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ORIGINAL PAPER

# Molecular markers show a complex mosaic pattern of wheat-*Thinopyrum intermedium* translocations carrying resistance to YDV

**Ligia Ayala-Navarrete · N. Thompson · H. Ohm · J. Anderson**

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**Abstract** *Thinopyrum intermedium* translocations derived from the wheat (*Triticum aestivum* L.) substitution line P-29 were previously characterized by RFLP. We have further analyzed these lines and additional related germplasm with publicly available STS and SSRs. Primers which showed a polymorphism between wheat and P-29, were tested in all recombinant and nulli-tetrasomic lines confirming their position on chromosome 7D. The resulting 7D/7E chromosome maps appeared as a mosaic of wheat and *Th. intermedium* chromatin sections. To verify the composition of the translocation lines suggested by the RFLP-PCR map, $F_2$ progeny of two crosses (CS/216-1 and CS/260-1) were analyzed with molecular markers. Both populations gave an unexpectedly diverse number of recombinant individuals, suggesting that interstitial translocations occur more frequently than previously thought. This analysis also showed that there is a wide range in the number and position of the interstitial translocations within a given line such as the mosaic chromosome in recombinant line 260-1/CS-26, which has four *Th. intermedium* chromosome segments. Phenotypic data of the two populations suggested the presence of one gene which we have called *Bdv3* to differentiate it from the previously reported orthologous gene *Bdv2*. Using the PCR-based molecular markers identified in this study, 5 out of 12 elite lines that showed good yields and no YDV symptoms contained *Th. intermedium* chromatin. Due to the multiple components involved in the YDV disease complex, combining selection for YDV resistance with the molecular markers and maps identified in this study will increase the efficiency of introgressing *Th. intermedium* chromatin containing YDV resistance or other beneficial traits into elite wheat germplasm.

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L. Ayala-Navarrete · H. Ohm · J. Anderson
Agronomy Department, Purdue University,
915 W State Street, West Lafayette, IN 47907-2054, USA

N. Thompson · J. Anderson
United States Department of Agriculture,
Agricultural Research Service (USDA-ARS),
915 W. State Street, West Lafayette, IN 47907-2054, USA

L. Ayala-Navarrete (✉)
Plant Industry CSIRO Clunies Ross and Barry Drive,
Canberra 2601, Australia
e-mail: Ligia.Ayala-Navarrete@csiro.au

N. Thompson
BSES Limited 50 Meiers Road,
Indooroopilly, QLD 4068, Australia

## Introduction

Barley yellow dwarf virus (BYDV) and cereal yellow dwarf virus (CYDV) are members of the *Luteoviridae* family causing the yellow dwarf virus disease (YDV) in a range of cereal crops. Reduced virus titer resistance to YDV (Cooper and Jones 1983) has not been found in wheat (*Triticum aestivum* L.) in contrast to other cereals such as barley (*Hordeum vulgare* L.) (Burnett et al. 1995). However, resistance derived from the wheat tertiary gene pool has been available for more than a decade (Banks et al. 1993, 1995; Larkin et al. 1995). YDV resistance in cultivated wheat was derived from several species of the genus *Thinopyrum* along with other beneficial traits (Sharma et al. 1984, 1989; Xin et al. 1988; Brettel et al. 1988). This source of resistance has been tested in several geographical areas to control a variety of YDVs (Barloy et al. 2003; Zhang et al. 2009).

Breeding for YDV resistance is one of the main objectives in the Cooperative USDA/ARS-Purdue University Winter Wheat Breeding Program. To achieve this objective, YDV resistance was introgressed into wheat by crossing with *Thinopyrum intermedium* and subsequent backcrossing the $F_1$ plants to wheat (Sharma et al. 1995). Molecular markers, bioassays, and cytogenetic analyses confirmed that resistance to the virus was due to substitution of chromosome 7E from *Th. intermedium* for 7D of wheat (Francki et al. 1997). Reduction of CYDV titers in plants carrying chromosome 7E was found to be 42–52% in leaves and stems when compared with the YDV susceptible wheat Abe (Anderson et al. 1998). In order to utilize this trait and avoid undesirable genetic drag, disomic substitution and monosomic addition lines were exposed to $\gamma$-irradiation. Selection of recombinants was based on virus bioassays and RFLP markers (Sharma et al. 1999), and a map was constructed showing the position of translocated fragments in a set of susceptible and resistant translocation lines (Crasta et al. 2000).

Several types of markers have been used to characterize *Th. intermedium* translocations in wheat backgrounds. Morphological markers allow the selection of individuals carrying foreign chromatin (Banks et al. 1995) but do not delineate their genetic constitution. Restriction fragment length polymorphisms (RFLPs) are the most abundant markers on the wheat map due to their consistency and their ability to determine heterozygotes. RFLP maps have, therefore, become the framework of choice for incorporating new markers. The main drawbacks of using RFLPs are that they typically identify just one or two polymorphisms, are laborious and time consuming. Simple sequence repeat markers (SSRs) are often genome specific, suitable for large-scale screening, and therefore are better suited for the study of traits in wheat breeding.

