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Identification of wheat-*Agropyron cristatum* monosomic addition lines by RFLP analysis using a set of assigned wheat DNA probes

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Abstract A non-radioactive digoxigenin-labelled DNA method was used successfully to identify RFLP markers in 54 *Triticum aestivum* cv 'Chinese Spring' – *Agropyron cristatum* (2n=28, genome PPPP) P-genome monosomic addition lines. Southern analysis using a set of 14 DNA probes identifying each homoeologous chromosome arm, combined with two restriction enzymes *Hin*dIII and *Eco*RI, indicated that six *A. cristatum* chromosomes (1P, 2P, 3P, 4P, 5P and 6P) and five *A. cristatum* chromosome arms (2PS, 2PL, 5PL, 6PS and 6PL) have been individually added to the wheat genome. The added chromosomes of three lines were *Agropyron* translocated chromosomes. It was also found that two addition plants possessed an *Agropyron*-wheat translocation. These results showed that RFLP analysis using the set of assigned wheat probes was a powerful tool in detecting and establishing homoeology of alien *A. cristatum* chromosomes, or arms, added to wheat, as well as in screening the alien addition material. The creation of the monosomic addition lines should be useful for the transfer of disease-resistance genes from *A. cristatum* to wheat.

Key words *Triticum aestivum* · *Agropyron cristatum* Alien addition · RFLP · Non-radioactive labelling

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Introduction

Agropyron Gaertn. is a perennial genus of the Triticeae consisting of 10–13 diploid (2n=14), tetraploid (2n=28) and hexaploid (2n=42) species, all with the same basic genome designated as P (Dewey 1984). It has been found to have useful traits of great potential for wheat improvement, including those for disease resistance and drought and cold tolerance (Chen et al. 1992). As a first step in these transfer of the valuable traits to wheat, intergeneric hybrids between bread wheat and some species of *Agropyron* have been recently produced (Chen et al. 1989, 1990; Limin and Fowler 1990; Ahmad and Comeau 1991; Li and Dong 1991; Jauhar 1992). Backcross progenies (BC_1, BC_2 and BC_3) of a wheat × *A. cristatum* hybrid were also obtained and analysed in detail (Chen et al. 1992). A systematic analysis of single chromosomes of the *Agropyron* P genome by screening a complete, or nearly complete, set of wheat-*A. cristatum* addition lines would be very useful for determining the genetic relationship between the *Agropyron* and wheat genomes. The set of addition lines would provide a starting point for chromosome substitution, translocation and recombination for the introgression of desirable traits.

The identification of added chromosomes in different sets of wheat addition lines has been so far achieved by using morphological (Miller and Reader 1987), cytogenetical (karyotype, different banding techniques, assessment of sporophytic and/or gametophytic compensation: Cauderon 1966; Dvorak and Knott 1974; Jewell and Driscoll 1983; Charpentier 1992), and biochemical (electrophoretic analysis of seed proteins, isozyme analysis: Cauderon et al. 1978; Forster et al. 1987; Morris et al. 1989) methods. At present, DNA restriction fragment length polymorphism (RFLP) markers offer greater potential by revealing more useful polymorphisms. Especially, the recent development of a set of RFLP probes identifying the 14 wheat homoeologous chromosome arms enables the presence of alien chromosomes in wheat-alien chromosome addition lines to be identified (Sharp et al. 1989).

The non-radioactive labelling technique for DNA analysis has now been used for RFLP analysis of rice, tomato and potato (Ishii et al. 1990; Panaud et al. 1993; Xu et al. 1993). Here, we report the identification of a series of wheat-*A. cristatum* monosomic addition lines using 14 assigned probes by the non-radioactive technique.

