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Plant breeding

Production of intervarietal substitution lines with improved interspecific crossability in the wheat cv Courtot

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Summary – In order to involve the semi-dwarf wheat, cultivar Courtot in the development of primary triticale, we have attempted to improve its crossability with rye. The Courtot chromosomes 5A, 5B, 5D were replaced by their counterparts from the highly crossable Japanese cultivars Norin 29 and Fukuhokomugi, using the monosomic back-crossing technique. It was confirmed that the most potent compatibility factor was on the chromosome 5B (kr_1); this allele was sufficient to achieve a seed set of about 50%, variable with environmental factors. Conversely, the effect of the 2 other chromosomes, whatever the donor parent, was hardly detectable: if they have the crossability alleles, these have a low level of expression, being inhibited by the Kr_1 allele. Recovering the Courtot genetic background during the subsequent back-cross generations did not alter the degree of crossability estimated in BC_1.

wheat / rye / triticale / crossability genes / substitution lines

Résumé – Production de lignées de substitution intervariétale dans le cultivar de blé Courtot et amélioration de son aptitude au croisement interspécifique. *Dans le but d'utiliser le cultivar de blé demi-nain Courtot dans les programmes de création de triticales primaires, on a cherché à améliorer sa capacité de nouaison en croisement avec le seigle. Pour cela, on a remplacé ses chromosomes 5A, 5B et 5D par ceux de 2 variétés japonaises hautement compatibles avec le seigle, Norin 29 et Fukuhokomugi, en utilisant les techniques du rétrocroisement sur des plantes monosomiques du parent récurrent. Il se confirme que l'allèle de compatibilité le plus puissant est bien sur le chromosome 5B* (kr_1)*: il permet l'obtention d'un taux de nouaison de l'ordre de 50%, variable selon les conditions environnementales. L'effet des 2 autres chromosomes, quel que soit le parent de départ, n'est guère perceptible : s'ils portent des allèles de compatibilité, ceux-ci sont peu puissants et inhibés par l'allèle* Kr_1*. La récupération d'un fonds génétique «Courtot» au fur et à mesure du nombre de rétrocroisements ne modifie pas le niveau de compatibilité évalué en* BC_1*.*

blé tendre / seigle / triticale / compatibilité au croisement interspécifique / lignées de substitution

INTRODUCTION

For more than a century (Rimpau, 1891), breeders have sought to combine the hardiness of rye (*Secale cereale* L) with the productivity of common wheat (*Triticum aestivum* L Thell ssp *vulgare* Mac Key). Wheat-rye hybrids are generally difficult to obtain and consequently the resulting number of hybrid combinations is limited. It is therefore necessary to develop methods aimed at enlarging the genetic variability of triticale (x *Triticosecale* Wittmack). The discovery of genetic factors which control the crossability of the species concerned (Backhouse, 1916) as well as that of the diploidizing role of colchicine have been decisive steps for the creation of this new spe-

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cies. In wheat, Lein (1943) showed the effects of the alleles Kr_1 and Kr_2 as crossability inhibitors. He proposed the following relationships between genotypes and phenotypes for wheat used as a female:

Kr_1 Kr_1 Kr_2 Kr_2	less than 5% aptitude for crossability
Kr_1 Kr_1 kr_2 kr_2	from 10 to 30%
kr_1 kr_1 Kr_2 Kr_2	from 30 to 50%
kr_1 kr_1 kr_2 kr_2	more than 50%

Riley and Chapman (1967) have located the loci of the genes on the homoeologous chromosomes of group 5: Kr_1 on the long arm of chromosome 5B and Kr_2 on the long arm of chromosome 5A. Kr_1 and Kr_2 were mapped by Sitch *et al* (1985); Kr_1 is at 44.8 ± 3.3 centimorgans from the centromere on the 5B and Kr_2 is in a similar position on chromosome 5A. These results confirm that Kr_1 and Kr_2 are located at homoeologous loci. Another allele, Kr_3, has been located on chromosome 5D by Krolow (1970).

Other factors of minor importance are involved in obtaining hybrid embryos between wheat and rye. Firstly, the D genome, especially chromosome 1D, is important in the formation of viable hybrid seed (Pienaar and Marais, 1976, 1986; Marais and Van Der Westhuizen, 1987, Marais, 1988). Secondly, the genotype of the rye parent also plays a role (Florell, 1931; Tozu, 1950; Kaltsikes, 1974; Marais and Pienaar, 1977; Taira *et al*, 1978; Scoles, 1983). Tanner and Falk (1981) demonstrated that some inbred rye lines partially circumvent one or even both inhibitor alleles present in wheat.

