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Study of androgenetic performance and molecular characterisation of a set of wheat-rye addition lines

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Abstract Rye chromosomes of wheat-rye addition lines were successfully identified by means of an RFLP analysis with 30 probes. Our results are in agreement with previous cytological data concerning the identity of lines F (+1R), D (+2R), C (+3R), A (+4R), E (+5R) and B (+7R). Two categories of chromosomal rearrangements have been distinguished, namely: (1) deletions: the current line D possesses a chromosome 2R deleted on its short arm and the line G a chromosome 3R deleted on its long arm; we have also noticed a deletion on the long arm of wheat chromosome 1A in line F61; and (2) evolutionary reciprocal translocations in rye relative to wheat which have been previously mentioned in the literature. The anther culture response of the different lines was studied. A significant difference between 'FEC 28' and the addition lines was observed for embryo production and plant regeneration. It appears that genes located on 'S 10' chromosome arm 3RL and on 'FEC 28' chromosome arm 1AL increase embryo frequency whereas gene(s) located on 'S 10' chromosome 5R reduce(s) it. Plant regeneration results suggest that genes increasing regeneration ability and green-plant frequency are located on 'S 10' chromosome 4R. The long arm of chromosome 1A seems to be involved positively in green-plant regeneration whereas chromosomes 1R and 3R limit plant regeneration.

Key words RFLP analysis · Wheat-rye addition lines Chromosomal rearrangements · Anther culture Regeneration

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Introduction

The development of alien chromosome addition lines in which single pairs of chromosomes from a related species are added to wheat are potentially of great value in wheat improvement in order to increase the germplasm resources of cultivated hexaploid wheat (*Triticum aestivum* L.) and to introgress useful genes from related species such as rye (*Secale sereale* L.) or wild *Triticum* species into wheat. These addition lines provide a starting point for chromosome substitution and translocation (Law 1981; Miller 1984). They are also of use for genetic analyses in order to locate genes, or else biochemical or molecular markers, on a specific chromosome (Sharp et al. 1989). Wheat-rye addition lines are particularly useful in the study of the genetic effect of rye chromosomes in the wheat×rye amphiploid *Triticale* (Bernard 1976). However, for some wheat-rye addition lines, it has been difficult to assign the additional chromosome to one of the seven homoeologous groups owing to the variability between and within populations (Darvey and Gustafson 1975; Bernard 1976; Miller and Reader 1987; Jouve et al. 1989). Many authors have provided evidence of different evolutionary translocations between rye and wheat, based on: the observation of meiotic chromosome pairing in wheat×rye hybrids (Naranjo and Fernandez-Rueda 1991); the location of biochemical markers (Ainsworth et al. 1986; Jouve and Diaz 1990); and the location of molecular markers (Liu et al. 1992). Recently, the construction of an RFLP genetic map of rye has shown multiple chromosomal rearrangements, except for chromosome 1R, in the rye genome relative to that of wheat (Devos et al. 1993 a).

Anther culture provides a rapid method of inducing homozygosity, which is of interest for the production of breeding lines and genetic studies. However, its use in the genetic improvement of cereals has been seriously restricted by the low in vitro response of most species. Much effort has been directed to overcome this problem and some breeders have released new wheat varieties such as 'Jinghua no. 1' (Hu et al. 1986) and 'Florin' (De Buyser et al.

1987) using this method. It has been recognized that several factors influence the response to in vitro culture, namely: the environmental growth conditions of the donor plant, the stage of microspore development, the culture medium composition (for review see Henry and De Buyser 1990) and the genotype, which remains a major factor. Genotypic effect on anther-culture ability has been observed in major cereal crops such as barley (Foroughi-Wehr et al. 1982), *Triticale* (Charmet and Bernard 1984), and wheat (Andersen et al. 1987; Foroughi-Wehr and Zeller 1990). Haploid plant production depends on: the rate of embryo induction, the embryo regeneration ability, and the ratio of green to albino plants. In *Triticale* and wheat, these three traits are independently inherited and controlled by mainly nuclear genetic systems (Charmet and Bernard 1984; Agache et al. 1988). It has been shown that there is a prevalence of additive gene action for embryo production whereas additive as well as non-additive gene effects seem to act on regeneration and green-plant formation (*Triticale*: Charmet and Bernard 1984; wheat: Lazar et al. 1984; Deaton et al. 1987; Agache et al. 1988; Tuvesson et al. 1989; rice: Quimio and Zapata 1990). Some authors have taken advantage of aneuploids and chromosome addition or substitution wheat lines to identify specific chromosomes which carry major genes involved in different steps of the androgenetic process (Zhang and Li 1984; Szakacs et al. 1988; De Buyser et al. 1992). Very little is known about the influence of rye chromosomes when they are added to wheat. Anther culture in rye appears quite difficult (Wenzel and Thomas 1974; Daniel 1993) whereas *Triticale* gives good results (Bernard 1989). Henry and De Buyser (1985) and Henry et al. (1993) showed that chromosome arm 1RS, present in several translocated 1BL-1RS wheat cultivars, carries a gametophytic gene acting positively on regeneration capacity. Lazar et al. (1987), using 'Chinese Spring'–'Imperial' chromosome addition lines, showed a positive effect of chromosomes 1R and 4R on the production of androgenetic calli and of chromosome 4R on shoot initial ('green spot') formation from calli.

