Theor Appl Genet (1997) 97: 147–153

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Mapping of three self-fertility mutations in rye (*Secale cereale* L.) using RFLP, isozyme and morphological markers

Received: 8 October 1997 / Acepted: 26 November 1997

Abstract Three mutations determining self-fertility at the *S*, *Z* and *S5* self-incompatibility loci on chromosomes 1R, 2R and 5R of rye, respectively, were mapped using three different F_2 populations. There was a close linkage of one isozyme and four RFLP markers, and no recombinant plants were detected. These markers are *Prx7*, *Xiag249* and *Xpsr634* for the *S* locus (1R), *Xbcd266* for the *Z* locus (2R) and *Xpsr100* for the *S5* locus (5R). Linkage data for markers associated to the self-fertility mutations at the *S*, *Z* and *S5* loci were calculated and compared with genetic maps computed by MAPMAKER multipoint analysis.

Key words Genetic mapping · Isozyme · RFLP · *Secale cereale* L. · Self-fertility

Introduction

Self-incompatibility in rye is caused by the interaction of pollen and stigma and is genetically controlled by two multiallelic loci – *S* and *Z* – that are inherited independently of each other (Lundqvist 1954, 1956, 1957). The incompatibility reaction results from the matching of alleles at these loci in both pollen and stigma. Mutations at the *S* or *Z* loci break down the system of self-incompatibility and lead to self-fertility (Lundqvist 1960). The two loci *S* and *Z* have been found to be linked to the isozyme markers *Prx7* on chromosome 1R (Wricke and Wehling 1985) and *β-Glu* (*β*-Glucosidase) on chromosome 2R (Gertz and Wricke 1989), respectively.

The possibility that self-fertility in rye may result from mutations at loci other than *S* and *Z* was suggested by Lundqvist (1968). Melz et al. (1990) subsequently described genes for self-fertility on chromosomes 1R, 4R, 5R and 6R. A mutation at a third locus different from *S* and *Z* and designated *S5* was identified by Voylokov et al. (1993) and localized on chromosome 5R by Fuong et al. (1993). A third class of allelic mutations at a *T* locus and not linked to *S* and *Z* were also described in *Phalaris coerulescens* Desf., a highly self-incompatible grass in the *Poaceae* (Hayman and Richter 1992). Mutations at both the *S5* (*Secale cereale*) and *T* (*Phalaris coerulescens*) loci determine gametophytic expression i.e. pollen grains carrying the mutations are self-compatible.

For the three loci of rye (*S*, *Z* and *S5*) self-fertile mutations were identified by linkage with the isozyme markers *Prx7*, *β-Glu* and *AadhNADP*, respectively, by Voylokov et al. (1994). The detection of such mutations is possible, because in selfed progenies that are heterozygous for the mutation, a deviation from the Mendelian segregation ratio is observed for markers linked to the incompatibility loci.

In the study described here inbred lines having known mutations for self-fertility at the *S*, *Z* or *S5* locus were used for mapping the mutants more precisely using restriction fragment length polymorphism (RFLP) markers. The use of molecular markers will offer possibilities for comparative mapping studies in other species of the *Poaceae* and for map-based cloning of incompatibility loci in the future.

Communicated by G. Wenzel

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Materials and methods

Plant materials

The three mutant loci were mapped in three different F_2 populations, produced by crossing the self-incompatible variety 'Volhova'

(V) with the self-fertile lines No. 6 (l6) carrying the mutation for self-fertility at the *S* locus (chromosome 1R), No. 454 (l454) carrying the mutation for self-fertility at the *Z* locus (chromosome 2R) and No. 2 (l2) carrying the mutation for self-fertility at the *S5* locus (chromosome 5R). The F_1 hybrids selected for the production of F_2 seeds were heterozygous for the isozyme or morphological markers *Prx7* (peroxidase) and *Lap1* (leucinaminopeptidase) in the cross V × l6, *al* (absence of ligules) in the cross V × 1454 and *AadhNADP* (NADP-dependent aromatic alcohol dehydrogenase), *Est3/5/6/7* and *Est2* (esterase) in the cross V × l2. The designation of esterase loci is in accordance with Wehling (1985). From one individual F_1 plant per combination 80, 73 and 82 F_2 seeds were obtained for the V × l6, V × l454 and V × l2 crosses, respectively.

