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Genetic studies of self-fertility in rye (*Secale cereale* L.). 2. The search for isozyme marker genes linked to self-incompatibility loci

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Abstract. The segregation of several isozyme marker genes has been studied in F_2 inbred families from hybrids between self-sterile and five self-fertile inbred lines (nos. 2, 3, 4, 5, and 8) as well as from interline hybrids. Self-pollination of F_1 hybrids between self-sterile forms and lines 5 and 8 gave an F_2 segregation ratio of 1 heterozygote:1 homozygote for the gene *Prx7* (chromosome 1R) against the allele from the line. This is interpreted as a result of tight linkage of the *Prx7* gene with the *S1* gene in chromosome 1R (recombination at a level of 0–1%). The self-pollination of such hybrids with lines 2, 3 and 4 gave normal segregation for the *Prx7* gene (1:2:1). This means that these lines carry a self-fertility allele which is not on chromosome 1R. Interline hybrids 5 × 2, 5 × 3 and 5 × 4 had self-fertility alleles for the two S genes and in inbred F_2 progenies gave the expected deviating segregation for the *Prx7* gene in a ratio of 2:3:1. The segregation of interline hybrid 5 × 8 was normal, 1:2:1, as expected. Highly-deviating segregation in an inbred F_2 family of a hybrid with line 5 has also been obtained for another gene from chromosome 1R – *Pgi2* (recombination with the *S1* locus of 16.7%). By using the same method it has been estimated that line 4 has a self-fertility allele of the *S2* locus from chromosome 2R and that the genes *β-Glu* and *Est4/11* are linked with it (recombination 16.7% and 17.5–20% respectively). Lines 2 and 3 have a self-fertility allele of the *S5* locus from chromosome 5R which is linked with the *Est5-7* gene complex (recombination at a level of 28.8–36.0%).

Key words: *Secale cereale* L. – Incompatibility genes – Linkage groups – Isozyme genes – Self-fertility

Introduction

Self-incompatibility loci may be revealed and mapped on the basis of deviations in the pattern of segregation of genes tightly linked to such loci. Isozyme marker genes are especially convenient for this purpose because they are co-dominantly inherited and their chromosomal localization is known. However, data of this type are very scant in the literature on rye genetics. The gene *Prx7* (chromosome 1R) has been shown to be tightly linked with the S-locus (recombination 0–2%; Wricke and Wehling 1985) and the *β-Glu* gene is linked with the *Z* locus on chromosome 2R (recombination 14%; Gertz and Wricke 1989).

In the present study we have used a simple and very efficient method for revealing such linkage. We have discovered cases of linkage between some isozyme marker genes and S loci and evaluated their frequencies of recombination. The genotypes of the inbred lines studied were characterized according to mutant (*Sf*) alleles of different S loci.

Materials and methods

Plant material

Five self-fertile inbred lines (nos. 2, 3, 4, 5 and 8) from a genetic stock collection have been used in this study. The origin of these lines has been described elsewhere (Voylokov et al. 1993). Self-fertile lines were crossed with each other and with self-sterile plants of the Russian variety Volkhova. F_1 plants of both types of

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hybrids were self-pollinated and segregation in F_2 inbred progenies has been studied.

Isozyme analyses

The isozyme analyses were carried out with 14–20-day-old seedling leaf blades which were extracted in 0.1 M Tris-HCl buffer (pH 8.0) containing 0.5 M sucrose, 0.1% ascorbic acid and 0.1% hydrochloride cysteine (Sako and Stachmann 1972). Extracts were centrifuged at 10,000 g and 0 °C for 15 min. The supernatant was directly used for electrophoresis and isoelectric focusing. The following isozymic systems were studied using 6% polyacrylamide-gel electrophoresis: aconitase, endopeptidase, beta-glucosidase, aromatic alcohol dehydrogenase and phosphoglucose isomerase; and with 7.5% polyacrylamide-gel electrophoresis: aspartate aminotransferase and superoxide dismutase. Polyacrylamide slabs were prepared using Tris-HCl (pH 8.9) as the gel buffer and Tris-glycine (pH 8.3) as the electrode buffer (Maurer 1968). Peroxidase and esterase isozymes were separated by flat-bed isoelectric focusing (IEF) in a pH 3.5–10 gradient according to Wehling (1986).

