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Y. B. Wang · H. Hu · J. W. Snape

The genetic and molecular characterization of pollen-derived plant lines from octoploid triticale × wheat hybrids

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Abstract Six doubled-haploid (DH) lines, derived by anther culture from octoploid triticale × wheat hybrids, were characterized using cytological, biochemical and molecular techniques. Lines varied in their wheat and rye genome composition, and were either wheat-rye chromosome multiple-addition lines or had spontaneous substitutions and/or wheat-rye translocations. Most of the lines contained a pair of 4R chromosomes, whereas 1R or 7R were present in others. The results are similar to those previously obtained with hexaploid triticale × wheat crosses and indicate that it is possible to produce alien (wheat/rye) addition, substitution, and translocation lines directly from the anther culture of intergeneric hybrids.

Key words Doubled haploids · Octoploid triticale · Wheat · Wheat/rye translocations · Wheat/rye addition · Genomic *in situ* hybridization (GISH) · SDS-PAGE · RFLP

Introduction

The transfer of alien genes into cultivars is an important method of agronomic improvement in wheat, particularly with respect to increasing pest and disease resistance (Gale and Miller 1987). Wheat/alien chromosome substitutions and translocations are generally derived from either backcrossing the interspecific hybrid or the amphiploid to wheat, or by first developing monosomic or disomic alien additions, and then bringing about homoeologous or non-homoeologous recombination by irradiation treatment or the induction of pairing. These methods, although undoubtedly successful, require extensive crossing programmes and cytological analysis, and take many generations. An alternative approach has been proposed though the use of anther culture (Hu et al. 1988), where wheat/alien recombinant doubled-haploid (DH) lines can be derived in one generation from interspecific hybrids. A number of doubled-haploid lines with different rye constitutions have been obtained in this way from crosses between hexaploid triticale × wheat (Tao 1990; Wang et al. 1993) and have been characterized using cytological and marker techniques. In the present paper we extend these observations by a study of six pollen-derived plant lines from heptaploid hybrids of octoploid triticale × bread wheat.

Materials and methods

Materials

Two secondary octoploid triticales, AH1095 and AH1001, derived from crosses between octoploid triticales and primary amphiploids, were crossed with bread wheat. Six pollen-derived plants, B77, V209, VS38, B91, B92 and B95, were obtained. B77 was derived from the cross of triticale line AH1095(F_4) × wheat cv "Kedong 58" (K58). The others were obtained from the cross AH1001(F_5) × K58. Seeds of the second or third generations, following selfing of the original DH plants, were examined in this study.

Methods

Mature seeds of the DH lines and parental varieties were cut into two halves. The endosperm halves were retained for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis and the embryo halves were germinated and root-tips removed for the cytological examination of mitotic chromosomes. The embryo halves were then planted in pots in a greenhouse. Six to eight weeks after sowing, leaf tissue was removed from plants of each line to permit DNA extraction for restriction fragment length polymorphism (RFLP) analysis.

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Y. B. Wang[1] · H. Hu
Institute of Genetics, Chinese Academy of Sciences, Beijing 100101, China

Y. B. Wang · J. W. Snape (✉)
John Innes Centre, Colney Lane, Norwich NR4 7UH, UK

Present address:
[1] The Norman Borlaug Institute for Plant Science Research, De Montfort University, Scraptoft, Leicester LE7 9SU, UK

Table 1 Chromosomal locations of the RFLP markers used

Probe	Chromosomal location in rye	Chromosomal location in wheat			Loci
PSR596	1RS	1AS	1BS	1DS	*Xpsr596*
PSR544	1RL	1AL	1BL	1DL	*Xpsr544*
PSR162	1RL	1AL	1BL	1DL	*Xpsr162*
PSR584	4RS	4AL	4BS	4DS	*Xpsr584*
PSR167	4RL	6AS	6BS (5B)	6DS	*Xpsr167*
PSR128	5R	5AL	5BL	5DL	*Xpsr128*
PSR627	6RS	6AS	6BS	6DS	*Xpsr627*
PSR154	6RL	6AL	6BL	6DL	*Xpsr154*
PSR115	7RS	4AL	5BL	5DL	*Xpsr115*
PSR129	7RL	7AL	7BL	7DL	*Xpsr129*

Genomic in situ hybridization (GISH)

In situ hybridization using labelled total rye DNA as the probe and wheat DNA as the blocking DNA was performed as described by Schwarzacher et al. (1992) with modifications according to King et al. (1993). Mitotic chromosomes in the root-tip preparations were analyzed by epifluorescence microscopy using a Nikon Microphot-SA microscope.