The present investigation is aimed at: (1) completing the identification of a set of wheat-rye addition lines by using molecular markers; and (2) evaluating their androgenetic ability, compared to that of the wheat recipient line, in order to provide further information about the chromosome location of genes affecting in vitro androgenesis.

Materials and methods

Plant materials

The materials examined consisted of: (1) the spring hexaploid wheat line 'FEC 28' (*Triticum aestivum* L.) derived from the cross 'Frontana'×'Etoile de choisy', (2) the self fertile rye inbred line no. 10 (hereafter denoted 'S 10') selected from the cultivar 'Petkus', from *Secale cereale* L., (3) 'S 10' rye chromosome addition lines to 'FEC 28' produced in the laboratory (Bernard 1976) according to the classical method described by Riley and Chapman (1958). Of about 100 plants obtained having 2n=43 chromosomes, 13 (which gave fertile disomic addition lines, 2n=44) were selected and their progenies were distributed into six separate classes. The different addition lines were first identified by means of morphological traits of the plant and by cytological studies of meiotic chromosome pairing which permitted the designation of the different lines as follows: A (4R/7R), B (7R/4R), C (3R), D (2R), E (5R), F (1R) (Bernard 1976) and G [classified in group 3R on morphological data, but tentatively identified by Darvey and Gustafson (1975) as 6R using the C-banding technique]. Eventually, the chromosome constitution was determined by C-banding (Bernard, unpublished data). Following the workshop on the nomenclature and homoeology relationships of rye chromosomes (Sybenga 1983), lines A, B, C, E and F present typical rye chromosomes 4R, 7R, 3R, 5R and 1R respectively. Due to instability and misdivision of additional chromosomes, available stocks of lines D and G are ditelosomic for rye chromosomes. Telosomes from line D have a prominent band of telomeric heterochromatin; the standard 2R is a near-median chromosome and has a telomeric heterochromatic block on both arms, so it is not possible to conclude whether the telosome corresponds to the 2RS or 2RL arm. The telosome pair from line G is relatively large and has a telomeric heterochromatin band and a proximal intercalary band. Two lines in the family F (F56 and F61) are different from each other as line F61 carries a non identified pair of telosomes.

RFLP analysis procedure

DNA extraction was performed according to the method described by Rogers and Bendich (1985). Enzyme digestion, electrophoresis, Southern blotting, probe labelling, filter hybridisation and autoradiography were as described by Sharp et al. (1988) except that Hybond-N^+ membranes (Amersham) were used. Thirty probes from wheat DNA libraries were employed. Twenty-three cDNA and six genomic DNA clones were kindly provided by Dr. M. D. Gale (Cambridge Laboratory, UK). One genomic DNA clone (WG605) obtained by Dr. M. E. Sorrells (Cornell University, USA) was kindly provided by Dr. D. Hoisington (CIMMYT, Mexico). These probes, and the chromosome arms of the homoeologous groups they identify in wheat and rye, are listed in Table 1.

Anther culture

Tillers from plants grown in the greenhouse (5–15 plants per genotype) were collected when most microspores were at the mid-to-late uninucleate stage of development and stored for 4–8 days at 4–5°C in the dark. Following this treatment, anthers (from a minimum of 21 spikes per genotype) were aseptically excised and plated onto Petri dishes containing a Potato-2 medium (Chuang et al. 1978) gellified with 5.5 g/l of agarose. Cultures were incubated at 27°C in the dark. The embryos which appear 4–7 weeks after anther plating were transferred onto a gellified (5.5 g/l agarose) regeneration medium described in earlier studies (Bernard 1977; Charmet and Bernard 1984) supplemented with 0.2 mg/l NAA, 1 mg/l IAA, and incubated at 22°C with a 16 h photoperiod.

The following traits were evaluated:

- embryo production rate, expressed as the number of embryos per 100 anthers plated;
- plant regeneration rate, as the number of plantlets (green and albinos) obtained per 100 embryos transferred to the regeneration medium;
- green-plant regeneration rate, as the number of green plantlets obtained per 100 embryos;
- green-plant ratio, as the number of green plantlets per 100 plantlets obtained.

Statistical analyses were performed on these parameters. In order to normalize the distribution, embryo production data were transformed by the square-root function prior to a variance analysis conducted in a complete randomised design. One replicate corresponds to one Petri dish. Regeneration data were submitted to a chi-square test. Data presented in the tables are untransformed means.