Isozyme analysis

Isozyme analysis was carried out with leaf extracts of 2-week-old seedlings. For resolution of peroxidases and esterases, isoelectric focusing in a pH 3.5–10 gradient of ampholines was used. The acrylamide concentration was 5% T/3%C. 0.5 *M* H_3PO_4 and 0.5 *M* NaOH were used as electrode solutions. The electrophoretic separation of leucinaminopeptidase and NADP-dependent aromatic alcohol dehydrogenase was performed on a 6% polyacrylamide gel (Davis 1964). For the staining of isozymes standard procedures were used as described by Shaw and Prasad (1970).

RFLP analysis

DNA was extracted from 5-to 6-week-old seedlings by the procedure of McCouch et al. (1988) and cut with the restriction enzymes *Hin*dIII, *Dra*I, *Eco*RI or *Eco*RV. Preparation of Southern blots, probe labeling and filter hybridization followed the method described by Devos et al. (1992). The probes used were cDNAs and genomic DNAs from various rye, wheat, barley and oat libraries developed at the Institute of Applied Genetics, University of Hannover, Germany (IAG clones), John Innes Centre, Norwich, UK (PSR clones), Institute for Resistance Genetics, Grünbach, Germany (MWG clones), Cornell University, Ithaca, USA (BCD, WG, CDO clones) or IPK Gatersleben, Germany (SCB clones). The probes are located on *Triticeae* homoeologous groups 1, 2 and 5.

Statistical analysis

In the case of selfing an F_1 hybrid that is heterozygous for a self-fertility mutation, only one-half of pollen grains grow (pollen grains having the mutation). Therefore, all markers linked to the self-fertility mutation show a distorted segregation ratio in the F_2. The range of distortion depends on the value of recombination frequency (r) between the locus carrying the mutation and the marker analyzed. For example, after selfing an F_1 with the constitution SfA_1/SA_2, where Sf and S are inactive and active alleles of an incompatibility locus, respectively, and A_1 and A_2 are alleles of a marker locus, it is expected that the segregation ratio for the marker will be $0.5(1\text{-}r)A_{11} : 0.5A_{12} : 0.5rA_{22}$. This ratio is a consequence of the combination of female gametes [$0.5\ A_1 : 0.5A_2$] and male gametes [$(1\text{-}r)A_1 : rA_2$] after selection of pollen grains on the stigma having self-fertility alleles and corresponds to gametic selection in one sex (Wagner et al. 1992). In this case the frequency of heterozygous genotypes for the marker locus is equal to 0.5, and a significant excess of homozygous genotypes for the allele that originated from the self-fertile line (A_{11}) would be obtained.

Tests for gametic selection were performed by χ^2 component analysis at a significance level of 5% (Wehling 1991). For testing whether the frequencies of $A_{11} + A_{22} = A_{12} = 0.5$, the ${\chi_1}^2$ component was calculated by $(n_1 + n_3 - n_2)^2/n$, whereas for testing whether the frequencies of $A_{11} = A_{12}$ the ${\chi_2}^2$ component was calculated by $2(n_1 - n_3)^2/n$. In both cases n_1, n_2 and n_3 are the numbers of A_{11}, A_{12} and A_{22} genotypes, respectively, and $n = n_1 + n_2 + n_3$. If the segregation for the marker analyzed was significantly different from the 1 : 2 : 1 ratio and the frequencies of $A_{12} = 0.5$ and $A_{11} > A_{12}$ ($P > 0.05$, $v = 1$) the recombination value was calculated as described by Weber and Wricke (1994). With co-dominant markers, the value of r is equal to $n_3/n_1 + n_3$ and the standard error to $n_1 x\ n_3/(n_1 + n_3)^3$.

