

# Genetic Association of Gene *Bdv1* for Tolerance to Barley Yellow Dwarf Virus with Genes *Lr34* and *Yr18* for Adult Plant Resistance to Rusts in Bread Wheat

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## ABSTRACT

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Genetic association between the gene *Bdv1*, which confers slow yellowing to barley yellow dwarf (BYD) in adult plants, and genes *Lr34* and *Yr18*, which confer partial adult plant resistance to leaf rust and stripe rust, respectively, was investigated. Nearly 115 random F<sub>3</sub> and F<sub>5</sub> lines, derived from the crosses of susceptible cultivar Jupateco 73S with resistant (symptomatically tolerant for BYD) cultivars Jupateco 73R and Condor, and the parents were evaluated for reaction to the three diseases in separate experiments. Resistance to each disease in Jupateco 73R and Condor is conferred by a single gene. Using a joint classification of the F<sub>3</sub> and F<sub>5</sub> lines for the three diseases indicated that genes *Lr34*, *Yr18*, and *Bdv1* are either very closely linked or under pleiotropic genetic control. Because *Lr34* is known to be located on the long arm of chromosome 7D, it was concluded that *Bdv1* is also located on the same chromosome arm. It is suggested that Condor, Jupateco 73R, or Anza be used as a tester genotype for *Bdv1*, as well as for *Lr34* and *Yr18*. The three genes are common in the wheat germ plasm of the International Maize and Wheat Improvement Center.

Additional keywords: *Triticum aestivum*

Barley yellow dwarf (BYD) is the most economically important and widespread virus disease of small grains in the world, and the most common fungal diseases of wheat (*Triticum aestivum* L.) are the rusts. Development of resistant cultivars provides a means to control these diseases. Between 20 and 40 genes are known that confer resistance to each rust (9); however the rust pathogens have been able to overcome several of them by expressing new virulences. Resistance of some cultivars has not been overcome because of their durable resistance. Combinations of *Lr34* with other genes are associated with durable leaf rust (caused by *Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* Eriks & E. Henn.) resistance (5,6,14,21). Dyck (3) located *Lr34* on chromosome 7D of wheat. Singh (16) and McIntosh (10) reported that *Lr34* is genetically associated with gene *Yr18*, which confers a durable, partial adult plant resistance to stripe rust (caused by *Puccinia striiformis* Westend.). The two genes are also known to be linked with gene *Ltn*, which confers a leaf tip necrosis in adult plants (4,17) that could be used as a morphological marker for the identification of *Lr34* and *Yr18*.

In a recent study, Singh et al (18) found that tolerance to the MAV-Mex serotype of barley yellow dwarf virus in Anza and nine other CIMMYT-derived wheats is conferred by a common gene, *Bdv1*.

Quantitative enzyme-linked immunosorbent assays (ELISAs) (1) at CIMMYT (*unpublished results*) showed that the tolerant and susceptible wheats have similar virus titers. Hence, based on the classification of Cooper and Jones (2), *Bdv1* confers tolerance, but not resistance. Anza and all other *Bdv1*-carrying wheats studied by Singh et al (18) are either known or suspected to carry *Lr34* or *Yr18* (16,17,21) and display the leaf tip necrosis symptom. Therefore, a possible linkage between *Lr34*, *Yr18*, and *Bdv1* is suspected. The objectives of this study were to estimate the number of genes that confer BYD tolerance and to test the genetic linkage among *Lr34*, *Yr18*, and *Bdv1*.

## MATERIALS AND METHODS

BYD-tolerant Australian cultivar Condor (University of Sydney accession W3749) and cultivar Jupateco 73R (CIMMYT accession BW17890), and BYD-susceptible Jupateco 73S (BW-17891) were included for genetic analysis. Condor is derived from the cross WW15\*2/WW80; WW15 is a sister line of the U.S. spring wheat cultivar Anza, which is derived from CIMMYT germ plasm. Anza was also included as a check cultivar because Singh et al (18) reported that it carries *Bdv1*. Jupateco 73R and Jupateco 73S are reselections from the Mexican cultivar Jupateco 73 and were originally selected for leaf rust resistance (based on *Lr34*) and leaf rust susceptibility, respectively. Condor and Jupateco 73R also carry *Yr18*, whereas Jupateco 73S lacks this gene (16).

Between 115 and 117 F<sub>3</sub> and F<sub>5</sub> lines from the crosses of Jupateco 73S with Jupateco 73R and Condor were used in the study. The F<sub>3</sub> lines were derived from randomly harvested individual F<sub>2</sub> plants. The F<sub>5</sub> lines were obtained by harvesting a random plant from each of the F<sub>3</sub> and F<sub>4</sub> lines. F<sub>3</sub> or F<sub>5</sub> lines were used in these studies instead of F<sub>2</sub> or backcross populations because they allowed for the parallel evaluation of the same family in separate experiments or at different locations with only one, but a different disease.

