Expression of *Thinopyrum intermedium*-Derived *Barley yellow dwarf virus* Resistance in Elite Bread Wheat Backgrounds

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ABSTRACT

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Resistance to *Barley yellow dwarf virus* (BYDV) is not found in wheat but is available in a *Thinopyrum intermedium* translocation (*Ti*) carried on chromosome 7DL of bread wheat recombinant lines. We used one of those lines (TC14/2*Spear) to introgress the *Ti* into bread wheat cultivars and to determine the influence of wheat backgrounds, with and without known tolerance to BYDV, on the expression of resistance. Two single and three backcross populations, segregating for the presence of the alien fragment, were tested under field conditions and artificial inoculation with BYDV isolates MAV-Mex and PAV-Mex. Lines containing the fragment were identified using the microsatellite marker *gwm37*. Tillering, biomass, grain yield, thousand-kernel weight, and seed quality were evaluated in inoculated and noninoculated plots. Resistance was assessed by enzyme-linked immunosorbent assay. In early generations, the alien fragment followed expected Mendelian segregation, whereas in the advanced ones a slight bias against its transmission was observed. No positive nor negative effects of *Ti* on agronomic performance and quality were found. A significant optical density reduction in individuals carrying the fragment was observed after PAV infection in crosses with lines Anza and Baviacora but not with Milan. In addition, the fragment was associated with a lower frequency of infected plants for both PAV and MAV isolates. The reduced yield loss associated with the presence of the translocation was due largely to the lower infection rate.

Additional keywords: breeding, luteovirus, simple sequence repeat marker.

Barley yellow dwarf (BYD) is globally the most important viral disease of wheat. *Barley yellow dwarf virus* (BYDV) belongs to the genus *Luteovirus* and causes severe losses in cereals (7). Grasses and cereals are hosts for the virus, and transmission only occurs through aphid vectors. Symptom severity and the extent of losses largely depend on the crop species and cultivar infected, virus strain involved, time of infection, and prevalent environmental conditions (12).

The main traditional approaches to control BYD have been the use of insecticide applications to eliminate the vector and the use of cultivars with varying levels of tolerance. The use of insecticides is mainly aimed at preventing the secondary spread of BYDV; however, its increased use is leading to insecticide resistance among aphid vectors, severe environmental problems due to the excessive use of broad-range chemicals, and an increase in production costs (20). Tolerance has been frequently deployed because fewer symptoms and losses are observed, despite high virus concentrations (33), and because no germ plasm with proven BYD resistance (reduced virus titer) has been available in a useful form until recently. The use of tolerant cultivars allows the build-up of the virus in the field.

Resistance, in which the host restricts viral infection, replication and invasion (11), is a desirable plant response because it allows the reduction and elimination of hazardous pools of viral inoculum that could promote secondary spread in the field. It also prevents high virus concentrations that could lead to the development of new, possibly more aggressive virus strains (24).

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BYDV tolerance is present in adapted common bread wheat germ plasm; in cv. Anza, it has been associated with the *Bdv1* gene (31). However, resistance has only been reported in wild relatives of wheat, most frequently in the genus *Thinopyrum* (9,23). The mechanism of BYDV resistance conferred by *T. intermedium* is interference with virus multiplication (30) or reduced cell-to-cell movement (2).

A major factor of BYDV resistance located on homologous group 7 of *T. intermedium* was transferred to wheat (4,6). A set of recombinant lines (called TC lines) carrying the *T. intermedium* translocation was obtained and their BYDV resistance was confirmed by enzyme-linked immunosorbent assay (ELISA) (4). The molecular and cytogenetic characterization of the TC lines (18), and more specifically of the TC14 lines (36) carrying the smallest fraction of alien heterochromatin, allowed us to identify molecular markers for the translocation. One of these markers diagnostically identified the *T. intermedium* fragment in segregating populations derived from crosses with TC14/2*Spear (3) and is now successfully being applied in marker-assisted selection at the International Maize and Wheat Improvement Center (CIMMYT, Mexico).

The study of the behavior of TC14-derived resistance using molecular markers and ELISA confirmed an optical density (OD) reduction of 27 to 55% in F_3 populations inoculated with BYDV (PAV-Mex). In addition, the alien segment was associated with a significant reduction in the percentage of infected plants in populations inoculated with PAV and MAV isolates (3).

The expression of TC14-derived resistance has been confirmed in early generations (3,4,18,36), but the expression and behavior in later generations of recombination has not been examined. At CIMMYT, preliminary field tests of TC lines, in particular the TC14 lines, have demonstrated that despite lower virus titers, these lines showed a poor agronomic behavior accompanied by severe symptoms, when the infection occurred at an early growth

stage (17). It appears that in this case resistance is expressed in an otherwise sensitive background. This suggested that it would be desirable to combine BYDV resistance with tolerance to achieve lower levels of symptom expression and reduce related yield losses.

The objectives of this study were to observe the agronomic performance of advanced segregating populations of elite bread wheat lines carrying the *T. intermedium* translocation and to determine how different genetic backgrounds (both with and without BYDV tolerance) influence the expression of true virus resistance.

MATERIALS AND METHODS

Plant materials. Lines with and without the *T. intermedium* translocation and lines with and without known tolerance to BYDV were selected as parents to develop the populations used in this study.