In our investigation we added PCR markers to the RFLP-based map from *Th. intermedium*/wheat $M_4$ recombinant lines (Crasta et al. 2000). Additionally two $M_4$ lines, showing unexpected RFLP-marker segregation patterns were crossed with wheat. The $F_2$ and $F_3$ populations were examined for the presence of *Th. intermedium* segregating fragments and resistance to YDV, respectively. The diagnostic markers were used to identify and characterize plant selections in the field that contained chromatin derived from *Th. intermedium*.

## Materials and methods

### Plant material

The plant genotypes used in these experiments included *Th. intermedium* and several wheat/*Th. intermedium* derivatives; P-107, a wheat addition line carrying chromosome 7E; P-29, a wheat disomic substitution line carrying chromosome 7E instead of chromosome 7D; a set of recombinant lines, and the parental (*Triticum aestivum* L.) lines: Abe, Purdue line 81401, Caldwell, Compton and Chinese Spring (CS). The recombinant lines were derived from a cross of P-29 or P-107 and the parental wheat lines listed above following $\gamma$-irradiation of the $F_1$ seed (Sharma et al. 1999). In this work we used four susceptible recombinant lines: 177-1, 317-1, 635-2 and 103-2 plus seven resistant recombinant lines: 216-1, 255-1, 260-1, 331-1, 632-21, 82-1, and 283-1 (Crasta et al. 2000). Seeds from all the individuals were grown in the greenhouse and green tissue from 3-week-old plants was harvested for DNA extraction. Nulli-tetrasomic lines for chromosomes 7A, 7B, and 7D in a CS background were used to confirm the position of the SSR markers in wheat.

The segregation of *Th. intermedium* fragments was studied in $F_2$ plants from a CS/line 216-1 cross and a CS/260-1 cross. Resistance to the virus was studied in $F_3$ families of the cross CS/216. Several elite wheat lines derived from P-29 or P-107 were also tested with the polymorphic PCR markers to determine the presence and size of the alien fragment. Some of these lines were derived from the $M_4$ lines, which were selected in early generations with molecular markers (Crasta et al. 2000), then crossed to wheat and further evaluated, and selected under field conditions.

### Location of PCR markers in the wheat/*Th. intermedium* RFLP map

Fifty-seven SSR and STS primers (Roder et al. 1998; Pestsova et al. 2000; Gupta et al. 2002) were tested for polymorphisms in the parental lines. Primers which showed a polymorphism were tested in all the recombinant and nulli-tetrasomic lines. The genomic DNA was extracted from frozen tissue using the CTAB method (Saghai-Maroof et al. 1984). Approximately 30 ng of genomic DNA was used in a 20-µl PCR reaction containing 1.25 mM $MgCl_2$, 250 µM of each dNTP, 0.5 µM of each primer, 1× Taq reaction buffer [Mg free], and 1U of Taq enzyme (Promega). The amplification was carried out in a MJ-100 thermocycler with 1 cycle of: 95°C for 4 min, 35 cycles of: 94°C for 50 s, 50–58°C for 55 s, and 72°C for 1 min; one cycle of 72°C for 6 min. For the primer *Xgwm121*, a hot start Taq enzyme (Qiagen) was used.

### Segregation of *Th. intermedium* fragments from recombinant lines

PCR molecular markers previously mapped on susceptible and resistant $M_4$ recombinant lines (Crasta et al. 2000), were used to test the following: 44 individuals of the cross CS/line 260-1; 107 individuals of the cross CS/line 216-1,

and; 12 elite lines from the Purdue University USDA-ARS Small Grains Breeding Program.

DNA, from the two populations, was extracted in 200 µl PCR tubes from a piece of leaf 1 cm × 5 mm long with 30 µl of TPS buffer (100 mM Tris–HCl pH 9.5, 1 M KCl, 10 mM EDTA pH 8.0; Thomson and Heney 1995). The samples were boiled for 20 min at 95°C and stored at −20°C from 30 min to 24 h. The extract was diluted 1:10 in $ddH_2O$ and 4 µl was used in the PCR reaction. The same PCR reaction conditions as described above were used for the SSR analysis with the addition of PVP-40 [1 mg/ml], and BSA [1 mg/ml], to neutralize PCR inhibitors present in the leaf extracts (Xin et al. 2003).

### Evaluation of BYDV resistance

Resistance to the virus was evaluated with ELISA (Anderson et al. 1998) under artificial infestation. Seventy-four $F_3$ families of the cross CS/216-1 were infested with viruliferous aphids (*Rhopalosiphum padi* L.). At least ten viruliferous aphids containing CYDV-RPV were sprinkled in each plant growing in flats contained in growth chambers. The aphids were killed with insecticide Malathion after a 2-day feeding period, and the plants were incubated for an additional 14 days.

After the incubation period, plant tissue above the crown was harvested and sap extracted to perform ELISA with polyclonal antibodies against CYDV-RPV as described by Anderson et al. (1998). The Absorbance A460 values from two replicate wells for each plant were averaged and an absorbance <0.10 indicated resistance whereas those plants with a OD > 0.1 were susceptible. $F_3$ families containing three or more individual plants were considered for the analysis. Families in which all the individuals showed resistance were considered to be homozygous for resistance and similarly families with all susceptible individuals were considered to be homozygous for susceptibility. Families with at least one individual showing different reaction (resistance or susceptibility) were considered to be heterozygous, therefore segregating for the YDV resistance trait.

