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A diagnostic molecular marker allowing the study of *Th. intermedium*-derived resistance to BYDV in bread wheat segregating populations

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Abstract Barley yellow dwarf (BYD) is the most important viral disease of small cereal grains. True resistance to the disease is not found in wheat (*Triticum aestivum* L.), but it has been introgressed from *Thinopyrum intermedium* (*Ti*) on chromosome 7DL of recombinant wheat lines designated TC. The objectives of our study were to identify a high through-put scoring tool for the presence of the translocated *Th. intermedium* fragment and to assess its suitability for evaluating resistance to BYDV in segregating populations. Segregation of the *Ti* fragment was followed in the F_2 population of an Anza (bread wheat) by TC14/2*Spear (TC14) cross. Resistance to BYDV isolates PAV-Mex and MAV-Mex in F_3, F_4, and F_5 populations was evaluated under field and/or greenhouse conditions by measuring the virus titers of infected plants using ELISA, and the number of infected plants per plot. The SSR marker *gwm37* was polymorphic for the translocation. In F_4 lines it was associated with the physical presence of an intact translocation on chromosome 7DL and with low virus titers of BYDV-PAV. Reductions in virus titer of 27% and 55% in the F_3 and 18% and 45% in the F_5 populations were observed when the fragment was present in the heterozygous and homozygous states, respectively, confirming a dosage effect of the resistance allele. A lower proportion of infected individuals in the field was associated with the presence of the fragment, indicating a mechanism that may interfere with aphid feeding or virus translocation within infected plants. Despite significant differences between groups with and without the fragment, the OD values of infected lines overlapped, and it was not possible to definitively detect the fragment based solely on ELISA. We conclude that *gwm37* is a reliable marker for the *Ti* translocation that will allow efficient detection of the translocation in breeding populations and greatly assist in selecting BYDV-resistant wheats in the absence of the disease.

Keywords Alien-derived resistance · Barley yellow dwarf · ELISA · *Thinopyrum intermedium* · Translocation · SSR marker

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Introduction

Barley yellow dwarf (BYD) is the most widespread and serious viral disease of small grain cereals worldwide. The disease is caused by a complex of closely related luteoviruses referred to as barley yellow dwarf virus (BYDV) and transmitted in a persistent manner (Rochow 1970) by at least 25 species of aphids (Halbert and Voegtlin 1995). Barley yellow dwarf virus infects almost all members of the Gramineae (Poaceae) family, including the most important cereal crops and grass species (Conti et al. 1990).

The most economical and practical means of controlling BYDV in cereals is to use cultivars that carry genetic resistance and/or tolerance to the virus or the aphid vectors. Plants that express true BYDV resistance can be identified by low virus concentrations (Cooper and Jones 1983) as estimated by the enzyme-linked immunosorbent assay (ELISA). Tolerant plants, on the other hand, show fewer symptoms and other adverse effects despite being infected by the virus.

Different genes for BYDV resistance and/or tolerance have been identified in barley (*Hordeum vulgare* L.) (Burnett et al. 1995) and oat (*Avena sativa* L.) (McKenzie et al. 1985). In bread wheat, no major genes for BYDV

resistance have been found (Zhou et al. 1990), although Singh et al. (1993) demonstrated that the tolerance of wheat varieties such as Anza is due to a partially effective and dominant gene named *Bdv1*.

Resistance to the disease, expressed as low virus titers, has been reported in wild relatives of bread wheat (Sharma et al. 1984, 1989; Xin et al. 1988; Banks et al. 1992), such as *Thinopyrum intermedium*, *Th. ponticum*, and *Th. elongatum*. The resistance mechanism in these wild species is believed to be associated with an inhibition of virus replication (Shukle et al. 1987; Sharma et al. 1989). Crosses between these species and bread wheat have resulted in BYDV-resistant progeny (Banks et al. 1995; Sharma et al. 1995).