The recombinant line TC14/2*Spear (Spear*2/4/Vulcan.cms//L1/'Millewa'/3/Restorer R35733), accession 289B (referred to as "TC14"), carries BYDV resistance in a small translocation located on the distal portion of chromosome 7DL. TC14 was chosen as the resistance source because it showed the lowest OD values when compared with other TC lines and with tolerant and susceptible wheats using ELISA (17). The following CIMMYT bread wheat cultivars and lines were used: Anza, reported to carry the *Bdv1* tolerance gene (31); Milan, an advanced line identified as probably having two genes for BYDV tolerance (M. van Ginkel, *personal communication*); and Baviacora, a cultivar that is intermediately sensitive to BYDV but high-yielding in both irrigated and semi-arid environments.

The behavior of the translocation in early and advanced generations was studied in two single cross populations involving wheat cv. Anza: 93 F_3 lines of the cross Anza/"TC14" and 128 F_5 lines of the cross "TC14"/Anza were derived by single seed descent (SSD) (14,15). The identification of the *T. intermedium* fragment was done by polymerase chain reaction (PCR) with the simple sequence repeat (SSR) *gwm37* marker (3) in the F_2 and F_6 generation of the two single cross populations Anza/"TC14" and "TC14"/Anza, respectively.

The influence of the genetic background on the behavior and expression of the *T. intermedium*-derived resistance was studied in three populations with three distinct wheat genotypes: the BC_2F_2 generations of the Anza*3/"TC14" cross (=BC_2-Anza), Milan*3/"TC14" cross (=BC_2-Milan), and Baviacora*3/"TC14" cross (=BC_2-Baviacora). To develop these populations, the F_1 was backcrossed twice to each of the respective wheat cultivars, representing a breeding program aiming to introgress the resistance into adapted backgrounds. After the first backcross, the presence of the alien fragment in heterozygotic state was determined in 60 plants of each BC_1F_1 population by the *gwm37* marker. Individuals determined to contain the *T. intermedium* translocation were used for the second backcross. All BC_2F_1 plants were grown in the greenhouse under optimal conditions, sampled for PCR analysis, and selfed to obtain enough BC_2F_2 seed for field testing. The experiments were conducted with 111 BC_2-Anza, 93 BC_2-Milan, and 107 BC_2-Baviacora lines.

Field trials. The expression of BYDV resistance and tolerance was tested in field experiments with artificial virus inoculation in the summer of 1998 at El Batán, Mexico (19°N, 100°W, 2,249-m elevation, and 650-mm mean annual rainfall). The number of lines used in the field experiment was slightly reduced due to the insufficient amount of seed harvested in the greenhouse.

The five populations were planted together in an alpha lattice design with two replications. Each line was sown in a plot consisting of an 80-cm long double row planted on a bed 75-cm wide, with 8 plants per row totaling 16 plants per plot, leaving 20 cm between rows. The three treatments were noninoculated, inoculated with BYDV-PAV-Mex, and inoculated with BYDV-MAV-Mex. Each treatment was planted in a separate block in the field and isolated by two rows of oats to avoid cross-contamination of the BYDV isolates.

Virus inoculation. The BYDV isolates used in the experiments belong to the PAV and MAV serotypes described by Rochow (28). They were Mexican isolates maintained in CIMMYT's greenhouses since 1993. At approximately the three-leaf stage (stage 13) (37), plants were inoculated by infesting them with aphids reared in the greenhouse on BYDV-infected oat plants. Approximately 10 aphids (*Rhopalosiphum padi* for PAV-Mex and *Metopolophium dirhodum* for MAV-Mex) were deposited at the base of each seedling with a calibrated mechanical dispenser (25). The success of infection was determined 12 days after infestation by performing ELISA on 100 leaf samples taken at random. Because only 60% of the plants were infected, a second inoculation was carried out 18 days after the first one, following the same procedure. The whole experiment was subsequently kept aphid free by fortnightly insecticide (Metasystox) applications starting 1 week after the second inoculation. The noninoculated plots were additionally protected with insecticide after emergence, the day before the inoculation of the rest of the trial.

Phenotypic evaluation. Six plants per family were tagged with colored plastic ribbons to ensure the same individuals were used during repeated sampling. Twenty-four days after the second inoculation, the flag leaf-one was sampled to measure OD by ELISA. At 60 and 75 days after inoculation, the intensity of yellowing was visually evaluated at the plot level, using a 0 to 9 scale as described by Bertschinger (5). The total number of tillers per plant was counted 100 days after planting. At maturity the main tillers of all six tagged plants were harvested jointly to determine biomass, grain yield, thousand-kernel weight (TKW), and harvest index (HI = grain yield/biomass).

To determine whether the alien fragment had an undesirable effect on industrial quality when backcrossed to common wheat, three commonly used quality parameters were measured on the 10 best lines of each backcross population selected on the basis of their field performance. Grain samples (6 g) of five lines with and five lines without the alien fragment were ground with a cyclone mill (0.5-mm sieve) (Udy, Ft. Collins, CO) and used to determine grain protein and grain hardness by NIR (Infralyzer 350; Technicon (Ireland) Ltd., Dublin) analysis. The 1.0-g sample method described by Peña et al. (26) was used to determine grain sodium dodecyl sulfate (SDS)-sedimentation. The tests were conducted in CIMMYT Industrial Quality Laboratory.