The Kr_1 and Kr_2 genetic system is also decisive in the crossability of common wheat with bulbous barley (*Hordeum bulbosum* L) used to produce common wheat haploids (Falk and Kasha, 1981; Fedak and Jui, 1982), but is not involved in the intergeneric crosses between bread wheat and maize (*Zea mays* L) (Laurie and Bennett, 1987, 1988; Inagaki and Tahir, 1990), which had made this latter method useful to obtain haploids with any common wheat genotype used to date.

The recessive alleles of the genes (kr_1, kr_2) are present in some wheat genotypes originating from China, Japan, Eastern Siberia, and Iran (Zeven, 1987) whereas European bread wheat varieties have the dominant alleles Kr_1 and Kr_2, with a few exceptions, such as cv Roazon, which is derived from an interspecific cross (Stefanowska and Cauderon, 1983).

For this reason, we are developing wheat genotypes with the recessive alleles kr_1, kr_2, kr_3, well adapted to local agronomic conditions, either by conventional pedigree breeding or by chromosome substitution. The latter case involves replacing chromosomes 5A, 5B, 5D from a non-crossable, well-adapted genotype by the corresponding chromosomes from highly crossable genotypes.

Creating such substituted lines in a known wheat background would also reduce empiricism in obtaining primary triticales. The best genetic wheat × rye combinations for characters such as ear fertility, chromosome stability, agronomic traits (size, lodging and disease resistance) and technological factors (shrivelling, α-amylase activity) could then be selected from amphiploid populations developed in this way. The substitution lines would thus be used as a genetic basis for screening rye genotypes.

MATERIALS AND METHODS

Two Japanese wheat varieties, Norin 29 and Fukuhokomugi, known for their high crossability with rye, were chosen as donors of group 5 chromosomes.

Monosomic lines of cv Courtot, developed by INRA laboratories in the 1970s (Denis *et al*, 1982) were used as recipient material. Courtot was the first semi-dwarf wheat variety released in France (1974). It has the dwarfing genes Rht_1, Rht_2 of Norin 10 and is highly resistant to lodging. It is of semi-winter type and has good baking quality. It is, however, susceptible to numerous fungal diseases.

The method employed for creating substitution lines was described by Unrau *et al* (1956) and uses Courtot monosomics as female parent and monosomic individuals originating from back-crossing onto Courtot as the male parent (fig 1). Throughout the back-crosses, the substituted chromosome was maintained in the monosomic condition, and therefore could not be subject to recombination. The back-cross process was continued up to BC_4 for chromosomes 5A and 5B, and only up to BC_1 for chromosome 5D. After 4 back-crosses, monosomic plants were selfed, and the plants with $2n = 42$ were selected in the progeny. Chromosome number was checked at each generation by squashing, using the Feulgen staining technique. The meiotic behaviour of each plant

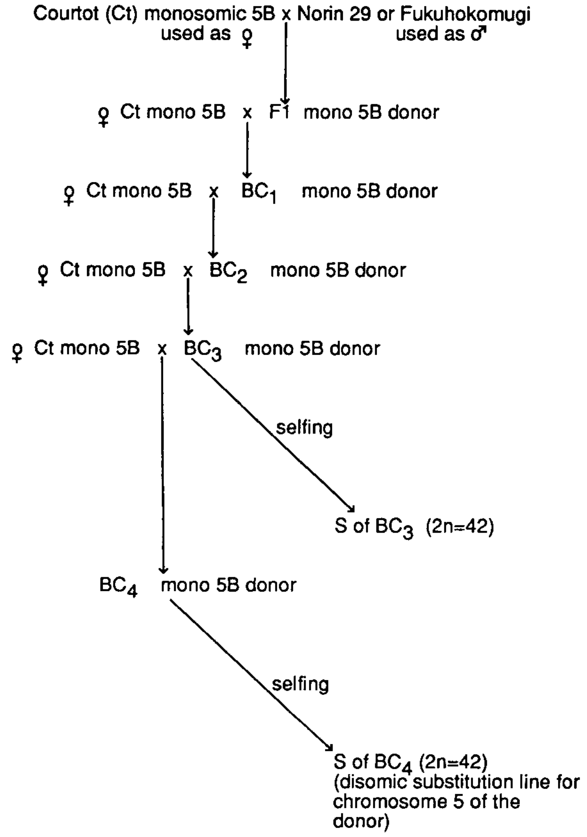

Fig 1. Construction of genotypes (as exemplified for chromosome 5B).

was observed on 25 pollen-mother cells stained with aceto-carmin (Cauderon and Gay, 1992).