Using the disomic addition line L1, derived from the TAF-46 octoploid (Cauderon et al. 1973), Brettell et al. (1988) identified a major BYDV resistance factor located on the long arm of homoeologous group 7 of *Th. intermedium* (2n=6x=42, $E_1E_1E_2E_2XX$). Using tissue culture techniques, Banks et al. (1995) transferred chromosome segments containing BYDV resistance from the L1 addition line to wheat. They obtained eight lines, commonly referred to as TC lines, containing *Th. intermedium* translocations (TC5, TC6, TC7, TC8, TC9, TC10, TC14 and 5395) carrying BYDV resistance, as determined by a decrease in virus titer. Hohmann et al. (1996) showed that line TC14 had the smallest *Th. intermedium* translocation, located in the distal 44% of the long arm of chromosome 7D.

Evaluation of some TC lines was initiated in CIMMYT in 1994; data were collected under field, greenhouse, and laboratory conditions (Bertschinger et al. 1994a,b). Later Henry (1997) showed that of the TC lines available at CIMMYT, TC14/2*Spear showed the lowest virus titers when infected with the BYDV isolates PAV-Mex and MAV-Mex; however, despite their low virus concentration, the TC lines were affected by BYDV in the field.

Transferring BYDV resistance to agronomically suitable germplasm has been slow, partly due to the laborious screening and evaluation methods that must be used when testing populations under disease pressure and different environmental conditions. Molecular markers offer an alternative screening method for monitoring the transfer of alien resistance because the technique does not require the presence of the pathogen and relies instead on identified markers linked to genes conferring resistance.

The alien fragment present in TC lines has been studied at the molecular level using restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) techniques, and at the cytogenetic level using C-banding and genomic in situ hybridization (GISH) (Banks et al. 1995, 1996; Hohmann et al. 1996; Wang and Zhang 1996). However, none of the reported markers – RFLPs: *ipsr129*, *cslH81* and *Acc2* (Banks et al. 1995); random amplified polymorphic DNA (RAPD): OPAO7 (Banks et al. 1996) – is suitable for large-scale screening. Thus, there is a need to identify markers involving easy and reliable techniques if the reported resistance is to be used more effectively in breeding programs.

The objectives of this study were (1) to identify a high through-put scoring tool for the presence of the translocated *Th. intermedium* fragment; (2) to evaluate the effectiveness of the alien resistance to Mexican BYDV isolates in bread wheat breeding populations.

Materials and methods

Plant materials

Lines carrying the *Thinopyrum intermedium* translocation (TC lines) and CIMMYT bread wheat lines were included in this study. Recombinant line TC5 and three TC14 accessions were kindly provided by Dr. Phillip Banks, CSIRO, Australia. Each of the three TC14 accessions has a different wheat parent: the Australian cultivars Spear, Hartog, and Tatiara (Banks et al. 1995).

The TC14 progenitors used for parental screening were *Th. intermedium*, the bread wheat cv. Vilmorin, and the L1 addition line which conveys the *Th. intermedium* chromosome 7Ai. Seven CIMMYT bread wheat cultivars without the alien fragment were also included: Anza, Bagula, Milan, Bobwhite, Prinia, Baviacora, and Bacanora.

We developed populations from two reciprocal crosses using TC14/2*Spear accession 289B [Spear*2/4/Vulcan.cms//L1/Millewa/3/Restorer R35733] (TC14) as the resistant parent and Anza as the tolerant parent. Of the cross Anza/TC14, a segregating F_2 population of 93 individuals was used to identify potentially useful molecular markers for the *Th. intermedium* translocation. This F_2 population was advanced to the F_3 generation in the greenhouse. The F_3 families were evaluated in field trials using BYDV inoculation. The harvested F_4 families were used for greenhouse inoculation experiments. An F_5 population of the cross TC14/Anza was developed in the greenhouse by single-seed descent (Grafius 1965; Gourmet et al. 1997). The 128 F_5 lines were tested in the field under virus inoculation, and an F_6 generation was derived to be planted in the greenhouse.

Virus isolates and BYDV inoculation

The BYDV isolates used in the experiments were collected in Mexico in 1993 and maintained in CIMMYT greenhouses. They belong to the PAV and MAV serotypes described by Rochow (1970).

Inoculations in the greenhouse were performed by infesting seedlings 7 days after planting [Zadoks' 10] (Zadoks et al. 1974) with ten viruliferous aphids that had acquired BYDV by feeding on infected plants for 48 h. Seedlings were isolated from each other by transparent plastic tubes. After a 3-day inoculation period, aphids were killed by insecticide (Metasystox®) application.

