

# Cytological and Molecular Characterization of Wheat Lines with *Thinopyrum intermedium* Chromosome Additions, Substitutions and Translocations Resistant to Barley Yellow Dwarf Virus

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**Summary** Barley yellow dwarf virus (BYDV) is the most serious viral disease affecting wheat and genes for BYDV resistance have not been found in wheat. BYDV-resistant alien addition and alien substitution lines produced from a wheat×*Thinopyrum intermedium* (species of *Agropyron* complex) cross were characterized. Chromosome pairing in the hybrids between two substitution lines showed that they had the same *Th. intermedium* chromosome. Likewise, two addition lines involved the same alien chromosome. *In situ* hybridization of chromosomes, confirmed that line P29 is a disomic substitution line. Double monosomic seeds and self-pollinated seeds from monosomic addition plants were irradiated to induce translocations between wheat and *Th. intermedium* chromosomes. Putative translocations were selected on the basis of BYDV resistance and studied by chromosome analysis, Southern hybridization using *Thinopyrum* specific probe and RFLP markers. A BYDV-resistant translocation was identified.

Barley yellow dwarf virus (BYDV) is a serious disease of wheat (*Triticum aestivum*,  $2n=6x=42$ , AABBDD). Resistance genes have not been found in wheat or other *Triticum* species (Comeau and Plourde 1987, Conti *et al.* 1990, Zhong *et al.* 1994, Sharma *et al.* 1997). Wheatgrasses (*Agropyron* complex, syn. *Thinopyrum*) are sources for transferring BYDV resistance into wheat (Sharma *et al.* 1989). To transfer alien genes into wheat, chromosome engineering is commonly used in which the alien chromosomes are introgressed by wide crossing followed by backcrossing to wheat and then the alien chromosome segments carrying useful genes are integrated into wheat chromosomes by translocations induced by irradiation or other methods (Li and Hao 1992, Friebe *et al.* 1996). We derived BYDV-resistant alien substitution lines (P12, P29) ( $2n=42$ ) and alien addition lines (P25, P114) ( $2n=44$ ) independently by spontaneous chromosome substitution and by selfing monosomic alien additions ( $2n=43$ ), respectively, from the same BYDV-resistant backcross-3 plant with 45 chromosomes from a wheat×*Thinopyrum intermedium* (intermediate wheatgrass,  $2n=6x=42$ , E1E1E2E2StSt) cross (Sharma *et al.* 1995). Although restriction fragment length polymorphism (RFLP) analysis indicated that in the substitution lines chromosome 7D was replaced by a chromosome from *Th. intermedium*, it remained uncertain whether the same alien chromosome is present in different lines.

Alien addition lines have been used to transfer *Thinopyrum* chromosome segments into wheat by radiation (Driscoll and Jensen 1963, Sharma and Knott 1966). Sears (1993) suggested that

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monosomic alien substitution lines (double monosomics) should be preferred, and Kimber (1971) suggested irradiation of selfed seed from monosomic alien addition lines if substitution lines are not available. Apart from classical chromosomal analysis, more recently, molecular methods have been useful to augment the identification of chromosome addition, substitution and translocation lines (Fedak 1998).

This paper is on the cytological and molecular characterization of the above substitution and addition lines, and potential translocations developed from them by irradiating monosomic alien substitution seed and selfed seed from monosomic alien additions.

### Materials and methods

The substitution line, P29, was crossed to substitution line P12 and chromosome pairing behaviour was studied in pollen mother cells of the hybrids. Similarly, addition lines P114 and P25 were crossed and chromosome pairing was studied in the hybrids. The presence of *Th. intermedium* chromosomes was confirmed by fluorescent *in situ* hybridization (FISH) of somatic cells of root-tips in P29 using *Th. intermedium* total genomic DNA as probe. The FISH procedure was the same as described by Francki and Langridge (1994) except that the hybridization solution had 40% formamide, 10% dextran sulfate, 20  $\mu$ g sheared salmon sperm DNA, 200 ng labeled probe and 10  $\mu$ g unlabelled sheared wheat DNA (probe : block ratio = 1 : 50).