Assessment of the alien translocation. A SSR marker, *gwm37*, mapping to 7DL and identified to be diagnostic for the *T. intermedium* translocation (*Ti*) (3), was used to assess the presence or absence of the translocation in segregating lines of F_2, F_6, and BC_2F_1 populations. Because of its codominant nature, the marker allowed us to differentiate among the three classes: *TiTi* = alien fragment in homozygous state, *Titi* = alien fragment in heterozygous state, and *titi* = no alien fragment present. DNA extraction, PCR amplification, and separation of the amplified products on agarose gels were described by Ayala et al. (3).

Assessment of resistance. In all tested populations, resistance to BYDV was assessed as a reduction in OD by ELISA. In the three backcross populations, OD was measured in two subsets of BC_2F_2 lines; some with and some without the alien fragment, as determined in the BC_2F_1 mother plants with the *gwm37* marker. Each subset included lines with high and low intensity of yellowing, typical of BYDV infection. On the 0 to 9 scale (5), some lines showed similar yellowing values when infected with both virus isolates, whereas others reacted differently. Therefore, different lines were tested for PAV and MAV isolates. In total, ELISA was performed on at least 40 lines per population.

Double antibody sandwich ELISA (DAS-ELISA) with polyclonal antibodies against PAV and MAV from the United States (provided by K. Perry, Purdue University) was carried out as described by Ayala et al. (3). Optical density was measured at

405 nm, after 1 and 2 h of incubation at room temperature by a microplate reader (MR 700; Dynatech Laboratories, Chantilly, VA).

A sample was considered infected if the OD value was higher than a fixed threshold. The threshold for each population was calculated with the ODs of all individuals as described by Sutula et al. (35). The resulting threshold value was usually between two to three times the average OD of the noninfected controls, depending on the population and the quality of the ELISA. The average OD of the infected plants (IOD) and the number of infected plants per family were calculated.

Data analysis. Phenotypic data were first tested for normality. For yellowing, the higher value of the two notes taken for each line was considered for subsequent analysis. Adjusted means were obtained by the alpha lattice design by PROC MIX (SAS Institute, Cary, NC). Data for each population were analyzed by grouping the lines based on the presence or absence of the alien fragment as detected by *gwm37* (*TiTi*: alien fragment in homozygous state; *Titi*: alien fragment in heterozygous state; and *titi*: no alien fragment present).

The analysis of variance to obtain adjusted trait means for the genotypic groups (*TiTi*, *Titi*, and *titi*) was conducted with PROC GLM over two replications (SAS Institute). The differences between adjusted means by genotypic groups were tested by Tukey's test (34).

Losses in biomass, TKW, grain yield, and HI were calculated by comparing performance of the noninoculated control (*C*) and the infected plot (*I*) in each genotypic group by the formula: percent loss = 100 – [(*I*/*C*) × 100]. The relative differences in performance between the lines containing the fragment (*TiTi* and *Titi*) and those without it (*titi*) were calculated as percent gain = [(performance of *TiTi* or *Titi*/performance of *titi*) × 100] – 100. The resulting percent value was considered as the advantage (percent gain) of lines carrying the fragment over those not carrying it.

RESULTS

Behavior of bread wheat breeding populations carrying a *T. intermedium* translocation under noninoculated conditions. In the F_2 population of the single cross Anza/"TC14", the segregation ratio obtained for the genotypic groups *titi*/*Titi*/*TiTi* was 1:2:1, which coincides with the Mendelian segregation of one genetic character with codominant effects (Table 1). In the F_5 population of the "TC14"/Anza cross, a segregation ratio of 12:2:8 was found for groups *titi*/*Titi*/*TiTi*, with the homozygous dominant genotype *TiTi* being less frequent than expected. This was not within the expected (P = 0.025) Mendelian ratios (15:2:15) for one segregating factor in the F_5 generation.

In all three backcross populations, the BC_1F_1 generations had segregation ratios very close to 1:1 for genotypic groups *titi*/*Titi*, in accordance with the expected Mendelian segregation for one genetic factor in a backcross. However, in the BC_2F_1 generation, two populations (BC_2-Anza and BC_2-Baviacora) had segregation ratios significantly different (P = 0.025 and 0.005, respectively) from Mendelian expectations for a backcross, expressing some bias against transmission of the alien fragment.

In the absence of BYDV infection, there was no significant difference between the genotypic groups in the agronomic characteristics measured (number of tillers, biomass, TKW, grain yield, and HI) in the two single-cross populations and in the populations backcrossed to cvs. Milan and Baviacora (Table 2). However, in the backcross to cv. Anza, biomass and yield were significantly higher (P = 0.05) in the group carrying the alien fragment. Hence, the alien fragment had no significant negative effect on the performance of any of the five populations with regard to the agronomic characteristics measured.

As for grain quality parameters, the TC14 parent was intermediate in hardness (50%), relatively lower in protein (11.6%), and poor in SDS (10.3 ml) value when compared with the wheat recurrent parents (Table 3). Within a backcross population, there were no differences in most quality parameters between lines carrying the alien fragment and those that did not. In general, protein levels of the progeny were equal or slightly better than those of the common wheat progenitors. In all cases, seed was harder (lower values) among the progeny than the parents, and SDS values of the progeny exceeded those of the parents (Table 3).