Isozyme activities were detected by standard methods (Tang and Hart 1975; Levites 1986; Wehling 1986).

The search for isozyme markers linked to S loci

According to Lundqvist (1956, 1958), self-fertile forms have an inactive (*Sf*) allele of at least one of the S loci. The hybrids between self-sterile plants and a self-fertile line contain a self-fertility allele of either one (*Sf/Sn*) or two (*S1f/S1n S2f/S2n*) S loci depending on the number of loci which are represented by self-fertility alleles in the particular inbred line chosen for study. When such hybrids are self-pollinated only 50% or 75% of the pollen grains take part in fertilization – those which carry an *Sf* allele of at least one S gene. This explains why deviations from Mendelian ratios are expected for genes linked with the S locus which happen to be heterozygous for the *Sf* allele (*Sf/Sn*). If some gene A is linked with the *S1* locus and the frequency of recombination between them is p, three different types of results may be obtained in inbred F_2 progenies from F_1 self-sterile A^2A^2 × self-fertile A^1A^1 hybrids (Table 1).

If the inbred line used for the cross contains an *Sf* allele of a locus which is not linked to gene A then a normal 1:2:1 segregation for this gene is expected. If, however, the inbred line contains an *S1f* allele then a deviation from the Mendelian ratio is expected: homozygotes for allele A^1 from the line will be in excess while homozygotes for allele A^2 from the self-sterile parent will be in deficit. In the case of complete linkage between A and *S1* (p = 0) only two genotypes are expected in F_2 progenies – $1A^1A^1:1A^1A^2$. When the inbred line used in a cross contains self-fertility alleles of two S genes (and one of them is an *S1* gene linked to the A gene) similar deviations are expected in F_2 inbred progenies. In the case of complete A-*S1* linkage a ratio of $2A^1A^1:3A^1A^2:1A^2A^2$ is expected.

For the interline hybrids, the Mendelian 1:2:1 ratio is expected when both lines carry an *Sf* allele of the same S gene or of two different S genes excluding the *S1* gene linked with the A gene. If the interline hybrid is a double heterozygote for *Sf* alleles and *S1f/S1n* heterozygosity is present then the expected segregation ratio is $(2-p)A^1A^1:3A^1A^2:(1+p)A^2A^2$ or 2:3:1 in case of complete A-*S1* linkage. Homozygotes for allele A^1 which comes from the line with the *S1f* allele will be in excess and homozygotes for the A^2 allele from the second line will be in deficit.

Thus the results of such types of crosses permit us to differentiate among the genotypes of inbred lines according to the S genes represented by *Sf* alleles.

Localization of S loci

The S loci are localized on the basis of their linkage with isozyme genes with an established chromosomal localization: *Pgi2* and *Prx7* are on chromosome 1R; *β-Glu, Sod2* and *Est4/11* are on chromosome 2R; *Aat3(Got4)* is on chromosome 3R; *Aadh1, Aco2, Est1, 2, 3* and *Est5-7* are on chromosome 5R and *Aco1, Ep* and *Est8/9* are on in chromosome 6R (see Melz et al. 1992). The frequencies of recombination between S loci and isozyme genes are calculated according to Leach (1988).

Results

Segregation for the Prx7 gene

The segregations for the *Prx7* gene in F_2 inbred progenies from interline F_1 hybrids and the hybrids between self-sterile plants of the Volkhova variety and some self-fertile lines are presented in Tables 2 and 3.