For induction of translocations, 1012 monosomic substitution seeds were produced by crossing P29 and P12 to wheat, and 151 seeds were produced by selfing the monosomic version ( $2n=43$ , determined from the root-tip cells) of P114. The seeds were irradiated by gamma rays ( $^{60}\text{Co}$ ) using 12 kR radiation dose based on LD50 determination on a sample of seed.  $^{60}\text{Co}$  has been effective in irradiating wheat seed at 10–20 kR (Konzak 1987). M2 seeds from 74 monosomic substitution-derived M1 plants and 50 monosomic addition-derived M1 plants were followed. Up to 11 M2 seeds per M1 plant were grown. Seedlings were analyzed for reaction to PAV isolate of BYDV by enzyme-linked immunosorbent assay (ELISA) and an ELISA value of  $<0.3$  was considered a resistant reaction as described previously (Sharma *et al.* 1995). Putative translocation lines (PTLs) were selected on the basis of M2 segregation for BYDV resistance and characterized in subsequent generations by ELISA, chromosome analysis (number and pairing), and Southern hybridization using *Thinopyrum* specific probe and RFLP markers. Chromosome numbers of 1–3 seedlings of nine PTLs were determined from root-tip cells. Chromosome pairing in a randomly selected PTL and its heterozygote was studied in the pollen mother cells. Procedures of Southern hybridization have been described before (Sharma *et al.* 1995, Francki *et al.* 1997). Total genomic DNA was isolated from homozygous PTLs (M4, M5 generations), alien substitutions, alien additions, wheat cultivars Caldwell, Abe, Compton, Chinese Spring and 81401, *Th. intermedium*, and nullisomic-tetrasomic (NT) stocks of Chinese Spring for group 7 chromosomes. For Southern hybridization with a *Thinopyrum* specific probe A600, total genomic DNA was digested with restriction endonuclease *EcoRV*. Similarly, for Southern hybridization with RFLP clones for group 7 chromosomes (Gale *et al.* 1993), DNA samples were digested with *EcoRV* or *BamHI*. Equal amounts (10  $\mu$ g) of the digested DNAs were electrophoresed in 1% agarose gels and transferred to nylon membranes. The DNAs fixed to the membranes were prehybridized and hybridized with either A600 or RFLP clones labeled with  $^{32}\text{P}$  by nick translation, and non-specifically bound probe was washed. Hybridization of A600 to genomic DNA digested with *EcoRV* results in two bands. The top band is present in wheat and *Thinopyrum* but the bottom band is specific to *Thinopyrum* chromatin (Sharma *et al.* 1995, Banks *et al.* 1995, Hohmann *et al.* 1996). Two RFLP markers, CD0475 and PSR311, were used which map to wheat group 7 chromosomes and the *Thinopyrum* chromatin in our BYDV resistant lines (Sharma *et al.* 1995, Francki *et al.* 1997).

Table 1. Mean (and range) chromosome pairing in hybrids of substitution lines P29 and P12, in hybrids of addition lines P114 and P25, in a putative translocation line PTL 601 and its hybrid with wheat

Material	No. of cells	I	Ring II	Rod II	III	Ring IV	Rod IV	Xmata
P29×P12	62	0.12 (0–2)	19.43 (17–21)	1.49 (0–4)	—	0.01 (0–1)	—	40.39 (38–42)
P114×P25	29	0.10 (0–1)	20.66 (18–22)	1.14 (0–4)	0.10 (0–1)	—	—	42.66 (40–44)
PTL601	24	0.12 (0–2)	19.25 (16–21)	1.29 (0–2)	0.04 (0–1)	0.17 (0–1)	—	40.55 (37–42)
PTL 601× wheat F1	41	1.58 (0–4)	18.20 (17–20)	1.39 (0–3)	0.12 (0–1)	0.12 (0–1)	0.10 (0–1)	38.81 (36–41)