SDS-PAGE analysis

SDS-PAGE was performed on endosperm protein extracts using the methods described by Payne and Corfield (1979) and Lawrence and Shepherd (1980). Unreduced protein extracts [DTT (dithiothreitol) free] were used to analyze variation at gliadin and *Sec-1* loci, while reduced protein extracts (from adding DTT) were used to analyze the high-molecular-weight glutenin subunit constitution of each line.

RFLP analysis

RFLP analysis was carried out as described by Devos et al. (1992). To establish appropriate probe/enzyme combinations for revealing polymorphisms between the parental lines, four restriction enzymes, *Hin*dIII, *Eco*RI, *Eco*RV and *Bam*HI, were used. Initially, ten low copy number wheat probes, which previously had shown polymorphisms between wheat and rye (Sharp et al. 1988; Devos et al. 1993), were chosen to identify individual rye chromosomes. The locations of these ten probes are summarized in Table 1, according to Devos et al. (1993) and Gale et al. (1995). The probes numbered PSR115–PSR167 are cDNA clones from the library described by Chao et al. (1989). PSR544–PSR627 are genomic DNA clones from a *Pst*I genomic library (Devos et al. 1992).

Results

GISH analyses

GISH was applied to detect the total rye genomic contribution to the DH lines. Fig. 1 a indicates that one of the parental triticale lines, AH1095, contained 14 rye chromosomes (in yellow) with a total chromosome number, as expected, of 2n=56. The original B77 DH plant and its first-generation selfed progenies contained 46 chromosomes. In the four third-generation plants examined by GISH, three plants showed four rye chromosomes (Fig. 1 d), and were 2n=46, whilst one plant had only three rye chromosomes (data not shown).

The other triticale, line AH1001, the parent of the other DH plants, was also examined by GISH. Three plants studied showed 14 rye chromosomes with a chromosome number of 2n=56. However, telocentric rye chromosomes were found in one other plant. The original VS38 plant was 2n=44+t. Among its eight third-generation plants studied, four types of rye chromosome constitution were observed: 2.R+2t.R (1 plant), 2.R+t.R (four plants), 2.R (two plants) and 1.R (1 plant). All eight V209 plants, derived from one selfed seed of the original pollen-derived plant, contained a single pair of a wheat/rye centric translocated chromosomes, where one wheat short arm was substituted by one rye short arm (Fig. 1 c).

The predominant chromosome constitution of the original B91 plant was 2n=47. In its first-generation progenies, plants which contained either 44 or 44+2t chromosomes were propagated. In the progeny of one of these 44 chromosome plants, five plants had the same chromosome number and contained one pair of a complete rye chromosome (Fig. 1 b); another plant had only one rye chromosome. The single plant from the 44+2t B91 parent had a rye constitution of 2.R+2t.R, the same as the one B92-derived plant. Two rye chromosomes were observed in the single B95 plant examined, whose original pollen-derived parent contained 46 chromosomes.

SDS-PAGE analyses

SDS-PAGE was used to identify the genotype at loci encoding high-molecular-weight glutenin subunits, *Glu-1*, located on the long arms of 1A, 1B, 1D and 1R; and gliadin monomers, *Gli-1*, on the short arms of 1A, 1B and 1D. *Sec-1* is located on the short arm of 1R (Payne 1987, Lawrence and Shepherd 1981 a, b). Figure 2 shows SDS-PAGE patterns of reduced protein extracts from DH lines B77 and V209 as well as those of their original parents. AH1001 plants were polymorphic for *Glu-D1*, indicating that heterozygosity existed in the original AH1001 stock. V209 plants did not have a 1RL or 1BS band although 1BL bands were present. Because no telocentric chromosomes were found in any V209 plants, a wheat/rye 1BL.1RS translocated chromosome is presumed to be present. B77 progenies obviously carry *Glu-R1* on the 1R long arm. Combining this with the result from GISH, we can infer that B77 plants have a 1R pair, and another unidentified rye pair. Most are thus double addition individuals. Some B77 plants, however, had lost the whole of their 1B chromosome (Fig. 2, far right lane), and so reduced to (1B)1R whole-chromosome substitution lines.