The data from Table 2 show that the hybrids between self-sterile plants and lines 5 and 8 give segregation of the 1:1 type for the *Prx7* gene in F_2 inbred progenies (almost complete absence of homozygotes with an allele from the self-sterile parent). This result means that lines 5 and 8 carry a self-fertility allele of same locus and that this locus is almost completely linked with the *Prx7* gene from chromosome 1R. This conclusion is confirmed by the results of a study of the segregation of the *Prx7* gene in F_2 inbred progeny of the hybrid 5 × 8 (Table 3). As expected segregation does not differ from the theoretical 1:2:1. In the hybrids of self-sterile plants with lines 5 and 8 all studied isozyme genes from chromosomes 3R, 5R and 6R demonstrated the expected Mendelian segregations of 1:2:1 or 3:1 in inbred progenies (Table 4). Only the

Table 1. Expected A gene segregations in inbred F_2 progenies from F_1 self-sterile A^2A^2 × self-fertile A^1A^1 hybrids (recombination between A and *S1* equals to p)

Genotype of self-fertile line	Genotype of F_1	Segregation ratio in F_2
A^1 *S1n*/A^1 *S1n S2f/S2f*	A^1 *S1n*/A^2 *S1m S2f/S2n*	$1A^1A^1:2A^1A^2:1A^2A^2$
A^1 *S1f*/A^1 *S1f S2n/S2n*	A^1 *S1f*/A^2 *S1m S2n/S2m*	$(1-p)A^1A^1:1A^1A^2:pA^2A^2$
A^1 *S1f*/A^1 *S1f S2f/S2f*	A^1 *S1f*/A^2 *S1m S2f/S2n*	$(2-p)A^1A^1:3A^1A^2:(1+p)A^2A^2$

Sn, Sm – any active alleles of S loci; *Sf* – mutant inactive allele

Table 2. Segregation for the *Prx7* gene in inbred F_2 progenies from self-sterile plants × self-fertile line hybrids

Self-fertile line	Number of F_2 plants			χ^2 (1:2:1)
	Self-fertile line	Hetero-zygote	Self-sterile plant	
2	17	53	24	2.57
3	77	128	59	2.69
4	33	65	39	0.88
5	23	25	0	22.13*
8	47	44	1	46.17*

* $P < 0.01$

Table 3. Segregation for the *Prx7* gene in inbred F_2 progenies from interline hybrids

Hybrid	Number of F_2 plants			χ^2 (1:2:1)	χ^2 (2:3:1)
	Line 5	Heterozygote	Other lines (2, 3, 4 or 8)		
5 × 2	71	88	30	18.68*	1.53
5 × 3	30	55	10	10.78*	3.42
5 × 4	36	50	10	14.25*	2.38
5 × 8	32	41	23	3.73	4.08

* $P < 0.01$

Pgi2 gene in a hybrid with line 5 demonstrated evident deviation from 1:2:1 with a deficit of homozygotes for the *Pgi2* allele from the self-sterile plant and an excess of homozygotes for the allele from line 5. This is perfectly understandable because the *Pgi2* gene is also localized on chromosome 1R as are *Prx7* and the gene with the *Sf* allele in line 5.

Lines 2, 3 and 4 contain self-fertility alleles of other S loci and not of that from chromosome 1R because the segregation for *Prx7* is perfectly Mendelian, 1:2:1, in the F_2 progenies of all hybrids between these lines and self-sterile plants (Table 2). Therefore, interline hybrids 5 × 2, 5 × 3 and 5 × 4 must be heterozygotes for *Sf* alleles of least two S genes including the *S1f* gene from chromosome 1R. As has been mentioned earlier the *Prx7* gene is almost completely linked with the *S1* gene. Therefore, we might expect segregation for this gene in F_2 progenies of the hybrids 5 × 2, 5 × 3 and 5 × 4 in a ratio 2:3:1 and this type of segregation is indeed found in the three inbred progenies studied (Table 3).

Segregations for the other isozyme genes

The segregations for isozyme genes other than *Prx7* in the hybrids between self-sterile plants and self-fertile lines are shown in Tables 4, 5 and 6. It has already been mentioned that the isozyme genes from chromosomes 3R, 5R, and 6R did not show deviations from Mendelian segregation in hybrids with lines 5 and 8 (Table 4) because these lines carry a self-fertility allele of the *S1* gene from chromosome 1R.