All the parent genotypes, except Courtot monosomic 5A, were checked for their crossability with rye. At each back-cross generation, the substituted monosomic plants were crossed with rye to check for the presence of crossability genes and to ensure that no change in univalent had occurred.

We used the Beaulieu synthetic variety as the rye tester as it consistently provided abundant pollen. The monosomic wheat plants were grown in soil, either in a tunnel (F_1, BC_1, BC_2) or in a greenhouse (BC_3, BC_4, and progenies issued from BC_3 and BC_4).

RESULTS

The results obtained varied considerably due to environmental factors. Nevertheless, interspecific crossability of the parental genotypes, Norin 29, Fukuhokomugi and Courtot, is very contrasted (table I): Norin 29 and Fukuhokomugi showed a very high seed set rate (95 and 86%), much higher than Courtot (7%). Of the Courtot monosomics, only monosomic 5B showed a relatively high seed set (18%) (table I). The 22 grains obtained from this cross were put to germinate. Nine gave viable plantlets, 7 with 27 chromosomes and 2 with 28 chromosomes.

The pollination tests with rye carried out on monosomic F1s showed a high seed set only when the monosomic 5B was used as the parent. This rate was very low or zero with monosomics 5A or 5D (table I).

The crossability tests on BC_1, BC_2, BC_3, BC_4 plants showed seed setting ranging from 30 to 60% when the chromosomes 5B from the 2 Japanese wheat varieties were involved, whereas it was zero or almost zero with chromosomes 5D and 5A (table II). The seed set of crosses on plants from BC_3 and BC_4 progenies with 42 chromosomes, disomic for chromosomes 5B of Norin 29 and

Table I. Crossability level with rye of the parents, the Courtot monosomic 5B, 5D (5A not tested) and the monosomic F1 progenies.

Identity of the genotypes	*Presumed allelic composition*						*No of grains/ No of flowers pollinated*	*Estimate of the seed set and confidence interval*	
	Kr1	*kr1*	*Kr2*	*kr2*	*Kr3*	*kr3*			
Courtot (Ct)	++		++		++		7/100	0.07	±0.05
Norin 29 (N29)		++		++		++	57/60	0.95	±0.06
Fukuhokomugi (Fuk)		++		++		++	52/60	0.86	±0.09
Ct mono 5B	+		++		++		22/120	0.18	±0.07
Ct mono 5D	++		++		+		0/100	0.00	±0.04
F1 (Ct mono 5A×N29) × rye	+	+		+	+	+	6/200	0.03	±0.02
F1 (Ct mono 5A×Fuk) × rye	+	+		+	+	+	0/20	0.00	±0.15
F1 (Ct mono 5B×N29) × rye		+	+	+	+	+	126/180	0.70	±0.07
F1 (Ct mono 5B×Fuk) × rye		+	+	+	+	+	123/260	0.47	±0.06
F1 (Ct mono 5D×N29) × rye	+	+	+	+		+	24/200	0.12	±0.05
F1 (Ct mono 5D×Fuk) × rye	+	+	+	+		+	1/100	0.01	±0.04

+ : presence of the allele.

Table II. Crossability with rye of the different back-cross progenies, monosomics for chromosomes 5A, 5B, 5D.

