab.), *Schizaphis graminum* (Rond.), respectively, while BYDV-PAV by both *R. padi* and *S. avenae* (Rochow, 1969; Power *et al*., 1991).

The genome of BYDV, like that of all *Luteoviridae* consists of a single-stranded messenger-sense RNA molecule (Waterhouse *et al*., 1986; Miller *et al*., 1988). The genome of BYDV-PAV consists of 5677 nucleotides and contains six open reading frames (ORFs) (Miller *et al*., 1988). BYDV isolates vary in capsid protein (CP) properties, including CP molecular weight (Vincent *et al*., 1990), and the presence or absence of epitopes recognized by monoclonal antibodies (Chay *et al*., 1996). The BYDV-PAV coat protein (22 kDa) is encoded by ORF3 (Kelly *et al*., 1994). Contained within ORF3, but in a different reading frame, is ORF4, which encodes a 17 kDa protein. Using reverse genetics approach, Chay *et al*. (1996) were able to show that BYDV 17 kDa protein is required for the systemic infection of plants.

In Morocco, BYDV occurs epiphytotically in cereal and graminaceous plants, but the molecular variation with respect to the location as well as to the host plants is not known for the different isolates. Barley and oat were previously found as species for which the relationships between symptoms and virus level determined by ELISA were significant (Jedlinski, 1977). New technologies such as polymerase chain reaction (PCR) facilitate detection and analysis of sequence polymorphism among virus isolates. In this study, we sought to determine the extent of the capsid protein-coding sequence variability within PAV-type isolates and the evolution of virus content in plant. The BYDV-PAV isolate population was found to differ depending on geographical region and index symptom severity.

Materials and Methods

Virus isolates

Twelve Moroccan BYDV-PAV isolates were examined: MA9415, MA9501, MA9502, MA9504, MA9505, MA9508, MA9511, MA9512, MA9513, MA9514, MA9516, and MA9517 (Bencharki *et al*., 1999). The BYDV-PAV isolates showing varying symptom severity against eight genotypes of barley and an oat cultivar and representing different locations within the cereal growing areas of the country, were maintained and propagated in Clintland 64 oat by serial transfers with *R. padi*, which was the most efficient vector at intervals of 5-6 weeks.

Plant material and virus infection

Three genotypes of barley: Atlas 57, Atlas 68 (California, sister lines), and line 80-81-BQCB-10 were used to study the evolution of the virus content of isolate MA9501 (moderate) and MA9514 (severe). These genotypes were selected from previous germplasm screening studies in Morocco (El Yamani *et al*., 1994). The barley genotypes were obtained from ICARDA (International Center for Agricultural Research in the Dry Areas)/CIMMYT (International Maize and Wheat Improvement Center) collections. The Atlas 68 variety carried the Yd_2 gene for resistance to BYDV-PAV (Schaller, 1984). Line 80-81-BQCB-10 was identified as tolerant, whereas Atlas 57 was used because of their high sensitivity to virus. Virus infection was performed using *R. padi* in the growth chamber maintained at 18 ± 2˚C with a photoperiod of 16 h of light. Ten viruliferous adult *R. padi* were placed on seven-days-old seedlings for a 5-day inoculation access period (IAP). After the IAP, all plants were sprayed with insecticide. The virus content of plant extract during six weeks after IAP, and the test was repeated three times.

RNA extraction and sequencing

Total RNAs were extracted from infected fresh leaves of Clintland 64 oat essentially as described by Reutenauer *et al*. (1993). First strand cDNA synthesis was performed using the primer 5'-*TAGGATCC*-^{3466}GTCTACCTATTTGG^{3453}. The cDNA region corresponding to the coat protein sequence was then amplified by the *Taq* DNA polymerase using the former primer and the oligonucleotide 5'-*GCACCATGG*-^{2862}ATTCAGTAGGTCGTAGAGG^{2880}. The PCR product was digested by *Nco*I and *Bam*HI, and then cloned into a modified pBluescript II SK (-) (Stratagene). For each isolate, four clones were submitted to automated sequence analysis using Amplitaq FS polymerase with rhodamine dye terminators. The sequencing reactions were analyzed using an Applied Biosystems 373 sequencer.

Results and Discussion

Molecular variation

Figure 1 present dendrograms derived from the multiple alignment of the CP and 17 kDa amino acid sequences of the twelve PAV-type isolates sequenced in this study, and the sequences of an additional five PAV isolates for which the sequences are available from Genbank. The analysis shows that the CP's of all the isolates were identical in size: 603 nucleotides and 200 amino acid residues (data not shown). Sequence homology grouping also yielded two clusters CPI and CPII which correlated with the clusters formed by

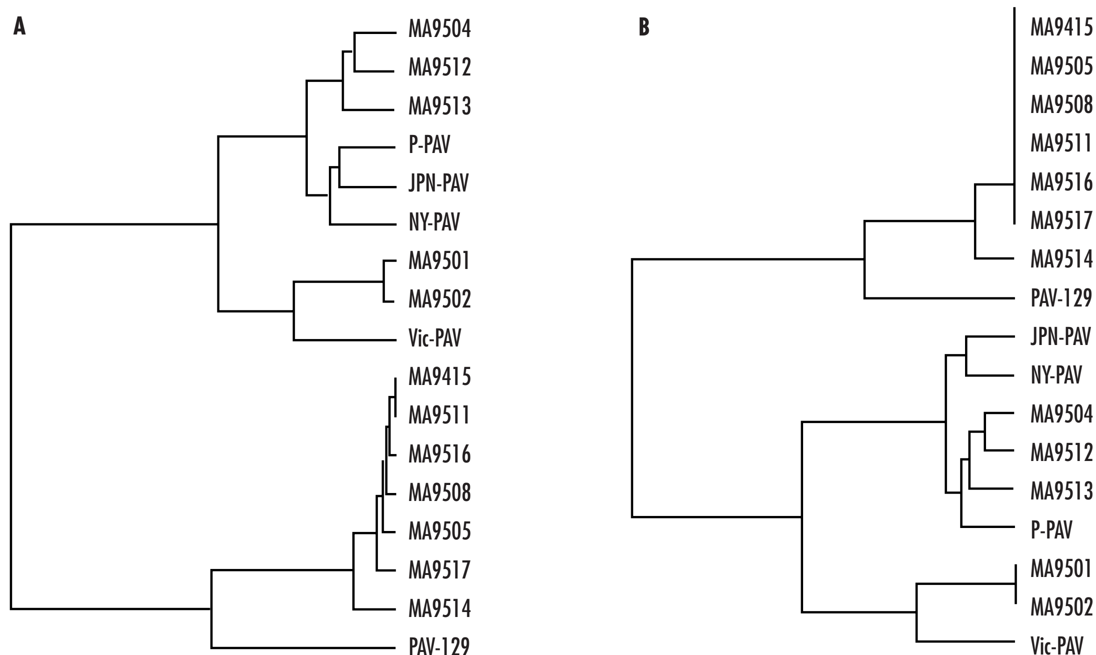


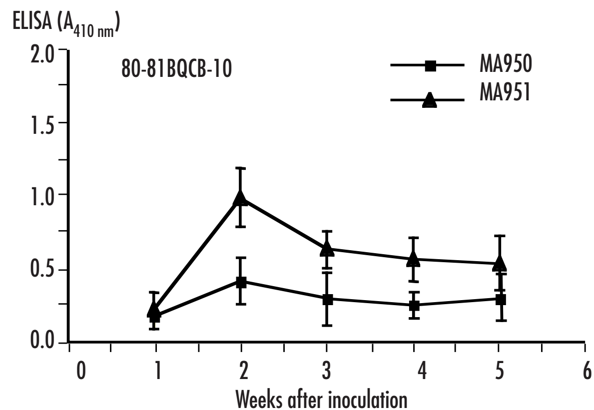
Figure 1. Dendrogram showing phylogenetic relationships of the coat protein (A) and 17 kDa protein (B). The scheme is based on cluster analysis of the sequences performed with the PILEUP program.

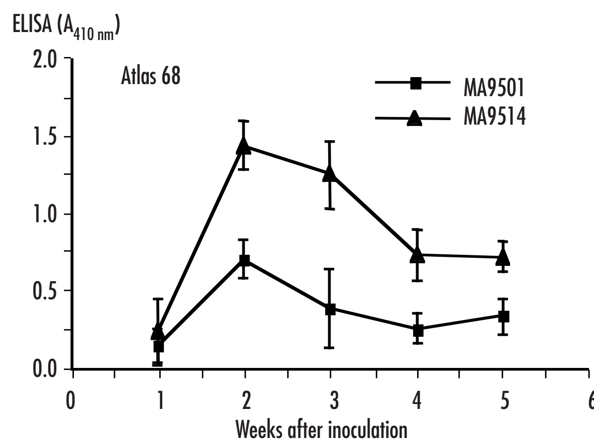
symptom indexing (Bencharki *et al*., 1999). Cluster CPI grouped the isolates MA9512, MA9504, MA9502, and MA9501, and cluster CPII the MA9508, MA9511, MA9415, MA9517, and MA9514 isolates. The comparisons of the CP's between the two clusters revealed 87.4% to 90.5% nucleotide sequence identity and 82.6% to 87.6% amino acid sequence identity. Comparison between the CP of the Moroccan isolates of cluster I to cluster II showed 28 and 83 amino acid and nucleotide changes respectively (data not shown). The CP's of cluster I were closely related to the Australian (Vic-PAV), the North American (NY-PAV, P-PAV), and Japanese (JPN-PAV) PAV-type isolates, while the CP's of cluster II were closely related to the North American PAV-129 isolate. Results presented here for the Moroccan isolates confirm the observations made by Chay *et al*. (1996) and Mastari *et al.* (1998) on the absence of geographical restriction of the New York and French BYDV-PAV isolates.

Dendrogram derived from the multiple alignment of the 17 kDa protein of the Moroccan BYDV-PAV (Figure 1) showed different clustering than the one based on symptom phenotypes and deduced amino acid sequences of CP's. The main cluster, which comprises isolates MA9516, MA 9517, MA9415, MA9508, MA9511, MA9505 and MA9514 corresponds to the former cluster II, and is now called subset 3. The former cluster I, however, is now split into two distantly related subsets, named subsets 1 and 2, the former containing isolates MA9501 and MA9502 and the latter MA9504, MA9513 and MA9512.

Evolution of virus content in plants during growth

The evolution of the virus content of isolate CPI MA9501 (moderate) and CPII MA9514 (severe) was investigated in barley (Figure 2). The resistant genotypes Atlas 68 and 80-81-BQCB-10 significantly reduced virus accumulation in aerial part of the plant. However, the level of resistance varied according to the genotype and isolate of the BYDV-PAV. When comparing resistant and sensitive genotypes, we noted that the titer of the virus in the resistant plant leaves is lower than sensitive genotype. Moreover, the isolate MA9501 is less concentrated than MA9514. The isolates of the BYDV-PAV reached their maximal titer in the plants two weeks after inoculation. Four weeks after inoculation, reduction of the virus content in the resistant genotypes compared to the sensitive ones for the MA9501 was 48% and 46%, in 80-81-BQCB-10 and Atlas 68, respectively. For the isolate MA9514, reduction in the accumulation was 64% and 54%, respectively. The simultaneous presence of the two viral clusters in one host was not yet investigated, but could have various and unpredictable consequences in terms of symptomatology.





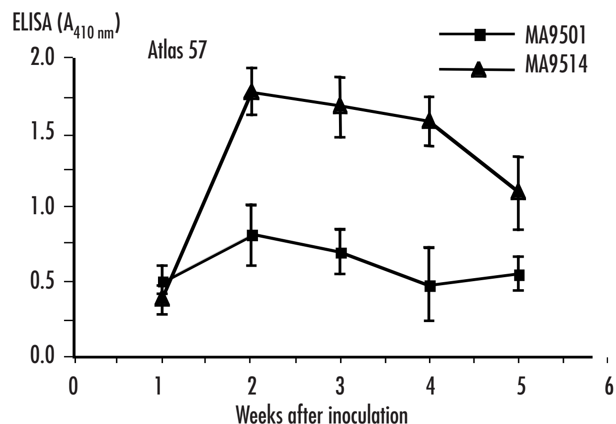


Figure 2. Sequential measurements of virus content (absorbance at 410 nm) in shoot extracts of three barley genotypes inoculated with MA9501 and MA9514 isolates of BYDV-PAV in the growth chamber.

Acknowledgments

We gratefully acknowledge financial support of International Foundation for Science – Sweden, under grant no. C2863-1.

References

Bencharki, B., Mutterer, J., El Yamani, M., Ziegler-Graff, V., Zaoui, D., and Jonard, G. 1999. Severity of infection of Moroccan barley yellow dwarf virus PAV isolates correlates with variability in their coat protein sequences. *Annals of Applied Biology* 134:89-99.

Chay, C.A., Smith, D.M., Vaughan, R., and Gray, S.M. 1996. Diversity among isolates within the PAV serotypes of barley yellow dwarf virus. *Phytopathology* 86: 370-377.

El Yamani, M., Amri, A., Comeau, A., St-Pierre, C. A. and Bencharki B. 1994. Resistance of barley yellow dwarf virus in Morocco. In: *Conference Aridoculture: Acquis et Perspectives.* El Gharrous, M., Karrou, M., and El Mourid, M. (eds) Rabat, Morocco. pp 442-448.

Gray, S.M. 1996. Plant virus proteins involved in natural vector transmission. *Trend in Microbiology* 4:259-264.

Jedlinski, H. 1972. Tolerance to two strains of barley yellow dwarf virus in oats. *Plant Disease Reporter* 56:230-234.

Kelly, L., Gerlach, W.L., and Waterhouse, P.M. 1994. Characterization of the subgenomic RNAs of the Australian isolates of barley yellow dwarf luteovirus. *Virology* 202:565-573.

Lister, R. M., and Ranieri, R. 1995. Distribution and economic importance of barley yellow dwarf. n : *Barley Yellow Dwarf: 40 Years of Progress.* D'Arcy, C.J. and Burnett, P.A. (eds.). American Phytopathological Society, St. Paul, MN. pp 29-53

Mastari, J., Lapierre, H., and Dessens, J.T. 1998. Asymmetrical distribution of barley yellow dwarf virus PAV variants between host plant species. *Phytopathology* 88:818-821.

Miller, W.A., Waterhouse, P.M., and Gerlach, W.L. 1988. Sequence and organization of barley yellow dwarf virus genomic RNA. *Nucleic Acids Research* 16:6097-6112.

Power, A.G., Seaman, A.J., and Gray, S.M. 1991. Aphid transmission of barley yellow dwarf virus: Inoculation access period and epidemiological implications. *Phytopathology* 81:545-548.

Reutenauer, A., Ziegler-Graff, V., Lot, H., Scheidecker, D., Guilley, H., Richards, K., and Jonard, G. 1993. Identification of beet western yellows luteovirus genes implicated in viral replication and particle morphogenesis. *Virology* 195: 692-699.

Rochow, W.F. 1969. Biological properties of four isolates of barley yellow dwarf virus. *Phytopathology* 59:1580-1589.

Schaller, C.W. 1984. The Genetics of Resistance to Barley Yellow Dwarf Virus in Barley. In: *Barley Yellow Dwarf. A Proceedings of the Workshop.* Mexico, D.F.: CIMMYT. pp. 93-100.

Vincent, J.R., Ueng, P.P., Lister, R.M., and Larkins, B.A. 1990. Nucleotide sequences of coat protein genes for three isolates of barley yellow dwarf virus and their relationships to other luteovirus coat protein sequences. *Journal of General Virology* 71:2791-2800.

Waterhouse, P.M., Gerlach, W.L., and Miller, W.A. 1986. Serotype-specific and general luteovirus probes and cloned cDNA sequences of barley yellow dwarf virus. *Journal of General Virology* 67:1273-1281.

Preliminary Study on the RNA-Binding Properties of BYDV-GAV and BYDV-GPV Movement Proteins

Z. Xia, Y. Zhang, K. Liu, and D. Wang

Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China

We expressed the cDNAs of the genes encoding the movement proteins (MPs) of BYDV-GAV and BYDV-GPV in bacterial cells. The over-expressed MPs were purified using gel fractionation and electro-elution. In in vitro *RNA binding assays, the bacterially expressed MPs of BYDV-GAV and BYDV-GPV were found to bind single stranded RNA (ssRNA) molecules in a sequence independent manner.*

Barley yellow dwarf virus (BYDV) is known to possess many serologically different strains (Mayo and Ziegler-Graff, 1996; Miller and Rasochova, 1997). More recently, based on differences in genome structure as well as variations in nucleic acid and protein sequences, some of the BYDV strains are considered to be distinct, but related, viruses of the *Luteoviridae* (Barker and Smith, 1999). BYDV-MAV and BYDV-PAV are members of the genus *Luteovirus*, whereas CYDV-RPV (formerly BYDV-RPV) belongs to the genus *Polerovirus*. Owing to the lack of sufficient information, the taxonomic status of several BYDV strains (such as BYDV-GPV, BYDV-RMV and BYDV-SGV) in the *Luteoviridae* remains unassigned (Barker and Smith, 1999).

In China, researchers have identified several BYDV species that are prevalent in small grain crops, two of which, BYDV-GAV and BYDV-GPV, possess dual vector specificity (Wang *et al*., 1998; Wang *et al*., 2001). While BYDV-GAV is transmitted by both *Schizaphis graminum* and *Sitobion avenae*, BYDV-GPV is transmitted by both *Schizaphis graminum* and *Rhopalosiphum padi* (Wang *et al*., 1998; Wang *et al*., 2001). Comparisons of nucleotide sequences of CP, MP, and/or RTP genes show that BYDV-GAV is similar to BYDV-MAV (Wang *et al*., 2001). In contrast, BYDV-GPV is more closely related to BYDV-RPV (Wang *et al*., 1998). In general, our understanding of BYDV-GAV and BYDV-GPV is not as advanced as that for BYDV-PAV or BYDV-MAV.

To provide more information on BYDV-GAV and BYDV-GPV, we initiated studies on the molecular and cell biological aspects of the infection caused by the two viruses. At present, the focus of our investigations is on the function of MP in the interaction between virus and host cell.

The MP of luteoviruses and poleroviruses is commonly encoded by the open reading frame 4 (ORF 4) of the virus genome (Barker and Smith, 1999; Mayo and Ziegler-Graff, 1996; Miller and Rasochova, 1997). This gene is entirely nested within the coat protein (CP) gene (ORF 3). The MP of BYDV-PAV has been shown to be required for viral systemic movement (Chay *et al*., 1996) and to be associated with viral RNA in infected cells (Nass *et al*., 1998). The MP of *Potato leaf roll virus* (PLRV) has been found to accumulate in plasmodesmata and to bind single stranded RNA or DNA molecules in a sequence nonspecific manner in *in vitro* assays (Schmitz *et al*., 1997; Tacke *et al*., 1991). In *Beet western yellows virus* (BWYV, a polerovirus), two modes of virus movement have been proposed: one involves the function of CP, and the other requires the participation of MP (Ziegler-Graff *et al*., 1996).

From the available data, it would appear that the function of luteovirus and polerovirus MP might be similar to that of the MPs of other better-studied RNA viruses (such as *Tobacco mosaic virus*, TMV) (Lartey and Citovsky, 1997; Wolf and Lucas, 1994). However, more studies are needed to reveal details of the action of

luteovirus and polerovirus MPs. In this paper, we report the expression of the genes encoding BYDV-GAV and BYDV-GPV MPs in bacterial cells and the demonstration of RNA binding activities of the purified MPs in *in vitro* assays.

Materials and Methods

Wheat leaf materials infected with BYDV-GAV were collected from the field, and those infected with BYDV-GPV were obtained from the Institute of Plant Protection of the Chinese Academy of Agricultural Sciences. For amplifying and cloning the cDNAs of the ORF 4 genes from BYDV-GAV and BYDV-GPV, specific PCR primers (Table 1) were designed based on published BYDV sequences (Wang *et al*., 1998; Wang *et al*., 2001). Total RNA from infected leaf materials was prepared using the Trizol reagent (Life Technologies) and reverse-transcribed using the M-MLV reverse transcriptase (Life Technologies).

For PCR reactions using the reverse transcription mixture, the high fidelity ExTaq polymerase (TaKaRa) was employed. The resulting DNA fragments were cloned into plasmid vector (pGEM-T, Promega) and completely sequenced (Sambrook *et al*., 1989). Two clones, pGAV-MP6 and pGPV-MP2, were found to harbor full-length cDNAs for the ORF 4 genes of BYDV-GAV and BYDV-GPV, respectively. To prepare the bacterial expression constructs pET-Gavmp and pET-Gpvmp, the inserts in pGAV-MP6 and pGPV-MP2 were re-amplified using either the primer combination GavmpF1 and GavmpR1 (Table 1, for ORF 4 cDNA of GAV) or the combination GpvmpF1 and GpvmpR1 (Table 1, for ORF 4 cDNA of GPV). The resulting PCR fragments were cleaved with *Bam*HI followed by cloning into the polylinker region of the bacterial expression vector pET-30a (In Vitrogen). After induction with IPTG, the over-expressed MPs were purified by gel fractionation and electro-elution using an Electro-Eluter (Bio-Rad) (Zhang *et al*., 1999).

Table 1. A list of PCR primers used in the study.

Primer	Sequence	Usage
GavmpF	5' GAGCCGCGGCCCAAGGAGAGCAA 3'	Amplification of cDNA of BYDV-GAV ORF 4
GavmpR	5' TGTAGGATCCGTTCACCGTACTCTCC 3'	
GpvmpF	5' TTAGCCGCGGCAATGGTCCGC 3'	Amplification of cDNA of BYDV-GPV ORF 4
GpvmpR	5' ACCGGATCCTCATCTTCGCTGGGAAG 3'	
GavmpF1	5' TGACATATGGCCCAAGGAGAGCAAGG 3'	Introducing *Nde* I and *Bam*HI sites to GAV ORF 4 cDNA
GavmpR1	5' TGTAGGATCCGTTCACCGTACTCTCC 3'	
GpvmpF1	5' GCGCATATGGTCCGCGTAGACGAC 3'	Introducing *Nde* I and *Bam*HI sites to GPV ORF 4 cDNA
GpvmpR1	5' ACCGGATCCTCATCTTCCTGGGAAG 3'	

For RNA binding assays, the non-radioactive Northwestern blot method described by Rodriguez and Carrasco (1994) was followed with some modifications. The ssRNA probes were prepared by *in vitro* transcription reactions in the presence of digoxigenin-11-UTP (Roche). The template for the transcription reactions was either cDNA clone pGAV-MP6 (for the synthesis of a sequence specific ssRNA probe, probe S) or the cDNA clone of the RNA1 of *Pea early browning virus* (PEBV, for the production of a sequence nonspecific ssRNA probe, probe NS) (MacFarlane *et al*., 1989). After MP-ssRNA probe reaction, the binding was visualized using the anti-digoxigenin Fab-alkaline phosphatase conjugate (1:3000 dilution, Roche) and the substrate solution containing BCIP and NBT (Sigma). The results of the RNA binding assays were recorded using a digital camera (Coolpix 990, Nikon).

Results and Discussion

DNA sequencing showed that the inserts in pGAV-MP6 and pGPV-MP2 represented full- length cDNA copies of the ORF 4 genes of BYDV-GAV and BYDV-GPV, respectively. In bacterial expression experiments, the two cDNAs directed the synthesis of MPs of expected molecular mass after IPTG induction (Figure 1). After gel purification of the over-expressed MPs (Figure 2a), RNA binding assays were conducted using ssRNA probes S and NS. We found that the MPs of BYDV-GAV and BYDV-GPV bound ssRNA probes S and NS with equal efficiencies (Figure 2b), indicating that the RNA binding activities of the two MPs were sequence nonspecific. In a previous investigation, the MP of PLRV, which is related to various BYDV species, has been found to bind ssRNA in a sequence independent manner (Tacke *et al*., 1991). This suggests that RNA binding activity may be a common property of the MPs encoded by luteoviruses and poleroviruses.

Although at present it is not known how important RNA binding property is to the function of BYDV MPs, molecular and cell biological studies with other RNA viruses have suggested that RNA binding activities are essential to the formation of the

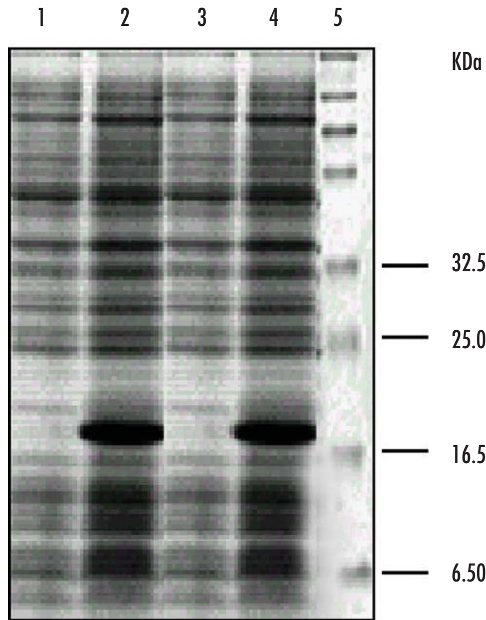


Figure 1. Over-expression of ORF 4 cDNAs in bacterial cells.
Notes: The ORF 4 cDNA of BYDV-GAV directed the synthesis of MP (17 kDa) in IPTG induced (lane 2), but not the uninduced (lane 1), bacterial cells. Similarly, The ORF 4 cDNA of BYDV-GPV gave rise to MP (17 kDa) in IPTG induced (lane 4), but not the uninduced (lane 3), bacterial cells. The size of the protein marker (kDa, lane 5) is shown on the right side of the graph.

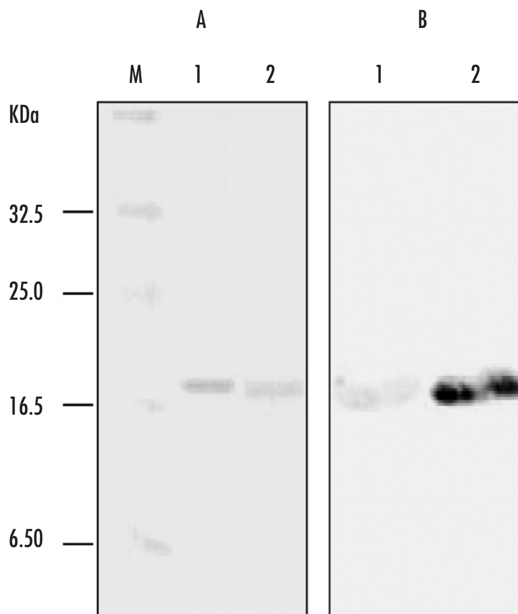


Figure 2. Purification (a) and RNA binding activity assay (b) of the MPs of BYDV-GAV and BYDV-GPV.
Notes: In both a and b, lane 1 was loaded with the MP of BYDV-GAV, whereas lane 2 was with the MP of BYDV-GPV. The result shown in b was obtained with the ssRNA probe NS. A similar result was recorded when the assay was performed using the ssRNA probe S. The size of the protein marker (kDa, lane M) is shown on the left side of the graph.

ribonucleoprotein complex between MP and viral genomic RNA, which is the entity that moves from cell to cell (Lartey and Citovsky, 1997; Wolf and Lucas, 1994). Structurally, it will also be important to investigate the motif(s) within BYDV MPs that determines RNA binding activities. In this respect, it is interesting to note that the C-terminal regions of the MPs of both BYDV-GAV and BYDV-GPV are rich in basic amino acid residues. Therefore, further studies will be directed towards understanding the potential involvement of the C-terminal regions of BYDV MPs in RNA binding activities.

References

Barker, H., and Smith, H.G. 1999. *The Luteoviridae.* CAB International.

Chay, C.A., Gunasinge, U.B., Dinesh-Kumar, S.P., Miller, W.A., and Gray, S.M. 1996. Aphid transmission and systemic plant infection determinants of barley yellow dwarf luteovirus-PAV are contained in the coat protein readthrough domain and 17-kDa protein, respectively. *Virology* 219:57-65.

Lartey, R., and Citovsky, V. 1997. Nucleic acid transport in plant pathogen interactions. *Genet Eng (N Y)* 19:201-214.

MacFarlane, S.A., Taylor, S.C., King, D.I., Hughes, G., and Davies, J.W. 1989. Pea early browning virus RNA1 encodes four polypeptides including a putative zinc-finger protein. *Nucleic Acids Res* 17:2245-2260.

Mayo, M.A., and Ziegler-Graff, V. 1996. Molecular biology of luteoviruses. *Crit Rev Plant Sci* 46:413-460.

Miller, W.A., and Rasochova, L. 1997. Barley yellow dwarf viruses. *Annu Rev Phytopathol* 35:167-190.

Nass, P.H., Domier, L.L., Jakstys, B.P., and D'Arcy, C.J. 1998. *In situ* localization of barley yellow dwarf virus-PAV 17-kDa protein and nucleic acids in oats. *Phytopathology* 88:1031-1039.

Rodriguez, P.L., and Carrasco, L. 1994. Nonradioactive north-western analysis using biotinylated riboprobes. *Biotechniques* 17:702-705.

Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. *Molecular Cloning: A Laboratory Manual.* New York: Cold Spring Harbor Laboratory Press.

Schmitz, J., Stussi-Garaud, C., Tacke, E., Prufer, D., Rhode, W., and Rohfritsch, O. 1997. *In situ* localization of the putative movement protein (pr17) from potato leaf roll luteovirus (PLRV) in infected and transgenic plants. *Virology* 235:311-322.

Tacke, E., Prüfer, D., Schmitz, J., and Rohde, W. 1991. The potato leaf roll luteovirus 17K protein is a single-stranded nuclei acid binding protein. *J Gen Virol* 72:2035-2038.

Wang, M.B., Cheng, Z., Keese, P., Graham, M.W., Larkin, P.J., and Waterhouse, P.M. 1998. Comparison of the coat protein, movement protein and RNA polymerase gene sequences of Australian, Chinese, and American isolates of barley yellow dwarf virus transmitted by *Rhopalosiphum padi. Arch Virol* 143:1005-1013.

Wang, X., Chang, S., Jin, Z., Li, L., and Zhou, G. 2001. Nucleotide sequence of the coat protein and readthrough protein genes of the Chinese GAV isolate of barley yellow dwarf virus. *Acta Virol* 45:249-252.

Zhang, J.-F., Zhao, H., Gui, J.-G., Liu, K.-F., and Wang, D.-W. 1999. Molecular characterization of a Chinese soybean mosaic virus isolate by RT-PCR, cDNA sequence analysis and direct expression of PCR products in bacteria. *Acta Bot Sin* 41:932-935.

Wolf, S., and Lucas, W.J. 1994. Virus movement proteins and other molecular probes of plasmodesmal function. *Plant, Cell and Environment* 17:573-585.

Ziegler-Graff, V., Brault, V., Mutterer, J.D., Simonis, M., Herrbach, E., Guilley, H., Richards, K.E., and Jonard, G. 1996. The coat protein of beet yellows luteovirus is essential for systemic infection but the viral gene products of P29 and P19 are dispensable for systemic infection and aphid transmission. *Mol Plant-Microbe Interact* 6:501-510.

Progress in Characterizing the Chinese GAV Isolate of the BYDV Luteovirus

X. Wang, LiLi, and G. Zhou

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China

Barley yellow dwarf virus (BYDV), a member of the *Luteovirus* genus, can cause significant economic losses in cereal grain yields. The luteoviruses are phloem-limited and obligatory transmitted by cereal aphids in a circulative/persistent manner. Rochow (1969) and Rochow & Muller (1971) characterized and differentiated five distinct BYDV isolates based on the efficiency of transmission by four aphid species and serological assays (Rochow, 1982). In China, BYDV is currently divided into four isolates according to Rochow's system. One isolate (GAV) is transmitted nonspecifically by the aphids *Sitobion avenae* and *Schizaphis graminum*, and has a strong serological reaction with BYDV-MAV-NY-specific antiserum, but the MAV-NY isolate is transmitted specifically by *S. avenae*. The nucleotide sequences of some BYDV isolates have been determined, and the genome structure has become clear (Ueng *et al.,* 1992). There are six open reading frames (ORF) in the BYDV genome. There is also some evidence suggesting that the read-through protein (RTP) is related to the specificity of vector transmission (Wang *et al.*, 1995). In recent years, the BYDV-GAV isolate has become a dominant isolate in China (Zhou *et al.*, 1984); therefore, it is very important to study its characterization.

Nucleotide Sequences of the Coat Protein and Read-through Protein Genes of the Chinese BYDV-GAV Isolate

The nucleotide sequences of the GAV isolate's CP and RTP genes were determined from four recombinant clones (Acc. No. AF338909). The CP and RTP genes consisted of 600 and 1374 nucleotides (nts), respectively, identical to MAV-PS1. The nucleotide sequences of the GAV isolate showed a low similarity to those of other BYDVs (52.2-76.6% for CP and 41.7-60.8% for RTP). The very high (97.2%) similarity of the CP genes of isolates GAV and MAV-PS1 suggested that the CP gene might not be the reason for the difference in transmission phenotypes between isolates GAV and MAV-PS1. Since the RTP gene showed a lower similarity between GAV and MAV-PS1 isolates (87.8%), and each gene performs its function through its protein product, a multiple sequence alignment was conducted with the deduced amino acid sequences of RTP of GAV and MAV-PS1. A similarity of 87.09% was obtained, and 59 of a total of 457 amino acids were substituted in the GAV isolate. These substitutions were located mainly in the C terminus region (amino acid positions 238–457). There were 40 deduced amino acids expressed substituted from a total of 215 amino acids in this region. Continuous substitution occurred at amino acid positions 282–294; in MAV-PS1, the amino acid sequence was EMDAGSPIDTASL, while in the GAV isolate it was GMDSRSPVEPPSP. These amino acid substitutions might be the reason for the differences in GAV and MAV-PS1 transmission phenotypes.

The region immediately downstream of the ORF3 stop codon is very rich in proline residues and shows considerable homology in the readthrough domain sequences of all luteoviruses. This proline hinge may serve as a loose tether joining CP (presumably anchored in the virion capsid) and the rest of the readthrough domain. However, the C-terminal region of RTP is more diverse; some researchers have proposed that this region is involved in the specificity of vector transmission. The results presented here support this hypothesis.

Identification of Proteins Associated with Circulative Transmission of BYD Luteoviruses from *S. graminum and S. avenae*

To detect whether BYDV showed affinity to protein components from its aphid vectors, we separated whole-body extracts of the aphids by SDS-polyacrylamide gel electrophorese (SDS-PAGE), and transferred the proteins to nitrocellulose. The proteins were reacted with purified GAV virus and probed with IgG labeled with alkaline phosphatase. In this way, two proteins of 31Kda (p31) and 50 Kda (p50) displayed high virus-binding capacities, and were identified in the whole-body extracts of *S. graminum (Sg)* and *S. avenae (Sa)*. GAV-binding proteins were not found in the whole-body extracts of *R. padi*, which do not transmit the GAV isolate. We also did not find any affinity between these proteins and *Beet black scorch virus* (BBSV) transmitted by a soil fungus. Furthermore, upon dissecting the accessory salivary glands (ASG) and gut of *Sg*, and analyzing the protein contents, p31 and p50 were found in the ASG and not in the gut.

Isolation of p31 and p50 from *Sg* was carried out by electro-elution from gel slices after SDS-PAGE of whole extracts. Samples of purified p31 and p50, emulsified in Freund's incomplete adjuvant, were injected into rabbits for antiserum production respectively. Using these antisera as the first antibodies to probe the proteins of the aphids (Western blotting), p31 and p50 were found in *Sg* and *Sa*, but not in *R. padi*.

Through membrane feeding technique, *Sg* and *Sa* were first fed on 500x antiserum to p50KDa protein through Parafilm, and then transferred on to GAV-infected leaves in dishes for acquisition feeding, before being placed on healthy seedlings of Coast Black Oats for inoculation. The results showed that the transmission efficiencies of *Sg* and *Sa* were reduced from 73.44% to 26.92% and from 72.55% to 46.88%, respectively, when fed first on the p50 antiserum, confirming that the p50Kda protein is a transmission-associated protein in both aphid vectors.

Using the two-dimensional gel electrophoresis technique, we obtained pure p50Kda protein of *Sg*. The 15 amino acids sequences of the N terminal of the pure protein was analyzed using Applied Biosystems 491 protein sequencing equipment.

Comparison of the CP and RTP of GAV and MAV

The GAV isolate was transmitted continuously for five generations by *Sg* (GAV-Sg) and *Sa* (GAV-Sa), respectively. The results showed that transmission efficiencies and serological reactions to MAV antibody were similar regardless of which aphid species were used for GAV transmission. Comparison of the nucleotide sequences of the CP and RTP genes between GAV-Sg and GAV-Sa reached 100% and 99.4% similarity, respectively, with only seven nucleotides differences in the RTP gene. The comparison of the nucleotide sequences of the CP of GAV-Sg and GAV-Sa and MAV CP showed 97.5% homology, while the nucleotide sequences of the RTP of GAV-Sg and GAV-Sa and MAV-RTP had 87.8% and 87.7% homology, respectively. Fourteen and eight nucleotides have changed in the RTP gene sequence at 856-886 nt. It is suggested that these changes may be the reason for the difference in transmission between GAV and MAV isolates.

References

Rochow, W.F. 1969. Biological properties of four isolates of barley yellow dwarf virus. *Phytopathology* 59:1580-1589.

Rochow, W.F. 1982. Identification of barley yellow dwarf virus: Comparison of biological and serological methods. *Plant Dis.* 66:381-384

Rochow, W.F. and Muller, I. 1971. A fifth variant of barley yellow dwarf in New York. *Plant Dis.* 55:874-877

Ueng, P.P., Vincent, J.R., Kawata, E.F., Lei, C.H., Lister, R.M., and Larkin, B.A.1992. Nucleotide sequence analysis of the genomes of the MAV-PS1 and P-PAV isolates of barley yellow dwarf virus. *J. Gen. Virol.* 73:487-492

Wang, J.Y., Chay, C., Gildow, F.E., and Gray, S.M. 1995. Readthrough protein associated with virions of barley yellow dwarf luteovirus and its potential role in regulating the efficiency of aphid transmission. *Virology* 206: 954-962

Zhou Guang-he, Cheng Zhuo-min, Zhang Shu-xiang, and Qian You-ting 1984. Serological identification of luteoviruses of small grains in China. *Plant Disease* 68:710-713

Serial Passages of a BYDV-PAV Isolate Modify Its Biological Properties

F. Chain, G. Riault, O. Valles, G. Mauvais, S. Tanguy, C. Kervarrec, C.-A. Dedryver, M. Trottet, and E. Jacquot

Institut National de la Recherche Agronomique, Domaine de la Motte, Le Rheu, France

Barley yellow dwarf viruses (BYDVs, *Luteoviridae*) cause one of the most important viral diseases among small grain cereals (D'Arcy and Burnett, 1995). The main symptoms of this world-distributed viral disease (i.e., dwarfing and yellowing of infected plants; Bruehl, 1961) cause important yield losses (Lister and Ranieri, 1995). Rochow (1970) distinguished five distinct BYDV serotypes based on their transmission by different aphid species. One of them, BYDV-PAV, is widespread in Western Europe and causes the most severe symptoms (Signoret and Maroquin, 1990).

Breeding for BYDV tolerance/resistance is the best means of controlling barley yellow dwarf disease, given that it is cost-reducing and least damaging to the environment. However, little resistance/tolerance is available in wheat. Some *Thinopyrum* species (*Th. intermedium* and *Th. ponticum*) are known to express resistance against BYDV-PAV. Breeding programs have therefore started to introduce such resistance into different wheat germplasms.

New BYDV-resistant wheat cultivars should be developed within the next ten years. Breeders have already produced new wheat lines carrying alien resistance genes such as TC14 (translocated line; Banks *et al.*, 1995), P29 (addition line; Sharma *et al.*, 1997), Zhong ZH, a ditelosomic addition line derived from Zhong 6 x Mission (Larkin *et al.*, 1995; Barloy *et al.*, 2002), and OK7211542 (partial amphiploid line, Comeau *et al.*, 1994). However, very few data are available on the efficiency and durability of these resistances/tolerances when confronted by the variability of BYDV-PAV isolates.

Our objectives were: 1) to assess the different behaviors of two wheat lines showing resistance when inoculated with BYDV, and 2) to study the effect of serial passages on the biological properties of a BYDV-PAV isolate inoculated into various wheat hosts.

Materials and Methods

Virus isolate

BYDV-PAV4 was used in the experiments. Originally collected on barley in 1989 in western France, it has been maintained on barley cv. Express. This variety is known to be a susceptible host for BYDV (Sadeghi *et al.*, 2000). Infected barley plants were used as the original virus source in inoculation experiments.

Host plants

Four BYDV hosts, a barley line (cv. Express), a wheat line (cv. Sunstar, a susceptible Australian cultivar), and the two resistant lines, Zhong ZH and TC14, were used in the experiments. Healthy and infected plants were grown in a temperature-controlled chamber at 20°C, L: 16/D: 8.

Aphid clones

Third or fourth instar larvae of a *Rhopalosiphum padi* clone were used for all BYDV-PAV transmission experiments. Virus-free and viruliferous aphids were obtained by parthenogenic reproduction of aphid females on healthy and infected barley plants, respectively.

Inoculation and detection of BYDV-PAV by DAS-ELISA

Three sets of ten to fourteen 10-day-old plants (1- or 2-leaf stage) of each test host were inoculated separately with the BYDV-PAV4 isolate. Three viruliferous aphids were reared at the base of each test plant. The latter were then covered with perforated cellophane bags for a 5-day inoculation access period (IAP). At the end of IAP, aphids were killed by spraying plants with an insecticide (deltamethrin). Each plant was sampled at 7, 11, 14, 18, and 21 days after inoculation (DAI). Samples were kept at –20˚C until analyzed.

Leaves were ground in PBS-T-PVP using Fastprep [Bio101] at maximum speed for 45 sec. Virus coat proteins present in sampled plants were detected by DAS-ELISA using a polyclonal serum raised against BYDV-PAV (IgG PAV52, H. Lapierre, INRA, France). One hundred ml of each sample was used to perform the ELISA assay. Standard samples corresponding to serial dilutions of an infected control plant or purified BYDV made possible the semi-quantitative detection of BYDV-PAV coat proteins in all tested samples. Plants were considered infected when the optical density at 405 nm (OD_{405}) obtained by DAS-ELISA was above 0.12.

Serial passage of BYDV-PAV4 and characterization of host-dependent BYDV isolates

At DAI 21, the four plants from each plant/isolate combination showing the greatest DAS-ELISA OD_{405} values were used to initiate a second passage of the isolate to the same host line. Ten virus-free third or fourth instar larvae of *R. padi* clones were reared on each of the four selected infected plants for a 3-day acquisition access period (AAP). At the end of the AAP, viruliferous aphids were transferred to healthy plants (3 aphids/plant) for a 5-day IAP before being killed. BYDV accumulation in plants was monitored as previously described. Six passages were made following this protocol. After the sixth passage, the BYDV-PAV4 isolate had been separately maintained on different host plants for an 18-month period. Isolates maintained on Express, Sunstar, Zhong, and TC14 were renamed BYDV-PAV4E, -PAV4S, -PAV4Z, and -PAV4T, respectively. Biological properties of these host-dependent isolates were checked by inoculating the four host plants used in this study with each isolate.

Results and Discussion

Semi-quantitative DAS-ELISA tests, performed from DAI 7 to DAI 21, gave the percentage of infected plants for all the isolate/host/passage combinations tested. This allowed us to estimate the virus load in plants. The resistance of the TC14 and Zhong lines was characterized based on the collected data, which were also used to study the effect of isolate passage on the virus' ability to infect susceptible and resistant hosts.

Percentage of infected plants

Serial passage of BYDV-PAV4 on wheat lines resulted in a strong increase of the percentage of infected plants, as well as earlier detection of the virus, particularly for Sunstar and Zhong lines (Figure 1 a and b). It seems that replication of the barley-derived BYDV isolate on wheat lines leads to select "adapted" isolate after serial passage. TC14 follows a similar behavior pattern in response to inoculation passage-based process. However, this line exhibits a high percentage of plants escaping infection (Figure 1 c). Lastly, the maximum percentage of TC14-infected plants only reached 67% (Figure 1 a, passage 5), and virus particles were detected at DAI 7 only after 6 passages of BYDV-PAV4 on TC14 plants.

Results of DAS-ELISA performed on Sunstar and Zhong plants inoculated with isolates from the 55th passage reveal percentages of infected plants similar to those of the first passages. The return to a "wild type" (barley-derived) state for PAV4S and PAV4Z could be related to the change in protocol used to maintain isolates on wheat after the sixth passage. Such observation, in favor of a cost for selection in the viral population of a modified isolate "adapted" to Sunstar or Zhong will be discussed. In contrast, selection performed by the 56 passages of BYDV-PAV4 on TC14 plants was able to select and maintain a variant adapted to this resistant host.

Comparison of virus load in test plants from different passages

Virus loads, corresponding to infected wheat plants from the six first passages, were compared for each test line. No significant effect was observed (not illustrated). However, a strong between-line effect was determined by statistical analysis of the OD_{405nm} data corresponding to passage 56 (i.e., $F=437.77$, $p<0.0001$

at DAI 21). This analysis confirmed the resistant behavior of Zhong and TC14 lines against BYDV-PAV infection.

These experiments highlighted differences between Zhong and TC14. BYDV-PAV4 serially passaged on Zhong and TC14 led to two distinct infection patterns, which could be interpreted as a difference in resistance mechanisms against barley yellow dwarf disease. The impact of such results on further breeding programs will be discussed.

Modification of biological properties

The four host plants were individually inoculated with BYDV-PAV4E, -PAV4S, -PAV4Z, and -PAV4T. These isolates did not show the same pattern of infection over time (Figure 2), although they have a common origin (barley-derived BYDV-PAV4).

Isolates maintained on resistant hosts were able to infect a greater percentage of plants ($F=93.06$; $p<0.0001$ at DAI 21) than isolates maintained on susceptible hosts, as shown by results at DAI 21 (Figure 2, DAI 21). Most resistant plants escaped infection when inoculated with BYDV-PAV4E or -PAV4S.

Isolates from resistant hosts required a longer time to accumulate sufficiently to be detected in plant by DAS-ELISA. However, they were able to infect both susceptible and resistant hosts efficiently. So the negative trait linked to the delay (compared to BYDV -PAV4E and -PAV4S) that resistant wheat line-derived isolates need to systemically infect hosts is compensated by the ability of BYDV-PAV4T and -PAV4Z to efficiently infect a larger range of plants, including TC14 and Zhong lines.

Data acquired with the semi-quantitative detection of virus particles in plants at different DAI allowed us to distinguish Express and Sunstar from Zhong and TC14, and revealed significant differences among isolates for the quantities of virus particles detected in infected plants ($F=34.17$; $p<0.0001$ at DAI 21). Regardless of the test line, BYDV-PAV4Z was the isolate with the highest virus load values from DAI 11 to DAI 18. However, between DAI 18 and DAI 21 there was a decrease in the amount of virus detected in plant, leading us to believe that BYDV-PAV4Z was the weakest concentrated virus in infected plants at DAI 21. Surprisingly, BYDV-PAV4T showed just the opposite behavior. The reason for this is not clear. Replicates are in progress.

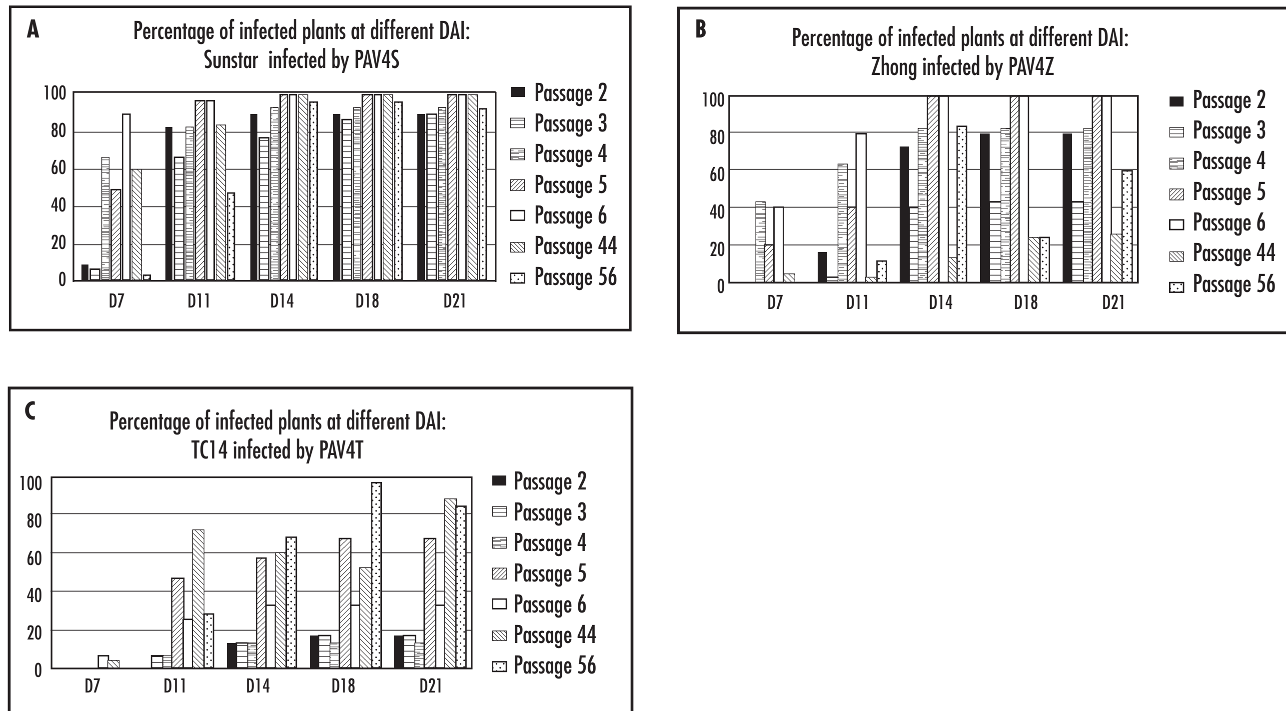


Figure 1. Effect of serial passages of a barley-derived BYDV on percentage of infected plants. The Y axis is the percentage of infected plants.

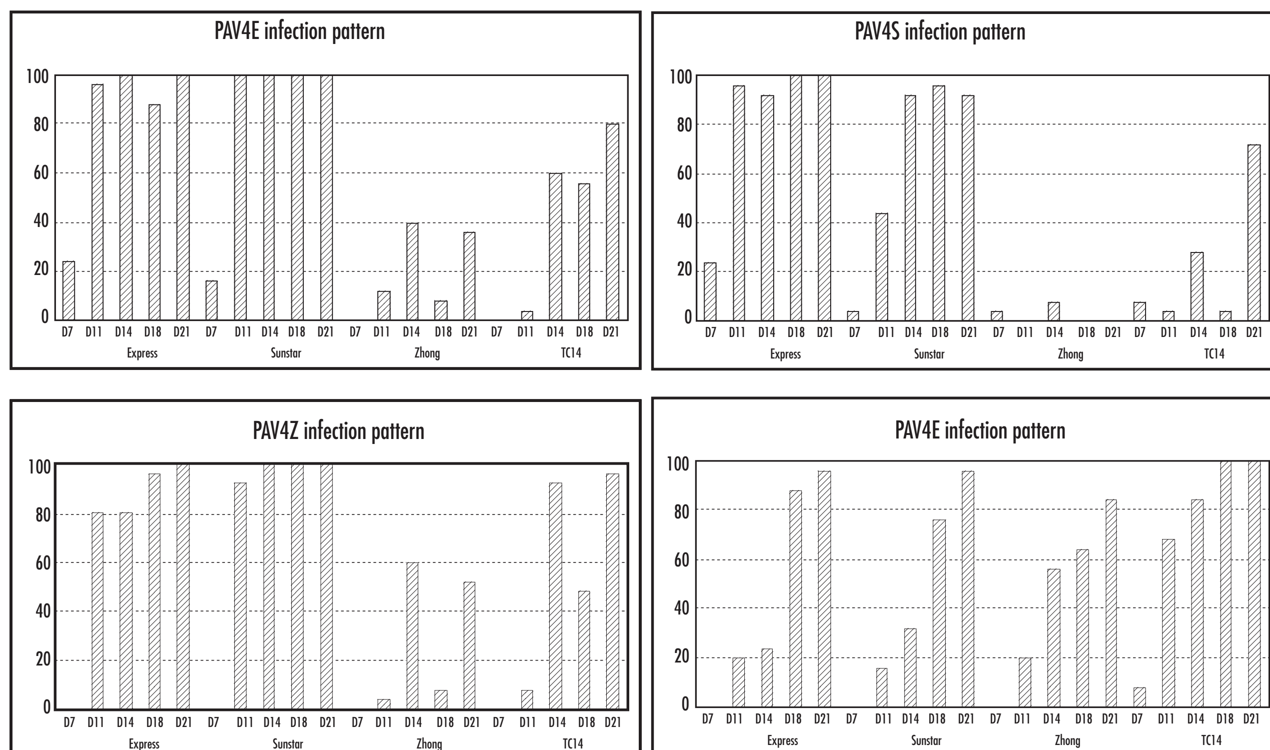


Figure 2. Infection patterns for the four "adapted" isolates at different DAI. The Y axis is the percentage of infected plants.

References

Banks, P.M., Larkin, P.J., Bariana, H.S., Lagudah, E.S., Appels, R., Waterhouse, P.M., Bretell, R.I.S., Chen, X., Xu, H.J., Xin, Z.Y., Qian, Y.T., Zhou, X.M., Cheng, Z.M., and Zhou, G.H. 1995. The use of cell culture for subchromosomal introgressions of barley yellow dwarf virus resistance from *Thinopyrum intermedium* to wheat. *Genome* 38:395-405.

Barloy, D., Etienne, C., Lemoine, J., Saint Ouen, Y., Jahier, J., Banks, P.M., and Trottet, M. 2002. Comparison of TAF46 and Zhong5 resistances to barley yellow dwarf virus from *Thinopyrum intermedium* in wheat. *Euphytica* (accepted).

Bruehl, G.W. 1961. *Barley Yellow Dwarf, A Virus Disease of Cereals and Grasses.* Monograph Number 1:1-52.

Comeau, A., Makkouk, F., Ahamad, F., and St Pierre, C.A. 1994. Bread wheat x *Agrotricum* crosses as a source of immunity and resistance to the PAV strain of barley yellow dwarf virus. *Agronomie* 2:153-160.

D'Arcy, C.J., and Burnett, P.A. (eds.). 1995. *Barley Yellow Dwarf: 40 Years of Progress.* APS Press, St Paul, MN.

Larkin, P.J., Banks, P.M., Lagudah, E.S., Appels, R., Chen Xiao, Xin Zhiyong, Ohm, H.W., and McIntosh, R.A. 1995. Disomic *Thinopyrum intermedium* addition lines in wheat with barley yellow dwarf virus resistance and with rust resistances. *Genome* 38:385-394.

Lister, R.M., and Ranieri, R. 1995. Distribution and economic importance of barley yellow dwarf virus. In: D'Arcy and Burnett (eds.). *Barley Yellow Dwarf: 40 Years of Progress.* APS Press, St Paul, MN. pp29-53.

Rochow, W.F. 1970. Barley yellow dwarf virus: phenotypic mixing and vector specificity. *Science* 167:875-878.

Sadeghi, S.E., Dedryver, C.A., Riault, G., and Tanguy, S. 2000. Variation in virus content among individual leaves and roots of barley and wheat infected with a BYDV-PAV isolate. *J Agr. Sci. Tech.* 2:151-160.

Sharma, H.C., Ohm, H.W., and Perry, K.L. 1997. Registration of barley yellow dwarf virus resistant wheat germplasm line P29. *Crop Sci.* 37:1032-1033.

Signoret, P.A., and Maroquin, C. 1990. The barley yellow dwarf virus situation in Western Europe. In: Burnett, P.A. (ed.). *World perspectives on barley yellow dwarf.* Mexico, D.F.: CIMMYT.

A Single Copy of Virus-Derived, Transgene-Encoding Hairpin RNA Confers BYDV Immunity

D. Abbott, M.-B. Wang, and P. Waterhouse

CSIRO Plant Industry, Canberra, Australia

The PAV serotype of Barley yellow dwarf virus *(BYDV-PAV) is the most damaging virus of cereals worldwide. Natural resistance genes against the virus give inadequate control, and previous attempts to introduce synthetic resistance genes have been disappointing. The transformation of cereals with BYDV coat protein or polymerase genes has produced a few virus-resistant plants, but the resistance has been weak, unstable, or has failed to segregate as expected. In an attempt to generate barley with protection against BYDV, Golden Promise barley was transformed with a transgene that was designed to produce hairpin (hp)RNA from BYDV-PAV sequences. The majority of the resulting lines contained a single copy transgene insert and were rated as immune because the virus could not be detected in the challenged plants by ELISA nor recovered by aphid feeding experiments. In the field, BYDV-PAV is sometimes associated with the related luteovirus* Cereal yellow dwarf virus *(CYDV-RPV). When the transgenic plants were challenged with BYDV-PAV and CYDV together, the plants were susceptible to CYDV but immune to BYDV-PAV. This shows that the immunity is sequence-specific and not broken down by the presence of CYDV. It also suggests that the immunity will be robust in the field and very useful in minimizing losses in barley production due to BYDV-PAV.*

Barley yellow dwarf disease comprises two subgroups of viruses: BYDV (*Barley yellow dwarf virus*) and CYDV (*Cereal yellow dwarf virus*). It is the major viral disease of barley in Australia, reducing yield by an average of 15% per annum (Lister and Ranieri, 1995). Barley yellow dwarf disease can also affect plant height, grain size, and grain quality, such that grain is suitable only for animal feed rather than malting.

Sources of natural resistance to BYDV and CYDV are rare (for reviews see Barker and Waterhouse, 1999, and Burnett *et al*., 1995). In barley, the *Yd2* gene (Paltridge *et al.,* 1998), originally identified in Ethiopian concessions (Schaller *et al.,* 1964), can confer resistance against BYDV-PAV, but its effectiveness varies depending on the genetic background of the plant and growth conditions (Larkin *et al.,* 1991). BYDV still replicates in plants containing the *Yd2* gene.

Recently, we discovered that virus immunity and posttranscriptional gene silencing (PTGS) can be induced in plants using transgenes that encode double stranded (ds) or self-complementary "hairpin" (hp) RNA (Waterhouse *et al.,* 1998; Wang and Waterhouse, 2000; Smith *et al.,* 2000). This protection/silencing appears to operate through sequence-specific RNA degradation similar to that of RNA interference (RNAi) in *Drosophila* (Zamore *et al.,* 2000). Here we describe the transformation of barley plants with a construct that encodes hpRNA containing the polymerase gene sequences from the major BYDV serotype, PAV. Our results indicate that this transgene confers immunity to BYDV-PAV on the plants and, where it occurs as a single locus, it is inherited in a simple Mendelian manner.

Materials and Methods

A gene construct (hpBYDVpol) was made in which hairpin RNA containing BYDV-PAV polymerase gene sequences is transcribed under the control of the maize ubiquitin promoter (Figure 1). Further details for the construction of hpBYDVpol and the transformation of *Hordeum vulgare* L. cv. 'Golden Promise' with this gene are provided by Wang *et al.* (2000). Aphids

(*Rhopalosiphum padi*) were used to infect barley plants with an Australian isolate of BYDV-PAV, an Australian isolate of CYDV-RPV, and an Australian B/CYDV-MIX isolate which contained both BYDV-PAV and CYDV-RPV (Waterhouse *et al.*, 1986). Virion accumulation was measured by enzyme-linked immunosorbent assay (ELISA) (Xin *et al.*, 1988).

Southern analysis (Lagudah *et al.*, 1991) used a radio-labeled probe from a 1.1 kb *hpt* (hygromycin resistance gene) fragment. PCR analysis was performed using oligonucleotide primers amplifying a 626 nt fragment from the spacer loop between sense and antisense sequences of the transgene.

Results and Discussion

Transformation and analysis of T_0 plants

Using this construct and an *Agrobacterium*-mediated transformation system, an overall transformation efficiency of 13% was achieved resulting in 38 independent transgenic barley plants. Southern analysis indicated that 19 plants carried a single transgene copy, 12 contained two copies, and 7 had 3 or more copies (data not shown). When 25 of the T_0 plants were inoculated with BYDV-PAV, 9 of them appeared highly resistant as they supported little or no virus replication when measured by ELISA (data not shown). Two single-copy lines (2 and 4) were selected to investigate the inheritance of the hpBYDVpol transgene and of BYDV-PAV resistance.

Transgene inheritance and virus immunity in hpBYDVpol lines 2 and 4

Fourteen T_1 plants of lines 2 and 4 were challenged with BYDV-PAV and monitored for virus symptoms and virion accumulation 21, 28, and 42 days after inoculation. The progeny of lines 2 and 4 conformed to a segregation ratio of 3 : 1 (highly resistant : susceptible), suggesting the presence of a single dominant transgene locus in each line. Southern analysis (Figure 2) revealed that each locus appears to contain a single transgene. The inheritance of the hpBYDVpol transgene in these plants was examined by PCR amplification of a 626 bp fragment of DNA from the transgene. In both lines (Figure 3a and b), the

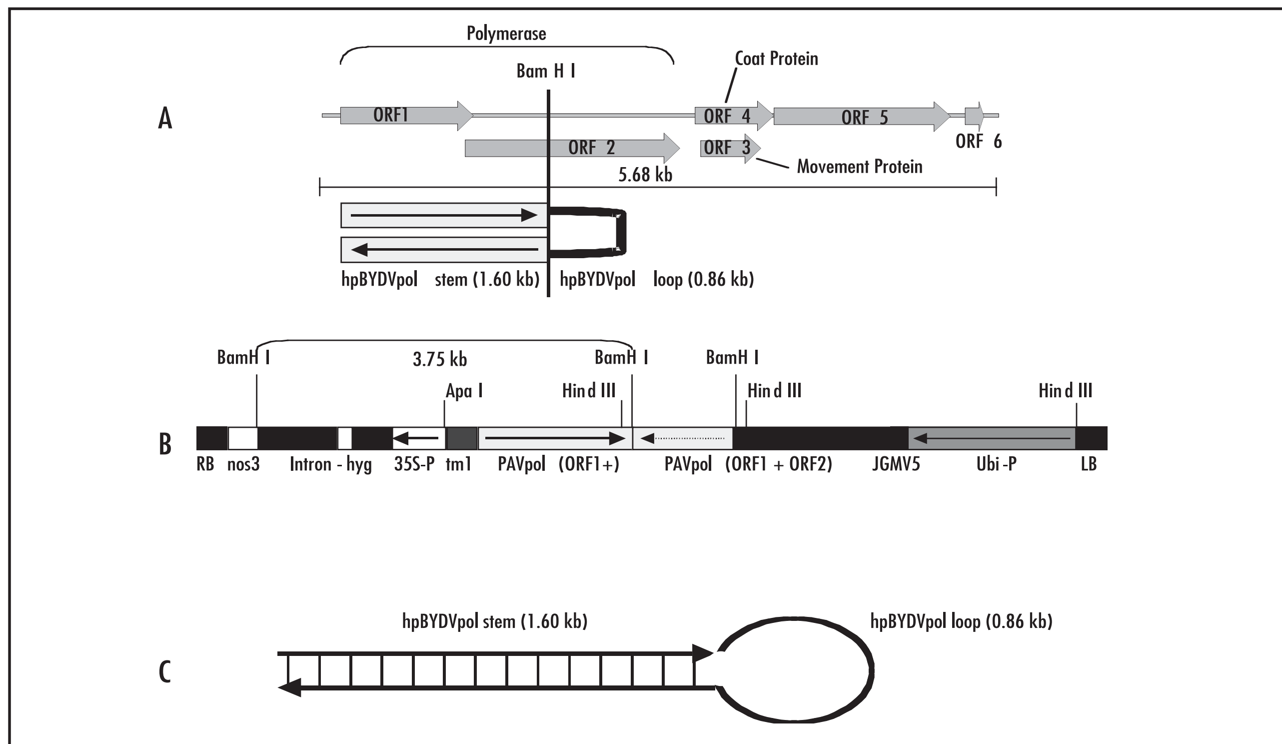


Figure 1. (A) Genome map of BYDV-PAV showing regions used to generate hpBYDVpol. (B) Design of hpBYDVpol construct. (C) Diagram of self-complementary (hairpin) RNA produced by hpBYDVpol.
Note: RB: right border; nos 3′: nopaline synthase 3′ region; 35S-P: cauliflower mosaic virus 35S promoter; tm1′: tumor morphology large gene 3′ region; PAVpol: BYDV-PAV polymerase gene sequence; JGMV5′: Johnson grass mosaic virus 5′untranslated region; Ubi-P: maize polyubiquitin gene promoter; LB: left border.