In the field, plants at the three-leaf stage [Zadoks' 13] were infested with aphids reared in the greenhouse on BYDV-infected plants. To avoid contamination, we sprayed the non-inoculated treatments with insecticide (Metasystox®) soon after emergence, whereas in the other treatments, approximately ten aphids were deposited at the base of each seedling using the calibrated mechanical dispenser (''bazooka'') described by Mihm (1983). Aphid species *Rhopalosiphum padi* and *Metopolophium dirhodum* were used for inoculation with PAV-Mex and MAV-Mex, respectively. A second inoculation was conducted 18 days later, and aphid movement was controlled starting 1 week after that by means of 2-weekly insecticide applications (Metasystox®), including the non-inoculated plots.

Evaluation of BYDV resistance

ELISA (enzyme-linked immunosorbent assay)

Double Antibody Sandwich-ELISA (DAS-ELISA) was used as described by Clark and Adams (1977), with modifications in the incubation times, antibody concentration, and sample dilution. The volume of sample and incubating solutions used was 100 µl instead of 200 µl in all steps. Polystyrene microtiter plates (NUNC) were incubated at 37°C for 3 h with a coating of polyclonal antibodies (1 ng/µl) directed against US PAV or MAV isolates (provided by K. Perry, Purdue University). Plant sap (1:10, in 0.1 *M* phosphate buffer pH 7.0) was incubated for 3 h at 37°C. Controls used consisted of phosphate buffer (blank), sap from non-inoculated plants, and infected PAV or MAV plant extract. Alkaline phosphatase-labeled polyclonal antibodies (1:1000), either anti-PAV or anti-MAV, were incubated overnight at 4°C; P-nitrophenyl phosphate substrate (Sigma) was then added. Optical density (OD) was measured at 405 nm after 1 and 2 h of incubation at room temperature using an MR 700 Microplate reader (Dynatech Laboratories).

A sample was considered infected when the OD value was higher than a fixed threshold, calculated for each population based on the method described by Sutula et al. (1986). Usually the threshold was two to three times the average OD of the non-infected samples, depending on the population and the quality of the test. Using that OD as the reference value, we calculated the number of infected plants (Nb+) per family and the average OD of the infected plants (IOD).

Molecular markers for the *Th. intermedium* translocation

The search for markers suitable for assessing the presence of the *Th. intermedium* translocation in our bread wheat material was carried out with DNA from *Th. intermedium*, L1, TC5, three TC14 accessions, and the eight wheat varieties mentioned above. Thirty RFLP probes and 30 microsatellites (SSRs) known to map at group 7 were used (Lagudah et al. 1991; Nelson et al. 1995; Röder et al. 1995; Hohmann et al. 1995; Korzun et al. 1997). Some of the RFLPs were shown by Banks et al. (1995) and Hohmann et al. (1996) to detect the *Ti* translocation in TC families. We wanted to verify that the polymorphism held up in our material and to transform these RFLPs into PCR-based markers if no reliable SSR marker was found.

RFLPs

DNA was extracted using the CTAB method on lyophilized ground tissue as described by Saghai-Maroof et al. (1984) and modified by Hoisington et al. (1994). Four restriction endonucleases (*Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I), were used for RFLP analysis with the digoxigenin-CSPD chemiluminescence method (Hoisington et al. 1994).

SSRs

Since small amounts of DNA are required for PCR analysis, we used a fast sap extraction method based on Clarke et al. (1989) when genomic DNA extracted by the lyophilized ground tissue method was not available. In the latter method, sap was extracted from a single freshly harvested leaf with CTAB buffer using a sap extractor (MEKU Erich Pollahne). A 30-min incubation period at 65°C was followed by chloroform-octanol extraction and DNA precipitation. Four microliters of sap-extracted DNA (approximately 20 ng/µl DNA) was used in a 20 µl PCR reaction (2.5 m*M* $MgCl_2$, 0.2 µ*M* of each dNTP, 0.25 µ*M* of each primer, 1× *Taq* buffer [Mg free], and 1 U of *Taq* enzyme). Amplification was performed in an ERICOMP, TwinBlock™ System thermocycler, as follows: one 1-min cycle at 93°C; 30 cycles of 1 min at 93°C, 2 min at 52°, 55°, or 60°C (depending on the individual microsatellite marker), and 2 min at 72°C; and a final 5-min extension step at 72°C. The annealing temperature was varied for each SSR, based on published reports (Plaschke et al. 1995; Röder et al. 1995; Korzun et al. 1997). Amplification products were separated on 3% agarose gels using a mixture of 1:1 Metaphor® and Seakem® and visualized with ethidium bromide under UV light.

