Theor Appl Genet (1985) 71:289–291

Linkage between an incompatibility locus and a peroxidase isozyme locus (*Prx 7*) in rye

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Accepted March 15, 1985
Communicated by H. F. Linskens

Summary. Under controlled growth chamber conditions of 30 °C, seed set after selfing is possible in normally self-incompatible rye plants. Within selfed progenies produced by this method, plants homozygous at the peroxidase isozyme locus *Prx 7* were crossed to heterozygous individuals. Segregation at the *Prx 7* locus in progenies of these crosses provides clear evidence of a close linkage between *Prx 7* and one of the two incompatibility loci in rye. A recombination fraction in the range of 0–2% was calculated from the segregation data. In rye, *Prx 7* is linked with a phosphoglucoisomerase locus (*Pgi*). The similarity between the observations in *Secale cereale* and those made in *Lolium perenne* is discussed.

Key words: *Secale cereale* L. – Incompatibility loci – Peroxidase locus – Linkage – Isoelectric focusing

Introduction

In rye the highly efficient system of incompatibility depends on two loci, *S* and *Z*. The incompatibility specifities are formed by some kind of complementary interaction between the *S* and *Z* locus and each pair of an *S* and *Z* allele forms a unique specifity (Lundqvist 1956). As the genes *S* and *Z* are functionally not distinguishable, a mapping of the incompatibility loci would enable us to distinguish between them. Such a distinction could be of importance for obtaining a deeper insight into the mechanism of two-factor incompatibility. The linkage to a convenient marker gene is, however, not only important from the theoretical point of view, but also for practical purposes, especially in connection with recent proposals to use the incompatibility mechanism for the production of hybrid varieties (Wricke 1984, 1985). In such a case, a knowledge of linkage relations to other genes could be valuable.

Materials and methods

Individual self-incompatible plants of the cultivars 'Dankowskie' and 'Animo', which were heterozygous at *Prx 7* and several other isozyme loci, were selfed in the growth chamber under a constant temperature of 30 °C during flowering time (Wricke 1978). Within each pseudocompatible selfed progeny, plants homozygous for one of the electrophoretically distinguishable *Prx 7* alleles (hom 1 or hom 2) were crossed to heterozygous individuals (het). In each cross a maximum number of two different alleles per incompatibility locus could be involved, e.g. S_1/S_2 and Z_3/Z_4, respectively. If the selfed parent was heterozygous at both incompatibility loci *S* and *Z*, then 9 different genotypes can be found among the selfed progeny. These can be combined in fertile hom × het pairwise crosses as shown in Table 1.

It is supposed that *Prx 7* (= *P* in Table 1) is linked closely to the incompatibility locus *Z*, with P_1 being linked to Z_3. *Prx 7* will then be expected to segregate with a large excess of heterozygous genotypes in crosses of type 1–5. In two cases (cross types 6 and 7) the expectation is about 1 : 2 (hom : het), and only in two crosses (types 8 and 9) is a 1 : 1 ratio (hom : het) expected. The cross types listed in Table 1 are the same for the linked alleles P_2 and Z_4.

If *P* and *Z* segregate independently, a 1 : 1 ratio is expected in all crosses. Thus, with the exception of the last two crosses, a close linkage of *Prx 7* to one of the incompatibility loci would clearly be discovered by the segregation of *Prx 7* in the progeny of hom × het crosses.

Reciprocal het × hom crosses will, if they yield any seed at all, exhibit a 1 : 1 segregation of *Prx 7* in all cases.

As a control the segregation of four different isozyme loci (*Est 1, Est 5, Est 8, Est 10;* Schmidt-Stohn and Wehling 1983; Wehling and Schmidt-Stohn 1984) unlinked to *Prx 7* was also examined. At least one of these four esterase loci segregated in every progeny. Since these control loci always segregated

according to expectation, only the *Prx 7* data are given in Table 2. Segregation ratios are pooled from several ears per plant. Families 7000 and 7003 originated in 'Dankowskie' individuals, 7008 was derived from an 'Animo' plant.

Peroxidase isoenzymes were separated by isoelectric focusing in polyacrylamide flat gels having a pH 3.5–10 gradient (LKB ampholytes). For a more detailed description of separation and staining techniques, see Wehling et al. 1985.

Table 1. Possible fertile crosses of homozygous × heterozygous *Prx 7* genotypes and the segregation of progeny in the case of linkage between *Prx 7* and one incompatibility locus

Type no.	Type of cross ♀ × ♂	Expected segregation of progeny under complete linkage; homozygous in *P*: heterozygous in *P*
1	$S_{11}Z_{33}P^{a}_{11} \times S_{11}Z_{34}P_{12}$	0:1
2	$S_{12}Z_{33}P_{11} \times S_{11}Z_{34}P_{12}$	0:1
3	$S_{12}Z_{33}P_{11} \times S_{12}Z_{34}P_{12}$	0:1
4	$S_{12}Z_{33}P_{11} \times S_{22}Z_{34}P_{12}$	0:1
5	$S_{22}Z_{33}P_{11} \times S_{22}Z_{34}P_{12}$	0:1
6	$S_{11}Z_{33}P_{11} \times S_{12}Z_{34}P_{12}$	1:2
7	$S_{22}Z_{33}P_{11} \times S_{12}Z_{34}P_{12}$	1:2
8	$S_{11}Z_{33}P_{11} \times S_{22}Z_{34}P_{12}$	1:1
9	$S_{22}Z_{33}P_{11} \times S_{11}Z_{34}P_{12}$	1:1

[a] *P* stands for the *Prx 7* locus

Results and discussion

In Table 2 the results of 22 crosses between heterozygous and homozygous peroxidase genotypes are given. Yate's correction for continuity was used for the calculation of χ^2-values. Progeny of the crosses ♀ 'homozygous' × ♂ 'heterozygous' can be divided into three classes. Most of the progeny in the first class (crosses no. 1, 3, 5, 7–11, 14, 15) show either zero or only few homozygous genotypes, indicating a strong linkage between *Prx 7* and one of the incompatibility loci. The crosses underlying these progenies can be ascribed to be of the types 1–5 in Table 1.