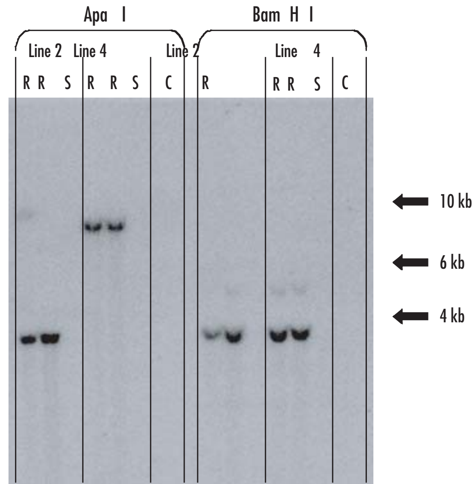


Figure 2. Southern blot analysis of T1 progeny of hpBYDVpol lines 2 and 4.
Note: DNA from two resistant (R) and one susceptible (S) T1 plants for lines 2 and 4, and for non-transgenic barley (C), was digested with Apa I or BamH I, separated by electrophoresis, blotted to Hybond N+' membrane and hybridized with a radioactively-labelled hpt probe. The number of bands in the Apa I lanes and the intensity of the 3.75 kb bands for the BamH I lanes should indicate copy number.

inheritance of hpBYDVpol correlated perfectly with lack of virus symptoms and resistance to BYDV infection. Plants that were symptomless and contained undetectable virus levels 21days after inoculation remained this way throughout the six weeks of analysis (Figure 3a), whereas those plants without the hpBYDVpol transgene showed virus symptoms and virion accumulation at all three time-points.

The co-segregation of virus accumulation with the absence of the transgene was also evident in the grain yield from the individual progeny plants. In line 2, the average grain yield from the nine hpBYDVpol-containing T1 progeny was 29.7±1.8 g, compared to a yield of 20.9±1.5 g from the six transgene-free progeny. Twenty-eight days after inoculation, the ELISA level in plant 12 of line 4 (Figure 3b) suggested that some virus accumulation had occurred. However, the plant had undetectable levels of BYDV-PAV both one week before and two weeks after this time-point, and never

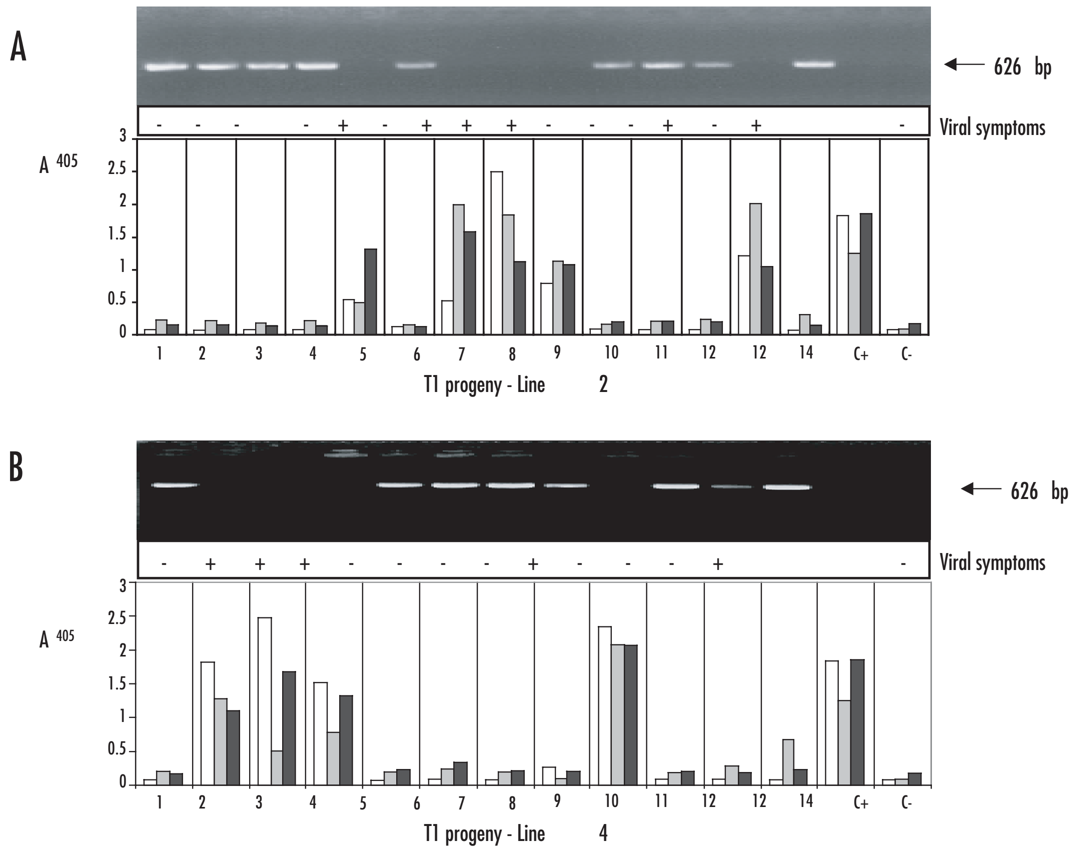


Figure 3. Relationship between virion accumulation and inheritance of the hpBYDVpol transgene.
Notes: Virus levels detected by ELISA 21 (white bar), 28 (grey bar) and 42 (black bar) days after inoculation in (a) 14 T1 progeny of Line 2 and (b) 12 T1 progeny of Line 4. Displayed above each histogram is a symbol representing the severity of viral symptoms in mature plants and an agarose gel containing PCR products from the corresponding plant samples; the presence of a 626bp product indicates the amplification and detection of the hpBYDVpol transgene.

developed virus symptoms. This suggests that 1) there might have been a low level of virus replication, peaking at 28 days, which was then overcome by the transgene-induced resistance, or 2) there was some contamination of the sample during analysis.

Specificity and robustness of virus resistance

Although BYDV-PAV is the major virus pathogen of cereals in the field, CYDV-RPV can also be present. Indeed the two viruses can occur as a complex and appear to have a synergystic effect on symptom severity. The viruses share common hosts, a common insect vector, and many aspects of their biology. However, they are at opposite ends of the luteovirus spectrum in terms of sequence homology and genome organization. To test whether the hpBYDVpol transgene also conferred protection against CYDV-RPV, approximately 15 T_1 progeny from each of hpBYDVpol lines 2 and 4 were challenged with BYDV-PAV and another 15 of each line with CYDV-RPV. Measuring the virus accumulation in these plants by ELISA revealed that, as before, both lines segregated 3:1 for BYDV-PAV resistance. However, all of the plants inoculated with CYDV-RPV were fully susceptible to the virus. These results indicate that the resistance conferred by the hpBYDVpol transgene is specific to BYDV-PAV. This might have been expected, as there is only 34% homology between the nucleotide sequence in hpBYDVpol and the corresponding region in CYDV-RPV.

It has recently been shown that some viruses have the capacity to inactivate PTGS (Voinnet *et al.* 1999). Therefore, it was important to determine whether infection by CYDV-RPV could inactivate the hpBYDVpol-mediated BYDV-PAV resistance. To test this, 15 T_1 progeny from hpBYDVpol line 2 were inoculated with the B/CYDV-MIX isolate, a virus complex containing BYDV-PAV and CYDV-RPV. The plants were subsequently tested using species-specific ELISA for accumulation of BYDV-PAV and CYDV-RPV and by PCR for inheritance of the transgene. The results showed that CYDV-RPV replicated to high levels in all 15 plants but that the 12 plants inheriting the hpBYDVpol transgene were resistant to BYDV-PAV (data not shown). This indicates that the resistance to PAV is not compromised by replication of CYDV-RPV and further confirms the 3:1 (BYDV-PAV resistance: susceptible) segregation ratio.

Recovery of virus from virus-challenged hpBYDVpol plants

Although BYDV-PAV-challenged hpBYDVpol-plants contain extremely low levels of BYDV-PAV antigen, they might contain sufficient virus to be acquired by aphids and thus be of ecological significance. To examine this, we attempted to recover infectious virus from T1 progeny plants from lines 2 and 4 that had been previously challenged with either BYDV-PAV or B/CYDV-MIX. Virus-free aphids were fed (for three days) on the plants 10 weeks after the initial challenge and then transferred to healthy test plants. Whereas the test plants became infected with BYDV-PAV from aphids fed on BYDV-PAV or B/CYDV-MIX challenged wildtype or non-transgene segregant plants, none of them was infected with BYDV-PAV from aphids fed on similarly challenged plants containing the hpBYDV-PAVpol transgene. However, aphids did recover CYDV-RPV from hpBYDVpol plants challenged with the B/CYDV-MIX mixture. Taken altogether, the data show that BYDV-PAV-challenged hpBYDVpol plants contain no biologically active virus and should be regarded as immune to BYDV-PAV (Figure 4).

Conclusion

We have generated barley plants containing transgenes encoding hpRNA derived from BYDV-PAV polymerase sequences. Over one-third of these independently transformed plants have extreme resistance to BYDV-PAV. Furthermore, some of the plants have a single transgene that is inherited, along

Figure 4. Reaction to BYDV of transgenic and non-transgenic barley.
Note: Two T1 hpBYDVpol immune plants of line 2 (left) and two nontransformed plants (right).

with virus immunity, in a simple Mendelian manner. This is a significant advance over previous attempts to produce transgenic cereals with protection against BYDV. While some attempts (McGrath *et al.,* 1997; Koev *et al.,* 1998; Wang *et al.,* 2001) have produced oat or barley plants with resistance (reduced virus replication) or tolerance (reduced virus symptoms but unimpeded virus replication) to BYDV, the inheritance of the resistance/tolerance has been variable. This has been further complicated by the complex transgene insertion patterns in such plants, especially those obtained using biolistic transformation.

Our BYDV-immune barley plants may have great potential for deployment in the field. However, a feature that must be considered before contemplating their widespread use is their interaction with other viruses. Some viruses have the capacity to enhance the replication and/or spread of co-infecting viruses and to inactivate PTGS (for example Vance 1991; Voinnet *et al*, 1999). Therefore, it was possible that infection by CYDV-RPV, which can co-infect with BYDV-PAV in the field, could enhance the replication and spread of BYDV-PAV or inactivate the hpBYDVpol-mediated BYDV-PAV immunity, thus disarming the plant's newly conferred protection. However, in our experiments, the protection against BYDV-PAV was not compromised by co-inoculation of the plants with CYDV-RPV and BYDV-PAV.

While it is reassuring that our plants should maintain their protection against BYDV-PAV in the field, it is our future goal to provide cereals with simultaneous protection against both BYDV and CYDV using hpRNA technology. Consequently, we have made several new hairpin constructs that we are presently using to transform barley. These constructs possess a number of features to increase their effectiveness and environmental safety. For example, they incorporate small segments of sequence from both BYDV-PAV and CYDV-RPV, selected on the basis of homology to provide protection across a range of isolates. In the unlikely event that one of these segments recombines with another invading virus, this would provide no advantage because the segments do not encode a whole protein, or even a protein domain. In fact, not only should our new genes for transformation be highly effective against both BYDV and CYDV, they will not encode any additional protein in the plant. The new constructs also incorporate a recently developed technique to remove the selectable marker gene (usually either antibiotic or herbicide resistance) from transformed plants.

Acknowledgments

We thank Jake Jacobsen for his support and the Grains Research and Development Corporation (GRDC) for its financial assistance.

References

Barker, H. and Waterhouse, P.M. 1999. In: *The Luteoviridae.* Smith, H.G. and Barker, H. (eds.). CABI publishing, UK. pp. 169-210.

Burnett, P.A., Comeau, A., and Qualset, C.O. 1995. In: *Barley Yellow Dwarf: 40 Years of Progress.* D'Arcy, C.J. and Burnett, P.A. (eds.). APS Press, MN. pp. 321-343.

Koev, G, Mohan, B.R., Dinesh-Kumar, S.P., Torbert, K.A., Somers, D.A., and Miller, W.A. 1998. *Phytopathology* 88:1013-1019.

Lagudah, E.S., Appels, R., Brown, A.H.D., and McNeil, D. 1991. *Genome* 34:375-386.

Larkin, P.J., Young, M.J., Gerlach, W.L., and Waterhouse, P.M. 1991. *Ann. Appl. Biol.* 118:115-125.

Lister, R.M., and Ranieri, R. 1995. In: *Barley Yellow Dwarf: 40 Years of Progress.* D'Arcy, C.J., and Burnett, P.A. (eds.). APS Press, MN. pp. 29-53.

McGrath, P.F., Vincent, J.R., Lei, C.H., Pawlowski, W.P., Torbert, K.A., Gu, W., Kaeppler, H.F., Wan, Y., Lemaux, P.G., Rines, H.R., Somers, D.A., Larkins, B.A., and Lister, R.M. 1997. *Eur. J. Plant Pathol.* 103:695-710.

Paltridge, N.G., Collins, N.C., Bendahmane, A., and Symons, R.H. 1998. *Theor. Appl. Genet.* 96:1170-1177.

Schaller, C.W., Qualset, C.O., and Rutger, J.N. 1964. *Crop Sci.* 4:544-548.

Smith, N.A., Singh, S.P., Wang, M-B., Stoutjesdijk, P., Green, A., and Waterhouse, P.M. 2000. *Nature* 407:319-320.

Vance, V.B. 1991. *Virology* 182:486-494.

Voinnet, O., Pinto, Y.M., and Baulcombe, D.C. 1999. *Proc. Natl. Acad. Sci. USA* 96:14147-14152.

Wang, M-B., Abbott, D.C., and Waterhouse, P.M. 2000. *Mol. Plant Pathology* 1:347-356.

Wang, M-B., and Waterhouse, P.M. 2000. *Plant Molecular Biology* 43:67-82.

Wang, M-B., Abbott, D.C., Upadhyaya, N.M., Jacobsen, J.V., and Waterhouse, P.M. 2001. *Aust. J. Plant Physiol.* 28:149-156.

Waterhouse, P.M., Gerlach, W.L., and Miller, W.A. 1986. *J. Gen. Virology* 67:1273-1281.

Waterhouse, P.M., Graham, M.W., and Wang, M.-B. 1998. *Proc. Natl. Acad. Sci. USA* 95:13959-13964.

Xin, Z.Y., Brettell, R.I.S., Cheng, Z.M., Waterhouse, P.M., Appels, R., Banks, P.M., Zhou, G.H., Chen, X., and Larkin, P.J. 1988. *Genome* 30:250–257.

Zamore, P.D., Tuschl, T., Sharp, P., and Bartel, D.P. 2000. *Cell* 101:25-33.

Genetically Engineered Wheat for *Barley Yellow Dwarf Virus* Resistance

P. Dupré,[1-2] M. Henry,[1] G. Posadas,[1] A. Pellegrineschi,[1] M. Trottet,[2] and E. Jacquot[2]

[1] CIMMYT, Mexico, D.F., Mexico
[2] INRA, Le Rheu, France

Barley yellow dwarf virus-PAV (BYDV-PAV) causes substantial losses (Lister and Ranieri, 1995) throughout the world in wheat (17%), barley (15%), and oats (25%). Similar to conventional breeding methods, genetic engineering is a potentially important tool to develop virus resistance. In this case, pathogen-derived resistance is the strategy commonly used (Lomonossoff, 1995). This strategy involves using as a gene, a part of the virus genome in order to disrupt infection by the invading virus, according to the process observed in cross-protection.

BYDV resistance has been reported in oats and barley transformed with the polymerase (Koev *et al*., 1998) and coat protein (McGrath *et al*., 1997) sequences and recently, immunity of barley plants to BYDV-PAV has been obtained by using a transgene that encodes a self complementary "hairpin" RNA containing the polymerase-gene sequence of BYDV-PAV (Wang *et al*., 2000). In wheat, transgenic resistant lines obtained by the use of the CP gene were reported once (McCarthy *et al*., 1996).

In this study we evaluated the pathogen-derived resistance strategy in the case of the couple BYDV/wheat by using 7 constructs harboring the BYDV-PAV ORF encoding for replicase, coat-protein, movement protein and a non-coding sequence corresponding to the promoter of sub-genomic 2 RNA.

Materials and Methods

BYDV constructs

The ORFs encoding for the replicase (Pol), coat protein (CP), movement protein (MP), or a non-coding sequence (NCS) corresponding to the promoter of sub-genomic RNA_2 were amplified from the cDNA clone of a French BYDV-PAV isolate (PAV 13). Fragments were cloned in the unique *Bam*HI site of the pAHC17 plasmid (Christensen and Quail, 1996), in sense and antisense orientation, between the UBI promoter and *Nos* terminator.

Plant transformation

Three bread wheats (Bobwhite SH-98-26, Kambara, Pastor) were transformed by microprojectile bombardment according to CIMMYT protocol (Pellegrineschi *et al*., 2001). The pAHC25 plasmid harboring the *bar* gene encoding for PPT resistance was used for selection.

Molecular analyses

Specific BYDV primers were used in PCR to identify T0 and T1 generations containing the BYDV genes. Southern blot analyses were also performed on T0 and T1 transgenic plants (10 to 20 mg DNA) according to CIMMYT protocol. The restriction enzymes used were *Hind*III (cut once in the plasmid) and *Bst*EII (not cut in the plasmid).

Testing for BYDV resistance

T1 progeny seeds were sowed in a completely randomized experimental design. Fifteen-day old seedlings were inoculated with the Mexican BYDV-PAV isolate (8 aphids/plant). Twelve and 26 days after inoculation, tips of all the leaves of the plant were harvested and virus titer was measured by DAS-ELISA. Optical densities (OD) were compared to OD obtained with a standard dilution of a BYDV-infected oat extract. Virus titers are expressed as dilution equivalents of this standard.

Results and Discussion

Transformation efficiencies (T0 generation)

Transformation efficiencies (by PCR amplification of the BYDV fragment) are variables, depending on the construct used. High efficiency was obtained with Bobwhite SH-98-26 (up to 12%), the variety commonly transformed at CIMMYT because of its high regeneration efficiency, and with Pastor (up to 15%), a high-yielding wheat, which is not normally used in transformation (Table 1).

Southern blots performed with 2 restriction enzymes (*Hind*III and *Bst*EII) on T0 generation show complex integration patterns (data not shown), with a copy number varying from 4 to15 and number of insertion sites varying from 1 to 14.

Transgene inheritance and response to BYDV-PAV inoculation in the T1 generation

Inheritance of the BYDV transgene was examined (Table 2) on 4 T1 progenies (1 with the CP in sense, 1 with the CP in anti-sense, 2 with the NCS in sense) by PCR amplification of BYDV fragment. Only one progeny (with the CP in sense orientation) show a Mendelian T1 segregation, and we can assume that in this case the 2 transgene insertions are very closed.

Virus levels in the transgenics (Table 2) are represented as variables ranging from 0.15 to 1.66 suggesting either a modification of the transgene integration pattern in the segregating progenies or a somaclonal variation.

Molecular analyses (Southern-blot and RT-PCR semi-quantitative) are in process on these plants in view to determine the respective influences of the integration pattern and the gene expression level in the virus titers obtained.

References

Christensen, A.H., and Quail, P.H. 1996. Ubiquitin promoter-based vectors for high-level expression of selectable and/or screenable marker genes in monocotyledonous plants. *Transgenic research* 5:213-218.

Koev, G., Mohan, B.R., Dinesh-Kumar, S.P., Torbert, K.A., Somers, D.A., and Miller, W.A. 1998. Extreme reduction of disease in oats transformed with the 5' half of the barley yellow dwarf virus-PAV genome. *Phytopathology* 88:1013-1019.

Lister, R.M., and Ranieri, R. 1995. Distribution and economic importance of barley yellow dwarf virus, In: *Barley Yellow Dwarf: 40 Years of Progress.* D'Arcy, C.J. and Burnett, P.A. (eds.).

Lomonossoff, G.P. 1995. Pathogen-derived resistance to plant viruses. *Annu. Rev. Phytopathol.* 33:323-343.

McCarthy, P.L., Hansen, J., Shiel, P.J., Zemetra, R.S., Wyatt, S.D., and Berger, P.H. 1996. Cp-mediated resistance to wheat streak mosaic and barley yellow dwarf viruses in soft white winter wheats. Poster abstract at International. Congress of virology, Jerusalem 10th. pp. 184.

McGrath, P.F., Vincent, J.R., Lei, C.H., Pawlowski, W.P., Torbert, K.A., Gu, W., Kaeppler, H.F., Wa, Y., Lemaux, P.G., Rines, H.R., Somers, D.A., Larkins, B.A., and Lister, R.M. 1997. Coat protein-mediated resistance to isolates of barley yellow dwarf in oats and barley. *European Journal of Plant Pathology* 103:695-710.

Pellegrineschi, A., McLean, S., Salgado, M., Velazquez, L., Brito, R.M., Noguera, L.M., Medhurst, A., and Hoisington, D. 2001. Transgenic wheat plants: A powerful breeding source. *Euphytica* 119:133-136.

Wang, M., Abbott, D.C., and Waterhouse, P.M. 2000. A single copy of a virus-derived transgene encoding hairpin RNA gives immunity to barley yellow dwarf virus. *Molecular Plant Pathology* 1:347-356.

Table 1. Number of BYDV transgenic wheat plants obtained after bombardment of 200-300 embryos per construct and variety.

	Coat protein		Movement protein		Non-coding		Replicase
	(sense)	(anti-sense)	(sense)	(anti-sense)	sequence (sense)	sequence (anti-sense)	(sense)
Bobwhite SH-9826	1[a] / 12[b] 0/3	20 /63	2/12	2/10	5/62	14/49	1/28 5/49
Kambara	1/5	6/72	1/6	3/29	5/16	2/30	NT
Pastor	0/9	NT	NT	NT	10/33	18/65	NT

[a] Number of transgenics holding the BYDV gene of interest.
[b] Number of transgenics obtained (putatively harboring the *bar* gene for herbicide resistance).
NT: Not transformed.

Table 2. Testing T1 transgenic plants for BYDV resistance in ELISA.

Lines tested	Number of insertion sites in T0 generation	T1 segregation (PCR)	Number of T1 plants with an ELISA titer		
			<control[a]	=control	>control
102-1(Coat protein sense)	2	45/60	14	17	5
33-2 (Coat protein anti-sense)	4	36/59	14	22	9
401-1 (non coding sequence sense)	ND	19/29	9	5	5
405-1 (non coding sequence sense)	ND	7/17	3	3	1

Control = Bobwhite SH-98-26 not transformed; Titer: 0.7+/- 0.1.
ND: not determined.

Nucleotide Sequence Analysis of the BYDV-GPV Isolate Genome, and Transgenic Wheat Obtained via Pollen Tube Pathway

Z. Cheng,[1] M. Wu,[1] X. He,[1] L. Xu,[1] G. Zhou,[1] and P.M. Waterhouse[2]

[1] Institute of Plant Protection, CAAS, Beijing, China
[2] Division of Plant Industry, CSIRO, Canberra, Australia

Barley yellow dwarf virus (BYDV), the type member of the luteovirus group, is considered to be of economic importance in most cereal-producing countries. BYDV is probably the most economically important virus of cereals. So far, no natural forms of resistance have been described in common wheat. At least five isolates (PAV, MAV, SGV, RPV, and RMV) of BYDV have been described previously. GPV is a Chinese isolate of BYDV that has no reaction with antiserum of common isolates and is transmitted efficiently by both *Schizaphis graminum* and *Rhopalosiphum padi.* Its genome is a single-stranded RNA with a molecular size of around 6 Kb.

Genetic engineering of plants for virus resistance has provided a potentially powerful strategy for control of virus disease. Since the first transgenic tobacco plants with increased resistance to *Tobacco mosaic virus* (TMV) resulted from the expression of the viral coat protein (CP) gene (Powell-Abel *et al*., 1986), it has been found that the equivalent sequences from tobraviruses, luteoviruses, cucumoviruses, potyviruses, and tenuiviruses could also confer resistance.

Various methods of transformation have been developed, allowing many of the world's important crop plants to be transformed. Among the various methods used for the transformation of cereals, an increasing number of fertile transgenic rice, maize, barley, and wheat plants have been obtained by the direct delivery of DNA into protoplasts by electroporation or PEG-mediated gene transfer, or into intact cells or immature embryos by high velocity microprojectile bombardment and *Agrobacterium tumefaciens*-mediated transformation.

We report here transformation methods mediated by the pollen tube pathway to transfer GPV coat protein gene (ORF3) and replicase gene (ORF1 and ORF2) to common wheat. Transgenic wheat plants resistant to BYDV were obtained by successfully introducing the GPV coat protein gene and replicase gene into wheat plants.

Materials and Methods

The BYDV GPV isolate was maintained in the greenhouse in oats (Hua Bei No.2) and infected oats tissue was stored at –70°C. Common wheat variety Beijing 837, which is susceptible to BYDV, was used for generation of transgenic plants.

A cDNA library representing the GPV genome was constructed in pUC8 and pUC18. A restriction map representing the viral genome was generated by single and double enzyme restriction digests of the cloned cDNA and by Southern hybridization between different restriction fragments.

Plasmid DNA isolated by an alkaline lyses technique was sequenced by the dideoxy-chain termination method with a modified T7 DNA polymerase or Taq DNA polymerase. RT-PCR was used for gap sequencing.

Based on the BYDV-GPV ORF3, ORF1, and ORF2 sequences, several primers were designed (Cheng *et al*., 1996). The cDNAs of CP or replicase gene were produced by RT-PCR. The full length cDNAs were inserted into expression plasmids pJ3x35SN or pEmu-mcs-n. The recombinant plasmids were checked by using an a-^{32}P-dATP labeled CP probe or

replicase probe and a r-^{32}P-ATP labeled GPV RNA probe, and were sequenced to confirm the presence of the unmodified GPV CP or replicase gene cDNA in the plasmids.

Pollen tube pathway transformation was used in the research. The nptII was used as selectable markers to screen transgenic seedlings. Molecular analysis such as PCR, Southern blots, and Western blots were used to detect foreign genes in transgenic wheat plants.

For resistance assay, the T1 transgenic wheat seedlings derived from all T0 lines as well as untransformed wheat seedlings were inoculated with aphids, which had been given a 2-day acquisition feeding on sap of GPV-infected plants at 15˚C through stretched Parafilm membranes. Each seedling (at the 3-leaf stage) was inoculated with more than 10 aphids for an inoculation access period of 5 days in the greenhouse. The aphids were then killed with insecticide and the seedlings were transferred into an aphid-proof room at 18˚C under artificial light (12 hours per day, 20000 lux).

In the field test, 100 split-plots consisting of three rows of transgenic plants, one row of untransformed controls and one blank row. In each row, 20 seeds were sown in the winter. All plants were inoculated at the tillering stage with aphids given a 5-day acquisition feeding period on leaf-pieces infected with GPV (more than 20 aphids/seedling). For the T2, T3, T4 generations, plant used for resistance assay was selected from resistant T1, T2 and T3 generation transgenic wheat respectively.

Results

The sequence of the GPV isolate of BYDV was identified and its amino acid sequence was deduced (Cheng *et al*., 1996). Genome organization has six (+ sense) open reading frames (ORFs), similar to those of CYDV-RPV (Vincent *et al*., 1991; Miller *et al*., 1988, 1995). In the RNA-dependent RNA polymerase coding region, the length of ORF1 is 1953nt and encodes a 71.4KD protein and ORF2 is 1872nt and encodes a 70.1KD protein. There is a 601nt overlap between ORF1 and ORF2. The coat protein of GPV (molecular weight 22.2 KD) is encoded by ORF3, with a length of 603 nt. As MAV, PAV and RPV, GPV contained a second ORF within the coat protein coding region. This protein of 17.0KD is thought to correspond to the VPg. The length of ORF4 is 453nt long. ORF5, 1326nt of length, is immediately after the coat protein termination codon, and in the same reading frame as the coat protein gene. This protein is associated within the intact virus as a 72.2KD protein.

The nucleotide and amino acid sequence homology of GPV has a greater identity to the sequence of RPV than those of PAV and MAV (Ueng *et al*., 1992). The GPV ORF1 sequence shared 69% of nucleotide similarity and 57% of deduced amino acid similarity with RPV-ORF1 whereas ORF2 shared 81% and 81%, ORF3 84% and 77%, ORF4 87% and 86%, ORF5 69% and 69% respectively.

The cDNAs of the coat protein or replicase genes of BYDV GPV were obtained by RT-PCR and were inserted into different plasmids to construct expression plasmids named pPPI1 to pPPI20 based on different promoter. The recombinant plasmids were identified by molecular analysis to confirm the presence of the GPV coat protein gene or replicase gene cDNAs in plasmids.

An efficient pollen tube pathway-mediated transformation protocol was developed for the generation of transgenic wheat plants that express coat protein (CP) of BYDV GPV. Molecular analysis of the CP gene in transgenic plants confirmed the stable integration of the CP gene into the wheat genome and inheritance of the gene to T_1, T_2, T_3 and T_4 generations. The CP gene segregated as a dominant Mendelian trait in T1 selfed plants. Upon inoculation with GPV in greenhouse tests, transgenic plants that expressed the CP gene exhibited a significant delay in symptom development and reduced virus accumulation (as measured by ELISA) compared with control plants. In the field, our results showed an increased in level of resistance to virus infection in T2, T3 and T4 plants indicating that the resistance trait was stably transmitted to transgenic T1, T2, T3 and T4 plants.

Resistance evaluation of the T1 generation of transgenic wheat plants carrying GPV replicase gene, were carried out in both field and greenhouse conditions. Compared with non-transgenic plants, two transgenic lines of Shan 160 showed high level of resistance with no or very weak symptoms on the leaf apex after virus infection. The transgenic line of Longjian 127 also showed delayed symptom development; when the leaves of the non transgenic seedlings were all yellow, the flag leaves of the transgenic line were still green, or with a yellowing area less than 1/3. Two transgenic lines of Jianmai 47 transformed with pPPI8 showed delay in disease development with mild symptoms.

Discussion

We have developed an improved protocol for the rapid and efficient production of transgenic wheat with resistance to BYDV by using no selectable markers and the pollen tube pathway to transfer the BYDV replicase gene into the common wheat varieties, then sowing transgenic seeds into the field for resistance screening and molecular assay. Transgenic wheat plants resistant to BYDV GPV were obtained.

Two transformation methods were tested to transfer the CP gene to wheat. PCR was used to examine the integration of the foreign DNA in the transformed plants. The first successful PCR amplification of the CP bands came by using the pollen tube pathway transformation procedure. The frequency of transformation with the CP gene resulting from application of pPPI1 (35S Promoter) and pPPI5 (Emu Promoter) was about 1% and 5% respectively. Resistance experiments showed that expression of the CP gene in transgenic wheat plants significantly reduced infection and delayed symptom development by 1 to 3 weeks compared to the control plants. Four situations were observed: 1) some lines were resistant to BYDV GPV at all growth stages, but some leaves still showed weak symptoms; 2) some lines expressed resistance to BYDV in an earlier stage (before heading) but were very susceptible in the heading period when the flag leaf and lower leaves showed symptoms; 3) some lines were susceptible at young stages but resistance appeared at heading; and 4) seedlings were very susceptible at all stages of growth, and the plants were stunted. It is possible that the position of insertion of the CP gene into the chromosomes and possibly the number of copies of the CP gene affected the expression of resistance. Microprojectile bombardment was also successfully used to transform another variety of wheat, but all transgenic wheat plants were susceptible.

References

Cheng, Z., He, X., Wu, M., and Zhou, G. 1996. Nucleotide sequence of coat protein gene for GPV isolate of barley yellow dwarf virus and construction of expression plasmid for plant. *Science in China* (Series C) 39(3):534-543.

Miller, W.A., Dinesh-Kumar, S.P., and Paul, C.P. 1995. Luteovirus gene expression. *Critical Reviews in Plant Sciences* 14(3):179-211.

Miller, W.A., Waterhouse, P.M., and Gerlach, W.L. 1988. Sequence and organization of barley yellow dwarf virus genomic RNA. *Nuclec Acids Research* 16:6096-6111.

Powell-Abel, P.A., Nelson, R.S., De, B., Hoffman, N., and Rogers, S.G. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein. *Science* 232:738-743.

Ueng, P.P., Vincent, J.R., and Kawata, E.E. 1992. Nucleotide sequence analysis of the genomes of the MAV-PS1 and P-PAV isolates of barley yellow dwarf virus. *Journal of General Virology* 73:487.

Vincent, J.R., Lister, R.M., and Larkins, B.A. 1991. Nucleotide sequence analysis and genomic organization of the NY-RPV isolate of barley yellow dwarf virus. *Journal of General Virology* 72:2347-2355.

Real-Time RT-PCR Quantification of Yellow Dwarf Virus Accumulation and Defense Gene Expression

B. Balaji,[1] D.B. Bucholtz,[2] H.W. Ohm,[1] and J.M. Anderson[2]

[1] Agronomy Department, Purdue University, Lilly Hall of Sciences, West Lafayette, USA
[2] USDA-ARS, Purdue University, Lilly Hall of Sciences, West Lafayette, USA

Yellow dwarf virus (YDV)* is one of the principal diseases of wheat causing considerable losses in world food grain production (Lister and Ranieri, 1995). This problem is exacerbated by the fact that wheat does not contain very effective YDV resistance or tolerance genes. High levels of YDV resistance have been introgressed into wheat by the introduction of a wheatgrass (*Thinopyrum intermedium*) chromosome into wheat (Sharma *et al.*, 1995). A YDV resistant wheat substitution line, P29, was previously developed as a result of this effort in which the 7D chromosome has been substituted with a 7E wheatgrass chromosome from wheat grass (Sharma *et al.*, 1997). When inoculated with the *Barley yellow dwarf virus* (BYDV), P-PAV, the titer of the virus in leaf and stem tissue of P29 was significantly reduced (42 to 52%) and the plant showed complete resistance to the *Cereal yellow dwarf virus* (CYDV) NY-RPV (Anderson *et al.*, 1998).

YDV infection is typically quantified by preparing a sap extract from the harvested tissues and using either a double Antibody Sandwich (DAS) or triple Antibody Sandwich (TAS) enzyme linked immunosorbent assay technique (Rochow, 1979; Rochow and Carmichael, 1979). Reverse Transcription-Polymerase Chain Reaction (RT-PCR), however, is more sensitive than ELISA and can detect even a low viral titer in infected plants. This increased sensitivity was demonstrated by the improved detection of YDV in individual aphids by RT-PCR (Canning *et al.,* 1996). Recently, real-time RT-PCR was introduced to study the quantification of gene products precisely and reproducibly and has the potential for analysis of gene expression studies (Heid *et al.*, 1996). An increase in fluorescence of a DNA binding dye is associated with an increasing amount of PCR product in the reaction, which is directly correlated with the amount of cDNA template (Bustin, 2000). By using SYBR Green I as a double stranded DNA binding dye and BYDV and CYDV specific PCR primers we followed the progression of yellow dwarf virus accumulation in a YDV resistant wheatgrass (*Th. intermedium*), YDV susceptible oat (Clintland 64), YDV resistant wheat (P29), and a susceptible wheat line, 8138.

Total RNA from each harvest and random hexamers were used to generate first strand cDNA, which was used as the template for quantitative Real-time PCR. Primers for PCR were designed from the coat protein gene of P-PAV and NY-RPV and also from 18S rRNA. Relative quantification was used to measure the viral titer during the progression of YDV disease in all the plants. When data from real time PCR reactions were analyzed, the kinetics of PAV accumulation was significantly different from that of RPV in susceptible oat, Clintland 64, after a 2-hour infestation period with viruliferous aphids containing both PAV and RPV. These data showed that PAV replicated faster and to a higher level than RPV in susceptible oat. There was a 15-fold increase in PAV titer by 2 hours post infestation (hpi) and the level of PAV increased significantly until reaching a plateau at 8 days post infestation (dpi) and then decreased till 12 dpi, the final sampling date. However, RPV increased more slowly through 24 hpi and then increased significantly for 12 dpi. In the resistant wheatgrass (*Th. intermedium*) PAV and RPV titers were significantly lower or absent when compared to susceptible oat. Our results with wheat using the susceptible line 8138 (Figure 1) and

* YDV stands for either BYDV or CYDV.

resistant line P29 validated our findings with oat and wheatgrass. There was an appreciable increase in PAV titer in 8138 at 2 days post infestation and the maximum amount of PAV accumulation was approximately 100 fold higher then RPV. The progression of PAV accumulation in P29 was similar to that observed in 8138 but as expected, the level of PAV in P29 was ten-fold less. Furthermore, there was a little or no RPV replication in P29 compared to 8138.

Using this information about the progression of virus replication, our second objective was to understand the molecular mechanism underlying the wheatgrass-derived YDV resistance in P29. Although progress has been made in the resistance mechanism of other viruses, not much focus has been placed on YDV and their elicitation of host defense genes. Plants have developed a plethora of defensive systems to evade the infecting pathogens including the production of chemicals and proteins. Also, treating wheat plants with certain chemicals, like the salicylic homologue BTH (Benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester) induces systemic acquired resistance (SAR) against powdery mildew infections (Gorlach *et al.*, 1996). This induced resistance was correlated with the induction of a number of wheat chemically induced (WCI) genes (Gorlach *et al.*, 1996). To determine if there was a correlation between YDV resistance and WCI gene expression we profiled the expression of these genes in P29 and its parent 8138 during the course of disease development following YDV inoculation.

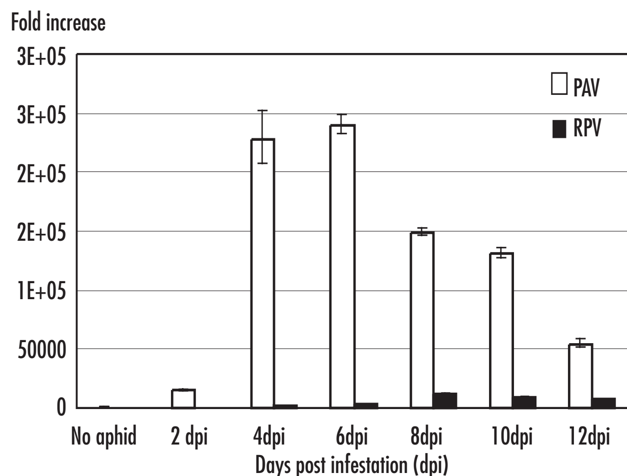


Figure 1. Progression of P-PAV and NY-RPV during disease development in a susceptible wheat 8138.

Degenerate primers were designed from wheat chemically induced genes (WCI-1 to WCI-5, Gorlach *et al.*, 1996). Primers from wheat 18S rRNA were used as an endogenous control. The resistant line, P29 and the susceptible line, 8138 were infested with PAV and RPV viruliferous aphids and as a control with nonviruliferous aphids for 24 hours. Plants were collected at regular intervals for 14 days post infestation. The results show that WCI genes were induced in both the resistant, P29 and susceptible, 8138 plants infected with YDV. These data indicate that YDV infection of plants results in marked up-regulation of systemic acquired resistance genes with YDV inoculation and replication. Further characterization of these and other candidate defense response genes is in progress.

References

Anderson, J.M., Bucholtz, D.L., Greene, A.E., Francki, M.G., Gray, S.M., Sharma, H., Ohm, H.W., and Perry, K.L. 1998. Characterization of wheatgrass-derived barley yellow dwarf virus resistance in a wheat alien chromosome substitution line. *Phytopathology* 88:851-855.

Bustin, S.A. 2000. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *J. Mol. Endocrinology* 25:169-193.

Canning, E.S.G., Penrose, M.J., Barker, I., and Coates, D. 1996. Improved detection of barley yellow dwarf virus in single aphids using RT-PCR. *J. Virol. Methods* 56:191-197.

Gorlach, J., Volrath, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K.H., Oostendorp, M., Staub, T., Ward, E., Kessmann, H., and Ryals, J. 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *Plant Cell* 8:629-643.

Heid, C.A., Stevens. J., Livak, K.J., and Williams, P.M. 1996. Real time Quantitative PCR. *Genome Res* 6:986-994.

Lister, R.M., and Ranieri, R. 1995. Distribution and economic importance of barley yellow dwarf. In: *Barley Yellow Dwarf: Forty years of progress.* C.J. D'Arcy and P.A. Burnett (eds.). The American Phytopathological Society, St. Paul, MN. pp. 29-53.

Rochow, W.F. 1979. Field variants of barley yellow dwarf virus: Detection and fluctuation during twenty years. *Phytopathology* 69:655-660.

Rochow, W.F., and Carmichael, L.E. 1979. Specificity among barley yellow dwarf virus in enzyme immunosorbent assays. *Virology* 95:415-420.

Sharma, H.C., Ohm, H., and Perry, K.L. 1997. Registration of barley yellow dwarf virus resistant germplasm line P29. *Crop Sci.* 37:1032-1033.

Sharma, H.C., Ohm, H., Goulart, L., Lister, R., Appels, R., and Benlhabib, O. 1995. Introgression and characterization of barley yellow dwarf virus resistance from *Thinopyrum intermedium* into wheat. *Genome* 38:406-413.

BYDV: The Heat Is On

R. Harrington

Rothamsted Research, Harpenden, Hertfordshire, UK

Seven years have passed since the publication of D'Arcy and Burnett's extremely useful edited volume *Barley Yellow Dwarf: 40 years of Progress* (D'Arcy and Burnett, 1995). Thus, we are approaching half a century of progress. While an enormous amount of science of the most excellent quality has answered many fundamental questions relating to all aspects of the virus and the disease it causes, *Barley yellow dwarf virus* (BYDV) (including *Cereal yellow dwarf virus*, or CYDV, throughout this text) is probably as big a problem now as it has ever been. Thus, the heat is on to do something practical about it. Epidemiologists, perhaps with breeders, probably come in for as hot a grilling as anyone in this respect. Their research is "nearer the market" than that of many others, and often depends on industry funding. Fundamental scientific understanding has to be combined with clever technologies in order to produce practical solutions.

The heat is also on in another sense. The world is getting warmer (I.P.C.C., 2001) and this is sure to affect arable agriculture. Whether the effect is positive or negative will depend on location and crops grown. It will depend as well on how changes not only in temperature, but also in other factors (perhaps especially rainfall) affect: 1) crops and their competitors, 2) the pests and diseases of those crops and their competitors, and 3) the natural enemies of those pests. Indeed, even this is a gross oversimplification. The interactions are hugely and horribly complex.

This presentation is in four parts. First I will summarize some of the complications alluded to above, with the purpose of drawing attention to their likely impact on attempts of applied epidemiologists to model disease spread and deliver decision support systems (DSSs). Then I will make things worse by introducing the potential impacts of climate change. Next I will describe the development of a UK DSS. Finally I will discuss whether, in the light of all the complexity, this and other DSSs have any prospect of being applicable over a wide area or of standing the test of time.

BYDV Epidemiology

Several authors have drawn attention specifically to the complexities of BYDV epidemiology (e.g., Irwin and Thresh, 1990; Burgess *et al.*, 1999). Here I will present just a brief summary. The so-called disease triangle, made into a pyramid by adding interactions with the environment to those between viruses, vectors, and host plants, is a bit hackneyed. However, it still serves a useful purpose in drawing attention to some of the issues that need to be addressed when devising DSSs.

The viruses

There are at least two species and a whole range of strains and isolates involved in barley yellow dwarf, each of which may interact differently with vectors, host plants, and the environment.

The vectors

Being a persistent, phloem-limited virus, aphids have to feed to acquire BYDV, rather than just probe for host

plant identification cues. However, at least 28 species can do this, although only a handful is probably important. Nonetheless, this brings more than a handful of complications. Aphids have different life cycles, fly at different times and in different places, and have different behavior patterns. Different clones within species vary in some or all of these characteristics.

The host plants

There is, of course, a whole range of crops and other Poaceae that are hosts to BYDV. All are potential reservoirs of infection.

Virus-vector interactions

Some species of aphid can transmit more than one virus strain efficiently, and some virus strains can be transmitted efficiently by more than one aphid species. Different clones of aphids differ in their vectoring efficiency (Guo *et al*., 1996) and behavior. Therefore, finding two known vectors does not necessarily mean that they are contributing equally to the development of disease. Furthermore, if an aphid is found to be carrying a virus, this does not necessarily mean that it will be capable of transmitting it. Therefore, testing a population of aphids by ELISA, for example, for the presence of virus does not necessarily provide a figure that can be used in a simulation model to describe the proportion of that population that will transmit the virus.

To add to the difficulties of the applied epidemiologist, a given virus isolate can be transmitted with different efficiency by different clones of a given aphid species, and a given aphid clone can transmit different isolates with different efficiencies. Yet another complication is that the species of aphid which inoculated the virus may have a significant effect on the efficiency with which different aphid species can subsequently transmit virus (Gray pers. comm.). It is possible that such effects are even seen at aphid clonal level.

Virus-host plant interactions

Symptoms in host plants differ with virus strain or isolate, making it difficult to assign resistance ratings to cultivars. Also, within this interaction, the latent period in the plant must be considered, i.e., the time between the plant becoming infected and itself acting as a source. This is affected by growth stage and virus strain.

Vector-host plant interactions

Different aphids have different host plant preferences, and this will clearly affect disease epidemiology. Also, crop growth stage will affect aphid feeding behavior.

Virus-vector-host plant interactions

Considering just the virus, the host range of each BYDV strain is similar. However, in the field there are differences in which virus strains are prevalent in different hosts. For example, *Rhopalopsiphum maidis* tends not to feed on oats, and so RMV is not usually a problem in oats.

Environmental Considerations and Climate Change

Clearly, environmental factors interact strongly at every point discussed above but, having suggested that all of the complications tied up in the disease pyramid make modelling and decision support highly complex tasks, even if a system was put into place that works now, would it work in the future with new crop cultivars, new aphid clones, new virus isolates, and a changed climate?

In the UK, general experience and some empirical evidence suggest that BYDV problems are greatest following two consecutive mild winters with a wet summer in between. The first mild winter leads to a high proportion of anholocyclic clones of *R. padi* in the following year (Figure 1). The anholocyclic clones are those which over-winter in the active stage on grasses, including cereals, rather than as an egg on their

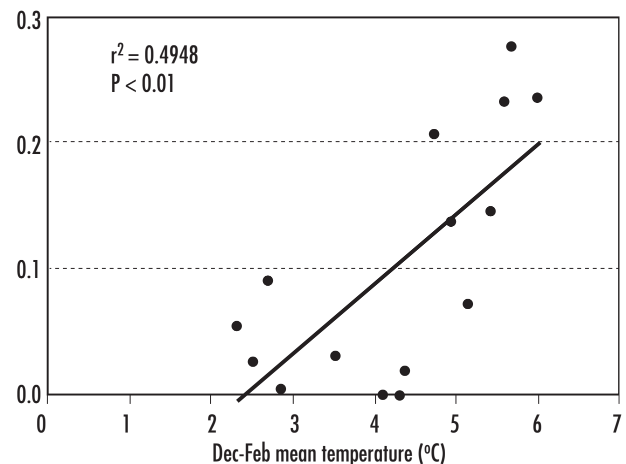


Figure 1. Relationship between previous winter temperature and proportion of cereal-colonizing forms of the aphid *Rhopalosiphum padi* trapped at Rothamsted in the fall, 1986-2000.

primary host, *Prunus padus*. Therefore, only the anholocyclic clones are important in transmission of BYDV in fall-sown crops. The active forms are far less tolerant of low temperature than are the eggs, and a tendency can be seen for the proportion of anholocyclic forms in autumn to be related to temperature in the previous winter, with a higher proportion following a mild winter. If, in the meantime, the summer is wet, there is a plentiful supply of good quality grasses to tide the aphids over between the drying out of one crop and emergence of the next.

In the UK, there is a weak but significant relationship between numbers of *R. padi* in fall and summer rainfall. A second mild winter allows all these aphids of the right morph to move around the crop in question, spreading virus. Thus, two mild winters with a wet summer in between sets the scene for trouble. Figure 2 shows how the probability of getting two consecutive mild winters (defined as December to February mean temperature exceeding 4.5^0C), with a wet summer in between (defined as precipitation from June to August exceeding 190 mm), is likely to change in different parts of the UK according to climate predictions to the end of this century. The BYDV risk is hence predicted to rise, particularly in northwest and southwest England. In other parts, although the criterion of warm winters may be met more often in the future, the summers are expected to be particularly dry.

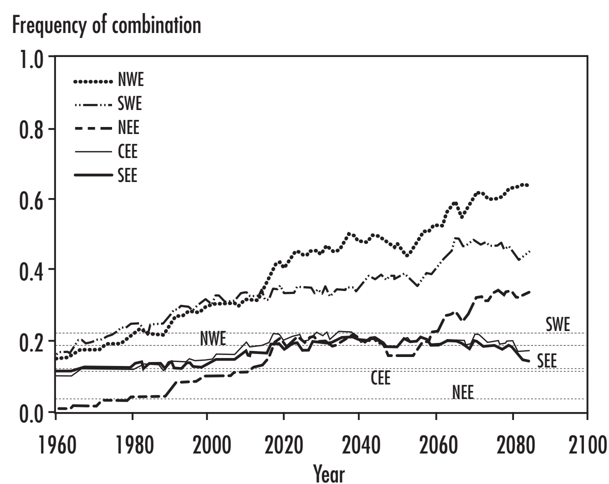


Figure 2. Probability of two mild winters with an intervening wet summer.

Notes: Dotted lines represent current risk and solid lines of the same color represent predicted risk for Northeast England (NEE), Southeast England (SEE), Central and Eastern England (CEE), Northwest England (NWE) and Southwest England (SWE).

Drought stress during the growing season can increase risk of spread. In laboratory experiments in which trays of plants were subjected to different levels of drought stress, aphids visited more plants at higher temperatures and at higher drought stress levels, and this was reflected in the number of plants infected with BYDV (Smyrnioudis *et al.*, 2000). There was temperature x drought stress interaction: high levels of drought stress had a particularly strong effect at higher temperatures.

Climatic change may influence the prevalence of particular virus isolates in a region and the efficiency of their transmission by particular aphid species. For example, the RMV strain of BYDV is currently unimportant in small grain cereals in the UK. It is transmitted by *R. maidis*, which is uncommon compared to other vectors. With climate warming, maize may become more widespread, as may the maize-preferring strain of the aphid, which does not produce an egg and hence is not tolerant of cold winters. At higher temperatures, the more common vectors in the UK may become capable of transmitting the RMV strain to cereals such as wheat and barley (Lucio-Zavaleta *et al.*, 2001). Therefore, warmer conditions, through their effect on interactions between host plants, aphids, and viruses, may render the maize strain of BYDV important in wheat and barley for the first time in the UK.

These examples serve to remind us that changes in climatic variables may have unexpected effects on BYDV epidemiology. Thus, the extrapolation based on mild winters and wet summers (above) could turn out to be grossly over simplistic, while modelling could turn out to grossly complex.

What can the distribution of BYDV incidence around the world today tell us about expected impacts of climate change in a given region? Can we find areas where the climate today is similar to that expected in our particular area of interest 50 years hence and infer that BYDV problems will be similar? Again, this is too simplistic. There are numerous reasons why such a so-called climate-matching methodology is fraught with dangers. For example, even though climate may be matched, photoperiod may not, and that influences plant growth, insect life cycles, and hence epidemiology in a range of ways. However, the review by Lister and Ranieri (1995) shows clearly that where there are cereals there is BYDV and, although the prevalent strains and isolates may change in a given area, the disease is likely to be sufficiently adaptable to continue to be ubiquitous no matter how the climate changes.

A UK Web-based Decision Support System

In the light of what has been said above about the complexities and dynamic nature of barley yellow dwarf epidemiology and the potential impacts of climate change, the longevity of DSSs may be limited unless they receive continual review and upkeep. Nonetheless, I will play the optimist, outline a decision support system being developed in the UK (http://bydv.csl.gov.uk/) (Harrington *et al*., 1999; Northing *et al.*, in press), and hope that support for its validation and upkeep is forthcoming.

The system was built with two practical assumptions in mind. First, farmers are keen to rationalize insecticide usage. Second, farmers are not keen to count aphids in the field. Indeed, having spent much time doing this myself, I feel it is totally unreasonable to expect them to do so, especially in winter. The system is based on a weather-driven stochastic simulation model of secondary spread, initialized on the basis of aphid captures in suction traps. This provides a regional risk estimate, which is made field specific on the basis of relationships between virus incidence and field characteristics.

Suction traps are in place, and aphids, including BYDV vectors, are identified routinely for a range of purposes at 15 sites throughout the UK (Woiwod and Harrington, 1994). Based on known relationships between numbers of aphids caught in the traps and numbers caught on sticky wire traps placed over the crop (Figure 3), the number of aphids landing per unit

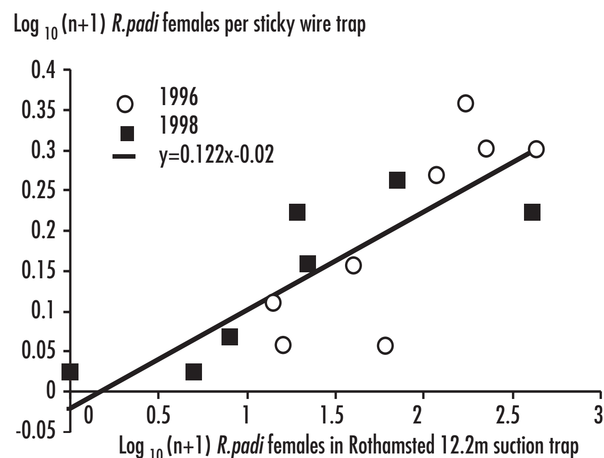


Figure 3. Comparison of numbers of the aphid *Rhopalosiphum padi* in a 12.2 m suction trap and on sticky wire traps in cereal crops.

area of crop is estimated from routine suction trap data on a regional basis. Estimates of the proportion of aphids that are from anholocyclic clones and will hence stay in the crop, the proportion likely to be infective, and the number of flights made by winged aphids within the crop are taken into account, so that values for the density of foci of infection and of aphids capable of spreading virus from those foci are obtained. These values are then used in a simulation model (Harrington *et al.*, 1999) which spreads the virus on the basis of algorithms describing the effects of temperature on aphid development, reproduction, mortality, and movement. This gives a continually updated estimate of incidence in an average field in a given suction trap region.

On the basis of a survey of more than 600 unsprayed plots throughout the UK over a three-year period, field characteristics were determined which influence whether an individual field has more or less virus than would be expected from the regional model (Harrington *et al.*, 1999; Foster *et al.*, in press). Sowing date is taken into account on the basis of the time that the model run begins, but field specific conversions are made on the basis of, at present, distance from the sea and whether or not arable land dominates the area.

Regional risk assessments are generated by running the model for each sowing date/crop type combination in each region, and the results are stored in the system database. They are then accessed by inputting data defining the region, sowing date category (of which there are five), and crop type of interest. Two crop types sown in five time periods in fifteen regions produce a total of 150 combinations provided by 75 different model runs. (Runs for wheat and barley are the same, with a different multiplication factor based on virus/yield loss relationships.) Twenty runs are done for each region/sowing date combination and the mean result taken. These models are run on a weekly basis and the results summarized on a map of Great Britain using a colored grading system for five different risk categories. The risk categories are based on the levels of virus incidence in crops, related to break-even points if controls are applied. The break-even virus incidence is the treatment cost divided by: the yield multiplied by the grain price multiplied by the virus/yield loss relationship.

The virus/yield loss relationship was calculated as the mean of various trial results available and is $y = 0.62\ x$ for barley and $y = 0.17\ x$ for wheat, where y is yield loss

(%) and x is virus incidence (%). The regional risk assessment map is presented on the web site, allowing users to assimilate quickly the distribution of BYDV across the whole country. This map is provided alongside a second map containing the regional risk assessment for the equivalent period of the previous year or, on request, the ten year mean. By clicking on the relevant spot, users can then pinpoint their locality, which will automatically provide an indication of "distance from the sea," one of the relevant field characteristics. They will be asked whether or not the area is dominated by arable land. On finally submitting the query, a risk category will be presented. The system has performed well in small plot validation trials in England, but not in Scotland, where aphid mortality, and hence virus incidence, were underestimated, probably due to the current omission of the influence of rainfall from the model. The system remains to be validated in commercial fields.

Other web-based DSSs are being developed, for example in Western Australia (http://www.agric.wa.gov.au/bydv/) and New Zealand (http://www.aphidwatch.com). In Western Australia, the epidemiology is somewhat simpler than in the UK. *Rhopalosiphum padi* and *R. maidis* are the only significant vectors and are exclusively anholocyclic, removing the need to distinguish between aphids that may and may not colonize cereals. Also, there is a much stronger relationship between late summer rainfall and vector arrival in crops in the fall than in the UK, probably because there is a much greater range of rainfall volume than in the UK. The mediterranean climate means that in some years there is no rain, and very little green vegetation survives over the four months between growing seasons. The WA system is based on these statistical relationships coupled with a simulation model, which is similar in concept to that used in the UK. Inputs required from users are location, crop variety, sowing date, and planting density. The models reliably predicted the time of arrival of aphids in the crops in all regions tested over a four year period. Predictions of BYDV incidence were accurate in high and medium rainfall zones but, in low rainfall zones, incidence tended to be lower than predicted. The model has been adapted to provide an internet-based DSS with maps of BYDV risk throughout high and medium rainfall areas of the grain belt.

Conclusions and Prospects

A major concern with any DSS, computer-based or otherwise, is its upkeep following development and launch. This is particularly important in the case of computer-based systems which, if left alone, can become scientifically outdated surprisingly quickly while continuing to present a beguiling façade to users. We have seen that a BYDV DSS has to account for a huge number of biotic and abiotic components of the disease system and for primary and lower level interactions between them. If any of these change, the model parameters may become inappropriate. New cereal varieties may interact in different ways than current varieties with the aphids and the viruses. In the longer term, environmental changes may have important effects. If a new DSS is found to be successful, growers should be prepared to reinvest some of the money saved through its use in its continued scientific development.

Full-scale evaluation of any system is clearly a prerequisite of its commercial release. Even if successful, developers should not hide any lingering doubts they may have. One notable failure can quickly bring the whole concept of computer-aided decision support into disrepute. Even the best systems are unlikely to better a local expert with intimate experience of the wrinkles of his patch. The challenge for DSS developers is to provide systems that take account of the huge amount of variation that goes with any pest and disease problem and hence produce a system with wide and enduring applicability.

Acknowledgments

I am grateful to my colleagues in the Rothamsted Insect Survey, to Keith Walters, Phil Northing, Derek Morgan, and Ian Barker (Central Science Laboratory, York, UK), to Garth Foster and colleagues (Scottish Agricultural College) and to Steve Tones and colleagues (ADAS), collaborators in the UK BYDV decision support system, and to the UK Home-Grown Cereals Authority, the UK Department for Environment, Food and Rural Affairs and the Lawes Agricultural Trust for funding development of the system. I am also most grateful to Stewart Gray (Cornell University), Martin Parry (Jackson Environment Institute, University of East Anglia), Ilias Symrnioudis (University of Thessaloniki), Debbie

Thackray (CLIMA and Agriculture Western Australia) and Dave Teulon (Lincoln University, New Zealand) for collaboration or discussions resulting in data or visuals. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom.

References

Burgess, A.J., Harrington, R., and Plumb, R.T. 1999. Barley and cereal yellow dwarf virus epidemiology and control strategies. Pp. 248-261 in H.G. Smith and H. Barker (eds.). *The Luteoviridae.* CABI.

D'Arcy, C.J., and Burnett, P.A. (eds) 1995. *Barley Yellow Dwarf: 40 Years of Progress.* APS Press.

Foster, G., Blake, S., Barker, I., Harrington, R., Taylor, M., Walters, K., Northing, P., and Morgan, D. (in press) Decision support for BYDV control in the UK: can a regional forecast be made field specific? *Proceedings 6th International Aphid Symposium, Rennes, France, 3-7 September 2001.*

Guo, J.Q., Moreau, J.P., and Lapierre, H. 1996. Variability among aphid clones of *Rhopalosiphum padi* L. and *Sitobion avenae* Fabr. (Homoptera: Aphididae) in transmission of three PAV isolates of barley yellow dwarf viruses. *Canadian Entomologist* 128:209-217.

Harrington, R., Mann, J.A., Burgess, A.J., Tones, S.J., Rogers, R., Foster, G.N., Blake, S., Morrison, S.F., Ward, L., Barker, I., Morgan, D., and Walters, K.F.A. 1999. Development and validation of decision support methodology for control of barley yellow dwarf virus. *HGCA Project Report No. 205.* Home-Grown Cereals Authority, UK.

I.P.C.C. 2001. Climate Change 2001: The Scientific Basis. Summary for Policymakers and Technical Summary of the Working Group I Report. WMO, UNEP.

Irwin, M.E., and Thresh, J.M. 1990. Epidemiology of barley yellow dwarf: a study in ecological complexity. *Annual Review of Phytopathology* 28:393-424.

Lister, R.M., and Ranieri, R. 1995. Distribution and economic importance of barley yellow dwarf. Pp. 29-53 in: C.J. D'Arcy and P.A. Burnett (eds.). *Barley Yellow Dwarf: 40 Years of Progress.* APS Press.

Lucio-Zavaleta, E., Smith, D.M., and Gray, S.M. 2001. Variation in transmission efficiency among barley yellow dwarf virus-RMV isolates and clones of the normally inefficient aphid vector, *Rhopalosiphum padi. Phytopathology* 91:792-796.

Northing, P., Walters, K., Barker, I., Foster, G., Harrington, R., Taylor, M., Tones, S., and Morgan, D. (in press) Use of the internet for provision of user specific support for decisions on the control of aphid-borne viruses. *Proceedings 6th International Aphid Symposium, Rennes, France, 3-7 September 2001.*

Smyrnioudis, I.N., Harrington, R., Katis, N., and Clark, S.J. 2000. The effect of drought stress and temperature on spread of barley yellow dwarf virus (BYDV). *Agricultural and Forest Entomology* 3:161-166.

Woiwod, I.P., and Harrington, R. 1994. Flying in the face of change - The Rothamsted Insect Survey. Pp. 321-342 in: R.A. Leigh and A.E. Johnson (eds.). *Long Term Research in Agricultural and Ecological Sciences.* CABI.

Vector Dynamics and BYD Disease Progress on Barley and Maize: Two Contrasting Cases and Consequences for Control

L. Haack,[1] F. Leclercq-Le Quillec,[1] M. Henry,[2] H. Albert,[3] and C.-A. Dedryver[1]

[1] INRA/ENSA, Unité Mixte de Recherche Biologie des Organismes et des populations appliquée à la Protection des Plantes, Le Rheu, France
[2] CIMMYT, Mexico, D.F., Mexico
[3] Bayer S.A., Puteaux, France

In France barley yellow dwarf (BYD) epidemiology is characterized mainly by an alternation of vectors and viruses between summer hosts (mostly maize) and winter hosts (mostly barley and wheat). At the beginning of autumn, winged aphids leave maize and infest young winter cereals, potentially transmitting viruses. When winter frost is moderate, they over-winter parthenogenetically in cereal fields and multiply again when temperature rises. During spring, aphids leave maturing barley and wheat and infest young maize crops, potentially transmitting viruses. At the end of summer, aphid multiplication on maize leads to large populations of vectors, especially *Rhopalosiphum padi* L. The stability of the pathosystem depends therefore mainly on the conditions of infestation of both crops by aphids, of viral multiplication in the crops, and of vector production by maturing crops.

Once introduced into cereal fields by winged aphids (primary infection), viruses can be propagated from primary foci by wingless aphids multiplying in the fields and moving from plant to plant. The intensity and pattern of this dissemination, or secondary spread, depends on interactions between host-plants, vectors, and climate. It is a major component of yield losses, but also of inoculum production for further infection. In this paper, we compare: 1) the shapes of BYD disease progress curves on winter barley and on maize, and 2) for both crops, the consequences for vector infestation and virus infection levels of an insecticide seed treatment (imidacloprid) to prevent aphid multiplication.

Materials and Methods

All field experiments were conducted at the INRA Research Centre of Le Rheu (western France). Barley trials (cv. Express) were carried out over three cropping seasons, from September 1989 to July 1992, in a Fisher block experiment (4 rep.) including control plots and plots treated with imidacloprid in seed dressing (70 g/100 kg of seed).

Maize trials (cv. Dea) were carried out in 1987 in an untreated field, and from 1997 to 1998 in a Fisher block experiment (4 rep.) comparing control plots and plots treated with imidacloprid in seed dressing (70 g/ 50,000 seeds).

Viruses and aphids were monitored at different intervals from crop emergence to maturity (weekly or fortnightly for maize, fortnightly or monthly for barley). For barley in 1989-1992 and maize in 1987, plants were randomly selected in the plots on each sampling date; for maize in 1997-1998, the same plants were regularly sampled on a grid throughout the cropping season. A total of 720 barley plants and 448 maize plants were sampled on each date, except in 1987 (50 plants only). The number of plants infested by each aphid species (*R. padi*, *Sitobion avenae*, and *Metopolophium dirhodum*) was assessed in the field. For each sampled plant, the upper well-developed leaf (for barley) or a piece of the flag leaf (for maize) were subsequently cut and placed individually in plastic bags for virus detection with TAS-ELISA, as described by Leclercq-Le Quillec *et al.* (2000). PAV-type and MAV-type viral capsids were respectively recognized by the monoclonal antibodies Mac 91 and MAFF2

(ADGEN diagnostic systems, Ayr, Scotland, UK). Statistical analyses of the data were done in ANOVA, using GLM procedures of SAS (SAS, 1988).