Results

Characterisation of wheat-rye addition lines with RFLP probes

Each probe was hybridised to blots of *Eco*RI, *Hin*dIII- and/or *Eco*RV, *Dra*I-digested DNA from the wheat 'FEC 28', the rye 'S 10' and the 'FEC 28'–'S 10' addition line series (namely lines F, D, C, A, E, G and B). Then, DNA fragments present in 'S 10' and not present in 'FEC 28' were searched for among the addition lines. The wheat background of these lines was also checked by comparing DNA hybridisation patterns from 'FEC 28' and from addition lines. The probes were selected so as to cover the seven homoeologous groups. With respect to wheat, they detected fragments specific to one homoeologous group except in the case of clones PSR56 and PSR78 (Table 1). Some of them have been mapped on the wheat and rye genomes (Chao et al. 1989; Wang et al. 1991; Devos et al. 1993 c). Although these probes originated from cDNA or gDNA wheat libraries, they provided a strong signal in rye and in most cases revealed one or two major bands with occasionally one or several minor bands. For each probe, addition lines characterised by rye-specific fragments are reported in Table 1.

According to the probe used, four different situations have been observed as illustrated in Fig. 1:

The probe detected a rye-specific DNA fragment in only one addition line (Fig. 1 a and b): probe PSR161, specific to homoeologous group 1, revealed a rye DNA locus in the addition line F (Fig. 1 a), previously identified as the ad-

Table 1 DNA clones, chromosomal location in wheat, rye, and loci mapped in A, B, D and (or) R genomes (according to the data collected from the papers of M. D. Gale and coworkers, except for the PSRX and WG605 clones), and characterisation of the 'FEC 28'-'S 10' addition lines

DNA clone	Chromosomal location in wheat, rye				Genomes mapped	Addition line with a rye specific locus	Proposed homoeologous group[c]
PSR161	1AS	1BS	1DS	1RS	A, R	F	1R
PSR158	1AL	1BL	1DL	1RL	R	F[d]	1R
PSR159	1AL	1BL	1DL	1R		F[d]	1R
PSR162	1AL	1BL	1DL	1RL	R	F[d]	1R
PSR601[a]	1AL	1BL	1DL	1RL	R	F[d]	1R
PSRX[ab]	1AL	1BL	1DL			F[d]	1R
WG605[a]	1AL	1BL	1DL			F[d]	1R
PSR135	2AS	2BS	2DS	2R	B	None	
PSR107	2AS	2BS	2DS	2RS	A, R	None	
PSR101	2AL	2BL	2DL	2R		D	2R
PSR123	3AS	3BS	3DS	3R		C and G[e]	3R
PSR598[a]	3AS	3BS	3DS	3RS	A, D, R	C and G[e]	3R
PSR902[a]	3AS	3BS	3DS	3RS	B, R	C and G[e]	3R
PSR56	3AL	3BL	3DL	3RL	A, R		
	7AL	7BL	7DL	7RL	R	C and B[f]	3R and 7R
PSR78	3AL	3BL	3DL	3RL	A, B, R		
	1A		1D	1RS	R	C and F[f]	3R and 1R
PSR156	3AL	3BL	3DL	3RL	B, D, R	C	3R
PSR144	4AL	4BS	4DS		A	A	4R
PSR163	4AS	4BL	4DL	7RS	D, R	B	7R
PSR118	5AS	5BS	5DS	5R	B, D	E	5R
PSR628 a	5AS	5BS	5DS	5RS	D, R	E	5R
PSR128	5AL	5BL	5DL	5R	A	E	5R
PSR113	6AS	6BS	6DS	6RS, 1R	D	None	
PSR167	6AS	6BS	6DS	4RL	B, R	A	4R
PSR312 a	6AS	6BS	6DS	6RS	A, B, R	None	
PSR141	6AS	6BS	6DS	6R	D	None	
PSR154	6AL	6BL	6DL	6RL	A, B,R	None	
PSR142	6AL	6BL	6DL		B, D	None	
PSR65	7AS	7BS	7DS	7R	B, D	F and B[f]	1R and 7
PSR103	7AS	7BS	7DS		D	A	4R
PSR129	7AL	7BL	7DL	7RL	B, D, R	B[g]	7R

[a] Genomic DNA clones, others are cDNA

[b] *PSRX* was provided by Dr. M. D. Gale as the probe PSR305 (3L) but, due to the specific hybridisation to the presumed addition line 1R, we have hybridised the probe with digested DNA from wheat 'Chinese Spring' and its nullitetrasomic lines and have confirmed that this probe detected DNA fragments specific for homoeologous group 1 of wheat

[c] In the last column is noted the previously assumed additional chromosome of rye

[d] The DNA fragments specific to the 'FEC 28' genome are present in line F56 and absent in line F61

[e] The rye-specific markers detected are of the same size in the two lines

[f] The rye-specific markers detected differ in size in the two lines

[g] PSR129 detected polymorphic DNA fragments in line B relative to wheat DNA fragments that are consistent with 'FEC 28' DNA patterns and relative to rye DNA fragments that disappear in this line