SDS-PAGE on unreduced protein extractions were also performed to detect the 1R short arm by observation of *Sec-1*-encoded products which were apparent in most, but not all, plants of both octoploid triticale parents, AH1001 and AH1095. However, surprisingly, none of the DH lines B77, V209 or VS38 had typical *Sec-1* bands even though, in the case of B77, independent evidence from C-banding

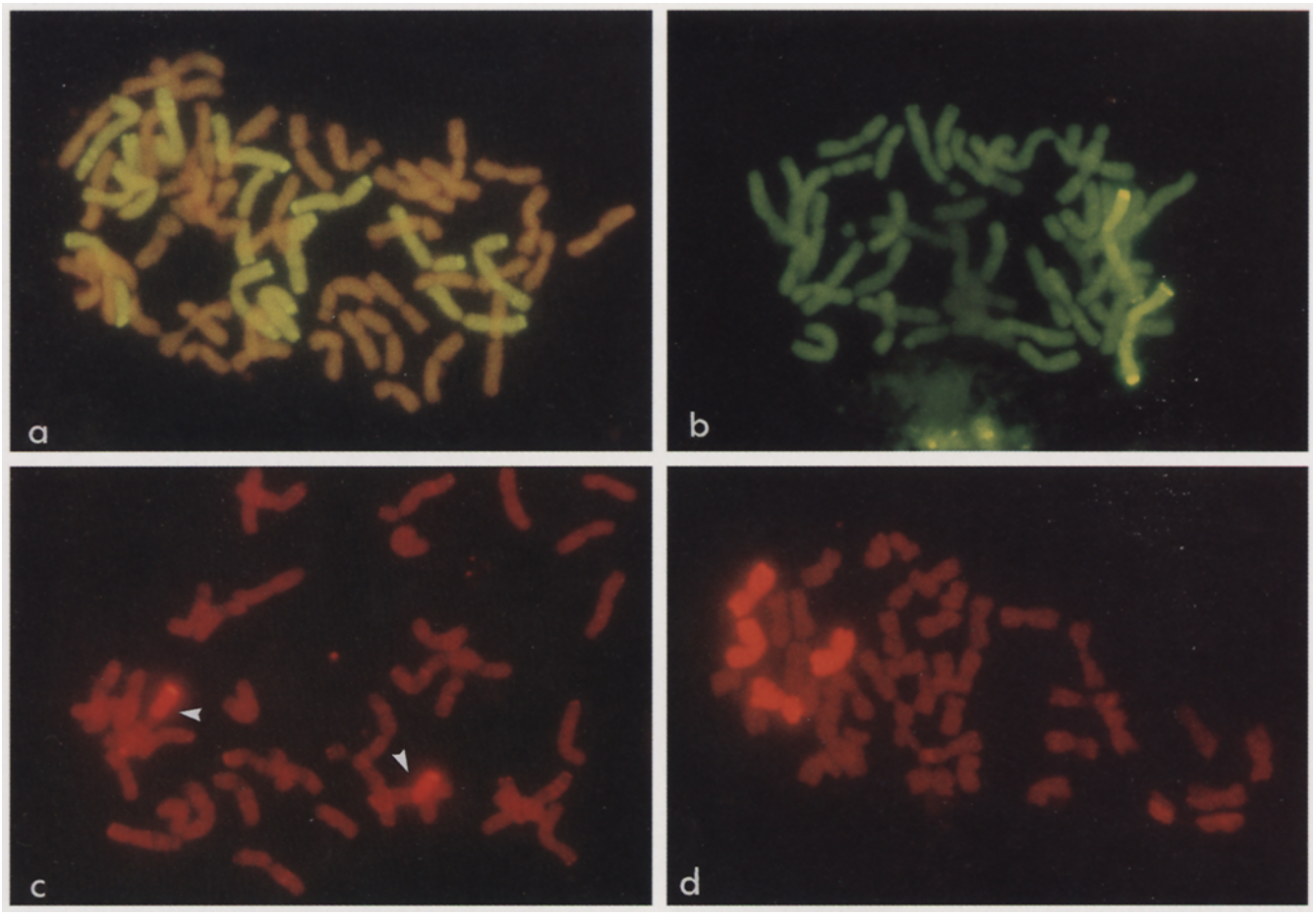

Fig. 1a–d Genomic in situ hybridization, using total genomic rye DNA as a probe, to chromosome preparations of DH lines and octoploid triticale. Rye DNA was labelled with digoxigenin (**a** and **b**) or rhodamine (**c** and **d**). **a** Octoploid triticale, AH1095, 2n=56, containing 14 rye chromosomes (in yellow); **b** a plant of B91, 2n=44, containing one pair of rye chromosomes (in yellow); **c** a plant of V209, containing one pair of a wheat/rye translocation chromosomes (*arrowed*); **d** a B77 plant, containing two pairs of rye chromosomes (in bright-red)

(data not shown) and RFLP analysis confirmed the existence of 1RS (Fig. 3). Thus perhaps the octoploid parents contain polymorphisms for *Sec-1* bands which were not distinguishable from other wheat bands.