Table 4. Segregation for the isozyme marker genes in F_2s from the self-sterile plants × self-fertile lines 5 and 8 hybrids

Chromo-some	Gene	Line no.	Number of F_2 plants			χ^2 1:2:1 (3:1)
			Line	Hetero-zygote	Self-sterile plant	
1R	*Pgi2*	5	20	24	4	10.66*
3R	*Aat3*	8	6	27	11	3.40
5R	*Aadh1*	5	11	25	11	0.21
	Aco2	8	14	19	11	1.23
	Est2	5	8	27	13	1.79
	Est5/7	5	7	41		2.78
	Est6	5	32		16	1.78
6R	*Est8/9*	5	10	27	11	0.45
	Aco1	5	10	26	9	1.13

* $P < 0.01$

Table 5. Segregations for the isozyme marker genes in F_2 from self-sterile plants × self-fertile lines 2 and 3[a] hybrids

Chromo-some	Gene	Line no.	Number of F_2 plants			χ^2 1:2:1 (3:1)
			Line	Hetero-zygote	Self-sterile plant	
2R	*β-Glu*	3	51	82	46	1.54
	Est4	2	13	34		0.18
	Est11	2	35		12	0.01
3R	*Aat3*	2	44	81	63	7.44**
		3	53	90	47	0.91
5R	*Aco2*	2	28	49	19	1.73
	Est2	2	71	193		0.51
		3	31	93		0.00
		Σ	102	286		0.34
	Est5/7	2	228		48	8.52*
		3	100		24	2.11
		Σ	328		72	10.45*
	Est6	2	64	116		10.69*
6R	*Aco1*	2	50	95	40	1.22
		3	27	48	17	2.34
		Σ	77	143	57	3.18
	Est8/9	2	68	137	70	0.03
		3	29	68	27	1.23
		Σ	97	205	97	0.30
	Ep	2	26	66		0.54

* $P < 0.01$; ** $P < 0.05$

[a] The test of heterogeneity ($P > 0.05$) shows that the segregation ratios for several genes in F_2 progenies from hybrids with lines 2 and 3 are homogeneous which permits us to pool the data

Considering the data presented in Table 5 we can see significant deviations from the Mendelian ratio in segregations for the genes *Est5-Est7* from chromosome 5R (they are completely linked with each other). All F_2 progenies with segregation for these genes had plants

with the allele from the self-fertile line in excess compared to theoretical expectation. This is the type of deviation in segregation which is expected in cases of linkage of the genes with the S locus and implies that lines 2 and 3 carry a self-fertility allele of the S locus localized on chromosome 5R. We designate this locus as *S5*. A deviation from Mendelian segregation is also observed for the gene *Aat3* in F_2 progenies from the self-sterile plant × line 2 hybrid. But this is not expected in the case of linkage of the *Aat3* gene with the S locus and, therefore, this deviation is caused by some other, unknown, factor. In hybrids between these lines and self-sterile plants there were perfectly normal Mendelian segregations in F_2 families for the *Prx7* gene of chromosome 1R (Table 2) as well as for the genes *β-Glu*, *Est3* and *Est5* on chromosome 2R (Table 5). The pedigrees of lines 2 and 3 diverged only after eight generations of inbreeding. Thus both lines possess a self-fertility allele of the same *S5* gene on chromosome 5R.

In the hybrid of self-sterile plants with line 4 the segregation for the *Prx7* gene was normal, 1:2:1 (Table 2). This means that the self-fertility allele in this line is not the *S1* gene from chromosome 1R. Among hybrids with this line the deviating segregations in inbred F_2 progenies were obtained only for the genes *β-Glu*, *Est4* and *Est11* of chromosome 2R (Table 6). In the case of these genes the deviations are also of the type expected for the linkage of genes with the *Sf* mutation: there is a definite excess of plants with alleles of the isozyme loci obtained from line 4. This line therefore appears to carry the self-fertility allele of the *S2* gene on chromosome 2R. This conclusion is supported by normal Mendelian segregations for the genes from chromosomes 5R and 6R in the same type of hybrids with line 4 (Table 6).

