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Genetics and molecular mapping of a male fertility restoration locus (*Rfg1*) in rye (*Secale cereale* L.)

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Abstract A gene determining the restoration of cytoplasmic genic male sterility (CMS) caused by the Gülzow (G)-type cytoplasm was mapped by analyzing an F_2 and F_3 population comprising 140 and 133 individual plants, respectively. The target gene, designated *Rfg1*, was mapped on chromosome 4RL distally to three RFLP (*Xpsr119*, *Xpsr167*, *Xpsr899*) and four RAPD (*XP01*, *XAP05*, *XR11*, *XS10*) loci. *Xpsr167* and *Xpsr899* are known to be located on the segment of chromosome 4RL which was ancestrally translocated and is homoeologous to the distal end of other Triticeae 6S chromosomes. It is suggested that *Rfg1* may be allelic to the gene determining the restoration of rye CMS caused by the Pampa (P) cytoplasm (chromosome 4RL) and to *Rfc4* that on rye addition lines of chromosome 4RL restores male fertility of hexaploid wheat with *T. timopheevi* cytoplasm. Homoeoallelism to two loci for cytoplasmic-male-sterility restoration on chromosomes 6AS and 6BS in hexaploid wheat is also suggested.

Key words Genetic mapping · CMS · Fertility restoration · RAPD · RFLP · Rye · *Secale cereale* L.

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Introduction

The identification and development of cytoplasmic genic male sterile (CMS) and restorer lines was a major step in the success of hybrid breeding programs in rye (*Secale cereale* L.). Putt (1954), who was the first to discover male sterility in rye, suggested that it was controlled by an interaction of genes located in the nucleolus and cytoplasm. Complete CMS systems, including male sterile, maintaining and restoring genotypes, were described in the early 1970s by Geiger and Schnell (1970) and Kobyljanskij (1971). To date, Lapinski and Stojalowskij (1996) estimate that 20 or more sterility-inducing cytoplasms are available.

However, in rye breeding, it is the P (Pampa) cytoplasm, originating from an Argentinean *Secale cereale* accession and discovered by Geiger and Schnell (1970), that is the most extensively used. The restoration is determined by at least three major genes localized on chromosomes 1RS, 4RL and 6R and additional genes with smaller effects discovered on chromosomes 3RL, 4RL, 5R and 1RS of different mapping populations (Miedaner et al. 1997).

In order to prevent cytoplasmic uniformity, we need to study alternative CMS sources. One of these, designated as the Gülzow (G) type, was described by Adolf and Winkel (1985). Genetic analysis of the restoration of the G-type male sterility led to the conclusion that at least three genes are involved. One major gene, *ms1* is located on chromosome 4R, whereas two modifying genes, *ms2* and *ms3*, were found to be located on chromosomes 3R and 6R, respectively (Melz and Adolf 1991). Because all three genes determine fertility restoration of male sterility caused by the G-type cytoplasm from now they will be designated *Rfg1*, *Rfg2* and *Rfg3*, respectively. The aim of the study presented here was to map the nuclear male fertility restoration gene *Rfg1* on chromosome 4R using random amplified polymorphic

DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers.

Materials and methods

Plant materials and DNA isolation

A mapping population of 152 F_2 plants was produced from one single F_1 plant heterozygous for *Rfg1* by crossing the two self-fertile lines R1620 (male sterile) and R347/1 (restorer). The same population was used by Korzun et al. (1996) for mapping the dwarfing (*Ddw1*) and hairy peduncle (*Hp*) genes on chromosome 5R. The F_2 plants were grown in the greenhouse, and the spikes were bagged just before flowering in order to determine seed set. Plants having on average ≤ 5 and ≥ 20 grains per spike were classified as male sterile and male fertile, respectively, whereas plants having 6–19 grains per spike (12) were excluded from the mapping analysis. One F_3 progeny of the single F_2 plant no. 194, heterozygous for the RAPD marker *XR11*, was selected, and 150 F_3 plants were again grown in the greenhouse and scored for seed set of bagged spikes. Leaf DNA was extracted from 5- to 6-week old F_2 and F_3 seedlings according to the procedure of McCouch et al. (1988).