## Results

### Location of PCR markers in the wheat-*Th. intermedium* RFLP map

Of the 57 SSR and STS primers specific for chromosome 7D of wheat, 12 SSRs, and one pair of STS primers showed polymorphism between wheat and the substitution line P-29. All 12 SSRs were confirmed to be located on chromosome 7D as tested on the nulli-tetrasomic lines. In each case, the diagnostic band for chromosome 7D was present in the N7AT7B and N7BT7A and absent in N7DT7B lines (data not shown). The majority of SSRs behaved as dominant markers for wheat. Consequently, the absence of the wheat-specific band in the substitution line P-29 and in the recombinant lines was recorded as presence of *Th. intermedium* chromatin. Two of the 12 SSRs were co-dominant (*Xgwm295* 7DS and *Xgwm37* 7DL) amplifying two different size bands, for the 7D and 7E genomes (Fig. 1).

In most cases the position of the SSR markers along the chromosome 7D (Fig. 2) confirmed the results obtained by RFLP (Crasta et al. 2000). However, some markers revealed regions of wheat DNA embedded within *Th. intermedium* chromatin: lines 177-1, 317-1, 635-2, and 331-1 on the short arm, and lines 632-21 and 82-1 on the long arm. Likewise, the PCR markers have revealed *Th. intermedium* segments not evident on the RFLP map, for example, on the short arms of lines 103-2 and 283-1.

Lines 320-1 and 331-1 appeared to have a similar genetic constitution, based on the RFLP map (data not shown). Differences between them were revealed when the PCR markers were integrated into the map. New interstitial regions containing wheat or *Th. intermedium* chromatin, not previously identified with RFLPs, were identified in all the recombinant lines, except for 260-1, 216-1 and 255-1. Of the newly identified translocations, this analysis identified a higher number of wheat sections compared to *Th. intermedium* sections.

### Segregation of *Th. intermedium* fragments from translocation lines

To verify the position of the *Th. intermedium* blocks suggested by the RFLP-PCR map, we analyzed with molecular

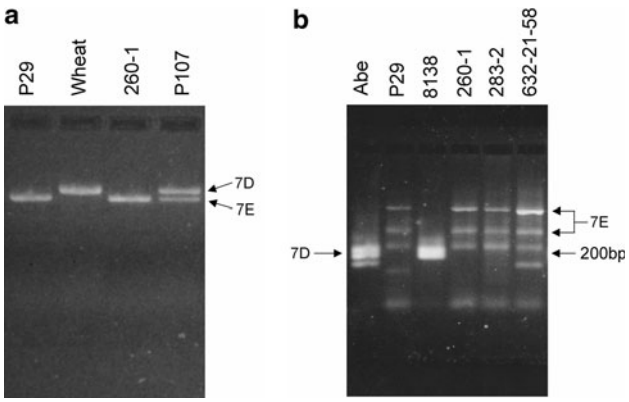


**Fig. 1** Codominant microsatellites showing diagnostic bands for 7E and 7D chromosomes. **a** *gwm295* shows the presence of both 7D and 7E bands indicated by *arrows* in the addition line P107 indicating the co-dominant character of this marker. **b** *gwm37* is diagnostic for *Th. intermedium* chromatin present in P29 and P29 derived lines: *arrows* indicate the wheat 7D diagnostic bands present in *wheat lines* Abe and 8138 and *Th. intermedium* 7E diagnostic bands in P29 and three *recombinant lines*