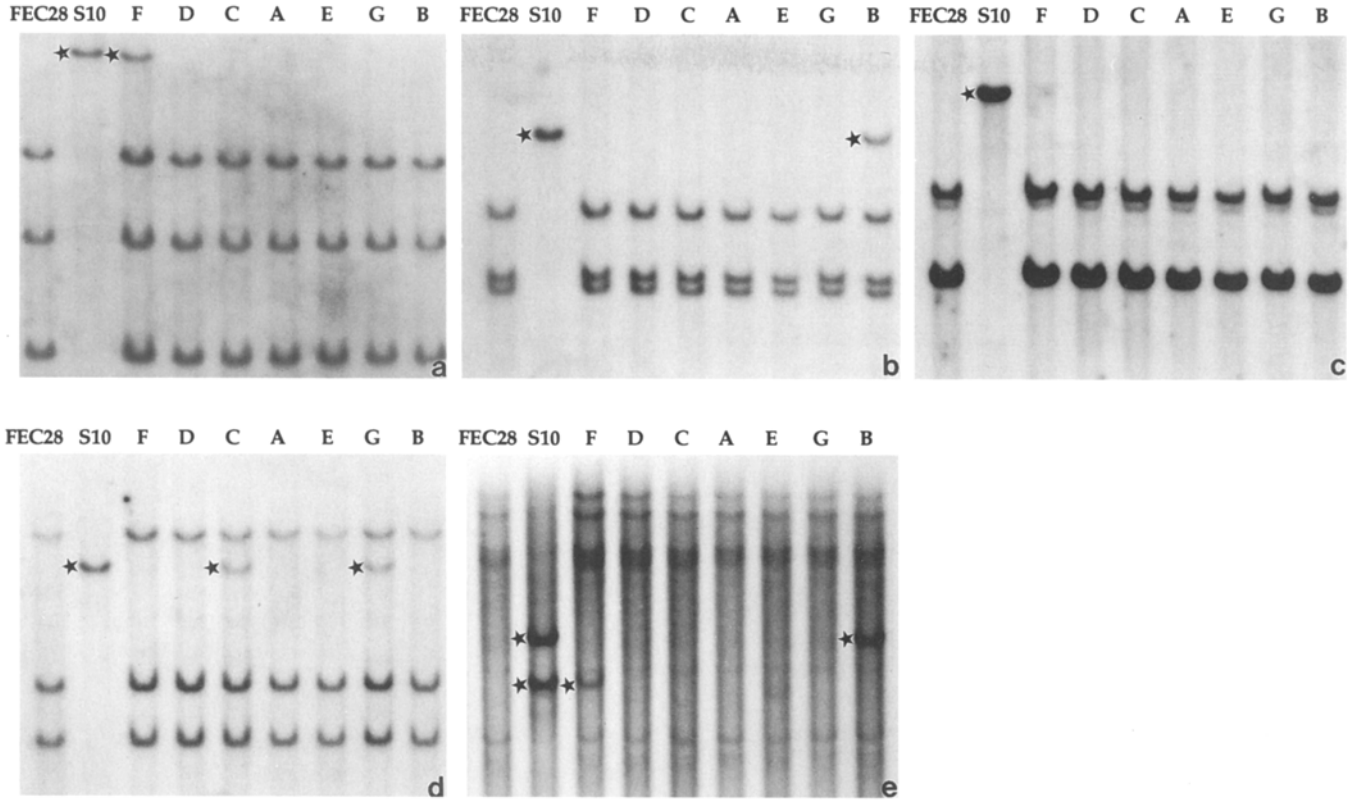

Fig. 1a–e Hybridisation patterns obtained with different probes for digested DNA from wheat 'FEC 28', rye 'S 10' and wheat-rye addition lines (F, D, C, A, E, G and B): **a** PSR161/*Hin*dIII combination; **b** PSR163/*Eco*RV combination; **c** PSR142/*Eco*RV combination; **d** PSR598/*Eco*RV combination; **e** PSR65/*Dra*I combination. The *stars* indicate rye fragments

dition line of chromosome 1R; PSR163, specific to the homoeologous group 4 in wheat, revealed a rye DNA locus on the 7RS present in addition line B (Fig. 1 b), which is consistent with the proposed structure of chromosome 7R (Rognli et al. 1992).

The probe did not detect a rye-specific DNA fragment in any addition line whatever the enzyme used; this was the case for probe PSR142 (located on homoeologous group 6, long arm, Fig. 1 c).

The probe revealed the same rye DNA fragment in several addition lines as was the case for PSR598 (located on homoeologous arm 3RS) which characterises lines C and G (Fig. 1 d).

The probe revealed two different size fragments in the 'S 10' rye genome and each of them was found in a different addition line, as exemplified for PSR65 giving one fragment (4.8 kb) present on addition line F and the other (6 kb) on addition line B (Fig. 1 e).