In the case of dominant markers the χ^2 test for a 3 : 1 segregation ratio was performed. The deviation from the 3 : 1 ratio must be in the direction expected from the way in which the self-fertility mutation and the analyzed marker entered the cross. If the H_o hypothesis was rejected, the recombination value was calculated for coupling by $2\ n_2/n$ or for repulsion by $(n_1 - n_2)/n$. In both cases s_r was calculated as the square root of the variance by $\sqrt{4n_1 \times n_2/n^3}$, where n_1 is a number of A**.** and n_2 is the number of aa genotypes. Coupling (sf-A) leads to a large excess of A**.** in the selfed progeny (no segregation in the case of complete linkage) and repulsion (Sf-a), to an excess of aa homozygotes (in the case of complete linkage the segregation ratio is 1 A.: 1aa).

Apart from the linkage of the self-fertility mutations with different types of markers, the linkage relationships between markers themselves were analyzed. This was possible because the deviation from a monohybrid segregation for markers has no effect on the estimation of the recombination value if at least one marker from two analyzed shows co-dominance (Heun and Gregorius 1987, Wagner et al. 1992). The linkage maps were constructed with the aid of the MAPMAKER 2.0 computer program (Lander et al. 1987).

Results

Linkage of single-marker loci with the self-fertility mutations

The segregation data for 16 markers mapped on chromosome 1R are presented in Table 1. There is a tendency for an excess of self-fertile genotypes *(A_{11})* in all cases, but only the segregation ratios of 11 markers fully agree with criteria for gametic selection. The frequency of recombination between the *S* locus and these markers varied from zero (*Prx7, Xiag249*, *Xpsr634*) to 19.4% (*Xmwg506*). The segregation ratios for the markers not closely linked to the *S* locus either conformed to the expected 1 : 2 : 1 segregation (*Xmwg938*, *Xpsr937/1*) or showed a significant excess of heterozygotes (*Xcdo99*, *Xbcd98*, *Lap1*). For *Lap1* an excess of homozygotes A_{11} was also observed.

A large excess of homozygous genotypes A_{11} characteristic for the self-fertile line was detected in all 6 marker segregations studied on chromosome 2R (Table 2). However, this excess was accompanied by significant greater numbers of heterozygotes. Only the segregation ratio for *Xbcd266* was consistent with the ratio expected in the case of gametic selection as the main cause of distortion. In this segregating population plants having a recombinant genotype (self-incompatible) were absent, i.e. 0% recombination between *Xbcd266* and *Z*. For the recessive marker gene *al* and *Z* the recombination frequency was $8.3 \pm 5.7\%$.

Table 1
Segregation data and recombination frequencies for markers in relation to the *S* locus on chromosome 1R (*n.c.* not calculated)

Marker	A_{11}	A_{12}	A_{22}	*n*	χ_1^2	χ_2^2	χ^2	$r \pm s_r$
Xmwg938	24	43	12	79	0.62	3.65	4.27	n.c.
Xpsr937/1	28	43	13	84	0.05	5.36*	5.41	n.c.
Xcdo99	22	51	11	84	3.86*	2.88	6.74*	n.c.
Xbcd98	22	51	11	84	3.86*	2.88	6.74*	n.c.
Xcdo580	38	42	4	84	0	27.52*	27.52	9.5 ± 4.5
Xmwg913	38	42	4	84	0	27.52*	27.52	9.5 ± 4.5
Xscb190	34	48	2	84	1.71	24.38*	26.09*	5.6 ± 3.8
Xbcd1072	34	48	2	84	1.71	24.38*	26.09*	5.6 ± 3.8
Xpsr161	34	48	2	84	1.71	24.38*	26.09*	5.6 ± 3.8
Xpsr634	33	46	0	79	2.14	27.57*	29.71*	0
Xiag249	36	48	0	84	1.71	30.86*	32.57*	0
Prx7	30	35	0	65	0.38	27.70*	28.08*	0
Xpsr544	34	48	2	84	1.71	24.38*	26.09*	5.6 ± 3.8
Xmwg506	29	47	7	83	1.46	11.66*	13.12*	19.4 ± 6.6
Xpsr601	30	47	7	84	1.19	12.59*	13.78*	18.9 ± 6.4
Lap1	18	46	4	68	8.47*	5.76*	14.23*	n.c.