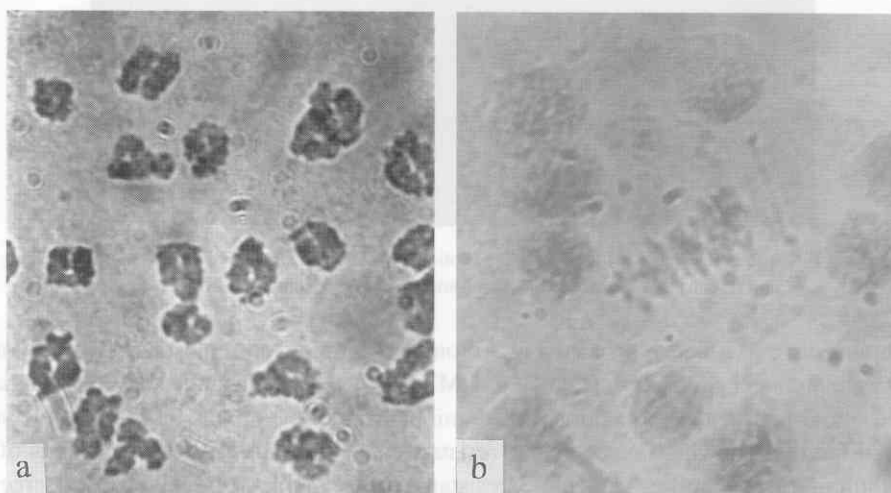


Fig. 1. Chromosome pairing in F1 hybrid between substitution lines P29 and P12. a) Diakinesis, 20 Ring II+1 Rod II (100×). b) Metaphase-I, 20 Ring II+1 Rod II (40×).

### Results and discussion

Chromosome pairing of the hybrids between substitution lines P29 and P12 showed that they contained the same *Th. intermedium* chromosome. The hybrids had 21 bivalents with only 0.12 univalents/cell (Table 1, Fig. 1a, b), and no laggards or micronuclei were observed. Similarly, the chromosome pairing behavior of hybrids between addition lines P114 and P25 showed that they both carried the same alien chromosome (Table 1). FISH results demonstrated that *Th. intermedium* genomic DNA hybridized to only one pair of chromosomes while all others had no hybridization signal (Fig. 2). This confirmed our previous results based on RFLP analysis, chromosome pairing in hybrids of P29 with wheat and FISH using rye telomeric probe pAW161 (Sharma *et al.* 1995, Francki *et al.* 1997) that P29 is a substitution line containing a pair of *Th. intermedium* chromosomes. FISH of P29 and chromosome pairing behaviour of P29×P12 hybrids established that P12 is also a substitution line and the same as P29.

From the monosomic substitution material, M2 families with translocations were expected to be homogeneously resistant and not segregating. From the monosomic addition material, M2 families giving 3 resistant : 1 susceptible segregation would potentially represent the translocation event as these would come from M1 translocation heterozygotes while those giving homogeneously susceptible, homogeneously resistant or a low proportion of resistant plants would result from 42-, 44-



Fig. 2. Fluorescent *in situ* hybridization of a mitotic cell of substitution line P29 showing hybridization (green) to a *Th. intermedium* chromosome pair (incomplete cell, 100 $\times$ ).

and 43-chromosome M1 plants, respectively. Among M2 families of monosomic substitution material, 16% were homogeneously resistant. Among M2 families of monosomic addition material, 10% segregated into 3 resistant : 1 susceptible. M3 families from 57 M2 plants of monosomic substitution-derived families showing homogeneous resistance and from 16 M2 resistant plants of monosomic addition-derived families showing 3 resistant : 1 susceptible segregation were screened for BYDV resistance. Families with small sample size or instability for resistance were dropped. Selfed seed of one plant per selected family was followed and at M4, these PTLs were called 601, 602, 603...