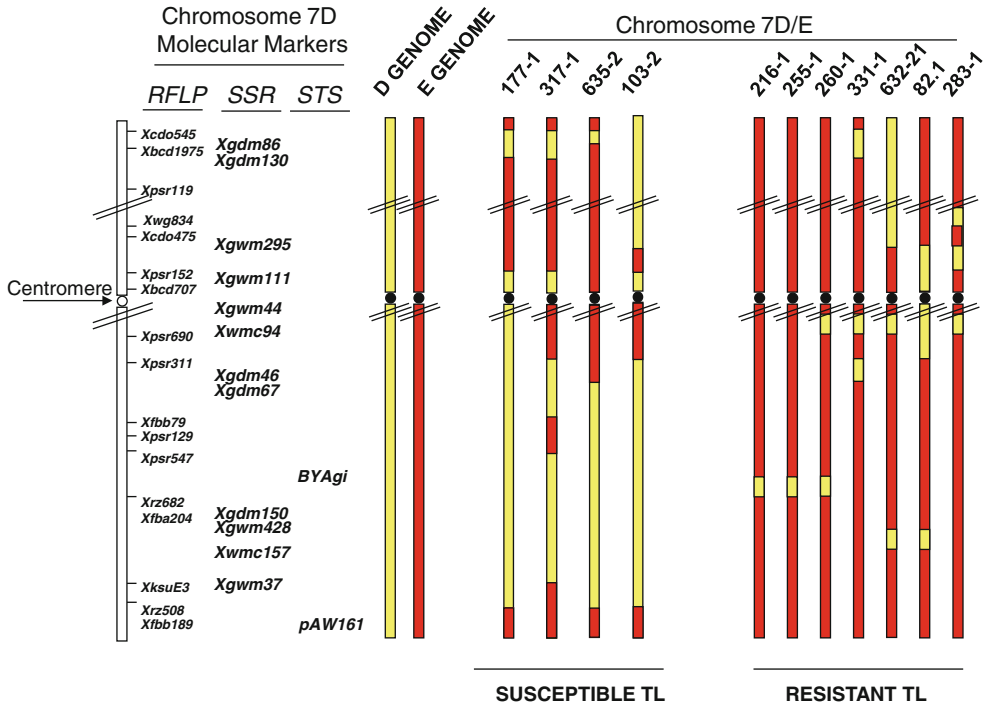


**Fig. 2** Location of *Th. intermedium* and wheat segments along chromosome 7D of M4 translocation lines characterized for introgressing resistance to BYDV. RFLP markers shown at the *left* were taken from Crasta et al. (2000). PCR derived markers used in this study are shown in *bold* at the *right*. The genomic composition of each individual has been schematized by representing the presence of *wheat markers* (*grey*/*yellow*) and *Th. intermedium* markers (*dark*/*red*) as different segments on the chromosome. Susceptible and resistant translocation lines (TL), as determined by ELISA, are grouped together. Transversal lines along the schematized chromosome indicate shortened regions in the whole representation

markers the $F_2$ progeny of two crosses: CS/216-1 and CS/260-1. These lines were chosen because both contained mainly *Th. intermedium* chromatin according to the map RFLP-SSR (Fig. 2) and previous preliminary data showing surprisingly high levels of recombination (JM Anderson, unpublished data). Seven polymorphic PCR markers covering chromosome 7D were selected and used to identify recombinant individuals in both $F_2$ populations.

The four dominant wheat and two co-dominant markers, analyzed in the 107 individuals of the $F_2$ population CS/216-1 showed a Mendelian segregation for the *Th. intermedium* and wheat fragments (Fig. 3). However, the segregation ratio for *Th. intermedium* dominant marker in this population was not Mendelian. The number of *Th. intermedium* individuals detected with this marker was lower than expected for a dominant marker (Fig. 3). The segregation ratios in the other population, CS/260-1, were significantly skewed against wheat for all markers (Fig. 4). The Mendelian expectation in these analyses was based upon the assumption that the amount of wheat and *Thinopyrum* chromatin was large enough to allow pairing and meiotic recombination to be normal as was the case in the CS/216-1 population (Figs. 3, 4).

Among non-parental genotypes an unexpected number of complex recombinants for an $F_2$ population were obtained (Table 1). A pattern of recombination not suggested by the RFLP-SSR map was observed among the recombinant individuals of both populations (Figs. 2, 3, 4).

Yellow dwarf virus resistance in the $F_2$ population was determined by measuring the virus titer in $F_3$ individuals. In both crosses the $F_3$ bioassays demonstrated that the resistance in the $F_2$ conformed to a 1:2:1 ratio, consistent with the phenotype being controlled by a single gene (Table 2).

## Characterization of elite wheat lines, carrying *Th. intermedium* translocations

Twelve high-yielding wheats, derived from the $\gamma$-irradiated $M_4$ recombinant and addition lines (Crasta et al. 2000; Sharma et al. 1999) were tested for the presence and size of alien fragments using polymorphic markers and YDV resistance (Fig. 5). Seven of the 12 lines showed no evidence of alien chromatin except for the pAW161 marker a *Th. intermedium*-derived subtelomeric repeat (Crasta et al. 2000). These seven lines also had high ELISA values and were rated as susceptible to YDV. Among the remaining five lines containing the alien fragments, four were derived from the addition line P-107 and one from the translocation line 283-1. These lines clustered in three *Thinopyrum* and wheat arrangement along the long arm of the chromosome