Linkage analysis

The microsatellite and RFLPs found to be polymorphic in previous tests were used to genotype the Anza/TC14 F_2 population. Molecular and phenotypic (field) data were jointly analyzed using single factor analysis of variance (PROC GLM, SAS, 1997) in order to determine the association between the variation observed in the field experiments and the presence of the alien fragment.

Validation of the SSR marker with the physical presence of the translocation and BYDV resistance

To validate that the SSR marker was reliable and diagnostic for the presence of the introgression carrying resistance to BYDV, we carried out C-banding and fluorescent in-situ hybridization (FISH) on some F_4 lines.

Nineteen families of the Anza/TC14 F_4 population with the alternative alleles of *Xgwm37* were chosen. Ten seeds per family were planted in the greenhouse, and root tips of 2 plants per family were sampled for cytogenetic detection of the alien fragment. Eight plants were inoculated with PAV-Mex, and each plant was sampled individually at 13 and 24 days after inoculation to measure virus concentration using ELISA. One leaf of each of the 10 plants was harvested for confirmation of the presence of the alien fragment by PCR using *gwm37*. The collected root tips of F_4 seedlings were processed according to the procedure of Mujeeb-Kazi and Miranda (1985). For C-banding the protocol of Jahan et al. (1990) was followed. The FISH procedures used were essentially those of Islam-Faridi and Mujeeb-Kazi (1995).

Phenotypic expression of resistance

Field testing

To test the expression of BYDV resistance under field conditions, we conducted trials with virus infection at CIMMYT's experiment station at El Batán, Mexico (19°N; 2,249 m elevation; 650 mm mean annual rainfall) during the 1998 summer season. Anza/TC14 F_3 and TC14/Anza F_5 populations were planted in an alpha-lattice design with two replications. Each line was sown in a plot consisting of an 80-cm double row, 8 plants per row, for a total of 16 plants, maintaining 75 cm between plots. Three treatments were applied: (1) non-inoculated, (2) inoculated with PAV-Mex, and (3) inoculated with MAV-Mex. Treatments were separated into three blocks and isolated by two rows of oat plants to avoid cross contamination.

Six and three plants per family of the F_3 and F_5 populations, respectively, were tagged using colored plastic ribbons. Twenty-four days after the second inoculation, sampling was done for virus titer measurement through ELISA by collecting the flag leaf–1 of the tagged plants.

F_6 seeds from TC14/Anza F_5 plants were grown in the greenhouse for tissue sampling and PCR testing. Bulk samples of six leaves per line (one leaf per plant) were collected for DNA extraction. Preliminary experiments showed that the alien fragment was detectable when diluted up to a 1:5 ratio (TC14: Anza).

Correlation between field and greenhouse resistance

A greenhouse experiment was conducted with 93 field-harvested F_4 families derived from the F_3 Anza/TC14 experiment to deter-

mine how virus titers obtained under field conditions correlate with titers obtained in the greenhouse from lines with and without the alien fragment. Ten seeds per F_4 family were planted in the greenhouse. Eight plants were infested with ten viruliferous aphids carrying PAV-Mex; 2 non-inoculated plants were used as healthy controls. Seventeen days after inoculation, each plant was sampled separately for ELISA, and two bulks of four leaves each (one leaf from each plant) were used to extract DNA for genotyping with *gwm37*.