Identity of genotypes	*Presumed allelic composition*						*No of grains/ No of flowers pollinated*	*Estimate of the seed set and confidence interval*	
	Kr$_1$	*kr$_1$*	*Kr$_2$*	*kr$_2$*	*Kr$_3$*	*kr$_3$*			
BC$_1$ (Ct mono 5A × N29) × rye	·+	··		+	·+	··	0/40	0	±0.08
BC$_1$ (Ct mono 5A×Fuk) × rye	·+	··		+	·+	··	0/40	0	±0.08
BC$_1$ (Ct mono 5B × N29) × rye		+	·+	··	·+	··	63/180	0.35	±0.07
BC$_1$ (Ct mono 5B×Fuk) × rye		+	·+	··	·+	··	102/240	0.42	±0.06
BC$_1$ (Ct mono 5D × N29) × rye	·+	··	·+	··		+	1/100	0.01	±0.04
BC$_1$ (Ct mono 5D×Fuk) × rye	·+	··	·+	··		+	1/80	0.01	±0.04
BC$_2$ (Ct mono 5A × N29) × rye	·+	··		+	·+	··	0/80	0	±0.04
BC$_2$ (Ct mono 5A×Fuk) × rye	·+	··		+	·+	··	7/120	0.06	±0.04
BC$_2$ (Ct mono 5B × N29) × rye		+	·+	··	·+	··	57/140	0.41	±0.08
BC$_2$ (Ct mono 5B×Fuk) × rye		+	·+	··	·+	··	108/200	0.54	±0.07
BC$_3$ (Ct mono 5A × N29) × rye	++			+	++		1/120	0.01	±0.04
BC$_3$ (Ct mono 5A×Fuk) × rye	++			+	++		0/40	0	±0.08
BC$_3$ (Ct mono 5B × N29) × rye		+	++		++		83/300	0.28	±0.05
BC$_3$ (Ct mono 5B×Fuk) × rye		+	++		++		119/380	0.31	±0.05
BC$_4$ (Ct mono 5A × N29) × rye	++			+	++		0/80	0	±0.04
BC$_4$ (Ct mono 5A×Fuk) × rye	++			+	++		0/40	0	±0.08
BC$_4$ (Ct mono 5B × N29) × rye		+	++		++		53/120	0.44	±0.09
BC$_4$ (Ct mono 5B×Fuk) × rye		+	++		++		74/120	0.62	±0.09

+: presence of the allele; ·: heterozygous condition and/or uncertain presence.

Fukuhokomugi, ranged from 50 to 60% (table III). Figure 2 summarizes seed set variations during the development of substitution lines involving chromosome 5B.

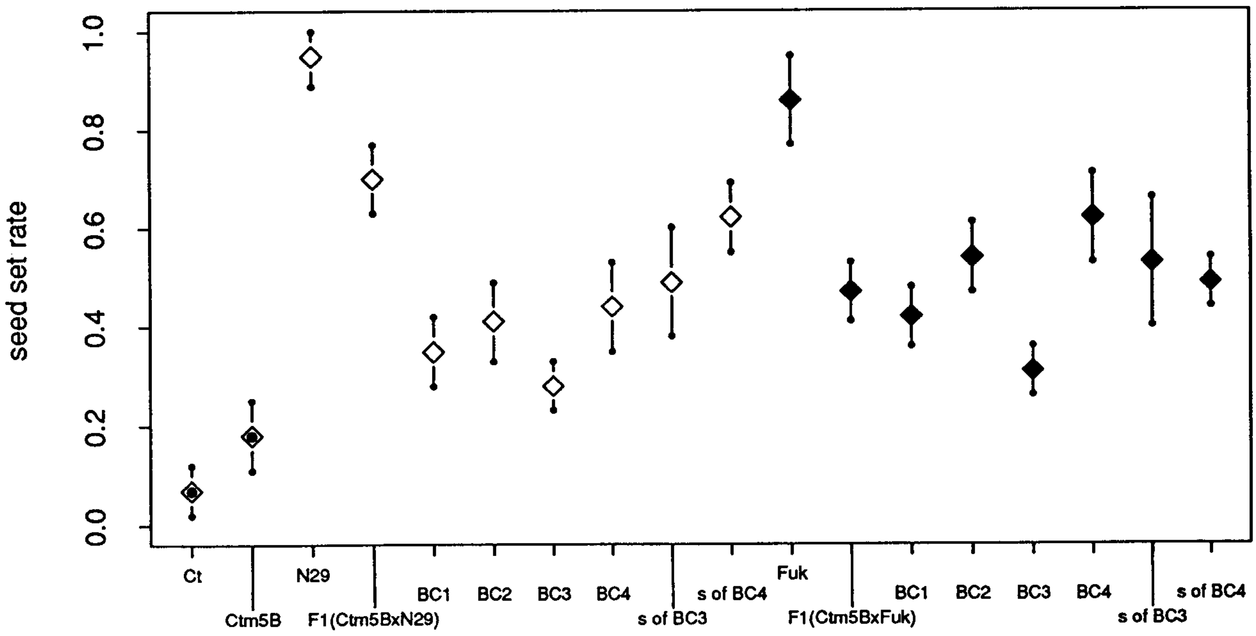

Fig 2. Evolution of seed set during the creation of substitution lines involving chromosome 5B.