* Significant at $P > 0.05$

Table 2 Segregation data and recombination frequencies for markers in relation to the *Z* locus on chromosome 2R (*n.c.* = not calculated)

Marker	A_{11}	A_{12}	A_{22}	n	χ_1^2	χ_2^2	χ^2	$r \pm s_r$
Xpsr107(S)	25	46	2	73	4.95*	14.49*	19.44*	n.c.
Xcmwg699	25	44	2	71	4.07*	14.90*	18.97	n.c.
al[a]	22	38	2	62	3.16	12.90	16.06	8.3 ± 5.7
Xbcd266	30	43	–	73	2.32	24.66*	26.98*	0
Xcmwg720	26	45	2	73	3.96*	15.78*	19.74*	n.c.
Xpsr609	25	47	1	73	6.04*	15.78*	21.82*	n.c.

* Significant at $P > 0.05$
[a]For *al*, F_3 data were used

Table 3 Segregation data and recombination frequencies for markers in relation to the *S5* locus on chromosome 5R (*n.c.* not calculated)

Marker	A_{11}	A_{12}	A_{22}	*n*	χ_1^2	χ_2^2	χ^2	$r \pm s_r$
Xpsr945a	41	36	5	82	1.22	31.61*	32.83*	10.9 ± 4.6
Xpsr326	44	35	4	83	2.04	38.35*	40.59*	8.3 ± 4.0
Xpsr120a(S)	50	31	1	82	4.88*	58.56*	63.44*	n.c.
Xpsr360(L)	51	32	1	84	4.76*	59.52*	64.28*	n.c.
Xpsr100	62	22	0	84	19.05*	91.52*	110.57*	n.c.
AadhNADP	29	28	5	62	0.58	18.58*	19.16*	14.6 ± 6.1
Xpsr574	39	35	9	83	2.04	21.69*	23.73*	18.8 ± 5.6
Xwg1026	33	32	17	82	3.95*	6.24*	10.19	n.c.
Est3/5/6/7	21	27	14	62	1.03	1.58	2.61	n.c.
Xscb35	25	31	26	82	4.88*	0.02	4.90	n.c.
Xpsr370	26	39	19	84	0.43	1.17	1.60	n.c.
Est2	20	25	16	61	1.98	0.32	2.50	n.c.

* Significant at $P > 0.05$

Twelve segregating markers located on chromosome 5R were analyzed (Table 3). Although 4 of these showed a normal segregation of 1:2:1 (*Est3/5/6/7*, *Xscb35*, *Xpsr370*, *Est2*), 8 deviated significantly from the expected. A strong agreement with gametic selection was found for the segregation of the 4 co-dominant markers *Xpsr945a*, *Xpsr326*, *AadhNADP* and *Xpsr574*. The other distorted segregations observed comprised an excess of homozygotes A_{11} and a significant deficit of heterozygotes. Since these cases cannot be treated solely as a result of gametic selection the value of recombination was not calculated. Figure 1 shows the segregation patterns for 3 RFLP markers (*Xpsr326*, *Xpsr574*, *Xscb35*) having increasing genetical distances from the *S5* locus (8.3, 18.8 and $> 50\%$, respectively). In contrast to *Xpsr326*, where only two types of alleles were detected, a normal segregation was observed for *Xscb35*.

Multipoint linkage analysis

For all three linkage groups a multipoint analysis was performed and compared with the maps created on the

basis of the deviations from the single locus segregation ratios. The three markers *Xpsr634*, *Xiag249* and *Prx7* (chromosome 1R), which showed no recombination with the *S* locus after linkage analysis had been performed with the gametic selection locus, were found to be closely linked after performing the multipoint analysis and to span a genetical distance of 5.5% recombination (Fig. 2). This region allegedly contains the *S* locus.

On chromosome 2R 6 markers were mapped in the centromere region. However, since only the segregations for *Xbcd266* and the morphological marker *al* agree with the criteria of gametic selection these are the only markers that could be analyzed. The *Z* locus was found to map distally to *al* and to be closely linked to *Xbcd266* (Fig. 3).

A comparison of the 5R maps presented in Fig. 4 shows that although the distances between common markers are slightly different the same order is maintained. The RFLP marker *Xpsr100*, which showed a highly distorted segregation, was excluded from the multipoint analysis. Although the segregation ratios for *Xpsr100*, *Xpsr120a* and *Xpsr360* did not agree with the criteria for gametic selection as the main cause of distortion, they are probably closely linked to the *S5* locus on chromosome 5R. The detection of a maximum of one recombinant plant indicates that *S5* is located in the centromeric region.