Materials and methods

Plant materials

A series of monosomic and double monosomic *A. cristatum* P-genome chromosome addition lines were isolated from a backcross program of an intergeneric hybrid of *Triticum aestivum* cv 'Chinese Spring' (2n=6x=42, AABBDD) and *Agropyron cristatum* (2n=4x=28, PPPP) produced by Chen et al. (1989). Two-hundred-and-five monosomic addition lines (2n=42+1) and 55 double monosomic lines (2n=42+2) were identified by somatic chromosome counts, and meiotic pairing studies of 280 progeny derived from 57 different BC_3 hyperploid plants, generally with one to three added chromosomes. Fifty-four monosomic or double monosomic addition plants were chosen on the basis of morphology and the meiotic behaviour of the mother plant for RFLP analysis in order to hopefully identify all seven *A. cristatum* chromosomes. The two parents 'Chinese Spring' and *A. cristatum* 4x, and the hybrid F_1 from which the addition lines derived, were used as controls. Since the original plant of *A. cristatum* employed in the hybridization died, the plant used in this study was from the open-pollination progeny of the original parental plant.

Cytological analysis

Root-tip squashes followed the procedure of Chen et al. (1989). For meiotic studies individual anthers were fixed in Carnoy's solution (3 ethanol: 1 acetic acid) and stored at 4 °C and were squashed in 1% aceto-carmine for cytological examination.

RFLP probes

The DNA probes used to reveal RLFP polymorphisms were produced by Sharp et al. (1989) and kindly supplied by Dr. M. D. Gale. These probes, together with their size and the homoeologous chromosomes which they identify in wheat, are listed in Table 1.

Genomic DNA extraction and Southern analysis

Leaf tissues used for DNA extraction were harvested from pot-grown plants in a greenhouse and immediately frozen in liquid nitrogen and then stored at –70 °C until DNA preparation. Genomic DNA was extracted and purified using a modification of the method employed by Dellaporta et al. (1983). About 5 g of leaf tissue was ground in a liquid nitrogen in mortar to a very fine powder and suspended in 15 ml of extraction buffer (100 mM Tris, pH 8.0, 50 mM EDTA, 0.5 M NaCl and 1% SDS) at 65 °C for 15 min. Then 5 ml of 5 M potassium acetate was added and the mixture was incubated for 20 min at 0 °C. After a 10-min centrifugation at 17400 g (rotor JA-20, Beckmann), the solution was poured into a second centrifuge tube containing 10 ml of isopropanol. The DNA pellet was transferred to an Eppendorf tube containing 500 µl of resuspending buffer (50 mM Tris, pH 8.0, 10 mM EDTA) and 20 µg/ml of RNAase. After 30 min incubation at 37 °C, the DNA solution was purified by two phenol/chloroform purifications and one chloroform/isoamyl alcohol extraction. DNA was then precipitated in an equal volume of isopropanol, pelleted by a 7-min centrifugation, rinsed with 70% ethanol, and resuspended in 300 µl TE (10 mM Tris, pH 8.0, 1 mM EDTA). The genomic DNA was diluted in TE to a concentration of 0.5 µg/l.

The procedures described by Lu et al. (1993) were followed for RFLP analyses, the Southern-blot preparation, the non-radioactive digoxigenin-labelling of the probes (Boehringer, Mannheim Co.), and the hybridization and the detection of hybridized probes. The filters were finally enclosed in a transparent plastic folder and were exposed to Amersham MP X-ray film for 6 h in a vinyl-covered exposure cassette (Sigma). If the hybridization signals were too strong, with a high background, or too weak, a secondary shorter (2 h) or longer (8 h) exposure was made on the following day (6–12 h later after the first exposure). The rate of signal production increases to a maximum 12–16 h after exposure to Lumigen-PPD and remains constant for several days.