Effect of the genetic background on the expression of *T. intermedium*-derived resistance to BYDV. Following BYDV infection, the range of OD values obtained by ELISA was wider with the PAV antiserum (0.190 – 1.165) than with the MAV antiserum (0.182 – 0.680). Therefore, the differentiation of the response between lines was easier with PAV than with MAV. All three cultivars used as recurrent parents showed higher IOD value than the resistant "TC14" (Table 4), and differences among the wheat parents were evident depending on the virus isolate used. Under PAV-Mex infection, the "TC14" parent's IOD was 21 to 68% lower than that of the common wheats, whereas under MAV-Mex infection, the IOD was lower by ≈15%.

In the presence of the *T. intermedium* fragment, there was a significant IOD difference between genotypic groups infected with PAV-Mex in BC_2-Anza and BC_2-Baviacora populations, but not in the BC_2-Milan (Table 4). Reductions in IOD value reached as much as 20 and 24% in the first two populations and only 7% in the latter. With MAV-Mex, there were no significant differences in the average IOD of the genotypic groups in the three backcrosses. Reductions in OD values of only 8, 8, and 1% were observed for BC_2Anza, BC_2-Milan, and BC_2-Baviacora, respectively (Table 4).

In all populations evaluated and regardless of the virus isolate used, the percentage of infected individuals (based on ELISA) was significantly lower in the group containing the alien fragment than in the group without it (Fig. 1). Between genotypic groups,

TABLE 1. Segregation of the *Thinopyrum intermedium* translocation in populations derived from TC14/2*Spear ("TC14") crossed with three bread wheats[z]

Population	Lines tested	Genotypes observed			Ratio		Chi square	*P*
		titi	*Titi*	*TiTi*	Observed	Expected		
F_2								
Anza/"TC14"	93	23	51	19	1:2:1	1:2:1	1.250	0.500
F_5								
"TC14"/Anza	128	72	12	44	12:2:8	15:2:15	8.667	0.025
BC_1F_1								
Anza*2/"TC14"	41	18	23	–	1:1.3	1:1	0.610	0.100
Milan*2/"TC14"	48	27	21	–	1:0.8	1:1	0.750	0.100
Baviacora*2/"TC14"	41	24	17	–	1:0.7	1:1	1.444	0.100
BC_2F_1								
Anza*3/"TC14"	111	66	45	–	1:0.7	1:1	3.973	0.025
Milan*3/"TC14"	93	52	41	–	1:0.8	1:1	1.301	0.100
Baviacora*3/"TC14"	107	67	40	–	1:0.6	1:1	6.813	0.005

[z] *titi* = no *T. intermedium* fragment; *Titi* = heterozygous for the *T. intermedium* fragment; *TiTi* = homozygous for the *T. intermedium* fragment. The presence of the alien fragment was determined with the simple sequence repeat marker *gwm37*.

percentage of reductions of infection of 20 to 28% were observed when the *T. intermedium* allele was present. The percentage of infection with MAV-Mex was slightly lower than with PAV-Mex.

Effect of the alien fragment on several agronomic traits under BYDV infection. The three wheat cultivars used as recurrent parents showed higher field losses under BYDV infection than the resistant TC14 (Table 5). In addition, the percentage of infection of TC14 was the lowest of all cultivars. Losses with both isolates were high except for TKW under MAV infection. Of all the cultivars, cv. Baviacora maintained the highest values for yield and yield components under MAV and PAV infection, in agreement with its high yield potential (Table 5).

Within the segregating populations, the yellowing associated with BYDV and the yield components under viral infection were mostly significantly different between genotypic groups (*TiTi*, *Titi*, and *titi*) (Table 6). However, there was no significant difference in the number of tillers (data not shown). In the F_3 Anza/"TC14" and F_5 "TC14"/Anza, the homozygous resistant group (*TiTi*) had significantly higher agronomic values and lower yellowing than the group homozygous for *titi*. The performance of the heterozygous group was in most cases intermediate. The same tendency was observed in the backcrosses BC_2-Anza and BC_2-Milan under both MAV-Mex and PAV-Mex infection. However, the effect of the presence of the translocation in reducing losses was seen in the BC_2-Baviacora population when inoculated with MAV, but not with PAV.

Yellowing was not significantly different among the genotypic groups in all the backcrosses under PAV inoculation, whereas highly significant differences were observed in single-cross populations (PAV and MAV) and backcross populations infected with MAV-Mex. Dwarfism, one of the other symptoms commonly used in BYDV evaluation, was not taken into account in this study, because the height-reducing *Rht* genes were segregating in all populations and confounded such observations.

Losses due to viral infection in the homozygous *titi* genotypic group in the single-cross populations were consistently higher than losses in the heterozygous (*Titi*) and homozygous (*TiTi*) genotypic groups (Table 6). Overall, losses were higher in the F_3 than in the F_5 population. Of the three backcross populations, BC_2-Milan suffered the highest losses, followed by BC_2-Baviacora, and then BC_2-Anza.