Results

Vectors and virus dynamics on barley

In any one year, PAV predominates in the pathosystem. The dynamics of PAV serotypes was bimodal in 1989-90 and 1990-91 (Figure 1). Infection levels increased during autumn and reached a plateau in November, then decreased during winter due to the death of infected plants. They increased again from March to May, suggesting a two-step infection process related to the seasonal activity of aphid vectors (Table 1a). The first peak corresponds to the overlap of: 1) primary infections by alate aphids, and 2) the beginning of secondary spread by wingless aphids. The second peak is due to infection by aphids disseminating in spring. In 1991-92, the PAV infection curve was monomodal and due mainly to primary infections by very large populations of *R. padi* (Table 1b).

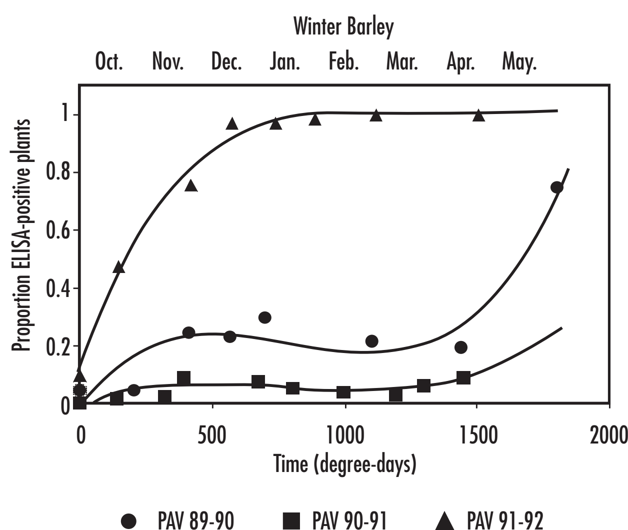


Figure 1. PAV progress curves on control barley in the three years of experimentation, estimated by a 4th degree polynomial.

Seed treatment with imidacloprid dramatically limited aphid populations in 1989-1990 (Table 1a), 1990-1991 (not illustrated), and 1991-1992 (Table 1b). No colonies were established in the treated plots, and the low percentage of infested plants in those plots in October-November corresponds to temporary infestations by the flow of migrating alates. Consequently, the percentage of viral infection remained very low in the treated plots compared to the controls (Table 1), and corresponded to the primary infection phase by some alates that succeeded in transmitting viruses before dying. Differences between control and treated plots are significant for infestation and infection ($p = 0.01$) during the whole sampling period, with the exception of 16/05/1990 for infestation (Table 1).

Vector and virus dynamics on maize

On maize, the dynamics of PAV and MAV serotypes was quite similar in 1997 and 1998, and very different from the situation in barley: maximum infection was reached very early in the growing period of maize, at the 6-leaf growth stage (GS 6). After this period, there was no further increase in the

Table 1. Infestation (mean percentage of infested plants by all aphid species confounded) and infection (mean percentage of infected plants by PAV and MAV confounded), in control and imidacloprid treated barley plots in 1989-1990 (a) and 1991-92 (b).*

a.

1989-90	Barley	5 Oct. 89	18 Oct. 89	12 Dec. 89	9 March 90	16 May 90
Infestation	Control	20.7 a	30.5 a	10.5 a	70.5 a	97.5 a
(in % infested plants)	Treated	0.7 b	2 b	0 b	0 b	94.1 a
Infection	*ControlT*	*7.0 a*	*7.7 a*	*35.8 a*	*32.3 a*	*99.2 a*
(in % ELISA-positive plants)	*reated*	*1.9 b*	*2.5 b*	*3.2 b*	*8.8 b*	*28.3 b*

b.

1991-92	Barley	9 Oct. 91	21 Oct. 91	20 Nov. 91	25 Feb. 92
Infestation	Control	91.9 a	91.1 a	75.3a	7.5 a
(in % infested plants)	Treated	8.1 b	5.6 b	1.7 b	0 b
Infection	*Control*	*10 a*	*47*	*75 a*	*92 a*
(in %ELISA-positive plants)	*Treated*	*3.3 b*	*Not tested*	*7.8 b*	*7.5 b*

* For each variable and date, numbers not followed by the same letter are significantly different.

percentage of infected plants, i.e. no more primary infections and no secondary spread (Figure 2, Table 2). In 1987, infected plants were detected later than in 1997 and 1998 (GS 7), and the levels of infection were much lower; however, the shape of the curve was roughly the same. Virus dynamics do not seem to be related to temporal changes either in aphid populations or in percentage of infested plants (Table 2).

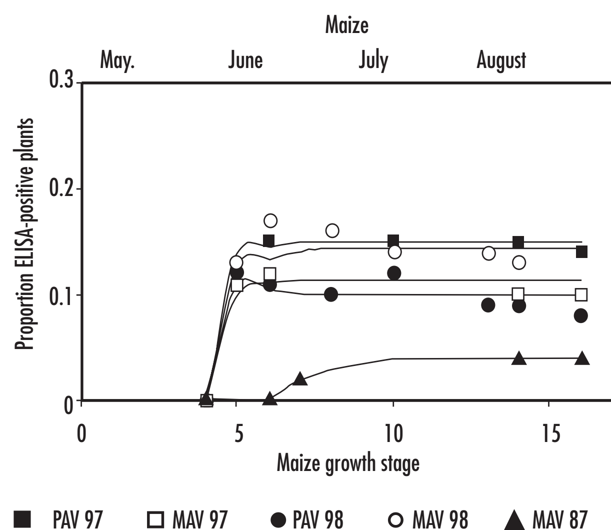


Figure 2. PAV and MAV progress curves on control maize in the three years of experimentation fitted visually.

In both 1997 and 1998, imidacloprid strongly reduced the mean numbers of aphids per plant (Table 2), but not the percentage of infested plants, which remained high until August in both treated and control plots. In treated plots, however, most aphids collected during this period were transient alates, as observed in barley, and no aphid colony developed.

Conversely to the situation in barley, there were no significant differences in infection percentages in control and treated maize plots.

Discussion and Conclusions

On barley, the course of BYDV infection follows a typically bimodal process with an autumn and a spring mode, excepted when the totality of the plants are infected during autumn, but this case seems uncommon (Dedryver, unpublished). Barley can be infected by BYDV at least until the end of stem extension (Jenkins, 1966). However, only autumn infections cause important losses, because autumn-infected plants have poor tillering and become more susceptible to winter frost. Although spring infections are certainly less damaging, they are nonetheless important from an epidemiological point of view, because they greatly increase the amount and availability of virus inoculum for winged aphids migrating from cereals to maize or other summer crops.

Table 2. Infestation (1: mean number of aphids per plants, and 2: mean percentage of infested plants, by all species confounded) and infection (mean percentage of infected plants by PAV and MAV confounded) in control and imidacloprid treated plots of maize in 1997 (a) and 1998 (b).*

a.

1997	Maize	17 June	24 June	1 July	12 Aug.	26 Aug.	29 Sept.
Infestation 1	Control	39.8 a	68.3 a	67.9 a	0.88 a	1.22 a	520 a
(N aphids/plant)	Treated	3.47 b	8.8 b	15.9 b	0.05 b	0.67 b	200 b
Infestation 2	Control	100 a	99.6 a	99.6 a	4 a	30 a	100 a
In % infested plants	Treated	85 b	99.4 a	99.4 a	2 a	29 a	100 a
Infection	*Control*	*0*	*22 a*	*23.4 a*	*19.5 a*	*22.8 a*	*Not tested*
(% ELISA-positive plants)	*Treated*	*0*	*19 a*	*19 a*	*21 a*	*16 a*	*Not tested*

b.

1998	Maize	15 June	22 June	30 June	20 July	24 Aug.	26 Oct.
Infestation 1	Control	2.45 a	5.17 a	7.40 a	16.34 a	2.68 a	360 a
(N aphids/plant)	Treated	0.2 b	1.84 b	2.53 b	5.28 b	0.15 b	170 b
Infestation 2	Control	69.5.5 a	90 a	88 a	95.5 a	43.5 a	100 a
In % infested plants	Treated	18.5 b	70. 5 a	64.5 a	89 a	6 b	100 a
Infection	*Control*	*0*	*23 a*	*17 a*	*17 a*	*10 a*	*Not tested*
(% ELISA-positive plants)	*Treated*	*0*	*21 a*	*13 a*	*20.5 a*	*14 a*	*Not tested*

* For each variable and date, numbers not followed by the same letter are significantly different.

The first infection mode is due to the addition of primary virus contamination and secondary spread, which overlap widely (Leclercq-Le Quillec, 1992). The imidacloprid seed treatment is clearly effective for avoiding autumn secondary spread and delaying post-winter re-infestations, which results in lower secondary spread in spring. Consequently, yields of treated plots were significantly higher than those of the controls, except in 1990-1991, when levels of infestation and infection were very low (data not shown), and the infectivity of winged aphids flying in May from treated plots was probably lower too.

On maize, the situation appears very different: a high proportion of plants (> 90%) is infested at an early stage (6 leaves) by winged aphids, and some plants are subsequently infected by viruses. However, neither the continuous re-infestation of maize plants by new alates until the end of July (Haack *et al*., 2000), nor the possible dispersion of wingless individuals leads to a further increase in infection. The reasons could be: 1) that maize very soon becomes resistant to virus inoculation (between GS 8 and 10, from Haack *et al*., 1999), and 2) that plant-to-plant dispersion of wingless aphids is difficult before summer due to the low density and small size of maize plants. From an epidemiological point of view, the infectivity of huge alate aphid populations leaving maize in autumn (Gillet *et al*., 1990) is probably completely dependent on a very short period in May or June during which aphid flight coincides with maize receptivity to infection.

As expected, the imidacloprid treatments prevented the development of aphid colonies on maize, and reduced the number of winged aphids infesting the crop (data not shown). Nevertheless, there were no differences between controls and treatments in percentage of infested plants, probably due to the high and long-time aerial flow of aphids in the spring of both 1997 and 1998. This could explain why, during periods when maize was susceptible to infection (roughly June), the number of infective alates was not limiting, even in the treated plots, resulting in no differences between infection rates in the control and treated plots. However, in conditions of lower contamination, imidacloprid has been reported to significantly reduce maize plant infection (Epperlein *et al*, 1997).

References

Epperlein, K., Schmidt, H.W., and Schwalbe, R. 1997. Untersuchungen zum Einfluss von Gaucho als Saatgutbeize am Mais auf das Virusauftreten, Schadinsekten und Epigäische Bodenfauna. *Archiv. Phytopathol. U. Pflanz.* 31:185-200.

Gillet, H., Dedryver, C.A., Robert, Y., Gamon, A., and Pierre, J.S. 1990. Assessing the risk of primary infection of cereals by yellow dwarf virus in autumn in the Rennes basin of France. *Ann. appl. Biol.* 117:237-251.

Haack, L., Courbon, R., Riault, G., Tanguy, S., Le Vilain, D., Henry, M., and Dedryver, C.A. 1999. A plant and field study of BYDV-PAV and -MAV distribution on maize in France. *Z. Pflanzenkr. Pflanzenschutz* 106:297-303.

Haack, L., Simon, J.C., Gauthier, J.P., Plantegenest, M., and Dedryver, C.A. 2000. Evidence for predominant clones in a cyclically parthenogenetic organism provided by combined demographic and genetic analyses. *Mol. Ecol.* 9:2055-2066.

Jenkins, G. 1966. Comparison of tolerance to barley yellow dwarf virus in barley and oats. *Ann. appl. Biol.* 57:163-168.

Leclercq-Le Quillec, F. 1992. Analyse de la dynamique spatio-temporelle du complexe BYDV (barley yellow dwarf virus) et effet d'un traitement de semences sur la distribution des vecteurs et des virus. Thèse de Doctorat. Univ. Paris XI.

Leclercq-Le Quillec, F., Plantegenest, M., Riault, G., and Dedryver, C.A. 2000. Analyzing and modeling temporal disease progress of barley yellow dwarf virus serotypes in barley fields. *Phytopathology* 90:860-866.

SAS Institute Inc. 1988. *SAS/STAT User's Guide*, release 6.03 Edition. Cary, North Carolina.

Use of Aphids' Abundance in Autumn to Predict BYD Yield Losses

F. Fabre,[1,2] C.A. Dedryver,[1] J.L. Leterrier,[2] and M. Plantegenest[1]

[1] INRA/ENSA, Unité Mixte de Recherche Biologique des Organismes et des Populations appliquée à la Protection des Plantes (Bio3P), Domaine de la Motte, Le Rheu, France
[2] Bayer Crop Science France, Lyon, France

The current tendency towards reducing the systematic use of insecticides has led to a strong need for reliable risk assessment tools. As emphasized by Hughes *et al.* (1999), algorithms based on logistic regression can be used to evaluate the risk of pest outbreaks or disease epidemics, and recent studies have demonstrated the relevance of this approach (Yuen *et al.*, 1996; Lindblad, 2001). Reliable forecasting systems are of special interest when crop damages are sporadic, varying from field to field and year to year. This is the case of barley yellow dwarf disease (BYD), one of the most severe cereal virus diseases in the world. BYD is caused by viruses belonging to the Luteoviridae family and transmitted persistently by various aphid species living on Poaceae. The most important of these aphids on autumn-sown cereals in France is *Rhopalosiphum padi* (L.). One method of BYD control relies on the use of insecticide sprays applied between mid-October and mid-November to kill aphid vectors established in the crop. Crops damage depends on many factors (cereal species and variety, virus species and isolate, environmental conditions) but, above all, on the time of infection (the younger the crop when infection occurs, the greater the loss will be) (Watson and Mulligan, 1960; Jenkins, 1966) and the proportion of infected plants (Kurppa, 1989; Banks *et al.*, 1995; Perry *et al.*, 2000). Though the spread of BYD viruses (BYDV) is strongly determined by vector population dynamics (Leclercq-Le Quillec *et al.*, 2000), it is generally considered that BYD epidemics cannot be predicted based on aphid density alone. This must be supplemented by the level of aphid infectivity, i.e., the aphids' ability to infect a host with BYDV (Plumb, 1983). However, aphid infectivity is difficult to assess in the field, in contrast to aphid population density, which can easily be assessed by visual count. This study examined to what degree a simple risk algorithm based only on autumnal population dynamics of *R. padi* can be useful for predicting BYDV yield losses and the need for insecticide treatment. The analysis was conducted using logistic regression on a large data set of 65 field experiments established in 18 sites of northern France. The experiments were sown with winter barley from mid-September to mid-October over a 10-year period. Results show a highly significant relationship between the explanatory variable chosen (the area under the curve of the percentage of plants infested by *R. padi* in autumn) and BYD yield losses. The proposed model gives a total of 80% of correct decisions. The results of this study suggest that a decision-making system aimed at improving *R. padi* control in autumn-sown cereals could be based on the prediction of the area under the curve of the proportion of plants infested by aphids. Further work is needed to develop such a decision-making system.

References

Banks, P.M., Davidson, J.L., Bariana, H., and Larkin, P.J. 1995. Effects of barley yellow dwarf virus on the yield of winter wheat. *Australian Journal of Agricultural Research* 46:935-946.
Hughes, G., McRoberts, N., and Burnett, F.J. 1999. Decision-making and diagnosis in disease management. *Plant Pathology* 48:147-153.
Jenkins, G. 1966. Comparison of tolerance to barley yellow dwarf virus in barley and oats. *Annals of Applied Biology* 57:163-168.
Kurppa, S. 1989. Damage and control of *Rhopalosiphum padi* in Finland during the outbreak of 1988. *Annales Agriculturae Fenniae* 28:349-370.
Leclercq-Le Quillec, F., Plantegenest, M., Riault, G., and Dedryver, C.A. 2000. Analyzing and modeling temporal disease progress of barley yellow dwarf virus serotypes in barley fields. *Phytophatology* 90:860-866.
Lindblad, M. 2001. Development and evaluation of a logistic risk model: predicting frit fly infestation in oats. *Ecological Applications* 11:1563-1572.
Perry, K.L., Kolb, F.L., Sammons, B., Lawson, C., Cisar, G., and Ohm, H. 2000. Yield effects of barley yellow dwarf virus in soft red winter wheat. *Phytopathology* 90:1043-1048.
Plumb, R.T. 1983. Barley yellow dwarf virus: A global problem. In: *Plant Virus Epidemiology* R.T. Plumb and J.M. Tresh (eds.). Blackwell Scientific Publications, Oxford. pp. 185-198.
Watson, M.A. and Mulligan, T.E. 1960. Comparison of two barley yellow dwarf viruses in glasshouse and field experiment. *Annals of Applied Biology* 48:559-574.
Yuen, J., Twengström, E., and Sigvald, R. 1996. Calibration and verification of risk algorithms using logistic regression. *European Journal of Plant Pathology* 102:847-854.

Incidence of *Barley Yellow Dwarf Viruses* in Symptom-Exhibiting Cereal Species

E. Pocsai,[1] I. Murányi,[2] M. Papp,[3] L. Szunics,[4] A. Tomcsányi,[5] and G. Vida[4]

[1] Plant Protection and Soil Conservation, Fejér County Service , Velence, Hungary
[2] Rudolf Fleischmann Agricultural Research Institute of the Szent István University, Kompolt, Hungary
[3] Cereal Research Non-Profit Company, Szeged, Hungary
[4] Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary
[5] Cereal Research Non-Profit Company, Táplánszentkereszt, Hungary

Barley yellow dwarf viruses (BYDV-MAV, -PAV, -RMV, -SGV) and *Cereal yellow dwarf virus* (CYDV-RPV) are the most economically important and widespread virus pathogens of cereal crops in the world. As members of the luteoviruses they are not mechanically transmissible, and are phloem-limited in host plants, but are transmitted in a persistent manner by numerous aphid species.

These viruses were first identified in The Netherlands by Oswald and Houston (1951) and were confirmed in the United Kingdom (Watson and Mulligan, 1957). Before the European identification of the disease, however, cereal diseases characterized by yellowing, dwarfing, and decreased yields had been observed in North America, sometimes in epidemic proportions. Widespread outbreaks with significant yield losses were probably caused by BYDVs in 1907 and 1949 (Hewings and Eastman, 1995). The first reports of barley yellow dwarf viruses in Australia and New Zealand were made more than 47 years ago by Smith (1955, 1957).

In Hungary, BYDV was first identified and described in winter barley by Szirmai (1967). Five years later, it was observed in winter wheat during the spring of 1972 by Szunics and Szunics (1980). This virus was later recorded in maize by Milinkó *et al.* (1984) and in rice by Pocsai *et al.* (1985). In 1982 a very severe epidemic occurred in barley. Yield losses caused by BYDV ranged from 27% to 100% in the different barley varieties (Pocsai and Kobza, 1983).

Systematic work on the frequency of BYDV strains in cereals in Hungary has been in progress since 1994. Pocsai *et al.* (1995) reported that all the five BYDV strains were present in Hungary. They demonstrated that, among the BYDV strains, the PAV strain was dominant in cereals.

Several strains or serotypes of BYDV have been differentiated on the basis of vector specificity, the efficiency of transmission by aphids, and serological and molecular biological properties. Rochow (1969) differentiated four strains of BYDV by their relative vector specificity in transmission to the oat variety Coast Black and in virulence on the host plant. These strains were named according to their predominant aphid vectors: RPV was specifically transmitted by *Rhopalosiphum padi*; MAV specifically by *Sitobion (Macrosiphum) avenae*; RMV specifically by *Rhopalosiphum maidis*; and PAV non-specifically by both *R. padi* and *S. avenae*. Gill (1969) described a fifth strain in Manitoba (SGV), which was specifically transmitted by *Schizaphis graminum.* The taxonomy of BYDV strains has been modified several times since the first classification based on vector specificity (Rochow, 1969).

Pringle (1998) summarized the new taxonomic proposals approved by the Executive Committee of the International Committee on the Taxonomy of Viruses, which included proposals for the family of the *Luteoviridae*. Fauquet and Mayo (1999) gave a list of virus names and their abbreviations and assigned the family and genus to which a given virus belonged. According to this list, BYDVs consist of five viruses (BYDV-RPV, BYDV-MAV, BYDV-PAV, BYDV-RMV and BYDV-SGV) belonging to the family *Luteoviridae*. Among these, BYDV-MAV and BYDV-PAV belong to the genus *Luteovirus*. The remaining three viruses were classified as unassigned within the family

Luteoviridae. The name of the BYDV-RPV strain was changed to *Cereal yellow dwarf virus* (CYDV-RPV) and placed in the genus *Polerovirus* within the family *Luteoviridae*.

In many countries where BYDV has been studied, efforts have focused on reducing yield losses, and very little is known about the incidence and dominance of BYDV strains or the role of particular aphid vector species. The aim of the present study was to determine the presence and dominance of BYDV-CYDVs in different cereal species in Hungarian cereal growing areas.

Materials and Methods

During 1994-2001 the incidence of barley yellow dwarf viruses (BYDV-MAV, BYDV-PAV, BYDV-RMV, BYDV-SGV) and cereal yellow dwarf virus (CYDV-RPV) was studied in cereal species for the determination of strain dominance. Surveys were carried out in four different regions of Hungary (Kompolt, Martonvásár, Szeged, and Táplánszentkereszt) in cereal species showing leaf yellowing and stunting symptoms. Samples were collected in the following cereal crops: winter wheat, durum wheat, winter and spring barley, triticale, and oat.

Virus diagnosis based on leaf samples exhibiting symptoms was carried out through DAS-ELISA using antibodies against BYDV-MAV, BYDV-PAV, BYDV-RMV, BYDV-SGV and CYDV-RPV from AGDIA, and antibodies against *Wheat dwarf virus* (WDV) from Sanofi. Serological reactions were evaluated by measuring optical densities at 405 nm using a Labsystems Multiskan Plus photometer.

Results and Discussion

Incidence rates of BYDV-MAV, -PAV, -RMV, -SGV, CYDV-RPV, and WDV in winter wheat samples exhibiting symptoms collected during 1994-2001 are illustrated in Figure 1. In 1994, BYDVs were present in 9.7% and CYDV-RPV in 1.0% of winter wheat samples. The distribution of BYDVs and CYDV-RPV in infected samples of winter wheat during 1994-2001 is presented in Table 1.

As the data show, WDV occurred at the highest ratio in winter wheat with the exception of 1999. Between 1996 and 1998, the incidence of WDV increased from 24.5% to 87.9% in winter wheat samples showing symptoms. At the same time the incidence of BYDV decreased from 18.7% to 8.2%. During the study period CYDV-RPV occurred only in 1996, with an incidence of 3.3%. In 1999, the proportions of BYDV and CYDV-RPV significantly increased compared with the data of previous years. In 2000 and 2001, WDV

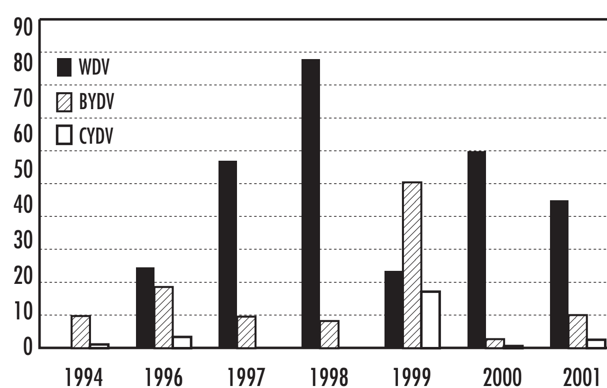


Figure 1. Incidence rates of BYDV-MAV, -PAV, -RMV, -SGV, CYDV-RPV and WDV in symptom-showing samples of winter wheat during 1994-2001.

Table 1. Distribution of BYDVs and CYDV-RPV in infected samples of winter wheat during 1994-2001.

Years	No. of samples tested	No. of samples infected by BYDV and CYDV	BYDV - MAV	BYDV - PAV	BYDV - RMV	BYDV - SGV	CYDV - RPV	Total
1994	297	32	0	17	12	1	3	33
1995	0	0	0	0	0	0	0	0
1996	240	53	19	29	20	10	6	84
1997	219	14	0	6	1	7	0	14
1998	133	11	2	9	0	0	0	11
1999	111	75	10	70	0	4	19	103
2000	150	4	3	1	0	0	0	4
2001	120	15	10	3	6	3	3	25
	1270	204	44	135	39	25	31	274

was dominant again in winter wheat samples showing symptoms. The incidence rate of BYDV and CYDV decreased to 10.0% and 2.5%, respectively. A contrasting tendency can be seen between the incidence rates of WDV and BYDV. With a rise in the incidence of WDV, the proportion of BYDV decreased, and viceversa.

The incidence rates of WDV, BYDV-MAV,-PAV,-RMV,-SGV and CYDV-RPV in symptom-showing samples of durum wheat during 1997-2001 are presented in Figure 2. BYDV occurred in varying degrees (4.0-17.7%). The highest incidence (17.7%) was detected in 1999. CYDV-RPV was only present in the last three years with a decreasing tendency (41.1–2.5%). In 1999 and 2000, the frequency of CYDV-RPV exceeded that of BYDV. Between 1997 and 2000, the incidence of WDV increased from 6% to 100%, while in 2001 it decreased to 23.75%. The distribution of BYDVs and CYDV-RPV in infected samples of durum wheat during 1997-2001 is presented in Table 2.

The incidence rates of BYDV-MAV -PAV, -RMV, -SGV, CYDV-RPV and WDV in symptom-showing samples of winter barley during 1994-2001 are illustrated in Figure 3. The incidence of BYDV was highest in winter barley compared to other cereal species. During the eight years, the incidence of BYDV varied from 5.1% to 32.4%. CYDV-RPV occurred in all years with the exception of 1998. Its incidence ranged from 0.7% to 31.7%. The incidence of WDV showed a decreasing tendency (from 58%-14.4%) from 1996 to 1999, but it increased again in 2000 and 2001. The distribution of BYDVs and CYDV-RPV in infected samples of winter barley during 1994-2001 is summarized in Table 3.

Incidence rates of BYDV-MAV, -PAV -RMV, -SGV, CYDV-RPV and WDV in symptom-showing samples of spring barley during 1999 and 2000 are presented in Figure 4. Surveys of spring barley varieties were carried out only at Táplánszentkereszt in West Hungary. BYDV strains occurred in 1999 with a high incidence (38.5%). The next year, both BYDVs and CYDV-RPV occurred at a relatively low rate (7.1% and 7.2%, respectively). The incidence of WDV varied from 2.0% to 2.7%, the lowest among the cereal species. The distribution of BYDVs and CYDV-RPV in infected samples of spring barley during 1999 and 2000 is shown in Table 4.

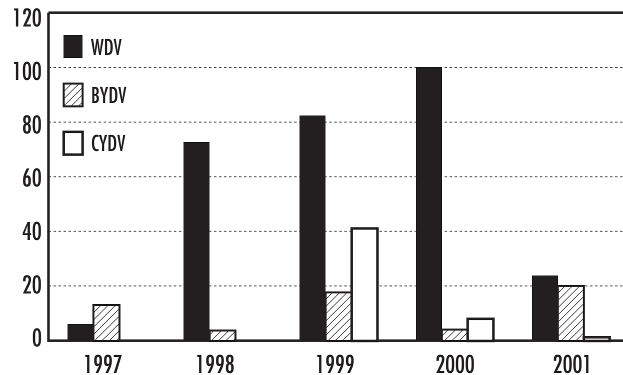


Figure 2. Incidence rates of BYDV-MAV, -PAV, -RMV, -SGV, CYDV-RPV and WDV in symptom-showing samples of durum wheat during 1997-2001.

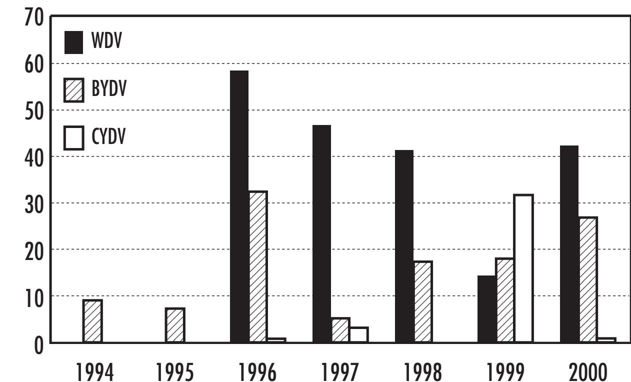


Figure 3. Incidence rates of BYDV-MAV, -PAV, -RMV, -SGV, CYDV-RPV and WDV in symtom-showing samples of winter barley during 1994-2001.

Table 2. Distribution of BYDVs and CYDV-RPV in infected samples of durum wheat during 1997–2001.

Years	No. of samples tested	No. of samples infected by BYDV and CYDV	BYDV - MAV	BYDV - PAV	BYDV - RMV	BYDV - SGV	CYDV - RPV	Total
1997	100	13	1	1	0	12	0	14
1998	80	3	1	3	0	0	0	4
1999	17	10	6	4	5	5	7	27
2000	50	6	3	0	1	4	5	13
2001	80	17	17	0	2	0	1	21
	327	49	28	8	8	18	12	74

The incidence rates of BYDV-MAV, -PAV, -RMV, -SGV, CYDV-RPV and WDV in symptom-showing samples of triticale during 1994-2001 are illustrated in Figure 5. The occurrence of BYDVs varied from 4.0% to 30.7%. CYDV-RPV was only present in 1996 with an incidence of 10%. As the data show, among the cereal species, the incidence of WDV was the highest in triticale, ranging from 80.0% to 100%. The distribution of BYDVs and CYDV-RPV in infected samples of triticale during 1994-2001 is presented in Table 5.

Spring oats were tested in only one location (Martonvásár) with a relatively low number of samples in 1996, 1999, 2000, and 2001. The incidence of WDV, BYDV-MAV, -PAV, -RMV, -SGV and CYDV-RPV in symptom-showing samples of spring oats is shown in Figure 6. In 1996, only BYDV was present in the samples of spring oats tested, and the following two years only WDV occurred. In 1996, BYDV-PAV alone was present in infected samples. In 2001, the incidence of BYDVs increased to 50%. BYDV-MAV, BYDV-RMV and BYDV-SGV were present, and BYDV-MAV was the dominant virus.

Table 3. Distribution of BYDVs and CYDV-RPV in infected samples of winter barley during 1994-2001.

Years	No. of samples tested	No. of samples infected by BYDV and CYDV	BYDV - MAV	BYDV - PAV	BYDV - RMV	BYDV - SGV	CYDV - RPV	Total
1994	119	9	0	9	1	0	0	10
1995	180	13	1	4	6	6	0	17
1996	290	96	48	4	75	50	2	179
1997	158	13	1	1	6	0	5	13
1998	150	26	0	16	11	4	0	31
1999	233	98	41	1	5	5	67	119
2000	170	42	30	5	1	1	9	46
2001	250	67	25	2	25	56	2	110
	1550	364	146	42	130	122	85	525

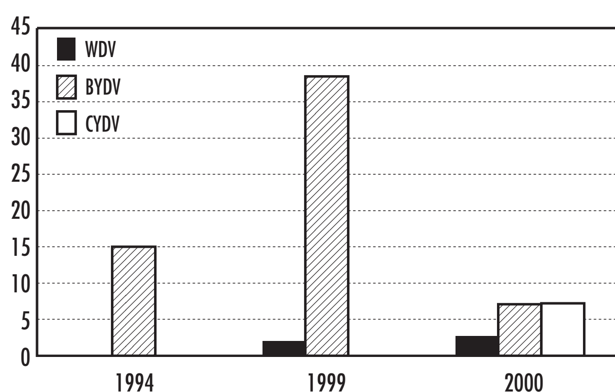


Figure 4. Incidence rates of BYDV-MAV, -PAV, -RMV, -SGV, CYDV-RPV and WDV in symptom-showing samples of spring barley during 1994-2001.

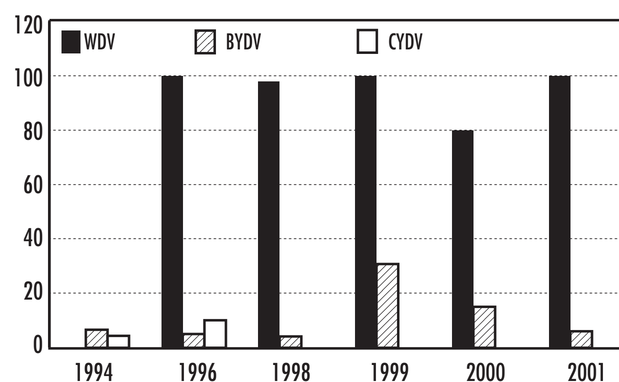


Figure 5. Incidence rates of BYDV-MAV, -PAV,-RMV, -SGV, CYDV-RPV and WDV in symptom-showing samples of triticale during 1994-2001.

Table 4. Distribution of BYDVs and CYDV-RPV in infected samples of spring barley during 1994-2000.

Years	No. of samples tested	No. of samples infected by BYDV and CYDV	BYDV - MAV	BYDV - PAV	BYDV - RMV	BYDV - SGV	CYDV - RPV	Total
1994	20	3	0	3	0	0	0	3
1999	200	77	37	50	7	10	0	104
2000	180	28	10	1	3	5	13	32
	400	108	47	54	10	15	13	139

Among the cereal species the incidence of BYDVs was the highest in spring oats, followed by spring barley and winter barley. The incidence of CYDV-RPV in cereal species was variable, the degree of infection being the highest in 1999.

In the last six years the incidence rate of WDV in winter cereal species exceeded that of BYDV. In winter wheat, durum wheat, and winter barley, the incidence of BYDV was generally higher in 1999 than that of WDV. It is possible that ecological conditions in 1999 differed from those of the previous years.

From the data of the eight-year survey, it can be concluded that the importance of WDV is increasing in cereal-producing regions of Hungary. The incidence of WDV was higher in winter cereal species than in spring ones. The highest degree of infection was detected in triticale. The results obtained for cereal species showing symptoms in the four regions demonstrate that virus incidence varied in each region and for each cereal species over the eight years.

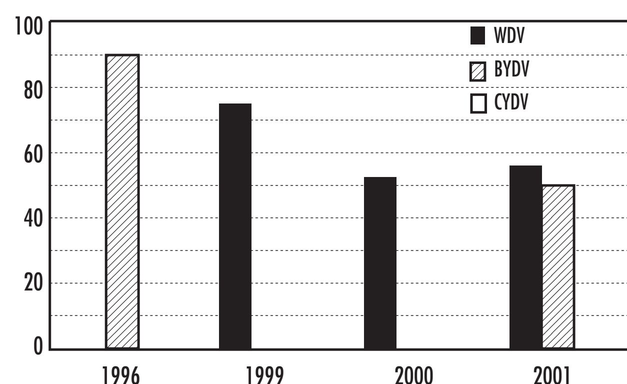


Figure 6. Incidence of BYDV-MAV, -PAV, -RMV, -SGV, CYDV-RPV and WDV in symptom-showing samples os spring oats during 1996-2001.

Table 5. Distribution of BYDVs and CYDV-RPV in infected samples of triticale during 1994–2001.

Years	No. of samples tested	No. of samples infected by BYDV and CYDV	BYDV - MAV	BYDV - PAV	BYDV - RMV	BYDV - SGV	CYDV - RPV	Total
1994	92	10	0	9	1	0	0	10
1996	20	3	1	1	0	1	2	5
1998	50	2	0	2	0	0	0	2
1999	13	4	0	3	0	1	0	4
2000	40	6	0	2	4	0	0	6
2001	50	3	2	0	0	1	0	3
	265	28	3	17	5	3	2	30

References

Fauquet, M.C., and M.A. Mayo. 1999. Abbreviation for plant virus names-1999. *Arch. Virol.* 44:1249-1273.

Gill, C.C. 1969. Annual variation in strains of barley yellow dwarf virus in Manitoba and the occurrence of greenbug-specific isolates. *Can. J. Bot.* 47:1277-1283.

Hewings, A.D., and C.E. Eastman. 1995. Epidemiology of barley yellow dwarf in North America. In: D'Arcy, C.J. and P.A. Burnett (eds.). *Barley Yellow Dwarf: 40 Years of Progress.* APS Press, St. Paul, Minnesota. pp. 75-106.

Milinkó, I., P. Nagy, Z. Rakk, and M. Dezséry. 1984. Elõzetes közlemény egy hazánkban új kukorica patogén vírusról. *Növényvédelem* 8:350-352.

Oswald, J.W., and B.R. Houston. 1951. A new virus disease of cereals, transmissible by aphids. *Plant Dis. Rep.* 35:471-475.

Pocsai, E., and S. Kobza. 1983. Epidemiological occurrence of barley yellow dwarf virus in Hungary. *Proceedings of the International Conference of Integrated Plant Protection.* pp. 50-57.

Pocsai, E., I. Kiss-Simonné, Z. Basky, and M. Dezséry. 1985. Árpa sárga törpeség virus fellépése rizsen. *Abstr. Növényvédelem* 7:308.

Pocsai, E., G. Kovács, I. Murányi, Á. Orosz, M. Papp, and L. Szunics. 1995. Differentiation of barley yellow dwarf luteovirus serotypes infecting cereals and maize in Hungary. *Agronomy* 15:401-408.

Pringle, C.R. 1998. Virus taxonomy. *Arch. Virol.* 143:1449-1459.

Rochow, W.F. 1969. Biological properties of four isolates of barley yellow dwarf virus. *Phytopathol.* 59:1580-1589.

Smith, H.C. 1955. Notes on new diseases in New Zealand. *Commonw. Phytopathol. News* 1:10-11.

Smith, H.C. 1957. Cereal yellow dwarf virus seen in Australia. *Commonw. Phytopathol. News* 3:10-11.

Szirmai, J. 1967. Új virusbetegség gabonaföldjeinken: A sárga törpeség. *Magyar Mezõgazdaság* 22:19.

Szunics, L., and Lu. Szunics. 1980. Vírus a búzán. *Magyar Mezõgazdaság* 35:9.

Watson, M.A., and T.E. Mulligan. 1957. Cereal yellow dwarf virus in Great Britain. *Plant Pathol.* 6:12-14.

Importance of BYDV and Its Vectors in Central Germany

A. Habekuß and E. Schliephake

Federal Centre for Breeding Research on Cultivated Plants, Institute of Epidemiology and Resistance, Aschersleben, Germany

The cereal viruses of the family *Luteoviridae* can cause considerable yield losses in the central regions of Germany especially in winter barley but also in winter wheat, depending on climatic conditions, vector incidence, and cultivation practices. In the last ten years, investigations were carried out to study the incidence of the *Barley yellow dwarf viruses* (BYDV-PAV, -MAV, -RMV) and the *Cereal yellow dwarf virus* (CYDV-RPV). For this purpose, plants were collected from cereal fields in autumn and spring. The main emphasis was on winter cereals. Transmission tests with *Rhopalosiphum padi*, *Sitobion avenae*, *Rhopalosiphum maidis*, and *Metopolophium dirhodum* aphids and serological tests (DAS-ELISA) were used for the identification of the different virus strains or viruses. The attack by *Wheat dwarf mastrevirus* (WDV) was analyzed using ELISA. This virus causes symptoms quite similar to those of BYDV/CYDV.

The incidence of BYDV predominated over that of WDV in 1995, 1997, 1998, 2001, and 2002, whereas most samples collected in 1994, 1999, and 2000 were infected with WDV. In all test years, BYDV-PAV was the predominant BYDV-strain. We found BYDV-MAV in only one sample of 1992. BYDV-RMV and CYDV-RPV were not observed in the geographical region around Magdeburg/Aschersleben. In about 15% of the samples a mixture of PAV/MAV was analyzed.

Beginning more than 15 years ago, comprehensive observations were done to gather information on the importance of different aphid species. A suction trap type Rothamsted and yellow pans were used to collect the aphids. *Rhopalosiphum padi* and *S. avenae* are the main aphid vectors for the BYDVs and CYDV. They were found in rates of 41% and 5% (mean values), respectively, of trapped individuals over the last 14 years. The highest number of *S. avenae* individuals were recorded during the summer period; in autumn only single individuals were detected. *Rhopalosiphum padi* showed a first peak of flying individuals also in summer, but in most years the highest flight activity occurred between September and early November. The majority of this autumnal flight is the migration of the gynoparae to the primary host. These individuals are thus not important for barley yellow dwarf epidemiology; however, the remaining number of virgins are of importance for virus transmission.

Incidence of *R. maidis* was very rare in the suction trap. It seems that this species possesses only a low importance for the virus epidemiology in our region.

There was good correspondence between virus infestation in winter/spring time over the last ten years and the number of days with temperatures lower than -5 °C during the November to April period.

Report on Barley Yellow Dwarf Disease Incidence in Latvia

M. Bisnieks,[1,2] A. Kvarnheden,[3] and R. Sigvald[2]

[1] Department of Plant Biology and Plant Protection, Latvia University of Agriculture, Jelgava, Latvia
[2] Department of Ecology and Crop Production Science, Swedish University of Agricultural Sciences, Uppsala, Sweden
[3] Department of Plant Biology, Swedish University of Agricultural Sciences, Uppsala, Sweden

The epidemiology of world-distributed barley yellow dwarf (BYD) disease has previously not been studied in Latvia, Baltic region. Thus a qualitative and quantitative approach was undertaken to investigate the status of BYD disease in this area. Field surveys were started to determine BYD incidence in spring cereals in 1999. Symptomatic leaves of spring barley and oats were sampled and assayed with triple antibody sandwich-enzyme linked immunosorbent assay (TAS-ELISA) to confirm the presence of the virus. Isolates of PAV, MAV, and RPV were detected from collected samples. In 2001 BYD disease was observed and detected by TAS-ELISA in samples from all 111 spring cereal fields surveyed. Preliminary results revealed BYDV-MAV was the most prevalent strain found in spring cereals in 2000 and 2001. The coat protein gene was sequenced for five isolates of both BYDV-PAV and BYDV-MAV. Sequence comparisons show that there is a low level of genetic diversity for BYDV-MAV, but that distinct BYDV-PAV variants are occurring. This study is part of a project aimed at providing knowledge for assessing the risk of BYD disease spread and the need to control aphid vectors. Preliminary data obtained suggest that BYD disease is sporadic, but potentially an important virus disease in cereal production in Latvia.

Direct measurements of barley yellow dwarf (BYD) disease incidence are not easy to obtain, as the newly infected plants are usually symptomless (Kendall and Chinn, 1990), and expression of disease symptoms in plant tissue occurs several weeks after infection (Burgess *et al*. 1999). Considering forecasting and control measures for viruses causing BYD disease, it is important to identify the initial sources of infection from which the virus spreads into or within a crop. The known host range includes more than 150 species of the Poaceae family (D'Arcy, 1995) that provide a reservoir for virus infection. However, most grasses are tolerant and do not show obvious definitive symptoms of BYD disease; thus laboratory methods for detecting the virus and appropriate sampling techniques for estimating field infection levels are required. This report is based on preliminary data on BYD, a potentially severe disease for cereal production in Latvia. Large, widespread patches with BYD symptoms were visually observed and serologically identified as BYD disease in spring cereals in 1999. Field surveys of BYD disease incidence in spring cereals and pastures were initiated in summer of 2000. The objective is to measure the incidence of BYD disease in spring cereals and pastures, identify the prevalent virus strains, and relate that with other factors such as vector occurrence, plant development stage, and distance to grasslands.

Materials and Methods

Field surveys

Field surveys of BYD disease incidence and prevalent virus strains were carried out in spring barley (*Hordeum vulgare*) and oat (*Avena sativa*) fields in regions of Cesis and Saldus in 2000. A few spring cereal fields were surveyed in 1999, and also a few pastures in 2000. In 2001, 50 spring cereal fields were assessed for BYD, number of vectors, plant growth stage, and distance to grasslands in each of the Cesis and Saldus regions and 11 fields in the Jelgava region. Symptomatic leaf samples of barley and oats were collected from commercial plantings, inserted into plastic bags, and kept on ice during transport to the laboratory of Department of Plant Protection, Latvia University of Agriculture in Jelgava, where they were put into a freezer at –20°C until tested by ELISA.

Leaf samples of six of the most common grass species (*Phleum vulgare*, *Lolium perenne*, *Poa pratensis*, *Dactylis glomerata*, *Festuca pratense* and *Bromus inermis*) in pastures and meadows were sampled from the experimental sites of breeding stations near stationary suction traps. Sampling strategy for estimating virus incidence in pastures involved collecting six plants of each 5 to 6 grass species at 10 positions along a diagonal in each field.

TAS-ELISA test

Leaf samples were assayed for the presence of BYDV-PAV, -MAV and -RPV using triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) (D'Arcy *et al.*, 1992), with the modification that alkaline phosphatase conjugates were incubated at 37˚C for 2 h. The tertiary antibody used in TAS-ELISA was rabbit anti-rat IgG conjugated to alkaline phosphatase (Sigma). Leaf samples were tested after storage at –20˚C for one day to several months. For the test, approximately 0.1 g of leaf tissue was extracted in a leaf extraction press with 0.5 ml of phosphate-buffered saline.

Samples were assayed by using polyclonal antibodies for trapping and monoclonal antibodies for detection of the PAV, MAV and RPV serotypes in Nunc Microtitre plates (Nunc Immunoplate Maxisorp™). Samples with absorbance at 405 nm, greater than twice the mean absorbance of 8 control wells, were considered positive.

IC-RT-PCR

To analyze the nucleotide sequence similarity of the BYDV-PAV and BYDV-MAV isolates, portions of the coat protein gene were amplified by immunocapture-reverse transcription- polymerase chain reaction (IC-RT-PCR). The primers Lu1 and Lu4 (Robertson *et al.*, 1991) were used for amplifying BYDV-PAV isolates, and four new primers were designed for amplifying BYDV-MAV isolates. The coat protein regions were sequenced after cloning from PCR products of five isolates of each serotype.

Results

Isolates of BYDV-PAV, -MAV and CYDV–RPV have been identified as causal agents of BYD disease in Latvia. Preliminary data show that the BYDV-MAV serotype was most often detected in samples collected in spring cereals and grasses in 2000 and 2001. Primary spread of BYD disease was found by visual observation in all 111 spring cereal fields surveyed in 2001; virus presence in symptomatic leaf samples were confirmed serologically by TAS-ELISA tests. Investigations on the frequency of these viruses in experimental sites of breeding stations and incidence in fodder grass species in different regions revealed a range between 0 and 7% for PAV, and 0 to 45% for MAV serotypes. CYDV-RPV has been found in barley, but was rarely detected.

Among isolates of each serotype collected in 2000 and 2001, a high level of nucleotide sequence similarity was observed for the coat protein region. The sequenced regions of the viruses were in general more than 98% identical. However, two variants of one BYDV-PAV isolate showed only 90% identity to the other BYDV-PAV isolates.

Concluding Remarks

Sequence comparisons showed that there is a low level of genetic diversity for BYDV-MAV, but that different distinct BYDV-PAV variants are occurring.

Further field surveys are planned to study the importance of common grass species at pastures as virus reservoirs. To obtain data for virus spread risk assessment and model building such variables as region, field size, crop, crop development stage, number of aphid-vectors, and distance to grasslands were identified and will be analyzed by logistic regression.

Acknowledgment

This work is part of a project supported by Royal Swedish Academy of Agriculture and Forestry.

References

Burgess, A.J., Harrington, R., and Plumb, R.T. 1999. Barley and cereal yellow dwarf virus epidemiology and control strategies. In: *The Luteoviridae*. Smith, H.G., and Barker, H. (eds.). CAB International. pp 211-279.

D'Arcy, C.J. 1995. Symptomatology and host range of barley yellow dwarf virus. In: *Barley Yellow Dwarf: 40 Years of Progress*. D'Arcy, C.J., and Burnett, P.A. (eds.). APS Press, St. Paul, MN, USA. pp 9-28.

D'Arcy, C.J., Hewings, A.D., and Eastman, C.E. 1992. Reliable detection of barley yellow dwarf viruses in field samples by monoclonal antibodies. *Plant Disease* 76:273-276.

Kendall, D.A., and Chinn, N.E. 1990. A comparison of vector population indices for forecasting barley yellow dwarf virus in autumn sown cereal crops. *Annals of Applied Biology* 116:87-102.

Robertson, N.L., French, R., and Gray, S.M. 1991. Use of group-specific primers and the polymerase chain reaction for the detection and identification of luteoviruses. *Journal of General Virology* 72:1473-1477.

Barley Yellow Dwarf Virus in Argentina

G. Truol

Instituto de Fitopatología y Fisiología Vegetal (INTA IFFIVE), Córdoba, Argentina

Argentina produces 15.5 million tons of wheat on 6.3 million hectares distributed over five different ecological as well as marginal regions. Among the virus diseases that attack wheat, BYDV (*Barley yellow dwarf virus*), BSMV (*Barley stripe mosaic virus*) and MRCV (*Mal de Rio Cuarto virus*) are the most important economically. BYDV was first identified in the country in 1964 mainly on wheat, oat, and barley. In 1995, MRCV was detected in Manfredi (Province of Cordoba) on maize crops in mixed infections with BYDV that caused symptoms such as reddening and enations on leaves. A severe attack of BYDV on triticale in Jesus Maria (Province of Cordoba) was recorded in 1997, and its presence on rice was also suspected. Barley is often affected by BYDV and BSMV, mostly when vector population is high.

The exploration of weeds as possible reservoirs of BYDV determined that *Digitaria sanguinalis* (annual species) presents the highest percentage of plants with the virus, followed in decreasing order of importance by *Paspalum notatum* and *P. dilatatum* (both perennial). The predominant strains of the virus in the weeds were PAV, RPV, and RMV, which could be related to the abundance of the aphid *Rhopalosiphum padi,* found to be an efficient vector of those strains in our transmission studies.

The incidence of the disease was 6-37%. The maximum value was detected in Roque Sáenz Peña, a marginal area in the Province of Chaco. This situation is probably related to climatic conditions that favor the aphid vector's activity. Serological tests (DAS and TAS ELISA) done on samples collected during surveys demonstrated the existence of five BYDV strains (PAV, MAV, SGV, RPV, and RMV) in all areas sampled in different years. The PAV strain was the most frequent in the study areas. In the marginal region, high frequency of mixed infections by different combinations of strains was detected. It was possible to isolate and to maintain in culture several PAV-like isolates, including several from Marcos Juárez (Province of Cordoba) and Roque Sáenz Peña (Province of Chaco), plus one RPV-like isolate from the Province of Cordoba and a mixed PAV+RPV infection (MIX), also from Cordoba.

In artificial infections, the damage caused by BYDV (local strain MIX = PAV+RPV) on cultivar Prointa Isla Verde was severe (a 66% yield reduction).

Characterization studies showed that local isolates with successful multiplication are in general quite similar to those described by other authors, though there are small differences probably due to local traits related to the epidemiology of the disease. The more notable difference was observed in the symptoms produced by the PAV-like strain, which among all the studied strains (RPV-like and MIX) turned out to be the least severe, although its virus concentrations were higher. Also, it was corroborated that symptom severity can vary according to the isolates; this was evident with the Roque Sáenz Peña isolate of the PAV-like strain that turned out to be the most severe.

Serology studies carried out via DAS ELISA and TAS ELISA with foreign antisera to determine the serological characteristics of the test isolates revealed that the local strains that were multiplied reacted only with their homologous antibodies, which was consistent with the results obtained using monoclonal antibodies in TAS ELISA. The MIX isolate reacted to PAV and RPV with both polyclonal and monoclonal antisera, and proved to be a mixture of strains of groups 1 and 2.

After the PAV-like isolate Marcos Juárez was purified, it was determined that to achieve higher virus concentrations in the purification process, plant materials should have been harvested 15 days after inoculation.

Also, an enzymatic treatment should be included in the processing of the material (SOLVAI, mix of pectinolithic and celulolithic enzymes, 1/1 v/v) to liberate a greater number of virus particles into the phloem, thereby improving final performance in mg/ml (virus concentration). A local antiserum of the PAV-like strain was obtained, with title 1:32.000 for NCM ELISA (with adsorption of normal plant proteins), 0.1ug/m IgG for DAS ELISA, and 1/1600 of the conjugated antibody (with adsorption of normal proteins).

The migration pattern of the replicative forms of the BYDV PAV-like strain was determined, and the molecular weight of the dsRNA's genomic fragments estimated. They proved to be similar to those of group 1 strains (foreign) in regards to the first and second band. The fifth band was not observed. Likewise, a small difference in the migration distance of the second band, and thus in its molecular weight, was detected, but it is not sufficiently significant to categorize this strain within another group.

Using electronic microscopy, three phases were observed in the evolution of the disease: premature, intermediate, and late. Cytopathological alterations of local isolates were similar to those described by other authors for groups I and II of BYDV. Nonetheless, infections with the PAV isolate Roque Sáenz Peña showed vesicles with double membrane, a cytopathology similar to what is common in specific isolates.

Virus concentration of the above mentioned isolates does not seem to be related to symptom severity. The local isolates turned out to be different among themselves, in terms of virus concentration and the moment they manifest peaks. The strain with the highest concentration was PAV-like, followed in order of importance by RPV and MIX. In relation to this last, the interaction between PAV + RPV had a synergistic effect that caused greater symptom severity in both wheat and oat.

Studies on variation of the relative density of main vector species (*Rhopalosiphum padi, R. maidis, Schizaphis graminum, Metopolophium dirhodum*, and *Sitobion avenae,* among others) determined that density increases when the weekly mean temperature is higher than 11˚C and relative humidity is high. This allowed us to recommend modifying the sowing date as a measure to escape the disease.

Estimating Yield Losses in Cereals Infected with *Barley Yellow Dwarf Virus*

K.M. Makkouk and W. Ghulam

Virology Laboratory, Germplasm Program, International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria

Using a prediction model to estimate yield losses due to barley yellow dwarf virus (BYDV, family Luteoviridae*) infection, thus avoiding the need for a healthy control, could facilitate germplasm screening and reduce evaluation costs. In the 1999/2000 growing season, 69 barley, 58 spring and winter/facultative bread wheat, and 39 durum wheat lines derived from the ICARDA (barley) and CIMMYT/ICARDA (wheat) improvement programs were evaluated for their resistance/tolerance to BYDV in artificially inoculated plots. Similarly, in the 2000/2001 growing season, 24 barley, 45 spring and winter/facultative bread wheat, and 31 durum wheat lines were evaluated. Many of these lines yielded well in response to infection when compared with control plots (non-inoculated and protected by insecticide treatment). A model developed earlier for spring bread wheat in Canada to predict yield losses due to BYDV infection based on symptom score (0-9) and plant height (cm) [predicted yield index (PYI) = 600 (0.2 – symptom score/height)] was evaluated. Experiments conducted during 1999/2000 indicated that correlation between PYI and percent yield loss was r= –0.665, –0.080 and –0.304 for barley, bread wheat, and durum wheat, respectively. In the 2000/2001 trials, correlation between PYI and percent yield loss was –0.88, –0.31 and –0.41, for the three crops respectively. It can be concluded that this prediction model is reliable for BYDV screening in barley but less so in bread or durum wheat, under growing conditions in northern Syria.*

To assess yield losses in cereal crops infected with barley yellow dwarf virus (BYDV), researchers used a few criteria such as symptomatology, grain yield, spikes weight, biomass, harvest index, and plant height, or a combination of more than one factor (Comeau *et al*., 1989; Cheour *et al*., 1989). The more precise measure to estimate yield loss is by comparing BYDV-infected plots with appropriate healthy controls. However, such assessment is costly in terms of space and labor. Thus it would be useful to develop a reliable assessment model that does not require healthy control plots. Haber *et al.* (1997) developed a yield prediction model for spring bread wheat following BYDV infection. In this study we evaluated the usefulness of this model for predicting yield losses in response to BYDV infection in barley, bread and durum wheat under growing conditions in northern Syria.

Materials and Methods

During the 1999/2000 and 2000/2001 growing seasons, selected barley, bread wheat and durum wheat lines derived from ICARDA (barley) and CIMMYT/ICARDA (wheat) materials were planted in small plots with 4x1 m rows. For each entry, one plot was BYDV-inoculated and another one was kept as healthy control. BYDV-inoculation was done through viruliferous *Rhopalosiphum padi*, using 10-15 aphids per plant. The virus species used was BYDV-PAV (BYDV-PAV, genus *Luteovirus*, family *Luteoviridae*). The control plots were separated from the inoculated plot by a 5-m buffer zone, and cereal seeds were treated with imidacloprid (Gaucho®) before planting. In addition, control plots were sprayed three times with a selective aphicide (Pirimicarb), starting two months after seed germination. Control plots remained symptomless until May, and all plots were harvested in June.

Results and Discussion

Several barley and wheat lines derived from the breeding projects were found to be tolerant to BYDV infection and yielded well in comparison to healthy (insecticide-protected) controls (Table 1). Yield loss data for entries evaluated in the two seasons were more-or-less comparable. Correlation between the predicted yield index and yield loss in barley was highly significant (P=0.01) in both years (Table 2), more significant than that between disease score and yield loss. In durum and bread wheat, the correlation

Table 1. Performance of selected barley lines evaluated for their reaction to barley yellow dwarf virus (BYDV) infection during two growing seasons after artificial inoculation with the virus, based on symptom disease score, biomass, plant height, grain weight, predicted yield index, and grain yield loss.

Entry	Disease score	Biomass	Height (cm)	Grain weight	PYI	Yield loss (%)
1999/2000 growing season						
BIT-98-403	5.0	284	68	152	76	1.8
BIT-98-451	5.0	338	78	171	81	4.2
BPT-98-104	6.0	348	63	137	62	4.0
BPT-98-171	5.0	332	70	157	77	0.0
SCR-98-188	6.0	351	53	163	51	11.3
SCR-98-204	6.0	385	55	160	55	0.0
WBCB-98-183	5.5	302	63	124	67	19.0
WBCB-98-24	2.0	349	73	167	104	0.1
WBCB-98-52	3.0	356	70	167	94	0.0
HBON-98-16	5.0	275	65	108	74	0.7
HBON-98-18	6.0	226	60	94	60	18.6
IFBYT-98-17	4.0	352	73	160	87	23.6
IWBYT-97-6	4.0	267	68	129	84	29.9
IWBYT-98-17	4.5	281	63	135	77	0.0
RWA-BA-31	4.0	301	70	122	86	16.3
WSS-BA-13	4.0	309	65	136	83	0.8
Corris	4.0	447	70	194	86	0.6
BQ-813.2	5.0	361	78	163	81	0.2
BON-MRA-89-4	5.0	335	70	152	77	9.3
Sutter	4.0	284	70	113	86	11.4
Abee	8.0	155	43	44	7	49.2
Cyclon	9.0	29	18	7	-188	96.2
2000/2001 growing season						
OF2-1-P1	3.0	709	103	384	102	31.5
Sutter//Sutter*2/Numar	2.5	598	108	226	106	36.4
Gustoe/Nk 1272	2.0	787	108	332	109	30.4
OF2-19-P2	2.5	524	100	263	105	42.6
IBSCGP2000-13	3.0	745	105	370	103	14.6
IBSCGP2000-21	3.0	694	110	268	104	9.3
IBPMGP2000-4	4.0	598	103	328	97	17.1
RWA-Ba-99-15	6.5	565	90	211	77	36.1
RWA-Ba-99-32	4.0	751	88	391	93	21.2
Corris	5.0	534	98	346	89	2.7
BKL-85-273	3.5	736	113	307	101	22.5
BQ-813.2	2.5	699	115	360	107	23.8
BQCB-10	3.0	617	98	321	102	11.0
Sutter	2.0	809	118	312	110	0.7
Wysor	3.0	801	103	408	102	11.3
Alanda-01	9.0	223	53	112	17	80.2
Cyclon	9.0	154	38	27	-24	91.8

PYI= 600*[0.2-(symptom/height)], where symptom (0-9) is the visual symptom score, and height is determined (in cm) for BYDV-infected plants.

BIT= Barley Initial yield Trial, SCR= Syrian and Jordanian landraces, WBCB= Winter Barley Crossing Block, HBON= Winter & Facultative Hulless Barley Observation Nursery, RWA= Russian Wheat Aphid Nursery, WSSF= Wheat Stem Sawfly Nursery, IWBYT & IFBYT = International Winter & Facultative Barley Yield Trials, IBPMGP= International Barley Powdery Mildew Germplasm Pool, IBSCGP=International Barley Scald Germplasm Pool, BKL= Barley Key Location disease nursery.

between the PYI and yield loss was not significant in the first year (r=–0.30 and –0.08, respectively), but significant (P=0.05) in the second year (r=–0.41 and –0.31, respectively). It can be concluded that the prediction model was reliable for screening barley lines for BYDV resistance, and less so for durum or bread wheat. It is interesting to note that the adopted prediction model found earlier to be a reliable predictor of spring wheat yield in Canada (Haber *et al*., 1997) was not reliable for predicting spring wheat yield in northern Syria under artificial BYDV-PAV inoculation conditions.

Table 2. Correlation coefficient (r) between grain yield loss (%) and the predicted yield index in barley, durum wheat and bread wheat artificially inoculated with barley yellow dwarf virus (BYDV) in trials conducted during two growing seasons.

Growing season	Crop	Lines tested (no.)	Correlation coefficient (r)
1999/2000	Barley	69	-0.665**
	Durum wheat	39	-0.304
	Bread wheat	58	-0.080
2000/2001	Barley	23	-0.882**
	Durum wheat	31	-0.414*
	Bread wheat	45	-0.311*

** Significant at P=0.01, * Significant at P=0.05.

Growing conditions during the two growing seasons varied, and that may have influenced measurement of the different factors evaluated. For example, annual rainfall during 1999/2000 was 260.7 mm, whereas during 2000/2001 it reached 428.6 mm. This increase in rainfall resulted in a significant increase in biomass, height, and grain weight, but had less impact on disease score. However, the effect on yield loss values was small, since these values were derived after comparison with a healthy control, which was also affected by rainfall. Nevertheless, the differences in conditions between the two growing seasons could explain why the correlation between PYI and yield loss was not significant for durum and bread wheat in the first season and significant in the second season.

References

Cheour, F., A. Comeau, and A. Asselin. 1989. Genetic variation for tolerance or resistance to barley yellow dwarf virus in durum wheat. *Euphytica* 40:213-220.

Comeau, A., J. Collin, and F. Cheour. 1989. Barley yellow dwarf virus symptoms and ELISA data in relation to biomass and yield loss. In: Barley yellow dwarf in West Asia and North Africa. A. Comeau and K.M. Makkouk (eds.). ICARDA, Aleppo, Syria.

Haber, S., R.I.H. Mckenzie, and A. Comeau. 1997. Barley yellow dwarf virus (BYDV) tolerance in wheat as a predictor of yield. *Canadian Journal of Plant Pathology* 19:324.

Spring Oat and Soft Winter Wheat Lines with BYDV Resistance

H. Ohm, J. Anderson, H. Sharma, L. Ayala-Navarrete, and D. Bucholtz

Purdue University and USDA-ARS, West Lafayette, Indiana, USA

One of the overall goals of the Purdue University-USDA/ARS Small Grains Research Program is to develop soft winter wheat (*Triticum aestivum*) and spring oat (*Avena sativa*) germplasms with resistance to BYDV, with emphasis on strains PAV and RPV. In oat, resistance of a number of BYDV resistance/tolerance donor lines, mostly *A. sativa*, and at least one accession of *A. sterilis* have been combined, resulting in effective resistance. In wheat, we have developed a number of translocation lines in which parts of chromosome 7E of *Thinopyrum intermedium*, including resistance to BYDV, have replaced parts of chromosome 7D.

BYDV symptom scores in oat cultivars INO9201, Classic, Jay, and susceptible check Clintland 64 are typically 5, 3, 4, and 9, respectively (0-9 scale; 0 = no symptoms to 9 = severe plant stunting and leaf discoloration) in tests involving inoculation in early seedling growth stages. We have advanced oat lines that typically develop symptom scores of 2 to 2.5 in similar tests. We think it will be difficult to develop oat lines with BYDV resistance from within *A. sativa* resulting in BYDV scores less than 2, which can lead to production loss under severe BYDV epidemic conditions. Thus, it would be useful to transfer BYDV resistance from other species, like *A. strigosa*, or other more unrelated donor species.

We have transferred BYDV resistance from a vegetative clone of *Th. intermedium*, cv. Oahe, into soft winter wheat. We have released a wheat substitution germplasm line, P29, in which chromosome 7D was replaced by chromosome 7E (Sharma *et al.*, 1997; Crasta *et al.*, 2000), and are releasing a wheat translocation line, P98134, selected from progeny of irradiated F_1 seeds of the cross P29/Caldwell (Sharma *et al.*, 2002). The BYDV resistance of P98134 is subterminal on the long arm of 7E (Crasta *et al.*, 2000). BYDV resistance of the TC wheat lines developed in Australia (Banks *et al.*, 1995; Hohmann *et al.*, 1996) is derived from the addition line L1 (Cauderon *et al.*, 1973), and the gene conditioning this BYDV resistance was designated *Bdv2* (McIntosh *et al.*, 1998). Of the several TC lines, TC14 was reported to have the smallest segment of 7X (Banks *et al.*, 1995). Zhang *et al.* (1999) reported the development of a BYDV resistant wheat translocation line derived from TC14 that has an even smaller segment of 7X than TC14. It has been reported that L1 has chromosome 7St from *Th. intermedium* and P29 has chromosome 7E (Crasta *et al.*, 2000). We have developed a number of soft winter wheat lines derived from the translocation lines, that have BYDV resistance from 7E, as determined by DNA markers, ELISA, and field tests. They also have quite good agronomic characteristics and are in performance trials for the first time in 2002. Grain from these trials will also be milled for pastry quality testing.