Results

DNA samples isolated from 'Chinese Spring' wheat, *A. cristatum* and the F_1 hybrid were firstly digested with the restriction enzyme *Hin*dIII, and subsequently hybridized to the 14 probes with the aim of detecting polymorphism between the two parents and monitoring the transmitted *Agropyron*-specific markers in the F_1 hybrid. All of the 14 probes used detected polymorphisms between the two parents. But only 11 of them displayed the same polymorphisms in *Agropyron* and the F_1 hybrid but which were absent in wheat cv 'Chinese Spring' (Fig. 1). The presence of heterozygosity in *Agropyron* is the most likely reason for the difference between the F_1 and *Agropyron*. In the case of the three other probes, *Xpsr*162 (wheat group 1, long arm), *Xpsr*135 (wheat group 2, short arm) and *Xpsr*129 (wheat group 7, long arm), use of a second enzyme, *Eco*RI, revealed the transmitted *Agropyron*-specific markers in the F_1 hybrid (Table 1). On average 2.3 bands were found in *A. cristatum* per enzyme – probe combination and most of them (87.5%) did not overlap with those of wheat.

The following is a brief description of the RFLP patterns detected by the 14 probes in the 54 alien addition lines.

Addition lines marked with only one probe

Ten out of the 54 lines classified cytologically as monotelosomic additions revealed additional hybridization bands only by the probes *Xpsr*135 (2S), *Xpsr*101 (2L), *Xpsr*128 (5L), *Xpsr*167 (6S) or *Xpsr*463 (6L). So their added telocentric chromosomes were identified unequivocally as the 2S, 2L, 5L, 6S and 6L (Table 2).

Addition lines marked by two probes for one homoeologous group

Among 54 addition lines analysed, 32 lines were marked by only the two probes specific for a given homoeologous group, indicating that they had one complete P-genome chromosome of *A. cristatum*. As they were identified by the probes for the first six homoeologous groups, the added chromosome should be 1P to 6P (Table 2). The examination of the hybridization profiles of the hybrid showed that its hybrid fragments were exactly the same as those in the addition lines. For example, in Fig. 2 the probe *Xpsr*128,

Table 1 Wheat cDNA probes used and RFLPs detected in DNA of wheat cv 'Chinese Spring', *A. cristatum* 4x, and their hybrids, digested with two restriction enzymes

Probes	Insert size (kb)	Arm location in wheat	Enzymes	Number of hybrid bands in		
				CS	*A. cris*	Hybrid
*Xpsr*161	0.7	1S	*Hin*dIII	3	2 (1)[a]	4
*Xpsr*162	2.0	1L	*Eco*R1	3	3	5
*Xpsr*135	1.0	2S	*Eco*R1	3	1	4
*Xpsr*101	0.6	2L	*Hin*dIII	3	1	4
*Xpsr*123	1.0	3S	*Hin*dIII	3	3	4
*Xpsr*543	3.0	3L	*Hin*dIII	3	3	4
*Xpsr*144	1.3	4AS 4BL 4DL	*Hin*dIII	3	3	4
*Xpsr*163	0.5	4AL 4BS 4DS	*Hin*dIII	3	2	4
*Xpsr*118	0.7	5S	*Hin*dIII	3	2	4
*Xpsr*128	0.5	5L	*Hin*dIII	3	1	4
*Xpsr*167	0.6	6S	*Hin*dIII	3	4 (2)	4
*Xpsr*463	1.3	6L	*Hin*dIII	4	2 (1)	5
*Xpsr*152	0.8	7S	*Hin*dIII	3	2	4
*Xpsr*129	1.2	7L	*Eco*RI	3	3	4
Average				3.1	2.3	4.1

