

Marker-Assisted Selection for BYDV Resistance in Wheat

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Barley yellow dwarf (BYD) is the most important viral disease of cereals. It has a worldwide distribution and infects a wide range of gramineae, including the major cereal crops. The disease is caused by five insect-transmitted luteoviruses, collectively known as barley yellow dwarf virus. The serotypes are PAV, MAV, RPV, RMV, and SGV (Waterhouse et al., 1988). PAV is the most severe and most common serotype, followed in occurrence by MAV and RPV.

Control of the disease can be partially achieved through the application of insecticides, cultural practices (such as changes in sowing date, alternate cropping, and removal of virus reservoirs), and the use of germplasm with tolerance or resistance to the virus or its vectors. In the sense of Cooper and Jones (1983), tolerant lines present attenuated symptoms and lower yield losses even though they multiply the virus. In resistant lines, virus multiplication and spread are inhibited or reduced, which may or may not attenuate symptoms. Tolerance to BYD in bread wheat has been reviewed by Burnett et al. (1995). Though not present in bread wheat, true resistance has been identified in several wheat relatives, such as *Thinopyrum intermedium*. Banks et al. (1995) successfully transferred this alien-derived resistance to bread wheat and produced a series of translocated lines (so-called TC lines) using tissue culture.

Previous observations showed that lines in the TC14 group had the most potential for wheat breeding because they showed low virus concentrations after infection with BYDV-PAV, -MAV, or -RPV (Henry, 1997) and carried the smallest translocation (Hohmann et al., 1996). However, though resistant, these lines were poorly adapted to the Mexican environment and sensitive to the infection; they

would thus exhibit severe symptoms in the field when infected (Henry, 1997). In light of these findings and to achieve better BYDV control, our strategy has been to combine the alien-derived resistance with tolerance in good agronomic backgrounds.

Resistance induced by the *Th. intermedium* translocation was found to be associated with a reduction in virus titers of BYDV-PAV under field and greenhouse conditions and a lower infection rate when artificially inoculated with BYDV-PAV or MAV in the field (Ayala et al., 2001). A reduction in virus titers was also observed with BYDV-MAV and RPV under greenhouse conditions (Henry, unpublished data).

At CIMMYT, screening for BYD has been based mainly on observation of symptoms after natural or artificial inoculation and has focused on identifying tolerance to the disease. Screening for resistance is laborious, involving artificial inoculation in the field or greenhouse and measuring virus titers by ELISA (enzyme-linked immunosorbent assay); to combine resistance with tolerance, lines are screened for low symptoms under infection. To complicate matter even more, expression of tolerance and resistance is strongly influenced by environmental conditions.

To overcome some of the constraints of testing for BYD tolerance and resistance, a search for molecular markers for these traits was undertaken. An SSR (simple sequence repeat) marker, *gwm37*, identified by Ayala et al. (2001), shows polymorphism between resistant TC14 lines and bread wheat. It is a diagnostic co-dominant marker that differentiates individuals possessing the *Th. intermedium* introgression (Ti) in homozygosity or heterozygosity from those not carrying it. Tolerance proved to be

controlled by several QTLs with small effects, for which no diagnostic markers could be identified (Ayala et al., unpublished data). In this paper, we report the use of *gwm37* in a marker-assisted selection strategy to incorporate *Th. intermedium*-derived resistance into high yielding bread wheats. The strategy involved selecting tolerant progeny for BYD resistance as a way of overcoming some of the limitations of the resistant but sensitive TC14 parents.

Materials and Methods

Selection strategies

Resistant lines TC14/2*Spear and TC14/2*Hartog (CSIRO 289B, 289X), kindly provided by Philip Banks, CSIRO, Australia, were crossed to about 50 advanced CIMMYT wheats representing materials adapted to irrigated, high rainfall, and/or drought environments. The materials were shuttled between a coastal irrigated site, Cd. Obregon in northwestern Mexico, and a high rainfall location near Toluca, in the central highlands of Mexico. Diseases prevalent in the two sites are different, and include leaf, stripe, and stem rusts, *Septoria tritici*, fusarium head blight, and a complex of soil-borne diseases. The best F2 plants within a cross were harvested in bulk. Selection criteria included good agronomic type, durable disease resistance, synchronous tillering, desired spike type and size, good fertility, appropriate height and maturity, as well as well-filled grains. Within the F3 and F5 plots, the best plants were selected, harvested, threshed in bulk, and visually checked for grain characteristics.

In the F4 and F6 generations, individual plants or lines were grown in Toluca under high natural disease pressure. They also underwent selection with artificial BYDV-PAV (Mexican isolate) inoculation in El Batán, also in the central Mexican highlands. Outstanding plants were visually selected at heading based on reduced or no BYD symptoms. The presence of the introgression (resistance allele) was then assessed using the SSR molecular marker *gwm37*. Resistant plants or lines were confirmed by measuring virus titers using ELISA.