Chromosomes 1 and 2 of certain *Thinopyrum* spp. also carry resistance to certain strains of BYDV (Larkin *et al.*, 1995a, 1995b; Zhang *et al.*, 2001) and it is likely necessary that resistance QTL of two or more chromosomes be pyramided to achieve resistance to multiple strains of the virus (Francki *et al.*, 2001). Our results show that BYDV resistance of chromosomes 1 of *Th. pulcherimum* and 2 of *Th. intermedium* are additive. However, our preliminary results show that resistance of chromosomes 1 or 2, in hemizygous condition, do not result in lower ELISA values when in combination with 7E compared with 7E alone.

References

Banks, P.M., Larkin, P.J., Bariana, H.S., Lagudah, E.S., Appels, R., Waterhouse, P.M., Brettell, R.I.S., Chen, X., Xu, H.J., Xin, Z.Y., Qian, Y.T., Zhou, X.M., Cheng, Z.M., and Zhou, G.H. 1995. The use of cell cultures for subchromosomal introgressions of barley yellow dwarf virus resistance from *Thinopyrum intermedium* to wheat. *Genome* 38:395-405.

Cauderon, Y., Saigne, B., and Dauge, M. 1973. The resistance to wheat rusts of *Agropyron intermedium* and its use in wheat improvement. In: *Proceedings of the 4th International Wheat Genetics Symposium* (Sears, E.R. and Sears, L.M.S., eds.). pp. 401-407. University of Missouri, Columbia, USA.

Crasta, O.R., Francki, M.G., Bucholtz, D.B., Sharma, H.C., Zhang, J., Wang, R.-C., Ohm, H.W., and Anderson, J.M. 2000. Identification and characterization of wheat-wheatgrass translocation lines and localization of barley yellow dwarf virus resistance. *Genome* 43:698-706.

Francki, M.G., Ohm, H.W., and Anderson, J.M. 2001. Novel germplasm providing resistance to barley yellow dwarf virus in wheat. *Aust. J. Agric. Res.* 52:1375-1382.

Hohmann, U., Badaeva, K., Busch, W., Friebe, B., and Gill, B.S. 1996. Molecular cytogenetic analysis of *Agropyron* chromatin specifying resistance to barley yellow dwarf virus in wheat. *Genome* 39:336-347.

Larkin, P.J., Banks, P.M., and Chen, K. 1995a. Registration of six genetic stocks of wheat with rust and BYDV resistance: Z1, Z2, Z3, Z4, Z5, and Z6 disomic addition lines with *Thinopyrum intermedium* chromosomes. *Crop Sci.* 35:604.

Larkin, P.J., Banks, P.M., Lagudah, E.S., Appels, R., Xiao, C., Xin, Z.Y., Ohm, H.W., and McIntosh, R.A. 1995b. Disomic *Thinopyrum intermedium* addition lines in wheat with barley yellow dwarf virus resistance and with rust resistances. *Genome* 38:385-394.

McIntosh, R.A., Hart, G.E., Devos, K.M., Gale, M.D., and Rogers, W.J. 1998. Catalogue of gene symbols for wheat. In: *Proceedings of the 9th International Wheat Genetics Symposium.* Volume 5. Saskatoon, Saskatchewan, Canada. 2-7 August.

Sharma, H., Anderson, J., Bucholtz, D., and Ohm, H. 2002. Registration of barley yellow dwarf virus resistant wheat germplasm line P98134. *Crop Sci.* 42:(submitted).

Sharma, H.C., Ohm, H.W., and Perry, K.L. 1997. Registration of barley yellow dwarf virus resistant wheat germplasm line P29. *Crop Sci.* 37:1032-1033.

Zhang, Z., Xin, Z., Ma, Y., Chen, X., Xu, Q., and Lin, Z. 1999. Mapping of a BYDV resistance gene from *Thinopyrum intermedium* in wheat background by molecular markers. *Science in China, Ser.* C 42:663-669.

Zhang, Z.Y., Xin, Z.Y., and Larkin, P.J. 2001. Molecular characterization of a *Thinopyrum intermedium* Group 2 chromosome (2Ai-2) conferring resistance to barley yellow dwarf vurus. *Genome* 44:1129-1135.

Utilizing *Bdv2*, the *Thinopyrum intermedium* Source of BYDV Resistance, to Develop Wheat Cultivars

P.J. Larkin,[1] S. Kleven,[1] and P.M. Banks[2]

[1] CSIRO Plant Industry, Canberra, Australia
[2] Leslie Research Centre, Toowoomba, Australia

Barley yellow dwarf (BYD) is of considerable economic significance worldwide, particularly in higher rainfall regions where the aphid vector itself is problematic. Yield losses in wheat have been reported to be as high as 40-50% (El Yamani, 1990; Banks *et al*., 1995a; McKirdy and Jones 1997; Herbert *et al*., 1999; Riedell *et al*., 1999). We have sought to find sources of resistance that can be made available for wheat improvement. *Thinopyrum intermedium* (host) Barkworth and Dewey, also known as *Agropyron intermedium*, *Elytrigia intermedia*, or intermediate wheatgrass, carries BYDV resistance genes on a group 7 chromosome (7Ai1, Brettell *et al*., 1988; Friebe *et al*., 1992) and a group 2 chromosome (Larkin *et al*., 1995; Tang *et al*., 2000). The group 7 chromosome carrying BYDV resistance was first identified in a 2n=44 disomic addition line called L1; the resistance is located on the long arm (Brettell *et al.,* 1988).

Genomic *in situ* hybridization (GISH) analysis suggests that *Th. intermedium* consists of three distinguishable groups of chromosomes given genomic names J, J^s, and S (Chen *et al*., 1998). However, it remains disputed whether the L1 additional chromosome (7Ai1) should be designated 7J (Chen *et al*., 1999) or 7S (Wang and Zhang, 1996). We utilized cell culture of the monosomic addition tissues to induce recombination or translocation between the alien and wheat chromosomes (Banks *et al*., 1995). A series of 7D-7Ai1 recombinants (TC5, TC6, TC8, TC9, TC10, and TC14) and one 1BS.7Ai1L translocation (TC7) were identified (Banks *et al*., 1995; Hohmann *et al*., 1996). Also identified was another recombinant called 5395, derived from crosses involving homoeologous pairing suppression (Banks *et al*., 1995b). The TC translocations have been analyzed by GISH in a number of laboratories and found to comprise *Thinopyrum* chromatin distally on the long arm of 7D; TC14 appears to be the smallest translocation (Banks *et al*., 1995b; Hohmann *et al*., 1996). Wang and Zhang (1996) found TC14 did not involve a centromeric break and had a short distal segment of 7Ai1 terminally attached to 56% of the proximal 7DL. Perhaps surprisingly it has been possible to utilize the TC sources of *Bdv*2 in the breeding of new cultivars of wheat, both spring and winter.

In breeding *Bdv2*-carrying wheats, we have developed and utilized a molecular marker for the translocations. The BYDV bioassay in the laboratory can be reliable but is very laborious requiring the maintenance of a clean population of aphids, a pure source of virus of known serotype, antisera against that virus, and prepared ELISA plates. It takes about eight weeks and considerable labor to raise viruliferous aphids, infest individual plants with aphids, grow the plants after inoculation in appropriate controlled environments, extract leaf saps, and conduct ELISA determinations of virus titres. We have developed a sequence-characterized, PCR-based molecular marker (SCAR) for the *Bdv2*-associated translocations (Stoutjesdijk *et al*., 2001) that was used to facilitate our breeding programs.

Results and Discussion

The sequence characterized PCR marker described by Stoutjesdijk *et al.* (2001) is a dominant marker. The PCR reaction with all susceptible control wheats yielded no product. However, with wheat lines

carrying any of the *Bdv2* translocations (TC5, TC6, TC8, TC9, TC10 or TC14), a single product was amplified of the size predicted by the RAPD product sequence, namely 566 bp. Segregating families could be scored readily for the *Bdv2* segment. In addition a high throughput PCR methodology (Zhang *et al*., 2001) has been developed which allows reactions to be developed in multiwell plate format and determined using an ELISA plate reader, without the need to electrophorese the DNA on agarose gels. This *solid-phase, anchored* or *capture* PCR technology may further facilitate future incorporation of *Bdv2* into breeding programs.

CIMMYT has developed a microsatellite marker for TC14, gwm137 (Ayala *et al*., 2001) which is codominant. It has the important advantage that homozygotes can be distinguished from hemizygotes for the translocation. However, the codominant marker is unsuitable for the high throughput PCR technology described above.

Mackellar (LH64C), an Australian dual-purpose grazing winter wheat incorporates the TC14 translocation. TC14 has the pedigree Vulcan.cms // L1 / Millewa /3/ Restorer R35733. A cycle of cell culture and regeneration was imposed on the monosomic addition line hybrid L1 x Millewa and the translocation first identified following the testcross to Sunstar. Mackellar has the pedigree Tatiara / TC14 // Beaver /3/ Soisson /4/ B1073.

Glover spring wheat incorporates the TC6 translocation following backcrossing into Hartog. The pedigree of TC6 is Sunstar // L1 / Millewa. A cycle of cell culture and regeneration was imposed on the monosomic addition line hybrid L1 x Millewa and the translocation first identified following the testcross to Sunstar. The pedigree of Glover is TC6 / 5* Hartog. Hartog is a selection from CIMMYT's Pavon. The backcrosses to Hartog were made in 1990-2, and the BC_4F_{2-4} were grown with selection for low BYDV titre in each generation. F3 families were tested by virus bioassay, and families were identified as homozygous for BYDV resistance. One of these, QT8733, was selected in the field in Queensland for its high yield and is to be released as Glover. It has agronomic attributes and grain yield very similar to its recurrent parent, Hartog, suggesting that the TC6 translocation does not convey a yield nor agronomic penalty.

Milling quality (flour yield and Minolta color), dough rheology (farinograph, extensogram and mixograph), and end product evaluation (yellow alkaline noodles and pan bread) of Glover and several other BYDV resistant lines were evaluated in comparison to recurrent parent lines. The only consistent trend in milling quality of BYDV resistant lines compared to recurrent parents was an increase of approximately 1 Minolta unit of flour b* value (yellowness) (Martin and Banks, unpublished). The increase in flour color was evident in TC14-derived lines, as well as the longer translocation-derived lines. No trends were observed in dough rheological properties. In BYDV resistant lines the Minolta b* value of yellow alkaline noodles was slightly higher compared to recurrent parents, and a small increase in pan breadcrumb color was also observed. The marginal increase in yellowness of end products is consistent with the increased flour color. We understand that the small increase in flour color is acceptable for all but highly discriminating international wheat markets. Glover has been classified as suitable for inclusion in the 2nd highest class of Australian hard white export wheat, Australian Hard.

Mackellar and Glover show substantial virus resistance, though not immunity. The resistance is evident as: reduced % infection in the field; reduced average ELISA readings in the field; and reduced ELISA readings in the laboratory with controlled infections (Figure 1).

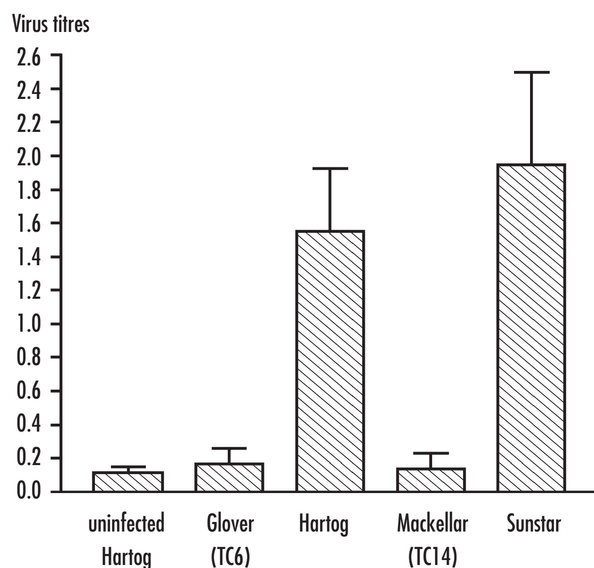


Figure 1. BYD virus titres of Glover, Mackellar and susceptible parents following controlled inoculation with PAV serotype.

Mackellar was on average 20% higher yielding across eight sites than the best of a number of current winter wheats including Declic, Currawong, Brennan, Tennant, Dennis, and Whistler (Figure 2). That experiment did not determine the extent to which the yield difference was due to the presence of BYDV. Furthermore a total of 130 winter wheat breeding lines were characterized for the segregation of *Bdv2* by PCR and their yields determined in 2000 after a season with evident widespread natural BYDV infection. Substantial yield protection was evident when *Bdv2* was present (64 lines) compared to sister lines without *Bdv2* (66 lines). This was evident when all lines were grouped (Figure 3) and also when segregants of individual families were compared. Averaged across all the lines, there was a 1.04 mt increase in yield (23%) with *Bdv2*. Results at CIMMYT have also demonstrated substantial yield protection with the TC14 translocation in a number of backgrounds using two serotypes of the virus (Ayala *et al*., 2001). On average one dose of the translocation gave a 13% increase and two doses of the translocation gave a 26% yield increase.

Trials in Queensland have established, in the apparent absence of BYDV infection, no difference in yield between Glover and its recurrent parent, Hartog [56 years*sites; 8 years*regions]. Glover has also been classified as tolerant in the field to root lesion nematode (RLN) *Pratylenchus thornei*. In addition it was classified as moderately resistant to RLN in replicated glasshouse trials conducted over two years; it had a similar level of resistance to Gatcher Selection 50a (Thompson *et al*., 2000, Thompson *et al*., 2001). It remains to be confirmed if this enhanced RLN resistance is associated with translocation TC6.

The 5395 translocation originally appeared to be a translocation because a red coleoptile marker on the short arm of 7Ai1 was missing (Banks *et al*., 1995). Despite this, Hohmann *et al.* (1996) presented some evidence suggesting it was a whole chromosome substitution. Xin *et al.* (2001) have now taken 5395 through further crosses to produce lines designated Yw642,Yw443, and Yw243. Molecular analysis of these lines indicated the presence of a 7Ai1 translocation smaller than TC14. Molecular mapping indicated the breakpoint was between Xpsr965 and Xpsr680, about 90cM from the 7D centromere, whereas the TC14 breakpoint was near Xpsr129 at about 45 cM from the centromere. It may be that there has been further recombination to yield the smaller translocation.

Acknowledgments

ACIAR (Australian Centre for International Agricultural Research) generously supported the development of *Bdv2* resistance germplasm. The germplasm was developed with our colleagues in the Chinese Academy of Agricultural Sciences, Institute of Crop Breeding and Cultivation [Xin Zhiyong, Zuang Qiaosheng, Chen Xiao, Xu Shujun, Xu Huijun], and the Institute of Plant Protection [Zhou Guanghe, Cheng Zhuomin, Qian Youting, Zhou Ximing]. GRDC (Grains Research and Development Corporation) supported the initial stages of breeding.

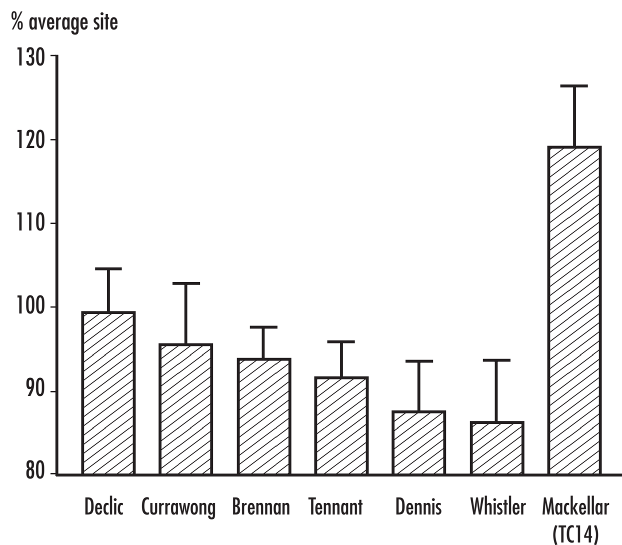


Figure 2. Long season wheats - 2000 data.
LH64C BYDV resistant line (8 sites, triplicate plots)

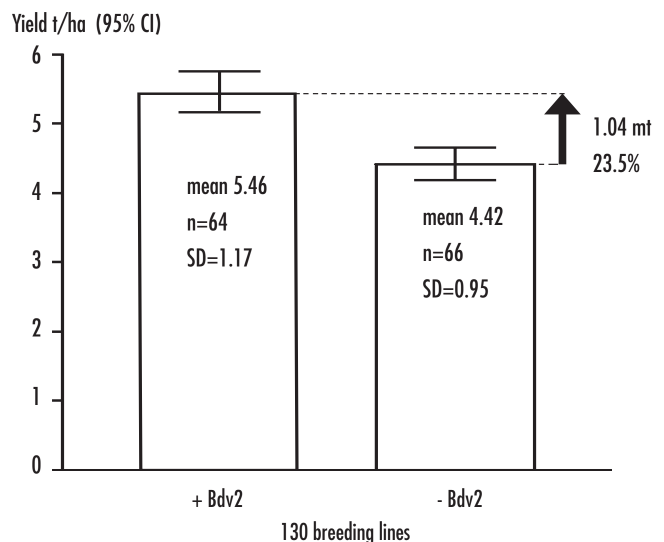


Figure 3. Yield of 130 breeding lines with or without *Bdv2* in presence of natural BYDV infection during 2000.

References

Ayala, L., van Ginkel, M., Khairallah, M., Keller, B., and Henry, M. 2001. Expression of *Thinopyrum intermedium*-derived Barley yellow dwarf virus resistance in elite bread wheat backgrounds. *Phytopathology* 91:55-62.

Banks, P.M., Davidson, J.L., Bariana, H., and Larkin, P.J. 1995a. Effects of Barley Yellow Dwarf Virus on the yield of winter wheat. *Australian Journal of Agricultural Research* 46:935-946.

Banks, P.M., Larkin, P.J., Bariana, H.S., Lagudah, E.S., Appels, R., Waterhouse, P.M., Brettell, R.I.S., Chen, X., Xu, H.J., Xin, Z.Y., Qian, Y.T., Zhou, X.M., Cheng. Z,M,, and Zhou, G.H. 1995b. The use of cell culture for sub-chromosomal introgressions of barley yellow dwarf virus resistance from *Thinopyrum intermedium* to wheat. *Genome* 38:395-405.

Brettell, R.I.S., Banks, P.M., Cauderon, Y., Chen, X., Cheng, Z.M., Larkin, P.J., and Waterhouse, P.M. 1988. A single wheatgrass chromosome reduces the concentration of barley yellow dwarf virus in wheat. *Annals of Applied Biology* 113:599-603.

Chen, Q., Conner, R.L., Laroche, A., Ji, W.Q., Armstrong, K.C., and Fedak, G. 1999. Genomic in situ hybridization analysis of *Thinopyrum* chromatin in a wheat - *Th. intermedium* partial amphiploid and six derived chromosome addition lines. *Genome* 42:1217-1223.

Chen, Q., Conner, R.L., Laroche, A., Thomas, J.B., and Qin, C. 1998. Genome analysis of *Thinopyrum intermedium* and *Thinopyrum ponticum* using genomic in situ hybridization. *Genome* 41:580-586.

El Yamani, M. 1990. Identification and importance of barley yellow dwarf virus in Morocco. *Plant Disease* 74:291-294.

Friebe, B., Mukai, Y., Gill, B.S., and Cauderon, Y. 1992. C-banding and in situ hybridization analyses of *Agropyron intermedium*, a partial wheat x *Ag. intermedium* amphiploid, and 6 derived chromosome addition lines. *Theoretical and Applied Genetics* 84:899-905.

Herbert, D.A., Stromberg, E.L., Chappell, G.F., and Malone, S.M. 1999. Reduction of yield components by barley yellow dwarf infection in susceptible winter wheat and winter barley in Virginia. *Journal of Production Agriculture* 12:105-109.

Hohmann, U., Badaeva, K., Busch, W., Friebe, B., and Gill, B.S. 1996. Molecular cytogenetic analysis of *Agropyron* chromatin specifying resistance to Barley Yellow Dwarf Virus in wheat. *Genome* 39:336-347

Larkin, P.J., Banks, P.M., Lagudah, E.S., Appels, R., Xiao, C., Xin, Z.Y., Ohm, H.W., and Mcintosh, R.A. 1995. Disomic *Thinopyrum intermedium* addition lines in wheat with Barley Yellow Dwarf Virus resistance and with rust resistances. *Genome* 38:385-394.

Lodhi, M., Ye, G.-N., Weeden, N.F., and Reisch, B.I. 1994. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitus* species. *Plant Molecular Biology Reporter* 12:6-13.

McKirdy, S.J., and Jones, R.A.C. 1997. Effect of sowing time on barley yellow dwarf virus infection in wheat – Virus incidence and grain yield losses. *Australian Journal of Agricultural Research* 48:199-206.

Riedell, W.E., Kieckhefer, R.W., Haley, S.D., Langham, M.A.C., and Evenson, P.D. 1999. Winter wheat responses to bird cherry-oat aphids and barley yellow dwarf virus infection. *Crop Science* 39:158-163.

Stoutjesdijk, P., Kammholz, S., Kleven, S., Matsay, S., Banks, P.M., and Larkin, P.J. 2001. PCR Based Molecular Marker for the Bdv2 *Thinopyrum intermedium* Source of Barley Yellow Dwarf Virus Resistance in Wheat. *Aust. J Agric. Res.* 52:1383-1388.

Tang, S., Li, Z., Jia, X., and Larkin, P.J. 2000. Genomic in situ hybridization (GISH) analyses of *Thinopyrum intermedium*, its partial amphiploid Zhong 5, and disease-resistant derivatives in wheat. *Theoretical and Applied Genetics* 100:344-352.

Thompson, J.P., Sheedy, J.G., and Clewett, T.G. 2000. Root-lesion Nematodes. *In* Northern Region Variety Trials 1999. QDPI Publication: Toowoomba. pp. 98-126.

Thompson, J.P., Sheedy, J.G., and Clewett, T.G. 2001. Root-lesion Nematodes. *In* Northern Region Variety Trials 2000. QDPI Publication: Toowoomba. (in press).

Wang, R.R.C., and Zhang, X.Y. 1996. Characterization of the translocated chromosome using fluorescence in situ hybridization and random amplified polymorphic DNA on two *Triticum aestivum Thinopyrum intermedium* translocation lines resistant to wheat streak mosaic virus or Barley Yellow dwarf Virus. *Chromosome Research* 4:583-587.

Xin, Z.Y., Zhang, Z.Y., Chen, X., Lin, Z.S., Ma, Y.Z., Xu, H.J., Banks, P.M., and Larkin, P.J. 2001.. Development and characterization of common wheat-Thinopyrum intermedium translocation lines with resistance to barley yellow dwarf virus. *Euphytica* 119:161-165.

Zhang, W.-J., Carter, M., Matsay, S., Stoutjesdijk, P., Potter, R., Jones, M., Kleven, S., Wilson, R., Larkin, P., Turner, M. and Gale, K. 2001. Implementation of probes for tracing chromosome segments conferring BYDV resistance. *Aust. J Agric. Res.* 52:1389-1392.

Evaluating Resistance to BYDV-PAV, BYDV-MAV, and CYDV-RPV in *Thinopyrum intermedium*-Derived Wheat Lines

M. Henry, G. Posadas, J. Segura, and S. Rajaram

CIMMYT, Mexico, D.F., Mexico

Barley yellow dwarf is a disease caused by a complex of viruses recently reclassified as *Barley yellow dwarf luteovirus* (serotypes BYDV-PAV and BYDV-MAV) and *Cereal yellow dwarf polerovirus* (CYDV-RPV) (D'Arcy *et al*., 1999). This disease has a wide host range and a worldwide distribution, and is regarded as the most significant viral pathogen affecting small grain cereals in the world, including wheat, barley, oat and triticale.

Management of the disease is mainly achieved through control of the vectors, the cereal aphids, by insecticide application, even though cultural practices, if properly applied can also reduce to a certain degree the yield losses due to this disease. However, the most effective and sustainable control method is the use of genetic resistance/tolerance to the virus complex.

Tolerance has been identified in several wheat lines, including CIMMYT wheat lines, while true resistance (reduction in virus concentration) has not been found in the wheat primary gene pool. However, it has been found in wheat relatives such as *Thinopyrum intermedium* and has been introgressed into breadwheat by several groups (Anderson *et al*., 1998; Banks *et al*., 1995; Chen *et al*., 1998; Francki *et al*., 2001; Larkin *et al*., 1995).

At CIMMYT, we undertook to incorporate resistance from *Th. intermedium* from the recombinant lines TC14 (Banks *et al*., 1995) into high yielding breadwheat. Resistance to BYDV-PAV, -MAV and CYDV-RPV in TC14 derived material was undertaken.

Materials and Methods

Plant materials

The resistant TC14/2*Spear (accession 289B), carrying the *Th. intermedium* translocation (*Bdv2*) on chromosome 7D (Hohmann *et al*., 1996) was kindly provided by P. Larkin, CSIRO, Australia and crossed with the tolerant breadwheat Anza to develop segregating populations (Ayala *et al*., 2001a). The studies of resistance were carried on 90 F4 lines of the cross Anza/"TC14" obtained through single-seed descent. The presence of the *Th. intermedium* fragment (*Bdv2*) was detected using PCR with the microsatellite marker *gwm37,* as described in Ayala *et al*. (2001a, 2001b). Families were classified as homozygous for the introgression (*Bdv2Bdv2*), heterozygous (*Bdv2bdv2*) or not having the introgression (*bdv2bdv2*).

Virus isolates and BYDV inoculation

The Mexican virus isolates used in these experiments were BYDV-PAV, BYDV-MAV, and CYDV-RPS. These isolates have been maintained in CIMMYT's greenhouses since their collection in 1993. Artificial inoculation was performed by depositing 10 aphids per single one-week old seedlings for a 48-h transmission period. Aphids had acquired the virus on BYDV/CYDV infected oat for a 48-h acquisition period. The bird cherry oat aphid *Rhopalosiphum padi* was used to transmit BYDV-PAV and CYDV-RPS and the rose grain aphid, *Metopolophium dirhodum* was used to transmit BYDV-MAV. At the end of the transmission period, aphids were killed by application of a contact insecticide (Pirimor).

Evaluation of virus titers in leaves and roots

Resistance to BYDV was assessed as a reduction in virus titers (Optical Density, OD) measured by ELISA, in both roots and leaves of the tested plants. ELISA was performed on the flag-1 leaf, 10, 20 and 31 days after inoculation and on roots, 5, 10 and 20 days after inoculation. Plants for which the OD was lower than the positive threshold were considered as not being infected. Two repetitions, each consisted of 4 infected plants + one non-infected control per family were done. The average OD (aver. OD) of the inoculated plants and the average OD of the plants for which the ELISA was higher than the positive threshold (IOD) were calculated. The resistant parent TC14 and the tolerant parent Anza were used as controls, together with the susceptible breadwheat Bagula.

The positive threshold was calculated for each repetition and each BYDV serotype, and was equal to the average ODs obtained with the non-infected control + twice standard variation.

ELISA

Double antibody-sandwich ELISA (DAS-ELISA) with polyclonal antibodies against PAV, MAV and RPV from the United States (provided by K. Perry, Purdue University) was carried out according to Ayala *et al*. (2001a). Optical densities were measured at 405nm after 1 and 2 hours incubation at room temperature using a microplate reader (MR 700; Dynatech Laboratories).

Results

Effect of the translocation on BYDV-PAV, MAV, and CYDV-RPV infection rates

In the conditions used for the inoculation, there is no difference between the numbers of plants inoculated (as evaluated 10 days after inoculation) with BYDV-PAV in the different genotypic groups (with or without *Bdv2*) (Figure 1). However, with BYDV-MAV and CYDV-RPV, the success of inoculation is less in the heterozygous group or the group homozygous for the translocation than in the group not carrying the translocation. In the case of RPV, a very limited percentage of plants got infected in the homozygous resistant group (17.5%) compared to 97.5% in the wheat type. Heterozygous were intermediate in their response. The same tendency was observed with BYDV-MAV with slightly higher infection rates (30%).

Effect of the translocation on BYDV-PAV, MAV, and CYDV-RPV virus titers

For the three viruses tested, virus titers in the leaves were lower in the homozygous plants carrying the *Bdv2* translocation than in the one that did not have the translocation (Figure 2). In all cases, the titer in the heterozygous was intermediate.

In the roots, the difference still existed but was far less important, especially in plants infected with BYDV-PAV. Virus titers reached high levels 5 days after inoculation with BYDV-PAV (OD>1.0). Very few plants homozygous for *Bdv2* got infected with CYDV-RPV and only in a few of these cases virus titers reached moderate level (OD>0.5).

Discussion

In field experiments under natural or artificial inoculation with BYDV-PAV, we have observed a lower rate of infection in material carrying the *Th. intermedium* translocation (*Bdv2*) (Ayala *et al*., 2001b, Henry unpublished). However, in the glasshouse experiment described here, there were no real differences in inoculation rate between the different genetic groups. This could be related to the optimum conditions used for this experiment (very young seedlings, high number of aphids). The number of plants homozygous for *Bdv2* that got infected with CYDV-RPV was closed to nil. In a few cases, infection was detected specially in the roots where levels were still lower than in the other genotypic groups. This suggest higher level of resistance for CYDV-RPV than

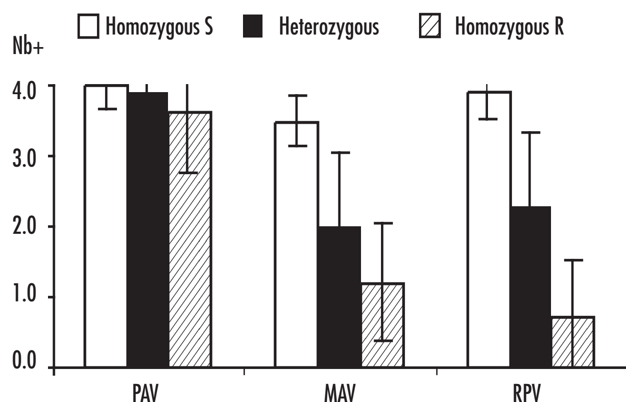


Figure 1. Number of infected plants (average of 2 repetitions) in the genetic groups (segregating for *Bdv2*) of the AnzaxTC14 F4 population, 10 days after artificial inoculation with BYDV-PAV, -MAV and CYDV-RPV assessed by ELISA.

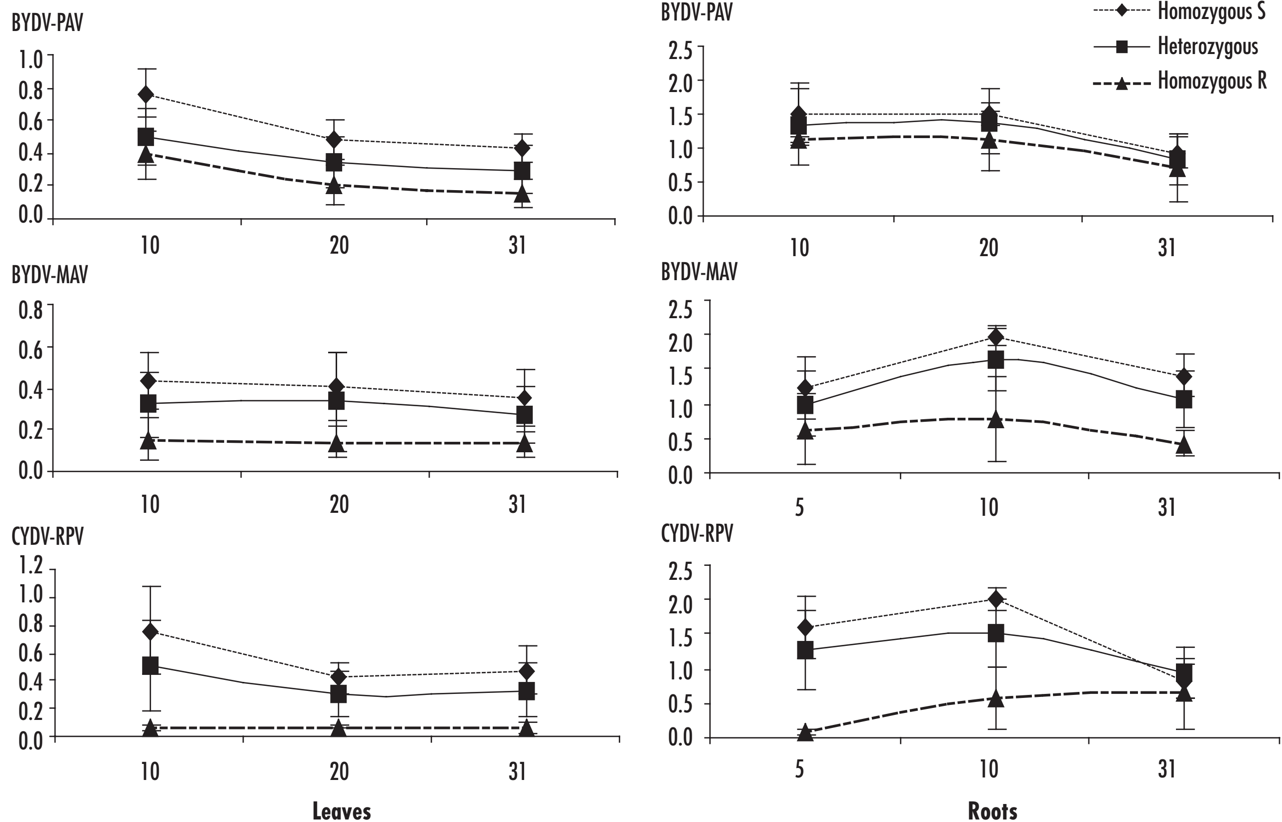


Figure 2. Average optical densities obtained in ELISA in leaves and roots of plants infected (OD>threshold) with BYDV-PAV, -MAV and CYDV-RPV at different times after inoculation.

BYDV-PAV, a situation opposite to what has been observed with resistance conferred in barley with the *Yd2* gene, where resistance is superior for BYDV-PAV than CYDV-RPV (Herrera, 1989).

Virus titers did not differ greatly in the roots of plants carrying or not the *Bdv2* gene and infected with BYDV-PAV, suggesting that resistance to BYDV could be expressed in the leaves only.

References

Anderson, J.M., Bucholtz, D., Greene, A.E., Francki, M.G., Gray, S.M., Sharma, H., Ohm, H.W., and Perry, K.L. 1998. Characterization of wheatgrass-derived barley yellow dwarf virus resistance in a wheat alien chromosome substitution line. *Phytopathology* 88(8):851-855.

Ayala, L., Henry, M., González de León, D., van Ginkel, M., Mujeeb-Kazi, A., Keller, B., and Khairallah, M. 2001a. A diagnostic molecular marker allowing the study of *Th. intermedium* derived resistance to BYDV in bread wheat segregating populations. *Theor. Appl. Gen*, 102:942-949.

Ayala, L., van Ginkel, M., Khairallah, M., Keller, B., and Henry, M. 2001b. Expression of *Thinopyrum intermedium*–derived Barley yellow dwarf virus resistance in elite bread wheat backgrounds. *Phytopathology* 91:55-62.

Banks, P.M., Davidson, J.L., Bariana, H.S., and Larkin, P.J. 1995. Effects of barley yellow dwarf virus on the yield of winter wheat. *Aust. J. Agri. Res.* 46:935-946.

Chen, Q., Collin, J., Comeau, A., St-Pierre, C.A., and Fedak, G. 1988. Comparison of various sources of resistance to barley yellow dwarf virus in wheat-*Thinopyrum* amphiploid lines. *Can. J. of Pl. Path.* 19:414-417.

D'Arcy, C.J., Domier, L.L., and Mayo, M.A. 1999. Luteoviridae. In: *Virus Taxonomy. Classification and Nomenclature of Viruses. Seventh Report of the International Committee on Taxonomy of Viruses.* van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon, S.M., Maniloff, J., Mayo, M.A., McGeoch, D.J., Pringle, C.R., and Wickner, R.B. (eds.). Academic Press. pp. 775-784.

Francki, M.G., Ohm, H. W., and Anderson, J.M. 2001. Novel germsplasm providing resistance to barley yellow dwarf virus in wheat. *Aust. J. Agric. Res.* 52:1375-1382.

Herrera, M.G. 1989. Interactions between host plants and British isolates of barley yellow dwarf virus. Thesis, University of London.

Hohmann, U., Badaeva, K., Bush, W., Friebe, B., and Gill, B.S. 1996. Molecular cytogenetic analysis of Agropyron chromatin specifying resistance to barley yellow dwarf virus in wheat. *Genome* 39:336-347.

Larkin, P.J., Banks, P.M., and Chen, X. 1995. Registration of six genetic stocks of wheat with rust and BYDV resistance: Z1, Z2, Z3, Z4, Z5, and Z6 disomic addition lines with *Thinopyrum intermedium* chromosomes. *Crop Science* 35:604.

Advances in Breeding Wheat for BYDV Resistance Using Biotechnology

Z.Y. Xin, Z.Y. Zhang, Z.S. Lin, X. Chen, H.J. Xu, Y.Z. Ma, D.L. Wu, Q.F. Xu, L.P. Du, P.M. Banks, and P.J. Larkin

Key Lab. of Crop Genetics and Breeding of Ministry of Agriculture, Institute of Crop Breeding and Cultivation, CAAS, Beijing, China

Barley yellow dwarf is a serious disease of wheat caused by Barley yellow dwarf virus *(BYDV). All wheat collections tested have been proven vulnerable to BYDV infection. However,* Thinopyrum intermedium *(Host) Barkworth and Dewey (syn.* Agropyron intermedium *(Host) P.B.), a hexaploid perennial Triticeae species ($2n=6x=42.E_1E_2X=JJ^sSt$), contains at least two genes for BYDV resistance located on the chromosome of group 7 or group 2, respectively. The wheat-*Th. intermedium *addition line L1 carries the BYDV resistance gene (*Bdv2*) located on the alien chromosome 7X, showing good resistance to PAV serotype of BYDV. Meanwhile, the wheat-*Th. intermedium *partial amphiploid Zhong4 Awnless carries another BYDV resistance gene, different from that of L1, located on the chromosome of group 2. It shows better resistance to GAV and GPV serotypes of BYDV. Amphidiploid CPI 113500 derived from* Ag. pulcherrimum *also shows good BYDV resistance. Some derivative lines from these resistance sources were developed and they can be used for wheat breeding program for BYDV resistance. Molecular markers for the resistance were developed and applied.*

Resistance from Addition Line L1

Screening results from the field tests and ELISA showed that *Th. intermedium*, partial amphiploid TAF46, and one disomic addition line L1, both developed by Cauderon *et al.* (1973), had good BYDV resistance.

Development of translocation lines from L1 using CSph mutant

We developed wheat-*Th. intermedium* translocation lines Yw642, Yw443, Yw243, and Yw1029 showing good BYDV resistance from L1 by induced homoeologous pairing using CSph mutant (Xin *et al.*, 2001; Zhang *et al.*, 1998). Spring wheat line Yw642 was developed from the F_6 progeny of the cross Zhong8601x4//Zhong 7902/5395-12. Winter wheat line Yw443 was developed from the F_6 progeny of the cross Zhong7902/5395-12//Shan 7859/3/Fengkang8. Line 5395-12 was selected from the cross of CSphx2/L1//CSN5BT5D. They showed good resistance to GPV and GAV serotypes of BYDV in field tests and ELISA analyses with 42 chromosomes and 21 pairs at meiotic metaphase. We found that a single dominant gene controlled their BYDV resistance.

In order to effectively use these wheat germplasms for wheat breeding, the wheat lines derived from L1 were characterized by GISH and RFLP analysis. GISH results showed that the wheat lines Yw642, Yw243 and Yw443 are wheat-*Th. intermedium* translocation lines with 40 wheat chromosomes and 2 wheat-*Th. intermedium* translocated chromosomes (Zhang *et al.*,1998; Xin *et al.*, 2001). The *Th. intermedium* chromosome segment, carrying the BYDV resistance gene, was located on the terminal of wheat chromosome. They are wheat-*Th. intermedium* translocation lines (Zhang *et al.*,1998; Xin *et al.*, 2001).

RFLP analysis

It was reported that the BYDV resistance gene in the addition line L1 was located on the chromosome 7X of *Th. intermedium*. Nine probes on the wheat homoeologous group 7 were selected for Southern hybridization. The results indicated that the probe psr680 with *Hind*III and the probe psr687 with *EcoRI* could show 7XL specific bands in all BYDV resistant materials but absent in all susceptible materials, while 7DL marker bands were absent in the translocation lines and individual resistant plants except L1 but present in susceptible wheat varieties. The results

indicated that the markers of psr680/*Hin*dIII and psr687/*EcoR*I were closely linked with the BYDV resistance gene *Bdv*2 that was mapped near the locus of Xpsr680 and Xpsr687 on the chromosome 7XL of *Th. intermedium*, and the 7XL segments including Xpsr680 and Xpsr687 replace the chromosome 7DL segments of wheat in the lines Yw642 and Yw443. They were T7DS.7DL-7XL translocation lines (Zhang *et al*.,1999; Xin *et al.*, 2001).

As probe psr965 on the long arm of the homoelogous group 7 chromosome could not detect the 7XL specific band, the breakpoint of the translocation should be located on the distal end of 7DL between Xpsr965 and Xpsr680 about 90~99cM from the centromere.

RAPD and SCAR marker analysis

In order to better use molecular markers for breeding, we also screened 2 RAPD markers of $OPR19_{940}$ and $OPD04_{1300}$, and developed 2 SCAR markers of SC-D04 and SC-W37. OPR19 could amplify a specific band of about 940bp that was present in all the resistant materials carrying the BYDV resistance gene of *Bdv*2 but absent in all wheat parents and BYDV resistance materials of Z1 and Zhong4 Awnless. $OPR19_{940}$ band could be used as a marker to detect the 7XL segment carrying the *Bdv*2 gene (Zhang *et al*., 2002).

OPD04 could amplify a specific band of about 1300bp that was present in all the resistant materials carrying the *Bdv*2 gene but absent in all wheat parents, susceptible lines and BYDV resistance materials of Z1, Z2, and Zhong4 awnless. $OPD04_{1300}$ band could be used as a marker to detect the 7XL segment carrying the Bdv2 gene (Zhang *et al*., 2002a).

Two SCAR markers of SC-D04 and SC-W37 were developed. Both specific PCR markers could amplify a single band present in all resistant materials carrying the *Bdv*2 gene but absent in all materials without *Bdv*2. The linkage between these PCR markers and the resistance gene *Bdv*2 was analyzed by using F_2 population plants of Yw642/Zhong 8601. The results indicated that SC-D04 and SC-W37 were co-segregated with *Bdv*2 gene. The SCAR markers of SC-D04 and SC-W37 have been used for MAS of BYDV resistance for wheat breeding program (Zhang *et al*., 2002a, b).

Resistance from Zhong4 Awnless

Development of substitution lines from addition line Z6

Zhong4 Awnless is an octoploid amphiploid (2n=56) between wheat and *Th. intermedium*, developed by Sun *et al*., Heilongjian Academy of Agricultural Sciences, China. By backcrossing Zhong4 Awnless to wheat, 3 wheat-*Th. intermedium* disomic addition lines with BYDV resistance, Z1, Z2 and Z6, were developed (Larkin *et al*., 1995). The substitution lines ZD28, N431, N432, and N439 were further developed from the young embryo culture of Z6/Shan7859 or Z6/Zhong 8601.

The chromosome construction of Zhong4 Awnless

GISH analysis showed that Zhong4 Awnless contains 42 wheat chromosomes and 14 *Th. intermedium* chromosomes. The 14 *Th. intermedium* chromosomes composed of 2 pairs of St chromosomes, 2 pairs of St-E intercalary translocated chromosomes, 1 pair of St-E Robertson translocated chromosomes, and 2 pairs of E^{st} chromosomes. The addition lines of Z1, Z2 and Z6 derived from Zhong4 Awnless were composed of 42 wheat chromosomes and 2 *Th. intermedium* chromosomes (2Ai-2). The substitution line ZD28 derived from Z6 carries 40 wheat chromosomes and 2 *Th. intermedium* chomosomes (2Ai-2). The 2Ai-2 chromosome mostly belongs to St genome but about one third of the long arm in the middle belonging to the E genome. The GISH pattern of 2Ai-2 may be used as a molecular cytogenetic marker (Zhang *et al*., 2000).

RFLP analysis

By using 12 probes on the long arm and short arm of wheat group 2 chromosome, the RFLP analysis indicated that the 2Ai-2 chromosome extensively homoeologous to the wheat group 2 chromosome with some different internal structure. Twenty RFLP markers specific to the 2Ai-2 chromosome, twenty combinations of 11 probes with some restriction enzymes were identified and could detect the 2Ai-2 chromatin in the wheat background (Zhang *et al*., 2001).

RAPD analysis and SCAR markers

Out of 600 10-mer RAPD primers, 5 RAPD molecular markers of $ORR16_{340}$, $OPH09_{1580}$, $OPC01_{500}$, $OPO05_{650}$, $OPM04_{1400}$, were identified specific to the 2Ai-2 chromatin. The RAPD markers of $OPR16_{340}$, $OPO04_{650}$ and $OPM04_{1400}$ were cloned, sequenced. 3 SCAR markers converted from the above RAPD markers (Zhang *et al*., 2001, 2002b).

Resistance from *Agropyron pulcherrimum*

Amphidiploid CPI 113500(2n=70), *Triticum turgidum* (2n=28) x *Agropyron pulcherrimum* (2n=42) produced by A. Mujeeb-Kazi, CIMMYT, showed good BYDV resistance. Some derivative lines were obtained by backcrossing CPI 113500 to common wheat as well as selfing. Two of them, the wheat-7 *Ag. pulcherrimum* disomic addition line 96S16-11, and the disomic substitution line 96W14-9 were resistant to BYDV. This BYDV resistance was linked with the presence of hairy glume (Hg) present in *Ag. pulcherrimum* but absent in the susceptible wheat parents. Since the Hg gene is located on group 1 chromosome, the BYDV resistance gene carried by *Ag. pulcherrimum* is most likely located on the group 1 chromosome.

The leaf peroxidase isozyme analysis showed that a specific band at pH5.0 was observed in CPI 113500, 96S16-11 and 96W14-9 but absent in their susceptible wheat parents. PER-1 is located on the short arm of the homoeologous group 1 chromosomes. Therefore the resistance gene carried by *Ag. pulcherrimum* is on the short arm of the group 1 chromosome (Wu *et al*., 1999).

References

Cauderon, Y., Saigne, B., and Dauge, M. 1973. In: *Proc. 4th. Inter. Wheat Genet. Symp.* Columbia, Missouri, USA. Pp. 401-407.

Larkin, P.J., Banks, P.M., Lagudah, E.S., Appels, R., Chen, X., Xin, Z.Y., Ohm, H.W., and McIntosh, R.A. 1995. Disomic *Thinopyrum intermedium* addition lines in wheat with barley yellow dwarf virus resistance and with rust resistances. *Genome* 38:385-394.

Wu, D.L., Z.Y. Xin, X. Chen, H.J. Xu, Y.Z. Ma, and Z.Y. Zhang. 1999. Development and identification of wheat-*Ag. pulcherrimum* addition line and substitution line with BYDV resistance. *Science in China* (Series C) 42(2):178-184.

Xin, Z.Y., Z.Y. Zhang, X. Chen, Z.S. Lin, Y.Z. Ma, H.J. Xu, P.M. Banks, and P.J. Larkin. 2001. Development and characterization of common wheat-*Thinopyrum intermedium* translocation lines with resistance to barley yellow dwarf virus. *Euphytica* 119:161-165.

Zhang, Z.Y., Y.Z. Ma, Z.Y. Xin, X. Chen, D.L. Wu, and Z.S. Lin. 1998. Analysis of the chromosome constitute of wheat lines resistant to barley yellow dwarf virus by genomic *in situ* hybridization. *Scientia Agricultura Sinica* 31(3):1-4.

Zhang Z.Y., Z.Y. Xin, Y.Z. Ma, X. Chen, Q.F. Xu, and Z.S. Lin. 1999. Mapping of a BYDV resistance gene from *Thinopyrum intermedium* in wheat background by molecular markers. *Science in China* (Series C) 42(6):663-669.

Zhang, Z.Y, Z.X. Xin, Z.S. Lin, X. Chen, and X.P. Wang. 2000. Identification of molecular markers for the *Thinopyrum intermedium* chromosome 2Ai-2 with resistance to barley yellow dwarf vius. *Acta Botanica Sinica* 42:1051-1056.

Zhang, Z.Y., Z.Y. Xin, and P.J. Larkin. 2001. Molecular characterization of a *Thinopyrum intermedium* group 2 chromosome (2Ai-2) conferring resistance to barley yellow dwarf virus. *Genome* 44:1129-1135.

Zhang, Z.Y., J.S. Xu, Q.J. Xu, Z.Y. Xin, L.P. Du, and Z.S. Lin. 2002a. Development of PCR markers for the BYDV Resistance (*Bdv2*)in wheat and their application for assisted selection in wheat breeding. In: These Proceedings.

Zhang, Z.Y., Wang, L.L., Xin, Z.Y., and Lin, Z.S. 2002b. Development of new PCR markers specific to a *Thinopyrum intermedium* chromosome 2Ai-2 and cloning of the St-specific sequences. *Acta Genetica Sinica* 29(7).

Development and Application of PCR Markers for Breeding Wheat for BYDV Resistance

Z. Zhang, J. Xu, Q. Xu, L. Du, Z. Lin, and Z. Xin

Key Lab. of Crop Genetics and Breeding, Ministry of Agriculture, Institute of Crop Breeding and Cultivation, Chinese Academy of Agriculture Sciences, Beijing, China

*Several wheat-*Thinopyrum intermedium *translocation lines with BYDV resistance gene* Bdv2*, including HW642, YW443, YW243, and Y991029, were developed by using the addition line L1 as the resistance parent. Out of 600 RAPD primers screened, the primers of OPD04 and OPR19 can amplify a specific band for* Bdv2 *present in resistant materials with* Bdv2*, but absent in susceptible wheat parents and other addition lines Z1, Z2, and Z6 without* Bdv2*. The specific band of OPD04-$_{1300}$ for* Bdv2 *was cloned, analyzed, and sequenced. Based on the sequence, a pair of primers were designed, synthesized and can amplify only one band present in the resistant materials with* Bdv2 *but nothing in materials without* Bdv2*. Results indicated that RAPD marker OPD04-$_{1300}$ was converted a sequence characterized amplified region (SCAR) marker SC-D04 to facilitate detecting materials with* Bdv2 *in wheat breeding populations. Another SCAR marker* SC-W37 *converted from gwm37$_{-450}$ was developed for* Bdv2*. After linkage analysis between these PCR markers and Bdv2 among F_2 population plants of HW642/ Zhong8601, results indicated that* SC-D04 *and* SC-W37 *co-segregated with* Bdv2 *and were more reliable and easily scored. Four generations of plants of the crosses and backcrosses among HW642 and synthetic wheat M53 with resistance to powdery mildew, wheat varieties Zhongmai 16 and Wan7107 were identified by SCAR markers. Results indicated that SCAR markers could be used in assisted selection of wheat, and 300 individual plants with resistance to BYDV and powdery mildew were selected from the backcross populations.*

Barley yellow dwarf virus (BYDV) is a group of luteoviruses, vectored by several aphids. BYDV is the most significant virus pathogen of wheat, other important cereal crops, and grass species (Brettell *et al.*, 1988; Conti *et al.*, 1990).

The most economical and practical means of controlling BYDV is to breed and make use of cultivars carrying genetic resistance to the virus or the aphid vector (Ayala *et al.*, 2001). So far, no BYDV resistance genes have been found in all wheat collections tested. However, *Thinopyrum intermedium* ((Host) Barkworth and Dewey), a wild relative of wheat, is highly resistant to BYDV due to resistance genes located on homoeologous group 7 and group 2 chromosomes (Sharma *et al.*, 1984; Brettell *et al.*, 1988; Xin *et al.*, 1988, 1991; Banks *et al.*, 1993; Larkin *et al.*, 1995). A wheat-*Th. intermedium* disomic addition line L1, derived from a partial amphiploid TAF46, was developed by Cauderon *et al.*, (1973) and showed good resistance to BYDV (Xin *et al.*, 1988). The *Th. intermedium* chromosome in L1 designated as 7Ai1 (7X) (Friebe *et al.*, 1992) resistance gene, known as *Bdv2* and carries a BYDV (Zhang *et al.*, 1999; McIntosh *et al.*, 1998; Stoutjesdijk *et al.*, 2001) on the long arm (Brettell *et al.*, 1988).

Several T7DS.7DL-7Ai1L translocation resistance lines with *Bdv2*, including Yw642, Yw443, and Yw243, etc., were developed using L1 as the resistance donor through CSph mutant-inducing homoeologous pairing and characterized by GISH, RFLP, and isozyme analysis (Xin *et al.*, 1991; Zhang *et al.*, 1999; Xin *et al.*, 2001). These translocation lines with small segment of *Th. intermedium* chromosome 7Ai1 were used as BYDV resistance parents to improve wheat.

In the prospect of transferring BYDV resistance gene *Bdv2* into elite wheat varieties, the resistance must be evaluated. Results of a traditional field test are often influenced by aphids inoculating BYDV, environment and evaluator experience, and are only carried out

once per year. The ELISA bioassay is so complicated, labor-intensive, and time-consuming that plant breeders are generally reluctant to do it (Stoutjedijk *et al*., 2001). Molecular markers could solve these problems.

Although some RFLP and RAPD markers for *Bdv2* were reported (Banks *et al.*, 1995; Hohmann *et al.*, 1996; Wang *et al.*, 1996; Zhang *et al.*, 1999), they are hardly suitable for large-scale selection in breeding program. Ayala *et al.* (2001) identified an SSR marker *gwm37* for *Bdv2*, however, it is more difficult and expensive to selection for high-output by SSR marker than by SCAR marker (Paran and Michelmore, 1993). In addition, developing more molecular markers in the 7AiL region containing *Bdv2* would be beneficial for genomic study and gene isolation.

In the paper, we identified two new RAPD markers OPD04-$_{1300}$ and OPR19-$_{940}$ for the resistance *Bdv2*, and converted one RAPD marker OPD04-$_{1300}$ and one SSR marker gwm37-$_{450}$ into SCAR markers. After the linkage relationship among the SCAR markers and *Bdv2* was analyzed, the SCAR markers were used to detect resistance and select BYDV-resistance genotypes in a large-scale wheat breeding program.

Materials and Methods

Plant materials

Wheat-*Th. intermedium* translocation lines Yw642 (3xZhong8601/PP9-1), Yw443 (PP9-1/Shan7859// Fengkang8), Yw243 {(PP9-1/Shan7859//Fengkang8) // (3xFengKang13/ Khapli)} and Yw1029 (PP9-1/ Shan7859x2//(M.Hustman/Yangmai3)/3/Shan7859) were developed by the Institute of Crop Breeding and Cultivation, CAAS (Xin *et al.*, 1991, 2001; Zhang *et al.*, 1999). The combination of PP9-1 is Zhong8601/4/ Zhong7902/3/CS phx2 /L1//CSN5BT5D. A wheat-*Th. intermedium* partial amphiploid TAF46 and an addition line L1 were kindly supplied by Dr. Cauderon, INRA, France. A ditelosomic addition line of 7Ai1 long arm (7Ai1L) was developed and kindly supplied by Dr. Larkin. *Th. intermedium* was collected and conserved by Institute of Crop Breeding and Cultivation, CAAS. These materials show good resistance to BYDV.

M53, a synthetic wheat (2n=42) with resistance to powdery mildew was supplied by Mujeeb-Kazi, CIMMYT.

The wheat cultivars susceptible to BYDV Chinese Spring (CS), Zhong7902, Zhong8601, Shan7859, Wan7107 and Zhongmai16 were collected or developed by the Institute of Crop Breeding and Cultivation, CAAS. The susceptible line Yw641 was derived from Yw642, a sib line.

In an F_2 population of 255 plants from the cross between the translocation line Yw642 (resistant parent) and Zhong8601 (susceptible parent), the resistant (R) pool and the susceptible (S) pool consisting of 10 individuals each were used in bulk segregant analysis. F_2 plants were selfed to generate F_3 lines.

Zy20195 is an F_1 plant from the cross Yw642 x M53 possessing both resistance to BYDV and powdery mildew. Py1-Py6 plants are F_1 plants of the cross Zy20195 x Zhongmai16 and Bcy1-Bcy630 are obtained by backcross of Py1-Py6 or their progeny with multi-resistance to BYDV and powdery mildew and Zhongmai16.

Tests for BYDV resistance

Resistance to BYDV was tested in field. At the three-leaf stage, the F1, F2 and F3 plants of as well as their parents were infested with approximately 10 aphids carrying BYDV-GAV serotype. After one week the aphids were killed by insecticide application. When the susceptible wheat plants show typical BYDV symptoms (about 30 days later), the resistance was evaluated.

DNA extraction and RAPD analysis

DNA was extracted by the modified SDS-Phenol method of Sharp *et al.* (1988). A half of a newly grown leaf was frozen and ground in liquid nitrogen and extracted by SDS-Phenol. RAPD-PCR reaction was performed by the method of Zhang *et al.* (2001). The amplified products were fractionated on 1.0 % agarose gels and detected by ethidium-bromide staining.

SSR analysis

The SSR primer pair of *gwm37* was selected to be synthesized based on Ayala *et al.* (2001) and Röder *et al.* (1998). SSR reaction was carried out by the modified method described by Ayala *et al.* (2001) with the annealing temperature modified to 50˚C.

Cloning, analyzing, and sequencing RAPDs and SSRs Diagnostic RAPD bands and the diagnostic SSR fragment of *gwm37* were purified, cloned, analyzed, and sequenced following the method described by

Zhang *et al.* (2001). The amplified products were fractionated on 0.8% agarose gels and detected by ethidium-bromide staining.

SCAR primers and SCAR-PCR amplification

Two pair of SCAR primers were designed and synthesized based on the sequence of the cloned RAPD diagnostic fragment. The SCAR fragment from the RAPD marker was amplified in a 25μl containing 10 m M/l Tris-HCl (pH 9.0), 50 mM/l KCl, 2.0 mM/l $MgCl_2$, 200 μM/l each dNTP, 1 U *Taq* polymerase, 40 ~100 ng genomic DNA, 0.2μM each SCAR primer. Amplification reactions were carried out in a DNA thermal cycler (Perkin Elmer Cetus 480), programmed at 96˚C 1 min, followed by 35 cycles of 94˚C 1 min, 64˚C 1 min, 2˚C 1 min and final extension of 72˚C 10 min. The amplified products were fractionated on 0.9% agarose gels and detected by ethidium-bromide staining.

The SCAR-PCR from SSR marker wmg37, *SC-W37* was amplified in the same program and similar condition as that of the SCAR-PCR from RAPD. But the concentration of $MgCl_2$ increased to 2.5 mM/l. The amplified products were fractionated on 3.5% agarose gels and detected by ethidium-bromide staining.

Results and Discussion

Identification of RAPD markers linked to *Bdv2*

A preliminary screening for genetic polymorphism among wheat parent Zhong 8601, *Th. intermedium*, and Yw642 as well as R pool and S pool was carried out by using 600 arbitrary 10-mers primers that were included in 30 groups of A-Z, AA, AB, AG, and AK group, 20 primers per group. Although four primers of OPD04, OPR19, OPF01, and OPAB01 could respectively amplified a polymorphic band between the resistant and susceptible materials, only the primers of OPD04 and OPR19 showed the same amplification diagnostic band in several independent repeats using different batchs of *Taq* polymerase.

The primer of OPD04 could amplify a specific band of about 1300 bp present in all the resistance materials containing *Bdv2* gene, including translocation lines Yw642, Yw443, Yw243, and Yw1029, L1 and *Th. intermedium*, but absent in the susceptible line Yw641, wheat parents as well as Z1 resistant to BYDV without *Bdv2* gene (Figure 1). Results showed that the DNA fragment of OPD04-$_{1300}$ could be used as a marker for *Bdv2* gene.

Similarly, the primer OPR19 amplified a specific DNA fragment of about 940 bp present in all the resistance materials containing *Bdv2* gene but absent in the materials without *Bdv2* (Figure 2). The band of OPR19-$_{940}$ is a RAPD marker for *Bdv2* gene.

Conversion of RAPD markers into SCAR markers

The cloning of OPD04-$_{1300}$ fragment from *Th. intermedium* and the translocation line Yw642 resulted in 28 clones. After *Eco*RI restriction and PCR analysis,

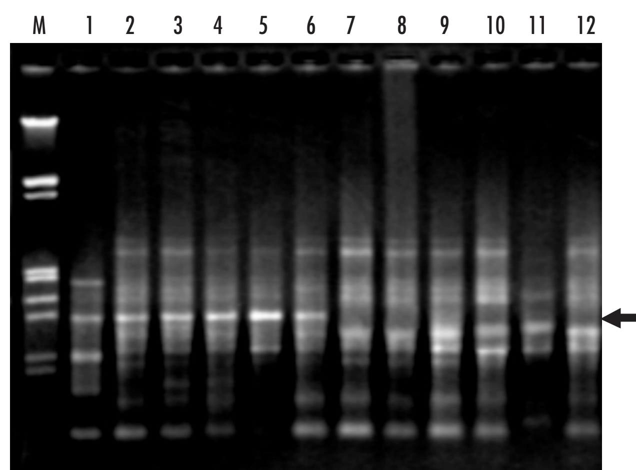


Figure 1. RAPD PCR products amplified with the RAPD primer OPD04.
Note: M,λ DNA\EcoRI+HindIII molecular marker; 1,*Th. intermedium*; 2,L1; 3,Yw642; 4,Yw443; 5,Yw243; 6,Yw1029; 7,Yw641; 8,Zhong7902; 9,Zhong8601; 10,CS; 11,Z1; 12.Wan7107. Arrow indicates position of marker specific to the *Bdv2*.

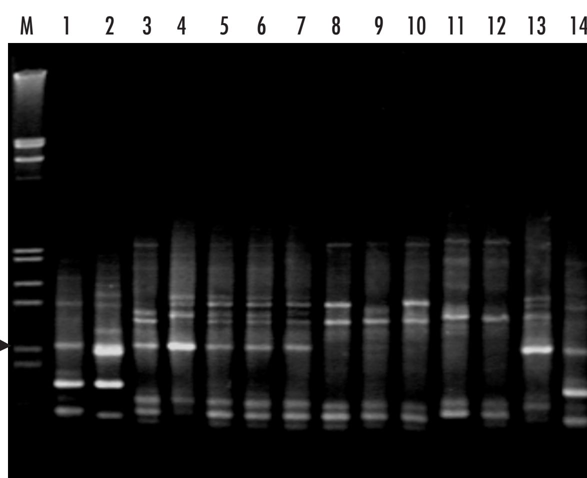


Figure 2. RAPD PCR products amplified with the RAPD primer OPR19.
Note: M,λ DNA\EcoRI+HindIII molecular marker; 1,14,*Th. intermedium*; 2,TAF46; 3,L1; 4,13,Yw642; 5,Yw443; 6,Yw243; 7,Yw1029; 8,H641S; 9.Zhong7902; 10,Zhong8601; 11,CS. 12,Z1. Arrow indicates position of marker for the *Bdv2* gene.

11 clones of the 28 clones had the expected fragment with size of 1300 bp corresponding to the original amplification products. These clone insertions were used as probes to Southern hybridize with the RAPD-PCR products amplified by primer OPD04. Results showed that the insertions in two clones of Tidz4 and Tidz8 only hybridized with the OPD04-$_{1300}$ specific band in resistant materials and were homologous with the OPD4-$_{1300}$ specific band (Figure 3).

The insert DNA fragments of the two clones Tidz4 and Tidz8 were sequenced from two ends. The sequencing results indicated that the insertion sequences were identical and bordered by 10-mers sequences that corresponded exactly to the primer OPD04. Based on the sequences, two pairs of primers were designed, synthesized, and used to amplify genomic DNA of the resistant materials with and without *Bdv2*. Results showed that only one pair of primers, SC-D04U and SC-D04L containing the original 10 bases of OPD04, could generate a single robust band of about 1300 bp present in the resistant materials but absent in the materials without *Bdv2* (Figure 4). Results indicated that the SCAR primers were available for *Bdv2*, and the SCAR marker, *SC-D04*, is dominant.

The F_2 population of 256 plants from a cross between the Yw642 and Zhong8601 was used to investigate the linkage between *SC-D04* and *Bdv2*. Results showed that 183 resistant plants completely amplified the *SC-D04* marker, while none of 73 susceptible plants generate the band.