Analysis of the rye genome.

With regard to the results obtained for all the probe/enzyme combinations (Table 1), we have found at least one rye DNA-specific sequence in each addition line. The current structure of rye chromosomes in 'FEC 28'–'S 10' addition lines is shown in Fig. 2. Our results agree with cytological data for addition lines F, D, C, A, E and B which carry rye-specific DNA sequences respectively located on chromosomes 1R, 2R, 3R, 4R, 5R and 7R. Further, none of the six probes specific to the homoeologous group 6 have detected a rye-specific fragment in addition line G. The three probes located on the short arm of chromosome 3, PSR123, PSR598 and PSR902, are present in addition lines C and G (see Fig. 1 d). The loci *Xpsr56*, *156* and *78*, mapped on 3RL (Devos et al. 1993 a), are found only in line C. Therefore, it is very likely that the additional chromosome present in line G corresponds to the short arm of chromosome 3R, and lacks a major part of the long arm, since the locus *Xpsr56-3RL*, mapped near the centromere at 2 cM from *Xpsr902-3RS*, is absent. Recent C-banding data (G. Gay, personal communication) confirm this hypothesis. Another rye chromosomal deletion has been identified in addition line D (+2R). Chromosome 2R of this line is deleted on the short arm since the two specific markers of 2S present in 'S 10' are absent in this line. This deletion involves a major part of the arm according to the proximal location of the *Xpsr107* locus (Devos et al. 1993 b).

Another interesting point is apparent from the hybridisation of probe PSR129. This probe, specific to homoeologous chromosome arm 7L, detected an RFLP between 'S 10' and line B for chromosome 7R whatever the enzyme used (Table 2). It seems that there is a deletion in the region of the *Xpsr129* locus on the line B chromosome 7R. This chromosome presents a standard C-banding pattern with two telomeric heterochromatic bands on both arms;

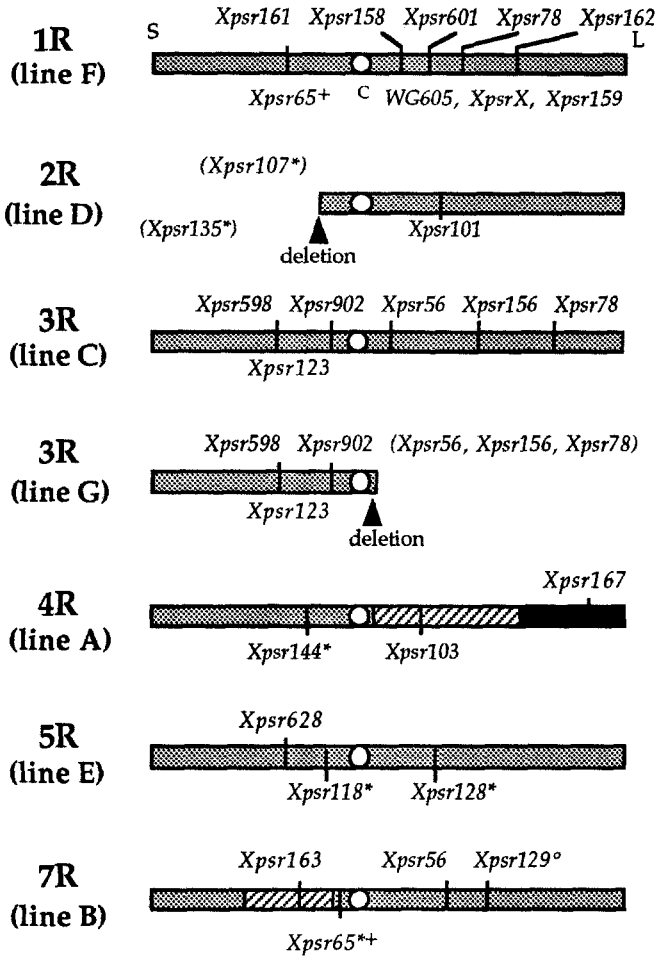

Fig. 2 The present structure of chromosomes 1R, 2R, 3R, 4R, 5R and 7R in the different wheat-rye addition lines. This representation does not reflect chromosome size and genetic distances but represent RFLP markers orders when known. Above and under the chromosomes are indicated mapped and unmapped loci, respectively; absent loci are indicated in parentheses. ▨, indicates markers of the translocation 4R/7R. ■, indicates marker of the translocation 6R/4R. +, indicates a duplication on 7R and 1R. *, means that the relative position of the locus is supposed with regard to the map of genomes A, B and D of wheat. °, indicates a small deletion within the sequence homologous to the probe PSR129