Progeny test of 36 PTLs, including five susceptible sister lines for comparison, showed that the resistant M4 were homogeneously resistant and susceptible M4 were homogeneously susceptible. Chromosomes number in each of the nine resistant PTLs (601, 602, 604, 605, 607, 608, 609, 610, 611) counted was 42. Chromosome pairing in the PTL 601 studied was normal with preponderance of bivalents and a high frequency of rings (Table 1, Fig. 3a). However, PTL 601  $\times$  wheat hybrid had an average of 1.58 univalents per cell, and the small frequency of quadrivalents was not higher than that of PTL 601 (Table 1, Fig. 3b). This indicated that PTL 601 was more likely a whole *Thinopyrum* chromosome substitution. Southern hybridization with A600 probe resulted in one band in wheat but two bands in *Th. intermedium*, addition and substitution lines, and PTLs showing thereby the presence of *Thinopyrum* chromatin in the PTLs (Fig. 4a, b). The intensity of the diagnostic (lower) band possibly indicating the relative amount/length of *Th. intermedium* chromosome segment appeared to be less in some PTLs (631, 632, 633, 635, 625) compared to *Th. intermedium*, addition and substitution lines and other PTLs suggesting that they might not have the whole *Thinopyrum* chromosome. Using RFLP markers, CD0475, specific to wheat chromosome arm 7DS, and PSR311, specific to wheat chromosome arm 7DL (Gale et al. 1993), the PTLs were scored for the presence of *Th. intermedium* chromosome specific band (present in P29 and *Th. intermedium* but absent in wheat) and 7D chromosome specific band (absent in P29 and N7DT7B but present in wheat and N7AT7B and N7BT7A). Of the 15 PTLs digested with *EcoRV* and probed with CD0475,

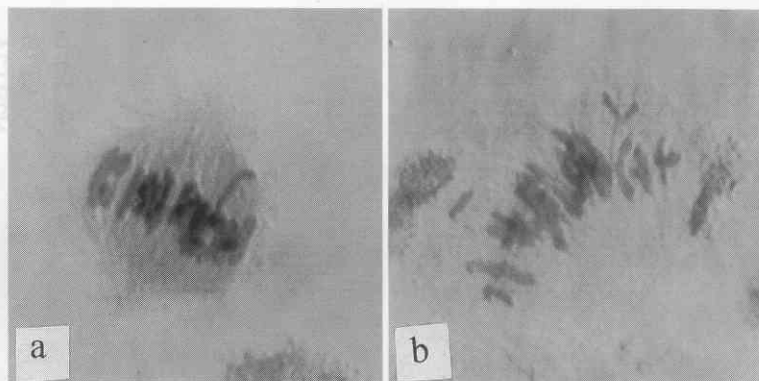


Fig. 3. Chromosome pairing in a) PTL 601 showing 20 Ring II+1 Rod II (40×). b) PTL 601 × wheat showing 4 I and no IV (40×).

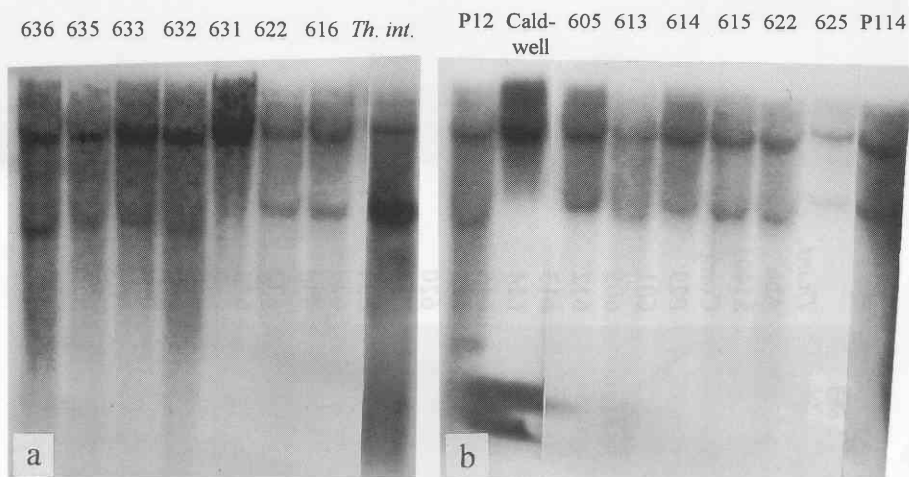


Fig. 4. Southern hybridization blots of *EcoRV* digested DNA from putative translocations using *Th. intermedium* specific probe A600. a) 20 h exposure at  $-70^{\circ}\text{C}$  to X-ray film. b) 5 h exposure at room temp to X-ray film.