[a] Number of hybrid bands of *A. cristatum* that overlapped with those of CS

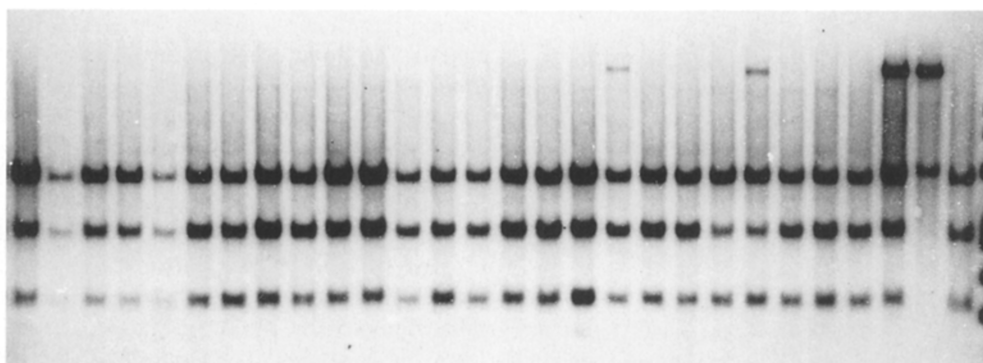

Fig. 1 *Xpsr*161 (1L)-probed Southern hybridization of the *Hin*dIII-digested genomic DNAs of a series of wheat-*A. cristatum* monosomic addition lines (*lanes 1–25*), F_1 hybrid (*lane 26*), *A. cristatum* 4x (*lane 27*) and 'Chinese Spring' wheat (*lane 28*). Two addition lines (*lanes 18 and 22*) displayed one hybridization band of about 12 kb which was present in the hybrid and *A. cristatum* 4x but was absent in 'Chinese Spring' and the addition lines carrying other *A. cristatum* chromosomes

specific for the long arm of homoeologous group 5, revealed a fragment of about 4.0 kb in all *A. cristatum*, the F_1 hybrid and three addition lines. So, at least for the loci detected, the added chromosomes should be identical to those transmitted to the F_1 hybrid by the paternal parent *A. cristatum*.

The two loci detected in the F_1 hybrid by the probes *Xpsr*152 (7S) and *Xpsr*129 (7L) were not found in any addition line.

Other addition lines

There were three addition lines that did not reveal any specific band of *A. cristatum*, while two addition lines that carried two *A. cristatum* chromosomes were marked by four probes of two homoeologous groups, 2 and 4, indicating that they were both double monosomic addition lines for chromosomes 2P and 4P.

There were two 43-chromosome plants marked by two probes of different homoeologous groups. One of them was found to possess two markers detected respectively by probe *Xpsr*161 (1S) and probe *Xpsr*123 (3S), suggesting a translocation between *A. cristatum* chromosomes 1P and 3P (1PS-3PS). The other line possessed two fragments of *A. cristatum* detected by probes *Xpsr*161 (1S) and *Xpsr*128 (5L), so this may be a translocation between *A. cristatum* chromosomes 1P and 5P (1PS-5PL).

One plant with 44 chromosomes was marked by the four probes specific for 4S, 4L, 5L and 6L. This indicated that the two chromosomes added were 4P and a translocated 5P-6P chromosome, possibly 5PL-6PL.

In an addition line with a confirmed 1P chromosome, a hybrid band contributed by a group-5 long arm of wheat was absent and a specific *A. cristatum* band revealed by the probe *Xpsr*118 (5L) was evident. Therefore, this line should be homozygous for a translocation between a wheat (W) group-5 chromosome and 5P, possibly 5WS-5PL.

Table 2 Identification of 54 wheat – *A. cristatum* addition lines by 14 RFLP probes

Class	No. plants	2n	Homoeologous group of wheat [a]							Alien chromosome added
			1S1L	2S2L	3S3L	4S4L	5S5L	6S6L	7S7L	
CS		42								
F_1		35	+ +	+ +	+ +	+ +	+ +	+ +	+ +	
Addition lines										
	1	42+t		+						2PS
	1	42+t		+						2PL
	4	42+t					+			5PL
	1	42+t						+		6PS
	3	42+t						+		6PL
	3	43	+ +							1P
	5	43		+ +						2P
	3	43			+ +					3P
	1	43				+ +				4P
	6	43					+ +			5P
	14	43						+ +		6P
	3	43								?
	2	44		+ +		+ +				2P, 4P
	1	43	+		+					1PS-3PS
	1	43	+				+			1PS-5PL
	1	44				+ +	+	+		4P, 5PL-6PL
	1	43	+ +				+(–)			1P, 5PL-5WS
	1	43							*	7P?
	2	43		+ +					(+–)	2P, nulli-tetra6W