RFLP analyses

RFLP analysis was used to identify the individual rye chromosomes or segments present in each DH line. To this end,

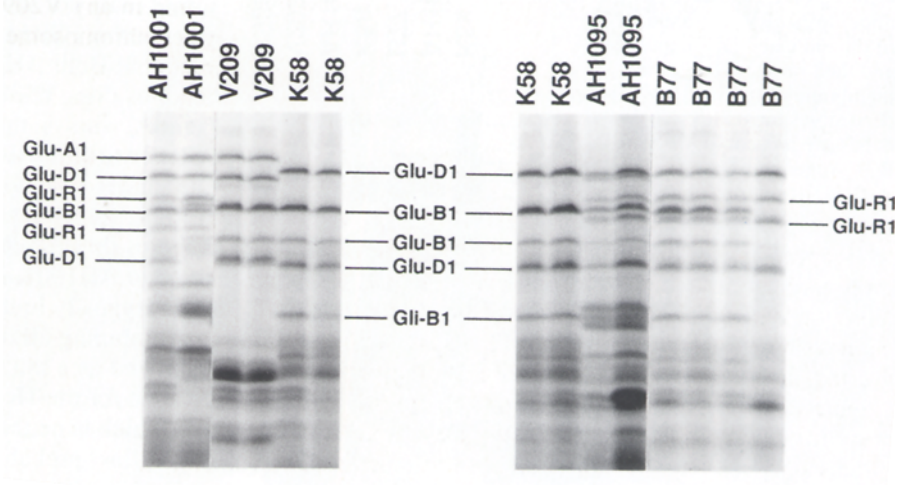

Fig. 2 SDS-PAGE patterns of reduced protein extracts from plants of DH lines V209, B77 and their parents. Phenotypes: V209, Glu-R1$^-$ Glu-B1$^+$ Gli-B1$^-$; B77 (three left lanes), Glu-R1$^+$; B77 (far right lane), Glu-R1$^+$ Glu-B1$^-$ Gli-B1$^-$

Table 2 RFLP analysis of the specific rye chromosome constitution of the DH lines

Probe	Location	Enzyme	B77	VS38			V209	B91		B92	B95
				R2	R3	R4		R2	R4		
PSR596	1RS	*Eco*RI	+								
		*Eco*RV	+								
		*Bam*HI	+	–	+	+	+(?)[a]	–	–	–	–
PSR544	1RL	*Eco*RI	+								
		*Eco*RV	+								
		*Hin*dIII						–	+		
PSR162	1RL	*Hin*dIII	+	–	–	–	–	–	+	+	–
PSR584	4RS	*Hin*dIII	–	–	–	–	–	+	+	+	+
PSR167	4RL	*Hin*dIII						+	+	+	+
PSR115	7RS	*Eco*RV	–			+	–				
PSR129	7RL	*Hin*dIII	–	+	+	+	–	–	–	–	–
Summary			B77	VS38			V209	B91		B92	B95
				R2	R3	R4		R2	R4		
Rye chromosome constitution			1R ?R	7R	7R 1RS	7R 1RS	1RS	4R	4R 1RL	4R 1RL	4R

[a] Band pattern is different from VS38(R2) and VS38(R4)