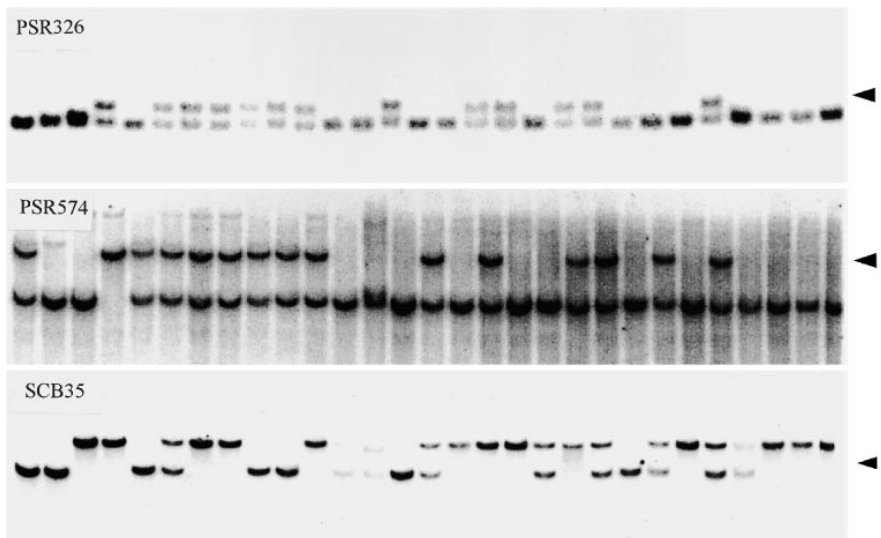

Fig. 1 RFLP patterns of the F_2 population of the cross V × l2 using the probes PSR326, PSR574 and SCB35 showing zero, one and seven homozygous plants, respectively, having alleles originating from the self-sterile parent (indicated by *arrows*)

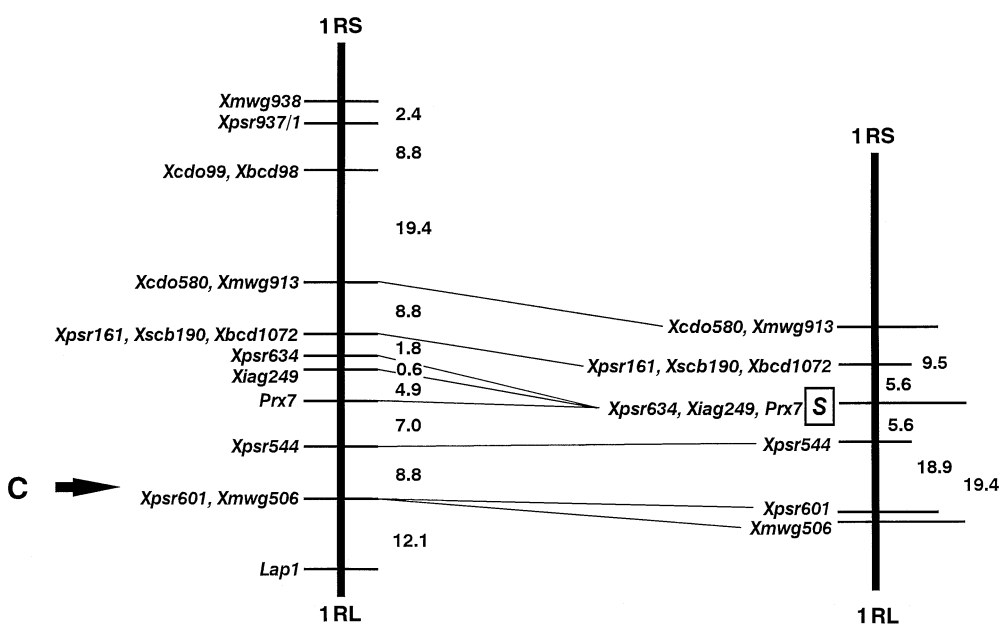

Fig. 2 Partial RFLP maps of rye chromosome 1R derived from multipoint analysis (*left*) or created on the basis of the deviations from single-locus segregation ratios (*right*) showing the position of the *S* locus. Genetic distances are given in percentage of recombination. *C* Centromere, *S* short arm, *L* = long arm