[a] +, presence of a band specific for *A. cristatum*; (–), missing a band specific for wheat; (+), a more dense band of wheat; *, presence of a band that is absent in both CS and the F_1 hybrid

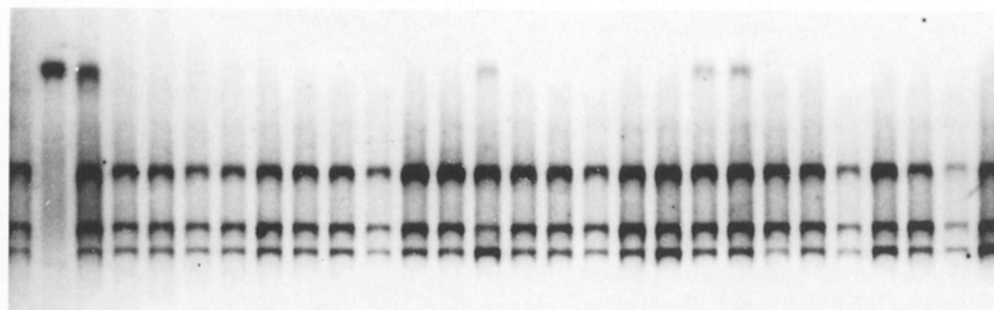

Fig. 2 *Xpsr*128 (5L)-probed Southern hybridization of the *Hin*dIII-digested genomic DNAs of a series of wheat-*A. cristatum* monosomic addition lines (*lanes 4–28*), F_1 hybrid (*lane 3*), *A. cristatum* 4x (*lane 2*) and 'Chinese Spring' wheat (*lane 1*). Three addition lines (*lanes 14, 20, 21*) diplayed one hybridization band of about 4.0 kb which was present in the hybrid and *A. cristatum* 4x but was absent in 'Chinese Spring' and the addition lines carrying other *A. cristatum* chromosomes

In one line with 43 chromosomes, the probe *Xpsr*129 (7L) displayed one band found neither in 'Chinese Spring' nor in the F_1 hybrid whereas a band (7L) characteristic of 'Chinese Spring' was missing (data not shown). It appeared that this line possessed a translocation between a wheat group-7 chromosome and 7P.

Two plants which had the 2P chromosome revealed a pattern typical of a nullisomic-tetrasomic of the wheat group 6 since one band specific for group 6 of 'Chinese Spring' was missing and another, also specific for this group, was more dense than in the 'Chinese Spring' profile.

Discussion

This study was initiated to characterize the *A. cristatum* P-genome chromosome addition lines in the wheat background by using RFLP markers. RFLPs were detected between wheat cv 'Chinese Spring' and *A. cristatum* 4x by 14 wheat probes located on each arm of each homoeologous group and two restriction enzymes, *Hin*dIII and *Eco*RI. Thirty-two addition lines were each identified by the two probes of one wheat homoeologous group. The added single *A. cristatum* chromosomes were 1P to 6P.

Consequently, there is as yet no evidence of structural rearrangements which differentiate the P genome from the A, B and D genomes of wheat. In future, the use of more known probes will provide an opportunity to establish the degree of colinearity of the genetic maps of the two species. This will be a prerequisite, as outlined by Devos et al. (1993) in rye, to fully exploit the *A. cristatum* gene pool for wheat improvement.

For most selected probe-enzyme combinations , two or three hybrid bands were displayed in the *A. cristatum* profile. This confirmed that the cross-pollinating autotetraploid *A. cristatum* (Dewey 1984) is a highly heterozygous species. In most enzyme-probe combinations, the hybridization profile of the F_1 hybrid displayed one more band than in 'Chinese Spring'. However the pentaploid F_1 hybrid possessed two P genomes (2n=35, ABDPP: Chen et al. 1989). Therefore any band characterizing the hybrids might not represent all, but only one to two, of the P genomes of the parent. This could result in addition lines without any marker. Indeed three of the 54 plants analysed presented no marker specific to *A. cristatum*. Hopefully, they could belong to homoeologous group 7. Moreover, concerning the group-7 probe, *Xpsr*129 (7L), one plant had a hybridization profile with a band present neither in the F_1 nor in 'Chinese Spring'. This might have resulted from intralocus recombination in an F_1 hybrid heterozygous for the locus concerned and the consequent extraction of the addition line for chromosome 7P with an unexpected hybridization profile. Further investigation with probes other than those used here will be required to characterize the lines without any marker or with an unexpected group-7 marker.