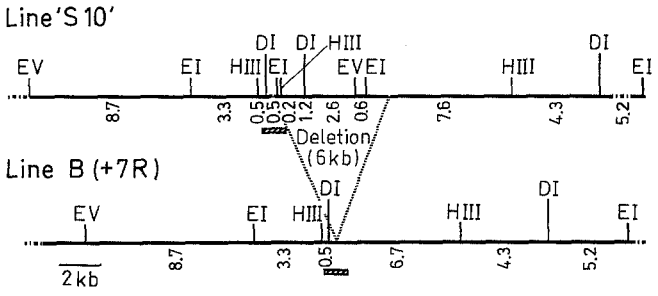

Fig. 3 Restriction maps proposed for the region homologous to probe PSR129 (located on chromosome arm 7RL) in lines 'S 10' and B (+7R). Below the maps are indicated distances in kilobases between two adjacent restriction sites. *EI*, *EV*, *HIII* and *DI* indicate restriction sites of endonucleases *Eco*RI, *Eco*RV, *Hin*dIII and *Dra*I, respectively. ▨ probe (1.2 kb)

Table 2 RFLP observed for the probe PSR129 between the parental rye line 'S 10' and the line B (+7R). The fragment sizes are indicated in kilobases (kb); 0 indicates that no fragment was observed

Enzyme	Line	
	'S 10'	Line B
*Eco*RI	4.3+4.6	20
*Eco*RV	17	0
*Hin*dIII	1.2+12	7.2
*Dra*I	1.9	11

the RFLP observed might be due to a small interstitial deletion, not detectable with C-banding. In Fig. 3, we propose, within the limits of our analysis, one of the hypotheses which could explain the data presented in Table 2: the deletion may be of about 6 kb including *Hin*dIII, *Dra*I, *Eco*RV and two *Eco*RI restriction sites.

The reciprocal 4L/7S translocation and the 6S/4L interchange are demonstrated by the results of hybridisation with probes PSR103, PSR163 (Fig. 1 b) and PSR167 respectively.

Analysis of the wheat genome

Four probes located on the long arm of chromosome 1, PSR158, PSR162, PSR601 and WG605, (Table 1), detected the absence of one or more DNA fragments belonging to the wheat genome in the addition line F61 (+1R) whatever the enzyme used. This indicates a large deletion in a group-1 chromosome. For the identification of the wheat genome involved, we have analysed digested DNA of 'Chinese Spring' and its nulli-tetrasomic lines with PSR158 and PSR601. This deletion concerns the long arm of chromosome 1A of the addition line F61. The C-banding analysis confirmed this fact. The wheat genetic content of the other six addition lines is in agreement with that of 'FEC 28'.

Anther culture response

Embryo-production rate

The data are reported in Table 3. A wide range of variation was observed between the lines in their frequency of embryo production: from 1.5% for line D (+2RL) to 63.5% for line C (+3R). The wheat recipient 'FEC 28' gave an embryo production rate of medium value (22.6%). Addition line C (+3R) produced significantly more embryos than all the other lines whereas the addition of the deleted chromosome 3RS (line G) gave a quite low value (13.6%), not significantly different from 'FEC 28'. On the other

hand, the embryo production rates of the lines D (+2RL) and E (+5R) were significantly lower than that of 'FEC 28'. However, the addition of some rye chromosomes may have an effect on agronomic characters such as plant vigor or pollen fertility and so indirectly influence androgenetic ability. For example, the addition lines of chromosome 2R have thin culms, narrow ears and usually very low pollen and female fertility (Miller 1984) as observed for line D (+2RL) (Bernard 1976). We have also noted that the two lines F56 (+1R) and F61 (+1R, 1AS) differ significantly for embryo-production rate (26.9 and 6.6%).

Embryo-regeneration ability

Data are presented in Table 4. The range of responsiveness between lines was relatively narrow: from 0 to 9.8% for the total plant regeneration rate and from 0 to 8% for the green-plant regeneration rate. The wheat recipient 'FEC 28' showed a total plant regeneration rate of 5.9% which is (1) lower than that of line A (+4R), (2) equivalent to lines B (+7R) and E (+5R), and (3) better than those of lines F56 (+1R), F61 (+1R, 1AS), C (+3R), G (+3RS) and D (+2RL). Embryos from lines F61 and D produced no plants. Results from line D were not considered because of the limited number of embryos produced. No difference was observed between lines C and G for embryo-regeneration ability.

Line ranking for green-plant regeneration was almost identical to that of total plant regeneration. The addition line F56 (+1R) reacted as did wheat 'FEC 28' for this trait. Concerning green-plant ratio, only four lines (A, 'FEC 28', C and G) produced a sufficient number of plants to permit statistical analysis of this trait. Line A (+4R) presented a green-plant ratio significantly better than that of 'FEC 28' (81 and 57%, respectively), whereas that of line C (+3R) was significantly lower (33%). Results obtained from lines C (+3R) and G (+3RS) did not differ significantly.

No significant correlations between embryo production and total plant regeneration, and between embryo production and green-plant regeneration were found.