Statistical analysis

For each population, data were analyzed by grouping lines based on the presence or absence of the *Th. intermedium* fragment as assessed with *gwm37* (*titi*: no alien heterochromatin; *Titi*: alien fragment in heterozygous state; and *TiTi*: alien fragment in homozygous state). Simple ANOVAs using PROC GLM (SAS 1997) were carried out to obtain averages by treatments (non-infected, infected with PAV-Mex, and infected with MAV-Mex) for the genotypic groups (*titi*, *Titi*, and *TiTi*) and to compare their adjusted means using Tukey's student test (Steel and Torrie 1981). A correlation analysis using the original data was performed between the IOD obtained in the field (Anza/TC14 F_3) and in the greenhouse (Anza/TC14 F_4) under PAV-Mex inoculation.

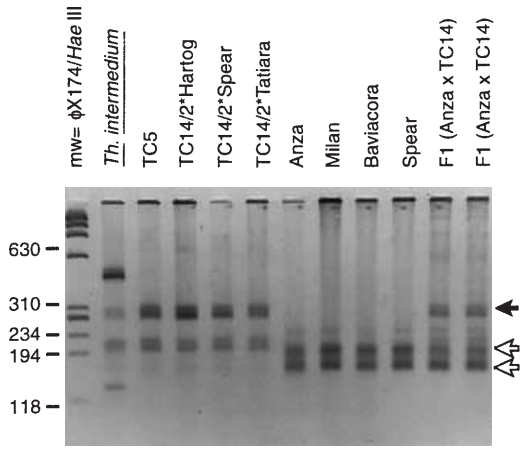

Fig. 1 PCR amplification products obtained using SSR marker *gwm37*, specific for chromosome 7DL, from genomic DNA. *Black arrow* indicates a band of approximately 300 bp amplified from the *Ti* translocation on chromosome 7DL; *white arrows* indicate bands of 194 and approximately 180 bp amplified from wheat chromosome 7DL. *mw* ϕx174/*Hae*III

Results

Molecular markers for the *Th. intermedium* translocation in bread wheat

Of the 30 RFLP probes tested, 13 detected *Th. intermedium*-specific fragments in TC5, and 7 detected them in TC14. The probes *psr548, ksuD2, fbb189,* and *ksuA1* were scored as dominant markers for the wheat allele. In the Anza/TC14 F_2 population, probes *psr129* and *cslH81* were scored as co-dominant markers. The probe *psr72* did not give a good hybridization signal in the population and was not analyzed further.

Of the 30 SSR primer pairs used, 3 detected polymorphisms between wheat and both TC5 and TC14 lines (*gwm37*, *gwm46*, and *gwm302*). Among those, the first amplified two bands in *Th. intermedium* materials and two other bands in common bread wheats (Fig. 1) and was scored as a co-dominant marker in the Anza/TC14 F_2 population. This marker was chosen for further population screening because it successfully detected the introgressed *Th. intermedium* segment in both homozygous and heterozygous plants and because PCR is a simpler test than RFLP assays.

Because of the favorable quality of this PCR marker, we did not proceed with our initial attempts at developing a PCR-based assay for the *psr129* locus.

Standard linkage analyses with the five markers (1 SSR: *gwm37*; 4 RFLPS: *psr129, psr548, ksuD2,* and *cslH81*) in the F_2 population showed a very tight linkage among them. The other two RFLP markers (*fbb189* and *ksuA1*) were not linked to the group of five markers.

The single factor analysis of variance showed high association of the five markers with both resistance traits, IOD, and number of infected individuals. The presence of the alien fragment was found to account for a decrease in virus titer (IOD) for PAV-Mex, but not for MAV-Mex, and explained about 35% of the phenotypic variation (σ_P^2) for such a trait (P = 0.0001). That percentage decreased to about 29% when the trait was measured in the greenhouse.

Another possible factor in the *Th. intermedium* translocation explained 43% and 48% of σ_P^2 for the percentage of infection with PAV-Mex and MAV-Mex, respectively (P = 0.0001).

Validation of the SSR marker with the physical presence of the translocation and BYDV resistance

In order to validate the PCR marker with the physical presence of the *Ti* translocation, we analyzed cytogenetically 2 plants from each of the 19 F_4 families (Anza/TC14) segregating for the *gwm37* marker. The 12 *titi*, 16 *Titi*, and 10 *TiTi* plants based on the *Xgwm37* alleles (*ti* = wheat allele, *Ti* = *Th. intermedium* allele) showed with both C-banding and FISH no translocation, one *Ti* fragment, and two copies of the translocation, respectively. Both C-banding and FISH showed that the distal portion of wheat chromosome 7DL was replaced (approx. 30%) by alien heterochromatin from *Th. intermedium* as described by Hohmann et al. (1996).