BYDV testing

BYDV testing was carried out during the summer cycle at CIMMYT, El Batán, Mexico, in June 1999 and 2000. Seedlings were inoculated at the 3-leaf stage (Zadoks'13, Zadoks et al., 1974). The F4s were space planted in a 4 x 5 m plot with double rows, while the F6s were sown in paired 1-meter double plots, one being infected and the other kept free of aphids through regular insecticide application.

ELISA (enzyme-linked immunosorbent assay)

Double antibody sandwich ELISA (DAS ELISA) was used as described by Ayala et al. (2001). Optical density (OD) was measured at 405 nm using a MR 700 Microplate reader (Dynatech Laboratories). A plant was considered infected when the OD was higher than twice the OD of the non-infected control. ELISA values were classified as follows: Low: $OD < 0.25$, Moderate: $0.25 < OD < 0.4$, High: $OD > 0.4$. In testing the F4s, the average OD value obtained for the resistant TC14/2*Spear was 0.131 ± 0.098 .

Determining the presence of the translocation

The presence of the translocation was assessed using the SSR marker as described in Ayala et al. (2001). Plants or lines were classified as homozygous resistant (TiTi), susceptible (titi) or heterozygous (Titi).

Results and Discussion

Four of the sixteen populations advanced to F4 were discarded after field evaluation in Toluca because of their high sensitivity to yellow rust. In El Batán, from the 12 remaining populations, 479 plants were selected based on their good appearance after BYDV inoculation. This group included plants not presenting any BYD-like symptoms and appeared to have escaped infection. As reported previously, this characteristic is also associated with *Th. intermedium* resistance (Ayala et al., 2001; Henry and Segura, 1999).

The range of OD values obtained in the selected lines with low symptoms levels was high (0.079-1.158), indicating that some plants were good hosts for the virus without presenting severe symptoms and thus could be qualified as tolerant.

PCR analysis was completed on 403 of the 479 plants selected. Of those, 34.5% were homozygous for the translocation (TiTi), 17.4% heterozygous (Titi) and 48.1% did not carry the fragment at all (titi).

A high proportion of the non-infected lines were either homozygous or heterozygous for the *Th. intermedium* fragment confirming the effect of the translocation on the incidence of infection (Table 1). Though the virus titers were distributed in three classes (low, medium or high), there was a tendency for the homozygous lines (TiTi) to have low or medium ODs, while the lines without the translocation (titi) had virus titers in the medium or higher classes.

Lines with reduced symptoms, low virus titers, or no infection, and shown to be homozygous for the translocation were selected and advanced to the F5. In total 156 lines were selected and tested for good agronomic characteristics in Obregon. Fifty-eight lines were advanced to the F6. In the F6, 10 were finally selected as having BYDV resistance (homozygous for the translocation, low virus titers) and some level of tolerance. Their resistance/ tolerance will be re-evaluated for an additional season.

In Toluca, selection among F4 populations was based on good agronomic characteristics, resistance

to stripe rust, and some level of BYDV tolerance. One hundred and ninety-six plants were selected from the 12 crosses mentioned above. The distribution of selected plants in the three genotypic groups was different from the one obtained in El Batán. There was a higher proportion of heterozygous individuals in Toluca (41.8%) than in El Batán (14.8%), and a lower proportion of individuals not carrying the translocation in Toluca (9.7%) than in El Batán (40.5%). This indicates that during initial selection for BYD tolerance, a high proportion of plants not carrying the translocation were chosen, possibly because the translocation is not associated with tolerance.

A total of 177 plants (missing data, TiTi, and Titi) selected in Toluca were advanced to the F5. Forty lines were tested as F6s and nine were finally selected as carrying both resistance and tolerance to BYDV.

The data suggest that selection based on the presence of the translocation as detected with the molecular marker *gwm37* can be used in early generations, thus avoiding the need for special field screening. This should be followed by one or two cycles of testing under BYDV infection and estimation of virus titers to make sure resistance is still expressed. Final evaluation of true resistance as expressed in reduced virus titers can also be done in the greenhouse. However, to combine resistance and tolerance, screening under BYD pressure is recommended at least every other cycle. In addition, because our task at CIMMYT is to provide germplasm adapted to different

Table 1. Distribution of selected F4 individuals among genotypic groups (based on *gwm37*) and classes of virus titers under BYDV pressure in El Batán.

Virus titer	Percentage of individuals			
	Homo-zygous (TiTi)	Hetero-zygous (Titi)	No translocation (titi)	Missing data
Not infected	28.8	8.6	2.6	4.0
Low	41.7	37.1	27.3	37.3
Medium	21.6	42.9	40.2	37.3
High	7.9	11.4	29.9	21.3

Table 2. Distribution of selected F4 individuals in three genotypic groups (based on *gwm37*) under selection with (El Batán) or without BYDV pressure (Toluca).