Table 3 Embryo production from the anthers of the wheat 'FEC 28' and the different wheat-rye addition lines

Genotype	No. of anthers plated	No. of embryos produced	Embryo frequency %[a]
Line C (+3R)	4287	2720	63.5 a
Line F56 (+1R)	1718	462	26.9 b
Line A (+4R)	1665	439	26.4 bc
FEC 28	2800	632	22.6 bc
Line G (+3RS)	9525	1295	13.6 bcd
Line B (+7R)	1201	92	7.7 cde
Line F61 (+1R, 1AS)	3360	221	6.6 cde
Line E (+5R)	1671	118	7.1 de
Line D (+2RL)	918	14	1.5 e

[a] The number of embryos obtained per 100 anthers plated; means followed by the same letter are not significantly different at P=0.05 according to the Newman Keuls Least Significant Range Test

Discussion

In this study, disomic wheat-rye addition lines were used to indicate the chromosomal location of genes involved in anther culturability and more particularly to investigate the rye genome which is a component of *Triticale*.

Characterisation of wheat-rye addition lines

The availability of at least one RFLP marker on each chromosome arm, allowed for the identification of each rye additional chromosome. This technique was also very useful to refine the chromosome characterisation, and complements the morphological, cytological and biochemical analyses.

Table 4 Regeneration ability of embryos obtained from the wheat 'FEC 28' and the wheat-rye addition lines

Genotype	No. of cultured embryos	No. total plantlets produced	No. green plantlets produced	Plant regeneration rate		Green-plant ratio %[c]
				Total %[a]	Green %[b]	
Line A (+4R)	439	43	35	9.8 *	8.0 *	81*
FEC 28	632	37	21	5.9	3.3	57
Line B (+7R)	92	3	3	3.3	3.3	(100)
Line E (+5R)	118	3	2	2.5	1.7	(67)
Line F56 (+1R)	462	11	10	2.4 **	2.2	(91)
Line C (+3R)	2720	63	21	2.3 ****	0.8 ***	33*
Line G (+3RS)	1295	25	12	1.9 ****	0.9 ****	48
Line F61 (+1R,1AS)	221	0	0	0	0	
Line D (+2RL)	14	0	0	0	0	

*, **, ***, **** Significantly different from 'FEC 28' in χ^2 tests at P=0.05; 0.01; 0.005 and 0.001 respectively (line D was not considered)

[a] Total plantlets produced per 100 embryos cultured

[b] Green plantlets produced per 100 embryos cultured

[c] Green plantlets produced per 100 total plantlets produced. Means in parentheses are not considered because of the low number of plants produced

We provide evidence that the current addition lines set is not complete and lacks chromosome 6R. We can hypothesize either (1) that 6R was missing among the 100 plants (2n=43) selected during the production of the addition series, or (2) that 6R monosomic addition plants did not give progenies with 44 chromosomes. Moreover, disomic addition lines are not completely stable: the added chromosome can be lost or broken, due to meiotic irregularities. This is the case for chromosomes 3R and 2R: addition line C possesses the complete chromosome 3R and line G the 3RS. A deletion on the short arm of chromosome 2R of line D was also identified. More amazing is the case of the deletion for chromosome 1A in line F61 (+1R), bearing a double 1AS telosome.

Results with PSR129 (Tables 1 and 2) lead to the hypothesis of an interstitial deletion of 6 kb (Fig. 3) on chromosome 7R of line B: the availability of other markers tightly linked to the both sides of the *Xpsr129-7R* locus, as well as double digestions of genomic DNA with two different restriction endonucleases, are necessary to confirm this.

Devos and Gale (1993 c) published a synthesis of the Cambridge laboratory genetic map of wheat (genomes A, B and D), showing some 800 loci (corresponding to RFLP and known-function gene probes), and made a comparison of the synteny of probes among the A, B, D and R genomes. There is strong evidence that the rye genome is extensively rearranged relative to the basic wheat constitution. Comparisons between the genetic maps confirmed Naranjo's and Fernandez-Rueda's (1991) recent pairing analyses as well as many other results. It is evident that only rye chromosome arms 1RS, 1RL, 2RL, 3RS, 4RS and 5RS remain homoeologous with the corresponding wheat arms. Our hybridisation results are in agreement with those of Devos et al. (1993 a) and previous results published by the same workers using these molecular markers with other rye genotypes. We have detected translocations inherent to the *Secale cereale* genome constitution: 4L/7S with the *Xpsr103-4RL* and *Xpsr163-7RS* loci and 6S/4L with the *Xpsr167-4RL* locus. Our results with PSR65 (Fig. 1 e), suggest an interchromosomal duplication of loci on 7R and 1R. This duplication was not previously mentioned in the literature. We have also observed the pattern of line 'S 10' on two other rye lines (no. 51 from 'Herzog' and no. 571 from 'Petkus', unpublished data).