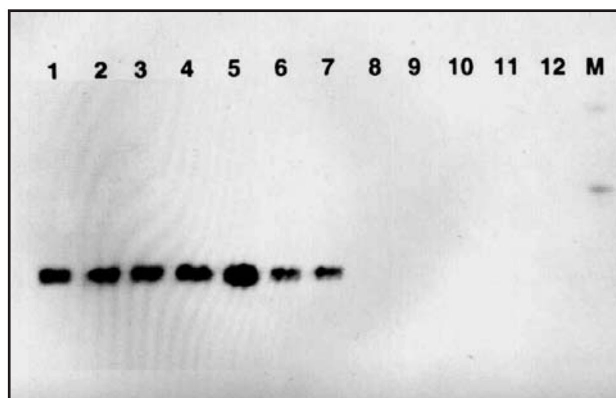


Figure 3. PCR-Southern pattern by using OPD04 as a primer and Tidz8 as a probe.
Note: 1.*Th. intermedium*; 2.L1; 3.7Ai1L; 4.Yw642; 5.Yw443; 6.Yw243; 7.Yw1029; 8.Yw641; 9.CS; 10.Zhong8601; 11.Zhong7902; 12.Vi; Mλ.DNA/*Hind*III+*Eco*RI molecular marker.

SSR analysis and SCAR converted from SSR marker

The SSR primer gwm37 could amplify a specific band of about 450 bp present in all the resistant materials containing *Bdv2* gene, including translocation lines L1 and *Th. intermedium*, but absent in the susceptible line Yw641, the wheat parents, as well as Z1 resistant to BYDV without *Bdv2* gene (Figure 5). But the SSR- PCR products must be fractionated by at least 3% agarose gels, while the specific band of gwm37$_{-450}$ was not always stable.

To facilitate detection of *Bdv2* gene by PCR markers in breeding, it is necessary to convert the SSR marker into a reliable SCAR marker. After the marker band was cloned and sequenced, one pair primer of SC-W37U and SC-W37L was designed and used to detect *Bdv2*. Results indicated that SCAR primers could amplify a single robust band of about 450 bp in the

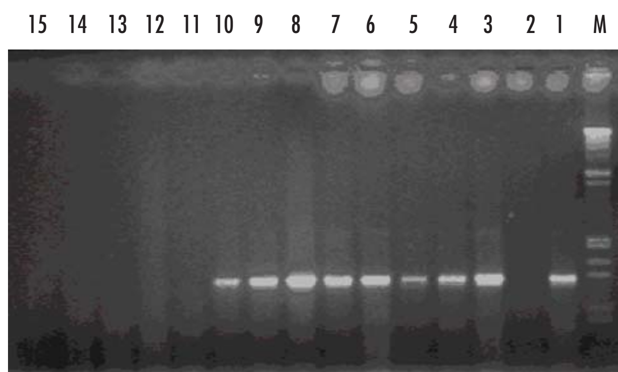


Figure 4. SCAR-PCR pattern of the resistant translocation lines and their parents amplified by the Primers SC-D4U and SC-D4L.
Note: M, λDNA\EcoRI+HindIII molecular marker; 1,*Th. intermedium*; 2,Zhong8601; 3,TAF46; 4,L1; 5,7Ai1L, 6,Yw642; 7,Yw443; 8,Yw243; 9,Yw1029; 10,R pool; 11,Yw641; 12.CS; 13, Vi; 14,Zhong7902; 15,Zhong8601.

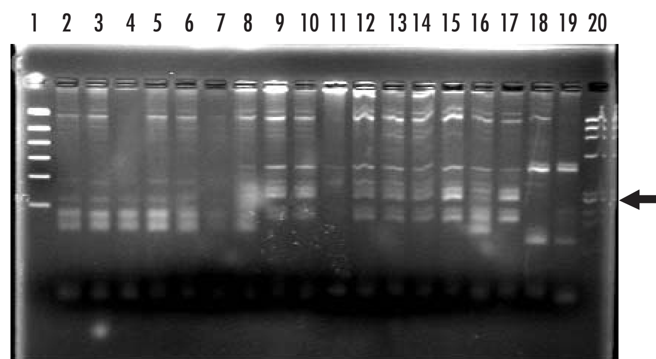


Figure 5. SSR-PCR products amplified with primer gwm37.
Note: 1,PCR marker; 2-7, susceptible materials; 8, ZY20195; 9-15, translocation lines; 16,7AiL; 17,L1; 18-19,*Th. intermedium*; 20, X174/*Hae*III marker. Arrow indicates position of marker for the *Bdv2* gene.

resistant materials with *Bdv2,* but nothing in the materials without *Bdv2*. The detection results of F_2 plants indicated that the SCAR marker of *SC-W37* was co-segregated with *Bdv2.*

SCAR marker-assisted selection in a wheat breeding program

The SCAR markers of *SC-D04* and *SC-W37* were used to detect the resistance and select BYDV-resistant genotypes in five generations of plants of the crosses and backcrosses. From 636 plants, the specific bands of *SC-D04* and *SC-W37* were detected in 300 individuals but absent in other 336 individuals. The results of SC-W37 were in agreement with that of *SC-D04.*The results of SCAR detection were in agreement with that of field test for resistance in F_2, F_3 and BC_3 plants.

Acknowledgment

This study was supported by National "863" Program (2001AA211011) and the Special Fund of Transgenic Plants (J99-A-011).

References

Ayala, L., Henry, M., Gonzalez-de-Leon, D., van Ginkel, M., Mujeeb-Kazi, A., Keller, B., and Khairallah, M. 2001. A diagnostic molecular marker allowing the study of *Th. intermedium*-derived resistance to BYDV in bread wheat segregating populations. *Theor. Appl. Genet.* 102:942-949.

Banks, P.M., Xu, S.J., Wang, R.R.C., and Larkin, P.J. 1993. Varying chromosome composition of 56-chromosome wheat x *Thinopyrum intermedium* partial amphiploids. *Genome* 36:207-215.

Banks, P.M., Larkin, P.J., Bariana, H.S., Lagudah, E.S., Appels, R., Waterhouse, P.M., Brettell, R.I.S., Chen, X., Xu, H.J., Xin, Z.Y., Qian, Y.T., Zhou, X.M., Cheng, Z.M., and Zhou, G.H. 1995. The use of cell culture for subchromosomal introgressions of barley yellow dwarf virus resistance from *Thinopyrum intermedium* to wheat. *Genome* 38:395-405.

Brettell, R.I.S., Banks, P.M., Cauderon, Y., Chen, X., Cheng, Z.M., Larkin, P.J., and Waterhouse, P.M. 1988. A single wheatgrass chromosome reduces the concentration of barley yellow dwarf virus in wheat. *Ann. Appl. Biol.* 113:599-603.

Cauderon, Y., Saigne, B., and Dauge, M. 1973. The resistance to wheat rusts of *Agropyron intermedium* and its use in wheat improvement. In: *Proc 4th Int. Wheat Genet. Symp.* Sears, E.R. and Sears, L.M.S. (eds.). University of Missouri, Columbia, Missouri. pp. 401-407.

Conti, M.D., D'Arcy, C.J., and Jedlinski, H. 1990. The "yellow plague" of cereals, barley yellow dwarf virus. In: Burnett, P.A. (ed.). World perspectives on barley yellow dwarf. Mexico, D.F.: CIMMYT. pp. 1-6.

Friebe, B., Mukai, Y., Gill, B.S., and Cauderon, Y. 1992. C-banding and in-situ hybridization analyses of *Agropyron intermedium*, a partial wheat X *Ag. intermedium* amphiploid, and six derived chromosome addition lines. *Theor. Appl. Genet.* 84:899-905.

Hohmann, U., Badaeva, K., Busch, W., Fribe, B., and Gill, B.S. 1996. Molecular cytogenetic analysis of *Agropyron* chromatin specifying resistance to barley yellow dwarf virus in wheat. *Genome* 39:336-347.

Larkin, P.J., Banks, P.M., Lagudah, E.S., Appels, R., Chen, X., Xin, Z.Y., Ohm, H.W., and McIntosh, R.A. 1995. Disomic *Thinopyrum intermedium* addition lines in wheat with barley yellow dwarf virus resistance and with rust resistances. *Genome* 38:385-394.

McIntosh, R.A., Hart, G.E., Devos, K.M., Gale, M.D., and Rogers, W.J. 1998. Catalogue of gene symbols for wheat. In: *Proceedings of the 9th International Wheat Genetics Symposium.* Volume 5. Saskatoon, Saskatchewan, Canada. 2-7 August.

Paran, I., and Michelmore, R.W. 1993. Development of reliable PCR-markers linked to downy mildew resistance genes in lettuce. *Theor. Appl. Genet.* 85:985-993.

Röder, M.M., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.H., Leroy, P., and Ganal, M.W. 1998. A microsatellite map of wheat. *Genetics* 149:2007-2023.

Sharma, H.C., Gill, B.S., and Uyemoto, J.K. 1984. High levels of resistance in *Agropyron* species to barley yellow dwarf and wheat streak mosaic viruses. *Phytopathol. Z.* 110:143-147.

Sharp, P.J., Kreis, M., Shewry, P.R., and Gale, M.D. 1988. Location of beta-amylase sequences in wheat and its relatives. *Theor. Appl. Genet.* 75:286-290.

Stoutjesdijk, P., Kammholz, S.J., Kleven, S., Matsay, S., Banks, P.M., and Larkin, P.J. 2001. PCR-based molecular marker for the *Bdv2 Thinopyrum intermedium* source of barley yellow dwarf virus resistance in wheat. *Aust. J. Agric. Res.* 52:1383-1388.

Wang, R.R.C., and Zhang, X.Y. 1996. Characterization of the translocated chromosome using fluorescence *in situ* hybridization and random amplified polymorplic DNA on two *Triticum aestivum-Thinopyrum intermedium* translocation lines resistant to wheat streak mosaic or barley yellow dwarf virus. *Chromosome Research* 4:583-587.

Xin, Z.Y., Brettell, R.I.S., Cheng, Z.M., Waterhouse, P.M., Appels, R., Banks, P.M., Zhou, G.H., Chen, X., and Larkin, P.J. 1988. Characterization of a potential source of barley yellow dwarf virus resistance for wheat. *Genome* 30:250-257.

Xin, Z.Y., Xu, H.J., Chen, X., Lin, Z.S., Zhou, G.H., Qian, Y.T., Chen, Z.M., Larkin, P.J., Banks, P.M., Apples, R., Clarke, B., and Brettell, R.I.S. 1991. Development of common wheat germplasm resistant to barley yellow dwarf virus by biotechnology. *Sciences in China* (Series B) 34(9):1055-1062.

Xin, Z.Y., Z.Y. Zhang, X. Chen, Z.S. Lin, Y.Z. Ma, H.J. Xu, P.M. Banks, and P.J. Larkin. 2001. Development and characterization of common wheat-*Thinopyrum intermedium* translocation lines with resistance to barley yellow dwarf virus. *Euphytica* 119:161-165.

Zhang, Z.Y., Z.Y. Xin, Y.Z. Ma, X. Chen, Q.F. Xu, and Z.S. Lin. 1999. Mapping of a BYDV resistance gene from *Thinopyrum intermedium* in wheat background by molecular markers. *Science in China* (Series C) 42(6):663-669.

Zhang, Z.Y., Xin, Z.Y., and Larkin, P.J. 2001. Molecular characterization of a *Thinopyrum intermedium* group 2 chromosome (2Ai-2) conferring resistance to barley yellow dwarf virus. *Genome* 44:1129-1135.

A Map-based Approach towards Cloning the *Yd2* Resistance Gene in Barley

B. King, N. Paltridge, N. Collins, C. Ford, R. Li, R. Symons, and P. Langridge

University of Adelaide, Department of Plant Science, Waite Campus, South Australia, Australia

In an attempt to identify and characterize the Yd2 *gene, a map-based cloning strategy has been undertaken in our laboratory. Linkage maps in two barley populations have been constructed placing 29 molecular markers, the* Yd2 *gene, and the centromere on these maps. Twelve of these markers are within 1cM of* Yd2*. Physical mapping of the* Yd2 *region revealed a physical linkage between the two loci* Xbcd134 *and Ylp. Because these loci are separated by 0.5cM on the Proctor/Shannon map, a relationship between physical to genetic distance around this immediate area could be calculated as 1cM being less than 360kb. Screening of barley bacterial artificial chromosome filters identified a number of* Yd2 *linked BACs forming a partial contig over the* Yd2 *region. Further chromosome walks will continue until a complete contig over the* Yd2 *gene is constructed.*

A major research program aimed at elucidating plant-pathogen interaction occurring upon virus infection was initiated in our laboratory in 1991. The experimental system chosen was the interaction between the *Yd2* resistance gene and *Barley yellow dwarf virus* (BYDV). Unlike many other resistance genes, the interaction between host and pathogen cannot be described as a hypersensitive response. *Yd2* does not prevent the virus from spreading systemically from the point of infection, rather it appears to act by reducing the rate of replication of the virus in the phloem (Skaria *et al.*, 1985; Ranieri *et al.*, 1993). The *Yd2* resistance gene has provided durable resistance over the last 40 years to a large range of BYDV isolates (Lister and Ranieri, 1995). The cloning and characterization of the *Yd2* gene may identify a new class of genes with novel modes of action. Such a discovery may give us insight into targeting specific plant genes involved in host-pathogen interactions for the creation of novel and durable resistances to other pathogens.

The major focus of our project has been to attempt to clone *Yd2* by a map-based approach. For this approach, a genetic map is constructed of sufficient resolution to enable the identification of one or more DNA markers at a physical distance from the target gene, which is less than the average size of the genomic library being used. Thus, the markers are used to "land" on a clone containing the gene (Tanksley *et al.*, 1995).

Construction of a Linkage Map

The first step towards the isolation of *Yd2* was the generation of a linkage map of the *Yd2* region in barley. A number of DNA probes were mapped as restriction fragment length polymorphisms (RFLPs) to a population of 106 F2 segregants derived from a cross between the barley varieties Proctor (*Yd2*-minus) and Shannon (*Yd2*-plus). By the end of 1996 a detailed linkage map of the *Yd2* region of barley chromosome 3 had been constructed and published (Collins *et al.*, 1996). This completed the first stage of the map-based approach.

The second stage of the project involved the saturation of the *Yd2* region with markers closely linked to *Yd2* and the resolution of co-segregating markers from *Yd2*. Therefore, a larger mapping population of 572 F2 progeny in a new cross, between Atlas (*Yd2*-minus) and Atlas68 (*Yd2*-plus) was constructed, allowing us to accurately place *Yd2* on our map with our closest flanking markers spanning a genetic distance of 1.6cM. Importantly, recombination rates for this cross were five times greater than in the Proctor/Shannon cross, allowing us to resolve molecular makers previously

unresolvable from *Yd2*. Amplified fragment length polymorphisms (AFLPs) also helped with the saturation of the region and the placement of a new marker YLM, 0.7cM from *Yd2* and distal to the centromere (Figure 1).

In the analysis of 572 F2 individuals, 18 F2 individuals were identified that showed a recombination event within a 1.6cM interval between the markers YLM and MWG952. These recombinants were selfed and individuals homozygous for the recombination event identified. These individuals were then available for the creation of bulks of resistant and susceptible individuals for further marker development and high resolution mapping of the *Yd2* region.

The revised maps presented in Figure 1 consist of 29 *Yd2* linked markers spanning a genetic distance of 27.6cM. Two markers, YLM and YLP, were developed into PCR markers and have since been used in breeding programs to introgress the *Yd2* gene into suitable agronomic backgrounds (Ford *et al.*, 1998; Paltridge *et al.*, 1998).

Physical Mapping of the Region

The third stage of the map-based cloning strategy involved the generation of data about the relationship between physical and genetic distance from the *Yd2* region. This information is crucial to the success of our map-based strategy as the relationship can vary over 100-fold in different regions of the genome (Ganal *et al.*, 1989). Such information would tell us of the probability of finding a large genomic clone with our closest marker which may also contain the *Yd2* gene, or whether there would be a need for another round of marker saturation. Therefore, pulsed field gel electrophoresis (PFGE), in combination with restriction enzyme digestion of high molecular weight DNA, was used to establish a relationship between our closest molecular markers.

Using the rare cutter *Pvu*I, it was shown that one of our co-segregating markers YLP was physically linked to a flanking marker BCD134 by a maximum physical distance of 180kb (Figure 2). BCD134 is 0.5cm north of *Yd2*. Therefore, the relationship between physical and genetic distance around the *Yd2* region could be calculated as being approximately 1cM = 360Kb. This information suggested that the identification of a large genomic clone(s) spanning the *Ylp* locus would have a high probability of spanning the *Yd2* gene.

Screening a Barley BAC Library and Development of a Contig over the *Yd2* Gene

Filters from a barley bacterial artificial chromosome library (BAC) were imported from Clemson University, USA, and screened with our closest molecular markers. A number of BACs were identified. These clones were confirmed to be from the *Yd2* region by specific PCR and Southern hybridization. Based on PFGE analysis, genomic inserts ranged in size from 98kb to 150kb. Southern fingerprint analysis identified an overlap between a number of these BACs, which contain genetically distinct molecular markers. Two clones spanned the *Ylp* locus and co-segregated with *Yd2*. The

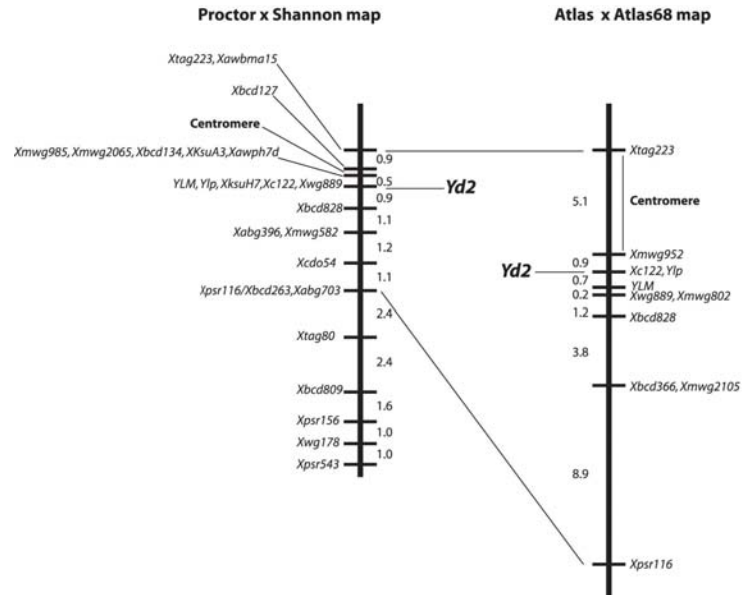


Figure 1. Linkage maps of the *Yd2* region of barley chromosome 3 developed in the two mapping populations Proctor x Shannon and Atlas x Atlas 68.

Notes: Linkage maps of the *Yd2* region of barley chromosome 3 developed in our two mapping populations. RFLP markers have the "X" prefix. Loci that perfectly co-segregated are separated by commas, and the PCR marker YLM was generated in this laboratory. The Proctor x Shannon population was constructed using 106 F2 individuals (Collins *et al.*, 1996), while the Atlas x Atlas68 cross was constructed using 572 F2 individuals. The maps comprise 29 molecular markers and the Yd_2 gene. Distances are in centiMorgans. Importantly recombination rates for the Atlas x Atlas68 cross were five fold greater than in Proctor x Shannon cross allowing us to resolve molecular makers previously unresolvable from *Yd2*.

other 4 clones span the *Xbcd134* locus, a molecular marker shown to be 0.5cM proximal of *Yd2*. Therefore, the first of a series of genomic walks with the aim of constructing a series of overlapping genomic clones (contig) across the *Yd2* region has been initiated.

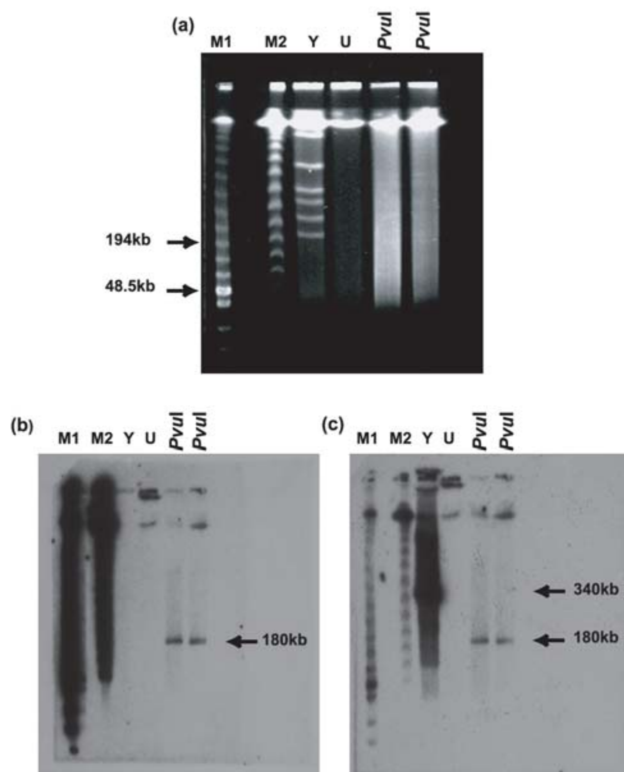


Figure 2. The co-hybridization of two genetically distinct markers closely linked with *Yd2*, to a single restriction enzyme fragment on a pulsed field gel.

(a) HMW DNA prepared from nuclei was digested with the restriction enzyme *Pvu*I and subjected to PFGE. Restricted DNA was run at 200 volts for 24 hours. A linear ramp of 15-50 seconds was used to resolve DNA from 6.55kb to 533.5kb in size. Low range (**M1**) and long range (**M2**) lambda concatemer ladders as well chromosomes of a rice yeast artificial chromosome (YAC) clone (**Y**) positive for one of the makers were used to help size the DNA. Undigested HMW DNA (**U**) was also included as a control.

(b) A Southern hybridization of the membrane described above with the probe BCD134 was performed and the membrane exposed to highly sensitive film at -80^{0}C for 48 hours. The genetic loci *Xbcd134* identified by the probe BCD134 is separated from *Yd2* by 0.5cM in genetic distance. Radiolabelled lambda DNA was used to visualize the concatemer ladder. BCD134 hybridized to a restriction fragment of approximately 180Kb in size, which was not present in the undigested control. BCD134 did not hybridize to the rice YAC. The membrane was washed for 10 minutes in 0.4M NaOH and then stripped 3 times with boiling stripping solution (2mMEDTA, 0.1% SDS) and re-exposed to highly sensitive film for 72 hours to check the probe had been successfully removed. The membrane was then used for re-hybridization.

(c) A subsequent hybridization to the same pulsed field membrane was made with the DNA probe YLP, which maps to the *Ylp* loci that co-segregates with *Yd2*. The probe hybridized to the rice YAC as well as to a restriction fragment of approximately 180Kb. The autoradiographs were overlaid using the concatemer ladders and autorad background to correctly align them. Both restriction fragments were found to hybridize to the same point therefore, suggesting physical linkage of both genetically distinct markers *Xbcd134* and *Ylp* to the single *Pvu*I restriction fragment.

Currently we are sequencing the ends of these clones so we can develop specific PCR markers which can then be used to confirm BAC overlaps and also amplify BAC end-probes. These BAC end-probes will be used as hybridization probes to identify more BAC clones close to the *Yd2* region as well as being mapped within a population of critical recombinants between the markers YLM and MWG952 (Figure 1). Based on physical-genetic relationships near the *Yd2* gene, we expect to complete construction of a contig over the *Yd2* area after a further 1-2 genomic walks.

Future Objectives

Once a series of overlapping clones are established across the *Yd2* gene, the entire BAC contig covering this region will be sequenced. Computer analysis of this sequence can be undertaken using gene prediction programs to identify putative candidate genes. Probes and DNA primers derived from the BAC contig based on this sequence information can be used for cDNA capture, Northern analysis, and Real-Time PCR. DNA probes can also be used for Southern analysis to delimit the physical area around the *Yd2* gene using individuals identified as recombinant between our two closest flanking markers in the Atlas/Atlas68 mapping population. The delimitation of the physical area will reduce the number of candidate genes to be analyzed. It is envisaged that proof of function of any candidate gene will be confirmed through complementation of phenotype by either transformation or mutagenesis.

References

Collins, N.C., Paltridge, N.G., Ford, C.M., and Symons, R.H. 1996. The *Yd2* gene for barley yellow dwarf virus resistance maps close to the centromere on the long arm of barley chromosome 3. *Theoretical and Applied Genetics* 92:858-864.

Ford, C., Paltridge, N.G., Rathjen, J.P., Moritz, R.L., Simpson, R.J., and Symons, R.H. 1998. Rapid and informative assays for *Yd2*, the barley yellow dwarf virus resistance gene, based on the nucleotide sequence of a closely linked gene. *Molecular Breeding* 4:23-31.

Ganal, M.W., Young, N.D., and Tanksley, S.D. 1989. Pulsed field gel electrophoresis and physical mapping of large DNA fragments in the *TM-2a* region of chromosome 9 in tomato. *Molecular and General Genetics* 215:395-400.

Lister, R.M., and Ranieri, R. 1995. Distribution and economic importance of barley yellow dwarf virus. In: D'Arcy, C.J. and Burnett, P.A. (eds.). *Barley Yellow Dwarf: 40 years of Progress.* The American Phytopathological Society, Minnesota. pp. 29-53.

Paltridge , N.G., Collins, N.C., Devaux, P., Baulcombe, D.C., and Symons, R.H. 1988. Development of a codominant PCR marker to *Yd2*, the barley yellow dwarf virus resistance gene. *Theoretical and Applied Genetics* 96:1170-1177.

Ranieri, R., Lister, R.M., and Burnett, P.A. 1993. Relationships between barley yellow dwarf virus titre and symptom expression in barley. *Crop Science* 33:968-973.

Skaria, M., Lister, R.M., Foster, J.E., and Shaner, G. 1985. Virus content as an index of symptomatic resistance to barley yellow dwarf virus in cereals. *Phytopathology* 75:212-216.

Tanksley, S.D., Ganal, M.W., and Martin, G.B. 1995. Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes. *Trends in Genetics* 11:63-68.

Exploitation of Detected BYDV Resistance Genes in Barley Breeding

J. Ovesna, V. Sip, J. Chrpova, J. Vacke, M. Skorpik, K. Polakova, and L. Kucera

Research Institute of Crop Production, Prague, Czech Republic

The resistance to Barley yellow dwarf virus *(BYDV) of barley cultivars, breeding lines, and resistance sources from world collections has been tested in RICP Prague-Ruzyne over the past 10 years. Most barley materials were found to be susceptible or moderately susceptible to BYDV. High resistance levels were associated with the presence of the* Yd2 *gene, but other genes non-allelic to* Yd2 *were also detected in moderately resistant spring and winter barley cultivars. Significant levels of resistance to BYDV were obtained in spring barley by combining* Yd2 *with genes detected in moderately resistant cultivars. The effect of* Yd2 *on barley quality traits was examined using different sets of double haploid (DH) lines and recombinant inbred lines (RILs). Analysis showed that the presence of the* Yd2 *gene did not affect malting quality traits or other important agronomic characters tested. The effect of the genetic background on* Yd2 *efficiency was investigated and found to be rather high, especially in spring cultivars. Valuable spring genotypes were developed using* Yd2 *lines, moderately resistant Czech lines, and traditional breeding approaches. Winter barley materials with good agronomic type and acceptable levels of BYDV resistance were obtained from crosses of non-*Yd2 *but moderately resistant genotypes.*

Infection with *Barley yellow dwarf virus* (BYDV) is a serious threat to the cultivation of barley in many parts of the world, including Central Europe. The virus was first identified in 1951 in the USA; in the Czech Republic the occurrence of this virus has been observed since the early 1960s. The virus has several strains with different properties but the PAV strain has the greatest importance in the Czech region. During the past 10-15 years, very strong outbreaks and at times even epidemic spread have been recorded in many regions of Czech Republic. Heavy attacks on barley and wheat crops by BYDV (and *Wheat dwarf virus*, WDV) have been recorded this year in warmer areas of the country.

There are several ways to minimize yield losses caused by BYDV. Early sown barley crops (late August – early September in Central Europe) and fields in which conservation tillage practices were applied are at particular risk in our conditions. Control is possible through aphicide applications (effective but costly) or by agronomic measures. The most effective means to limit damage caused by this pathogen is through the use of BYDV resistant varieties.

We have started a BYDV resistance breeding program aimed at developing cultivars with good agronomic traits, excellent end-use quality, and carrying BYDV resistance gene(s). These objectives have involved several steps such as genetic resources screening, genetic analysis, crossing and selection of the most promising progeny, and DNA marker application.

Field Tests and Results of BYDV Resistance Screening

Response to BYDV infection was studied in field tests using artificial inoculation, in which cultivars/lines were grown on two row 1 m-plots (usually with three replications). Earlier, field trials consisted of two blocks (infected and control) separated by a protective belt. More recently, the control and infected blocks have been adjacent (without a

protective belt), which helps to improve symptom classification (direct comparison of control and infected strains). During inoculation, control blocks are covered with sheets to protect them against infection.

At the beginning of tillering and, in the case of winter barley, in the autumn, artificial inoculation with the PAV strain of BYDV is carried out by placing greenhouse-reared aphids (*Rhopalosiphum padi*) on the plants. Reactions to the virus infection are recorded using the 0-9 scale developed by Schaller and Qualset (1980). Data on grain weight per spike and tiller number after infection are combined with visual symptom scores using the "susceptibility" index proposed for barley by Sip *et al.* (1997b).

Most spring and winter barley cultivars grown in Central Europe have been found susceptible or very susceptible to infection with the PAV strain prevalent in this region (Vacke *et al*., 1997, 1998). Among spring barleys, only the cultivars Malvaz, Atribut, and Madras were found to be moderately resistant to BYDV. Moderate resistance was also detected in the winter cultivar Perry from the USA and Sigra from Germany (Table 1). Test cultivar sets included breeders' lines, resistance sources originated mainly from CIMMYT/ICARDA collections, and cultivars registered in the Czech Republic. High resistance levels were associated with the presence of the *Yd2* gene (Ovesna *et al*., 2000a).

Results of Genetic and Molecular Analyses

Based on genetic analysis, new resistance gene(s) were detected in Perry, Sigra, and several local cultivars (Sip *et al*., 1997a; Ovesna *et al*., 2000b), which were non-allelic with *Yd2*. Analyses of segregation in the F3 showed that this resistance is likely to be polygenic in cultivars Malvas and Atribut (with Czech malting quality) and Madras. Genetic analyses in crosses with winter barley cultivars Perry and Sigra, however, showed that one gene with larger effect is likely to be responsible for resistance in Perry and two genes for resistance in Sigra. Some lines resulting from crosses between *Yd2* cultivar and Sigra or Perry were highly resistant (Fig. 1).

The absence of *Yd2* gene in the above mentioned moderately resistant cultivars was also confirmed with the use of the PCR diagnostic marker Ylp (Table 1).

Traits of Selected Materials Differing in BYDV Resistance

The characteristics of spring and winter barley lines selected for use in hybridization programs are given in Table 1. It is apparent that highly resistant *Yd2* gene carriers are predominantly 6-row barleys with compact

Table 1. Characteristics of test barley cultivars and lines, their average responses to BYDV infection, and evidence of *Yd2* gene presence obtained with the use of Ylp (Ford *et al.*, 1998) PCR diagnostic marker.

Cultivar/line	Origin	Number of rows	Response to BYDV*	Ylp	Plant height (cm)	Heading **	TGW (g)	% extract	Powdery mildew***
Spring barley									
Corris	CIM	6	2.5	+	100	46	45	75.5	MS
Giza 121/Pue	CIM	6	3.0	+	70	32	34	71.5	MS
Atlas 68	USA	6	3.4	+	75	26	39	74.3	S
Brea´S´/Ben	CIM	2	3.4	+	90	34	36	74.0	MR
Madras	DEU	2	5.1	-	90	37	43		R
Malvaz	CZ	2	5.1	-	90	41	44	74.1	S
Atribut	CZ	2	5.2	-	80	37	40	75.8	R
Akcent	CZ	2	8.5	-	85	36	37	75.2	R
Winter barley				o					
Wysor	CIM	6	2.5	+	80	10	34		MR
Sigra	DEU	6	4.9	-	90	18	35		MS
Perry	USA	6	5.2	-	90	19	35		MR
Luxor	CZ	6	8.9	-	90	18	46		MR

* Average response is based on the 0-9 scale, in which 0 = no disease symptoms.
** Days after 15th May in 1996.
*** R: resistant; MR: moderately resistant; MS: moderately susceptible; S: susceptible.
TGW= Thousand grain weight - % extract (malting quality)

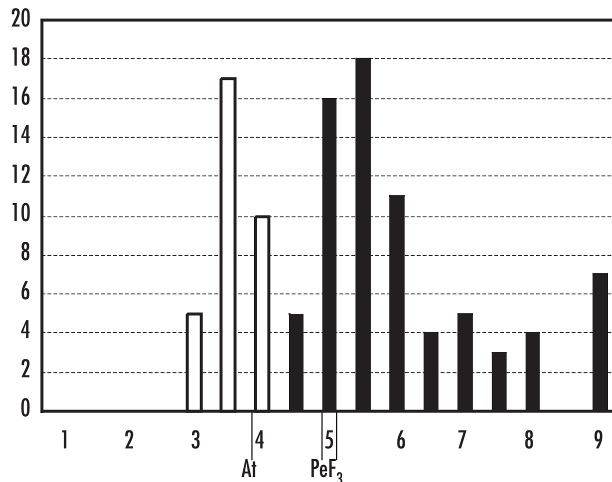


Figure 1. Frequency distribution for BYDV scores (0-9; 0 = no symptoms) from F3 lines of crosses between moderately resistant cultivar Perry and Atlas 68, resistant cultivar carrying *Yd2* gene, 1997-1999 field trials. Several transgressive lines were detected.

ears (not suitable for Czech spring barley breeding). These *Yd2* materials differed in their resistance levels, which indicates the effect of genetic background on *Yd2* efficiency. Other important traits were also examined. It was shown that these materials are mostly not suitable for direct use in Czech breeding. The most convenient material appeared to be two row CIMMYT barley line Brea 'S'/Ben.

In winter barley the available *Yd2* genotypes were found to be very distant from current genotypes. In addition to Wysor, we examined highly resistant lines from ICARDA W BON 96-116, W BON 123, and W BON 96–118. These materials have in our conditions a type similar to that of Wysor, i.e., highly unsuitable short, compact ears. To obtain more rapid progress in breeding, our efforts concentrated on winter barley, especially moderately resistant cultivars Perry and Sigra, which are likely to carry major genes different from *Yd2*.

Effect of *Yd2* on Different Traits in Doubled Haploid Lines

A set of doubled haploid (DH) lines was developed from a Igri/Atlas 68 cross to study the effect of genetic background on *Yd2* efficiency. Results obtained with groups of *Yd2* and non-*Yd2* lines of the Igri/Atlas 68 cross showed high agreement between DNA analysis (using Ylp marker) and field trials. The presence of *Yd2* did not significantly influence heading date, grain yield, thousand-grain weight (TGW), and % extract (malting quality). Only the winter DH lines carrying the *Yd2* gene were taller by about 5 cm than the respective non-*Yd2* lines. The variability of symptom scores in spring *Yd2* DH lines was much higher (2.22) than in winter lines (1.14) (Chrpova *et al*., 2001).

Results of Breeding Barley for BYDV Resistance

A high proportion of lines with acceptable resistance levels (VSS < 4) was obtained by crossing the *Yd2* gene carriers with moderately resistant malting barley cultivars (Atribut, Madras, or Malvaz) (Sip *et al*., 2001). The highest percentage of lines with high breeding value occurred in the cross Akcent/Brea'S' Ben, which is very important from a breeding standpoint.

In winter barley, three moderately resistant (VSS = 4.5 – 5.O) lines coming from the cross Sigra/Borwina/ /SG-L 74 are being yield tested this year in official breeding station trials. Resistance derived from Perry was also successfully exploited especially after crossing this cultivar with high yielding modern cultivar Luxor.

Further Breeding Strategies

Primary *Yd2* genotypes may be suitable for transferring resistance genes into adopted germplasm when broadening of genetic diversity is required. However, simple introgression of *Yd2* into elite genotypes requires multiple backcrossing and careful characterization of the resulting progeny. Evaluation of progenies with the Ylp marker and monitoring of saturation of genetic background by SSR or AFLP analyses may speed up the breeding process. We tested the reliability of YLM (Paltridge *et al*., 1998) and Ylp (Ford *et al*., 1998) markers to differentiate *Yd2* genotypes, and we demonstrated the advantages of DNA analysis over artificial field tests. Development of a DNA marker associated with Perry resistance gene is under way. DNA fingerprints of Czech elite barley cultivars are available, and the tool is being introduced into practical breeding.

Acknowledgment

This work was supported by the Grant Agency of the Czech Republic, Grant No. 0521/ 00/0332.

References

Chrpova, J., V. Sip, J. Ovesna, J. Vacke, and L. Kucera. 2001. Möglichkeiten der Resistenzzüchtung bei Gerste gegen Gerstengelbverzwergungsvirus mit Nutzung molekularer Marker. 52. Züchtertagung, Gumpenstein, Austria. (In press.)

Ford, C.M., N.G. Paltridge, J.P. Rathjen, R.L Moritz, R.J. Simpson, and R.H. Symons. 1998. Rapid and informative assays for *Yd2*, the barley yellow dwarf virus resistance gene, based on the nucleotide sequence of a closely linked gene. *Mol. Breed.* 4:23–31.

Ovesna, J., J. Vacke, L. Kucera, J. Chrpova, I. Novakova, A. Jahoor, and V. Sip. 2000a. Genetic analysis of resistance in barley to barley yellow dwarf virus. *Plant Breeding* 119:481-486.

Ovesna, J., V. Sip, L. Kucera, J. Chrpova, I. Novakova, A. Jahoor, and J. Vacke, 2000b: Genetic diversity of BYDV resistant barley cultivars determined by molecular markers. *Czech J. Genet. Plant Breed.* 37:17-22.

Paltridge, N.G., N.C. Collins, A. Bendahmane, and R.H. Symons. 1998. Development of YLM, a codominant PCR marker closely linked to the *Yd2* gene for resistance to barley yellow dwarf disease. *Theor. Appl. Genet.* 96:1170-1177.

Schaller, C.W., and C.O. Qualset. 1980. Breeding for resistance to barley yellow dwarf virus. In: Proc. Third Int. Wheat Conf., Madrid, Spain. University of Nebraska Agric. Experiment Station. MP 41:528-541.

Sip, V., J. Vacke, J. Chrpova, and M. Skorpik. 1997a. Genetic diversity and mode of inheritance of resistance to barley yellow dwarf virus in spring barley. *Genet. a Slecht.* (Prague) 33:261-279.

Sip, V., J. Radek, M. Skorpik, and J. Vacke. 1997b. Comparison of different index estimates of BYDV resistance in cereals. Advances in Biometrical Genetics. Proceedings of the Tenth Meeting of EUCARPIA Section Biometrics in Plant Breeding. pp. 265-270.

Sip, V., J. Chrpova, J. Vacke, and I. Novakova. 2001. Possibility of exploitation of the detected BYDV resistance genes in barley breeding. In: Proc. of the 50th Anniversary Conf. pp. 60-61.

Vacke, J., V. Sip, and M. Skorpik. 1997. Response of selected spring barley varieties and advanced breeding lines to the infection with barley yellow dwarf virus. *Genet.a Slecht.* 33:33-44.

Vacke, J., V. Sip, and M. Skorpik. 1998. Barley yellow dwarf virus harmfulness on winter barley crops infected at an early growth stage. *Czech J. Genet. Plant Breed.* 34:27-29.

Breeding for BYDV Tolerance in Wheat as a Basis for a Multiple Stress Tolerance Strategy

A. Comeau[1] and S. Haber[2]

[1] Agriculture Agri-Food Canada, SCRDC, Sainte-Foy Québec, Canada
[2] Agriculture Agri-Food Canada, CRC, Winnipeg, Canada

There are major theoretical and practical differences related to tolerance vs. resistance breeding. The correlation between disease resistance sensu stricto *and protection against crop losses is not what the layman would expect. It seems entirely logical that a resistance gene reducing virus levels should also protect against losses. Yet tolerance genes simply reduce economic loss factors, regardless of virus titers. Resistance* sensu stricto *(reflected in reduced ELISA titers) always leads to reduced virus spread in the field, but not necessarily to smaller losses in individually infected plants. In studies of the* Barley yellow dwarf virus *(BYDV), visual symptom data, somewhat based on loss of chlorophyll, were much less reliable in wheat than in other species. Moreover, in wheat, susceptibility* sensu stricto *(reflected in high ELISA titers) is not related to a higher probability of serious losses. Species certainly differ in the manner in which virus titers correlate with symptoms and damage. Correlation between ELISA titers, symptomatology and losses may be found in bulk plant population samples, in cases where infection occurred at an early stage and under growing conditions that increased damage. Yet in species like bread wheat, durum wheat, oats and triticale, the correlation between symptoms and damage might become significant only under the most severe epidemic conditions; in average conditions, up to 25% economic loss can occur in the presence of minimal or near invisible symptoms. In fact, durum wheat and bread wheat often give counterintuitive research results which appear at face value like a violation of the Koch postulate. In these species, reliable tolerance data necessitate a study for many years and at many contrasting sites, using quantitative data on many parameters. This is because growing conditions represent an important variable that can modify the losses caused by barley yellow dwarf virus (BYDV) infection, and this interaction is serious in the genus* Triticum*. In ideal growing conditions, some amount of phloem damage may have very little real effect on crop yield. In the presence of secondary stresses, the deleterious effects induced by BYDV may be increased a lot. In cases where secondary stresses are mild, BYDV-induced losses may also be minor, and this reduces the difference between tolerant and sensitive lines. Our current understanding of tolerance mechanisms is that diverse aspects of biotic and abiotic stress resistance can also improve virus tolerance, and that the tolerance mechanisms of one line or cultivar can be quite different from the mechanisms present in another. This has theoretical and practical consequences. Due to the complex nature of tolerance and to its interaction with environmental stress, tolerance data obtained at one site may correlate poorly with data from another site. The practical caveat is that BYDV information on bread wheat and durum wheat must be seen as much more site-specific and year-specific that the data on rusts for example, and that many test sites and/or years of data are needed for a valid evaluation of tolerance. A practical application of the current understanding of tolerance is that within breeding programs, BYDV can be used to increase the overall effects of secondary stresses in wheat and oats. Since yield stability and yield potential are both related to the capacity of plants to mitigate stress-induced losses, BYDV is used to make the response to secondary stress easier to evaluate. Instead of being simply a disease, BYDV is now one of the most essential tools in germplasm enhancement and cereal breeding, in order to identify stability of yield and quality, and also water and nutrient use efficiency.*

In the past, it has been commonly assumed that improving disease resistance *sensu stricto* also reduced crop losses, i.e. made plants also more tolerant. Yet this view may not apply to all diseases, and, as we shall discuss, especially not to BYDV. Tolerance and resistance are not one and the same. This paper will focus more attention on the merits of virus tolerance *sensu stricto*, in relationship with the efforts of our research groups to develop elite BYDV-tolerant germplasm and cultivars. We will also discuss current understanding of how the underlying mechanisms are involved at the cellular and physiological levels.

It is essential to apply rigorously the terminology of Cooper and Jones (1983), so as to develop a realistic understanding of the complex genetic nature of so-called “field resistance” phenomena. Field resistance at the individual plant level should be properly called tolerance; it is the desirable plant response to disease pressure, i.e. reduction or absence of pathogen-induced damage. Whether or not the pathogen is present in large or small amount is not relevant to the notion of tolerance. Lack of tolerance must be called “sensitivity”, and distinguished from “susceptibility”.

The use of the term “resistance” must be restricted to cases where pathogen amounts in individual plants were measured or assayed indirectly, as it means a reduced level of pathogen multiplication and/or translocation in individual plants, and represents the effect of the plant itself on the pathogen. Virus evaluation from bulked samples of many plants may lead to misinterpretation of what happens inside individual plants. The most common test for virus titer is also not direct; ELISA quantifies extractable viral protein, and correlation with viable virions is not necessarily demonstrated. The presence of high amounts of virus is “susceptibility” *sensu stricto* as defined by Cooper and Jones. The extreme case of resistance is immunity, in which case the pathogen cannot be found in the host plant. In BYDV research, resistance has been evaluated much less thoroughly than tolerance (Burnett *et al*., 1995). One might think that cultivars endowed with high resistance or immunity would be automatically very tolerant; but this idea, while seemingly logical and appealing, must be abandoned when using the Cooper and Jones terminology, and when confronting numerous cases where plants restrict pathogen multiplication but yet suffer important damage. One must learn to observe and discuss the two phenomena of resistance and tolerance as non-correlated traits, generally (but not always) controlled by totally different genes in wheat. The meaning of the term “symptoms” also needs discussion, as well as the use of symptom data vs. quantitative data on many traits in the decision-making. In practice, true resistance may limit virus spread. It may or may not reduce damage to individual plants. Tolerance, on the other hand, does reduce damage, but this may or may not be correlated to a reduction of virus content or ELISA values.

For the sake of discussion, symptoms will be separated into categories: those visible only under microscopy or through physiological measurements, and those visible by the naked eye; causal relationships will also be discussed.

Symptoms: Early and Less Obvious Events

Before considering the meaning of the visible symptoms, one should review what is known about the microscopic and physiological consequences of virus infection. Virus content reaches only a few parts per million. This tiny biomass of pathogen is known to cause heavy damage in certain cereal cultivars, and not in others. Viral biomass proportion is thus unrelated to damage. In the first electron microscopy studies of the internal symptoms (Esau, 1957a, 1957b), phloem degeneration and callose deposition inside the phloem were the first internal visible symptoms. James Chong (pers. comm.) later observed that callose deposition actually plugged plasmodesmata that connect phloem sieve elements. This might account for a series of observed consequences. If the plugging of plasmodesmata is the way by which translocation of photosynthate is rapidly impeded, then, the abundance of callose deposition and the way it plugs plasmodesmata might be called the primary symptom, the very first cause of all further damage. The difference in damage between two cultivars might have little relationship with the way the cultivar represses virus multiplication and spread, but relate more to differences in callose formation and deposition patterns. Thus, the overall efficiency of plants in reaching an optimal control of the callose formation mechanism might be an important aspect of tolerance mechanisms. Callose plays many roles in plants, and one of its roles is to reduce virus spread through plasmodesmata (Epel, 1994). Yet this resistance mechanism is due to have many deleterious effects on the plant.

It was hypothesized that cereal plants could perhaps become gradually divided into compartments, during plant growth, so that mid-season infection of one tiller might not lead to infection of other tillers. The validity of this hypothesis is supported by some data (Makkouk *et al*., 1994c), at least for oats and bread wheat, since the tiller-to-tiller virus movement is less likely to occur when plants are infected at later growth stages. This was shown using the tissue-blot immunoassay method (TBIA-ELISA). Yet, the tolerant bread wheat check Maringa was not different from the sensitive check Katepwa in that respect. Therefore, in bread wheat, mechanisms that prevent virus spread in other tillers remains another form of resistance without a proven benefit for tolerance.

The study of various interspecific hybrids of wheat with *Agrotricum* yielded a lot of the data that are the basis for the present discussion. For a series of Chinese-Australian interspecific-derived wheat lines, identified with the prefix "Zhong", the BYDV resistance was confirmed by many research teams, through the use of ELISA and TBIA-ELISA (Chen *et al*., 1997, 1998). Yet in field trials in Canada, in Quebec and in Winnipeg as well, the expected tolerance did not express (Comeau *et al*., 2001) (Figure 1), regardless of the fact that in some other countries, in which the growing season was longer, tolerance did express rather well. Another wheat interspecific hybrid derived from crosses to OK7211542 had the generally

Figure 1. Zhong 5 wheat, with (right) and without (left) BYDV. No virus was detectable by ELISA in the inoculated plant, thus Zhong 5 is resistant to BYDV, but not tolerant. Data from Haber.

expected behavior: its resistance was correlated to tolerance, in Canada as well as in other countries (Comeau *et al*., 1994). However, this was a winter wheat. To our knowledge, transfer of this resistance from winter wheat background to spring wheat background is not yet demonstrated. One should never assume that the genes that protect winter wheat would automatically protect spring wheat. In winter wheat, a lag time between infection and the triggering of the resistance and tolerance responses is permitted. In spring wheat, this lag time must be extremely short, at least in Canada; a long lag time leads to losses as those shown in Figure 1.

The reverse counterintuitive case was also common. Wheat lines with high ELISA values together with low losses were easy to find. Very useful genes of tolerance were found in lines that were totally devoid of resistance. For example, the interspecific Chinese *Triticum aestivum* line Long Miai 10 tolerates BYDV quite well, but 26 d after infection, Long Miai 10 had the highest ELISA titre of all wheat lines evaluated (Comeau *et al*., 1992). This proved that tolerance within individual plants had very little to do with resistance.

Physiological Symptoms and Visible Symptoms

The physiological effects of BYDV were recently summarized (Jensen and D'Arcy, 1995). Damage to the phloem and plasmodesmata leads to physiological effects of the virus which in turn lead to macro effects in terms of plant morphology, phenology, color, and quality. In brief, most of these effects result from reduced translocation. This causes carbohydrate accumulation which in turn increases leaf dry weight per unit area, inhibits photosynthesis by feedback, reduces chlorophyll, and increases respiration (Jensen, 1968).

What the virus does to root tips is a critical question. Root tips are far from the source of photosynthate, and thus suffer a lot from impaired translocation. The root tips of sensitive barley lines can be killed by BYDV after as little as 4 days (Comeau *et al*., 1990). Root tips are important in stress signaling. The first stress signals emitted by the root apex may be mediated by ABA; under severe stress, ethylene may also be involved. Such signals spread rapidly to the whole plant, potentially affecting all traits. In oats, root apex growth is reduced, and root

morphology is altered by the virus (Al Faiz, 1994). Wheat behaves in a similar way (Comeau and Al Faiz, unpublished).

The mechanisms that control the phenology, morphology and tiller rate are also distorted in rather unpredictable ways by the virus-induced hormonal disturbances. A biodiversity of patterns of plant response to virus-induced stresses can be identified within a group of lines that have poor tolerance. Some lines react to infection by developing a larger number of weak tillers; others grow fewer tillers. Because of this genetic variability in plant response, a more general approach to the description of symptoms is needed.

Isolates of BYDV differ in severity, and the environment modulates the effects of the virus. A rather weak strain in a difficult growing environment may cause severe losses, while a severe strain in a mild environment may cause only mild losses. Even the moderately severe isolates of BYDV (often PAV serotypes) may fail to produce typical visual symptoms in wheat grown in good soil under good conditions. Yet, even in absence of visual symptoms, a quantitative study may reveal significant economic damage. A total absence of symptoms would necessarily entail that no micro, macro or physiological effect reached the detectable level. But in practice, in most of the Gramineae, BYDV symptoms are expressed through changes in many parameters, which must also be considered in evaluations of tolerance:

A. Organ size and shape,
B. Organ number,
C. Phenology,
D. Quality parameters of plant tissues (color, texture, composition),
E. Reaction to other diseases and to other stresses.

Defining and Measuring Virus Tolerance

Tolerance relates to the effects virus infection exerts on the plant, and has little to do with ELISA titers or quantities of infectious virus. These effects are complex, and when the plant deals with a restricted amount of available photosynthate, a strong effect on one parameter can be counterbalanced by a smaller effect on another parameter. Thus, if quantitative measurements could be made for each factor, and if the data were normalized and adjusted, then equations that quantify global plant tolerance could look like the following:

$A_v*B_v*C_v*D_v*E_v \ / \ A_{ck}*B_{ck}*C_{ck}*D_{ck}*E_{ck}$, or else $((A_v+B_v+C_v+D_v+E_v) \ / \ (A_{ck}+B_{ck}+C_{ck}+D_{ck}+E_{ck}))$ in which the v suffix identifies transformed virus inoculated data of each parameter and ck identifies transformed data for non-inoculated checks. Using indexes always involves arbitrary choices. Some choices emerge as better practices, when the correlation of indexes with real losses of yield and quality is verified. The equation would be improved further by taking into account many organs of the plant (tillers, roots, florets, seeds), and also the number of days to boot, flowering or ripeness stage. This academic approach takes into account the fact that one line or cultivar could allocate more of its scarce resources to roots when infected, thus reducing the photosynthate available to tillers, florets or seeds. It also takes into account that another line could simply slow down its phenology, taking more time in an attempt to produce the same number of tillers, spikes, and florets for example. A first step is to calculate standardized values: (A_v –(mean of A))/(standard deviation of A), and so on for other parameters. The mean and standard deviation of the parameter may be taken from all pooled data (virus-infected and non-infected). Signs must be adjusted to give positive values to all desirable traits. An arbitrary constant must be added so that all values stand well above zero; a value of 10 can be suggested for a start. Tolerance equations could include weighing factors on each parameter, for practical reasons.

In practice, measurements of yield, biomass, harvest index, and seed quality (caliber and specific weight) can replace the very detailed approach suggested above. Selection indexes can be developed from data on many traits, and experimental validation of those indexes is possible using comparisons between inoculated and virus-free plots or plants. As an ultimate cost-cutting strategy, selection work is possible using solely an index based on data from virus-inoculated plants. Once validated with enough virus-free checks, such an index is efficient for the early screening of genotypes.

To complete these general guidelines, one must realize that height and lodging data must also be used in decision-making, and present a serious problem. Lodging is not normally distributed. Height has an

optimum value (80-95 cm); very tall and very short values are undesirable, because the tallest suffers lodging, and the ultra short always presents complex, severe defects. Height genes also interact strongly with virus tolerance. Therefore, it is essential to consider height in decision-making. Because of the problems related to height, one alternate approach is to make a 2-D graph of height versus a tolerance index based on other parameters. Another approach is to forget about using indexes, and take all decisions based on rotating 3-D plots with the shape and color of points added as fourth and fifth dimensions; this rapid and elegant approach can be implemented using Data Desk® software (Data Description Inc., Ithaca, NY).

Tolerance measurements based on one single criterion have the virtue of simplicity, but the major defect of low heritability, because over a few generations, it is unavoidable that improving one single organ's reaction to BYDV will lead to reduction of size and number of other organs, and to a longer life cycle. This understanding led to the development of selection indexes based on many parameters, routinely used by Comeau in field trials, and to the "QI-assay" used for trials under controlled conditions (Haber and Comeau, 1998). These methods have brought the art of screening and breeding for tolerance to a level of reproducibility and precision that increases the heritability of tolerance and thus allows for more rapid progress.

Virus Tolerance as a Phenomenon Related to General Tolerance

The complexity of breeding for tolerance might be viewed at first as a disadvantage. However, tolerance confers many additional benefits, which relate to how tolerance is intermingled with the overall physiological efficiency of the plant. Current knowledge of the physiology of plant response to stress supports the postulate of the existence of a central, common core of stress response mechanisms, explained in detail by Geiger and Servaites (1991). The concept of the common core is shown in a simplified manner in Figure 2. This common core is strongly related to genes and biochemical pathways related to signaling and stress response. However, signals and responses are dependent on constitutive traits that can modify physiological efficiency and must also be seen as part of the core mechanisms. There is evidence that genes can impart multiple resistance (Kasuga *et al*., 1999), but more research is needed on these central mechanisms. Examples might relate to sucrose transport and to abscisic acid and other elements of the signaling pathways. Central mechanisms modifying the reallocation of carbohydrate or photosynthate in response to a stress deserve attention. One example would be the mechanisms that lead to rapid allocation of most of the energy to roots when maize seedlings suffer from water deficit (Comeau and Nodichao unpubl.); nutrient deficiencies create a similar response.

The plant response to any stress signal can be described as a modification the redistribution of photosynthate between organs and biochemical pathways (Geiger and Servaites, 1991). This redistribution should allow the plant to make a better use of its environment, yet the intertwined signal and response mechanisms are genetically complex, and vary in their level of efficiency of use of the environment. Most of these mechanisms can be classified as tolerance rather than resistance.

Tolerance mechanisms, due to their complexity, are buffered in a way that makes them most unlikely to lose effectiveness due to a point mutation in the pathogen. Indeed, the effectiveness of tolerance to BYDV in the oat cultivar Ogle for example has lasted for over 35 years. Tolerance, being associated to a number of traits that make the plants more efficient under complex natural stress, tends to be related with high yield and yield stability (Haber *et al*., 1997), as well as with horizontal resistance to biotic stresses.

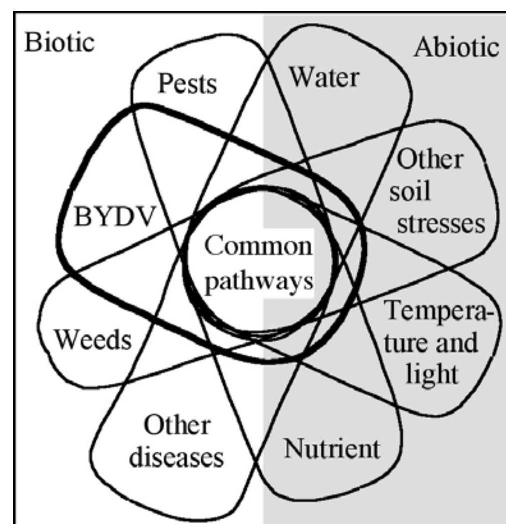


Figure 2. Hypothesis of a common core of stress response and stress tolerance mechanisms, related for example to sucrose reallocation mechanisms and various elements of the signaling and response pathways. A small part of the common core also relates to constitutively expressed genes.

Some resistance genes had an unavoidable yield-reducing side effect (Smedegaard-Petersen and Tolstrup, 1985). A resistance gene may have an energy cost or a pleiotropic effect making plant physiology somewhat less efficient. Plant pathologists should verify how common is the phenomenon with fungal and viral diseases. Yet in discussing this issue, it is important to remember that tolerance is not resistance, and that side effects related to resistance may not apply to tolerance. Tolerance might have many positive consequences related to various traits.

In a plant breeding perspective, BYDV is a very desirable stress component, whenever one intends to select plants that maintain physiological efficiency in the presence of complex natural stresses. Being itself a moderate, chronic stress, BYDV amplifies the effects of a number of other stresses. The first damage may be on roots; but through reduced absorption of minerals, it also reduces floret initiation, tiller number, floral peduncle elongation, and can increase plant sensitivity to root rots and other diseases (Comeau, 1992). Thus mechanisms affecting water and nutrient use efficiency become more critical after viral infection. In fact, through this complex damage, BYDV helps eliminate the lines that possess various physiological weaknesses in the segregating populations. It should not come as a surprise that BYDV data can be used as a reasonably good predictor of yield and yield stability in natural conditions, even in areas where BYDV is not a very frequent problem (Haber *et al*., 1997).

Roots are important in being one of the first targets of the virus, and in being a source of signals within the plant. Viral effects on roots cannot be neglected if one is attempting to breed for tolerance. The root apex can be compared to a nerve center involved in the control of plant growth and plant response to stress (Davies and Zhang, 1991, Tardieu *et al*., 1992). A reduction of the ability of the root apex to capture minerals may trigger one of the earliest signals of drought stress (Schmidhalter, 1995). Root damage from BYDV comes from reduced photosynthate translocation. However, by reducing apex growth, the virus indirectly reduces the plant's ability to capture minerals, which may lead to the same signal-inducing situation as drought.

Some apparent drawbacks of the tolerance approach are acknowledged. Tolerance will not reduce the pool of virus inoculum in the environment; in fact, it may do the reverse. Breeding for tolerance to BYDV is moderately labor-intensive, but necessitates special training about how to control stress severity. Data analysis is more complex. Natural inoculum is seldom uniform enough to be useful, except in the rare areas where BYDV is very severe every year. Even when using artificial inoculation, special care is needed to obtain uniform plantlet emergence and uniform vigor at the early stages, otherwise the effect of the virus will be to favor the plantlets that had an early advantage. In certain environments, tolerance correlates with excess plant height, which must be understood before taking decisions about parental selections for further crosses.

Breeding for Tolerance

Research focused on gene pyramiding for BYDV tolerance was pursued from 1980 to 1993. This focused research was mostly a failure in the sense that high tolerance was obtained, but it was in a background that made it poorly heritable and almost useless. All of the best sources of tolerance had very low gluten viscosity and other deleterious traits like excess height and mediocre grain quality. For unknown reasons, all the early attempt to transfer tolerance to medium height plants failed, despite major efforts. Height genes do modify the expression of tolerance to BYDV; in fact, selection on the basis of visual symptoms alone tends to lead to favor plants like QW 624.13, which is much too tall for practical use. Dwarfing genes tend to reduce BYDV tolerance in all cereal species. However, this interaction between height and tolerance is not expressed in the same manner in all ecological and climatic conditions. The interaction is very clear-cut in Quebec every year, but in other areas where soils are deep or the growing season much longer, the interaction is less obvious. This interaction might relate in part to effects of the dwarfing genes on root systems. In virus-free conditions, roots of a series of tall virus-tolerant lines grew about 10% longer than those of the best semidwarf lines, in an aerated hydroponic systems with intermittent pumping of nutrient solutions (St-Pierre and Comeau, unpublished). We concluded that dwarfing genes probably had some effect on root length.

Profound interactions between virus stress, soils and climate had been observed. Excellent growing conditions delayed the expression of symptoms and reduced their intensity; damage was also reduced in such conditions. In some replicated trials, symptomless or near symptomless damage exceeded 25% (Collin, pers. comm.). The integrity of

plasmodesmata and phloem is likely more critical when plants are under environmental stress. Therefore genotypes that possess a more efficient phloem, genotypes that develop lesser amounts of virus-induced damage to plasmodesmata , and genotypes that adjust in a more successful way to the resulting overall stress complex may become the only ones able to tolerate BYDV in a complex stress situation. The nature of the damage observed differs according to the species and stage of infection of plants. In bread wheat, midseason inoculation caused more damage than early inoculation, while producing less obvious symptoms (Comeau, 1987). Early infection affects all parameters but may have less effect on grain fill; late infection may have little effect on tillering and floret fertility, but can have profound effect on grain fill. Part of the damage may also take the form of phenology shift. The infected plants may ripen earlier, with disastrous effects on yield, or later than normal, in which case risks of frost may be increased.

Thus in some environments, instead of basing selection on symptoms, it is necessary to ponder data on many traits when taking decisions. The heritable and usable part of the tolerance components may be those that are not tightly linked or pleiotropic with excess height. Care must be taken not to keep resistance or tolerance genes that respond too slowly to virus infection, leading to a period of sluggish growth that modifies the phenology. The "QI-assay" method developed by Haber recognized those problems and based its selection criteria on notes about heading and flowering date (more often delayed by the virus), spike size, and flag leaf size. Provided a strict protocol is followed in growth cabinet trials, delay of heading can predict a major proportion of the viral effect on yield, and promising lines can be identified early enough to use them as crossing parents in the same plant growth cycle (Haber and Comeau, 1998). The field selections done by Comeau are based on height, symptoms, yield, biomass, harvest index, and specific weight of infected plots. Rotating 3-D plots (using Data Desk software) and indexes weighing the different traits are viewed as good ways to implement a multiple-trait tolerance selection system. These recommended methods are a bit complex, but not very costly, and seem to lead to major gains in efficiency of selection for tolerance genes.

Selection methods used after 1993 emphasized the study of correlation between traits in order to identify the heritable components of tolerance. As an example, in the examples in Table 1, the recent Ste-Foy line QG 2.1, which has perhaps the highest BYDV tolerance of all lines observed in 24 years, would have been discarded if judged on symptoms alone. For symptoms it would have been rated average, not exceptional. The cultivar Maringa, which has the best symptom rating, does confer tolerance when used as a parent in crosses, but it also contains a large number of alleles that give tall weak straw and others that are deleterious to hectoliter weight and bread-making quality. Thus, it has not led until now to easy progress in breeding for BYDV tolerance. The line QW 624.13 might be easier to use than Maringa, but suffers from a few similar defects. Crosses to QG 2.1 show that the good tolerance of this line is very heritable. Parents such as QG 2.1, which have tolerance without excess height, generally seem to provide a better heritability of BYDV tolerance.

Selection of tolerant or resistant cereal lines in plant breeding programs can be done on the basis of symptoms only if a number of conditions are met (Burnett *et al*., 1995). Virus infection with a severe strain must be done early with a heavy inoculum; this is best done through artificial inoculation. The use of ELISA for breeding purposes may work for barley (Makkouk *et al*., 1994a) but is not practical for oats, durum wheat, triticale or bread wheat, unless the material selected contained specific resistance genes of interspecific origin. All resistance genes must be assessed as to whether or not they confer tolerance in field situations; indoor tests are really not reliable for this goal, unless extreme precautions are taken. ELISA or preferably active virus titer assays are essential to distinguish resistance from tolerance. The textbook symptoms of barley yellow dwarf virus mostly relate to losses of chlorophyll, which are obtained only under specific conditions, i.e. with certain lines of some species inoculated early with severe isolates (Comeau *et al*., 1992). Some cereal genotypes may not lose chlorophyll even when suffering from severe stress (Thomas and Howarth, 2000; Silva *et al*., 2000). Thus, the best possible symptom evaluation must include many traits besides plant color, and the visual notation may still lead to inconclusive evidence. Symptoms are not acceptable for diagnosis and loss estimation purposes except in severe epidemics.