Genotypic groups	Percentage of individuals	
	El Batán	Toluca
Homozygous resistant (TiTi)	29.0	17.3
Heterozygous (Titi)	14.8	41.8
Homozygous susceptible (titi)	40.5	9.7
Missing data	15.7	31.1

environments with strong disease pressure, in particular rusts, it is important to alternate screening under BYD pressure with other diseases, such as leaf and stem rusts (Cd. Obregon) and yellow rust (Toluca).

We have obtained a set of 19 lines (Table 3) combining BYDV resistance and (apparently) a certain level of tolerance. Tolerance to BYD in wheat is polygenic in nature, based on the action of minor genes. It is more important to have uniform infection when selecting for minor gene resistance than for major gene resistance (Qualset, 1984). In crosses with *Th. intermedium*-derived material, escape from infection is more common than in susceptible wheat, resulting in non-uniform infection. A sensitive plant might be rated as tolerant if it did not get infected. To minimize the error due to the escape mechanism and because BYD expression is strongly associated with the environment, it is important to confirm the tolerance identified in this work through another cycle of testing.

The molecular marker *gwm37* has proven to be a reliable tool for incorporating BYDV resistance, accelerating the process, and reducing the need

for continuous testing under BYDV infection. This marker has been used successfully in selecting other populations, such as backcrosses with tolerant BYD germplasm.

Table 3. List of F6 lines carrying both resistance and tolerance to BYDV.

Pedigree	Selection history
TC14/2*HTG//HUITES	CMSS97M00087S-030M-020Y-10BYB-010Y
TC14/2*HTG//HUITES	CMSS97M00087S-030M-020Y-11M-010Y
TC14/2*HTG//HUITES	CMSS97M00087S-030M-020Y-13BYB-010Y
TC14/2*HTG//HUITES	CMSS97M00087S-030M-020Y-17M-010Y
TC14/2*HTG//HUITES	CMSS97M00087S-030M-020Y-3BYB-010Y
TC14/2*HTG//HUITES	CMSS97M00087S-030M-020Y-5BYB-010Y
TC14/2*HTG//HUITES	CMSS97M00115S-030M-020Y-22BYB-010Y
TC14/2*HTG//TRAP#1/BOW	CMSS97M00095S-030M-020Y-12M-010Y
TC14/2*HTG/3/VEE/PJN//2*TUI	CMSS97M00089S-030M-020Y-10M-010Y
TC14/2*HTG/3/VEE/PJN//2*TUI	CMSS97M00089S-030M-020Y-1BYB-010Y
TC14/2*HTG/3/VEE/PJN//2*TUI	CMSS97M00089S-030M-020Y-5BYB-010Y
TC14/2*SPEAR//HUITES	CMSS97M00115S-030M-020Y-16BYB-010Y
TC14/2*SPEAR//MILAN	CMSS97M00168S-030M-020Y-12M-010Y
TC14/2*SPEAR//MILAN	CMSS97M00168S-030M-020Y-16M-010Y
TC14/2*SPEAR//MILAN	CMSS97M00168S-030M-020Y-1BYB-010Y
TC14/2*SPEAR//MILAN	CMSS97M00168S-030M-020Y-3BYB-010Y
TC14/2*SPEAR/3/BOW/URES//KEA	CMSS97M00151S-030M-020Y-10M-010Y
TC14/2*SPEAR/3/BOW/URES//KEA	CMSS97M00151S-030M-020Y-14M-010Y
TC14/2*SPEAR/3/BOW/URES//KEA	CMSS97M00151S-030M-020Y-1M-010Y

References

- Ayala, L., Khairallah, M., González-de-Leon, D., van Ginkel, M., Mujeeb-Kazi, A., Keller, B., and Henry, M. 2001. Identification and use of molecular markers to detect barley yellow dwarf virus resistance derived from *Th. intermedium* in bread wheat. *Theoretical and Applied Genetics* (in press).
- 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.
- 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., Burnett, P.A. (eds.). St. Paul, MN, USA: APS Press. pp. 321-343.
- Cooper, J.I., and Jones, A.T. 1983. Responses of plants to viruses: Proposals for the use of terms. *Phytopathology* 73:127-128.
- Henry, M. 1997. Evaluation of resistance to BYDV in *Thinopyrum intermedium* translocated lines. In: *Barley Yellow Dwarf Newsletter*. No. 6. Henry, M. (ed.). Mexico, D.F.: CIMMYT. p 8.
- Henry, M., and Segura, J. 1999. Estimation of yield losses due to BYDV in wheat under artificial inoculation. *Phytopathology* 89:S33.
- 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.
- Qualset, C.O. 1984. Evaluation and breeding methods for barley yellow dwarf resistance. In: *Barley Yellow Dwarf*. A Proceedings of the Workshop. P. Burnett (ed.). Mexico, D.F.: CIMMYT. pp. 72-82.
- Waterhouse, P.M., Gildow, F.E., and Johnstone, G.R. 1988. Luteovirus group. C.M.I./A.A.B. Description of plant viruses 339.
- Zadoks, J.C., Chang, T.T., and Konzak, C.F. 1974. A decimal code for the growth stages of cereals. *Weed Research* 14:415-421.