Adult plant leaf rust studies in the field were carried out at CIMMYT's El Batan headquarters in Mexico. F<sub>3</sub> and F<sub>5</sub> lines were tested during 1991 and 1992, respectively. The *P. r. tritici* pathotype used was TBD/TM (15) with the avirulence-virulence formula: *Lr3ka*,-9,11,16,19,21,23,24,25,26,29,30,32,33,34/1,2a,2b,2c,3,3bg,10,13,14a,14b,15,17,18,-20,27+31,28. This pathotype was chosen because it lacked virulence for *Lr34* but was virulent for other named *Lr* genes in the cultivars (20).

Adult plant field studies involving stripe rust were carried out at CIMMYT's Toluca, Mexico, station. F<sub>3</sub> and F<sub>5</sub> lines were tested during 1991 and 1992, respectively. The El Batan and Toluca locations are in the highlands near Mexico City. Materials were planted at both locations during mid-May. The pathotype 14E14 (8) of *P. striiformis* used for the studies carries virulences for the stripe rust resistance genes *Yr2,3,6,7* as well as the resistance gene known in Australia as *YrA* (22). The cultivars display a susceptible reaction in seedlings with this pathotype.

For BYD evaluation, F<sub>3</sub> lines were planted at Toluca during late January 1992, and F<sub>5</sub> lines were planted at El Batan during mid-June 1992. The BYD serotype MAV-Mex was used in all studies.

Field plots of the parents and the lines in each study consisted of two 1-m rows seeded 20 cm apart with 70 cm between plots. Each plot consisted of approximately 40 plants for rust evaluations, and approximately 20 space-seeded (10 cm between) plants were used for BYD evaluation. The parents of the cross were alternated every tenth row and served as checks for the symptoms. The leaf rust epidemic at El Batan and the stripe rust epidemic at Toluca were both created by inoculating susceptible spreader rows

carrying an equal mixture of Morocco and Jupateco 73S planted as clumps at one end of each plot. The disease severity estimation on adult plants was according to the modified Cobb Scale (12). For each test row, the range of disease severity (disease of the most resistant and of the most susceptible plant) was recorded.

*Metopolophium dirhodum* (Walker), which transmits the MAV-Mex serotype most efficiently, was reared on infected oat (*Avena sativa* L.) cultivar Paramo in a greenhouse maintained between 16 and 20 C and at a relative humidity of 70% under natural daylight. The viruliferous aphids were collected by shaking the leaves onto paper sheets sprinkled with talcum powder to prevent the aphids from sticking together. The aphids were then mixed homogeneously with corncob grit in bottles, which screw onto an applicator (11). The applicator was calibrated to drop approximately 10 aphids at a time. Each plant in the field was infested with approximately 10 aphids when two or three leaves were unfolded. The plots were sprayed 2 wk later with the systemic insecticide

oxydemeton-methyl (Metasystox-R) to eliminate the aphids. Further sprays of insecticide were carried out every 3 wk to keep the plots aphid-free. Plots for BYD evaluations were also sprayed periodically with the systemic fungicide tebuconazole (Folicur) for rust control. A 0-9 scale, based on the percent leaf discoloration, was used for BYD evaluation because plant stunting rarely occurs in Mexico (0 = no disease symptom, 1 = 1-10% discoloration, 2 = 11-20%...8 = 71-80%, and 9 = 81-100%).

The F<sub>3</sub> and F<sub>5</sub> lines were classified into three groups: homozygous resistant or symptomatically tolerant (HR or HT), all plants with a parental-type low response; segregating (Seg), plants of low to high response; and homozygous susceptible (HS), all plants with a parental-type high response. The parental-response ranges for each disease are given in Table 1. A chi-square goodness of fit test was carried out to test the conformity of the observed distribution with that expected for segregation at a single locus. When segregation occurs at a single locus, F<sub>3</sub> lines are expected to occur in

a proportion of 0.25 HR (or HT):0.5 Seg:0.25 HS; whereas in the F<sub>5</sub> generation a ratio of 0.4375 HR (or HT):0.125 Seg:0.4375 HS is expected.

## RESULTS AND DISCUSSION

Jupateco 73S displayed high levels of leaf rust, stripe rust, and leaf discoloration due to BYD during both years or seasons (Table 1); whereas Jupateco 73R, Condor, and Anza had moderate and similar responses to all three diseases during both years (Table 1). Because Condor and Anza are related wheats, it was presumed that the same gene (*Bdv1*) conferred tolerance to BYD in both wheats.