An adequate supply of micronutrients is necessary for lignin formation and also for disease resistance and tolerance (Graham and Webb, 1991). Mid-season BYDV infection was observed to increase lodging (Comeau unpubl.), perhaps as a secondary effect of root damage which reduces all nutrients and may impede lignin

formation. The predisposition of cereals to other diseases after BYDV infection has also been discussed (Comeau, 1992). Screening for BYDV tolerance might theoretically favor plants that are more efficient for micronutrient uptake. Some of the recent BYDV tolerant parental selections have improved straw (Table 2), and some showed better than average manganese efficiency in muck soils (ex. AC Superb, QW 628.5 and BRS 120) (Comeau and Voldeng, unpublished). It seems plausible that the use of BYDV as a selection factor in segregating populations may help identify alleles for better straw and improved nutrient use efficiency, provided the tallest plants are eliminated.

A special note is needed about the use of specific weight and harvest index in the BYDV selection process. The use of specific weight was originally

Table 1. Example of the use of data on six traits of various lines or cultivars under BYDV inoculation, for the purpose of assessing the tolerance level.

line or cultivar	n	Height (cm)	Symptoms (0-9) [a]	Yield (kg/ha)	Biomass (kg/ha)	Harvest Index	Specific weight (kg/hl)	Tolerance class
Katepwa	>8	67	7,9	1488	6091	0,229	71,5	Sensitive
AC Voyageur	>8	76	5,0	2646	8851	0,294	73,7	Mod. tolerant
Maringa	>8	94	2,2	3683	11489	0,316	74,3	Mod. tolerant
QG 2.1	>6	77	4,4	4295	10550	0,389	76,5	Very tolerant
QW 624.13	>6	91	3,7	3902	11218	0,326	77,6	Tolerant
89 Mu2-c.EL-6	>5	87	3,2	3361	10937	0,306	79,8	Mod. tolerant
PF 70354	3	69	5,0	2454	7304	0,338	72,4	Mod. tolerant
Long Miai 10	3	74	5,4	2331	7862	0,296	73,4	Mod. sensitive
Mean		79	4,6	3020	9288	0,312	74,9	
F value [b]		14,73	19,18	8,77	8,05	7,82	11,36	

[a] The most tolerant would be rated 0, and the least tolerant 9.
[b] Data is summarized by a least squares analysis, with n data sets (sites or years). Note that virus damage tends to be more severe for shorter lines, and yet heritable components of tolerance are easier to obtain from shorter parents.

Table 2. Noteworthy traits observed in some of the favorite parents available within the currently known sources of BYDV tolerance for bread wheat, in Canadian germplasm development projects.

Cultivar or line[a]	Source	Tolerance - biotic	Tolerance – abiotic and other useful traits	Undesirable traits
Maringa (MT)	Brazil	Pythium, rusts, mildew, Fusarium head blight, root rots	Acid soils, hypoxia	Low specific weight, excess height, lodging, low harvest index, inferior gluten and bread-making quality
Pai Toborochi[b] (MT)	Bolivia(CIMMYT)	WSMV, rusts	Early drought and heat, good short straw, good gluten	Slightly inferior bread-making quality
Fundacep 29 (MT)	Brazil		Good short straw, yield	Inferior gluten
EMBRAPA 27(MT)	Brazil	Pythium, rusts	Good short straw	Inferior gluten
Kohika(MT-VT)	New Zealand		Good short straw, good root system	Inferior gluten, Fusarium head blight
QG 2.1 (VT)	Canada	Dryland root rot	Early drought and heat, Very good harvest index	Mildew, inferior gluten
QW 628.5(VT)	Canada	Mildew	Mn efficient, good flexible straw	inferior gluten
AC Superb(MT)	Canada	Wheat midge, Hessian fly, mildew, rusts	Good harvest index	

[a] BYDV tolerance is qualified as very tolerant (VT), or moderately tolerant (MT).
[b] Pai Toborochi is derived from PF 70354, a Brazilian line viewed as a source of BYDV tolerance. One must rate PF°70354 and Pai Toborochi as moderately tolerant, considering they are among the best in their height class.

recommended by I. Ramirez (INIA, Chile), during joint research supported by the International Development Research Center. It was confirmed over the years that this trait is one of the most useful ones to consider, because its use integrates the late effects of BYDV on grain fill and grain quality with the increased root rot damage that often accompanies BYDV infection (Comeau, 1992). However, in many environments, specific weight is generally lower for the shortest lines. Harvest index is a trait that tends to be higher for shorter germplasm. Harvest index, yield and biomass under infection are not as easy to evaluate as specific weight, and give less significant F values in ANOVAs. Thus, the final judgement about lines and parents must take into account the autocorrelation between traits and the statistical value of the observations.

A better understanding of the mechanisms that confer tolerance would be helpful. Circumstantial evidence originally led us to believe that root health and vigor traits were those that were the most correlated with BYDV tolerance. Reduced absorption of minerals by BYDV-infected oats was observed (Comeau and Barnett, 1979), and BYDV tolerant lines had, on average, slightly longer roots than sensitive lines in virus-free situations (Comeau and St-Pierre, unpubl.). Our present hypothesis is that tolerance might be the net result of the interaction of a group of traits that relate to the global theory of stress response proposed by Geiger and Servaites (1991): the most tolerant genotype is the one that has the plasticity needed to make best use of photosynthate in a given complex stress situation. In this case, indeed, what confers tolerance in one environment could differ from what confers tolerance in another environment.

Over years of using them as parental material, some of the outstanding traits of the preferred BYDV-tolerant parental lines have become known. Traits other than the virus tolerance of those lines can be quite noteworthy on their own (Table 2). It has been difficult in the past to keep the highest bread-making quality while breeding for BYDV tolerance, because much of the germplasm used came from Brazil and from other sources that had not selected for the gluten type judged essential in Canada and USA. In countries where gluten quality is judged on less stringent standards, this would be a lesser problem.

Conventional breeding in Western Canada has recently come up with one wheat cultivar that has improved BYDV tolerance (medium-good level) together with good bread-making value (AC Superb). Breeding using BYDV selected parents has led to the feed wheat line QW 628.5, soon to be released as a cultivar by Agriculture Agri-Food Canada, Ottawa (Voldeng, pers. comm.). This line embodies high yield and yield stability with other valuable traits. Part of the BYDV tolerance genes come from a Flicker's' line (CIMMYT source).

The best use of BYDV inoculum is to apply the stress during the segregating generations. In the Quebec oat breeding program (1971-1987), this approach has yielded many BYDV tolerant oats with improved yield, stability of yield and quality, and other good attributes. These cultivars were highly successful and occupied almost all of the oat area in Quebec for over a decade, replacing the sensitive ones that were also available. Recent research at Agriculture Agri-Food Canada, Winnipeg recently confirmed that the use of BYDV in the segregating generations can indeed help obtain germplasm with improved yield and yield stability as the end product (Haber, unpubl.). The new working hypothesis is that the use of BYDV could be combined with the use of a few other stresses in order to allow synergistic interactions leading to the best results in terms of rapid elimination of lines that have higher G x E (yield instability over environments) (Comeau *et al*., 2001). Research is under way to verify the hypothesis.

Combining stresses in very early generations led to a high discard rate of F_2 or F_3; in most populations, less than 2% of the plants were kept. Thus a complex crossing system became essential to create the higher genetic variability that is needed under complex stress. For example one may use F_1 / F_1 crosses including 3 or 4 different sources of resistance and tolerance genes, and the resulting F_1 plants can be submitted immediately to complex stress. The very high rate of rejection may upset traditions. However, severe natural selection can also happen in standard breeding. For example, in the early days of triticale breeding, a major epidemic of barley yellow dwarf virus (BYDV) destroyed a lot of the CIMMYT germplasm. This was viewed as a beneficial event, eliminating many genotypes with unstable yield, while the more stable ones survived (Comeau *et al*., 2001).

Within the genus *Aegilops*, some resistance *sensu stricto* was found (Makkouk *et al*., 1994b), and attempts to use this are still under way. Some promising tolerant lines were obtained but reconfirmation of resistance *sensu*

stricto in any wheat/*Aegilops* derivatives is still lacking. Over the last 20 years, ELISA was used in Quebec on approx. 200 genotypes representing very broad biodiversity and containing many of the wheat/*Aegilops* mentioned above. No true resistance was found except in the Chinese-Australian interspecific derivatives (which in Canada lacked tolerance) and in derivatives of *Agrotricum* line OK 7211542. True resistance thus seems very uncommon in cultivated wheat. Current data indicates that whatever was transferred from resistant *Aegilops* to wheat through selection pressures with BYDV in segregating generations was tolerance genes; the resistance genes *sensu stricto* may have been lost. In oats, some true resistance was found, mostly in alien species (Al Faiz, 1994); whatever was found in cultivated oats was poorly correlated with tolerance, confirming a similar conclusion by Lapierre and Gavrilovic (pers. comm.) about cultivated oats.

Conclusion

Virus tolerance must be measured using data about many traits, and decision-making must take the correlation between traits into account, otherwise the effects of selection may be quite different from the intended goal. Tolerance may relate to the efficiency of plant response to complex stresses, and to the ability to maintain photosynthate translocation after viral infection. The way BYDV interacts with other biotic and abiotic stresses is a promising research topic with practical applications for breeding and germplasm development purposes. Data from diverse sites have more meaning once extra care is given to data interpretation in relationship to local secondary stresses. Root tips suffer heavily from BYDV-induced phloem damage. Since root tips are part of a signaling system which can rapidly modify photosynthate allocation, a fine-tuned, robust stress response system is essential for tolerance to BYDV in the presence of secondary stresses. There is real interest in investigating drought, excess rain, insufficient or excessive light intensity, mineral deficiencies, root rot, and other viral diseases such as WSMV, in mixed stress situations where a moderately severe or severe BYDV inoculum is an added component at a given stage. It seems that this approach imitates the natural complex stresses and also makes selection of better plants easier. So far, this approach has led to germplasm with higher yield, more stable yield, and reduced G x E interactions in wheat and oats.

The concepts of resistance and tolerance may well be too complex for the layman. However, scientists should deal with those concepts, do the science accordingly, and use the correct terminology in scientific publication. Useful data on tolerance is obtainable in one year for oats and barley, and correlation between sites tends to be rather good. For bread wheat and durum wheat, good tolerance data implies a study for many years and at many contrasting sites. Tolerance also is more site-specific for wheat than for other cereals. As an example of potential problems, the BYDV data in the USDA-ARS germplasm bank does not clearly spell out the difference between BYDV resistance and tolerance (USDA-ARS, 2002). Evaluation may have focused strongly on visual symptoms. Some of the lines mentioned in the USDA-ARS collection have been tested in Quebec, and at least 6 lines listed as rather "resistant" would be viewed as rather inferior choices in breeding against BYDV in Canada, based on our tolerance tests. Table 1 provides vivid examples that justify a distrust of visual symptoms when used alone. Thus, we could not use the USDA-ARS data within a Canadian project.

Tolerance and resistance both have merits. There is less genetic complexity in resistance. Some of the mechanisms are partly understood. For example the virus transport rate is affected by Yd2 in barley, and plant defense mechanisms that reduce virus spread within a plant belong *ipso facto* to the resistance category. Using transgenic approaches, some resistance mechanisms can be improved upon or invented. However, most of the additional benefits associated with natural tolerance genes are not obtained by resistance breeding approaches, and those extra benefits, discussed below, seem important enough to make tolerance an excellent strategic choice in its own right.

Resistance *sensu stricto* must be evaluated as to its effects on other traits and as to its correlation (or lack of) with tolerance. One should never assume resistance leads to tolerance. Resistance may be a good choice in areas that have frequent severe epidemics; it also reduces the spread of inoculum to nearby plants.

Tolerance seems correlated to a number of valuable traits and may deserve a much more prominent place in the toolbox of the breeders. QTLs associated with yield and yield stability are accepted as having value in plant breeding; one would be hard pressed ignoring

a simple tool such as BYDV which provides similar benefits, using a rather simple technology. Tolerance to this virus has shown consistent and repeatable correlation with good and stable yield (Haber *et al* ., 1997, Comeau *et al*., 2001) and seems associated with many other tolerance traits.

BYDV remains the most important viral disease of cereals. Yet, only a small percentage of cereal breeding programs have included BYDV tolerance as a major goal. The benefits of breeding for BYDV tolerance are obvious for regions where BYDV epidemics are frequent (Burnett *et al*., 1995). Yet, it should be recognized that important benefits are also possible for other areas where BYDV is only a negligible problem. In those areas, controlled BYDV inoculation may help to increase in a synergistic manner the overall efficiency of the selection processes for tolerance to other biotic and abiotic stresses (Comeau *et al*., 2001). This is why we now label BYDV as a broad-purpose breeding tool, and not only as a disease (Haber *et al*., 1997).

References

Al Faiz, C. 1994. Effets du virus de la jaunisse nanisante de l'orge (VJNO) sur les racines de l'avoine [Effects of BYDV on oat roots]. Ph. D. thesis, 124 pp, Laval U., Quebec, Canada.

Burnett, P,A., Qualset, C.O., and Comeau, A. 1995. Host plant tolerance or resistance for control of barley yellow dwarf. Pages 321-343. In: D'Arcy, C. and Burnett, P.A. *Barley yellow dwarf: 40 Years of Progress.* Am. Phytopath. Soc. Press, St-Paul, Minn., USA.

Chen, Q., Ahmad, F., Collin, J., Comeau, A., Fedak, G., and St-Pierre, C.A. 1998. Genomic constitution of a partial amphiploid OK7211542 used as a source of immunity to barley yellow dwarf virus for bread wheat. *Plant Breed.* 117:1-6.

Chen, Q., Collin, J., Comeau, A., St-Pierre, C.A., and Fedak, G. 1997. Comparison of various sources of resistance to barley yellow dwarf virus in wheat-*Thinopyrum* amphiploid lines. *Can. J. Plant Pathol.* 19:414-417.

Comeau, A. 1987. Effects of BYDV inoculation at various dates for oats, barley, wheat, rye, and triticale. *Phytoprotection* 68:97-109.

Comeau, A., Caetano, V., St-Pierre, C.A., and Haber, S. 2001. Accelerated breeding: dream or reality? In: Bedo, Z. and Lang, L. (eds.). Wheat in a global environment. Kluwer Acad. Publ., The Netherlands. pp. 671-679.

Comeau, A. 1992. Interactions between Barley yellow dwarf virus, aphids, plants, and fungal diseases: an ecological model. In: Barley yellow dwarf virus in Western Asia and North Africa. A. Comeau A and Makkouk KM, eds. ICARDA, Aleppo, Syria. pp. 227-233.

Comeau, A., Collin, J., and Chéour, F. 1992. Barley yellow dwarf virus symptoms and ELISA data in relation to biomass and yeld loss. In: Barley yellow dwarf virus in Western Asia and North Africa. A. Comeau and Makkouk KM, eds. ICARDA, Aleppo, Syria. pp. 155-168.

Comeau, A., and Barnett, G. 1979. Effect of barley yellow dwarf virus on N, P, K fertilizer efficiency and on the harvest index of oats. *Can. J. Plant Sci.* 59:43-54.

Comeau, A, Dubuc, J.P., and Munoz, C. 1990. Effects of stress on the etiology of barley yellow dwarf virus. In: Riesselman, J. (ed.). Biotic stress of barley in arid and semi-arid environment (proceedings). Bozeman, Montana. pp. 107-114.

Comeau, A., Makkouk, K.M., Ahmad, F., and St-Pierre, C.A. 1994. Bread wheat x *Agrotricum* crosses as a source of immunity and resistance to the PAV strain of barley yellow dwarf luteovirus. *Agronomie.* 2:153-160.

Cooper, J.I., and Jones, A.T. 1983. Responses of plants to viruses: proposals for the use of terms. *Phytopathol.* 73:127-128.

Davies, W.J., and Zhang, J. 1991. Root signals and the regulation of growth and development of plants in drying soil. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 42:55-76.

Epel, B.L. 1994. Plasmodesmata: Composition, structure, and trafficking. *Plant Mol. Biol.* 26:1343-1356.

Esau, K. 1957a. Anatomical effects of barley yellow dwarf virus and maleic hydrazide on certain Poaceae. *Hilgardia* 27:15-69.

Esau, K. 1957b. Phloem degeneration in gramineae affected by the barley yellow dwarf virus and maleic hydrazide on certain Poaceae. *Am. J. Bot.* 44:245-251.

Geiger, D., and Servaites, J. 1991. Carbon allocation and response to stress. In: H. Mooney, W. Winner, E. Pell and Chu E., (eds.). *Response of plant to multiple stresses.* Acad Press. pp. 103-127.

Graham, R.D., and Webb, M.J. 1991. Micronutrients and disease resistance and tolerance in plants. In: Mortvedt, J.J., Cox, F.R., Shuman, L.M., Welch, R.M. (eds.). Micronutrients in agriculture. 2nd ed. Soil Sci . Soc. Am., Madison Wisc. USA. pp. 329-370.

Haber, S., McKenzie, R.I.H., and Comeau, A. 1997. Barley yellow dwarf virus (BYDV) tolerance in wheat as a predictor of yield. *Can. J. Plant Pathol.* 19:324 (abstr).

Haber, S., and Comeau, A. 1998. "Quantitative indoor assay" (QI-assay), a tool to identify select and develop cereal germplasm that performs well under barley yellow dwarf (BYDV) disease pressure and under other biotic and abiotic stresses. In: Int. Congr. Plant Pathol. Edinburgh. pp. 9-16.

Jensen, S.G. 1968. Photosynthesis, respiration and other physiological relationships in barley infected with barley yellow dwarf virus. *Phytopathology* 58:204-208.

Jensen, S.G., and D'Arcy, C. 1995. Effects of barley yellow dwarf on host plants. In: D'Arcy, C. and Burnett, P. (eds.). Barley yellow dwarf – 40 years of progress. Am. Phytopath. Soc. Press, St-Paul, Minn., USA. pp. 55-74.

Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K., and Shinozaki, K. 1999. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nature Biotechnology*, 17:287-291.

Makkouk, K.M., Comeau, A., and St-Pierre, C.A 1994a. Screening for barley yellow dwarf luteovirus resistance in barley on the basis of virus movement. *J. Phytopathol.* 141:165-172.

Makkouk, K.M., Comeau, A., and Ghulam, W. 1994b. Resistance to barley yellow dwarf virus in *Aegilops* species. *Can. J. Plant Sci.* 74:631-634

Makkouk, K.M., Comeau, A., and St-Pierre, C.A. 1994c. Evaluation of various methods for the detection of barley yellow dwarf luteovirus by the tissue-blot immunoassay and its use for BYDV detection in cereals inoculated at different growth stages. *European J. Plant Pathol.* 100:71-80.

Schmidhalter, U. 1995. Does soil nutrient uptake control drought perception in plants? Interdrought proc., III a,. INRA, ENSA, OSTOM, CIRAD, Montpellier, France.

Silva, S.A, Carvalho, F.I.F., Caetano, V.R., de Oliveira, A.C., de Coimbra, J.L.M., de Vasconcellos, N.J.S., and Lorencetti, C. 2000. Genetic basis of stay-green trait in bread wheat. *J New Seeds* 2:55-68.

Smedegaard-Petersen, V., and Tolstrup, K. 1985. The limiting effect of disease resistance on yield. *Ann. Rev. Phytopathol.* 23:475-490.

Tardieu, F., Zhang, J., and Davies, W.J. 1992. What information is conveyed by an ABA signal from maize roots in drying field soil? *Plant, Cell and Environ.* 15:185-191.

Thomas, H., and Howarth, C.J. 2000. Five ways to stay green. *J. Exp. Bot.* 51:329-337.

USDA-ARS. 2002. Descriptor form for wheat. www.ars-grin.gov/cgi-bin/npgs/html/desc_form.pl?65

Breeding for BYDV Tolerance/Resistance in CIMMYT Bread Wheats Targeted to Developing Countries

M. van Ginkel and M. Henry

CIMMYT, Mexico, D.F., Mexico

The occurrence of *Barley yellow dwarf virus* (BYDV) in certain developing countries is well documented (Lister and Ranieri, 1995). Mostly areas growing rainfed wheat are subject to losses associated with this disease that thrives under moderate temperatures and where precipitation is intermittent.

Typical areas include the highland regions of Kenya and similar climatic settings in neighboring countries in the Eastern African Highlands, such as Burundi, Ethiopia, Rwanda, and Uganda. Also the wetter regions in Mediterranean North Africa may encounter occasional epidemics, such as in Algeria, Tunisia, and the cooler Atlas Mountain region of Morocco (El Yamani and Hill, 1990). In Latin America BYDV is common in Argentina, Brazil, Uruguay, and Chile (Ramirez, 1990), and in the cooler highlands of Bolivia, Colombia, Ecuador, Peru, Mexico (Burnett and Mezzalama, 1990), and Guatemala.

Unlike most common wheat pathogens, the classic disease triangle of host, pathogen, and favorable climate in this case is expanded with a fourth very active player, the vector of the virus particles. This additional complexity, and its large sensitivity to environmental factors, has made the disease rather capricious in a number of ways. Its occurrence in any year is very unpredictable. Once observed in a young crop, it is still unclear whether it will spread during a particular cycle. Even if it spreads, yield losses range from barely noticeable to dramatic with stunting of the tillers and removal of synthesizing tissue due to extreme chlorosis.

This whimsical behavior has made it difficult for researchers especially in most developing countries to justify even moderate funding for studying this disease. In general in many affected countries, steady progress in developing germplasm with durable resistance in a background that is also agronomically desirable to the farmer has been slow or absent. Most cultivars for such environments express adaptation to the majority of local requirements but lack an appreciable level of tolerance or resistance to BYDV. Large advances remain to be made in controlling this disease.

Control measures such as the application of insecticides to limit aphid populations are costly, and if used are not always effective in combating the pathogen. Chemical control is only recommended if both vector and virus are annually endemic with a high level of certainty. Otherwise it is almost never an economically effective option. One of the main reasons for the lack of great effectiveness lies in the specific dynamics of the build-up of a vectored disease. Often alarming levels of the vector (mostly aphids) are not duly noticed until its passenger, the virus, has multiplied in infected plants, and symptoms obvious to the farmers have started to appear. By then control of the vector would clearly come too late. On the other hand, control of the virus itself is an impossibility, with no known compounds available that will slow its multiplication or spread in wheat tissue once it is infected.

As is clear from this introduction, the slogan "an ounce of prevention is worth a pound of cure" is very appropriate for this disease. Vectors can be controlled very early in the cycle (which undoubtedly would reduce any epidemic threat), but this may not be cost effective due to the uncertainty that the disease will

ever really spread, given its complex response to environmental factors. The main approach is to instill the crop itself up front with an inbuilt ability to avoid losses due to the disease.

Breeding for Tolerance to BYDV

Tolerance to this disease has been known since the 1950s (Burnett *et al*., 1995) and is defined as losses being low in the presence of an otherwise relatively large virus concentration in the plant. It is still one of the most promising methods to limit losses due to this disease in developing countries.

A partially effective gene, *Bdv1*, was identified in the variety Anza as conferring tolerance to BYDV-MAV (Singh *et al*., 1993) and is so far the only reported gene for BYD tolerance. It has been incorporated into CIMMYT germplasm, in particular through the association of the tolerance gene *Bdv1* with the widely present durable leaf rust gene *Lr34*. However, other germplasm from various geographical origins has also shown some tolerance to BYDV-PAV and MAV in our trials (Table 1). Recently, it was suggested that, as in oat, tolerance in wheat is multigenic (Ayala *et al*., 2002).

Since the early 1990s, testing for tolerance is done using artificial inoculation of mainly BYDV-PAV at an early growth stage, and then comparing symptoms and growth under disease and in protected plots. These conditions are quite drastic, mimicking a very severe infection.

Table 1. A few examples of lines developed at CIMMYT with confirmed tolerance to BYDV based on symptom evaluation during several cycles. Lines 1 to 5 have acceptable agronomic type.

Cross	Toluca winter 2001*	EL Batan summer 2001**
1 PF72640/PF7326//PF7065/ALD/3/KLT/4/DUCULA	3-2-3	3
2 NG8319//SHA4/LIRA	3-1-2	2
3 NANJING 8508/3/CHUM18//JUP/BJY	2-3-4	3
4 ALTAR 84/AE.SQUARROSA (221)//3*BORL95	1-2-3	3
5 MILAN	4-2-4	3
6 THB/CEP7780	1-2-2	3
7 ANZA	3-2-4	3
8 URES/BOW//OPATA (sensitive check)	5-3-5	6

* Data based on a 3-digit scale as described by Bertschinger (1994).
** Index 1-9 adapted from Qualset (1984), 1 being tolerant and 9 being sensitive.

Over the years at CIMMYT, several of these tolerant sources have been introgressed into better agronomic types and made available to the wider public through the international BYDV and other CIMMYT nurseries (Table 1). They combine reduced losses with acceptable to quite high yield, and resistance to other diseases prevalent in central Mexico, including stripe rust and *Septoria tritici*. The main deficits of these materials are often stable yield, wide adaptation, and acceptable bread-making quality.

It is particularly encouraging that synthetic hexaploids are involved in some of these crosses (e.g. ALTAR 84/ AE.SQUARROSA (221)//3*BORL95). Synthetic hexaploids are a rich new source of diversity in bread wheat breeding that have become more widely available and applied only in the past 10 years. While frequency of occurrence varies, synthetic hexaploid entries have been found that transmit resistance to one or more of the three major wheat rusts, *Septoria tritici*, *Fusarium* head blight, Karnal bunt, *Helminthosporium* leaf blotch, and root rots, and also tolerance to drought, heat, cold, and waterlogging. In addition their grain size is often large with thousand kernel weights of up to 55-60 g.

Further top- and limited back-crossing is in progress to combine these different tolerance sources not just into acceptable agronomic backgrounds but also into ones with high, stable yields and possessing better industrial quality. The latter trait is increasing in importance with many wheat research programs around the world, rightfully or wrongfully so. The schematic representation of such crosses is that of a tolerant source (T), though not the original sources but rather somewhat improved ones (Table 1), being crossed to one or more lines with good agronomic type and industrial quality (A): R/A or R/A_1//A_2, or R/2*A (limited backcross to A). The BYDV tolerant line is mostly used as the female, just in case some of the tolerance is maternally inherited. Table 2 lists some examples of such crosses, with the male parent(s) contributing yield and quality to the BYDV tolerant female parent.

Breeding for Resistance to BYDV

True resistance has not been reported in wheat, but exists in wild relatives such as *Thinopyrum intermedium.* This resistance has been successfully introgressed into bread wheat by several groups. When the first reportedly resistant lines (low virus titers) became

available for crossing in the mid 1990s, studies were undertaken to incorporate the resistance gene (*Bdv2*) into CIMMYT germplasm. TC lines from Australia (Banks *et al*., 1995), and in particular TC14, have been the most studied at CIMMYT. While the virus titer in these wild relatives and their derived lines was shown to be reduced (Ayala *et al*., 2001b), once infected, these lines still expressed considerable sensitivity with obvious dwarfing and biomass reduction. One of the most interesting features of the TC14-derived material is that under artificial or natural infection, levels of infection remain low (Ayala *et al*., 2001a).

A codominant microsatellite marker (gwm 37) was identified and used in the breeding program (Ayala *et al*., 2001a; Henry *et al*., 2001) (Table 3).

It was fortunate that this resistance did not have a strong negative effect on desired agronomic type (Ayala *et al*., 2001b). In some cases, some flecking of leaves was noted, but could effectively be selected against.

Thinopyrum-derived resistance was also introgressed into common wheats with better agronomic type (such as New Long Mai 15 and New Long Mai 19) by researchers in Harbin, China. This material has been used in crosses at CIMMYT for the past five years.

As for tolerance, attempts are underway to improve yield and yield stability, plus industrial quality in these resistant lines. The schematic representation of such crosses is that of a somewhat agronomically improved resistant source (R) (Table 3) being crossed to lines with high yield and outstanding industrial quality (A): R/A, or $R/A_1//A_2$, or R/2*A, or $R_1/R_2//A$ (see Table 4). In the last example listed, two sources of resistance (TC14 and New Long Mai 15) are first combined, prior to a common wheat being used to correct both agronomic type and quality. When the true genetic constitution of these various resistance sources is known, better targeted crosses can be made with the aim of pyramiding resistance mechanisms and genes. Again, the resistant parent is used as a female to include any cytoplasmic contribution to resistance, should there be any.

Segregating populations were alternated between a fully irrigated site in NW Mexico, Cd. Obregon (where BYDV is mostly absent, but selection is carried out for rust resistance and agronomic type) and a high rainfall site in the central Mexican highlands, Toluca or El Batan, where BYDV is naturally prevalent or artificially inoculated. Molecular analyses are being conducted to confirm the presence of the *Thinopyrum* segment (carrying *Bdv2*) in selected plants.

It has become clear from experience that without careful selection for the presence of this gene in early generations (F3/F4), it is easily lost as selection is practiced for other traits. This means that relatively large populations will need to be screened for the gene at these earlier generations. We aim to use the wheat breeding simulation software program QU-CIM to better determine where and when markers are most effectively and efficiently employed.

Table 2. Crosses made with the aim of combining tolerance with good agronomic type and industrial quality.

Entry	Cross
1	ALTAR 84/AE.SQUARROSA (224)//2*YACO/3/SHA4/CHIL/4/MILAN/PASTOR
2	MILAN/SHA7//2*FINSI

Table 3. Lines with gene *Bdv2* from TC14 with acceptable agronomic type from common wheats.

Entry	Cross
1	TC14/2*SPER/3/VEE/PJN//2*TUI
2	TC14/2*SPER//HUITES
3	TC14/2*SPER//MILAN
4	TC14/2*SPER/3/BOW/URES//KEA
5	MILAN*3//TC14/2*SPER
6	TC14/2*HTG//ESDA/LIRA/3/PRINIA

Combining Tolerance and Resistance

To get the full benefit of the alien resistance, it has to be combined with tolerance, since resistance alone does not give complete protection against losses under very severe infection.

Table 4. Crosses made to combine resistance derived from *Thinopyrum intermedium* with good agronomic type and industrial quality from common bread wheats.

Entry	Cross
1	NEW LONG MAI 15/FINSI//MILAN/PASTOR
2	TC14/2*HTG//MILAN/3/BAU/MILAN
3	TC14/2*SPER//MILAN
4	TC14/2*HTG//NEW LONG MAI 15/3/MILAN/PASTOR

This is probably best facilitated by crossing genotypes that have better agronomic adaptation plus good industrial quality, as are represented in the Tables 2 and 4. Those crosses are presently in progress and are schematically represented as: T/A1//R/A2, or T/A_1//A_2/3/R/A_3//A_4.

Earlier on crosses were made between tolerant and resistant sources that did not yet have the full complement of agronomic type and industrial quality. The objective is first to confirm performance under BYDV pressure and the presence of resistance genes (e.g. *Bdv2*) of the resulting lines. Only then will a final cross be made with parents expressing the desired agronomic type, high yield, and quality. The schematic representation of the first cross type is: R/T or T/R; with the final cross represented as: R/T//A or T/R//A (see Table 5). The last example listed is R_1/R_2//T. Applying this scheme, the tolerance by resistance crosses illustrated in Table 5 will later be top-crossed with lines providing better agronomic type and industrial quality.

Conclusions

Resistance brought about by *Th. intermedium* derived material is not easily monitored because in addition to a reduction in virus titer, it has also been shown to be associated with a reduced infection rate. While the latter is good news, a lot of research remains to be done in this area. The bad news is that the resistance often appears to go in tandem with increased sensitivity, at least against BYDV-PAV. It is obvious that any selection strategy has to be modified to ensure the accumulation of desired traits such as resistance and reduced infection rate, while reducing sensitivity to BYDV.

Furthermore, it is not yet clear whether segregating and advanced genotypes are best evaluated under natural infection, or moderate or even severe artificial inoculation.

Table 5. Crosses aimed at combining tolerance from common wheats with resistance derived from *Thinopyrum intermedium.*

Entry	Cross
1	ALTAR 84/AE.SQUARROSA (224)//2*YACO/3/NEW LONG MAI 15
2	NG8319//SHA4/LIRA/4/TC14/2*SPER/3/VEE/PJN//2*TUI
3	PGO//CROC_1/AE.SQUARROSA (224)/3/2*BORL95/4/TC14/2*SPER//MILAN
4	TC14/2*SPER//SHA7/VEE#5/3/MILAN/SHA7
5	TC14/2*HTG//NEW LONG MAI 15/3/NG8319//SHA4/LIRA

Finally, for any resulting germplasm to be accepted as a new variety by producers, industry, and consumers, it will need to have desirable agronomic type, high yield, good resistance to other diseases, and acceptable industrial quality.

Several crossing approaches are feasible, as described above, with additional permutations possible. With the recent advent of marker technology, fieldwork can be augmented with early generation laboratory confirmation of the presence of resistance-conferring chromosome segments.

We are hopeful that meetings such as this one will help us bring together the insights and tools that will result in varieties able to withstand BYDV being successfully released and accepted by farmers.

References

Ayala, L., Henry, M., González de León, D., van Ginkel, M., Mujeeb-Kazi, A., Keller, B., and Khairallah, M. 2001a. A diagnostic molecular marker allowing the study of *Thinopyrum intermedium* derived resistance to BYDV in bread wheat segregating populations. *Theor. Appl. Genet.* 102:942-949.

Ayala, L., van Ginkel, M., Khairallah, M., Keller, B. and Henry, M. 2001b. Expression of *Thinopyrum intermedium*-derived *Barley yellow dwarf virus* resistance in elite bread wheat backgrounds. *Phytopathology* 91:55-62.

Ayala, L., Henry, M. , van Ginkel, M., Singh, R., Keller, B., and Khairallah, M. 2002. Identification of QTLs for BYDV tolerance in bread wheat. Accepted for publication in *Euphytica*.

Banks, P.M., Larkin, P.J., Bariana, H.S., Lagudah, E.S., Appels, R., Waterhouse, P.M., Brettell, R.I.S., Chen, X., Xu, H.J., Xin, Z.Y., Qian, Y.T., Zhou, X.M., Cheng, Z.M., and Zhou, G.H. 1995. The use of cell culture for sub-chromosomal introgressions of barley yellow dwarf virus resistance from *Thinopyrum intermedium* to wheat. *Genome* 38:395-405.

Burnett, P.A., and Mezzalama, M. 1990. Barley yellow dwarf virus and aphids in Mexico. In: *World Perspectives on Barley Yellow Dwarf*. P.A. Burnett (ed.). Mexico, D.F.: CIMMYT. pp. 21-24.

Burnett, P.A., Comeau, A., and Qualset, C.O. 1995. Host plant tolerance or resistance for control of barley yellow dwarf. In: *Barley Yellow Dwarf, 40 Years of Progress*. D'Arcy, C.J. and Burnett, P.A. (eds.). APS, St. Paul, Minnesota, USA. pp. 321-343.

El Yamani, M., and Hill, J.H. 1990. Identification and importance of barley yellow dwarf in Morocco. *Plant Disease* 74:291-294.

Henry, M., van Ginkel, M., and Khairallah, M. 2001. Marker assisted selection for BYDV resistance in wheat. *Research Highlights of the CIMMYT Wheat Program, 1999-2000*. pp. 41-44.

Lister, R.M., and Ranieri, R. 1995. Distribution ands economic importance of barley yellow dwarf. In: *Barley Yellow Dwarf, 40 Years of Progress*. D'Arcy, C.J. and Burnett, P.A. (eds.). APS, St. Paul, Minnesota, USA. pp. 29-53.

Ramirez, A.I. 1990. A review of barley yellow dwarf in the Southern Cone countries of South America. In: *World Perspectives on Barley Yellow Dwarf*. P.A. Burnett (ed.). Mexico, D.F.: CIMMYT. pp. 29-34.

Singh, R.P., Burnett, P.A., Albarran, M., and Rajaram, S. 1993. *Bdv1*: A gene for tolerance to barley yellow dwarf virus in bread wheats. *Crop. Sci.* 33:231:234.

Qualset, C.O. 1984. Evaluation and breeding methods for barley yellow dwarf resistance. In: *Barley Yellow Dwarf. A Proceedings of the Workshop*. Mexico, D.F.: CIMMYT. pp. 72-82.

Coat Protein Sequence of an Egyptian BYDV-PAV Isolate

H.M. Waziri,[1] M. Abd El Gaffar,[2] E.K. Allam,[2] and A.S. Gamal El Din[1]

[1] Plant Virus Dept., Plant Pathology Institute, Agriculture Research Center, Cairo, Egypt
[2] Microbiology Dept., Faculty of Agriculture, Ain Shams University, Shobra El Kima, Cairo, Egypt

BYDV-PAV is the most common strain in Egypt. Immunocapture IC/RT-PCR procedure was used to amplify fragments specific for BYDV-PAV. The system was further validated by sequencing fragments amplified. The cDNA of CP was inserted into plasmid to construct expression plasmid. The recombinant plasmid was identified by using restriction enzymes and sequenced to confirm PAV-CP gene in plasmid. The sequence of the coat protein of the PAV strain was identified and its amino acid sequence was deduced. Sequence comparison of the CP coding region between the PAV strains of BYDV have been done, and revealed the highest identity with the Moroccan strain (99%).

Barley yellow dwarf viruses (BYDV) are phloem limited, transmitted in persistent manner by several species of aphids to over 100 species of cultivated and wild grasses. Cereal grain crops are important throughout the world. Since barley yellow dwarf viruses (BYDVs) have a very wide host range, in some locations losses due to BYDV infection can be very serious. Disease severity in a crop depends on various factors, including the cultivars and viruses involved, the time of infection, vector numbers, and environmental conditions (Irwin and Thresh, 1990). In Egypt BYDV is common. Five strains of BYDV were identified. Most of the work focused on the disease distribution and identification methods.

Materials and Methods

Virus source, isolation, and propagation

Virus was isolated from wheat plants at Agriculture Research Center, Giza, Egypt. The strain identified as BYDV-PAV was maintained on oat plants. Viruliferous *Rhopalosiphum padi* aphids reared on the infected oats were transferred at intervals of 5-10 days to healthy seedlings at two-leaf stage. The plants were covered with glass cylindrical cages and cheesecloth fixed with rubber. Plants were harvested, and the tissue was ground to a fine powder under liquid nitrogen and kept at –80°C until use for subsequent studies.

The primers

Two primers were synthesized based on published data sequence of BYDV-PAV (Miller *et al*., 1988): the upstream primer Lu1 (5′CCAGTGGTTRTGGTC′3) bases 2938 to 2952, and the downstream primer Lu4 (5′GTCGTACCTATTTGG′3) bases 3455 to 3468 of the PAV strain. The PCR predicted to be 530 bp (Robertson *et al*., 1991).

Immunocapture RT-PCR (IC/RT-PCR)

One hundred of BYDV–PAV polyclonal antibody diluted 1:1000 in coating buffer (Na_2CO_3, $NaHCO_3$, NaN_3, pH 9.6) was added to 0.5 ml thin wall eppendorf tubes and incubated at 37°C for 2 h. The tubes were washed first time with washing buffer (PBS Tween phosphate buffer saline with 0.05% Tween 20), and three times with PBS tween containing 2.0% egg albumin.

Healthy and infected tissues were extracted in extraction buffer (PBS, polyvinyl pyrolodin [PVP], Tween 20, pH 7.4). The samples were centrifuged at 10.000 for 5 min, and clarified extract was collected. One hundred µl of the clarified extract was added to each antibody-coated tube, and incubated overnight at 4 °C.

The tubes were washed three times with PBS Tween, then washed with deionized autoclaved DEPC (diethyl pyrocarbonate treated water). To each tube was added

25 µl of DEPC water and 1.5 µl (10 pmol) of reverse primer Lu4. The tubes were heated at 75-80 °C for 5 min, then cooled in ice. RT–PCR reaction was carried out in 50 µl total reaction volume, using the Titan. One tube RT-PCR system kit (Boehringer). RT mix was 1.5 µl Lu1 primer (10 pmol) 1.0 µl dNTPs (10 mM), 2.5 µl RNase inh, 10 µl 5X RT-PCR reaction buffer with 7.5 mM $Mgcl_2$, 1.0 µl enzyme mix. The volume of the mixture was increased to 50 µl with distilled water; reagents were mixed by tapping, spinning briefly, and placing in a thermocycler (HYBAID) programmed to give one cycle at 45 °C (30 min), one cycle 94 °C (2 min), 35 cycles 94 °C (30 sec), 41 °C (45 sec), 68 °C (1 min) with a final cycle of 68 °C (10 min).

Analysis of PCR products

PCR reaction products (5 µl of PCR amplified cDNA) were analyzed by electrophoresis through 1% agarose gel in TAE buffer at 100 volts for 2 h. Gels were stained in ethidium bromide (0.5 µg/ml) for 10 min, rinsed in water, and photographed on a UV transiluminator; 1 kb DNA ladder (GIBCO-BRL life technologies) was used as marker to estimate sizes of PCR products.

cDNA cloning and nucleotide sequencing analysis

Amplified cDNA fragments were cloned to the pTAdv vector using the AdvanTAgeä PCR cloning kit (CLONTECH) following the manufacturer's instructions, and used to transform *Escherichia coli* cells.

Ampicillin screening and blue/white selection were used to select transformed *E. coli* containing recombinant plasmid. DNA minipreparations from recombinant plasmids were screened for the insert by restriction enzymes digestion using Hae III (GIBCO-BRL life technologies). Sequencing of the cDNA clones was carried out using M13 primers at Bioresource Center, Cornell University, New York State, USA. Nucleotide sequence data were compiled and analyzed using the DNASIS version 2.5 program, and database searches of National Center for Biotechnology Information (NCBI, National Institute of Health).

Results

Immunocapture RT-PCR

In the infected samples, an amplified DNA product of 526 bp long was observed while not present in amplification of healthy controls (Figure 1).

Restriction analysis of DNA

Restriction digestion of presumptive recombinant DNA plasmids with Hae III restriction endonucleases identified several colonies containing inserts with the expected size of BYDV-PAV fragment.

Coat protein sequencing

DNA sequencing for the coat protein of the PAV strain shown in Figure 2 indicate the presence of 526 bp for the coat protein of BYDV-PAV Egyptian strain. This is not the full sequence for the coat protein. Computer analysis of the predicted amino acids sequence showed an amino acid residue of 175. Nucleotide sequence and amino acid sequence alignment between the other BYDV-PAV strains were performed and revealed the highest identity with the Moroccan strain 99%.

Discussion

The primers Lu1 (15 mer) and Lu4 (14 mer) were derived from sequences conserved among sequenced strains of BYDV-PAV, BWYV and PLRV (Robertson *et*

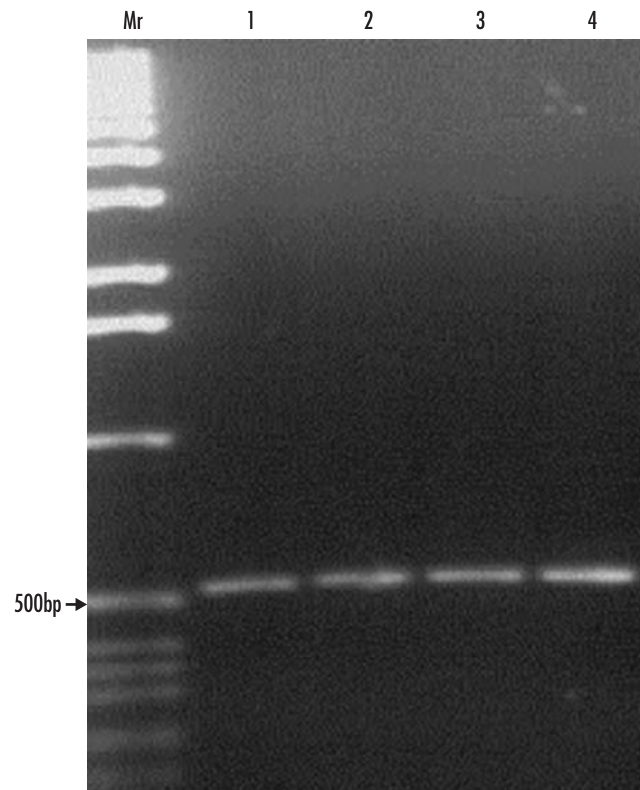


Figure 1. Agrose (1%) gel electrophoresis for PCR products.
Note: The DNA marker (Mr) is shown (1 Kb ladder, Gibco-BRL). From lane 1 to lane 3: different samples from the Egyptian PAV strain; lane 4, the PAV-NY strain.

```
  1 CCAGTGGTTGTGGTCCAACCCAATCGAGCAGGACCCAGACGACGAAATGGTCGA    54
    P V V V V Q P N R A G P R R R N G R
 55 CGCAAGGGAAGAGGAGGGGCAAATCCTGTATTTAGACCAACAGGCGGGACTGAG 108
    R K G R G G A N P V F R P T G G T E
109 GTATTCGTATTCTCAGTTGACAACCTTAAAGCCAACTCCTCCGGGGCAATCAAA   162
    V F V F S V D N L K A N S S G A I K
163 TTCGGCCCCAGTCTATCGCAATGCCCAGCGCTTTCAGACGGAATACTCAAGTCC   216
    F G P S L S Q C P A L S D G I L K S
217 TACCATCGTTACAAGATCACAAGTATCCGAGTTGAGTTTAAGTCACACGCGTCC   270
    Y H R Y K I T S I R V E F K S H A S
271 GCCACTACGGCCGGCGCTATCTTTATTGAACTCGACACCGCGTGCAAGCAATCA   324
    A T T A G A I F I E L D T A C K Q S
325 GCCCTGGGTAGCTACATTAATTCCTTCACCATCAGCAAGACCGCCTCCAAGGTC   378
    A L G S Y I N S F T I S K T A S K V
379 TTCCGGTCAGAGGCAATTAACGGGAAGGAATTCCAGGAATCAACGATAGACCAA   432
    F R S E A I N G K E F Q E S T I D Q
433 TTTTGGATGCTCTACAAGGCCAATGGAACCACCTCTGATACGGCAGGACAATTC   486
    F W M L Y K A N G T T S D T A G Q F
487 ATCATCACGATGAGTGTCAGTTTGATGACGGCCAAATAG                  526
    I I T M S V S L M T A K *
```

Figure 2. Nucleotide sequences (DNA) for the coat protein for the PAV strain of BYDV. The deduced amino acid sequences are indicated below the nucleotide sequence.

al., 1991). We succeeded in amplifying 526 bp DNA fragment from cDNA derived from IC/RT-PCR of BYDV-PAV for the Egyptian strain. Immunocapture RT-PCR was sensitive and effective for the detection and identification of the BYDV-PAV strain in this study. The PCR products obtained using Lu1, Lu4 primers was 526 bp, which did not correspond to the full sequence of the coat protein. However, this fragment can still provide a source of initial sequence information for the Egyptian PAV strain. The nucleotide sequence and amino acid alignment indicate 99% similarity with another African BYDV strain, the Moroccan BYDV-PAV strain. These sequence data could be used to design virus specific PCR primers for this strain. Barley yellow dwarf virus needs more study in Egypt, complete sequence for the coat protein and other genes, search for source of resistance. New methods to obtain host resistance to this virus are very important. The epidemiology of this virus in Egypt and neighboring countries must be studied.

Acknowledgments

The first author would like to thank Dr. Stewart M. Gray (Virology and Nematology Lab, Plant Pathology Dept., Cornell University, New York State, USA) for hosting him in his lab for three months, and for his supervision and valuable advice throughout this work. We would like to thank the UNESCO for funding this fellowship.

References

Irwin, M.E., and Thresh, J.M. 1990. Epidemiology of barley yellow dwarf: a study of ecological complexity. *Annu. Rev. Phytopathology* 28:393-424.

Miller, W.A., Waterhouse, P.M., and Gerlach, W.L. 1988. Sequence and organization of barley yellow dwarf virus genomic RNA. *Nucleic Acids Res.* 16:6097-6111.

Robertson, N.L., French, R., and Gray, S.M. 1991. Use of group specific primers and the polymerase chain reaction for the detection and identification of luteovirus. *Journal of General Virology* 72:1473-1477.

Diagnosis of *Barley Yellow Dwarf Virus* and *Cereal Yellow Dwarf Virus* Isolates in Infected Plants and Vectors by Group PCR and ELISA

B. Kulemeka,[1] W. Bliss,[1] A. Harness,[1] C. Sutula,[1] M. Bandla,[1] and S.M. Gray[2]

[1] Agdia Inc., Elkhart, Indiana
[2] USDA-ARS, Ithaca, New York

A group PCR test and a group ELISA test for *Barley yellow dwarf virus* (BYDV) and *Cereal yellow dwarf virus* (CYDV) were developed and optimized for the detection of CYDV-RPV, BYDV-MAV, -PAV, -RMV, and -SGV infections in plants and aphids. The group PCR test can detect CYDV-RPV, or BYDV-PAV in single oat aphids (*Rhopalosiphum padi*); MAV in single *Sitobion avenae* aphids; RMV in a pool of five corn aphids (*R. maidis*), and SGV in a pool of five greenbugs (*Schizaphis graminum*). The test does not show any reactivity to healthy barley, oat, tobacco, and *Chenopodium* leaf tissue nor to *R padi, R. maidis, S. graminum,* and *S. avenae* fed on healthy barley. The ELISA test can detect RPV, MAV, PAV, RMV and SGV in infected leaf samples. No reaction was observed to healthy wheat leaf extracts. Optimization of the ELISA test for BYDV detection in aphid vectors is underway.

Preparing Monoclonal Antibodies against BYDV Using Viruses Purified from Naturally Infected Plants

T.N. Erokhina,[1] T.B. Kastalieva,[2] and K.A. Mozhaeva[2]

[1] Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

[2] Russian Research Institute of Phytopathology, Moscow Region, Russia

After a severe epidemic of barley yellow dwarf disease (BYD) on small grain crops that took place in European Russia in 1988-1991, the need for a BYDV identification method became evident. As we did not have diagnostic kits for BYDV detection, we could only identify the disease based on visual symptoms or by electronic microscopy of purified virus preparations and their UV spectra. To solve this problem, we prepared monoclonal antibodies (MAbs) against the viral preparations purified from naturally infected plants. This task had special features and difficulties: 1) viral yields were usually low, and 2) generally, the viral preparations consisted of a mixture of different BYDVs, sometimes polluted with other cereal viruses. In any case, we were able to separate BYDV through zonal centrifugation in sucrose gradient.

Well-purified virus preparations from naturally infected barley plants of unknown BYDV strain composition were used to immunize mice. Later, using commercial polyclonal antibodies, the virus preparations were shown to contain mainly BYDV PAV and MAV, and smaller amounts of BYDV SGV and CYDV RPV. As a result, one panel containing five monoclonal antibodies were raised in 1993, but only two (Mabs 4B5 and 3C2) were chosen for further work. These Mabs were compared to polyclonal antibodies specific to BYDV PAV, BYDV SGV, and CYDV RPV (from Agdia Inc.). A purified virus preparation containing both BYDV PAV and CYDV RPV was used as antigen.

MAbs 4B5 bound to BYDV PAV, but not to CYDV RPV, when used as coating antibodies in DAS ELISA (Figures 1 and 2). MAbs 3C2 adsorbed CYDV RPV to the same degree as anti-RPV polyclonal antibodies (Figure 2). Mabs 3C2 reacted weakly to BYDV PAV, binding to this virus approximately 2-3 times less than Mabs 4B5 (Figure 1).

We conjugated MAbs 4B5 and 3C2 with horseradish peroxidase (HRP) (Tussen and Kurstak, 1984) and constructed a monoclonal test system for BYDV detection (Erokhina, 1995; Erokhina and Kastalieva, 1995). Specificity of MAb 4B5 to BYDV PAV was verified in experiments using lyophilized strain-specific plant extracts from Agdia (Figures 3 and 4)

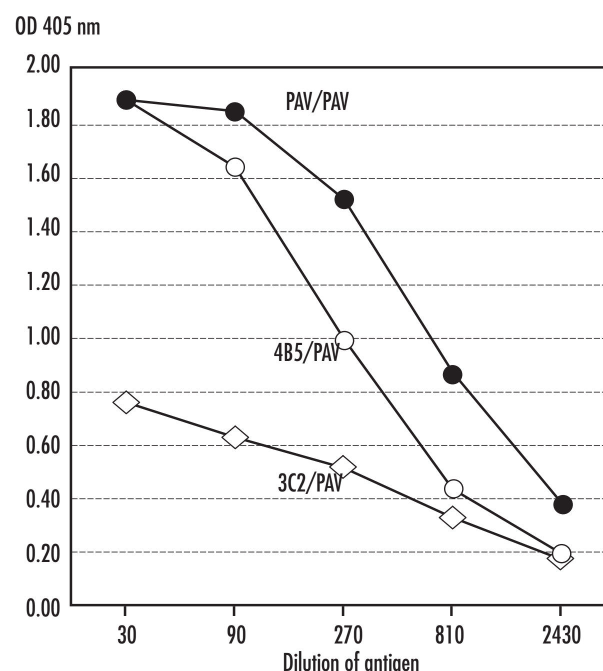


Figure 1. Detection of BYDV-PAV in purified virus samples by a panel of polyclonal antibodies (PAV from Agdia) and monoclonal antibodies (Mabs 4B5 and 3C2) in DAS-ELISA. The conjugate used is anti-PAV-AP (Agdia).

and several combinations of coating and conjugate antibodies. The 4B5-HRP conjugate detected BYDV PAV as well as the polyclonal anti-PAV conjugated with alkaline phosphatase (AP) (Figures 1, 3a, and 4), but failed to detect CYDV RPV (Figure 3b). 3C2-HRP detected CYDV RPV better than the anti-RPV polyclonal antibodies (Figure 2, 3b). 3C2-HRP detected BYDV PAV with a slightly lower sensitivity than 4B5-HRP (Figure 4).

It was shown that MAb 4B5 could also detect BYDV SGV in plant extracts (Table 1). 3C2 also reacted with BYDV-SGV but to a lesser degree than Mabs 4B5.

The combination 4B5/4B5-HRP allowed good detection of BYDV-PAV in plants extracts (Table 1). Mabs 3C2 trapped BYDV-PAV in plant extract when used as coating in the 3C2/4B5 system. However, in the system 3C2/3C2, PAV was not detected in plants extracts (Table 1).

MAbs 3C2 was not as specific as MAbs 4B5, for it weakly detected PAV in purified virus preparation, but not in plant extracts.

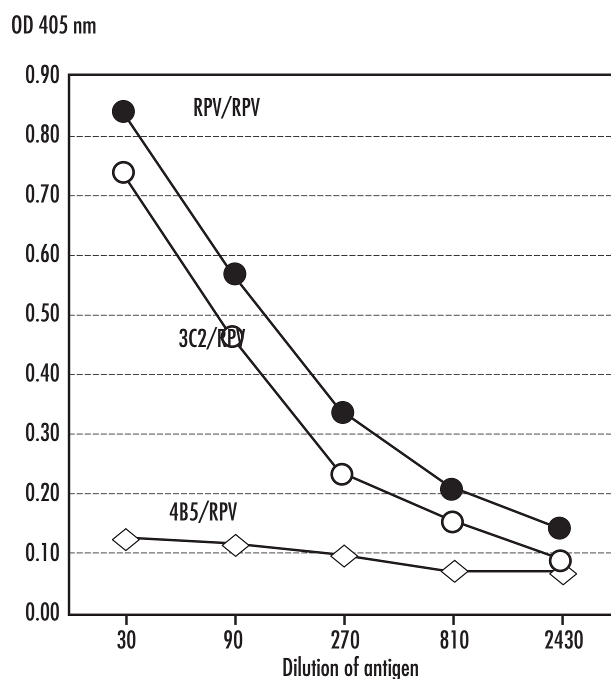
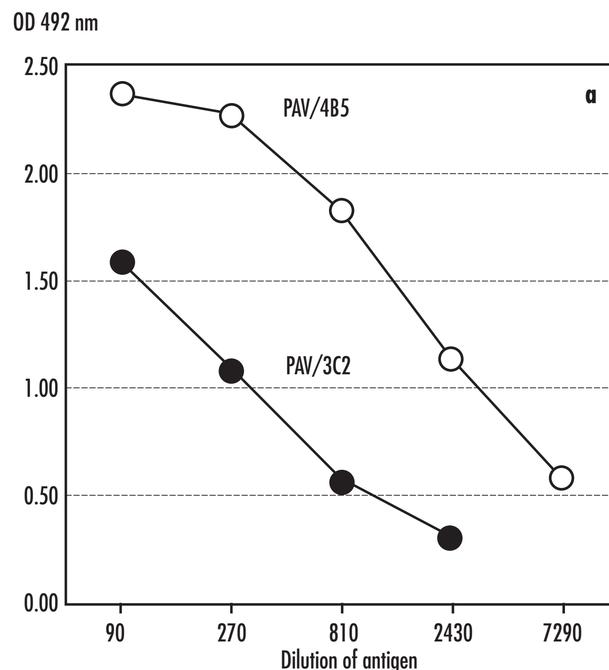


Figure 2. Detection of CYDV-RPV by a panel of polyclonal antibodies (RPV from Agdia) and monoclonal antibodies (Mabs 4B5 and 3C2) in DAS-ELISA. The conjugate used is anti-RPV-AP (Agdia).

To determine strain composition of plant samples collected in different regions of European Russia, we used both DAS-ELISA with polyclonal antibodies as capture antibodies, and the monoclonal test-system using conjugates with HRP. Analysis of composition of the samples showed that BYDV PAV and CYDV RPV are most abundant in European Russia. We were



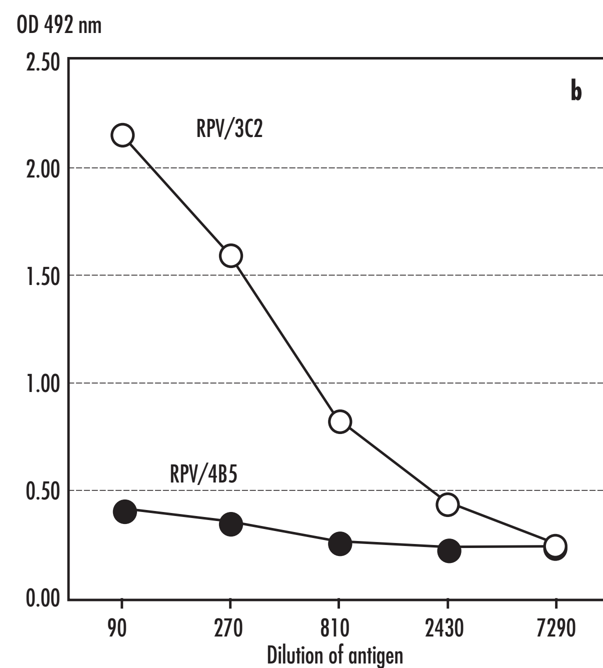


Figure 3. Detection of BYDV-PAV (a) and CYDV-RPV (b) in purified virus samples by DAS-ELISA, coating with the correspondent polyclonal antibodies PAV or RPV from Agdia and using the prepared Mabs conjugate, 4B5-HRP and 3C2.

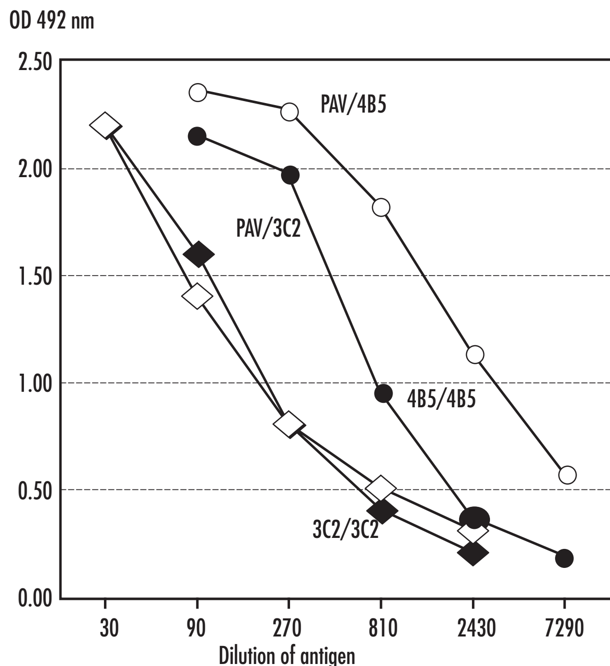


Figure 4. Comparison of the monoclonal antibodies conjugates (4B5-HRP, 3C 2-HRP) in the detection of BYDV-PAV. Coating antibodies are indicated before the slash (4B5, PAV and 3C2) and conjugate antibodies after the slash.

Table 1. Detection of BYDV-SGV and BYDV-PAV in plant extracts using the monoclonal test-system with different combinations of coating and conjugate (HRP) antibodies (OD measured at 492nm).

Plant extract	BYDV SGV		BYDV PAV			
Dilution	4B5/ 4B5-HRP	3C2/ 3C2-HRP	4B5/ 4B5-HRP	3C2/ 3C2-HRP	4B5/ 3C2-HRP	3C2/ 4B5-HRP
1	0.92	0.42	1.76	0.26	0.98	1.38
3	0.52	0.32	1.20	0.28	0.47	0.85
9	0.26	0.22	0.62	0.18	0.29	0.68
27	0.18	0.11	0.38	0.09	0.21	0.49

fortunate to detect BYDV concentrations as low as 8-10 ng with monoclonal DAS-ELISA in purified viral preparations. BYDV was also detected in 10% plants extracts diluted up to 1/16. At present, we are using the MAbs test-system for BYD monitoring in Russia.

References

Erokhina, T. 1995. Monoclonal antibodies to barley yellow dwarf virus: An immunoenzyme test-system for virus diagnosis. *Bioorganicheskaya Khimia* 21(4):256-260.

Erokhina, T., and Kastalieva, T. 1995. Production of monoclonal antibodies against barley yellow dwarf virus. Detection of BYDV in field samples from central Russia. *Zuchtungforschung* 1(2):71-74.

Tussen, P., and Kurstak, E. 1984. Highly efficient and simple methods for preparing peroxidase and active peroxidase-antibody conjugates for enzyme immunoassays. *Annals of Biochemistry* 136:451-457.

Improvement of BYDV-PAV Detection in Single Aphid by Using Real-Time RT-PCR Fluorescent Method

F. Fabre,[1,2] C. Kervarrec,[1] L. Mieuzet,[1,2] A. Vialatte,[1] and E. Jacquot[1]

[1] INRA/ENSA, Unité Mixte de Recherche Biologique des Organismes et des Populations appliquée à la Protection des Plantes, Le Rheu, France
[2] Bayer Crop Science, Lyon, France

Development of biological, serological and molecular tools is a required step for characterization and studying plant viruses. Barley yellow dwarf (BYD), one of the most important disease among small grain cereals (D'Arcy and Burnett, 1995), was observed for the first time in the early 50′ based on physiological disturbances of barley plants (Oswald and Houston, 1951). Characterization of the viral agent, *Barley yellow dwarf virus* (BYDV), involved in this disease was performed using biological, biochemical and biophysical techniques (Rochow and Brakke, 1964; see Smith and Barker, 1999), aphid transmission experiments (Rochow, 1970), serological assays (for a review, see French, 1995) and molecular analysis of the BYDV genome (Miller *et al.*, 1988; Bencharki *et al.*, 1999; Chaloub *et al.*, 1994). Taken together, collected data allow among other to identify six distinct species of BYDV including BYDV-PAV, the most widespread in Western Europe (Lister *et al.*, 1990). BYDV epidemiology studies had identified the level of aphids infectivity, i.e., the proportion of the migrant winged aphids that carry and are able to transmit the virus, as one of the major components determining the incidence of BYD on cereals (Plumb, 1990). At first, assessment of aphid infectivity data required infectivity assays. From the end of 70′, Enzyme-Linked Immunosorbant Assays (ELISA), a less costly and time-consuming technique, was used routinely for BYDV detection in single aphid (Lister and Rochow, 1979). However, the end-point of ELISA assays for virus detection is 1-10 ng or equivalently one billion of BYDV particles per sample (Canning *et al.*, 1996). This threshold induces problem of false negative for viruliferous aphids containing less virus than the end-point of this technique. Generalization, in the last decade, molecular techniques for virus detection such as dot-bot hybridization, reverse transcription polymerase chain reaction (RT-PCR) coupled or not with an immunocapture (IC) step made possible to detect approximately 10 fg of virus (equivalent to 1000 genome copies) in tested samples (Canning *et al.*, 1996). However, due to its simplicity, ELISA remains widely used for large scale testing. The more recently developed gelfree quantitative "real-time" PCR technology has proved to be able to detect as few as 10 copies of a target sequence (Rasmussen *et al.*, 1998). Therefore, it appears interesting to investigate if "real-time" PCR can improve previously described BYDV detection techniques. A one-step "real-time" reverse transcription-polymerase chain reaction assay using TaqMan chemistry was developed for the detection and quantification of the BYDV-PAV isolates. BYDV-PAV specific primers and TaqMan probe were designed from published sequences of PAV and tested over a worldwide isolate collection. The sensitivity, reproducibility and specificity of this new detection technique were tested and compared to previously published ELISA and RT-PCR techniques. Our results suggest that quantitative "real-time" RT-PCR reaction developed for BYDV-PAV is respectively 10 and 10^4-fold more sensitive than standard RT-PCR and TAS-ELISA performed simultaneously and pocesses an end-point below 100 copies of BYDV-PAV genome. From a technical point of view, "real-time" RT-PCR is less time consuming than classical RT-PCR or even than ELISA for small number of samples. An other interesting advantage of the technique described, is that the detection of BYDV-PAV is performed using a small fraction of the total acid nucleic extracted from a single aphid allowing other studies on the same sample. Finally, BYDV quantification using "real time" RT-PCR opens a way toward studies of plant infection kinetic or aphid transmission process.