the short arm segment of *Th. intermedium* chromosome (homoeologous to short arm of 7D) was absent in PTLs 629 and 632 but present in others (Fig. 5a). When these 15 PTLs were digested with *Bam*HI and probed with PSR311, the alien chromosome segment was absent in PTLs 629 and 633 but present in the rest (Fig. 5b). These results indicated that with the exception of 629, 632 and 633, the remaining PTLs analyzed might be substitutions or long translocations. PTLs 629 and 633 were susceptible while 632 was resistant to BYDV. Thus, we were able to isolate a BYDV-resistant translocation 632 from irradiation of monosomic substitution version of P29.

Other researchers have also introgressed and characterized BYDV resistance from *Th. intermedium* into wheat as group 7 chromosomes or their segments using L1 addition line (Mujeeb-Kazi *et al.* 1994, Banks *et al.* 1995, Hohmann *et al.* 1996). Addition line L1 developed by Caudeyron *et al.* (1973) involves wheat cultivars and *Th. intermedium* accession different from our Purdue University lines. L1 and P29 both contain a *Th. intermedium* group 7 chromosome (Banks *et al.* 1995, Sharma *et al.* 1995, Hohmann *et al.* 1996, Zhang *et al.* 1996). Dot blot hybridization showed that probe pAW161, which specifically hybridizes to the long arm telomere of *Th. intermedium* chromosome in P29 (Francki *et al.* 1997), hybridized to P29 genomic DNA but not to wheat or L1 genomic DNA (Anderson *et al.* 1998). This supports our conclusion based on RFLP analysis (Sharma *et al.*