Marker analysis

The first step in marker selection was a bulked segregant analysis of the F_2 population with RAPD primers. Two pools of DNA of ten male-fertile and ten male-sterile plants, respectively, were used. RAPD reactions were performed as described by Williams et al. (1990, 1993) and Michelmore et al. (1991) with 1000 different RAPD primers (Operon, Alameda, Calif.) using a Beckman Biomek pipetting robot. The polymerase chain reaction (PCR) was carried out in a volume of 25 µl in a Perkin-Elmer. The reaction mixture contained 10 m*M* Tris-HCl (pH 8.3 at 25°C), 50 m*M* KCl, 3.5 m*M* $MgCl_2$, 0.1 m*M* of each deoxynucleotide, 0.001% gelatin, 1 U *Taq* polymerase, 5–7 ng of template DNA and 4 pmol of primer. The amplification reaction consisted of 45 cycles of 30 s at 94°C, 30 s at 35°C and 2 min at 72°C followed by a final step of 7 min at 72°C. Amplification products were analyzed on 1% agarose gels stained with ethidium bromide and visualized on an ultraviolet light-transmitting transilluminator. RAPD markers indicating a close linkage to the target gene were used for analyzing all classified individuals of the F_2 and F_3 populations.

For RFLP analysis DNA was cut with the restriction enzymes *Hin*dIII *Dra*I, *Eco*RI and *Eco*RV. Restriction digests, gel electrophoresis, Southern transfer, probe labeling and filter hybridization were performed as described by Devos et al. (1992). Four RFLP probes (PSR119, PSR167, PSR392, PSR899) developed at the John Innes Centre, Norwich, UK and known to be located on the long arm of chromosome 4R (Devos et al. 1993) were used in the F_2 only.

A linkage map was constructed with the MAPMAKER 2.0 computer program (Lander et al. 1987) using the Kosambi map unit function.

Results

F_2 analysis

Of the 152 F_2 plants scored for seed set of bagged spikes 140 could be classified as sterile (≤ 5 grains per spike) or fertile (≥ 20 grains per spike) (Fig. 1). The analysis of this 140 plants gave a distorted Mendelian segregation ratio of 120 (fertile):20 (sterile) plants ($\chi^2 = 8.57$; $P > 0.001$).

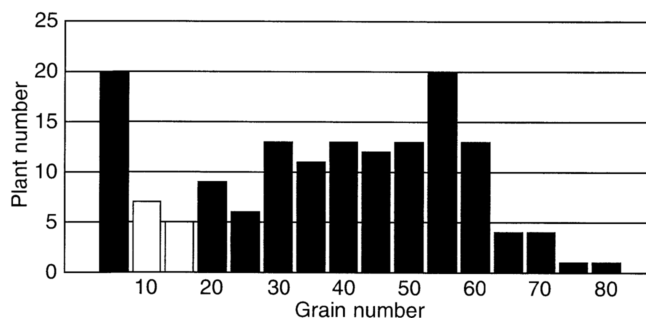

Fig. 1 F_2 segregation pattern for the trait grains per spike of the cross R1620 × R347/1. The *white columns* indicate genotypes excluded from the mapping analysis

From the F_2 bulked segregant analysis it was shown that of the 1000 RAPD primers 118 (11.8%) yielded at least one fragment which was polymorphic between the two pools. To investigate whether these differences were reproducible we re-analyzed the 118 primers using the same DNA bulks in another set of PCR reactions. Finally, 4 RAPD primers (P01, AP05, R11, S10) were confirmed to yield a polymorphic fragment for all ten selected male-sterile plants and consequently to be linked to *Rfg1*. All four markers were dominant. The other 114 primers yielded patterns of amplified fragments that were not closely linked or not reproducible.