References

Bencharki, B., Mutterer, J., El Yamani, M., Ziegler-Graff, V., Zaoui, D. and Jonard, G. 1999. Severity of infection of Moroccan barley yellow dwarf virus PAV isolates correltes with variability in their coat protein sequences. *Ann. Appl. Biol.* 134:89-99.

Canning, E.S.G., Penrose, M.J., Barker, I., and Coates, D. 1996. Improved detection of barley yellow dwarf virus in single aphids using RT-PCR. *J. Virol. Methods.* 56:191-197.

Chaloub, B.A., Kelly, L., Robaglia, C., and Lapierre, H.D. 1994. Sequence variability in the genome-3'-terminal region for 10 geographically distinct PAV-like isolates of barley yellow dwarf virus: analysis of the ORF 6 variation. *Archives of Virology* 139:403-416.

D'Arcy, C.J., and Burnett, P.A. (eds.). 1995. *Barley yellow dwarf virus: 40 years of progress.* APS Press, St Paul.

French, R. 1995. Barley yellow dwarf: Diagnostic procedures and reagents. In: *Barley Yellow Dwarf: 40 Years of Progress.* C.J. D'Arcy and P.A. Burnett (eds.). American Phytopathological Society, APS Press. pp. 293-306.

Lister, R.M., Fereres, A., Foster, J.E., and Castanera, P. 1990. Occurrence of barley yellow dwarf virus in three major cereal-growing regions of Spain. In: *Barley Yellow Dwarf: A Proceedings of the Workshop.* Burnett, P.A. (ed.). Mexico, D.F.: CIMMYT. pp. 49-54.

Miller, W.A., Waterhouse, P.M., and Gerlach, W.L. 1988. Sequence and organization of barley yellow dwarf virus genomic RNA. *Nucleic Acid Res* 16:6097-6111.

Lister, R.M., and Rochow, W.F. 1979. Detection of barley yellow dwarf virus by enzyme-linked immunosorbent assay (ELISA). *Phytophatology* 69:649-654.

Oswald, J.W., and Houston, B.R. 1951. A new virus disease of cereals, transmissible by aphids. *Plant Dis. Rep*, 15:471-475.

Plumb, R.T. 1990. The epidemiology of barley yellow dwarf in Europe. In: *Barley Yellow Dwarf: A Proceedings of the Workshop.* P.A. Burnett 8ed.). Mexico, D.F.:CIMMYT. pp. 215-227.

Rasmussen, R., Morrison, T., Herrman, M., and Wittwer, C. 1998. Quantitative PCR by continuous fluorescence monitoring of a double strand DNA specific binding dye. *Biochemica* 8-11.

Rochow, W.F. 1970. Barley yellow dwarf virus. In: CMI/AAB Description of plant virus. pp. 32-36.

Rochow, W.F., and Brakke, M.K. 1964. Purification of Barley yellow dwarf virus. *Virology* 24:310-322.

Smith, H.G., and Barker, H. (eds.). 1999. *The Luteoviridae.* Oxford: CAB International.

Risk Index for *Barley Yellow Dwarf Virus* and *Cereal Yellow Dwarf Virus* of Wheat in Alabama

K.L. Bowen,[1] K.L. Flanders,[1] P. Mask,[2] and J.F. Murphy[1]

[1] Dept. of Entomology and Plant Pathology, Auburn University, Alabama, USA
[2] Dept. of Agronomy and Soils, Auburn University, Alabama, USA

In the state of Alabama, USA, soft red winter wheat production is valued at as much as US$15 million. Wheat production throughout the state is limited, however, by the diseases of wheat caused by *Barley yellow dwarf virus* (BYDV, strain PAV, genus *Luteovirus*) and *Cereal yellow dwarf virus* (CYDV, strain RPV, genus *Polerovirus*). These viruses are vectored by several aphids commonly found in Alabama, notably greenbug (*Schizaphis graminum*), English grain aphid (*Sitobion avenae*), bird cherry-oat aphid (*Rhopalosiphum padi*), rice root aphid (*R. rufiabdominalis*), and corn leaf aphid (*R. maidis*) (Halbert and Voegtlin, 1995; van Riessen *et al*., 1998). Use of the insecticide imidacloprid, applied as a seed treatment, has been shown to reduce the incidence of these viruses and substantially increase wheat yields (van Riessen *et al*., 1998). In addition, this insecticide was shown to be effective for up to 90 days after planting, thus protecting against early infection by BYDV and CYDV. The expense of treatment with imidacloprid, however, keeps this product from being applied as a preventative for yellow dwarf (YD) problems. Given that the severity of YD varies from year to year, it would be desirable to have a means of forecasting seasons in which YD might become problematic. Such a forecast would allow growers to make better choices about the use of insecticide treatments on winter wheat. Thus, we have been attempting to develop a "risk index" for predicting conditions that might be favorable for outbreaks of YD viral disease problems of wheat in Alabama.

Several factors contribute to the severity of yellow dwarf disease problems. For example, earlier planting leads to greater YD severity (Sforza and Herbert, Virginia, personal communication). There is, however, contradictory evidence about the effects of rainfall prior to planting and subsequent YD severity on wheat. In Australia and the northwest USA, irrigation of cereal fields seems to have favored increases in YD (Briggle, 1984; Jones *et al*., 1990). Conversely, analysis of historical data from Alabama indicates that warmer weather with reduced rainfall prior to planting leads to greater disease severity (Bowen and Burch, 2001). Rainfall is usually not lacking in Alabama, and there are abundant perennial grass hosts along roadsides and in the 10 million ha of grass forage areas through the year. Thus, clarification of the effects of weather is needed relative to YD occurrence in Alabama.

We have been working with county agents and wheat producers throughout the state. Growers are being asked to characterize their wheat production sites relative to certain factors such as planting date, field history (previous crop, tillage), variety, aphid abundance, and weather patterns. The authors have been assessing YD intensity (disease incidence and severity) in fields during the soft dough stage (April-May), and leaf samples have been collected for ELISA assays to confirm diagnosis.

BYDV-PAV and CYDV-RPV were found throughout the state, occurring alone or in combination in 14.6% and 12.2% of samples in 2000 and 2001, respectively (Table 1). Further, the 1999-2000 winter wheat growing season was slightly warmer with substantially less rainfall than the second growing season (2000-2001) (Table 1). These observations provide some support of the results derived from

historical data in which more YD was observed in seasons preceded by warmer temperatures with less rainfall (Bowen and Burch, 2001). In addition, in 2000, BYDV-PAV was found at lower incidence than CYDV-RPV (4.3% and 9.9%, respectively). This is the first observation in the southeast USA in which CYDV-RPV occurred at greater incidence than BYDV-PAV, and these results may also be related to the abnormal weather during the 1999-2000 winter wheat growing season.

References

Bowen, K.L., and K.B. Burch. 2001. Influence of weather on barley yellow dwarf severity and yields of wheat in Alabama. *Phytopathology* 91:S10.

Briggle, L.W. 1984. The barley yellow dwarf research program in the USA. In: Barley Yellow Dwarf, A Proceedings of the Workshop. P.A. Burnett (ed.). Mexico, D.F.: CIMMYT. pp. 141-144.

Halbert, S., and D. Voegtlin. 1995. Biology and taxonomy of vectors of barley yellow dwarf viruses. In: Barley Yellow Dwarf: 40 Years of Progress. C.J. D'Arcy and P.A. Burnett (eds.). APS Press. pp. 217-258.

Jones, R.A.C., McKirdy, S.J., and Shivas, R.G. 1990. Occurrence of barley yellow dwarf viruses in over-summering grasses and cereal crops in Western Australia. *Aust. Plant Pathol.* 19:90-96.

van Riessen, H.W., P.L. Mask, K.L. Flanders, J.F. Murphy, and W.S. Gazaway. 1998. Barley Yellow Dwarf in Small Grains. *Ala. Coop. Exten. System Circ.* ANR 1082.

Table 1. Average temperatures and cumulative rainfall for periods indicated that are relevant to the winter wheat growing season for three locations and three seasons, as well as viral incidence in northern, central, and southern regions of Alabama.

		Wheat growing season (Oct-May)		Incidence (%)			
Location	Year	Temp (C)	Rainfall (cm)	PAV[x]	RPV [x]	Both [x]	Total
Belle Mina, Limestone Co.[y]	Normal (1963-1993)	12.1	120.0				
	1999-2000	12.4	59.0	3.1	6.6	0.3	10.0
	2000-2001	10.8	107.2	4.9	1.2	0.2	6.3
Marion Junction, Dallas Co.[y]	Normal	13.5	116.8				
	1999-2000	14.6	79.2	8.0	11.5	1.0	20.5
	2000-2001	13.2	124.0	15.0	3.5	6.0	24.5
Fairhope, Baldwin Co.[y]	Normal	16.2	116.8				
	1999-2000	16.5	44.8	2.3	14.9	0.0	17.2
	2000-2001	14.9	87.2	nd	nd	nd	
Statewide	2000			4.3	9.9	0.4	14.6
	2001			8.2	1.9	2.1	12.2

[x] PAV = Barley yellow dwarf virus strains PAV; RPV = Cereal yellow dwarf virus strain RPV; Both = mixed infections.

[y] Limestone County data represents northern third of the state; Dallas County data represents central portion of state; Baldwin county represents southern part of state. nd = no collections performed.

Identification of Cereal Viruses in Uzbekistan

Z.N. Kadirova, A.A. Zueva, and A.A. Abdukarimov

Institute of Genetics and Experimental Biology, Academy of Science of Uzbekistan

Several viruses affect wheat crop including *Barley yellow dwarf virus* (BYDV), *Barley yellow mosaic virus* (BYMV), *Brome mosaic virus* (BMV), *Wheat dwarf virus* (WDV), and *Wheat streak mosaic virus* (WSMV), among others.

Virus diseases of cereal crops were first observed in Uzbekistan in 1962 by Gorbunova *et al*. (1966), and WSMV was detected. More than 30 years later in 1998, other authors indicated that streaks on wheat was caused by viruses (Juraeva *et al*., 1999). During the field survey in spring, 2001, wheat and barley plants showed symptoms of yellowing leaf, streak mosaic, yellowing of flag leaf, and dwarfing. The identification of these viruses was undertaken and the results presented here.

Materials and Methods

A preliminary survey to identify virus disease affecting wheat in Uzbekistan was conducted during April-May, 2001. The survey covered wheat fields on the road Tashkent-Sirdarya-Jizzakh-Kashkadarya, as well as experimental wheat plots in the Institute of Plant, Tashkent region, and the Institute of Grain, Jizzakh district, Gallaorol region, representing different climatic regions in Uzbekistan. The survey was carried out on released and promising wheat varieties such as: Ulugbek-600, Umanka and others irrigated conditions and rainfed varieties at the heading and flowering stages.

The percentage of infecting plants was calculated according to Vlasov and Lantas (1962). The field was sampled in 15-20 places and all plants were evaluated on 1 m^2 square. In addition, infected plants were counted where samples were taken and weeds that could act as virus reservoirs were observed. Percentage of infected plants was calculated following the formula $P=n \times 100/N$, where P indicates the spread of disease, n the number of infected plants, and N the average number of plants observed.

Diagnostics of the virus presence was done using the double diffusion method in 1% agar, diluted in 0.1 M phosphate buffer pH 8.0 according to Ouchterlony (1953). Tissue blot immunoassay (TBIA) on nitrocellulose membranes was followed as published earlier by Makkouk *et al*. (in press). Color reaction on nitro-cellular membranes was read under a stereomicroscope.

Antiserum to *Maize dwarf mosaic virus* (MDMV), *Barley stripe mosaic virus* (BSMV), and BMV were kindly provided by V.K. Novikov from Laboratory of Biochemistry of Plant Viruses, Moscow State University, Russia. Antiserum to BYDV-PAV, WSMV, and BYSMV were received from the Virology Laboratory of ICARDA, Aleppo, Syria. Plants of Graminae, Chenopodiacea, and Solaneceae families were sap-inoculated with homogenates of infected leaves of wheat (1 g per 2 ml of 0.01 M potassium buffer, pH 7.5) using carborundum as abrasive.

Sitobion avenae F. aphids were gathered from disease wheats and placed on healthy plants. Aphids were killed with an insecticide after 48 h.

Results

Investigations showed that several types of viruses were identified from infected leaves of wheat and barley. Symptoms include weak stripe mosaic on leaves, yellow and green streaks, yellowing (reddening in some varieties) of infected leaves and yellowing of flag leaf. Serological tests showed that BYDV infected plants were characterized on some varieties by weak stripe mosaic of the infected leaves and on others by more severe yellowing and stunting. The green and yellow parallel mosaics caused by BMV or other viruses were also observed (Table 1). The stunted wheat plants with streak and yellow mosaic noticed in the experimental station of the Plant Institute, in the Tashkent region, were infected with more than one virus. Serological analyses showed that infections were caused by BYDV.

Attempts of mechanical transfer from virus infected wheat to healthy plants or other test plants were unsuccessful. However, yellowing of flag leaves was observed in places, where the aphid *S. avenae* was abundant in the field. The yellowing of flag leaves was observed on plants where the aphids *S. avenae* collected in the field were fed.

Weak stripe mosaic of infected leaves were more common on farms in the Kashkadarya region (Table 2), where percentage of infected plants varied ranged from 5 to 80%. The variety Ulugbek -600 was sensitive to the virus but did present such symptoms as stunting or dwarfing. Streaking of leaves was less common in this region. Wheat plants presenting streak mosaic were also stunted. Such symptoms were more common in the second year of sampling.

Serological tests with the use of double diffusion method showed that winter wheat in Kashkadarya region were infected with BMV and BYDV. The results obtained indicated that BYDV -PAV was the most common on the farm fields and experimental stations, followed by BYSMV, BMV, MDMV and WSMV.

References

Gorbunova, N.J., Uspenskay, N.V., and Shevakova, M.N. 1966. Streak mosaic of wheat in Uzbekistan. In Virus disease of Agricultural plants and Control methods. Proceedings of the 5th United meeting of virus diseases of plants. C.N. Moskoves (ed.). Kiev. pp. 378-381.

Juraeva, U.M., Yuldashev, J., and Vakhobov, A.K. 1999. To the question about expose cereal viruses in condition of Uzbekistan. Proceedings of the conference "Environment problems of Central Asia, regions of Amydarya". Abdullaev, N.A., Buriev, S.B., and A.S. Buhoro (eds.). pp. 51-52.

Vlasov, Y., and Lantas, E.C. 1962. Methodical indication to investigate agricultural plants infections virus disease. (Russian) in book. Leningrad. p. 280.

Ouchterlony, Y. 1953. Antigen-antibody reactions in gel. Types of reactions in coordinated systems of diffusions. *Acta Path. Microbial. Scand.* 32:231-239.

Makkouk, K.M., Kumar, S.G., Kadirova, Z., and Zueva, A. First record of Barley Yellow Striate Mosaic Virus infecting Wheat in Uzbekistan. Accepted for publication in Plant Disease.

Table 1. Detection of viruses using serological tests of infected plants.

Symptoms	Antiserum to BYDV-PAV	BSMV	WSMV	BMV	MDMV	BYSMV
Weak stripe mosaic on infected leaves	++	-	-	-	-	-
Green or yellow streaks	-	-	-	++	-	-
Yellow mosaic	+++	-	-	-	-	-
Yellowing of flag leaves	-	-	-	-	-	+
Dwarfing	+++	-	++	++	++	-

Note: " - " absence of reaction, " + "- presence of precipitation's line. "++" and " +++ "-indicate the severity of the reaction.

Table 2. Virus disease incidence in winter wheat based on symptoms observed in the Kashkadarya region of during May, 2001.

District	Farm	Varieties	Number of one meter square sites observed/ha	Symptoms (%)		
				Weak stripe mosaic	Streaks	Yellowing
Yakkabog	Juma Mukhliev	Sansar-8a	14	14	1	-
	"	Ulugbek-600	20	53	1	-
	"	Kroshka	18	18	3	-
	Kattaboy Ashurov	Kupava	10	21.5	-	-
	"	Sansar-8	14	10	-	15
	Karim Mayliev	Ulugbek-600	30	30	-	12.5
	Uzbekistan	Ulugbek-600	17	35	-	5
	Bolta Fozilov	Ulugbek-600	37	70	30	5
Shahrisabz	Uzbekistan	Umanka	24	5	20	5
Kitab	Meyliqul Boboev	Ulugbek-600	16	17.5	5	-
	"	Umanka	10	20.5	5	-
	Javli Turdiev	Ulugbek-600	14	20	2.7	3.5
	"	Sansar-8	25	5	1	-
	Shodmon Umarov	Kroshka	10	-	17.5	30
	"	Ulugbek-600	15	80	-	-

Distribution of *Barley Yellow Dwarf Virus* Vic-PAV Strain in South Korea

J.-C. Park, J.-H. Seo, J.-S. Choi, S.-L. Park, D.-Y. Suh, and M.-H. Lee

National Honam Agricultural Experiment Station, Iksan, South Korea

Barley yellow dwarf virus (BYDV) occurs in cereal cropping worldwide and brings severe damage especially in yield. In South Korea, the major winter crop is barley, and the study on cereal viruses has been focused on *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV) that showed higher incidence and caused the most severe damage. Woo (2000) reported that in Korea, BYDV is widely distributed and its incidence and damages may increase in wheat fields in future. There is a need for more investigation of its distribution and methods for its control. BYDV has five main strains named PAV, MAV, and SGV in subgroup I and RPV and RMV in subgroup II. In an earlier report, Vic-PAV strain was found to be dominant in this country with 52.6% infection against 3.4% for MAV (Woo, 2000) using RT-PCR detection method in cereal fields including barley and wheat.

Materials and Methods

A total of 41 samples were collected from 19 barley and wheat fields, nationwide from March to April, 2002 and tested for infection with Vic-PAV strain (Victoria, Australia) by RT-PCR detection. The collected regions, variety group and number of collections are listed in Table 1. For RT-PCR diagnosis, extracted total RNA was used for cDNA synthesis with reagents mixture included reverse primer, 5'-ATATTCGTTTTGCGAGTTGT-3', reverse transcriptase and RNA inhibitor etc. Reverse transcription was carried out in a total 20ul reaction volume at 48°C/60min. The PCR mixture (100ul) contained PCR buffer, MgCl2, Ampli-Taq, DNA polymerase, dNTP mix and forward primer 5'-GTACTTC GACGATCTTACGG-3'. The reaction was denatured for 30 sec at 94°C, annealed for 60 sec at 68°C, and then extended for 2 min at 60°C for a total of 40 cycles in a DNA thermal cycler. Amplified product was separated on agarose gel for identification of infection.

Results and Discussion

Typical BYDV symptom was seen usually in wheat fields in South Korea; however, recent climatic changes could effect on the vector's life cycle, and cause increasing incidence and damage other cereal crops such as barley. The RT-PCR results showed an amplified 112bp band that identified BYDV infection

Table 1. Sites of sampling for BYDV detection in South Korea.

Province	Region	Crop	Number collected
Jeonnam-do	Gangjin	Hulled barley	4
	Haenam		5
	Wando	Naked barley	1
	Hampyung		1
	Naju	Hulled barley	2
	Jangheung		1
	Mooan	Naked barley	1
Jeonbuk-do	Buan	Naked barley	2
	Gochang		2
	Gimje		1
	Namwon		1
	Imsil	Hulled barley	1
	Daeyalksan	Naked barley	1
		Hulled and naked barley	3
Chungnam-do	Yousung	Hulled and naked barley	2
Gyunggi-do	Suwon	Wheat, hulled and naked barley	11
Gawgwon-do	Samchuk	Hulled barley	2

(Figure 1). RT-PCR has been an effective method for diagnosis or detection of specific virus quickly and precisely, and this method could be use to identify the BYDV strain present in the infected samples. BYDV is a complex of several strains at the difference of other cereal viruses and their accurate identification requires precisely designated primers for RT-PCR.

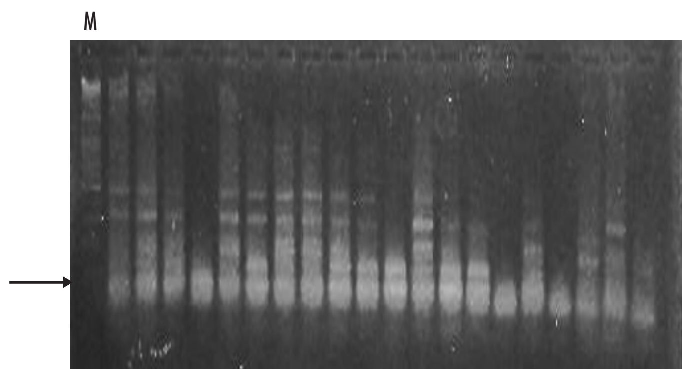


Figure 1. Detection of Vic-PAV strain by RT-PCR.
M: 100bp DNA ladder, tested materials show amplified viral cDNA at 112bp indicated by arrow.

Table 2. Distribution of Vic-PAV strain in South Korea, based on RT.PCR detection.

Province	No. of regions	No. of detection	Infection ratio (%)
Jeonnam-do	15	9	60.0
Jeonbuk-do	11	6	54.5
Chungnam-do	2	1	50.0
Gyunggi-do	11	7	63.6
Gangwon-do	2	2	100.0

Vic-PAV strain was identified in 25 regions among the 41 examined local areas and the distribution was even in national barley and wheat fields (Table 2). The 61.0% incidence identified in this study was higher than the 52.6% for PAV and 3.4% for MAV reported by Woo (2000). The PAV strain was identified in all of the tested provinces. These results were supported by earlier reports that Vic-PAV strain was dominant in this country and that the vectors of BYDV-PAV, *Rhopalosiphum padi* and *Sitobion avenae,* were frequent in cereal fields in Korea. Continuous investigations is needed to accumulate basic information about other strains distribution and developed a breeding approach for resistance to control BYDV.

References

Carrigan, L.L., Ohm, H.W., Foster, J.E., and Patterson, F.L. 1981. Response of winter wheat cultivars to barley yellow dwarf virus infection. *Crop Science* 21:377-380.

Creamer, R., and Falk, B.W. 1989. Characterization of nonspecifically aphid-transmitted CA-RPV isolated of barley yellow dwarf virus. *Phytopathology* 79:942-946.

Figuera, A.R., Domier, L.L., and D'Arcy, C.J. 1997. Comparison of techniques for detection of barley yellow dwarf virus-PAV-IL. *Plant Disease* 82:1236-1240.

Hoffman, T.K., and Kolb, F.L. 1998. Effects of barley yellow dwarf virus on yield and yield components of drilled winter wheat. *Plant Disease* 82:620-624.

So, I.Y. 1993. Transmission of barley yellow mosaic virus by the fungal vector, *Polymyxa graminis* Ledingham. *Korean J. Plant Pathology* 9(2):128-135.

Woo, M.O. 2000. Molecular identification of barley yellow dwarf virus spread in Korea and evaluation of wheat for its resistance. *Seoul Nation. Uni.* 30 pp.

Host Specialization in BYDV Vectors and Its Impact on Virus Spread

A. Vialatte, F. Fabre, E. Jacquot, J.-C. Simon, and M. Plantegenest

UMR INRA/ENSAR, Domaine de la Motte, Le Rheu, France

The *Barley yellow dwarf virus* (BYDV) pathosystem is a good model for the study of the evolution of interactions in a system with three biological components, a host, a pathogen and its vector. It allows in particular to assess the consequences of host specialization of the vector on the evolution of plant-virus interactions. The results presented here are part of this project. Host-specialization has been shown in various aphids such as the peach-potato aphid *Myzus persicae* (Zitoudi *et al.*, 2001) or the English grain aphid, *Sitobion avenae* (Haack *et al.*, 2000) and extensively studied in the pea aphid, *Acyrthosiphon pisum* (Via 1991a, 1991b, 1999). Similarly, a variability of different clones in their ability to transmit some viruses has been shown in *M. persicae* for the PLRV (*Potato leafroll virus*) by Bourdin *et al.* (1998) and Terradot *et al.* (1999) or in *S. avenae* for the BYDV-PAV (Papura *et al.*, 2002). However, host specialization and ability to carry a virus have never been studied simultaneously.

In the present study, aphid specialization regarding their host plant and their propensity to carry virus were assessed in the same time through the analysis of genetic differentiation between populations of the bird cherry-oat aphid, *Rhopalosiphum padi*, sampled from various host plants and according to whether they are viruliferous or not.

Materials and Methods

The analyses were performed in two steps. A large sample (N = 1458) of adults *R. padi* was collected in the Rennes Basin (Brittany, France) in autumn 2001 on six types of host plants: corn, barley seedlings, wheat and barley volunteers, ryegrass (*Lolium perenne*) and brome *(Bromus erectus*). First, a TAS-ELISA test was performed on all individuals to check for the presence of BYDV-PAV and BYDV-MAV within aphids. This first step allowed to constitute a sub-sample of 302 individuals containing an equal number of viruliferous and non-viruliferous individuals originating from each host plant. Then, the aphid and virus nucleic acids (RNA + DNA) were extracted at the same time from individual *R. padi*. A highly sensitive quantitative RT-PCR was used to confirm the presence of the virus and the aphid genotype was characterized using seven polymorphic microsatellite loci (see Simon *et al.*, 2001 for details on aphid genotyping).

Results

TAS-ELISA confirmed that barley volunteers are an important reservoir of BYDV in autumn (Henry *et al.*, 1991; Comas *et al.*, 1996) with about 24% of individuals from this host carrying BYDV. On the other host plants, the frequency of viruliferous aphids ranged from 0% (brome) to 10% (ryegrass) (Figure 1).

Despite a high genotypic diversity across populations of *R. padi*, genetic analysis showed neither significant differentiation between populations originating from different host plants (Fst = 0.00819; P = 0.14) nor significant differentiation between viruliferous and non-viruliferous aphids (Fst = -0.0007; P = 0.1).

Discussion

Our results contrast with those obtained on another vector of BYDV, *S. avenae*. In that species, both a genetic differentiation according to the host plant (Haack *et al.*, 2000) and a variability in the ability to transmit BYDV-PAV (Papura *et al.*, 2002) were observed. Though

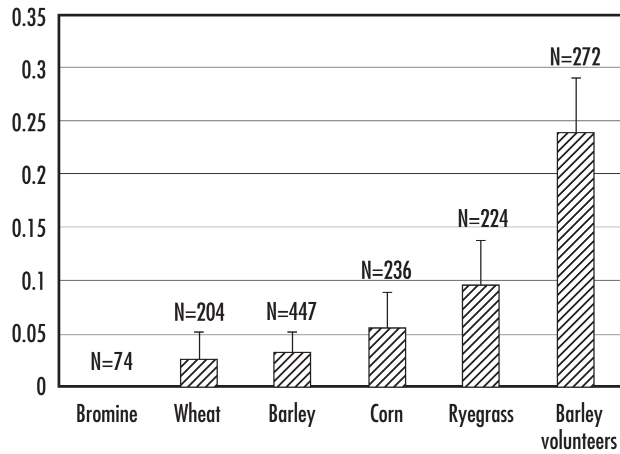


Figure 1. Frequency of viruliferous aphids obtained for each host plant.
Notes: Differences among host plants are significant (Generalized Linear Model, χ^2=119, ddl=5, P<0.0001).
N= number of aphids tested.

Sadeghi *et al.* (1998) also found a variability of *R. padi* clones in their ability to transmit BYDV-PAV in laboratory, our study seems to indicate that, if any, this variability is not associated to a variation in plant preferences. Both *S. avenae* and *R. padi* reproduce by cyclical parthenogenesis (alternate a single sexual generation and several parthenogenetic ones). However, the sexual phase is associated to an obligate host alternation in *R. padi* from Poaceae (summer hosts) to *Prunus padus* (winter host), a Rosaceae species. Conversely, *S. avenae* reproduces both sexually and parthenogenetically on the same plant family, the Poaceae (Figure 2). Host alternation can be suspected to favor a large genetic mixing in *R. padi* that could prevent any process of specialization on the summer hosts.

In contrast, the absence of host alternation in *S. avenae* could promote a reproductive isolation of

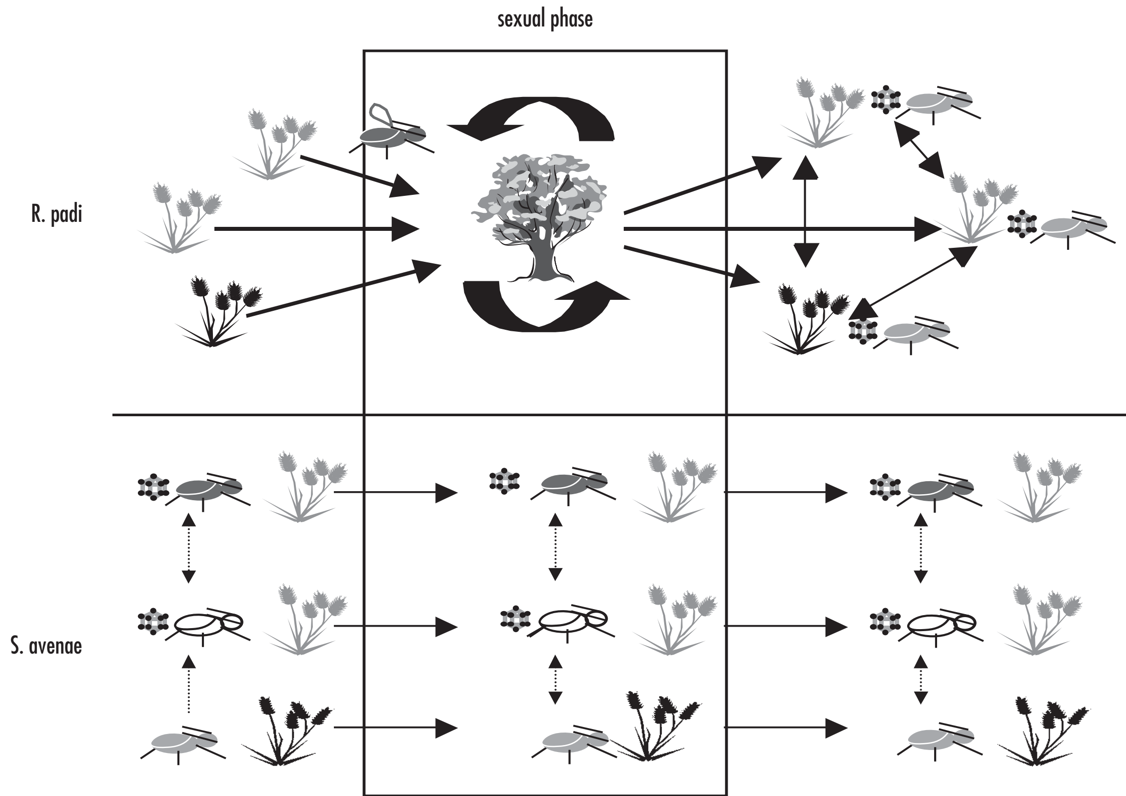


Figure 2. Life cycles of *Rhopalosiphum padi* and *Sitobion avenae* and hypothetical consequences on their level of host specialization and variability to carry BYDV.

populations according to the host plant species that is favourable to the evolution of genetically differentiated and reproductively isolated host races, as demonstrated for another aphid, *A. pisum* by Via (1991a and b; 1999). Variability in the ability to transmit the virus could then be a consequence of host specialization of aphids allowing a close adaptation of the virus to some particular clones living preferentially on the most suitable plant for the virus. This could finally result in a partial isolation of some virus strains and their specialization on a particular host species as it has been observed by Mastari *et al.* (1998). Obviously, host specialization in aphids and its consequences on BYDV could have a strong influence on the epidemics of the BYD disease through the risk of transmission from a reservoir to a target crop that has been until now largely neglected, justifying further studies.

References

Bourdin, D., Rouzé, J., Tanguy, S., and Robert, Y. 1998. Variation among clones of *Myzus persicae* and *Myzus nicotianae* in the transmission of a poorly and highly aphid-transmissible isolate of potato leafroll luteovirus (PLRV). *Plant Pathology* 47:794-800.

Comas, J., Pons, X., Albajes, R., and Plumb, R.T. 1996. Barley yellow dwarf luteovirus (BYDV) infectivity of alate aphid vectors in Northeast Spain. *Journal of Phytopathology* 144:273-276.

Haack, L., Simon, J.C., Gauthier, J.P., Plantegenest, M., and Dedryver, C.A. 2000. Evidence for predominant clones in a cyclically parthenogenetic organism provided by combined demographic and genetic analyses. *Molecular Ecology* 9:2055-2066.

Henry, M., and Dedryver, C.A. 1991. Occurrence of barley yellow dwarf virus in pastures of western France. *Plant Pathology* 40:93-99.

Sadeghi, E., Dedryver, C.A., and Gauthier, J.P. 1998. Role of acquisition and inoculation time in the expression of clonal variation for BYDV-PAV transmission in the aphid species *Rhopalosiphum padi* L. *Plant Pathology* 48:545-551.

Mastari, J., Lapierre, H., and Dessens, J.T. 1998. Asymmetrical distribution of barley yellow dwarf virus PAV variants between host plant species. *Phytopathology* 88:818-821.

Papura, D., Jacquot, E., Dedryver, C.A., Luche, S., Riault, G., Bossis, M., and Rabilloud, T. 2002. Two-dimensional electrophoresis of proteins discriminates aphid clones of *Sitobion avenae* differing in BYDV-PAV transmission. *Arch. Virol.* (in press).

Terradot, L., Simon, J.C., Leterme, N., Bourdin, D., Wilson, A.C.C., Gauthier J.P., and Robert, Y. 1999. Molecular characterization of clones of the *Myzus persicae* complex (Hemiptera: Aphididae) differing in their ability to transmit the potato leafroll luteovirus (PLRV). *Bulletin of Entomological Research* 89:255-263.

Simon, J.-C., Leterme, N., Delmotte, F., Martin, O., and Estoup, A. 2001. Isolation and characterization of microsatellite loci in the aphid species, *Rhopalosiphum padi. Molecular Ecology Notes* 1:4-5.

Via, S. 1991a. Specialized host plant performance of pea aphid is not altered by experience. *Evolution* 72:1420-1427.

Via, S. 1991b. The genetic structure of host plant adaptation in a spatial patchwork: demographic variability among reciprocally transplanted pea aphid clones. *Evolution* 45:827-852.

Via, S. 1999. Reproductive isolation between sympatric races of pea aphids. I. Gene flow restriction and habitat choice. *Evolution* 53:1446-1457.

Zitoudi, K., Margaritopoulos, J.T., Mamuris, Z., and Tsitsipis J.A. 2001. Genetic variation in *Myzus persicae* populations associated with host-plant and life cycle category. *Entomologia Experimentalis et Applicata* 99:303-311.

Barley Yellow Dwarf Virus in Egypt: Current Situation and Prospects

A.E. Aboulata

Plant Virus and Mycoplasma Res. Sec., Plant Path. Res. Inst., ARC, Giza, Egypt

Barley Yellow Dwarf Virus in Egypt

BYDV-like symptoms were first recorded on wheat in Egypt by Abdel-Hak (1984). The virus was isolated and identified (Aboul-Ata *et al*., 1992). The five serotypes (PAV, RMV, RPV, MAV, and SGV) were found in Egypt, with PAV being dominant (Lister *et al*., 1994). Sub-types cpA and cpB of BYDV-PAV (Aboulata *et al*., 2001) transmitted by *Rhopalosiphum padi* were found in PAV-infected wheat samples using capside protein-specific PCR products (unpublished data). The cpA-PAV type was less frequent and more aggressive on wheat plants.

Host Range

BYDV-PAV serotype was detected in bread wheat (*Triticum aestivum*), durum wheat (*T. durum*), and barley (*Hordum vulgare*). BYDV-RMV serotype was detected in maize (*Zea mays),* sorghum (*Sorghum bicolor*), and sugar cane (*Saccharum officinarum*) (Lister *et al*., 1994). Several graminaceous weeds found in cereal or non-cereal crops were found to be infected by BYDV-PAV using insect transmission and ELISA (Aboul-Ata *et al*., 1992).

BYDV Incidence

Field survey was performed under the Aphid/Virus Network-NVRSRP organized by ICARDA during March each year (1996-2002). Wheat fields of Middle Egypt (Fayium, Giza, Beni Suef, Minia, and Assuit) and Lower Egypt (Qualubia, Menufia, Gharbia, Behira, Kafr El-Shaikh, Ismailia, and Sharkia) were visited. Fifty stem samples were collected randomly at each stop (approximately at 10 km distance from each other) to be analyzed using DAS-ELISA and TBIA at the Laboratory of Plant Virus and Mycoplasma. BYDV-PAV fluctuated from year to year. The disease incidence was 5.4% in 1996, 13.6% in 1997, 11.6% in 1998, 11.6% in 2001 and the lowest in 2002 (0.4%). Qualubia, Menufia, and Giza had the highest infection.

Epidemiology

The aphid species; *R. padi*, *R. maidis*, *Schizaphis graminum*, and *Sitobion avenae* were found in Egypt. The BYDV isolate found in Egypt was transmitted by *R. padi* and *S. graminum* (Aboul-Ata *et al*., 1992).

Transmission of BYDV-PAV by aphids (*R. padi)* caught alive in the suction trap located at Giza Agricultural Research Station, ARC, was measured from February 1998 to June 1998 by feeding the aphids on barley bait plants. Average transmission of BYDV was 4.0%. From September 1998 to April 1999, aphids were tested by DAS-ELISA; an average of 4.2% were positive by ELISA. During this period, seven peaks of aphid flights were monitored (Figure 1). Virus was detected in as much as 60% of the aphids at some sampling dates. In comparison, level of transmission by aphids caught alive in the suction trap the previous year were low. The difference could be due to barriers of virus transmission by aphid vector (Power and Gray, 1995).

Estimation of Yield Losses

Both systemic insecticide-treated plots and non-treated plots left for natural infection were used to determine the yield losses caused by BYDV in four commercial wheat varieties (Gz164, Gz167, Sakha69, Sids7). They

ranged between 9.3 to 0.6% according to the percentage of virus infection, severity, and host tolerance (Table 1).

Screening for BYDV Resistance/ Tolerance in Wheat and Barley

Complete randomized plots and aphid inoculation was used under insect-proof plastic cage to screen for virus resistance during 1995-2002. Susceptible and tolerant checks were used in the insect-treated and non-insect treated plots (control). Different parameters (i.e., virus incidence, concentration, severity and yield losses) were measured on the wheat and barley genotypes tested (local, ICARDA's and CIMMYT's). Three genotypes did not get infected suggesting that they could be resistant, and 30 showed some level of tolerance on a total of 924 tested.

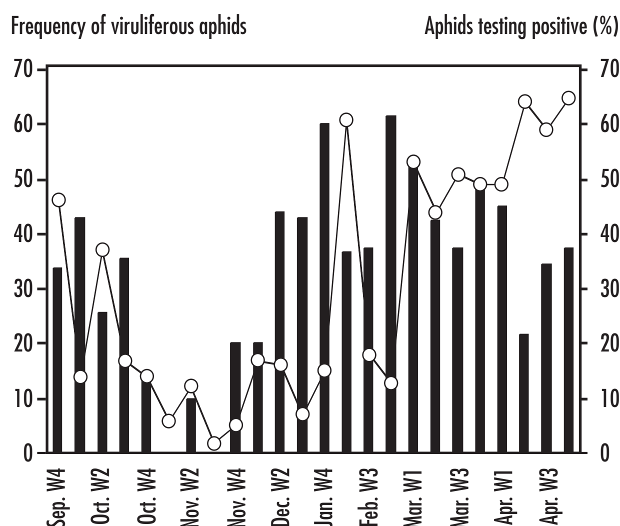


Figure 1. Detection of BYDV-PAV by TBIA in insects caught in an Aphtrak portable aphid samples (suction trap, Code BF00295, UK) at Giza Experiment Station between September 1998 and April 1999. Notes: Bars represent the % of aphids that tested positive by TBIA; line, the number of aphids caught.

Table 1. Yield losses caused by BYDV infection in commercial wheat varieties.

Wheat variety	% infection (assessed visually)*	Disease severity	% of infection (measured by ELISA)**	Yield losses %
Gz164	95	3.6	100	9.3
Gz167	82.5	2.8	33.3	3.9
Sakha69	82.5	2.5	66.6	7.5
Sids7	82.5	3.1	66.6	0.6

* = 2000 samples examined; ** = 60 samples tested.

The Future

Future prospects are to identify sources of resistance/ tolerance and make them available to farmers, pursue the seasonal monitoring of BYDV for prediction and strengthen collaboration with other institutions, including international institutes in the area of information and genetic material exchange, training and strain identification.

The work achieved is collaboration between Egypt and international institutions (i.e., Aphid/Virus Network, ICARDA; ORSTOM and INRA, France and Purdue University, USA). Constraints are lack of funding to carry out screening for virus resistance, lack of temperature-controlled green house, advanced technology in virus detection and strain recognition.

Acknowledgments

The author acknowledges K. Makkouk, R. Aboul-Enien, Maisa A. Awad, Lila M. Ibrahim, M. El-Hariry, N.H. Soliman, and H. Lapierre for their support in both laboratory and field work and for their encouragement. This study has been funded by the government of The Netherlands through the NVRSRP/Network (Aphid/Virus Network) and organized by ICARDA.

References

Abdel-Hak, T.M., and Ghobrial, E. 1984. Present status of virus disease of wheat and barley in Egypt and the Near East region. In: Burnett, P.A. (ed.). Barley Yellow Dwarf: Proceedings of the Workshop. Mexico, D.F.: CIMMYT.

Aboul-Ata, A.E., Thouvenel, J.-C., Makkouk, K.M., and Satour, M.M., 1992. Barley yellow dwarf virus in Egypt: Natural incidence, transmission, and wild hosts. Arab. Jour. Plant Protection 10(2):226-231.

Aboul-Ata, A.E., L. Ibrahim, M. Awad, M. El-Hariry, and H. Lapierre. 2001. PAV-BYDV occurrence and variability in Egypt. EFPP 2000. Biodiversity in Plant Pathology: Proceeding of the 5th Congress of the European Foundation for Plant Pathology. 605-607.

Lister, R.M., Aboul-Ata, A.E., El-Dawoudi, Y., Marshall, D., Makkouk, K.M., Satour, M.M., Ghanim, E., and Burnett, P. 1994. Serotyping of barley yellow dwarf virus isolates from Egypt. Phytopathologia Mediterrenea 33(2):152-157.

Power, A., and Gray, S. 1995. Aphid transmission of barley yellow dwarf viruses: interaction between viruses, vectors and host plants. In Barley Yellow Dwarf: 40 Years of Progress. C. D'Arcy and P.A. Burnett. APS Press. pp. 259-289.

Breeding BYDV Resistant Barley with an Agronomically Improved Background

F. Capettini,[1] M. Henry,[2] and H. Vivar[2]

[1] ICARDA/CIMMYT Barley Program for Latin America, Mexico, D.F., Mexico
[2] Wheat Program, CIMMYT, Mexico, D.F., Mexico

Barley yellow dwarf (BYD) is the most important viral disease of barley, having the potential to destroy entire crops in some Latin American countries and worldwide. Breeding for this devastating disease is one of the most important objectives of the ICARDA/CIMMYT barley breeding program, which has been successful in producing BYD resistant/tolerant germplasm in an agronomically enhanced genetic background (Table 1).

Table 1. Sample of elite germplasm produced by the ICARDA/CIMMYT barley breeding program resistant to BYDV-PAV,- MAV and CYDV-RPV in an agronomically improved background.

Genotype	Stem rust	Leaf rust	Scald	Grain type	Stem rust	Yield (t/ha)
Six-row						
EGYPT4/TERAN78//P.STO/3/QUINA	R	40S	TR	C		9.0
BELLA UNION	30S	TR	TR	C		8.2
ALPHA/DURRA//CORACLE/3/ALELI/4/MPYT169.1Y/LAUREL//OLMO/5/GLORIA-BAR..	R	TR	R	C		8.0
DC-B/SEN/3/AGAVE/YANALA//TUMBO/4/CEN-B/2*CALI92	5S	TR	MS	C		7.3
PETUNIA 1	5MS	TR	R	N	R	7.1
BBSC/CONGONA	R	TR	TR	N		6.8
CARDO/VIRDEN//ALOE	-	TR	-	C		6.7
PALTON	TR	TR	TR	C		6.6
DC-B/SEN/3/AGAVE/YANALA//TUMBO/4/CEN-B/2*CALI92	5MS	TR	TR	C		6.5
QUINN/ALOE//CARDO	TR	TR	TR	C		6.4
SEN/SLLO/3/RHODES/CI14100//LIGNEE527	30S	TR	R	C		6.4
MONROE/4/ASE/3CM//RO-B/3/SMA1/5/MATICO	R	TR	R	C		6.3
Two-row						
MADRE SELVA	R	TR	R	C	TS	7.1
ABN-B/KC-B//RAISA/3/ALELI	TR	TR	R	C		6.9
CONDOR-BAR/3/PATTY.B/RUDA//ALELI/4/ALELI	TR	TR	R	C		6.7
ARUPO*2/KC-B//ALELI	R	TR	S	C		6.7
LIMON	TR	TR	R	C	TS	6.6
INCIENSO	5MS	TR	TR	C	TS	6.5
COMINO/3/MATICO/JET//SHYRI/4/ALELI	R	TR	R	C		6.5
POROTILLO	R	TR	TR	N		6.3
HLLA/GOB//HLLA/3/CANELA	-	10MS	-	C		5.8
CALENDULA	R	TR	R	N		5.7
GOBERNADORA/HUMAI10//CANELA/3/ALELI	-	TR	-	C		5.4
DUMARI	10S	TR	TR	N	VS	5.3

T: Traces; R: Resistant; MR: Moderately Resistant; MS: Moderately Susceptible; S: Susceptible; VS: Very Susceptible.
N: Naked; C: Covered

Selection against susceptible genotypes has been carried out for more than 20 years in CIMMYT's Toluca Experiment Station in the Central Highlands of Mexico, where symptoms are frequent under natural conditions. More recently, artificial inoculation with greenhouse-reared aphids has been done in screening nurseries under field conditions to assure uniform infection, differentiate biotype reaction, and reduce the risk of escapes. The three BYD-causing viruses most commonly found in the Americas (BYDV-PAV, -MAV, and CYDV-RPV) were used in our research. Four plots were planted with each genotype, and three were inoculated with one serotype each. The fourth plot was a check plot, kept free of aphids by insecticide applications.

This screening methodology has proven useful for identifying genotypes with enhanced reactions to BYDV. The most common and effective source of resistance to BYDV in barley has been identified in Ethiopian barleys and found to be associated to the major semi-dominant gene*Yd2* (Rasmusson and Schaller, 1959). This gene has been reported to be associated with reduction in virus concentration with BYDV-PAV and BYDV-MAV but not with CYDV-RPV (Herrera, 1989). An assay based on a protein showing allelic variation that correlates with *Yd2* (Paltridge *et al*., 1998) and a PCR marker for the *Yd2* associated allele *Ylp* (Ford *et al*., 1998) has been used in the program to screen genotypes, finding that the *Yd2* gene was present in the genotypes showing field tolerance (Henry and Vivar, 2001). Several lines tested showed field tolerance to PAV and MAV, as well as to RPV, indicating that additional gene(s) conferring resistance to CYDV-RPV should also be present in the germplasm (Henry and Vivar, 2001). Future work will determine which additional genes also confer resistance to RPV.

Research carried out with ICARDA/CIMMYT germplasm was able to produce a large germplasm pool with high field tolerance to BYDV isolates PAV, MAV, and RPV in a high-yielding, agronomically improved germplasm resistant to several important diseases that is available to breeding programs worldwide.

References

Ford, C.M., Paltridge, N.G., Rathjen, J.P., Moritz, R.L., Simpson, R.J., and Symons, R.H. 1998. Rapid and informative assays for *Yd2*, the barley yellow dwarf virus resistance gene, based on the nucleotide sequence of a closely linked gene. *Molecular Breeding* 4:23-31.

Henry, M., and Vivar, H.E. 2001. Resistance and/or tolerance to BYDV: Recent advances in barley at CIMMYT. In: *Breeding Barley in the New Millenium: Proceedings of an International Symposium*. H.E. Vivar and A. McNab (eds.). Mexico, D.F.: CIMMYT. pp. 72-76.

Herrera, M.G.F. 1989. Interactions between host plants and British isolates of barley yellow dwarf virus. Thesis - University of London.

Paltridge, N.G., Collins, N.C., Bendahmane, A., and Symons, R.H. 1998. Development of YLM, a codominant PCR marker closely linked to the *Yd2* gene for resistance to barley yellow dwarf disease. *Theoretical and Applied Genetics* 96:1170-1177.

Rasmusson, D.C., and Schaller, C.W. 1959. The inheritance of resistance in barley to the yellow-dwarf virus. *Agronomy Journal* 51:661-664.

Problems and Solutions in the Development of New Sources of BYDV Tolerance for Bread Wheat

A. Comeau[1] and S. Haber[2]

[1] Agriculture Agri-Food Canada, SCRDC, Sainte-Foy Québec, Canada
[2] Agriculture Agri-Food Canada, CRC, Winnipeg, Canada

In bread wheat and durum wheat, good tolerance data implies a study for many years and at many contrasting sites. This is due to the fact that tolerance tends to be more site-specific for wheat than for other cereals. Tolerance data also shows contrast between years at a given site. Data on loss of chlorophyll is less reliable in wheat than in other species. Considering these constraints, collaborative research was initiated on wheat tolerance at contrasting test sites, Winnipeg and Quebec City. Lines were evaluated based on complex criteria. This was done recognizing the fact that one line or cultivar could attribute more of its scarce resources to roots when infected, thus reducing the photosynthate available to tillers, florets or seeds. It also acknowledged that another line could simply slow down its phenology, taking more time in an attempt to produce the same number of tillers, spikes, and florets for example. Index selection and rotating 3-D plotting software were used in decision-making. The height and lodging data must also be used in decision-making, and present a serious problem. Height has an optimum value; the tall are lodging-prone, and the ultra short always presents complex, severe defects. Height genes also interact strongly with virus tolerance in Quebec: short plants are more damaged. Information based on many years of data and confirmed at more than one site will be presented. It is concluded that through collaborative research, it is feasible to develop wheat with BYDV tolerance that expresses at many sites and in contrasting soil and climate conditions.

Barley Yellow Dwarf in Russia

K.A. Mozhaeva and T.B. Kastalyeva

Russian Research Institute of Phytopathology, Bolshie Vyaziomy, Moscow Region, Russia

Barley yellow dwarf (BYD) was first found in Russia during a viral epidemic on cereal crops in the Krasnodar Territory in 1961, and subsequently in Moscow Province. Severe damage of small grain crops by a disease of unknown etiology was reported again in European Russia between 1988 and 1991. Small grain yields were decreased by 90% during this epidemic. BYD was shown to be the cause of the epidemic.

The Virology Group of the Russian Research Institute of Phytopathology has been studying the causal agent of BYD since 1991. Before 1996, the only way to identify the pathogen was through visual inspection, which did not allow distinguishing among the different viruses that cause BYD. In 1995-1996, in collaboration with Dr. T. Erokhina from the Institute of Bioorganic Chemistry of the Russian Academy of Sciences, an ELISA-based detection method for PAV-BYD and CYDV-RPV was developed using monoclonal antibodies (Erokhina and Kastalieva, 1995). The data we present here were obtained since those years.

Methods

Oat and barley fields were sight inspected. Leaf samples were simultaneously collected from plants having typical symptoms of BYD damage. Some samples were also received from the Regional Stations of Plant Protection. All samples were analyzed for BYDV presence in double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with monoclonal antibodies specific against PAV (monoclone 4B5) and against RPV (monoclone 3C2). In some years BYDV MAV, SGV, and RMV were analyzed by means of polyclonal antibodies produced by Agdia Inc. Hammond's method of purification (Hammond *et al.*, 1983) was used to isolate BYDV (Kastal'yeva *et al.*, 1994).

Results and Discussion

More than 1000 samples from 20 provinces of eight European Russia Regions and from six provinces of four Asiatic Russia Regions were analyzed for BYDV presence between 1996 and 2002. BYDV was found in all of them (Fig. 1). In the Central Region of Russia (Moscow Province) 15 species of aphids known to be BYDV vectors were found. *Rhopalosiphum padi*, *Sitobion avenae*, *Schizaphis graminum*, and *Metopolophium dirhodum* were predominant in the Central Region of Russia. They transmit BYDV MAV, PAV, SGV and CYDV RPV. Viruliferous aphids of both *R. padi* and *S. avenae* species are effective BYDV vectors. They can infect almost 90% of plants with the virus. *Sitobion avenae* transfers not only BYDV MAV, but also BYDV PAV and CYDV RPV (Dzhama, 2000). As a result of the availability of several aphid species, 60-80% of diseased plants are usually infected with 2-3 BYDVs.

Analyses of BYDV PAV and CYDV RPV, conducted in Moscow Province in 1996-2001, showed that BYDV PAV was predominant. On average 44% of the samples tested over six years contained BYDV PAV. CYDV RPV was found in 28.6% of the samples (Mozhaeva and Kastalyeva, 2001).

In 1999-2001, the presence of BYDV PAV, MAV and SGV as well as CYDV RPV was measured in samples

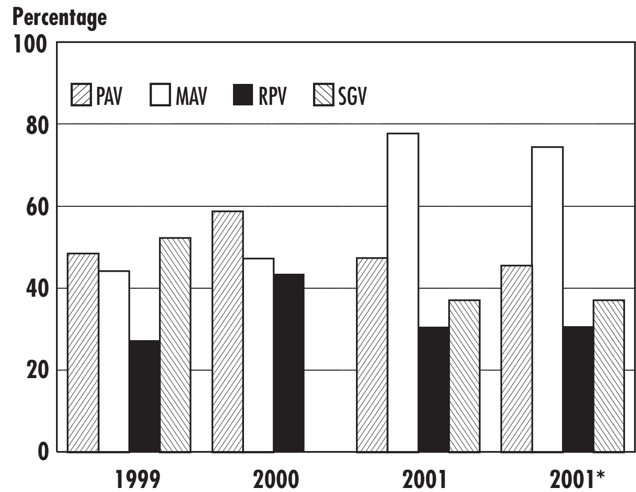
of grain crops of Moscow Province. On average, BYDV PAV, MAV and SGV were found in 50% of the analyzed samples, and CYDV RPV in 30% of the samples. In 2001, the frequency of BYDV PAV and CYDV RPV in Moscow Province was the same as in the other seven regions of European Russia. In the last years, higher incidence of BYDV MAV has been observed (Fig. 2).

The ratio between different BYDVs and CYDV changed not only from year to year, but also during the same year. It should be noted that oat and barley plants grown for green fodder have higher virus incidence than those grown for grain (Table 1).

In the Ural Region of Asiatic Russia, BYDV PAV, MAV and SGV were found. In 2001, BYD was first revealed in East and West Siberia (BYDV PAV, MAV and SGV as well as CYDV RPV). BYDV MAV and SGV were found in the Far East and so was RMV.

It is known that BYD severity depends upon the interaction between different factors within the "virus-vector-plant" system influenced by the weather conditions of the preceding year (Plumb, 1995).

Some aspects concerning BYD epidemics under the conditions of Moscow Province were clarified based on data on BYDV infection of grain crops, cereal grasses, and weeds in the dynamics during the vegetative phase. The degree of damage caused by BYDV on



* 2001 -European part of Russia.

Figure 2. Incidence of BYDVs and CYDV in grain crop samples within Moscow province in 1999-2001.

Table 1. Incidence of BYDVs and CYDV in oat and barley samples, Moscow Province, 1999.

	Number of samples			% of samples infected with			
Crop	Tested	Infection	% infected	MAV	SGV	PAV	RPV
Oats for grain	56	29	51.8	44.8	41.4	62.1	41.4
Oats for green fodder	14	12	85.4	66.6	75.0	58.3	50.0
Barley for grain	80	60	75.0	61.2	80.0	63.3	18.3
Barley for green fodder	4	4	100.0	50.0	75.0	100.0	75.0
Total 154	105	68.2	57.1	68.6	63.8	30.4	

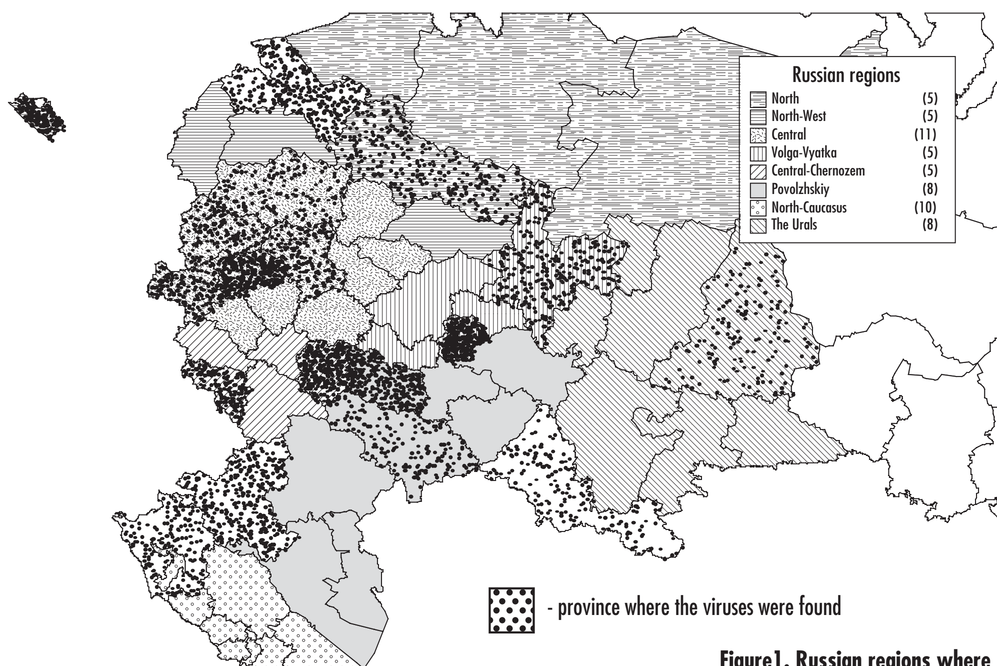


Figure1. Russian regions where BYDV and CYDV were identified.

spring grain crops depends upon the number of aphid vectors and species composition, as well as their ability to acquire the virus from the reservoirs. In spring, the main sources of BYDV are winter crops, perennial cereal grasses, and weeds. In summer, the main sources are cereal crops and grasses, small grain crops growing in mixture with leguminous plants, or maize.

Winter crops and their volunteer plants being infected in the fields in autumn, where there is no winter tillage, as well as perennial cereal grasses and weeds on the sides of the fields and fallow lands can act as sources of BYDV infectivity for the following year.

In the last 10 years, low but stable BYDV incidence has been observed annually on barley and oats in European Russia. Strain composition of BYDV and the degree of the disease damage seem to depend on available aphid species and weather conditions that influence the life and the spread of aphids. If conditions are favorable for the multiplication, infection, and migration of aphid vectors, disease outbreak can arise. Local at first, the disease can spread over individual regions and even whole countries, causing large economic losses, as was the case in the late 1980s.

Conclusions

- Damage of grain crops, especially small grain crops, by BYDV and CYDV was revealed in the eight regions of European Russia and four regions of Asiatic Russia. The incidence of viral strains depends on the aphid vectors available, geographic location, and weather conditions.
- In the central nearby regions of European Russia, the main BYDV and CYDV vectors were *R. padi*, *S. avenae* and *S. graminum* aphids. BYDV PAV, MAV, SGV and CYDV RPV were the predominant viruses.
- In the Central Region, the main sources of BYDV and CYDV can replace one another during the vegetative cycle. The sources may be perennial cereal grasses and weeds, winter crops, small grain crops growing in a mixture with legumes, volunteer plants, and regrowth of grain crops.

References

Erokhina, T., and Kastalieva, T. 1995. Production of monoclonal antibodies against barley yellow dwarf virus. Detection of BYDV in the field samples from Central region of Russia. *Zuchtungforschung* 1:71-74.

Dzhama M. 2000. The role of aphids from the *Aphididae* family in virus spread on wheat and *Triticum* x *Agropirum* hybrids. Ph.D. thesis, Moscow.

Hammond, J., Lister, R.M., and Foster, J.E. 1983. Purification, identity and some properties of an isolate of barley yellow dwarf virus from Indiana. *J. Gen. Virol.* 64:667-676.

Kastal'yeva, T.B, Vasil'yeva, T.Y., and Mozhaeva, K.A. 1994. Isolation of the barley yellow dwarf virus from naturally infected oat and barley plants. *Russian Agricultural Sciences* (*Doklady Rossiiskoi Akademii Sel'skokhozyaistvennykh Nauk*) 5:35-38.

Mozhaeva, K.A, and Kastalyeva, T.B. 2001. Barley yellow dwarf virus incidence and strains in Russia. Abstracts of the III International Conference "Bioresourses and viruses", Kyiv, Ukraine, p. 196.

Plumb, R.T. 1995. Epidemiology of barley yellow dwarf in Europe. In: *Barley Yellow Dwarf. 40 Years of Progress.* C.J. D'Arcy and P.A. Burnett, eds. APS Press. pp. 107-127.

Use of PCR Markers to Select *Barley Yellow Dwarf Virus* Resistant Plants

K.M. Makkouk, W. Ghulam, M. Baum, S. Ceccarelli, and S. Grando

Germplasm Program, International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria

Barley yellow dwarf virus *(BYDV) is considered the most economically important virus affecting cereal crops worldwide. The use of laboratory methods to identify plants resistant to BYDV at the seedling stage could make the selection of resistant plants in the* F_2 *population simple and fast. By using tissue-blot immunoassay (TBIA) to monitor BYDV concentration in the parents and* F_2 *populations of 21 barley crosses planted in pots in a plastic house, it was possible to identify resistant plants based on the absence of virus in phloem tissues five days after inoculation. Such differentiation was less clear when the test was conducted nine days after virus inoculation, and was not possible 14 days after virus inoculation. Using this approach, it was possible to eliminate susceptible plants five days after inoculation and transplant the resistant ones to the field for observation until harvest. When a similar study was conducted on the parents and the* F_2 *populations of 26 crosses planted directly in the field, it was possible to differentiate resistant plants three weeks after virus inoculation. Planting directly in the field avoids the need for transplanting, which promotes better plant development. However, preliminary screening in the plastic house requires much less space compared to direct planting in the field. Within the same crosses and populations, the usefulness of the* Yd2 *specific PCR primers was tested. The availability of allele-specific or cleaved amplified polymorphic sequence PCR markers makes it possible to quickly identify the presence of the* Yd2 *gene in the barley material and compare its presence with the expression of resistance in the field. In most cases analyzed, the* Yd2 *gene was found to be present in BYDV resistant plants. The use of virus movement and PCR markers proved to be useful in selecting barley genotypes resistant to BYDV at the seedling stage.*

Barley yellow dwarf virus (BYDV, family *Luteoviridae*) is considered one of the most economically important viruses affecting cereals worldwide, and genetic resistance is in general the most practical approach to reduce the losses it causes (Jones and Catheral, 1970). By screening barley genetic resources, it was possible to identify many barley lines resistant/tolerant to BYDV infection, which breeders can use in their crossing programs (Delogu *et al.*, 1995; Qualset, 1990). Barley parents with the *Yd2* gene located on chromosome 3H have been used extensively to produce barley breeding lines resistant to BYDV (Rasmusson and Schaller, 1959; Chalhoub *et al.*, 1995).

Resistant oat plants can be differentiated from susceptible ones in the F_2 segregating population based on symptom expression and virus multiplication level following artificial virus inoculation at the seedling stage under plastic house conditions (Gourmet and Kolb, 1992). In barley, BYDV resistant genotypes were differentiated from susceptible ones 4-6 days after inoculation (Makkouk *et al.*, 1994). The aim of this study was to evaluate the use of tissue blot immunoassay to differentiate between barley BYDV-resistant and susceptible genotypes following virus inoculation at the seedling stage in the plastic house and in the field, and compare it with the use of cleaved amplified polymorphic sequence (CAPS) PCR markers (Ford *et al.*, 1998) to quickly identify the presence of the *Yd2* gene in barley plants.

Materials and Methods

One objective of the Barley Improvement Project at ICARDA is to produce breeding lines resistant to

abiotic and biotic stresses, including BYDV-PAV. F_2 segregating populations of 20 crosses of a BYDV-resistant parent (QB813.3, released in Canada in 1995 as Acca; provided by A. Comeau, Agriculture Canada, St. Foy, Quebec, Canada) with 20 parents having resistance to different stresses and adapted to growing conditions in North Africa were evaluated. Seeds were planted in Jiffy pots (180/cross) and placed in 20x30 cm trays in a plastic house at 20-25^{0}C and under natural light in February 2000 at Tel Hadya, Syria. For comparison, 60 seeds of the different parents were also planted as a control. All plants were artificially inoculated with BYDV-PAV by viruliferous *Rhopalosiphum padi*, using 10-15 aphids/plant. Plants were tested for the presence of the virus at 3, 5, 9, and 14 days after inoculation by tissue-blot immunoassay (TBIA, Makkouk and Comeau, 1994). Plants in which BYDV-PAV was not detected five days after virus inoculation were transplanted to the field and monitored until harvest.