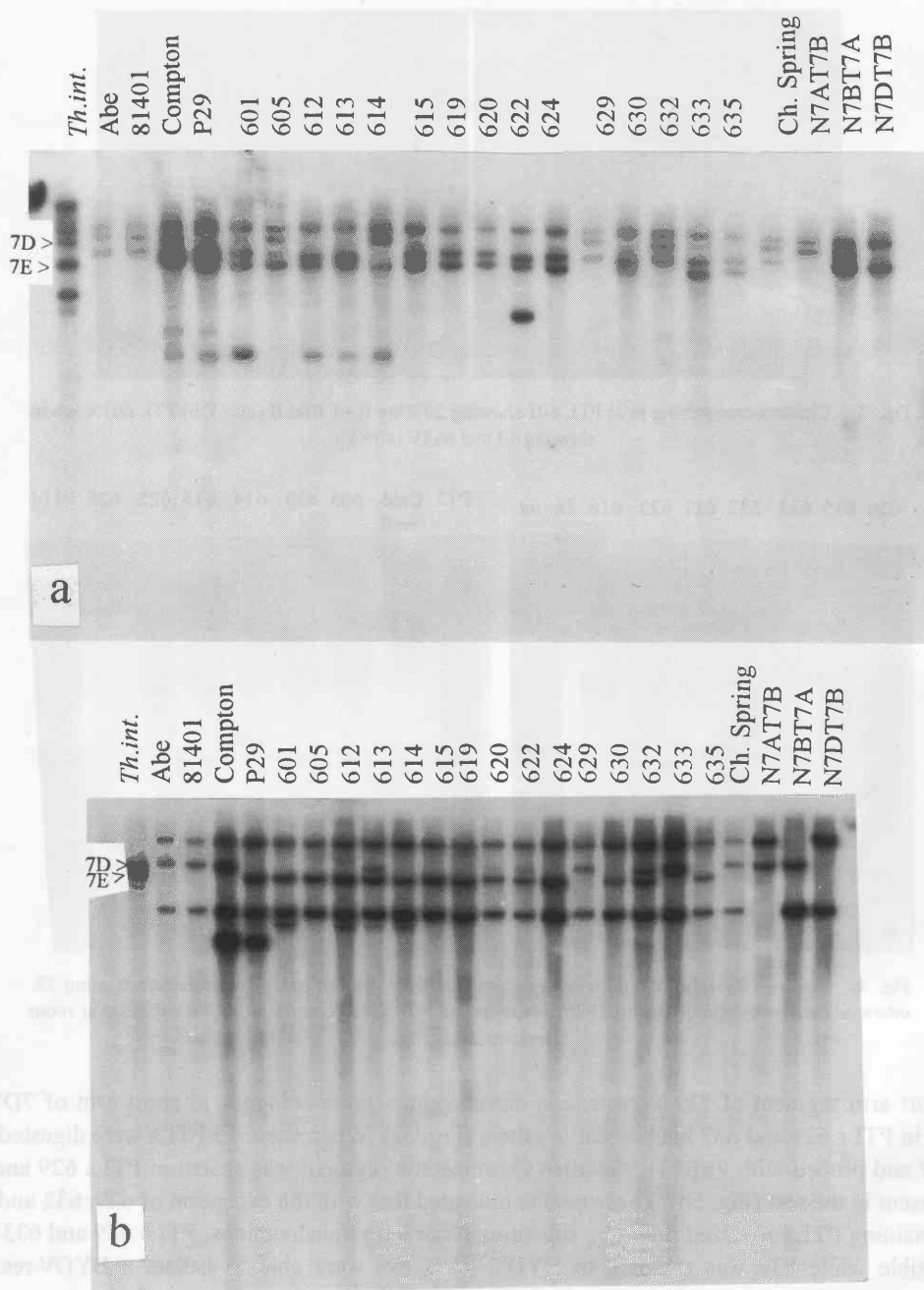


Fig. 5. Southern hybridization of DNA from putative translocations using RFLP markers. a) CD0475 as a probe of *EcoRV* digested DNA. b) PSR311 as a probe of *BamHI* digested DNA.

1995) and unpublished data on chromosome pairing in hybrids between L1 and Purdue University P lines that the wheatgrass chromosome in L1 is a different group 7 chromosome from that in P29 and other related P lines. According to Zhang *et al.* (1996), the wheatgrass chromosome in L1 is from the St genome with some E genome DNA. Although this suggests the possibility of rearrangements between homoeologous chromosomes of different genomes of *Th. intermedium*, the results of the study by Sharma *et al.* (1995) and Anderson *et al.* (1998) suggest that the wheatgrass chromo-

some in P29 is most likely from one of the E genomes. Among the L1-derived translocations, Hohmann *et al.* (1996) found that line TC14 had the smallest *Th. intermedium* segment. In the present study we used only one marker per chromosome arm. Thus it does not show the size of translocation in 632. Comparison of a large number of deletions/translocations, including 632, using FISH and several group 7 markers locating BYDV resistance is being published in a follow-up paper (Crasta *et al.*, submitted).