Likewise, gains in the single-cross populations were high for the homozygous *TiTi* group, especially in biomass and yield, reaching values as high as 32.9%. In the backcross populations, the largest gains associated with the presence of the alien fragment were for yield in BC_2-Anza after PAV (17.4%) and MAV (14.3%) infection and in BC_2-Milan (15.9%) with PAV-Mex infection. For all parameters, the lowest gains were obtained for the BC_2-Baviacora population following PAV-Mex infection.

DISCUSSION

Behavior of bread wheat breeding populations carrying a *T. intermedium* translocation under noninoculated conditions. The segregation ratios for the alien fragment recorded in early segregating populations fitted Mendelian expectations for one genetic character. As expected, segregating ratios of 1:2:1 in the F_2 population and of 1:1 in the BC_1F_1 populations were obtained. These results are in agreement with segregation ratios in other early generations reported for the TC lines (4). Knott (22) stated that generally genes transferred from wheat's close relatives to wheat tend to behave as simply inherited characters. Apparently the alien fragment carried by TC14/2*Spear is small enough that it does not interfere excessively with normal meiosis.

In advanced generations such as the F_5 of the "TC14"/Anza cross, the segregation ratio obtained (72:12:44) did not correspond

TABLE 3. Industrial quality parameters of the grain, evaluated in each genotypic group of the backcross populations and their progenitors (5 BC_2F_2 lines per genotypic group)[z]

Genotype	Genotypic group	Protein in grain	Grain hardness	Sodium dodecyl sulfate-value
Progenitors				
Anza	*titi*	13.0	50.5	12.8
Milan	*titi*	14.5	54.5	13.8
Baviacora	*titi*	13.1	49.5	13.0
"TC14"	*TiTi*	11.6	50.0	10.3
Generation-cross				
BC_2-Anza	*titi*	14.5 a	41.5 a	19.0 a
	Titi	14.2 a	41.6 a	16.5 a
BC_2-Milan	*titi*	14.8 a	43.4 a	16.4 a
	Titi	15.1 a	47.2 a	17.0 a
BC_2-Baviacora	*titi*	13.0 a	46.6 a	14.0 a
	Titi	12.3 a	46.3 a	13.1 a

[z] *titi* = no *Thinopyrum intermedium* fragment; *Titi* = heterozygous for the *T. intermedium* fragment; *TiTi* = homozygous for the *T. intermedium* fragment. Comparison by Tukey's test. Values followed by different letters within a column indicate significant differences between means at $P = 0.05$.

TABLE 2. The effect of the *Thinopyrum intermedium* fragment on the performance of two single-cross and three backcross populations evaluated under field conditions in the absence of *Barley yellow dwarf virus*

			Grams per 6 tillers[z]			
Population	Genotypic group[y]	No. of lines	Biomass	TKW	Yield	Harvest index
F_3-Anza/"TC14"						
	titi	23	25.7 a	33.1 a	10.6 a	0.41
	Titi	51	23.9 a	32.8 a	10.0 a	0.42
	TiTi	19	24.9 a	32.2 a	10.4 a	0.42
F_5-"TC14"/Anza						
	titi	63	25.8 a	36.0 a	11.0 a	0.43
	Titi	12	27.7 a	35.8 a	11.8 a	0.43
	TiTi	43	26.6 a	35.8 a	11.5 a	0.43
BC_2-Anza						
	titi	49	26.8 a	38.2 a	11.8 a	0.44
	Titi	36	27.7 b	38.6 a	12.4 b	0.45
BC_2-Milan						
	titi	44	31.7 a	39.1 a	13.4 a	0.42
	Titi	36	32.7 a	40.4 a	13.8 a	0.42
BC_2-Baviacora						
	titi	57	41.7 a	41.5 a	18.7 a	0.45
	Titi	34	42.8 a	42.1 a	18.4 a	0.43

[y] *titi* = no *T. intermedium* fragment; *Titi* = heterozygous for the *T. intermedium* fragment; *TiTi* = homozygous for the *T. intermedium* fragment.

[z] Comparison by Tukey's test. Values followed by different letters within a column indicate significant differences between means at $P = 0.05$. The values given are means of several lines in a genotypic group. TKW = thousand-kernel weight.

to Mendelian expectations (60:8:60) ($P = 0.025$). The numbers were skewed toward reduced frequencies of lines carrying the fragment in a homozygous status. The same tendency was observed in the BC_2F_1 populations, where the ratios for the crosses with cvs. Anza and Baviacora were significantly different from Mendelian expectations.

The observed shift in the advanced generations could be due to several reasons. The time period available to develop the SSD F_5 populations did not allow the inclusion of later flowering individuals, which may have slightly biased our host population, if presence or absence of the alien fragment is associated with maturity. However, this was not the case in the backcross population with cv. Milan as the recurrent parent. In this population, which had the highest numbers of individuals discarded for late flowering (12 of 93), the segregation ratio for the alien fragment was as expected (1:1). No shift in the ratio was observed in the Anza/"TC14" F_2 segregating data, perhaps because tissue was collected from all germinated plants (early, normal, and late maturing). A second reason may have been certation (competition of pollen tubes of different genotypes within the stigma) of the male gametes with or without the alien fragment during fertilization, a phenomenon often associated with intergeneric crosses (27). Individuals with aberrations (an additional or missing portion of heterochromatin) may prevent the completion of normal gamete formation (16), resulting in gametes of unequal fitness. Thirdly, relatively small population sizes may have resulted in genetic drift, further biasing the segregation in later generations.