Crosses no. 12, 16, 20 and 22 in Table 2 yielded progenies segregating in a 1:2 ratio. Although the progeny of cross no. 22 exhibits segregation which is also non-significant for the 1:1 model the almost ideal 1:2 ratio of 9:19 suggests that cross 22, together with cross 12, 16 and 20, belongs to cross type no. 6 or 7 (Table 1).

The third group comprises crosses no. 18 and 21 in Table 2 which produced a progeny segregating at *Prx 7* in a 1:1 ratio, as is expected when crosses of the type no. 8 or 9 (Table 1) are performed.

As expected, all progenies of het × hom crosses (crosses 2, 4, 6, 13, 17 and 19) segregate in a 1:1 manner.

Table 2. Segregation at the *Prx 7* locus after crossing *Prx 7* homozygous and heterozygous plants

Cross no.	Cross parents ♀ × ♂	*Prx 7* genotype of parents	Segregation of progeny		
			Prx 7 hom:het	χ^2 (1:1)	χ^2 (1:2)
1	7000/22 × 7000/2	hom 2 × het	1:13	8.64*	–
2	7000/2 × 7000/22	het × hom 2	24:25	0.00 n.s.	–
3	7000/25 × 7000/27	hom 2 × het	0:71	69.01*	–
4	7000/27 × 7000/25	het × hom 2	9:10	0.00 n.s.	–
5	7000/30 × 7000/51	hom 2 × het	0:53	51.02*	–
6	7000/51 × 7000/30	het × hom 2	45:49	0.10 n.s.	–
7	7000/6 × 7000/2	hom 1 × het	0:42	40.02*	–
8	7000/35 × 7000/2	hom 1 × het	3:70	59.67*	–
9	7000/50 × 7000/53	hom 2 × het	0:49	47.02*	–
10	7000/29 × 7000/53	hom 2 × het	1:41	36.21*	–
11	7000/5 × 7000/12	hom 2 × het	1:49	44.18*	–
12	7003/17 × 7003/32	hom 2 × het	35:69	10.47*	0.001 n.s.
13	7003/32 × 7003/17	het × hom 2	11:10	0.00 n.s.	–
14	7003/14 × 7003/32	hom 2 × het	1:40	35.22*	–
15	7008/39 × 7008/15	hom 1 × het	2:34	26.69*	–
16	7008/39 × 7008/9	hom 1 × het	5:18	6.26*	0.92 n.s.
17	7008/9 × 7008/39	het × hom 1	13:18	0.52 n.s.	–
18	7008/34 × 7008/9	hom 1 × het	39:47	0.57 n.s.	5.06*
19	7008/9 × 7008/34	het × hom 1	7:12	0.84 n.s.	–
20	7008/14 × 7008/21	hom 1 × het	12:25	3.89*	0.003 n.s.
21	7008/19 × 7008/4	hom 1 × het	48:64	2.01 n.s.	4.15*
22	7003/46 × 7003/7	hom 1 × het	9:19	2.89 n.s.	0.004 n.s.

* Significant for $\alpha = 0.05$

It should be mentioned, that the reciprocal crosses 1–2, 3–4, 5–6, 12–13, 16–17, and 18–19 show the expected differences. Thus, the results of all crosses (Table 2) confirm a close linkage between *Prx 7* and one locus of the incompatibility system.

From the progenies of crosses no. 1, 3, 5, 7–11, 14 and 15 a direct estimate for the recombination value can be obtained. If we take the sum of all these crosses we get 9 homozygous : 462 heterozygous, yielding a recombination value of p = 1.9%.

Another estimate of recombination values can be obtained by crosses 12, 16, 20 and 22. In these cases, a segregation of 1 homozygous : 2 heterozygous is expected with absolutely linked loci. Recombination would lead to more than one-third homozygous plants. Altogether these progenies give only 31.8% homozygous plants. The estimate for the recombination value p in this case is therefore zero. We may conclude, therefore, that the true value of p lies somewhere between 1.9% and zero.

Analysis of wheat/rye addition lines allowed us to locate the *Prx 7* locus on chromosome 1R in 'King II' rye (Wehling et al. 1985). Thus, the incompatibility locus linked with *Prx 7* must also be located on 1R. Linkage analysis performed at our institute indicated linkage between *Prx 7* and a locus coding for phosphoglucoisomerase (PGI) in rye (results unpublished). The *Pgi* locus was located on chromosome 1R in 'King II' rye by Chojecki and Gale (1982).

In *Lolium perenne,* Cornish et al. (1980) found linkage between a *Pgi* locus and one of the incompatibility loci which they defined as the *S* locus. Regarding the close evolutionary relationship between *Lolium* and *Secale* one could imagine a situation in rye similar to that in *Lolium.* However, since linkage between *Prx 7* and *Pgi* in rye is relatively loose we cannot yet decide whether *Prx 7* is linked to the *S* or *Z* locus in rye. Additional cross experiments involving the *Pgi* and *Prx 7* locus are necessary to answer this question.

The data obtained with the *Prx 7* isozyme locus reveal the importance of mapping the incompatibility loci. All loci linked to one of the two incompatibility loci in rye may show deviations from the expected segregational ratios in certain crosses which can lead to misleading interpretations.

Acknowledgement. We thank the Deutsche Forschungsgemeinschaft for its financial support.

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