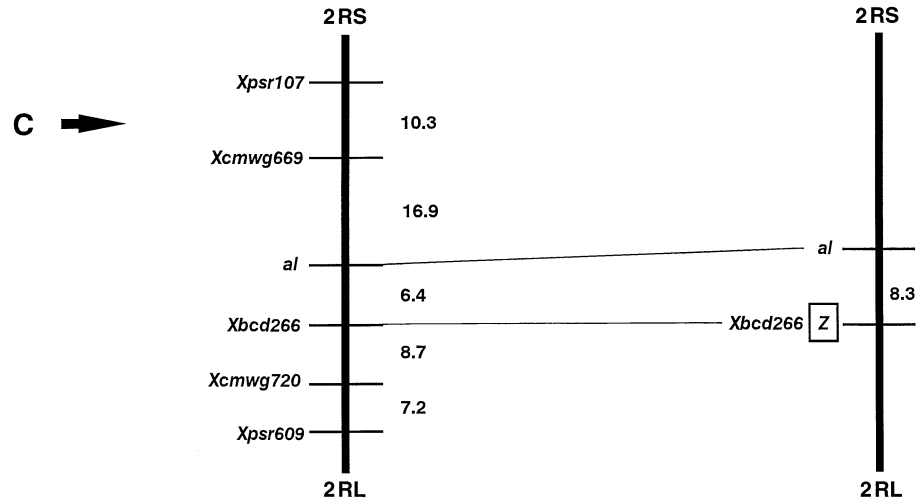

Fig. 3 Partial RFLP maps of rye chromosome 2R derived from multipoint analysis (*left*) or created on the basis of the deviations from single-locus segregation ratios (*right*) showing the position of the *Z* locus. Genetic distances are given in percentage of recombination, *C* = centromere, *S* = short arm, *L* = long arm

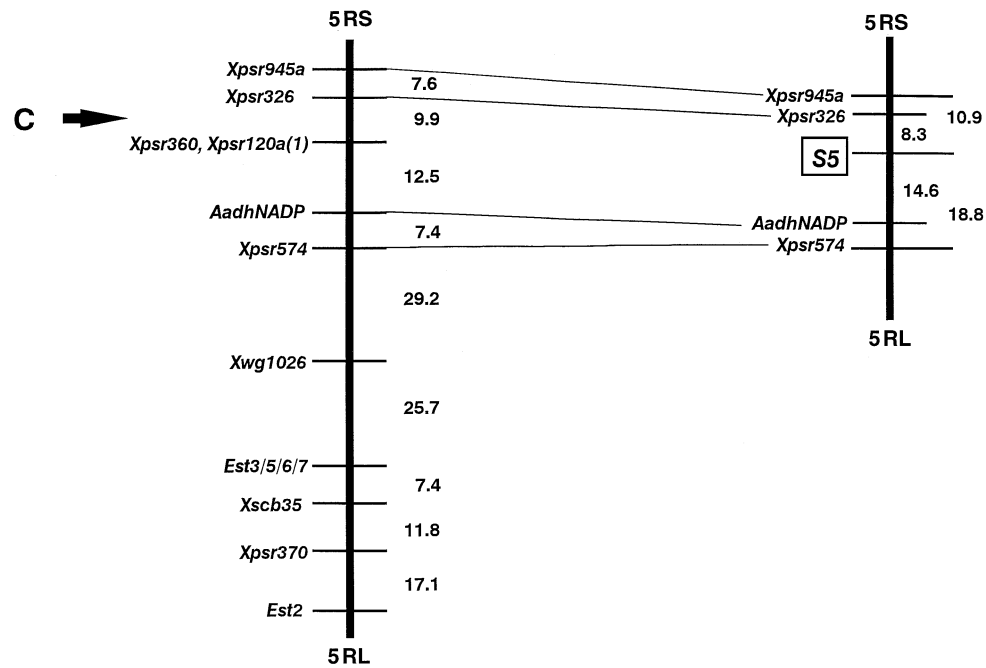

Fig. 4 Partial RFLP maps of rye chromosome 5R derived from multipoint analysis (*left*) or created on the basis of the deviations from single-locus segregation ratios (*right*) showing the position of the *S5* locus. Genetic distances are given in percentage of recombination. *C* Centromere, *S* short arm, *L* long arm