Anther culture

It has been well demonstrated in numerous studies that differences in androgenetic ability exist between species and between genotypes within the same species. Most of the *S. cereale* populations (2n=14 chromosomes) give no, or only low, in vitro response whereas many genotypes from *T. aestivum* (2n=42) and from *Triticale* (2n=42) react well to in vitro culture. Significant differences in androgenetic ability are also observed between hexaploid and tetraploid wheats: hexaploid wheat generally produces more embryos and gives rise to better green-plant regeneration (Orlov et al. 1993). Thus, it seems that the ploidy level of plant material might be one of the factors affecting androgenetic ability in wheat and related species. However, within a species, it is obvious that the response to culture is strongly genotype-dependent. Our results clearly demonstrate that foreign chromosomes can influence the responsiveness of a genotype in a particular species.

Anther-culture results from lines C, G and E suggest that the long arm of 'S 10' chromosome 3 carries a gene(s) with a positive effect on the embryo induction rate in the wheat 'FEC 28' background and that chromosome 5R possesses a gene(s) acting negatively on the same trait. We cannot confirm a direct negative effect of chromosome 2R on embryo production owing to the reduced fertility observed for this line. Results from lines F56 and F61 led us to assume that chromosome arm 1AL of 'FEC 28' has a major positive effect on embryo production. We did not find mention in the literature of any effect of wheat chromosome arm 1AL on the wheat anther-culture response. It would be interesting to test 'FEC 28' 1AL and 1AS ditelosomic lines. Previous studies were performed with wheat genotypes different from that used here, essentially with 'Chinese Spring'. Analysis of 'Chinese Spring' (CS) monosomic lines suggested that chromosome 5A and members of group 2 carry major genes limiting embryo production (Zhang and Li 1984) whereas chromosome 1D stimulates it (Agache et al. 1989). Chromosomal substitution analysis (CS substituted by 'Cheyenne') showed that 1B and 7A have an important effect on the same trait (Szakacs et al. 1988). De Buyser et al. (1992), using ditelosomic and nullitetrasomic CS lines, found that chromosome arms 3A, 5A, 5B and 7D stimulate embryo production whereas 1BS and 1BL reduce it. All these results support the hypothesis that chromosomes from groups 1, 3 and 5 are involved in this androgenetic trait.

Concerning regeneration capacity, our results have shown that rye chromosome 4 bears a gene(s) which positively affect(s) the total plant regeneration response of embryos and a gene(s) increasing the green-plant frequency in a 'FEC 28' background (Table 4). This is in agreement with a previous report on 'Chinese Spring'–'Imperial' disomic addition lines (Lazar et al. 1987). We also noticed negative effects of the addition of chromosomes 1R, 3R, and more precisely 3RS, on the total plant regeneration rate. The deletion of the long arm of chromosome 1A inhibits the regeneration response. In wheat, Szakacs et al. (1988) showed that chromosomes 3A, 5B and 4D influence the total plant regeneration, and that chromosome 2D influences green-plant formation. De Buyser et al. (1992) using CS aneuploids, suggested that genes acting on regeneration ability are located on chromosomes 5A and 1BL, the latter increasing albino plant frequency. The androgenetic studies from several translocated 1BL-1RS wheat cultivars by Henry and De Buyser (1985), Agache et al. (1989), Foroughi-Wehr and Zeller (1990) Lashermes et al. (1991), and Henry et al. (1993), suggested that a major gene located on rye chromosome arm 1RS increases embryo regeneration ability. The 'S 10' 1R has an opposite effect, probably owing to either a different origin or else to the presence of the long 1R arm.

Results from linear regression clearly demonstrated that the different features examined are independently inherited and might be mainly under nuclear polygenic regulation as has also been suggested for *Triticale* (Charmet and Bernard, 1984) and for wheat (Agache et al. 1988).

Conclusions

We have demonstrated that RFLP markers are a useful tool to identify chromosome rearrangements in wheat-rye addition lines as has been observed in previous reports for other wheat-alien chromosome addition lines (Sharp et al. 1989). The information concerning the rye or wheat DNA content of each addition line has proven very useful in explaining part of the anther-culture results. However, it must be kept in mind that, owing to chromosome reorganization between species, results obtained for a particular chromosome in one species do not give a direct indication of the effect of its apparent homoeologous chromosome(s) in another species, and that the results need to be accompanied by a precise identification of the genetic relationships between the chromosome segments involved. It is clear that the rye genome possesses genes which act positively in a wheat genetic background. This fact could explain the great androgenetic potential of some *Triticale* lines compared to wheat lines (Bernard et al. 1993). With regard to the positive effects of chromosomes 3R and 4R in a wheat background for embryo induction and green-plant regeneration traits respectively, it would be very interesting to develop subsets of '3R' and '4R' addition lines with different parts of rye chromosomes deleted in order to more precisely define the chromosome segments involved in the androgenetic response, and to test the F_1 hybrid between '3R' and '4R' addition lines.

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