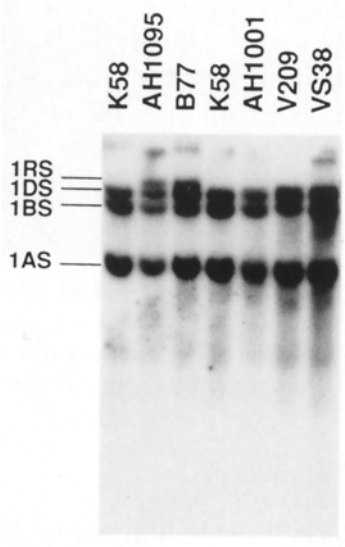

Fig. 3 Hybridization of probe PSR596 (homoeologous group-1 specific) to *Eco*RV-restricted DNA of the indicated DH plant lines and their parents. B77 has a 1R short arm. No 1R band can be seen in AH1001

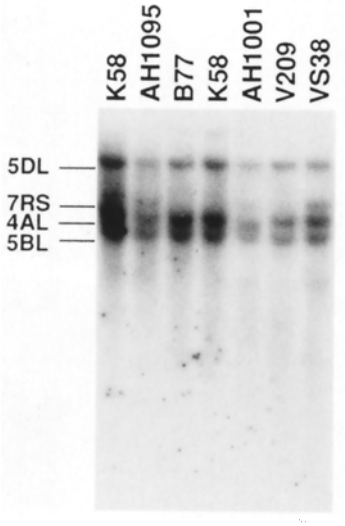

Fig. 4 Hybridization of probe PSR115 (4A, 5B, 5D long arms) to *Eco*RV-restricted DNA of the indicated DH plant lines and their parents (the same membrane as in Fig. 3). VS38 derivatives have 7RS. No 7RS bands are present in V209 or B77 progenies

ten wheat probes were hybridized to DNA extracts of the DH plants and the original parental lines, digested with four restriction enzymes. Not all hybridizations were informative due to the lack of polymorphism between the homoeologous rye and wheat chromosomes (R bands were sometimes not distinguishable from other wheat bands). The results from the RFLP analyses are summarized in Table 2.

The hybridization pattern of probe PSR596 shows that the 1R short arm is present in B77 and its triticale parent, AH1095 (Fig. 3). However, no 1R band can be observed in AH1001, and it is uncertain whether V209 or VS38 contain 1RS. The hybridization pattern of the probe PSR162 to *Hin*dIII-restricted DNA of the DH lines and their parents (as well as several other triticale and rye varieties) showed a high level of polymorphism for 1RL bands. B77 has the same 1RL band as its female parent AH1095. B91 and B92, which contain four rye chromosomes, have a 1RL band which is different from AH1095, but the same as their female parent AH1001. The 1RL band was absent in V209, VS38 and B95, and in B91 plants with two rye chromosomes (data not shown). Figure 4 shows the pattern of another probe, PSR115, hybridized to the same membrane as in Fig. 3. Both AH1001 and AH1095 have an extra 7RS band, as does VS38, but this is absent in B77 and V209. Figure 5 shows the hybridization pattern of the probe PSR129 to *Hin*dIII-restricted DNA of the parents and DH lines and it is obvious that AH1001 and AH1095 are polymorphic for 7RL bands. VS38 is the only DH line which contains this 7RL band, but surprisingly it has a different allele to AH1001, its female parent, probably because of heterozygosity in the original octoploid triticale stock.

Combining all of these analyses suggests that B77 has stabilized as a multi-addition line with 46 chromosomes, disomic for the 1R chromosome and another unidentified pair which is neither 4R nor 7R. V209 is a ?R/1B translocation line, probably 1BL.1RS. VS38 possesses a pair of