The decision to use the BYDV resistance-carrying translocation in an ongoing breeding program will depend on its behavior and secondary effects on crucial agronomic and quality traits. It is common for alien translocations to carry, along with desirable genes, other genes that negatively affect the performance of the recipient host. The *T. elongatum* translocation conferring leaf rust resistance (*Lr19*) (29), transmitted good agronomic characteristics including high yield potential (32), but also an undesirable yellow pigmentation of the flour (21).

In our study, few significant differences were found between sister genotypic groups with or without the alien fragment for the agronomic (grain yield, biomass, and TKW) and quality (grain hardness, protein, and SDS) traits measured. Despite the small plot size and the relatively low number of tillers sampled, this suggests that the fragment can be used with confidence in breeding superior BYDV resistant wheat germ plasm. In fact, in the populations derived from the cv. Anza backcross, the genotypic group carrying the alien fragment (*Titi*) had significantly higher biomass (3.4%) and grain yield (5.1%) than its sister derivatives without the fragment.

Effect of the background on the expression of *T. intermedium*-derived resistance to BYDV. In the backcross populations, PAV-infected lines carrying the alien fragment had significantly lower virus titers (7 to 24%) than noncarriers, indicating that the *T. intermedium* fragment carries a gene whose products interfere with virus multiplication (30) or with cell-to-cell movement (13,24). Comparing P29, a wheat substitution line in which chromosome 7D of wheat is replaced by the 7D chromosome of *T. intermedium* with the BYDV susceptible cv. Abe, Anderson et al. (2) observed OD reductions of 42 to 52%. Although the translocation in TC14 and the alien chromosome in P29 belong to different 7D homologous *T. intermedium* chromosome groups, they could carry homologous resistance genes. Using protoplasts, Anderson et al. (2) demonstrated that reduced cell-to-cell movement, rather than interference in virus multiplication, is the mechanism of resistance derived from *T. intermedium*.

No IOD differences were found between genotypic groups in the BC populations when infected with MAV-Mex. This could be

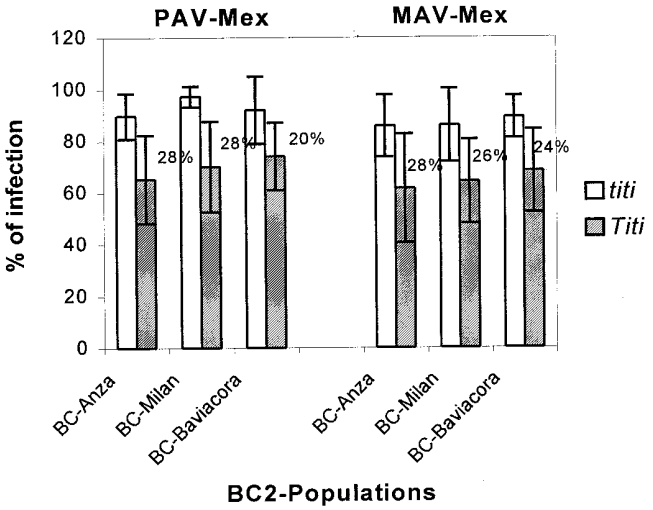

Fig. 1. Mean level of *Barley yellow dwarf virus* (BYDV) infection, determined by enzyme-linked immunosorbent assay in the two genotypic groups (*titi*/*Titi*) of the BC_2F_2 populations, inoculated in the field with BYDV-PAV or -MAV. The percentage of reduction between *titi* and *Titi* is indicated.

TABLE 4. Decrease in virus titers as measured by enzyme-linked immunosorbent assay in three backcross populations derived from crosses of TC14/2*Spear with three common bread wheats, tested under field conditions with *Barley yellow dwarf virus* (BYDV)-PAV and -MAV infection

			IOD PAV-Mex[z]			IOD MAV-Mex[z]		
Population/parental lines	Genotypic group[y]	No. of lines	Average	SD	% Reduction	Average	SD	% Reduction
BC_2-Anza	*titi*	29	0.681 b**	±0.239		0.381 a	±0.104	
	Titi	24	0.547 a	±0.172	20.0	0.351 a	±0.112	8.0
Anza	*titi*		0.514			0.322		
TC14	*TiTi*		0.267			0.278		
BC_2-Milan	*titi*	27	0.714 a	±0.175		0.548 a	±0.111	
	Titi	27	0.665 a	±0.244	7.0	0.506 a	±0.144	8.0
Milan	*titi*		0.812			0.443		
TC14	*TiTi*		0.256			0.375		
BC2-Baviacora	*titi*	20	0.389 b**	±0.101		0.266 a	±0.053	
	Titi	20	0.296 a	±0.184	24.0	0.264 a	±0.078	1.0
Baviacora	*titi*		0.329			0.214		
TC14	*TiTi*		0.255			0.184		

[y] *titi* = no *Thinopyrum intermedium* fragment; *Titi* = heterozygous for the *T. intermedium* fragment; *TiTi* = homozygous for the *T. intermedium* fragment.