In the course of isolation of a set of addition lines, chromosome rearrangements, mostly translocations, may occur. Evidence of translocations between *Agropyron* chromosomes and between wheat and *Agropyron* chromosomes was obtained in three and two lines respectively. The plants analysed were chosen in the F_2 of the third BC generation. Moreover, at each generation the translocations were counterselected on the basis of meiotic behaviour. This means that the frequency of translocations could be much higher than observed (9%). They most likely occurred mainly in the F_1 hybrid because of the complicated meiotic behaviour due to interference with the 5B homoeologous pairing restriction system of wheat by the *A. cristatum* genetic system (Chen et al. 1989, 1990). The translocations between wheat and *Agropyron* chromosomes might provide new cytogenetic stocks for the transfer of alien genes. One of them could have resulted from a centric fusion between 5PL and a short arm of a group-5 chromosome. If the *Ph* suppressor gene(s) of *A. cristatum* are on 5PL, this line may be significant for studying the effect of that suppressor.

The present study constitutes the first example of using RFLP analysis with the aim of identifying a complete set of addition lines from *A. cristatum* with a P genome. Our results confirm the conclusion of Sharp et al. (1989) that using RFLP markers to characterize some unknown alien addition lines enables minimization of time and effort in screening programs. If conventional techniques were used to achieve our result, i.e., the identification of at least six monosomic additions (1P to 6P) and five monotelosomic additions (2S, 2L, 5L, 6S, 6L), a much longer time would have been needed. Additionally, RFLP analysis was useful for identifying plants with translocated chromosomes.

The materials identified in this investigation will enrich the collection of wheat alien addition lines from the perennial Triticeae which is has been limited to a few species belonging to the E and H genomes (Cauderon et al. 1973; Dvorak and Knott 1974; Hart and Tuleen 1983; Gill and Morris 1987; Charpentier 1992).

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References

Ahmad F, Comeau A (1991) A new intergeneric hybrid between *Triticum aestivum* L. and *Agropyron fragile* (Roth) Candargy: variation in *A. fragile* for suppression of the wheat *Ph*-locus activity. Plant Breed 106:275–283

Cauderon Y (1966) Étude cytogénétique de l'évolution du matériel issu de croisement entre *Triticum aestivum* et *Agropyron intermedium*. Ann Amélior Plantes 16:43–70

Cauderon Y, Saigne B, Dauge M (1973) The resistance to wheat rusts of *Agropyron intermedium* and its use in wheat improvement. Proc 4th Int Wheat Genet Symposium, Columbia, Missouri, pp 401–407

Cauderon Y, Autran JC, Joudrier P, Kobrehel K (1978) Identification de chromosomes d'*Agropyron intermedium* impliqués dans la synthèse des gliadines, des α-amylases et des péroxydases à l'aide de lignées d'addition Blé × *Agropyron*. Ann Amélior Plantes 28:257–267

Charpentier A (1992) Production de lignées d'addition disomique et d'amphiploïdes partiels de *Thinopyrum junceum* sur le blé tendre. C R Acad Sci Paris Ser 3, 315:551–557

Chen Q, Jahier J, Cauderon Y (1989) Production and cytogenetical studies of hybrids between *Triticum aestivum* L. Thell. and *Agropyron cristatum* (L.) Gaertn. C R Acad Sci Paris Ser 3 308: 411–416

Chen Q, Jahier J, Cauderon Y (1990) Intergeneric hybrids between *Triticum aestivum* and three crested wheatgrasses: *Agropyron mongolicum*, *A. michnoi* and *A. desertorum*. Genome 33:663–667