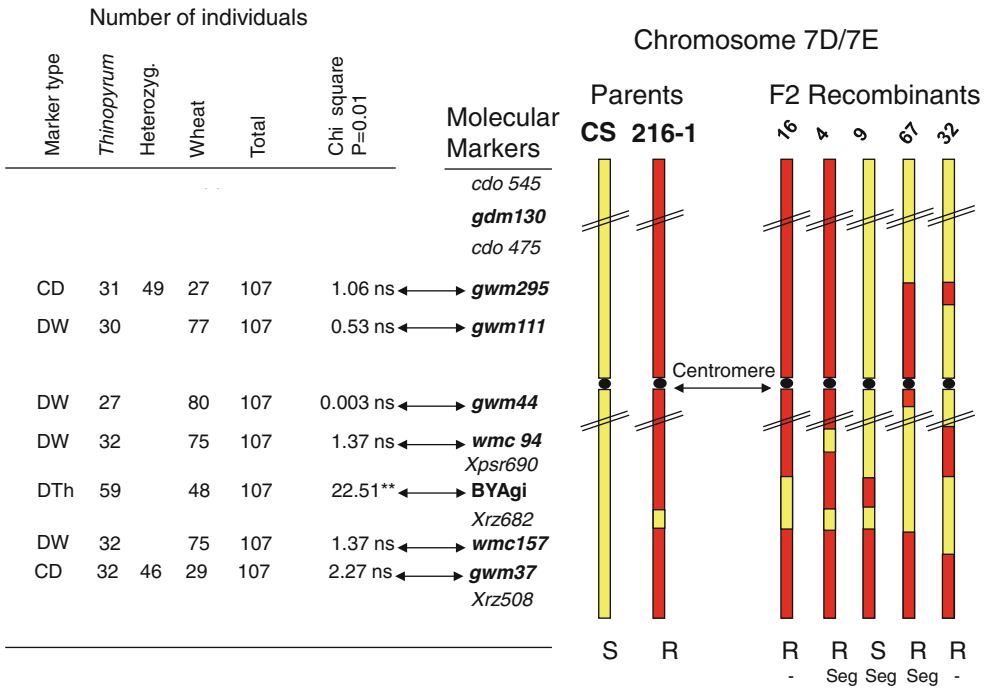


**Fig. 3** Segregation ratios and map positions for PCR markers on chromosome 7D/E for an $F_2$ populations of Chinese Spring/216-1 (CS/216-1). Depicted on the schematized chromosomes, at the *right*, are the recombinant genotypes deducted from the PCR markers on chromosome 7D/E; *yellow* (*grey*) = wheat, *red* (*dark*) = *Th. intermedium*. The RFLP *Xrz682* was placed as a reference marker. At the *left*, the *arrows* are connecting the segregation ratios for the corresponding molecular data: *DW* dominant wheat; *DTh* dominant *Th. intermedium*; *CD* co-dominant. The goodness of fit was tested using the Chi square as the segregation ratio for one gene with dominant or co-dominant effects: 3:1 (DTh), 1:3 (DW) or 1:2:1 (CD), respectively. **Significantly different at *P* 0.01 and ns = non significantly different. At the *bottom* is the response to YDV inoculation: Susceptible (S) and Resistant (R), as determined by ELISA

(Fig. 5): one of the four P-107 derived lines (number 4) had an interstitial *Th. intermedium* translocation with wheat at the distal portion of chromosome 7DL; the other three lines numbers 6, 7, and 30, had the distal portion of chromosome 7DL replaced by 7E, and; the line derived from 283-1 an $M_4$ P-29-derived plant number 9 had a good portion of the 7DL chromosome arm replaced by 7E *Th. intermedium* chromatin. The ELISA showed a low virus titer in most of the lines that had the terminal portion of chromosome 7DL arm replaced by *Th. intermedium* chromatin (Fig. 5). Line 30 was released as the germplasm line P961341 (Ohm et al. 2005).

## Discussion

### Location of PCR markers in the wheat-*Th. intermedium* RFLP map

We have previously developed wheat lines with *Th. intermedium* translocations carrying resistance to YDV (Sharma et al. 1999; Crasta et al. 2000). Subsequently, two parallel lines of research were followed: (a) characterized $M_4$ lines were subjected to a more detailed genetic analysis to identify markers to use in the breeding program; and, (b) recombinant lines with YDV resistance and 42 chromosomes were integrated into the breeding program.

The RFLP map described by Crasta et al. (2000) was used as the framework for locating PCR markers. The markers of choice were SSRs that mapped to chromosome 7 of wheat. In general, SSRs are considered to be very specific and mostly co-dominant markers (Roder et al. 1995). In our material the majority of SSRs tested behaved as dominant markers for wheat by showing the presence of a band in wheat chromosome 7D and absence in *Th. intermedium* chromosome 7E. From 12 polymorphic SSRs, two were found that showed co-dominance. Previous work demonstrated the usefulness of SSRs for the study of wheat/*Thinopyrum* translocation lines (Ayala et al. 2001). The absence of a wheat band was interpreted as presence of *Thinopyrum* as confirmed with C-banding and FISH studies (Ayala et al. 2001) and RFLP and GISH analysis (Crasta et al. 2000). This new map provides more information on the constitution of chromosome 7D/7E in the translocation lines. In most recombinant lines tested, the chromosome 7D/7E appeared as a mosaic of wheat and *Th. intermedium* chromatin sections. The lines used in this study were derived from double-monosomic substitution lines containing