[z] IOD = optical density of infected individuals (i.e., individuals with values above that of the healthy threshold). Values for the parents of each population are underlined. Comparison by Tukey's test. Values followed by different letters within a column indicate significant differences between groups at $^{**}P = 0.01$. Percentage of IOD reduction in *Titi* individuals relative to *titi* individuals.

due to the lower sensitivity of the MAV polyclonal antiserum used, for which the range of OD values obtained was lower than for the PAV polyclonal antiserum. This lower sensitivity might not have allowed good differentiation among individual lines. Alternatively, virus accumulation might not have been at its maximum on the MAV-infected lines at the time of sampling, because the rate of multiplication differs between PAV and MAV (33).

A common feature observed in all backcross populations, regardless of the virus strain infecting the crop, was the considerable number of noninfected plants that presumably escaped infec-

TABLE 5. Agronomic field performance under *Barley yellow dwarf virus* (PAV or MAV) infection of parents used in the backcrosses[y]

			Biomass		Thousand-kernel weight		Yield		
Variety	% Infection	Yellowing[z]	Inoculated	% Loss	Inoculated	% Loss	Inoculated	% Loss	Harvest index
PAV									
"TC14"	36 a	1.7 a	23.5 a	7.1	30.7 a	8.3	9.3 a	18.6	0.40
Anza	75 b**	3.9 b**	22.8 a	12.4	31.4 a	14.3	9.1 a	21.0	0.40
Milan	100 c**	2.8 ab	27.0 b*	28.6	33.4 ab	18.0	10.7 a	22.2	0.40
Baviacora	100 c**	2.3 a	33.0 b*	29.8	39.4 b**	10.2	14.9 b**	34.1	0.45
MAV									
"TC14"	49 a	1.6 a	23.6 ab	6.5	32.6 a	2.6	9.7 a	14.7	0.41
Anza	75 b**	3.1 b**	22.0 a	15.4	33.8 a	7.7	9.4 a	18.7	0.43
Milan	100 c**	2.8 b*	27.5 b**	27.2	37.0 ab	9.2	11.0 a	20.2	0.40
Baviacora	100 c**	2.8 b*	33.4 c*	29.0	41.0 b**	6.4	15.4 b**	32.3	0.46

[y] Yield parameters were measured on six main tillers per plot, and expressed in grams per six main tillers. Comparison by Tukey's test. Values followed by different letters within a column indicate significant differences between means at $*P = 0.05$ and $**P = 0.01$. % Loss = [100 – (inoculated/healthy) × 100]. Average of inoculated lines expressed in grams.

[z] Taken on a plot basis on a 0 to 9 scale (0 = green no chlorosis and 9 = completely chlorotic).

TABLE 6. Agronomic performance of TC14-derived populations under *Barley yellow dwarf virus* (BYDV)-PAV and BYDV-MAV artificial field infection[s]

			Means per genotypic group					% Loss[v]			% Gain relative to *titi*[w]		
Population/group[t]	Lines	% Infection[u]	Yellowing[x]	Biomass	TKW[y]	Yield	HI[z]	Biomass	TKW	Yield	Biomass	TKW	Yield
PAV													
F_3-Anza/"TC14"													
titi	23	90.0 a	3.4 a	20.3 a	26.9 a	7.0 a	0.34	21.0	18.5	34.0			
Titi	51	58.3 b*	3.2 a	21.5 a	27.6 a	7.9 a	0.37	10.0	16.4	21.0	5.9	2.6	12.9
TiTi	19	28.3 c*	2.0 b**	23.8 b**	29.8 a	9.3 b**	0.39	4.4	6.9	10.6	17.2	10.8	32.9
F_5-"TC14"/Anza													
titi	63	83.3 a	3.1 a	21.3 a	31.5 a	8.4 a	0.39	17.4	12.5	23.6			
Titi	12	76.7 b*	3.0 a	22.2 ab	31.8 a	9.5 ab	0.43	19.9	11.2	19.5	4.2	1.0	13.1
TiTi	43	46.7 c*	2.0 b**	24.2 b**	32.5 a	10.1 b**	0.42	9.0	9.2	12.2	13.6	3.2	20.2
BC_2-Anza													
titi	49	89.7 a	4.0 a	22.2 a	32.1 a	8.6 a	0.39	17.2	16.0	27.1			
Titi	36	65.3 b*	3.7 a	24.1 b**	33.7 b**	10.1 b**	0.42	13.1	12.7	18.5	8.4	5.0	17.4
BC_2-Milan													
titi	44	97.2 a	3.0 a	24.3 a	31.0 a	8.8 a	0.36	23.3	20.7	34.3			
Titi	36	70.1 b*	2.8 a	27.4 b**	34.2 b**	10.2 b**	0.37	16.2	15.3	26.1	12.8	10.3	15.9
BC_2-Baviacora													
titi	57	92.0 a	2.3 a	32.5 a	36.7 a	13.5 a	0.42	22.1	11.6	27.8			
Titi	34	74.0 b*	2.5 a	33.4 a	37.1 a	13.9 a	0.42	22.0	11.9	24.5	2.8	1.1	3.0
MAV													
F_3-Anza/"TC14"													
titi	23	78.3 a	2.8 a	19.8 a	28.8 a	7.3 a	0.37	23.0	13.0	31.1			
Titi	51	53.3 b*	2.0 b**	21.2 ab	30.4 a	8.2 a	0.38	11.3	7.3	18.0	7.1	5.6	12.3
TiTi	19	26.7 c*	1.6 c**	23.3 b*	31.0 a	9.5 b**	0.41	6.4	3.7	8.7	17.7	7.6	30.1
F_5-"TC14"/Anza													
titi	63	80.0 a	2.9 a	22.4 a	32.0 a	8.6 a	0.38	13.2	11.1	21.8			
Titi	12	60.0 b*	2.4 b**	24.7 ab	33.2 ab	9.8 ab	0.40	10.8	7.8	16.9	10.3	3.8	14.0
TiTi	43	36.7 c*	1.5 c**	25.4 b**	34.4 b*	10.4 b**	0.41	4.5	4.4	9.6	13.4	7.5	20.9
BC_2-Anza													
titi	49	85.8 a	3.2 a	21.6 a	33.0 a	9.1 a	0.42	19.4	13.6	22.9			
Titi	36	61.6 b*	2.5 b**	23.9 b**	35.1 b**	10.4 b**	0.44	13.7	9.1	16.1	10.6	6.4	14.3
BC_2-Milan													
titi	44	86.0 a	2.8 a	25.7 a	32.8 a	9.6 a	0.37	18.9	16.1	28.4			
Titi	36	64.3 b*	2.2 b**	27.1 b**	35.0 b**	10.2 b*	0.38	17.1	13.4	26.1	5.4	6.7	6.2
BC_2-Baviacora													
titi	57	89.2 a	2.7 a	31.3 a	38.0 a	13.4 a	0.43	24.9	8.4	28.3			
Titi	34	68.3 b*	2.1 b**	33.9 b**	39.8 b**	14.2 b*	0.42	20.8	5.5	22.8	8.3	4.7	6.0