Chen Q, Jahier J, Cauderon Y (1992) Production and cytogenetic analysis of BC_1, BC_2, and BC_3 progenies of an intergeneric hybrid between *Triticum aestivum* (L.) Thell. and tetraploid *Agropyron cristatum* (L.) Gaertn. Theor Appl Genet 84:698–703

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19–21

Devos KM, Atkinson MD, Chinoy CN, Francis HA, Harcourt RL, Koebner RMD, Liu CJ, Masojc P, Xie DX, Gale MD (1993) Chromosomal rearrangements in the rye genome relative to that of wheat. Theor Appl Genet 85:673–680

Dewey DR (1984) The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae. In: Gustafson JP (ed) Gene manipulation in plant improvement. 16th Stadler Genet Symposium. Plenum, New York, pp 209–279

Dvorak J, Knott DR (1974) Disomic and ditelosomic additions of diploid *Agropyron elongatum* chromosomes to *Triticum aestivum*. Can J Genet Cytol 16:399–417

Forster BP, Reader SM, Forsyth SA, Koebner RMD, Miller TE, Gale MD, Cauderon Y (1987) An assessment of the homoeology of six *Agropyron intermedium* chromosomes added to wheat. Genet Res 50:91–97

Gill BS, Morris KLD (1987) Assignment of the genomic affinities of chromosomes from polyploid *Elymus* species added to wheat. Genome 30:70–82

Hart GE, Tuleen NA (1983) Chromosomal locations of eleven *Elytrigia elongata* (=*Agropyron elongatum*) isozyme structural genes. Genet Res 41:181–202

Ishii T, Panaud O, Brar DS, Khush GS (1990) Use of non-radioactive, digoxigenin-labelled DNA probes for RFLP analysis in rice. Plant Mol Biol Rep 3:167–171

Jauhar PP (1992) Chromosome pairing in hybrids between hexaploid bread wheat and tetraploid crested wheatgrass (*Agropyron cristatum*). Hereditas 116:107–109

Jewell DC, Driscoll CJ (1983) The addition of *Aegilops variabilis* chromosomes to *Triticum aestivum* and their identification. Can J Genet Cytol 25:76–84

Li L, Dong Y (1991) Hybridization between *Triticum aestivum* L. and *Agropyron michnoi* Roshev. 1. Production and cytogenetic study of F_1 hybrids. Theor Appl Genet 81:312–316

Limin AE, Fowler DB (1990) An interspecific hybrid and amphiploid produced from *Triticum aestivum* crosses with *Agropyron cristatum* and *Agropyron desertorum*. Genome 33:581–584

Lu YH, Merlino M, Isaac PG, Staccy J, Bernard M, Leroy P (1994) A comparative analysis between [^{32}P] and digoxigenin-labelled single-copy probes for RFLP detection in wheat. Agronomie 14: 33–39

Miller TE, Reader SM (1987) A guide to the homoeology of chromosomes within the Triticeae. Theor Appl Genet 74:214–217

Morris KLD, Raupp WJ, Gill BS (1989) Isolation of Ht-genome chromosome additions from polyploid *Elymus trachycaulus* (StStHtHt) into common wheat (*Triticum aestivum*). Genome 33:16–22

Panaud O, Magpantay G, McCouch S (1993) A protocol for non-radioactive DNA labelling and detection in the RFLP analysis of rice and tomato using single-copy probes. Plant Mol Biol Rep 11:54–59

Sharp PJ, Chao S, Desai S, Gale MD (1989) The isolation, characterization and application in the Triticeae of a set of wheat RFLP probes identifying each homoeologous chromosome arm. Theor Appl Genet 78:342–348

Xu YS, Jones MGK, Karp A, Pehu E (1993) Analysis of the mitochondrial DNA of the somatic hybrids of *Solanum brevidens* and *S. tuberosum* using non-radioactive digoxigenin-labelled DNA probes. Theor Appl Genet 85:1017–1022