Discussion

The possibility of using data with distorted segregations for mapping was proposed by Heun and Gregorius (1987) and subsequently used for mapping in sugar beet (Pillen et al. 1992), carrot (Schulz et al. 1994) and rye (Philipp et al. 1994, Wanous et al. 1995). Most of such deviations are caused by gametophytic factors of unknown function.

In the present paper the distorted segregations caused by self-fertility alleles of three loci participating in the incompatibility reaction were studied. If a distortion in the segregation of markers only arises from the influence of a self-fertility mutation then two consequences can be expected. First, the distortion must decrease distally from the mutation. Second, the order of the markers on the multipoint linkage map, and to some extent the genetic distances between the markers, must correspond to the order of the markers and the estimates of recombination frequency, respectively, calculated on the basis of the deviations from single-locus segregation ratios.

For two loci (*Z* and *S5*), the segregation of the surrounding markers differed greatly from the theoretically expected situation, i.e. gametic selection based on the exclusive growth of pollen grains carrying the self-fertility mutation. The reason for such distortion may

be the segregation for additional loci in the analyzed intervals, which are under gametic or zygotic selection and could arise from the self-incompatible parent.

The map positions of the three loci *S*, *Z* and *S5* presented herein consistently agree with data already published. In addition to the close linkage of *S* (chromosome 1R) to the isozyme marker *Prx7* (Wricke and Wehling 1985, Fuong et al. 1993, Phillip et al. 1994), a linkage to the RFLP markers *Xiag249* (2.7 cM) and *Xpsr544* (4.5 cM) was described by Senft and Wricke (1996). The recombination frequency between *S* and *Lap1* was previously shown to be 11.8% (Voylokov et al. 1994), whereas from the multipoint analysis carried out in the present study, the marker is located on the short arm of chromosome 1R at a distance of 27.9% from *Prx7*. The latter marker co-segregates with *S*. The greater distance is probably due to the relatively low number of scorable plants (68) for that isozyme locus. The order of markers and the values of recombination frequency are very similar on both types of linkage maps shown in Fig. 2. The marker data are also comparable to those for chromosome 1R published by Wanous and Gustafson (1995).

The *Z* locus was mapped on the long arm of chromosome 2R closely linked to *Xbcd266*, which is located distally to the gene *al* determining the morphological trait 'absence of ligules'. A genetical relationship between *Z* and *al* has already been described by Smirnov and Sosnikhina (1984) who calculated a recombination frequency that ranged from 0 to 22%. Common RFLP markers for the map presented here and that of Senft and Wricke (1996), who located the *Z* locus on chromosome 2R without knowledge of the centromere position, is not available. The map position of *al* agrees with data published by Korzun et al. (1997) who mapped this mutant locus in rye and described its relationship to homoeologous loci within the *Gramineae*.

For the third self-fertility mutation mapped no RFLP data were available. However, Voylokov et al. (1994) reported that *S5* and the isozyme marker *AadhNADP* were linked, which was confirmed in the present studies. The *S5* locus is located in the centromeric region of chromosome 5R between *AadhNADP* and *Xpsr326*. A close linkage of *S5* to the RFLP markers *Xpsr360*, *Xpsr120a(1)* and *Xpsr100* is highly likely.

From the molecular mapping data presented here, we have been able to identify the chromosome regions carrying three self-fertility mutations in rye. A more precise location of the mutations will be achieved by choosing additional probes and increasing the mapping populations. Furthermore, a comparison with self-fertility mutations in other grass species will become possible.

Acknowledgments The authors like to thank Renate Voss, Sofia F. Priyatkina and Sergej Malyshev for their excellent assistance and INTAS Brussels (INTAS-93-355) for financial support.

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