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### References

- Anderson, J., Bucholtz, D., Greene, A., Francki, M., Gray, S., Sharma, H., Ohm, H. and Perry, K. 1998. Characterization of wheatgrass-derived barley yellow dwarf virus resistance in wheat alien chromosome substitution line. *Phytopath.* **88**: 851–855.
- Banks, P., Larkin, P., Bariana, H., Lagudah, E., Appels, R., Waterhouse, P., Brettell, R., Chen, X., Xu, H., Xin, Z., Qian, Y., Zhou, X., Cheng, Z. and Zhou, G. 1995. The use of cell culture for subchromosomal introgressions of barley yellow dwarf virus resistance from *Thinopyrum intermedium* to wheat. *Genome* **38**: 395–405.
- Cauderon, Y., Saigne, B. and Dauge, M. 1973. The resistance to wheat rusts of *Agropyron intermedium* and its use in wheat improvement. *Intl. Wheat Genet. Symp.* **4**: 401–407.
- Comeau, A. and Plourde, A. 1987. Cell, tissue culture and intergeneric hybridization for barley yellow dwarf virus resistance in wheat. *Can. J. Plant Path.* **9**: 188–192.
- Conti, M., D'Arcy, C., Zedlinsky, H. and Burnett, P. 1990. The Yellow "Plague" of Cereals: Barley Yellow Dwarf Virus. In: Burnett, P. (ed.) *World Perspectives on Barley Yellow Dwarf*. International Maize and Wheat Improvement Center (CIMMYT), Mexico. pp. 1–6.
- Crasta, O., Francki, M., Bucholtz, D., Sharma, H., Ohm, H. and Anderson, J. Molecular characterization of wheat-wheatgrass translocation lines and localization of BYDV resistance locus on the alien chromosome in wheat. *Genome*: Submitted.
- Driscoll, C. and Jensen, N. 1963. A genetic method for detecting induced intergeneric translocations. *Genetics* **48**: 459–468.
- Fedak, G. 1998. Procedures for transferring genomic traits from alien species. *Intl. Wheat Genet. Symp.* **9**(1): 1–7.
- Francki, M. and Langridge, P. 1994. The molecular identification of the midget chromosome from the rye genome. *Genome* **37**: 1056–1061.
- , Crasta, O., Sharma, H., Ohm, H. and Anderson, J. 1997. Structural organization of an alien group 7 *Thinopyrum intermedium* chromosome in US soft red winter wheat (*Triticum aestivum* L.). *Genome* **40**: 716–722.
- Friebe, B., Jiang, J., Raupp, W., McIntosh, R. and Gill, G. 1996. Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. *Euphytica* **91**: 59–87.
- Gale, M., Atkinson, M., Chinoy, C., Harcourt, R., Jia, J., Li, Q. and Devos, K. 1993. Genetic maps of hexaploid wheat. *Intl. Wheat. Genet. Symp.* **8**: 29–40.
- Hohmann, U., Badaeva, K., Busch, W., Friebe, B. and Gill, B. 1996. Molecular cytogenetic analysis of *Agropyron* chromatin specifying resistance to barley yellow dwarf virus in wheat. *Genome* **39**: 336–347.
- Kimber, G. 1971. The design of a method, using ionizing radiation, for the introduction of alien variation into wheat. *Indian J. Gen. Pl. Breed.* **31**: 580–584.
- Konzak, C. 1987. Mutations and Mutation Breeding. In: Heyne, E. (ed.) *Wheat and Wheat Improvement*. Agronomy Monograph 13, 2<sup>nd</sup> ed. pp. 428–433.
- Li, Z. and Hao, S. 1992. Chromosome engineering of wheat in China. *Critical Reviews in Pl. Sci.* **10**: 471–485.
- Mujeeb-Kazi, A., Cortes, A., Islam-Faridi, N. and Bertschinger, L. 1994. Cytogenetic characterization of bread wheat lines with resistance to BYDV luteoviruses derived from *Th. intermedium*. *BYD Newslett.* **5**: 19.
- Sears, E. 1993. Use of radiation to transfer alien chromosome segments to wheat. *Crop Sci.* **33**: 897–901.
- Sharma, D. and Knott, D. 1966. The transfer of leaf rust resistance from *Agropyron* to *Triticum*. *Can. J. Genet. Cytol.* **8**: 137–143.

- Sharma, H., Ohm, H., Lister, R., Foster, J. and Shukle, R. 1989. Response of wheatgrasses and wheat×wheatgrass hybrids to barley yellow dwarf virus. *Theor. Appl. Genet.* **77**: 369–374.
- , —, Goulart, L., Lister, R., Appels, R. and Benhabib, O. 1995. Introgression and characterization of barley yellow dwarf virus resistance from *Thinopyrum intermedium* into wheat. *Genome* **38**: 406–413.
- , — and Lister, R. 1997. Reaction of near relatives of wheat to P-PAV isolate of BYDV. *BYD Newslett.* **6**: 7–8.
- Zhang, X., Koul, A., Petroski, R., Oellet, T., Fedak, G., Dong, Y. and Wang, R. 1996. Molecular verification and characterization of BYDV-resistant germplasms derived from hybrids of wheat with *Thinopyrum ponticum* and *Th. intermedium*. *Theor. Appl. Genet.* **93**: 1033–1039.
- Zhong, G., McGuire, P., Dvorak, J. and Qualset, C. 1994. Cytological and molecular characterization of a *Triticum aestivum*×*Lophopyrum ponticum* backcross derivative resistant to barley yellow dwarf. *Genome* **37**: 876–881.
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