Although the bulked segregant analysis gave some indication for a very close linkage between the RAPD markers and *Rfg*1 the calculation including all 140 F_2 individuals gave a distance of about 20 cM to the target gene due to a distorted segregation at the *Rfg1* locus (120 fertile to 20 sterile plants). Figure 2 shows the pattern seen using the primer P01 with the DNA of 24 individual F_2 plants.

Of the four RFLP probes tested three (PSR119, PSR167, PSR899) detected polymorphism and were shown to be linked to the four RAPD markers and to the restorer gene.

F_3 analysis

When the progeny of F_2 plant no. 194 was analyzed, including 133 characterized plants, a 92 (fertile):41 (sterile) segregation was observed fitting the expected 3:1 ratio ($\chi^2 = 2.40$; $P > 0.10$) for a monogenic inheritance. The RAPD marker *XR11* again showed linkage to *Rfg1* with a distance of 9.2 cM. The map considering the F_2 and F_3 data is presented in Fig. 3 where *Rfg1* is located distal to the four RAPD and three RFLP markers on the long arm of chromosome 4R.

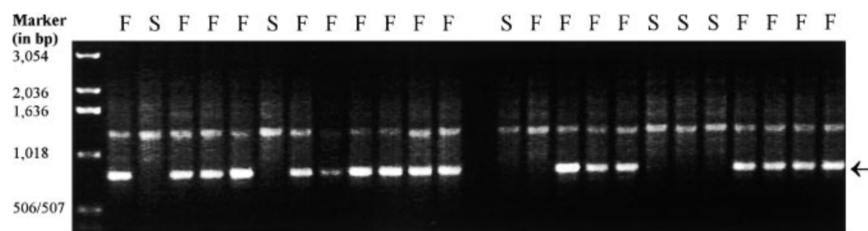

Fig. 2 Fragment patterns obtained from RAPD PCR using the primer P01 with the DNA of 24 individual F_2 plants (*F* male fertile, *S* male sterile) of the cross R1620 × R347/1. *Lane 1* shows the 1-kb DNA ladder (Gibco BRL). The *arrow* indicates the polymorphic band that is linked to the target trait

Discussion

In crops where the seed is used for consumption the exploitation of a CMS system requires the restoration of the CMS line. The G-type cytoplasm, which is 1 out of approximately 20 known sterility-inducing cytoplasms, was studied here. Using primary trisomics of the rye variety 'Esto', Melz and Adolf (1991) located a major gene responsible for the restoration of G-type CMS on chromosome 4R. The molecular mapping data presented here confirms the location of the major restoration gene on that chromosome. *Rfg1* was found to map distally on the long arm of 4R. From pairing (Naranjo and Fernandez-Rueda 1991) and RFLP analysis (Devos et al. 1993) it is known that 4RL comprises a proximal segment with homoeology to the short arms of other Triticeae group 7 chromosomes, whereas the distal segment is homoeologous to the distal ends of the short arms of the primeval Triticeae group 6 chromosomes. The data presented here provide clear evidence that *Rfg1* maps distal to the two RFLP markers *Xpsr167* and *Xpsr899* and therefore is located on that segment of chromosome 4RL which was ancestrally translocated and is homoeologous to other Triticeae 6S chromosomes. The order of the three mapped RFLP markers in our population is the same as described by Devos et al. (1993).

In addition, four RAPD markers that are closely linked to each other were also found to be linked to the target gene. Whereas in the F_2 the distance to the RAPD markers was about 20 cM it was less than 10 cM in the selected F_3 population. The reason for the higher distance in the F_2 may be due to the highly distorted segregation for *Rfg1* in that mapping population, probably caused by the effects of the two modifying genes located on chromosomes 3R and 6R (Melz