[s] Comparison by Tukey's test. Different letters indicate significant differences between means at $*P = 0.05$, $**P = 0.01$.

[t] *titi* = no *Thinopyrum intermedium* fragment; *Titi* = heterozygous for the *T. intermedium* fragment; *TiTi* = homozygous for the *T. intermedium* fragment.

[u] Data of the two single-crosses taken from Ayala et al. (3).

[v] % Loss = 100 – [(infected/healthy) × 100]. The values given are the means obtained for the lines of a genotypic group, based on data taken on six main tillers per plot.

[w] Percentage of gain of *TiTi* and *Titi* over *titi*.

[x] Taken on a plot basis, using a 1 to 9 scale, (0 = green, no chlorosis and 9 = completely chlorotic plants).

[y] Thousand-kernel weight.

[z] Harvest index.

tion in genotypic groups carrying the alien fragment (*Titi*), varying in proportion from 20 to 28%. This is not considered to be due to a lack of uniform inoculation because all lines, with and without the alien fragment, were randomized in two replications and treated equally. Within the lines not carrying the alien fragment (*titi*), an average of 90% of the individuals were positively infected by BYDV. Therefore, a mechanism of avoidance of infection caused by the influence of the alien fragment on aphid behavior may be involved. This phenomenon of a reduced percentage of infected individuals would represent another type of resistance associated with the "TC14" material.

Interestingly, even though significant IOD differences between genotypic groups were only observed in two of six cases when the populations were inoculated with BYDV, improvement in the agronomic traits was still observed in lines carrying the alien fragment of most crosses. This suggests that positive effects between resistance and tolerance, as well as reduced infection frequencies, primarily influenced the measured field traits in the populations derived from cultivars also carrying tolerance. In the population derived from cv. Baviacora, having no known tolerance genes, some of the lowest gains in field performance under BYDV infection were observed in the presence of the translocation, although the IOD values and the level of infection were both reduced.

Despite the low gains recorded for cv. Baviacora, this line and its derived population maintained their good characteristics under BYDV infection. This suggests that genes involved in adaptation and good agronomic performance might also be involved in BYDV tolerance as suggested by Comeau and Makkouk (10). However, more specific tolerance genes are needed to significantly reduce losses under BYDV infection allowing the full expression of the agronomic potential of elite lines. The low virus titers recorded in cv. Baviacora for PAV and MAV could indicate that this cultivar has some resistance to BYDV. However, in the absence of tolerance genes, reductions in losses were only significant with MAV.

In many virus–host systems reported in the literature (1,8,19), virus resistance (low virus titer) has been accompanied by what is commonly called field resistance (tolerance), expressed as reduced symptoms and losses. This is the case for the Yd_2 gene in barley. Our results suggest that the BYDV tolerance (low symptoms) and resistance (low virus titer) mechanisms studied on wheat in this report are independent. In fact, lines with high IOD values, as for example the ones derived from cv. Milan, did not always express the highest losses, and lines with low IOD values, as for example, the ones derived from cv. Baviacora, expressed losses as high as Milan and Anza populations.

In conclusion, the *T. intermedium* translocation did not negatively influence the agronomic characteristics of the recipient host plant and has the advantage of being inherited mostly in a simple Mendelian fashion. It significantly reduced the IOD values after PAV infection and was associated with lower frequencies of plants infected by both PAV and MAV. The bread wheat backgrounds tested in this study influenced the expression of the alien resistance differently. Significant reductions of yield losses were associated with the presence of the translocation, due to the synergism between tolerance and resistance and the lower infection rate in lines carrying the alien fragment. Further research should attempt to distinguish in more detail the distinct effects of the alien fragment on reducing IOD values and avoiding primary infection.

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