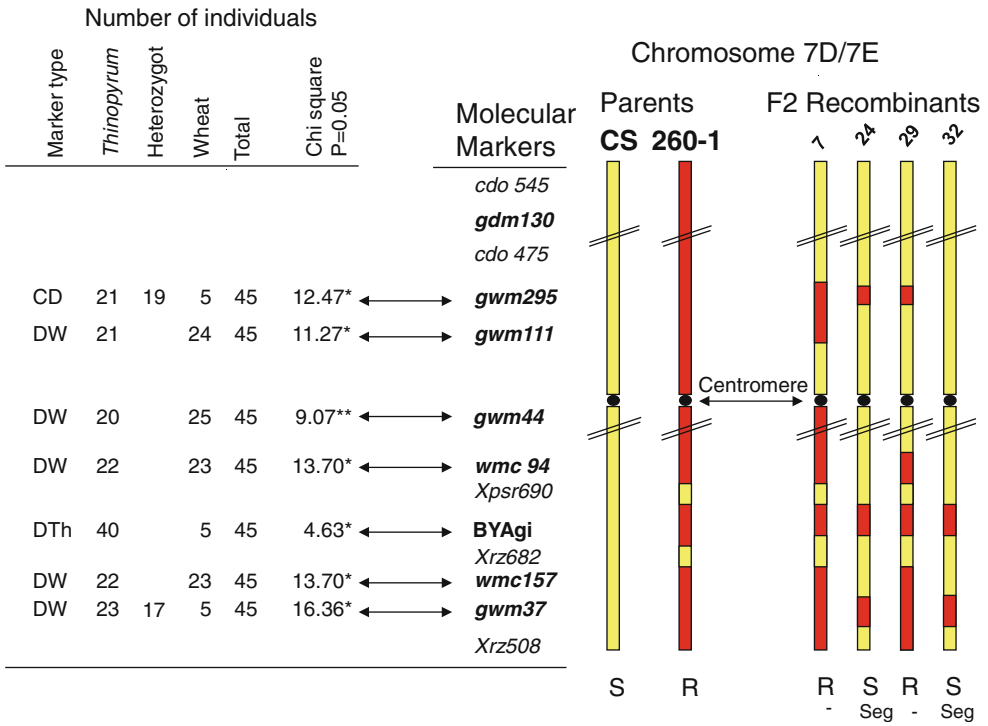


**Fig. 4** Segregation ratios and map positions for PCR markers on chromosome 7D/E PCR for an $F_2$ populations of the cross Chinese Spring/260-1 (CS/260-1). Depicted on the schematized chromosome, at the *right*, are the recombinant genotypes deducted from the PCR markers on chromosome 7D/E; *yellow* (*grey*) = wheat, *red* (*dark*) = *Th. intermedium*. The RFLP *Xrz682* was placed as a reference marker. At the *left*, the *arrows* are connecting the segregation ratios with the corresponding molecular markers: *DW* dominant wheat; *DTh* dominant *Th. intermedium*; *CD* co-dominant. The goodness of fit was tested using the Chi square for the segregation ratio of one gene with dominant or co-dominant effects: 3:1 (DTh), 1:3 (DW) or 1:2:1 (CD), respectively. Significantly different at $^{**}P$ 0.001 and $^{*}P$ 0.05. At the bottom is the response to YDV inoculation: Susceptible (S) and Resistant (R), as determined by ELISA

**Table 1** Percentage of parental lines and non-parental $F_2$ individuals deduced from the combination of dominant and co-dominant molecular markers for both populations CS/216-1 and CD/260-1

| Genotype | CS/216-1 | | CS/260-1 | |
|---|---|---|---|---|
| | Number of individuals | Percentage of total | Number of individuals | Percentage of total |
| Wheat[a] | 26 | 24 | 4 | 9 |
| Thinopyrum[b] | 20 | 19 | 19 | 42 |
| Heterozygote[c] | 32 | 30 | 15 | 33 |
| Recombinants | 29 | 27 | 7 | 16 |
| Total | 107 | | 45 | |
| $\chi^2$ | 2.7 ns | | 10.0** | |

*ns* non significant

** Significant at $P = 0.01$ from the chi square segregation ratio expected for one gene with dominant effects

[a] Wheat parental genotype, individuals showing the wheat band in all the wheat markers absence of Thinopyrum band in co-dominant and dominant Thinopyrum markers

[b] Thinopyrum parental genotype, individuals showing absence of wheat band in dominant wheat and co-dominant markers; and presence of Thinopyrum band in co-dominant and dominant Thinopyrum markers

[c] Non-Parental genotype, individuals showing presence of wheat and Thinopyrum bands in co-dominant markers, presence of wheat in all wheat dominant markers and Thinopyrum band in all Thinopyrum dominant markers

a 7D and a 7E chromosome (Sharma et al. 1999). It is possible that even though the two chromosomes belong to different genomes (homoeologous), they may associate with each other during the resting phase of the nucleus (Feldman, cited by Sears 1993). Studies of chromosome pairing in wheat/*Th. intermedium* hybrids have shown a

**Table 2** ELISA results of two $F_3$ wheat populations segregating for *Th. intermedium* fragments carrying resistance to BYDV

| | Cross | Phenotypic response | Number of $F_3$ families | ELISA average OD | Number of individuals tested | Reaction |
|---|---|---|---|---|---|---|
| | CS/216-1 | Resistant | 13 | 0.033 | 80 | R |
| | | Segregating | 40 | 0.553 | 370 | R/S |
| | | Susceptible | 16 | 1.749 | 90 | S |
| Total | | | 69 | | 540 | |
| $\chi^2$ | | | 2.01 ns | | | |
| | CS/260-1 | Resistant | 4 | 0.036 | 41 | R |
| | | Segregating | 14 | 0.330 | 97 | R/S |
| | | Susceptible | 2 | 2.420 | 11 | S |
| Total | | | 20 | | 149 | |
| $\chi^2$ | | | 3.6 ns | | | |

Test done with CYDV-RPV under controlled conditions

The goodness of fit was calculated with chi square for one gene with co-dominant effects 1:2:1