Table III. Crossability of the selfed progenies of BC_3, disomic 5A, 5B from N29 and Fuk and the selfed progenies of BC_4, disomic 5B from N29 and Fuk.

Identity of the genotypes	*Presumed allelic composition*						*No of grains/ No of flowers pollinated*	*Estimate of the seed set and confidence interval*
	Kr_1	*kr_1*	*Kr_2*	*kr_2*	*Kr_3*	*kr_3*		
Self of BC_3 (Ct diso 5A × N29) × rye	++			++	++		7/100	0.07 ± 0.05
Self of BC_3 (Ct diso 5A × Fuk) × rye	++			++	++		1/40	0.03 ± 0.12
Self of BC_3 (Ct diso 5B × N29) × rye		++	++		++		39/80	0.49 ± 0.11
Self of BC_3 (Ct diso 5B × Fuk) × rye		++	++		++		32/60	0.53 ± 0.13
Self of BC_4 (Ct diso 5B × N29) × rye		++	++		++		112/180	0.62 ± 0.07
Self of BC_4 (Ct diso 5B × Fuk) × rye		++	++		++		208/420	0.49 ± 0.05

+: presence of the allele.

DISCUSSION AND CONCLUSIONS

Although the results showed considerable variation due to environmental factors, they confirmed the strong inhibitory role of allele Kr_1 in wheat × rye crossability; a reduction in the dose of Kr_1 seemed beneficial by itself. Crossing Courtot monosomic 5B (with one Kr_1 allele) by rye (table I) gave a seed set of 18% compared with 7% with normal Courtot. This suggests an enhancement of the incompatibility effect with 2 doses of Kr_1. On the other hand, the effect of a reduction in the dosage of Kr_3 was not perceptible, as Courtot monosomic 5D was not different from Courtot. Furthermore, the presence of Kr_1 during backcrosses, even at 1 dose, as in F1 (table I), was sufficient to greatly limit seed-setting.

The substitution of Kr_1 by kr_1 brought an outstanding improvement. The seed set of substituted Courtot 5B, crossed with rye was close to 50%. In contrast, substitution of Kr_2 and Kr_3 by their recessive alleles kr_2 and kr_3 had no effect, at least in the presence of Kr_1. This result tends to confirm the active antagonistic effect of the dominant allele Kr_1. It would be interesting to refine this study by comparing the 5B nullisomic and $5B_S$ ditelosomic of Courtot with the 5B substitution lines and to evaluate the crossability of genotypes associating 2 and 3 crossability genes. Moreover, we plan to continue the back-cross process to obtain substitution lines as close to Courtot as possible, with less than 6% of genetic differences as expected in BC_4; the monosomic BC_5 generation is already available.

The comparison of the seed set in the crosses between Japanese wheat varieties or the substitution lines and rye showed a relatively strong inhibitory effect of Kr_2 and Kr_3 (fig 2). Analysing a diallel cross involving all possible substituted genotypes should make it possible to determine the genetic relationships between the different genes involved. While it is clear that partial dominance exists at each locus, the relationships between loci have not been entirely elucidated.

The cytological study of the 9 mono Courtot 5B × rye F1 plantlets showed a distribution close to the normal disjunction in the female gametogenesis of a 5B monosomic plant of Chinese Spring (70% of 20-chromosome gametes, 30% of 21-chromosome gametes). This observation means that the action of inhibitory alleles is sporophytic rather than gametophytic, since ovules with 20 and 21 chromosomes are fertilized as expected. This is in accordance with the conclusions of Lange and Wojciechowska (1976), who showed that the Kr_1 and Kr_2 alleles act as inhibitors of pollen tube growth at the micropyle.

From a practical point of view, using substitution lines of chromosomes 5B from Norin 29 and Fukuhokomugi into Courtot has major advantages. A seed set of 50% makes it easier to create primary octoploid triticale and therefore to enlarge the genetic variability of this species. The availability of a crossable wheat genotype with a satisfactory agronomic performance would enable an effective screening of rye populations for their aptitude to give triticale material with interesting biological and agronomical characteristics. In particular, since the Courtot genotype possesses the Rht_1 and Rht_2 dwarfing alleles, and is of good baking quality, we may obtain short triticales with satisfactory technological properties. The availability of a generalized labelling system of the *Triticeae* genome (RFLP, or other), should make it possible to identify in the rye genome the chromosome segments bearing genes which favour the expression of the above-mentioned characters, either directly or by favorable interaction with their homoeoalleles present in wheat.

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