Similarly, in the 1999/2000 growing season, seeds of the parents and the F_2 segregating populations of 26 crosses obtained from 18 parents adapted to North Africa growing conditions and three parents resistant to BYDV-PAV (Sutter//Sutter*2/Numar, Gustoe/NK 1272 and QB 813.2) were sown directly in the field. For each cross, 10 rows, each 2 m long, were sown with the F_2 seeds at a rate of 20 seeds/row, in addition to one row for each parent. Plants at the 2-3 leaf stage were inoculated with the virus, and plants were tested for the presence of the virus by TBIA three weeks after inoculation. BYDV symptoms were recorded two months after virus inoculation.

DNA extraction was performed according to the rapid NaOH DNA extraction method of Wang *et al.* (1993). A tissue homogenate was prepared by grinding 5 mg of leaf tissue in 50 µl of 0.5 M NaOH. One µl of the homogenate was diluted tenfold in 0.1 M TRIS-HCl pH 8.0. One µl was then used for PCR amplification (without quantification). DNA prepared in this way can be stored and used for up to four weeks.

The PCR-CAPS primers (Ford *et al.*, 1998) used for the amplification of the *Yd2* gene were: Ylp-MF (AATACAGGAATCTGTTGAAAGAA) and Ylp-MR (TCATCATGGCTCGGAGAAGGTGG).

Each PCR reaction included (besides the genomic DNA template) 25 ng of primer, 200 µM of each dNTPs, 5mM $MgCl_2$, and 1.0 U of *Taq* DNA polymerase (Roche). The PCR cycling profile used was 94 C for 2 min, 94 C for 30s, 60 C for 1 min, 72 C for 2 min, 72 C for 5 min, carried out for 35 cycles in a Perkin Elmer 9600 thermocycler. PCR products were electrophoresed in 6% denaturing polyacrylamid gels and silver stained according to Bassam *et al.* (1991).

Results and Discussion

When plants of the F_2 population of the different crosses grown in the plastic house were tested by TBIA for the presence of BYDV-PAV at 3, 5, 9 and 14 days after virus inoculation, it was possible to detect the virus in susceptible plants 3 days after inoculation. The optimal time to differentiate resistant from susceptible plants in the F_2 populations was five days after virus inoculation in most crosses (Table 1). In only one cross (P1 x P10), however, the differentiation was better at 3 days after virus inoculation. When one of the parents in the crosses had the *Yd2* gene, the proportion of plants with no detectable virus by TBIA in the population of the majority of the crosses ranged from 27% to 45% five days after virus inoculation, and then started to drop nine days after inoculation, to become negligible 14 days after inoculation. The correlation between the presence of *Yd2* gene in barley and virus movement has been reported (Makkouk and Ghulam, 1992; Makkouk *et al.*, 1994). Accordingly, BYDV-susceptible plants were discarded in the plastic house and only resistant plants were transferred to the

Table 1. Proportion of plants with no detectable virus in the phloem vessels in F_2 populations of selected crosses tested at 3, 5, 9, and 14 days following barley yellow dwarf virus inoculation under plastic house conditions, 2000.

Cross		% plants with no detectable virus at different days after virus inoculation			
Female	Male	3 days	5 days	9 days	14 days
P1	P2	-	45.7	14.3	0.0
P6	P1	-	37.7	0.0	3.6
P1	P8	-	28.6	0.0	0.0
P9	P1	29.1	27.8	5.8	-
P1	P10	24.0	2.2	4.2	-
P1	P11	80.0	38.2	1.9	-
P7	P1	45.5	38.2	5.4	-
P18	P1	66.7	35.7	21.8	-

Notes: All genotypes in bold have BYDV resistance gene: P1= **QB813.2**, P2= Arig 8, P6= 80-5145/N-Acc4000-301-80, P7= Algerian Selection DZ 21-3/3/**CM67**/Apro//Sv.02109/Mari, P8= Alanda-02//Ssn/Lignee 640, P9= Ager/3/Robust//**Gloria'S'/Copal'S'**, P10= **Lignee 527/NK1272**//UL76252/Jaidor, P11= Hma-02//11012-2/**CM67**/3/Arar, P18= Scottiall/3/Robust//**Gloria'S'/Copal'S'.**

field and observed until harvest. This preliminary screening in the plastic house at the seedling stage required little space to identify resistant plants to be evaluated further in the field.

In the F_2 populations evaluated directly in the field, it was possible to differentiate between BYDV-resistant and susceptible plants three weeks after inoculation (Table 2). This was expected, as temperatures in the field in February at Tel Hadya were much lower than temperatures in the plastic house, which had a negative effect on virus movement in the plant. There was a significant correlation (P=0.01) between the proportion of plants with no symptoms two months after virus inoculation, and the proportion of plants in the F_2 populations with no detectable virus by TBIA three weeks after virus inoculation (r=0.657). The results suggest that we can test the plants three weeks after virus inoculation and eliminate the susceptible ones, without waiting 8-10 weeks until BYDV symptoms become visible.

When resistant (symptomless) and susceptible (with typical BYDV symptoms) plants in the F_2 populations, as well as selected resistant plants in the F_{3-5} families were tested for the presence of the *Yd2* gene with the CAPS primers, it was possible in most cases to amplify the *Yd2* gene in the resistant plants but not in the susceptible plants (Figure 1). There was a strong association between resistant symptomless plants and the amplified *Yd2* fragment (88%); this association was stronger in the advanced lines (F_{3-5}) (93%) than in the F_2 segregating population (83%). The few symptomless plants that were identified by PCR as being susceptible (i.e. *Yd2* gene not amplified) need to be studied in detail in order to verify whether their resistance might be due to something other than the *Yd2* resistance genes. Susceptible plants that amplified the *Yd2* gene could be studied in order to verify the functionality of the gene. Nevertheless, the PCR screening method with CAPS markers provides a quick, efficient method that if optimized could be used to screen thousands of barley lines for BYDV resistance based on *Yd2* and possibly to identify resistances based on genes other than *Yd2*.

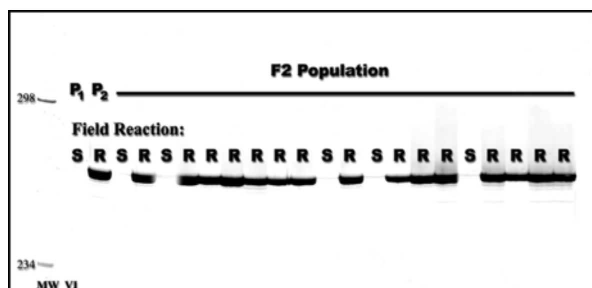


Figure 1. PCR screening of F_2 populations segregating for BYDV resistance with allele-specific PCR markers.
Notes: Top Left: lane 1: size marker VI Roche, lane 2: P1 Arbayan-01/CI07117-9/Deir-Alla, lane 3: P2 Sutter//Sutter*2/Numar, lane 4-23 individuals of F_2 population of P1x P2.

Table 2. Proportion of plants with no detectable virus as compared to symptomless plants in the F_2 segregating populations of selected crosses at three weeks after BYDV inoculation, 1999/2000.

Cross*		% plants with no detectable virus			% symptomless plants in the F_2 segregating population
Female	Male	Female parent	Male parent	F_2 segregating population	
P4	P1	43	86	46	37.4
P4	P2	77	100	36	40.7
P5	P1	0	90	22	12.4
P5	P2	0	100	17	11.5
P6	P1	0	83	15	12.2
P6	P2	0	100	21	10.4
P7	P1	0	69	6	3.4
P7	P2	0	63	6	15.2
P8	P1	0	70	23	15.2
P8	P2	0	100	30	8.8
P9	P1	0	50	12	5.4
P9	P2	0	44	19	4.2
P10	P1	0	50	15	2.3
P10	P2	0	29	6	7.2
P3	P14	22	13	21	17.6
P3	P15	17	0	3	7.8
P3	P16	53	0	10	17.4
P3	P17	29	0	12	20.0
P3	P18	33	0	8	25.8
P19	P3	0	17	6	23.8

* Pedigree of parents used in the crosses.
Notes: All genotypes in bold have BYDV resistance gene: P1= **Sutter//Sutter*2/Numar**, P2= **Gustoe/NK1272**, P3= **QB813.2**, P4= Rhn-13//**Lignee527/NK1272**/3/**Lignee527**/Chn, P5= Hamra/Arar, P6= Saida/N-Acc4000-172-80, P7= 80-5145/N-Acc4000-301-80, P8= Mtn-01, P9= Rhn-03, P10= Saida, P14= Hma-02//11012-2/**CM67**/3/**NK1272**//Manker/Arizona, P15= Aths/**Lignee 686**/4/Rhn-03/3/Bc/Rhn//Ky63-129, P16= Alanda-02/4/Arizona5908/Aths//Assc/3/F208-7, P17= Alanda/Hamra, P18= Alanda-01/Hamra, P19= Alanda//Ssn/**Lignee 640**.

Acknowledgment

We would like to acknowledge the excellent technical input of Mr. Amar Zannerni in the development of PCR screening tests for BYDV at ICARDA.

References

Bassam, B.J., G. Caetano-Anolles, and P.M. Gresshoff. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal. Biochem.* 196:80-83.

Chalhoub, B.A., A. Sarrafi, and H.D. Lapierre. 1995. Partial resistance in the barley (*Hordeum vulgare* L.) cultivar "Chikurin Ibaraki 1" to two PAV-like isolates of barley yellow dwarf virus: allelic variability at the *Yd2* gene locus. *Plant Breeding* 114:303-307.

Delogu, G., L. Cattivelli, M. Snidaro, and A.M. Stanca. 1995. The *Yd2* gene and enhanced resistance to barley yellow dwarf virus (BYDV) in winter barley. *Plant Breeding* 114:417-420.

Ford, C.M., N.G. Paltridge, J.P. Rathjen, R.L. Moritz, R.J. Simpson, and R.H. Symons. 1998. Rapid and informative assays for *Yd2*, the barley yellow dwarf virus resistance gene, based on the nucleotide sequence of a closely linked gene. *Molecular Breeding* 4:23-31.

Gourmet, C., and F.L. Kolb. 1992. Selection for barley yellow dwarf virus tolerance in F2 oat population in the greenhouse. *Crop Science* 32:1476-1479.

Jones, A.T., and P.L. Catherall. 1970. The relationship between growth rate and expression of tolerance to barley yellow dwarf virus in barley. *Annals of Applied Biology* 65:137-145.

Makkouk, K.M., and W. Ghulam. 1992. Resistance of barley genotypes with the *Yd2* gene to the movement of barley yellow dwarf virus. *Rachis Newsletter* 11(1/2):81-83.

Makkouk, K.M., A. Comeau, and C.A. St. Pierre. 1994. Screening for barley yellow dwarf luteovirus resistance in barley on the basis of virus movement. *Journal of Phytopathology* 141(2):165-172.

Makkouk, K.M., and A. Comeau. 1994. Evaluation of various methods for the detection of barley yellow dwarf virus by the tissue-blot immunoassay and its use for virus detection in cereals inoculated at different growth stages. *European Journal of Plant Pathology* 100:71-80.

Qualset, C.O. 1990. Genetics of plant resistance to barley yellow dwarf virus. In: World Perspectives on Barley Yellow Dwarf. P.A. Burnett (ed.). Mexico, D.F.: CIMMYT. pp. 368-382.

Rasmusson, D.C., and C.W. Schaller. 1959. The inheritance of resistance in barley to the yellow dwarf virus. *Agronomy Journal* 51:661-664.

Wang, H., M. Qi, and A.J. Cutler. 1993. A simple method of preparing plant samples for PCR. *Nucl. Acids Res.* 21:4153-4154.

Resistance to BYDV-PAV and CYDV-RPV in a Bread Wheat x *Agrotricum* Cross

G. Posadas and M. Henry
CIMMYT, Mexico, D.F., Mexico

Barley yellow dwarf is a disease caused by a complex of viruses recently reclassified as *Barley yellow dwarf virus* (serotypes BYDV-PAV and BYDV-MAV) and *Cereal yellow dwarf virus* (CYDV-RPV) (D'Arcy *et al*., 1999). The disease has a wide host range and worldwide distribution, and is regarded as the most significant viral pathogen affecting small grain cereals (wheat, barley, oat, and triticale) in the world today.

True resistance (reduction in virus concentration) has not been found in wheat's primary gene pool. However, it has been found in wheat relatives such as *Thinopyrum intermedium* and introgressed into bread wheat by several groups of researchers (Anderson *et al*., 1998; Banks *et al*., 1995; Chen *et al*., 1998; Francki *et al*., 2001; Larkin *et al*., 1995).

The *Agrotricum* line OK7211542 (amphiploid 2n = 56) was reported to be immune to a Canadian BYDV-PAV isolate (Comeau *et al*., 1994). To diversify the source of resistance used in CIMMYT's BYDV breeding program, we attempted to produce addition lines of OK7211542 x bread wheat (Henry *et al*., 2000) and studied the resistance to BYDV-PAV and CYDV-RPV in the resistant OK7211542 and the addition lines. Presented here are preliminary data on resistance to BYDV-PAV and CYDV-RPV in OK7211542.

Materials and Methods

Plant materials

The *Agrotricum* OK7211542 (2n=8x=56) line reported to be immune to BYDV-PAV by Comeau *et al*. (1994) was used in this study. It was compared to the *Th. intermedium*-derived line TC14/2* Spear and to susceptible bread wheats Sunstar, Bagula, and Prinia.

Virus isolates and BYDV inoculation

The Mexican virus isolates used in these experiments were BYDV-PAV and CYDV-RPS. These isolates have been maintained in CIMMYT's greenhouses since they were collected in 1993. Artificial inoculation was performed by depositing 10 aphids in each one-week-old seedling for a 72-hour transmission period. Aphids had acquired the virus on BYDV/CYDV-infected oat during a 72-hour acquisition period. The bird cherry oat aphid, *Rhopalosiphum padi*, was used to transmit BYDV-PAV and CYDV-RPS. At the end of the transmission period, aphids were killed by application of a contact insecticide (Pirimor).

Evaluation of virus titers in leaves and roots

Resistance to BYDV was assessed as a reduction in virus titers measured by ELISA in both roots and leaves of the tested plants. ELISA was performed at 7, 14, 21, and 28 days after inoculation in leaves and roots of different sets of plants on each date. Leaf samples consisted of the 2.5 cm tip of each leaf bigger than 5 cm. Ten plants were inoculated for each isolate and sampling date; two were kept virus-free to serve as non-infected controls. Plants for which the optical density (OD) was lower than the positive threshold were considered as not infected. The positive threshold was calculated for each repetition and each BYDV serotype, and was equal to the average ODs obtained with the non-infected control + 3 standard variation.

A standard dilution curve was prepared by diluting the concentrated sap (1/5, 1/10, 1/25, 1/50, 1/100, 1/200, 1/400, 1/800, and 1/1600) of oat plants inoculated with BYDV-PAV or CYDV-RPV and harvested 14 days after inoculation. Virus titers were

thus expressed as equivalent to a dilution of our concentrated sap and not as optical densities.

ELISA

Double antibody-sandwich ELISA (DAS-ELISA) with polyclonal antibodies against PAV, MAV, and RPV from the United States (provided by K. Perry, Purdue University) was carried out according to Ayala *et al*. (2001). Optical densities were measured at 410 nm after 30 minutes and 1 hour incubation at room temperature using a microplate reader (MR 700; Dynatech Laboratories).

Results

While inoculation rates in the susceptible lines were 90-100%, ODs obtained by ELISA for OK7211542 and TC14/2*Spear did not reach a level higher than the threshold for at least half the plants at 7, 14, and 28 days after inoculation. The virus could be detected in the roots of the same plants (Table 1).

With CYDV-RPV, levels of infection were lower (data not shown) at 7 days; however, they reached 100% in all susceptible lines at 14 days and later. At the same time, only a few plants of OK7211542 and TC14/2*Spear had ODs higher than the positive threshold in leaves (30-40%) and roots (0-20%).

Virus concentrations were lower in resistant lines OK7211542 and TC14/2*Spear than in the susceptible wheats Sunstar, Bagula, and Prinia at 7, 14, and 21 days after inoculation (Figure 1). At 28 days, virus concentration decreased, and differences between lines were attenuated.

Virus concentration in the roots did not differ from one line to the other and were similar in resistant and susceptible lines (Figure 1). Data for roots at 7 days are not presented because ODs were higher than the standard dilution curve, which did not allow us to calculate the concentration. However, the tendency was the same.

As there were very few cases of infection with CYDV-RPV in OK7211542 and TC14/2*Spear, virus concentration data are not presented.

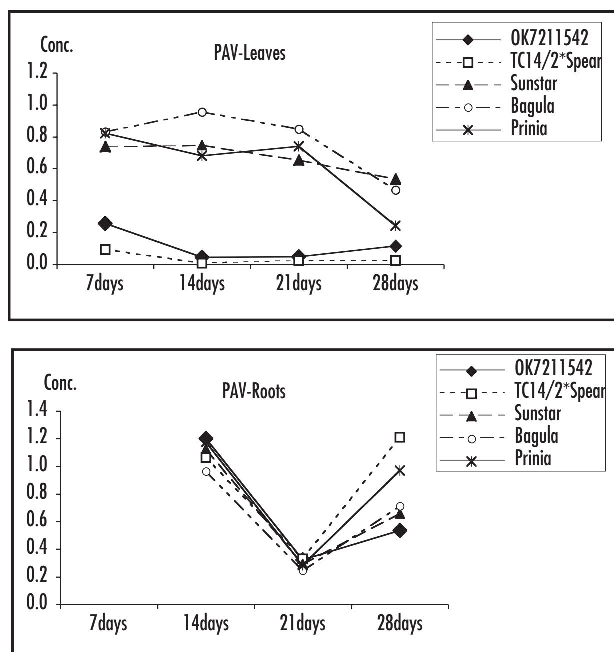


Figure 1. Virus concentrations of BYDV-PAV in leaves and roots of a *Th. intermedium* derived line and the susceptible bread wheat at different times after inoculation.

Table 1. Number of infected plants after artificial inoculation with BYDV-PAV.

Days after inoculation	7 days		14 days		21 days		28 days	
	Leaves[1]	Roots[1]	Leaves	Roots	Leaves	Roots	Leaves	Roots
OK7211542	4	10	5	9	9	10	5	10
TC14/2*Spear	4	10	7	10	10	10	2	10
Sunstar	9	10	10	10	10	10	10	10
Bagula	9	10	10	10	10	10	10	10
Prinia	10	10	10	10	10	10	10	10

[1] Different plants were tested on each collection date.

Discussion

Contrary to what has been reported by Comeau *et al*. (1994), OK7211542 was not immune to BYDV-PAV-Mex in our experimental conditions. However, when only the leaves were tested, the resistance was apparent. The roots accumulated virus levels equivalent to the leaves of susceptible wheat.

Resistance to CYDV-RPV was superior to resistance to BYDV-PAV in both OK7211542 and TC14/2*Spear, as reported by Henry *et al*. (these proceedings). It seems that resistances in OK7211542 and TC14/2*Spear are of similar types, with reduction in the success of infection, reduction in virus titers in leaves, and very strong resistance to CYDV-RPV.

References

Anderson, J.M., Bucholtz, D., Greene, A.E., Francki, M.G., Gray, S.M., Sharma, H., Ohm, H.W., and Perry, K.L. 1998. Characterization of wheatgrass-derived barley yellow dwarf virus resistance in a wheat alien chromosome substitution line. *Phytopathology* 88(8):851-855.

Ayala, L., Henry, M. , González de León, D., van Ginkel, M., Mujeeb-Kazi, A., Keller, B., and Khairallah, M. 2001. A diagnostic molecular marker allowing the study of *Thinopyrum intermedium* derived resistance to BYDV in bread wheat segregating populations. *Theor. Appl. Gen.* 102:942-949.

Banks, P.M., Davidson, J.L., Bariana, H.S., and Larkin, P.J. 1995. Effects of barley yellow dwarf virus on the yield of winter wheat. *Aust. J. Agri. Res.* 46:935-946.

Chen, Q., Collin, J., Comeau, A., St-Pierre, C.A., and Fedak, G. 1988. Comparison of various sources of resistance to barley yellow dwarf virus in wheat-*Thinopyrum* amphiploid lines. *Can. J. of Pl. Path.* 19:414-417.

Comeau, A., Makkouk, K.M., Ahmad, F., and Saint-Pierre, C.A. 1994. Bread wheat x *Agrotricum* crosses as a source of immunity and resistance to the PAV strain of barley yellow dwarf luteovirus. *Agronomie* 2:153-160.

D'Arcy, C.J., Domier, L.L., and Mayo, M.A. 1999. Luteoviridae. In: *Virus Taxonomy. Classification and Nomenclature of Viruses. Seventh Report of the International Committee on Taxonomy of Viruses*, edited by van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon, S.M., Maniloff, J., Mayo, M.A., McGeoch, D.J., Pringle, C.R., and Wickner, R.B. Academic Press. pp. 775-784.

Francki, M.G., Ohm, H. W., and Anderson, J.M. 2001. Novel germsplasm providing resistance to barley yellow dwarf virus in wheat. *Aust. J. Agric. Res.* 52:1375-1382.

Henry, M., Cortes, A., Rosas, V., Delgado, R., and Mujeeb-Kazi, A. 2000. *Agrotricum* (2n=8x=56): potential for barley yellow dwarf virus (BYDV) resistance and its cytogenetics. Annual Wheat Newsletter 46:73-74.

Larkin, P.J., Banks, P.M., and Chen, X. 1995. Registration of six genetic stocks of wheat with rust and BYDV resistance: Z1, Z2, Z3, Z4, Z5, and Z6 disomic addition lines with *Thinopyrum intermedium* chromosomes. *Crop Science* 35:604.

Resistance and Tolerance to *Barley Yellow Dwarf Virus* in Wheat Wild Relative *Aegilops geniculata*

M. Zaharieva, J. Segura, and M. Henry

CIMMYT, Mexico D.F., Mexico

Barley yellow dwarf virus (BYDV) is the most widespread and economically important viral disease of cereals worldwide (Plumb, 1983). Sources of resistance to the disease (expressed as reduction of virus multiplication) has been reported in *Thinopyrum* species and used in crossing programs to introgress resistance genes in wheat background (as reviewed by Ayala *et al*., 2001). Tolerance to BYDV, defined by Cooper and Jones (1983) as attenuation of symptoms and lower yield losses without reduction in virus multiplication, has been identified in bread wheat (Singh *et al*., 1993) and is present in several CIMMYT lines.

Search for new sources of resistance and/or tolerance to BYDV among wheat wild relatives is essential and represents the preliminary step in their further utilization in wide hybridization programs. Some promising sources of resistance to BYDV have already been reported in *Aegilops geniculata* Roth (Zaharieva *et al*., 2001). Moreover, this annual, allo-tetraploid species (2n=4x=28, MMUU), is a valuable reservoir of genes for improving wheat resistance to other important diseases. The aim of this study was to estimate *Ae. geniculata* potential for both resistance and tolerance to BYDV and to identify promising accessions to be used in wide hybridization programs.

Material and Methods

Aegilops geniculata accessions from different eco-geographic regions were tested in this study. They originated from Algeria (10), Bulgaria (18), Croatia (6), Cyprus (4), France (13), Greece (4), Italy (12), Jordan (8), Lebanon (6), Libya (8), Morocco (17), Portugal (8), Spain (14), Syria (10), Tunisia (4) and Turkey (15). Four high yielding bread wheat (Prinia and Baviacora) and durum wheat (Sooty 9/Rascon 37and Kucuk) cultivars were also tested. The controls were the *Thinopyrum intermedium* derived line TC14/2*Spear used as resistant check in BYDV resistance test, and the two bread wheat cultivars Anza and Bobwhite used as tolerant and susceptible checks, respectively, in BYDV tolerance test.

BYDV resistance test was performed in greenhouse conditions. Six plants per accessions were inoculated one week after sowing with 10 aphids (*Rhopalosiphum padi*) per plant, previously fed during 48 h on plants infected by a PAV-Mex isolate of BYDV. For each accession, one or two plants were kept free of aphids to serve as the non-inoculated controls. After 48 h, the aphids were eliminated by application of Metasystox, and 14 and 26 days after inoculation the penultimate leaf of each plant was harvested for the ELISA test, according to Clark and Adams (1977). The plants were considered as moderately resistant when the average optical density was less than 2.5 times the OD obtained with the line TC14/2*Spear.

Evaluation for BYDV tolerance was carried out in field conditions at Toluca CIMMYT experimental station during the winter cycle 2001/2002. Each accession was sown in plots of two 1m long rows in two replicates. Plants of one replicated plot were artificially infected with BYDV-PAV at three-leaf stage, with approximately 10 viruliferous aphids, while the adjacent two rows were kept free of BYDV and served as healthy check. Symptoms (tillering, dwarfing, biomass production) were evaluated during anthesis by using visual scoring system rated from 1 (no visible symptoms) to 9 (very susceptible). Tolerance was also evaluated by measuring the reduction of plant height,

tillers numbers and biomass production in the inoculated plants compared to the control, in a set of 26 accessions, randomly selected within the different scores. The data were assessed on three plants by accession. An accession was considered as tolerant when this reduction for all traits was in average no more than 25% (or 1-4 visual score).

Results and Discussion

Two ELISA tests were performed after 14 and 26 days of inoculation. Five lines out of the 157 tested were moderately resistant. In order to confirm these results a third test including bread and durum wheat high yielding cultivars was realized. The average OD was much lower in the tested five *Ae. geniculata* accessions than in the bread and durum wheat cultivars (Table 1). These accessions could consequently represent new sources of moderate BYDV resistance for wheat improvement. Two of them, originated from South of France (MZ 20 and MZ 21) were previously reported (Zaharieva *et al*., 2001). The remaining three accessions originated from Cyprus, Italy, and Greece. Introgression of the resistance traits coming from these accessions could contribute to reduce virus concentration in the plant and limit the dissemination of the virus.

Field trial revealed differences in BYDV response between Ae. *geniculata* accessions. The proportion of the plants with slight visual symptoms (1-4) was 10% and the proportion of plants showing the most severe symptoms (7-9) was 77%. Average percent reduction due to BYDV infection (Table 2) was high for biomass production (59.4%). Less marked was the reduction of tiller number (48.4%). The lower reduction rates were recorded in plant height (29%). Among the 26 accessions evaluated for these traits (Figure 1), MZ 57, MZ 7 and MZ 91, were the least affected. There was no relationship between the origin of the accessions and

Table 1. Comparison of virus titers in ELISA 14 days after inoculation with BYDV-PAV (Mex).

Genotype (origin)	Species	Average OD
Prinia	*Triticum aestivum*	1.429 ± 0.407
Baviacora	*Triticum aestivum*	1.907 ± 0.160
Sooty 9/Rascon 37	*Triticum durum*	1.469 ± 0.403
Kucuk	*Triticum durum*	1.124 ± 0.550
MZ 20* (France)	*Aegilops geniculata*	0.543 ± 0.123
MZ 21 (France)	*Aegilops geniculata*	0.503 ± 0.095
MZ 97 (Cyprus)	*Aegilops geniculata*	0.478 ± 0.160
MZ141 (Italy)	*Aegilops geniculata*	0.525± 0.147
MZ149 (Greece)	*Aegilops geniculata*	0.479± 0.139
TC14/2*Spear	Resistant check	0.229 ± 0.124

* *Ae. geniculata* accession number in CIMMYT Wide Crosses working collection.

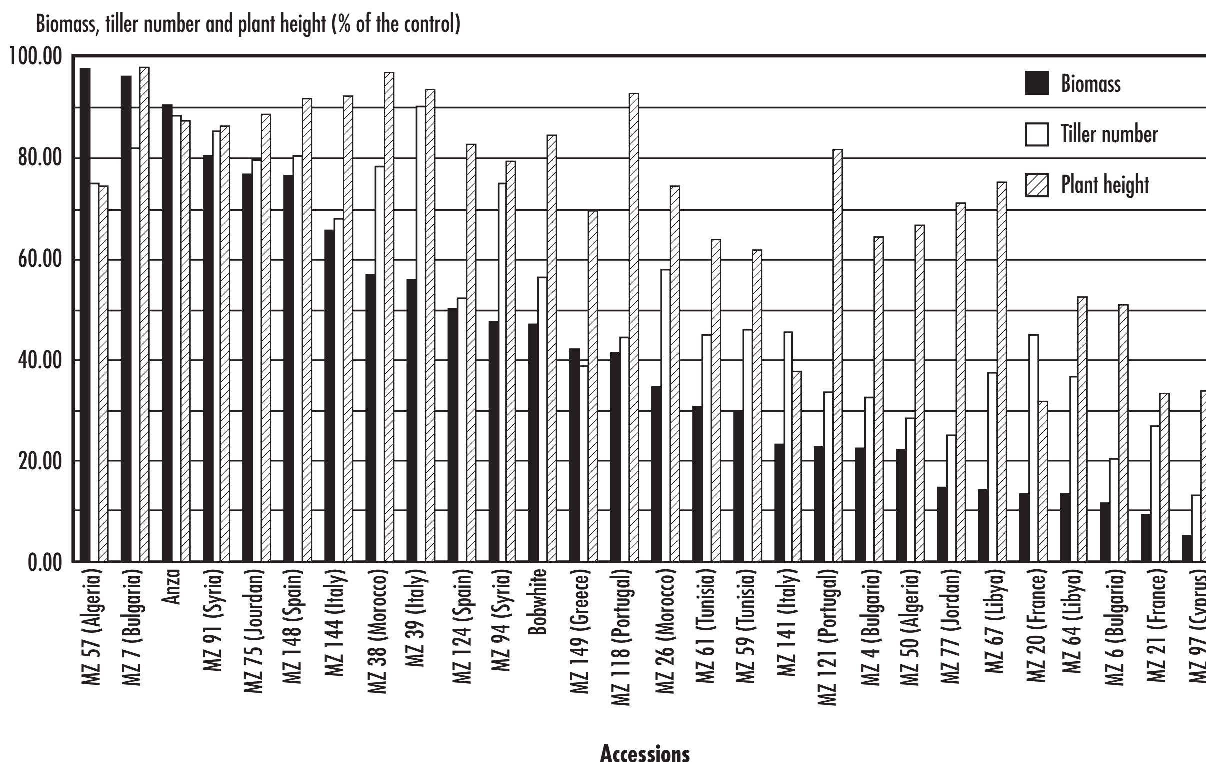


Figure 1. Effect of BYDV infection on biomass, tillering and plant height in *Ae. geniculata* accessions.

the tolerance level. Some non-resistant *Ae. geniculata* accessions had quite good tolerance while the most resistant were severely damaged (Table 2), suggesting a lack of association between resistance and tolerance in this species. Moreover, negative correlation was found between virus titers and tolerance level measured as reduction of biomass, tillering and plant height (r=-0.52**, -0.53** and –0.64***, respectively).

Knowing the strong environmental effect on the expression of tolerance to BYDV, it would be important to confirm these results for at least another cycle.

Table 2. Reduction in biomass production, tiller number and plant height in BYDV infected plants under field conditions in Toluca experimental station during the winter 2001/2002 cycle.

		Reduction (%)		
Accessions	**Number**	**Biomass**	**Tillers**	**Plant height**
Ae. geniculata (moderately resistant)	5	81.4 (58.0-94.9)	66.2 (54.6-86.9)	58.8 (30.4-68.3)
Ae. geniculata (tolerant)	5	14.1 (2.2-23.6)	19.8 (14.7-25.1)	12.6 (2.1-25.5)
Ae. geniculata (all)	26	59.4 (0.2-94.9)	48.4 (9.9-86.9)	29.0 (2.1-68.3)
Anza (tolerant check)	1	9.7	11.7	12.7
Bob White (susceptible check)	1	53.0	43.7	15.6

Ae. geniculata accessions moderate possessing resistance or tolerance traits to BYDV in addition to other abiotic/biotic stresses are presently used in CIMMYT wide hybridization program. They were crossed with susceptible high-yielding bread (Prinia and Baviacora) and durum wheat (Kucuk and Sooty9/Rascon37) cultivars in order to improve BYDV resistance/tolerance into cultivated wheat.

References

Ayala, L., Henry, M., Gonzalez de Leon, D., van Ginkel, M., Mujeeb-Kazi, A., Keller, B., and Khairallah, M. 2001. A diagnostic molecular marker allowing the study of *T. intermedium*-derived resistance to BYDV in bread wheat segregating populations. *Theor Appl Genet* 102:942-949.

Clark, M.F., and Adams, A.N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* 34:475.

Cooper, J.I., and Jones, A.T. 1983. Responses of plants to viruses : proposals for use of terms. *Phytopathology* 73:127-128.

Plumb, R.T. 1983. Barley yellow dwarf virus-a global problem. In: Plumb, R.T., Tresh J.M. (eds.). *Plant Virus Epidemiology.* Blackwell, London, UK.

Singh, R.P., Burnett, P.A., Albarran, M., and Rajaram, S. 1993. *BDV1*: a gene for tolerance to barley yellow dwarf virus in bread wheats. *Crop Science* 33:231-234.

Zaharieva, M., Monneveux, P., Henry, M., Rivoal, R., Valkoun, J., and Nachit, M.M. 2001. Evaluation of a collection of wild wheat relative *Aegilops geniculata* Roth and identification of potential sources for useful traits. *Euphytica* 119:33-38.

Development of 'Thelin' Wheat Lines with Genes *Lr19* and *Bdv2* from *Thinopyrum elongatum* and *Th. intermedium*

R.P. Singh, M. Henry, J. Huerta-Espino, A. Mujeeb-Kazi, and R.J. Peña

CIMMYT, Mexico, D.F., Mexico

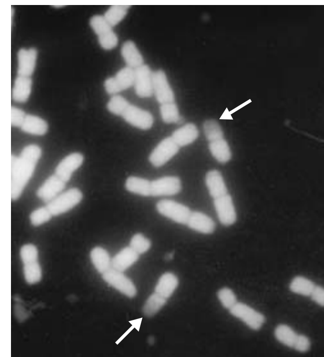
Leaf rust (caused by *Puccinia triticina*) and barley yellow dwarf (BYD) (caused by *Barley yellow dwarf virus*, BYDV) are important diseases of wheat in several wheat growing regions. Genetic resistance offers the most economical and environmentally safe control measure.

Sharma and Knott (1966) transferred a chromosome segment from *Thinopyrum elongatum* to chromosome 7DL of wheat (Figure 1a). This segment carries leaf rust resistance gene *Lr19*, which has had limited use in wheat improvement due to its linkage with a gene that causes yellowness of wheat flour. In a recent study Singh *et al*. (1998) found that the presence of this alien segment increases wheat grain yield by about 10%.

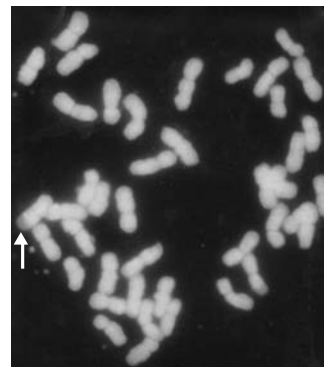
Using tissue culture, Banks *et al*. (1995) transferred a chromosome segment carrying BYDV resistance from *Th. intermedium* to wheat and obtained eight lines, commonly referred to as TC lines. The *Th. intermedium* fragment carried the only known BYDV resistance gene named *Bdv2*. Among TC lines, TC14 carries the smallest translocation that replaces the terminal part of wheat chromosome 7DL (Figure 1b).

The objective of our work was to recombine the two alien chromosome segments in a wheat background to identify recombinants that combine genes *Lr19* and *Bdv2*, and lack the gene for yellowness of flour. Status of the gene that enhances grain yield potential was also studied.

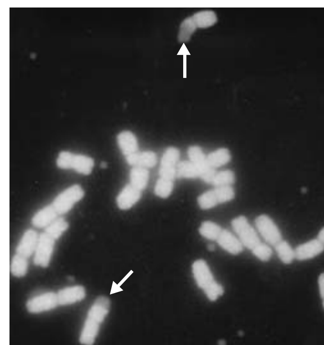
Figure 1. Fluorescent *in situ* hybridization (FISH) detail in partial mitotic cells of:



a) *Triticum aestivum* cv. Oasis 86 with *Lr19;*



b) *Triticum aestivum* cv. TC14/2*Spear with *Bdv2;*



c) Oasis 86//TC14/2*Spear with *Lr19* and *Bdv2.* Wheat DNA was biotin labeled and *Thinopyrum bessarabicum* DNA was used for blocking.

Materials and Methods

Two wheat varieties, 'Oasis 86' and 'Super Seri #2', carrying gene *Lr19*, were crossed with two other varieties, 'TC14/2*Spear' and 'TC14/2*Hartog' carrying *Bdv2*. Chromosome pairing was studied in meiosis of the F_1 plants. By testing with an *Lr19*-avirulent race of *P. triticina*, 118 individual F_2 plants derived F_3 lines from each of the four crosses were evaluated for homozygosity for *Lr19*.

The *Lr19* homozygous lines were evaluated for endosperm or flour yellowness by two methods: 1) visual evaluation of endosperm yellowness by cutting the seed in half, and 2) flour color determination using Minolta Color Meter, where "b" values were recorded. Acceptable "b" values are 8-12, while unacceptable (yellow) "b" values are 15-20. Lines showing non-yellow endosperm and flour were advanced to the F_5 generation by harvesting individual plants in the F_4 generation that showed good agronomic features.

The F_1 plants from the two crosses involving Oasis 86 described above were top-crossed with 'Yecora+*Lr34*', whereas the remaining two F_1s were top-crossed with 'Seri.1B'. Yecora+*Lr34* and Seri.1B are very similar to Oasis 86 and Super Seri#2 but do not carry any alien chromosome translocation. The top-crossed seedlings were first tested for resistance to PAV-Mex isolate of BYDV; plants with low virus titers in ELISA were retained, and then tested for the presence of *Lr19*-based resistance to leaf rust. Only those plants considered resistant to both diseases were grown and harvested. The leaf rust resistant F_2 progenies of these plants were advanced to F_3 and lines homozygous for gene *Lr19* were identified for further work as described above for the F_3 lines from simple crosses.

Cytological procedures for meiosis and fluorescent *in situ* hybridization (FISH) were similar to those of Mujeeb-Kazi *et al*. (1994) and Islam-Faridi and Mujeeb-Kazi (1995), respectively.

From 21 F_4 lines (representing at least 21 recombination events), 235 individual F_5 plants were selected that were homozygous for *Lr19* and had white endosperm. An SSR marker, *gwm37*, mapping to 7DL and identified to be diagnostic for the *Th. intermedium* translocation (Ayala *et al*., 2001), was used to assess the presence or absence of the translocation. Because of its co-dominant nature, the marker allowed us to differentiate if the alien fragment was present in homozygous (1) or heterozygous (10) state, or whether it was absent (0) (Figure 2). DNA extraction, PCR amplification, and separation of the amplified products on agarose gels were done as described by Ayala *et al*. (2001).

Five 7-day-old seedlings of a total of 41 selected F_5 lines were inoculated with 10 BYDV-PAV viruliferous aphids (*Rhopalosiphum padi)* for a 48-h inoculation period. After spraying with the insecticide Pirimor, plants were grown in the greenhouse for 30 days. Virus titers were assessed by double antibody sandwich ELISA (DAS ELISA) on the flag-1 leaf, 10, 20, and 30 days after inoculation. The test was repeated once. For each repetition a non-infected seedling was tested for each line as a control for ELISA.

Yield performance of a group of selected lines was determined at CIMMYT's research station in Ciudad Obregon in the northwestern Mexico in replicated yield trials. One year yield data for two lines and the parents are presented.

Results and Discussion

Meiotic chromosome pairing in F_1 plants
The presence of 21 chromosome ring bivalents in at least some cells (Table 1) in the F1 plants from the cross Oasis 86//TC14/2*Spear indicated that the two chromosomes with alien translocations paired at metaphase I, suggesting that recombinants could be

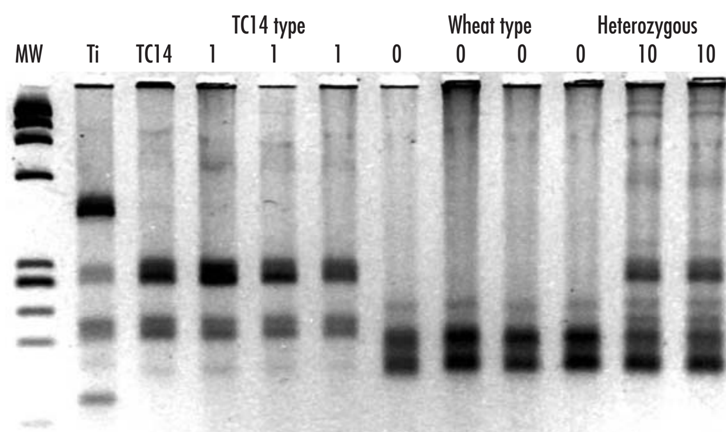


Figure 2. Agarose gel showing differentiation between lines homozygous for *gwm37* (1), heterozygous (10) or not carrying the diagnostic marker (0).

expected. Frequency of 21 ring bivalents in the other three crosses was much lower (data not presented).

Flour yellowness

The two crosses involving Super Seri#2 did not give any *Lr19* homozygous line with white flour. Of the 21 recombinants identified (Table 2), 16 were from the simple cross Oasis 86//TC14/2*Spear, plus 3 more when the above cross was top crossed with Yecora+*Lr34*. The remaining 2 white-floured recombinants were derived from the cross Oasis 86//TC14/2*Hartog/3/Yecora+*Lr34*.

Table 1. Mean meiotic metaphase I chromosomal associations observed in the F1 between two translocations germplasms (Oasis//TC14/2*Spear) with *Lr19* and *Bdv2* genes.

	Metaphase I chromosome association				
No. of cells	Univalent	Ring bivalent	Rod bivalent	Trivalent	Total bivalents
5	0	21	0	0	21
3	0	20	1	0	21
2	2	20	0	0	20
6	0	19	2	0	21
5	0	18	3	0	21
1	2	18	2	0	20
1	4	18	1	0	19
1	1	18	1	1	19
2	0	17	4	0	21
1	4	17	2	0	19
1	1	17	2	1	19
1	3	16	2	1	18
1	0	15	6	0	21

Table 2. Distribution of F_3 lines homozygous for *Lr19* and for flour color in each cross.

Lr19 homozygous F_3 lines (No.)		
Cross	Yellow flour	White flour
Simple:		
Super Seri #2//TC14/2*Hartog	37	0
Super Seri #2//TC14/2* Spear	30	0
Oasis 86//TC14/2*Hartog	28	0
Oasis 86//TC14/2*Spear	15	16
Top:		
Super Seri #2//TC14/2*Hartog/3/Seri.1B	3	0
Super Seri #2//TC14/2*Spear/3/Seri.1B	7	0
Oasis 86//TC14/2*Hartog/3/Yecora+*Lr34*	5	2
Oasis 86//TC14/2*Spear/3/Yecora+*Lr34* = Thelin	6	3

Status of molecular marker *gwm37* and BYDV resistance

Of the 235 F_5 lines (homozygous for *Lr19* and white floured) tested, 121 did not carry *gwm37*, 28 were heterozygous for this marker, and 85 were homozygous. In total, 41 F_5 lines were tested, 19 homozygous for the marker *gwm37* and the remaining 22 lines, not carrying it. All lines that did not carry *gwm37* were susceptible to BYDV (high virus titers in ELISA) (Table 3). Most lines where *gwm37* was present were highly or moderately resistant to BYDV indicating the presence of the *Bdv2* gene. However, in four cases, lines homozygous for *gwm37* were susceptible (high titers). These results suggest that probably recombination also occurred between the molecular marker and the *Bdv2* gene.

Comparison of grain yield potential

One year of yield data indicate that some lines from the simple cross Oasis//TC14/2*Spear and some more from the three-way cross Oasis//TC14/2*Spear/3/Yecora+*Lr34* (named as Thelin) may carry the gene that enhances yield potential. Grain yield results for two selections of Thelin, designated as Thelin#1 and Thelin#2, are given in Table 4.

FISH preparations

Each F_1 combination and several recombined lines where genes *Lr19*, *Bdv2* and *gwm37* were present together and possessed white flour were used for

Table 3. Examples of the F_5 recombinant lines with white flour and carrying *Lr19* (leaf rust resistance) and/or *Bdv2* (BYDV resistance) genes.

Cross	Line number	*gwm37*	BYDV response	IOD[3] – 10 days
Oasis 86//TC14/2*Spear	F5Lr19RG-34	1[1]	Resistant	0.211±0.062
Oasis 86//TC14/2*Spear	F5Lr19RG-74	1	Resistant	0.181±0.055
Oasis//TC14/2*Spear/3/Yecora+*Lr34*	F5Lr19RG -193	1	Resistant	0.216±0.067
Oasis 86//TC14/2*Spear	F5Lr19RG -27	1	Susceptible	0.986±0.127
Oasis//TC14/2*Spear/3/Yecora+*Lr34*	F5Lr19RG -233	1	Susceptible	1.399±0.327
Oasis//TC14/2*Hartog/3/Yecora+*Lr34*	F5Lr19RG -134	1	Susceptible	0.741±0.191
Oasis//TC14/2*Spear	F5Lr19RG-108	0	Susceptible	1.143±0.145
TC14/2*Spear (Check)	F5Lr19RG -237	1	Resistant	0.223±0.077
Oasis 86 (Check)	F5Lr19RG -236	0	Susceptible	0.637±0.132

[1] 1 = Homozygous for diagnostic marker, 0 = not carrying the diagnostic marker.
[2] IOD = average ODs of infected individual assessed by ELISA, 10 days after inoculation.

Table 4. Grain yield performance and flour yellowness of two Thelin selections and the parental checks in northwestern Mexico for one year.

Line	Grain yield kg/ha	% Yecora+*Lr34*	% Oasis 86	Flour yellowness Minolta 'b'
Thelin#1	8,935	112	97	10.1
Thelin#2	8,790	110	96	9.3
Yecora+*Lr34*	8,015	100	87	8.6
Oasis 86	9,170	114	100	14.3
Tc14/2*Spear	6.919	86	76	12.8
L.S.D.	903			

mitotic FISH preparations. The translocations present in this germplasm were characteristic of *Lr19* and *Bdv2* in the F_1 heterozygote (Figure 1c), and appeared to be of similar length as in the TC14 lines in the advanced progeny with white flour.

Conclusions

- The *Th. elongatum* and *Th. intermedium* chromosome segments were recombined successfully.
- Recombined alien segments possessing genes *Lr19*, *Bdv2*, and white flour with or without the molecular marker *gwm37* were identified.
- The recombined translocations could be useful for transferring the *Bdv2* gene using leaf rust resistance as a marker, or vice-versa by using the *gwm37* molecular marker.
- One year of yield results indicate that the gene that enhances yield potential may be present in some white-floured recombinant lines (e.g., Thelin#1 and Thelin#2) together with *Lr19* and *Bdv2*.

References

Ayala, L., Henry, M. , González de León, D., van Ginkel, M., Mujeeb-Kazi, A., Keller, B., and Khairallah, M. 2001. A diagnostic molecular marker allowing the study of *Thinopyrum intermedium* derived resistance to BYDV in bread wheat segregating populations. *Theor. Appl. Genet.* 102:942-949.

Banks, P.M., Larkin, P.J., Bariana, H.S., Lagudah, E.S., Appels, R., Waterhouse, P.M., Brettell, R.I.S., Chen, X., Xu, H.J., Xin, Z.Y., Qian, Y.T., Zhou, X.M., Cheng, Z.M., and Zhou, G.H. 1995. The use of cell culture for subchromosomal introgressions of barley yellow dwarf virus resistance from *Thinopyrum intermedium* to wheat. *Genome* 38:395-405.

Islam-Faridi, M.N., and Mujeeb-Kazi, A. 1995. Visualization of *Secale cereale* DNA in wheat germplasm by fluorescent *in situ* hybridization. *Theor. Appl. Genet.* 90:595-600.

Mujeeb-Kazi, A., Jahan, Q., and A. Vahidy. 1994. Application of a somatic and meiotic cytological technique to diverse plant genera and species in the Triticeae. *Pak. J. Bot.* 26:353-366.

Sharma, D., and D.R. Knott. 1966. The transfer of leaf-rust resistance from *Agropyron* to *Triticum* by irradiation. *Can. J. Genet. Cytol.* 8:137-143.

Singh, R.P., J. Huerta-Espino, S. Rajaram, and J. Crossa. 1998. Agronomic effects from chromosome translocations 7DL.7Ag and 1BL.1RS in spring wheat. *Crop Sci.* 38:27-33.

List of Participants

David C. Abbott
Division of Plant Industry
CSIRO
GPO Box 1600
Canberra, A.C.T. 2601, Australia
Phone: +61 2 6246 4911
Fax: +61 2 6246 5000
Email: David.C.Abbott@csiro.au

Aboulata Elnady Aboulata
Plant Virus and Mycoplasma Res. Sec.
Plant Pathology Research Institute
Agriculture Research Centre
P.O. Box 12619
Giza, Egypt
Phone: +20 2 6327 251
Fax: +20 2 5728 099
Email: aboulata_nady@hotmail.com;
aeaboulata@yahoo.com

Emad M. Al Maaroof
Agricultural and Biological Research Center
IAEC, P.O. Box 765, Twaitha
Baghdad, Iraq
Phone: +964 (1) 7738410
Fax: +964 (1) 718929
Email: bakirmarouf@uruklink.net;
teknologia@uruklink.net

Joseph M. Anderson
Research Molecular Biologist
Department of Agronomy
USDA/Crop Production & Pest Control Research
Purdue University
1150 Lilly Hall
West Lafayette, IN 47907, USA
Phone: +1 (765) 494 5565
Fax: +1 (765) 496 2926
Email: jmanders@purdue.edu

Murali Bandla
Director, Research & Development
Agdia Inc.
30380 County Road 6
Elkhart, IN 46514, USA
Phone: +1 (574) 264-2014
Fax: +1 (574) 264-2153
Email: mbandla@agdia.com

Bouchaib Bencharki
Faculté des Sciences et Techniques
Université Hassan 1er
P.O. Box 577
26000 Settat, Morocco
Phone: +212 23 40 07 36
Fax: +212 23 40 09 69
Email: bbencharki@hotmail.com;
benchark@uh1.ac.ma;
benchark@ibnsina.uhl.ac.ma;
bencharki2003@yahoo.fr

Maris Bisnieks
Latvia University of Agriculture
Liela iela 2
Jelgava LV 3001, Latvia
Phone: +371 30 21998
Fax: +371 30 7820607
Email: maris.bisnieks@evp.slu.se;
iturka@cs.llu.lv

Kira L. Bowen
Dept. Entomology and Plant Pathology
Auburn University
Auburn, AL 36849, USA
Phone: +1 (334) 844 1953
Fax: +1 (334) 844 5002
Email: kbowen@acesag.auburn.edu

Flavio Capettini
Head, Barley Program,
Latin American Regional Program
ICARDA c/o CIMMYT
Apdo. Postal 6-641
06600 Mexico, D.F., Mexico
Phone: +52 (55) 5804 7505; 5804 2004 ext. 1176
Fax: +52 (55) 5804 7558/59
Email: f.capettini@cgiar.org

Florian Chain
INRA/UMR BiO3P
BP 35627 Domaine de la Motte
35653 Le Rheu Cedex, France
Phone: +33 (2) 23 48 58 09
Fax: +33 (2) 23 48 51 80
Email: chain@rennes.inra.fr

Catherine Chay
Ceregen, Monsanto Co.
700 Chesterfield Village Pkwy
Chesterfield, MO 63198, USA
Phone: +1 (636) 737 6067
Fax: +1 (636) 737 7670
Email: Catherine.a.chay@monsanto.com

Cheng Zhuomin
Institute of Plant Protection
Chinese Academy of Agricultural Sciences
Beijing 100094, China
Phone: +86 (10) 62815906
Fax: +86 (10) 62890875
Email: zmcheng@95777.com;
zmcheng@public.east.cn.net

André I. Comeau
Centre de Recherches Agriculture and Agri Food Canada
2560 Hochelaga Bldg.
Sainte-Foy, QC G1V 2J3, Canada
Phone: +1 (418) 657 7980
Fax: +1 (418) 648 2402
Email: comeaua@agr.gc.ca

C.A. Dedryver
INRA/ENSAR UMR BiO3P
BP 35327 Domaine de la Motte
F-35653 Le Rheu Cedex, France
Phone: +33 (2) 23 485 151
Fax: +33 (2) 23 485 150
Email: cdedryve@rennes.inra.fr

Patricia Dupré
Wheat Program, CIMMYT
Apdo. Postal 6-641
06600 Mexico, D.F., Mexico
Phone: +52 (55) 5804 2004 ext. 2220
Fax: +52 (55) 5804 7558/59
Email: pdupre@cimmyt.exch.cgiar.org

Fréderic Fabre
INRA/UMR BiO 3P
BP 35327 Domaine de la Motte
35653 Le Rheu Cedex, France
Phone: +33 (2) 23 48 58 08
Fax: +33 (2) 23 48 51 80
Email: fabre@rennes.inra.fr

Kathy Flanders
Auburn University
208A Extension Hall
Auburn 36849-5629, AL, USA
Phone: +1(334) 844-6393
Fax: +1(334) 844-5002
Email: kflander@acesag.auburn.edu

Antje Habekuss
Federal Centre for Breeding Research on Cultivated Plants
Institute for Epidemiology and Resistance
Theodor-Roemer-Weg 4
D-06449 Aschersleben, Germany
Phone: +49 3473 879162
Fax: +49 3473 2709
Email: A.Habekuss@bafz.de

Richard Harrington
Plant and Invertebrate Ecology Division
IACR/Rothamsted
Harpenden, Herts AL5 2JG, UK
Phone: +44 (1582) 763 133 ext. 2452
Fax: +44 (1582) 760 981
Email: richard.harrington@bbsrc.ac.uk

Monique Henry
Wheat Program, CIMMYT
Apdo. Postal 6-641
06600 Mexico, D.F., Mexico
Phone: +52 (55) 5804 2004 ext. 2217
Fax: +52 (55) 5804 7558/79
Email: m.henry@cgiar.org

Julio Huerta
Campo Experimental Valle de México-INIFAP
Apdo. Postal 10
56230 Chapingo, Edo. de Méx., Mexico
Phone: +52 595 9542877
Fax: +52 595 9542277
Email: j.huerta@cgiar.org

Hwang Jong-Jin
CIMMYT
Apdo. Postal 6-641
06600 Mexico, D.F., Mexico
Phone: +52 (55) 5804 2004
Fax: +52 (55) 5804 7558/59
Email: jhwang@cimmyt.exch.cgiar.org

Emmanuel Jacquot
INRA/UMR BiO3P
BP 35327 Domaine de la Motte
35653 Le Rheu Cedex, France
Phone: +33 (2) 23 48 58 17
Fax: +33 (2) 23 48 51 80
Email: jacquot@rennes.inra.fr

Zarifa Kadirova
Genetics and Plant Experimental Biology
Institute of Genetics and Experimental Biology
Academy of Science of Uzbekistan
Yunus-obod 5-3A-27
Tashkent 700093, Uzbekistan
Phone: + 998 3712 64 22 30
Fax: + 998 3712 64 22 30
Email: Zarifakadirova@yahoo.com;
saidmurot@yahoo.com;
sai-baboev@yandex.ru

Kang Moon-Seok
Wheat and Barley Division
National Crop Experiment Station, RDA
209 Seodun-dong, Gwonseon-gu, Suwon Si
Gyeonggi-Do, 441-100, South Korea
Phone: +82 (31) 290-6682
Fax: +82 (31) 295-5410
Email: gr27@rda.go.kr

Mujeeb Kazi
Wheat Program, CIMMYT
Apdo. Postal 6-641
06600 Mexico, D.F., Mexico
Phone: +52 (55) 5804 2004 ext. 1336
Fax: +52 (55) 5804 7558/79
Email: m.kazi@cgiar.org

Tatyana Kastalyeva
Russian Research Institute of Phytopathology
VNIIF, Bolshie Vyaziomy
143050 Moscow Region, Russian Federation
Phone: +7 (095) 592 92 87
Fax: +7 (095) 334 09 02
Email: mozhaeva@vniif.rosmail.com

Brendon James King
University of Adelaide, Waite Campus
Glen Osmond, South Australia 5064
Australia
Phone: +61 83036501
Fax: +61 83037109
Email: brendon.king@adelaide.edu.au

Miriam Kinyua
Director
National Plant Breeding Research Center
Kenyan Agricultural Research Institute
P.O. Private Bag
Njoro, Kenya
Phone: +254 (37) 61 120/61528
Fax: +254 (37) 61 576
Email: mgkinyua@africaonline.co.ke

Kwon Yil-chan
Yeongnam Agricultural Experiment Station
1085 Naidong
Milyang City, Gyeungnam 627-130
South Korea
Phone: +82 (55) 350 1225
Fax: +82 (55) 352 3059
Email: kwonic@rda.go.kr

Philip Larkin
Subprogram Leader
Division of Plant Industry
CSIRO
P.O. Box 1600
Canberra, ACT 2601, Australia
Phone: +61 2 6246-5060
Fax: +61 2 6246-5000
Email: Philip.Larkin@csiro.au

Khaled M. Makkouk
Acting Leader
Germplasm Program, ICARDA
P.O. Box 5466
Aleppo, Syria
Phone: +963 (21) 2213-433; 2213-477;
IVDN: +1 (650) 833-6680 (USA Direct)
Fax: +963 (21) 2213-490; 5744-622
IVDN: +1 (650) 833-6681 (USA Direct)
Email: k.makkouk@cgiar.org

Ricardo Guilherme Matzenbacher
Fundacep-Fecotrigo
Rs 342, Km. 114, Caixa Postal 10
Cruz Alta , RS, Brazil
Phone: +55 3322 7900
Fax: +55 3322 7900
Email: fundacep@comnet.com.br

Cesar Antonio Michel
Fundacep-Fecotrigo
RS 342, Km 114, Caixa Postal 10
Cruz Alta , RS, Brazil
Phone: +55 3322 7900
Fax: +55 3322 7900
Email: fundacep@comnet.com.br

W. Allen Miller
Plant Pathology Department
Iowa State University
Ames, IA 50011-1020, USA
Phone: +1 (515) 294 2436
Fax: +1 (515) 294 9420
Email: wamiller@iastate.edu

Karina Mozhaeva
Russian Research Institute of Phytopathology
VNIIF, Bolshie Vyaziomy
143050, Moscow Region
Russian Federation
Phone: +7 (095) 592 92 87
Fax: +7 (095) 334 09 02
Email: mozhaeva@vniif.rosmail.com

Herbert Ohm
Department of Agronomy
Purdue University
1150 Lilly Hall of Life Sciences
West Lafayette, IN 47907-1150, USA
Phone: +1 (765) 494 8072
Fax: +1 (765) 494-6805
Email: hohm@purdue.edu

Jaroslava Ovesna
Department Head
Research Institute of Crop Production
Drnovska 507
CZ-16106 Prague, Ruzyne 6
Czech Republic
Phone: +420 2033 022 424
Fax: +420 2033 022 286
Email: ovesna@vurv.cz

Jong-Chul Park
Barley Breeding Lab
National Honam Agricultural Experiment Station
381, Songhag-dong
Iksan-Chonbuk, 570-080
South Korea
Phone: +82 (63) 840 2244
Fax: +82 (63) 840 2112
Email: pacc43@rda.go.kr

Tae Il Park
National Honam Agricultural Experimental Station
381, Songhag-dong
Iksan-Chonbuk 570-080
South Korea
Phone: +82 (63) 840 2239
E-mail: parktl@rda.go.kr

Emil Pocsai
Head of Department
Laboratory of Virology
Plant Protection and Soil Conservation
Fejér County Service
Ország út 230
H-2481 Velence, Hungary
Phone: +36 22 589 225
Fax: +36 22 589 211
Email: pocsai@fmnta.hu

Gabriel Posadas
Wheat Program, CIMMYT
Apdo. Postal 6-641
06600 Mexico, D.F., Mexico
Phone: +52 (55) 5804 2004 ext. 2220
Fax: +52 (55) 5804 7558/59
Email: gposadas@cimmyt.exch.cgiar.org

Calvin O. Qualset
Director
Genetic Resources Conservation Program
Division of Agriculture and Natural Resources
University of California Davis
One Shields Avenue
Davis, CA 95616-8602, USA
Phone: +1 (530) 754-8502
Fax: +1 (530) 754-8505
Email: coqualset@ucdavis.edu

Sanjaya Rajaram
Director, Wheat Program
CIMMYT
Apdo. Postal 6-641
06600 Mexico, D.F. Mexico
Phone: +52 (55) 5804 2004 ext. 1145
Fax: +52 (55) 5804 7558/7559
Email: s.rajaram@cgiar.org

Edgar Schliephake
Federal Centre for Breeding Research on
Cultivated Plants
Institute of Epidemiology and Resistance
Theodor-Roemer-Weg 4
D-06449 Aschersleben, Germany
Phone: +49 3473 879148
Fax: +49 3473 2709
Email: e.schliephake@bafz.de

Bent Skovmand
Wheat Program, CIMMYT
Apdo. Postal 6-641
06600 Mexico, D.F., Mexico
Phone: +52 (55) 5804 2004 ext. 2226
Fax: +52 (55) 5804 7558/79
Email: b.skovmand@cgiar.org

Peter Sforza
Virginia Tech
418 Price Hall
Blacksburg, VA 24061-0331, USA
Phone: +1 (540) 231 1867
Fax: +1 (540) 231 7477
Email: sforza@vt.edu

Roland Sigvald
Department of Ecology and Crop Production
Science
Swedish University of Agricultural Sciences
P.O. Box 7043
SE-75007 Uppsala, Sweden
Phone: +46 18 672366
Fax: +46 18 672890
Email: roland.sigvald@evp.slu.se

Ravi P. Singh
Wheat Program, CIMMYT
Apdo. Postal 6-641
06600 Mexico, D.F., Mexico
Phone: +52 (55) 5804 2004 ext. 2221
Fax: +52 (55) 5804 7558/9
Email: r.singh@cgiar.org

Graciela Ana María Truol
Instituto de Fitovirología y Fisiología Vegetal
Instituto Nacional de Tecnología Agropecuaria
Camino a 60 Cuadras -Km 5Ω
C.C. 70-Barrio Colon
5119 Cordoba, Argentina
Phone: +54 (351) 5 973636/4343
Fax: +54 (351) 4974 330
Email: gtruol@correo.inta.gov.ar

Maarten van Ginkel
Wheat Program, CIMMYT
Apdo. Postal 6-641
06600 Mexico, D.F., Mexico
Phone: +52 (55) 5804 2004 ext. 1135
Fax: +52 (55) 5804 7558/7559
Email: m.van-ginkel@cgiar.org

Vidal Velasco
Bioagricultural Science
Colorado State University
Fort Collins, CO 80523-1177, USA
Phone: +1 (970) 491 6407
Fax: +1 (970) 491 2804
Email: vvelasco@lamar.colostate.edu

Aude Vialatte
INRA/UMR BiO3P
BP 35327 Domaine de la Motte
35653 Le Rheu Cedex, France
Phone: +33 (2) 23 48 57 15
Fax: +33 (2) 23 48 51 80
Email: vialatte@rennes.inra.fr

Wang Daowen
Institute of Genetics and
Developmental Biology
Chinese Academy of Sciences
Beijing 100101, China
Phone: +86 (10) 64 889380
Fax: +86 (10) 64 854467
Email: dwwang@genetics.ac.cn

Wang Xifeng
Professor and Group Leader
Institute of Plant Protection
Chinese Academy of Agricultural Sciences
Yuan Ming Yuan Xi Lu #2
Beijing 100094, China
Phone: +86 (10) 6281 5928
Fax: +86 (10) 6289 6114
Email: xfwang319@yahoo.com.cn

Hoda Waziri
Plant Virus Department
Agric. Research Center
Plant Pathology Institute
9 El Gamaa St.
Cairo, Egypt
Phone: +202 5695077; 5692642
Fax: +202 3363582; 3372881
Email: h_waziri@yahoo.com

Xin Zhiyong
Director General
Institute of Crop Breeding and Cultivation
Chinese Academy of Agricultural Sciences
12 Zhongguancun South Street
Beijing 100 081, China
Phone: +86 (10) 6891-9718
Fax: +86 (10) 6897-5212
Email: zyxin@public3.bta.net.cn;
Xzy-42@163.com;
zwskyc202@mail.caas.net.cn

Maria Zaharieva
Wheat Program, CIMMYT
Apdo. Postal 6-641
06600 Mexico DF, Mexico
Phone: +52 (55) 5804 2004 ext.1137
Fax: +52 (55) 5804 7558
E-mail: m.zaharieva@cgiar.org

Zhang Zengyan
Institute of Crop Breeding and Cultivation
Chinese Academy of Agricultural Sciences
12 Zhongguancun South Street
Beijing 100081, China
Phone: +86 (10) 6891-8781
Fax: +86 (10) 6897-5212
Email: zyzh-68@163.com;
zengyanzhang@yahoo.com