The ELISA results on the 19 F_4 lines tested with PAV-Mex showed that lines carrying the alien fragment had lower virus titers and showed a slower increase in virus concentration than lines not carrying it (Fig. 2).

Association of the *Th. intermedium* translocation with BYDV resistance in segregating populations

Resistance to BYDV was evaluated in the two reciprocal populations Anza/TC14 and TC14/Anza, based on virus

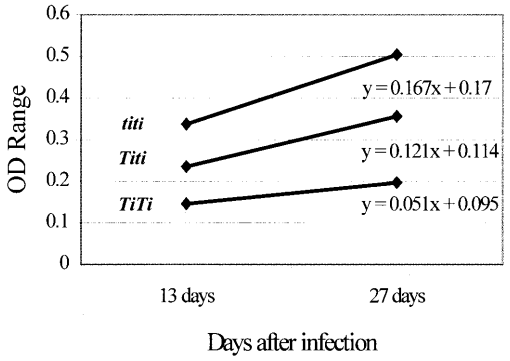

Fig. 2 Rate of virus titer increase in 19 families of the Anza/TC14 F_4 population inoculated with BYDV-PAV-Mex isolate under greenhouse conditions. Variation shown for three genotypic groups: *TiTi*, *Titi*, and *titi*. *Ti*, presence of *Thinopyrum intermedium*, fragment, *ti* absence of *Th. intermedium* fragment. The equation for each line is indicated

titers as measured by ELISA. In general, OD values were very variable among individuals of the same segregating line, and among lines of the same genotypic group (based on *Th. intermedium* content). Similar variation in virus concentration was found among individuals of the parental line TC14/2*Spear. Data of lines not containing the fragment (*titi*) and those carrying it in the heterozygous (*Titi*) and homozygous (*TiTi*) state (based on the allelic composition of locus *Xgwm37*) are presented in Table 1.

When Anza/TC14 F_3 and TC14/Anza F_5 individuals were inoculated with PAV-Mex under field conditions, the lowest IOD values were obtained for the *TiTi* group, followed by the heterozygous group and then by homozygous (*titi*) susceptible lines. Average IODs of the Anza/TC14 F_4 population evaluated under greenhouse conditions were also significantly different among the three groups, with the homozygous *TiTi* having the lowest IOD and the homozygous *titi* the highest. However, under MAV-Mex field infection, no significant IOD differences were observed among the three groups in both the F_3 and F_5 populations. When the IOD (PAV-Mex) obtained under field and greenhouse conditions was related to the presence of the alien fragment, a highly significant negative correlation was observed in both cases, -0.60 and –0.53, respectively (P = 0.0001) (Table 1). The correlation between field IODs and greenhouse IODs for 93 individuals was also quite high (r = 0.5; P = 0.0001).

Significant differences were observed in the percentage of infected plants (as determined by ELISA) among the three genetic groups, regardless of the virus isolate used (Table 1). There were more non-infected plants in the homozygous *TiTi* group than in the heterozygous group, and many more than in the homozygous *titi* group, even though all plots were inoculated twice. However, when the population Anza/TC14 was inoculated with PAV-Mex in the greenhouse, almost all plants in the three groups were infected (though with varying titers). There was a slight but significant difference among the *titi*, *Titi*, and *TiTi* groups (Table 1). A high

Table 1 Virus titers and percentage of infected individuals in two populations inoculated with BYDV isolates PAV-Mex and MAV-Mex under field conditions, and of one population tested with PAV-Mex under greenhouse conditions. Results are presented for three genotypic groups (*titi*, *Titi*, and *TiTi*) based on the presence/absence of the *Thinopyrum intermedium* fragment as determined by the allelic composition of locus *Xgwm37*