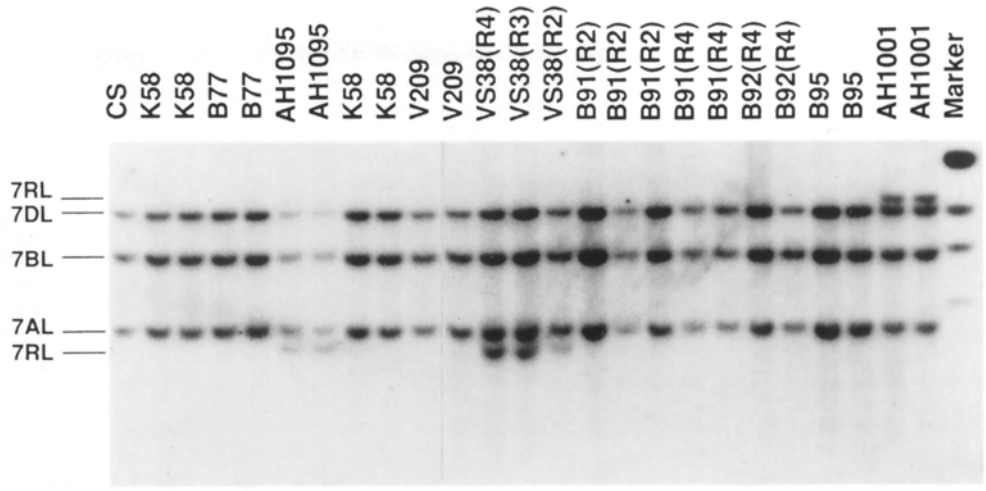

Fig. 5 Hybridization of probe PSR129 (homoeologous group-7 specific) to *Hin*dIII-restricted DNA of the indicated DH plant lines and their parents and Chinese Spring. Among the DH lines, only VS38 has 7RL. AH1095 and AH1001 give different 7R bands, showing polymorphism between different 7R chromosomes. *R2, R3 and R4* refer to progenies containing 2, 3 and 4 rye chromosomes, respectively

7R chromosomes, and some plants had one or two extra 1R short arms. Lines B91, B92 and B95 appeared, at their third generation, to be very similar wheat/rye addition lines with a chromosome number of 2n=44. Most plants of these lines contained a pair of 4R chromosomes but, additionally, some plants from B91 and B92 had one pair of extra 1R long arm telocentrics.

Discussion

These results show that wheat/rye translocations, such as that found in V209, can occur during male meiosis of the 49-chromosome hybrids between octoploid triticale and wheat. In a similar study, Wang et al. (1995) found wheat/rye Robertsonian and non-Robertsonian translocations from female meiotic products of such a cross. This indicates that wheat/rye pairing and genetic exchange may occur at male and female meiosis of interspecific hybrids even in the presence of *Ph1*.

These DH lines were derived from doubled male gametes, and thus should be homozygous. However, instabilities were observed, particularly with respect to chromosome number. These must be generated either during meiosis or during the culture period, sometimes at a high frequency. Among the pollen plants derived from the F_1 hybrids with a chromosome constitution of AABBDDR, the frequency of plants with chromosome variation was about 20% (Wang and Hu 1993). Four out of six DH plant lines analyzed in this study were derived from such DH plants. For example, the original B91 and B92 plants, regenerated from the same donor F_1 plant, had a chromosome constitution of 2n=47 in about 70% of root-tip cells. C-banding showed that both had a pair of rye chromosomes and a pair of rye telocentric chromosomes. In the following generation, segregation occurred with chromosome numbers ranging from 2n=44 to 2n=47. In the third generation, a stable disomic 4R addition line could be identified (Fig. 1 b). Similarly, the original VS38 plant had a 2n=44+t chromosome construction. After two generations of selfing, disomic additions could be selected and the telocentric chromosome was lost. The original V209 plant contained four telocentric chromosomes, but after two generations, no telocentric chromosomes remained. Thus, these DH plants, although initially chromosomally unstable, can set seeds and more stable progenies can be produced in only one or two generations.

Wheat chromosomes can also be lost during selfing. The original B77 plant contained 46 chromosomes. After three generations of selfing, some plants, however, had lost their 1B chromosome (Fig. 2, far right lane) while four rye chromosomes still remained. Thus stable substitution lines can be formed spontaneously by selfing the addition line. Although these DH lines may not initially be stable, after only two or three selfing generations, relatively stable addition lines, substitution lines, and translocation lines can be obtained.

Overall, these results and our previous studies indicate that novel and new wheat/rye chromosomal constitutions can be generated and stabilized following anther culture of triticale × wheat hybrids. However, it should be noted that a detailed genetic analysis using a combination of conventional and molecular cytogenetic analysis together with molecular-marker data is required to characterize the materials precisely, and ambiguities can arise which are difficult to resolve. Nevertheless, the genetical information obtained makes these lines an interesting and valuable resource for wheat improvement.

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