The responses of parents planted as checks between the plots of F<sub>3</sub>s and F<sub>5</sub>s were fairly consistent, with a maximum difference of 10%, which did not interfere in deciding the status of the F<sub>3</sub> or F<sub>5</sub> lines. Distributions of F<sub>3</sub> and F<sub>5</sub> lines for the three diseases are summarized in Table 2 for the crosses of Jupateco 73S with Jupateco 73R and Condor. The observed F<sub>3</sub> or F<sub>5</sub> distributions for each cross and for each disease conformed with the frequencies expected for a monogenic segregation. The pooled F<sub>3</sub> and F<sub>5</sub> distributions for the crosses of Jupateco 73S with Jupateco 73R and Condor were also in accordance with the single-gene segregation for resistance to leaf rust and stripe rust. The pooled F<sub>3</sub> distribution for BYD was significant at  $P = 0.05$ ; however, it was nonsignificant at  $P = 0.01$ . It could be attributed to misclassifications of a few HR or HS F<sub>3</sub> lines as Seg lines. Because the pooled F<sub>5</sub> distribution conformed with a monogenic ratio, it is concluded that a single gene also conferred tolerance to BYD.

The joint classifications of F<sub>3</sub> and F<sub>5</sub> lines for the three diseases are given in Table 3. Twenty-seven classes are

**Table 1.** Cultivars and the range of their adult plant field responses to leaf rust and stripe rust during 1991 and 1992 and to BYD during 1992-Toluca and 1992-El Batan

Cultivar	Leaf rust <sup>a</sup>		Stripe rust <sup>a</sup>		BYD <sup>b</sup>	
	1991	1992	1991	1992	Toluca	El Batan
Jupateco 73S	100S	100S	80-100S	60-100S	7-9	6-8
Jupateco 73R	15-40MSS	20-50MSS	5-30M	5-30M	2-5	1-4
Condor	10-30MSS	20-40MSS	10-30M	10-30M	1-4	1-3
Anza	10-30MSS	20-40MSS	10-30M	10-30M	1-4	1-3

<sup>a</sup>Disease estimation has two components: disease severity and infection type (IT). Numbers from 5 to 100 = percent severity based on modified Cobb Scale (12). M = moderately resistant to moderately susceptible IT (stripes with some necrosis and chlorosis), MSS = moderately susceptible to susceptible IT (moderate to large uredia without chlorosis or necrosis), and S = susceptible IT (large uredia or stripes without chlorosis or necrosis).

<sup>b</sup>BYD estimation is based on a 0-9 scale, where 1 = 1-10% leaf discoloration...8 = 70-80% discoloration.

**Table 2.** Distributions and  $\chi^2$ -analysis for a monogenic segregation model of F<sub>3</sub> and F<sub>5</sub> lines in the crosses of Jupateco 73S with Jupateco 73R and Condor when tested with leaf rust, stripe rust, and BYD

Cultivar crossed with Jupateco 73S	F <sub>3</sub>			$\chi^2$ and $P$ values <sup>b</sup>	F <sub>5</sub>			$\chi^2$ and $P$ values <sup>c</sup>
	No. lines with reaction <sup>a</sup>				No. lines with reaction			
	HR	Seg	HS		HR	Seg	HS	
Leaf rust								
Jupateco 73R	29	65	23	2.06, $P > 0.25$	54	16	46	0.81, $P > 0.50$
Condor	24	67	25	2.81, $P > 0.10$	43	19	53	2.69, $P > 0.25$
Total	53	132	48	4.34, $P > 0.10$	97	35	99	1.51, $P > 0.25$
Stripe rust								
Jupateco 73R	29	65	23	2.06, $P > 0.25$	54	16	46	0.81, $P > 0.50$
Condor	23	68	25	3.52, $P > 0.10$	43	19	53	2.69, $P > 0.25$
Total	52	133	48	4.81, $P > 0.05$	97	35	99	1.51, $P > 0.25$
BYD								
Jupateco 73R	26	68	23	3.24, $P > 0.10$	53	20	43	3.37, $P > 0.10$
Condor	23	69	24	3.80, $P > 0.10$	42	21	52	4.46, $P > 0.10$
Total	49	137	47	7.25, $P > 0.01$	95	41	95	5.80, $P > 0.05$

<sup>a</sup>Reaction categories are HR = homozygous resistant (homozygous symptomatically tolerant in case of BYD), Seg = segregating, and HS = homozygous susceptible.

<sup>b</sup>An expected F<sub>3</sub> line frequency of 0.25 HR:0.5 Seg:0.25 HS was applied to calculate the  $\chi^2$  values.  $P$  values higher than 0.05 indicate nonsignificant values of  $\chi^2$ .