*ns* non significant at $P = 0.05$

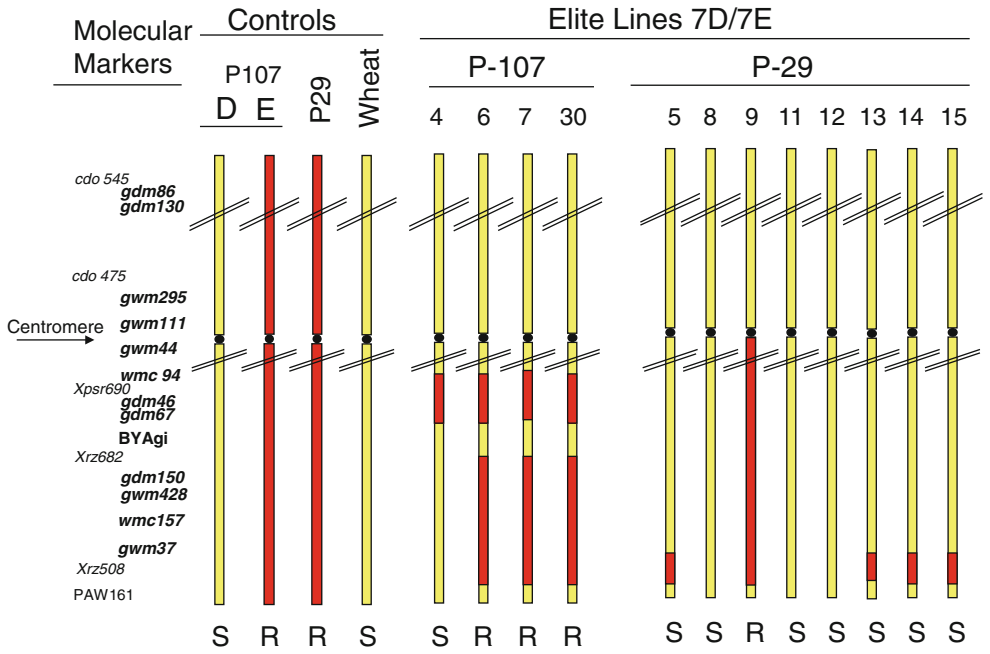


**Fig. 5** Genomic composition of chromosome 7D/E of elite wheat lines from Purdue-USDA Wheat Breeding Program. Characterization of *Th. intermedium* translocation and resistance to YDV with PCR-molecular markers and ELISA, respectively. The ELISA test was done in the greenhouse under artificial inoculation and the results depicted at the bottom of each chromosome as S = Susceptible and R = Resistant. The genetic composition of each line in depicted as: *yellow* (*grey*) = wheat, *red* (*dark*) = *Th. intermedium*. RFLP markers were placed as reference. Line P107 is an addition line and contained wheat and *Thinopyrum* chromosomes. At the *top* of each representation is the identification number of each line in the population. Line 30, derived from P107, is the YDV resistant germplasm line P961341 (Ohm et al. 2005)

close relationship between the chromosomes of the E-genome (or J) with the ABD genomes of wheat (Dvorak 1981; Jauhar 1995; Chen et al. 2001). This relationship is also evident at the nucleotide sequence level, since when wheat DNA is used for blocking homologous hybridization in GISH studies, hybridization to *Th. intermedium* chromatin can also be blocked (Crasta et al. 2000; N. Thompson, unpublished data).

To induce incorporation of the resistance carried on *Th. intermedium* chromosome 7 into wheat, Sharma et al. (1999) utilized $\gamma$-irradiation. This irradiation produces double-strand DNA breaks through discrete ionization events

that are essentially randomly distributed (Cornforth 1998). After γ-irradiation, the DNA of the cell is repaired by the dsDNA repair system (reviewed by Friedberg 1996; Nickiloff et al. 1998). There are two basic pathways the cell utilizes for double-strand DNA repair: (1) homologous recombination (HR) and (2) non-homologous end joining (NHEJ). Extensive research has demonstrated that the two systems exist in all organisms. In higher plants and mammals, breaks are repaired by NHEJ more frequently than through HR (West et al. 2002), possibly because the enzymes involved in NHEJ "out compete" those required for HR to process the double strand break (Britt 1999).

Because of their homology and position following γ-irradiation induced chromosome breakage and subsequent rejoining of dsDNA by NHEJ, there exists a high potential for *Th. intermedium* and wheat chromosome segments to interchange, resulting in the mosaic chromosomes depicted in Fig. 2. Similar mosaic chromosomes arising from multiple translocations involving two non-homologous chromosomes were previously reported (Jiang and Gill 1993). The chromosome was named "zebra" because of its striped pattern consisting of four chromosome segments derived from *Elymus trachycaulus* alternating with four chromosome segments from *Triticum aestivum* cv. Chinese Spring (Jiang and Gill 1993).

Only large translocations are observable using GISH on mitotic metaphase chromosomes. Small interstitial introgression, as is predicted for the mosaic chromosome (Fig. 2), is difficult to detect in wheat/*Th. intermedium* translocation lines by use of total genomic DNA as a probe (Wang et al. 2003).