Population	Genotypic group[a]	Number of lines	PAV-Mex		MAV-Mex	
			IOD[b]	Percentage infection[c]	IOD	Percentage infection
F_3 Anza/TC14 (Field)	*titi*	23	0.579±*0.161* a[e]	90.0±*14.5* a	0.485±*0.071* a	78.3±*17.7* a
	Titi	51	0.422±*0.126* b	58.3±*17.1* b	0.467±*0.057* a	53.3±*16.1* b
	TiTi	19	0.263±*0.113* c	28.3±*16.4* c	0.433±*0.100* a	26.7±*17.1* c
	Threshold		0.076		0.082	
	Correlation[d]		–0.60**	–0.78**	–0.16	–0.72**
F_4 Anza/TC14 (Greenhouse)	*titi*	22	0.400±*0.124* a	100.0±*3.7* a		
	Titi	51	0.330±*0.089* b	97.5±*5.4* a		
	TiTi	20	0.221±*0.079* c	95.0±*10.3* b		
	Threshold		0.080			
	Correlation		–0.53**	–0.25*		
F_5 TC14/Anza (Field)	*titi*	72	0.477±*0.154* a	83.3±*9.1* a	0.491±*0.169* a	80.0±*12.7* a
	Titi	12	0.381±*0.114* b	76.7±*10.9* a	0.477±*0.174* a	60.0±*9.1* b
	TiTi	44	0.288±*0.123* c	46.7±*13.6* b	0.459±*0.182* a	36.7±*13.6* c
	Threshold		0.136		0.136	
	Correlation		–0.52**	–0.60**	–0.07	–0.63**

[a] *Ti*, Presence of the *Th. intermedium* fragment; *ti*, absence of the *Th. intermedium* fragment

[b] IOD, Average optical density of infected individuals (i.e., individuals with values above the healthy threshold); standard deviation values in italics

[c] Percentage of infected individuals within each group; standard deviation values in italics

[d] Correlation with presence of the alien fragment * P = 0.05; ** P = 0.001

[e] Comparison by Tukeys test: values followed by the same letter indicate that there is no difference between groups at the 0.05% level of significance

negative correlation was found in the field between the percentage of infected plants and the presence of the *Th. intermedium* translocation ($r = –0.78$), as seen above for virus concentration. A lower correlation between the number of infected individuals and the presence of the alien fragment ($r = –0.25$, $P = 0.014$) resulted when greenhouse data were analyzed.

Discussion

Molecular markers linked to the *Th. intermedium* translocation

Five molecular markers were found to be useful in identifying the *Th. intermedium* translocation carrying BYDV resistance: four RFLPs and one SSR. The position of the four RFLPs (*psr129, psr548, ksuD2,* and *cslH81*) coincides with the reported physical map of chromosome 7DL (Hohmann et al. 1995), and three of them coincide with their genetic position in TC lines (Banks et al. 1995; Hohmann et al. 1996). SSR *gwm37,* which mapped at the same position as the four RFLP markers, has been reported as being in the distal portion of 7DL, close to the *ksuE3* probe (Röder et al. 1998).

The five markers were very tightly linked and inherited as a block. Incomplete chromosome pairing in heterozygous individuals of the Anza/TC14 population carrying a *Th. intermedium* segment is likely to be due to the relatively large phylogenetic distance between the X (= St) genome of the *Th. intermedium* translocated segment and the D genome of common bread wheat (Sharma et al. 1984; Einzega 1987; Friebe et al. 1992; Wang and Zhang 1996). In such cases, the entire alien fragment is inherited as a block of genes, as demonstrated by Schachermayr et al. (1995) while searching for molecular markers for the *Lr24* leaf rust resistance gene carried in an *Agropyrum elongatum* translocation in wheat.

The absence of wheat bands (as detected with *gwm37*) in homozygous resistant individuals was associated with the replacement of a portion of wheat chromosome 7D by *Th. intermedium* heterochromatin, as reported by Hohmann et al. (1996). There was a complete match between PCR results and the physical presence/absence of the translocation.

Although in general SSR markers have been reported to be species- and even genome-specific in wheat, *gwm37,* which was isolated from wheat, gave good amplification products with *Th. intermedium*-derived materials, especially when the annealing temperature was decreased from 60°C (Korzun et al. 1997) to 52°C. SSR marker *gwm37* could also be used to detect the *Th. intermedium* translocation in other TC families given that the RFLPs that clustered with the SSR in the F_2 population have been previously reported to identify the translocation in those TC families (Banks et al. 1995; Hohmann et al. 1996).