<sup>c</sup>An expected F<sub>5</sub> line frequency of 0.4375 HR:0.125 Seg:0.4375 HS was applied to calculate the  $\chi^2$  values.  $P$  values higher than 0.05 indicate nonsignificant values of  $\chi^2$ .

expected if the resistance to three diseases is conferred by independent genes. However, a majority of the lines was classified as HR (or HT), Seg, or HS to all three diseases, indicating that genes conferring resistance to all three diseases must be very closely linked. Occasional lines, which occurred in other classes, are believed to be due to the misclassification for one disease or the other. Population size, especially in the case of BYD evaluation, where only approximately 20 plants of each line were evaluated, could be one reason for the misclassifications. Furthermore, occasional escapes or over-infestation of plants through the natural aphid population could also lead to misclassifications. Because no line HS to leaf rust or stripe rust and HT to BYD, or vice versa, was obtained, recombination between the genes cannot be proven. Therefore, the possibility that resistances to all three diseases were under pleiotropic genetic control could not be ruled out. By using F<sub>3</sub> lines from the crosses of Jupateco 73S with Jupateco 73R and Condor, Singh (16) reported that gene *Lr34* for leaf rust resistance and gene *Yr18* for stripe rust resistance are very closely linked. Analysis of random F<sub>5</sub> lines again supported the above results.

The genetic associations (or pleiotropism) for genes conferring resistance to the three diseases have a major breeding implication. Genes *Lr34* and *Yr18* occur in numerous CIMMYT-derived wheats (16,17) and possibly are present in various other wheats of non-CIMMYT origin (4,5,14). Therefore, gene *Bdv1* should also be widespread. Older wheats, such as Chinese Spring and Frontana, which are known to carry *Lr34* (4,21), also show less BYD yellowing (R. P. Singh, unpublished).

Genes *Lr34* and *Yr18* when present alone confer a slow rusting type of adult plant resistance (16,19). Similarly, gene *Bdv1* confers slow yellowing of adult plants (18). Resistances (tolerance for BYD) to the three diseases conferred by the respective genes have remained effective to all tested pathotypes or serotypes in various laboratories (4,5,7,10,13,14,19,20) and are apparent in data from other parts of the world (CIMMYT database). Resistances conferred by *Lr34* or *Yr18* can be improved by adding other partially effective additive genes (4,16,21). Because gene *Ltn* is known to be linked with *Lr34* (4,17), the presence of *Lr34*, *Yr18*, and *Bdv1* could easily be followed in the segregating populations even in the absence of diseases or in the presence of other major or minor genes.

Because *Lr34* is located on chromosome 7DL (4; P. L. Dyck, unpublished), the linked gene *Bdv1* for tolerance to barley yellow dwarf virus must also be located on that chromosome. Condor, Anza, or Jupateco 73R could be used as a tester genotype for *Bdv1*. Genes

**Table 3.** Reaction and number of F<sub>3</sub> and F<sub>5</sub> lines from the crosses of Jupateco 73S with Jupateco 73R and Condor when classified for the three diseases

Reaction of lines <sup>a</sup>			No. lines			
			Jupateco 73S × Jupateco 73R		Jupateco 73S × Condor	
Leaf rust	Stripe rust	BYD	F <sub>3</sub>	F <sub>5</sub>	F <sub>3</sub>	F <sub>5</sub>
HR	HR	HT	25	52	21	40
HR	HR	Seg	3	1	2	2
HR	HR	HS	0	0	0	0
HR	Seg	HT	0	0	0	0
HR	Seg	Seg	1	1	1	1
HR	Seg	HS	0	0	0	0
HR	HS	HT	0	0	0	0
HR	HS	Seg	0	0	0	0
HR	HS	HS	0	0	0	0
Seg	HR	HT	0	0	0	0
Seg	HR	Seg	1	1	0	1
Seg	HR	HS	0	0	0	0
Seg	Seg	HT	1	1	2	2
Seg	Seg	Seg	61	14	63	13
Seg	Seg	HS	2	0	2	3
Seg	HS	HT	0	0	0	0
Seg	HS	Seg	0	0	0	0
Seg	HS	HS	0	0	0	0
HS	HR	HT	0	0	0	0
HS	HR	Seg	0	0	0	0
HS	HR	HS	0	0	0	0
HS	Seg	HT	0	0	0	0
HS	Seg	Seg	0	0	0	0
HS	Seg	HS	0	0	0	0
HS	HS	HT	0	0	0	0
HS	HS	Seg	2	3	3	4
HS	HS	HS	21	43	22	49
χ <sup>2</sup> (26 df) independence			725.1	513.2	672.9	452.0
P value			<0.001	<0.001	<0.001	<0.001

<sup>a</sup>Reaction categories are HR = homozygous resistant, HT = homozygous symptomatically tolerant, Seg = segregating, and HS = homozygous susceptible.

different from *Bdv1* should be searched for in wheats that do not show leaf tip necrosis symptoms to enhance the genetic diversity.

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