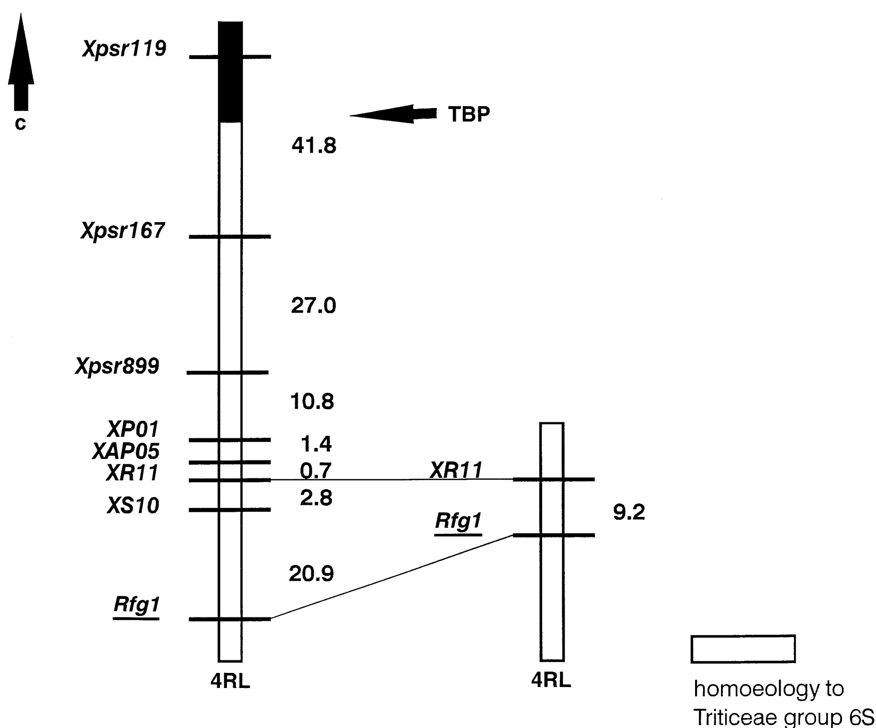

Fig. 3 Partial linkage map of chromosome 4RL of rye derived from F_2 (*left*) and F_3 (*right*) populations of the cross R1620 × R347/1, including the gene *Rfg1* that determines the restoration of the G-type male sterility in rye. Genetic distances are given in centimorgans (cM). *c* Centromere, *TPB* 7 S/6 S translocation breakpoint

and Adolf 1991) which may have been become homozygous in the selected F_3 population.

Interestingly, Miedaner et al. (1997) described loci for the restoration of CMS induced by the Pampa (P) cytoplasm, on 4RL. One of these loci was linked to *Xpsr899* by 5.9 ± 2.3 cM. This locus may be allelic to *Rfg1*. Curtis and Lukaszewski (1993) screened wheat-rye addition lines to localize genes in rye that restore the male fertility of hexaploid wheat with *T. timopheevi* cytoplasm. Two genes, *Rfc3* and *Rfc4*, were located on the long arms of the chromosomes 6R and 4R, respectively. Genetic mapping of *Rfc4* was inconclusive but suggested that it was located 16.1 cM from the telomere of 4RL and at least 8.0 cM from the centromere. Therefore, *Rfc4* may also be allelic to *Rfg1*.

In wheat five loci for the restoration of CMS induced by the *T. timopheevi* cytoplasm were described to be located on chromosomes 1 A (*Rf1*), 7D (*Rf2*), 1B (*Rf3*), 6B(*Rf4*) and 6D(*Rf5*) (McIntosh et al. 1995). Hart (1997) described two loci for cytoplasmic-male-sterility restoration on the short arms of chromosomes 6A and 6B, both designated *Rf6*. A homoeologous relationship to the restoration loci on chromosome 4RL of rye may be concluded.

Considering all this data it is probable that some genes controlling CMS restoration are conserved across the cereal species rye and wheat. Collinearity for genes affecting plant height and development has already been described by Börner et al. (1998). These authors clearly demonstrated that homoeology is present for two members of the gibberellic acid (GA_3)-sensitive dwarfing genes of wheat (*Rht12*) and rye (*Ddw1*), located on translocated segments of the long arms of chromosomes 5A and 5R, respectively, and for the Triticeae group 5 vernalization response genes of wheat, rye and barley. Further examples for the homoeology of mutant loci were described by Korzun et al. (1997) who analyzed genes determining the absence of ligules, waxless plant and waxy endosperm characters.

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