The sub-telomeric marker pAW161 was shown previously (Crasta et al. 2000) and in this study (Fig. 5) to be present in both susceptible and resistant lines indicating that recombination can readily occur between this locus and the resistance locus. This supports the conclusion made earlier (Figs. 4, 5) that initially there were many small γ-irradiation induced breaks in the generation of these lines, which are being resolved following backcrosses to wheat lines.

## Segregation and characterization of *Th. intermedium* translocations in wheat

Based upon the map generated after adding the SSR markers (Fig. 2), recombinant lines such as 216-1 did not show any differences to the previous RFLP map (Crasta et al. 2000). However, analysis of the $F_2$ populations from the crosses 216-1 and 260-1 by wheat revealed more recombinant phenotypes than the ones predicted from the RFLP-SSR map (Figs. 2, 3, 4, 5).

There are at least two possible explanations for our results: first, and perhaps more likely, the marker density was not sufficient to detect small interstitial *Th. intermedium*-embedded translocations. If this is the case, considerably more homeologous joining occurred during the repair process following γ-irradiation than expected. In a second explanation, some breakpoints (exchanged chromatin blocks) may have been present in the initial generation but were masked by the presence of compensating chromatin and only in the backcross population were the recombinant fragments resolved and detected.

Irradiation may induce recombination pathways by epigenetic processes or other unknown mechanisms. After α-particle irradiation, chromosomal instabilities are demonstrated in the descendants of un-irradiated stem cells (Lorimore et al. 1998; Morgan et al. 2002). Associated with that, radiation induces conditions and/or factors that stimulate the production of reactive oxygen species (ROS) promoting chromosomal recombination and other phenotypes (Morgan et al. 2002). Analysis of recombinants involving a human chromosome in a hamster genome background exposed to γ-radiation, found a significantly higher number of recombinants than normally expected (Wright and Coates 2006).

Dong et al. (2004) established that in the production of octoploids, in their case the wheat × *Th intermedium* partial amphiploid Zhong 3, there were important changes due to the "lability" of the nascent polyploid and demonstrated that those changes were inherited in the following generations. In characterizing the addition and recombinant lines derived from Zhong 3, Dong et al. (2004) found multiple "cryptic" translocations that were detectable by RFLP but not by GISH, as was the case with our materials (Crasta et al. 2000).

In summary, it is possible that exposure to ionizing radiation caused DNA strand breaks which were repaired by one or more of the mechanisms mentioned above resulting in high levels of recombination. The actual composition of the chromosomes only became evident following a backcross to wheat. Interestingly, this recombination occurred in the apparent presence of the 5B *Ph1* gene which inhibits non-homeologous pairing. In such a case the observed segregation would likely be the product of recombination between stretches of wheat that carried along adjacent *Thinopyrum* segments, as is the case in translocations lines (Knott 1980; Lukaszewski 2000; Ayala-Navarrete et al. 2007). Another factor influencing recombination might be related to the reported effect of genes promoting homeologous gene pairing carried by *Thinopyrum* species (Chen et al. 1998; Jauhar 1995; Zhang 1992).

The evaluation of virus titer by ELISA showed the $F_3$ families of the two crosses were segregating for a single gene for resistance to BYDV. In both populations, about 50% of the $F_3$ families contained both resistant and susceptible plants whereas the other half of the $F_3$ families were either fully susceptible or fully resistant. However, by using

the molecular data of the cross CS/216-1 we separated the parental genotypes; all 24 families with all the molecular markers showing the presence of wheat genotype corroborated the susceptibility data obtained in the ELISA test. Semi-dominance or dosage dependence was previously reported for *Bdv2* (Ayala et al. 2001), another gene for resistance to BYDV derived from *Th. intermedium* chromosome 7 (Banks et al. 1995). Since both *Bdv2* (Stoutjesdijk et al. 2001) and the gene conferring resistance in our materials are derived from chromosome 7 and from different genomes of *Th. intermedium* (Anderson et al. 1998; Crasta et al. 2000), they can be considered as orthologous. Consequently, we will refer to the *Th. intermedium*-derived YDV resistance gene examined in this study as *Bdv3* for future reference.

Seven out of 12 elite lines that showed good yields and no YDV symptoms possessed only one *Th. intermedium* marker, namely PAW161. This clearly demonstrates that selection for resistance to YDV under natural infection was a difficult task due to the multiple components involved in the disease complex. Pathogen, vector, plant genotype, and environmental conditions play an active role in the expression of the disease, especially in wheat (reviewed by D'Arcy and Burnett 1995). The position and size of the translocation was related to YDV resistance as measured by ELISA. Line #4 (Fig. 5), for example, had high virus titers. Although this line contained the smallest alien fragment, it did not have the terminal portion of the chromosome previously linked with resistance in other *Th. intermedium*-derived materials (Banks et al. 1995; Ayala et al. 2001) as shown by the lack of *gwm37* and the surrounding markers.

The molecular markers and maps provided in this study constitute valuable information about *Th. intermedium* translocations in chromosome 7D that will be applicable in wheat breeding. The use of those markers and the various translocations identified in this study will help to increase the efficiency of selection for *Th. intermedium*-mediated YDV-resistance into elite wheat germplasm. The characterization of recombinants also provides another example of the elasticity of the wheat genome and the value of wild relatives as trait donors.

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