Table 6. Segregation for the isozyme markers genes in the F_2 from self-sterile plants × self-fertile line 4 hybrids

Chromosome	Gene	Number of F_2 plants			χ^2 1:2:1
		Line	Heterozygote	Self-sterile plant	
2R	*β-Glu*	35	52	7	17.74*
	Est4	66	94		22.53*
	Est11	144		16	19.20*
	Sod2	23	37	21	0.70
5R	*Est2*	21	33	26	3.07
	Est3	22	58		0.27
	Est5/7	16	64		1.07
	Est6	125		35	0.83
	Aco2	27	43	24	0.87
6R	*Aco1*	17	51	26	2.40
	Est8/9	48	78	34	2.70

* $P < 0.01$

Thus, among the five inbred lines of rye studied we have revealed three different incompatibility loci – *S1*, *S2* and *S5* – based on the expected deviations in the segregation of the genes linked to these loci. These data permitted us to calculate the frequencies of recombination between the S loci and isozyme markers (Table 7). We can also identify the genotypes of the lines studied in terms of their S genes: lines 5 and 8 are *S1f/S1f S2n/S2n S5m/S5m*, lines 2 and 3 are *S1n/S1n S2m/S2m S5f/S5f*, and line 4 is *S1n/S1n S2f/S2f S5m/S5m* the designation *Sn*, *Sm* implies the presence of the active incompatibility allele).

Discussion

In this paper we demonstrate a most efficient and simple method of searching for genes linked with self-incompatibility loci. The method has been applied to reveal the linkage of the genes determining ligule (*el*) and ear type (*mo*) with one of the S loci (Smirnov and Sosnikhina 1984). The results which we obtained by this method are in good agreement with the data described in other publications by studying segregations after the crossing of different self-sterile I_1 plants with each other. The very low frequency of recombination between *S1* and *Prx7* (0–1%) is in accordance with similar data of Wricke and Wehling (1985) concerning the linkage between *S* and *Prx7*. The recombination frequency between the genes *S2* and *β-Glu* (16.6 ± 5.6%) is also quite similar to the data obtained by Gertz and Wricke (1989) for the genes *Z* and *β-Glu*. The *S1* gene is probably identical to the *S* gene of Wricke and Wehling and the *S2* gene to the *Z* gene of Gertz and Wricke.

The procedure used in the present study has some advantages over the method of crossing self-sterile plants. By including several isozyme genes in crosses one can identify cases of linkage of some of them with S loci. One can also include several inbred lines of independent origin in the crosses and so find several differ-

Table 7. Recombination frequencies between isozyme markers and self-incompatibility loci

Chromosome	Loci pair	Recombination frequency (%)
1R		
	S1 – Prx7	0–1.00
	S1 – Pgi2	16.66 ± 7.60
2R	*S2 – β-Glu*	16.66 ± 5.75
	S2 – β-Est4	17.50 ± 3.00
	S2 – Est11	20.00 ± 3.16
5R	*S5 – Est5/7*	36.00 ± 2.40
	S5 – Est6	28.88 ± 3.38

ent S loci. Finally, the results of studying segregation patterns in F_2 inbred progenies permit one to differentiate among different genotypes of inbred lines according to the S loci represented by self-fertility alleles. There is no doubt that such an approach may also be successfully applied to genetic studies of the gametophytic incompatibility system of other cross-pollinating species.

Not only we have obtained additional proof for the linkage of genes *Prx7* and *β-Glu* with incompatibility loci but we have also found several new linkages of that type: *Pgi2* with *S1*, *Est4* and *Est11* with *S2*, and *Est5-Est7* with *S5*. The genes *Est4* and *Est11* are known to be completely linked as are the *Est5-Est7* genes (Wehling et al. 1985; Wehling 1986). Therefore, the real value of recombination between *S2* and *Est4/11* is 17.5–20.0% and between *S5* and *Est5/6/7* is 28.8–36.0%. These differences in the evaluation of recombination frequencies may be related to the different fractions of the F_2 progenies used for calculating recombination.

The lines of rye with established genotypes for their *S1*, *S2* and *S5* genes may be used in further studies for testing the genotypes of other lines.

There are also some suggestions for using crosses with inbred lines in order to improve traditional procedures of rye breeding (Smirnov and Voylokov 1990). The use of lines similar to lines 5 and 8, which carry a self-fertility allele of the *S1* locus on chromosome 1R, is probably the most convenient for this purpose because the almost complete linkage of the *Prx7* gene with *S1* facilitates the tracing of the *S1f* allele by means of studying the segregation for the *Prx7* gene.

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