Association of the *Th. intermedium* translocation with BYDV resistance in segregating populations

The single point analysis of variance showed that the region of the *Th. intermedium* translocation in the Anza/TC14 population influences four of the five traits analyzed (IOD in both field and greenhouse with PAV-Mex, and number of infected plants following both PAV-Mex and MAV-Mex infection).

The IOD values of individual lines in the three genotypic groups (*titi, Titi,* and *TiTi*) showed considerable overlap. The range of IOD under field conditions was *titi* = 0.205–1.061, *Titi* = 0.222–0.680, and *TiTi* = 0.123–0.579. Therefore, it was not possible to determine whether or not an individual carried the *Th. intermedium* fragment based solely on virus titer. However, when average IODs for lines inoculated with the PAV-Mex isolate were computed and compared for the three genotypic groups, differences among the groups were evident, significant, and consistent across populations. It is clear, then, that the identified markers made it possible to select individuals carrying the *Th. intermedium* translocation.

The three groups (*titi, Titi, TiTi*) of lines did show significant OD differences when infected with PAV-Mex, but not MAV-Mex. This suggests that resistance carried in the alien translocation is more effective against PAV-Mex than MAV-Mex and that it may be strain-specific, or that the antibodies for MAV were less able to discriminate differences in virus concentrations compared to antibodies for PAV.

The differences in virus titers observed with PAV-Mex confirm the dosage effect of the alien heterochromatin suggested by Banks et al. (1995). Resistance (low virus titer) was more evident in lines homozygous for the alien fragment than in heterozygous lines, with both groups being more resistant (lower titers) than lines lacking the entire fragment. In that respect, our results also agree with those of Fraser (1990) and Sharma et al. (1989), who indicated the possible dosage effect of BYDV resistance genes derived from wheatgrass species. According to Fraser (1990), when the gene dosage effect is operative, the virus will be partially localized or allowed to spread throughout the plant but will be inhibited in its overall multiplication.

Besides resistance being expressed as reduced virus titer, a lower number of infected individuals were associated with the alien fragment under both PAV-Mex and MAV-Mex infection. The percentage of phenotypic variance explained by the *Th. intermedium* fragment was highest (48% and 43%, respectively) ($P = 0.0001$) for the number of infected individuals. This phenomenon was previously observed in TC14 lines, but in bigger plots (Henry and Segura 1999), and suggests that plants carrying the alien fragment are more likely to escape infection, or that translocation of the virus within plants is limited by the resistance factor. This relationship seems to indicate the existence of a mechanism that interferes with aphid transmission and, possibly, feeding. A similar

mechanism was reported by Shukle et al. (1987), who observed a low infection rate in *Agropyron* species inoculated with BYDV in greenhouse experiments. In this case, the alien fragment may make plants unpalatable to aphids, which as a result merely probe the plants but do not feed on them. This may be the mechanism by which plants escape infection, since BYDV is not acquired nor transmitted during short feeding periods. On the other hand, the high number of infected plants in the greenhouse may be explained by the 3-day period of aphid confinement on individual plants. This is also in agreement with D'Arcy (1995), who, when referring to studies of insect host range, notes that aphids are more selective when free than when confined to a certain grass.

We have confirmed that the BYDV resistance reported in TC14/2*Spear is effective against Mexican isolates of BYDV as evidenced by a reduction in virus titer and a lower number of infected plants. The reduction in virus titer was effective with the PAV-Mex isolate, which is the most aggressive one in the study area (Henry and Segura 1999). The PAV strain is also the most common globally (Lister and Ranieri 1995).

The *gwm37* SSR marker reported in this study is a reliable selection tool for manipulating the *Th. intermedium*-derived resistance in backgrounds carrying tolerance genes. The marker would facilitate large-scale screening that does not require the presence of the pathogen and is thus less complicated than conventional screening methods. Finally, since tests can be conducted at early stages of crop growth, breeders would have time for making crossing and selection decisions without worrying about environmental conditions that may affect BYDV resistance selection in the field.

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