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The Inheritance of Rye Seed Peroxidases

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Summary. Genetic analyses were conducted on peroxidase of the embryo and endosperm of seeds of one open pollinated and six inbred lines of cultivated rye (*Secale cereale* L.), and one line of *Secale vavilovii* Grossh. The analyses of the individual parts of the *S. cereale* seed yield a total of 14 peroxidase isozymes. Isozymes m, a, b, c, d, e, f and g (in order from faster to slower migration) were found in the embryo plus scutellum, while isozymes 1, 2, 3, 4, 5 and 6 (also from faster to slower migration) were peculiar of the endosperm. *S. vavilovii* has isozymes m, c_1, d, e, f and g in its embryo plus scutellum, and isozyme 2 in the endosperm. Segregation data indicated that at least 13 different loci would be controlling the peroxidase of *S. cereale*. Isozymes a and b are controlled by alleles of the same locus, all the other loci have one active and dominant allele coding for one isozyme, and other null and recessive allele. The estimation of linkage relationships shows that five endosperm loci are linked, and tentative maps are shown. A possible dosage effect and the existence of controlling gene(s) for endosperm isozyme 4 is reported. All these data and the high frequency of null alleles found are discussed in relation to recent reports.

Key words: *Secale cereale* – *S. vavilovii* – Rye – Peroxidases – Linkage

Introduction

Rye (*Secale cereale* L.) is one of the best known species of higher plants from the cytogenetical point of view. After the classic review by Jean (1960) numerous papers dealing with B-chromosomes, reciprocal translocations, c-banding, etc. in rye have been published. However, its genetics is poorly developed in comparison with other crop plants, especially other cereals such as barley, maize and wheat. The interest in rye is now increasing since a greater knowledge of this species might contribute to the breeding of superior triticales.

Recently, Tanksley and Rick (1980) stated that a classic approach to Genetics, such as linkage mapping in flowering plants, continues to have a basic significance, and can facilitate research in many other areas. Electrophoretic techniques have revealed a large number of genetics markers. The codominant nature of isozyme markers (as it will be shown later, this statement is not true for peroxidases), the absence of epistatic interactions, and the unlimited number of markers which can be incorporated into a single stock makes marker-analysis a valuable technique.

The peroxidase system is one of the most utilized isozymatic systems, because of its great variability among higher plants, the general high number of isozymes present, and the good results reached with relatively easy and non-expensive staining techniques. Peroxidases are usually characterized by a monogenic control, by monomeric enzyme behaviour, and by the presence of null alleles (Benito et al. 1980; Brown and Allard 1969; Clegg and Allard 1973; Felder 1976; Hoess et al. 1974; Marshall and Allard 1969; Rick et al. 1974; Smith 1972; Yen and Sadanaga 1977). A modified gene has been postulated by Rick et al. (1979) in the peroxidase system of *Lycopersicon pimpinellifolium*, and controlling genes were proposed by Shahi et al. (1969) in *Oryza*.

The purpose of the present paper is to analyze the genetic control and the linkage relationships of the peroxidase isozymes of two seed components, embryo plus scutellum, and endosperm. To date, no linkage maps for isozyme markers have been reported in rye; this article is the first attempt to construct linkage maps in this species. The results are compared with recent wheat seed peroxidase linkage maps reported by Benito et al. (1980).

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Materials and Methods

The materials used in the present work were: 12 F_2-like progenies obtained after self-pollination of heterozygous plants of the open pollinated rye line JNK, and 5 F_2-like progenies of different heterozygous plants of the inbred line P, and F_2 of the inbred lines E, P, R and V of rye (*Secale cereale* L.). All the inbred lines had, at least, 16 or more generations of selfing. Seeds of *S. vavilovii* Grossh. were used as control.

The seeds were excised into two different parts: embryo plus scutellum, and endosperm. Each individual part was crushed, and the enzymes were extracted by adding 5 µl (embryos) and 40 µl (endosperms) of sodium acetate 0.1 M, pH 7.2, during 1 h 30 min at 0 °C. After that, paper wicks (Whatman 3M) were soaked with the crude extract and then electrophoresed in 2 mm thick polyacrylamide slab gels. The migration was to the cathode. Electrophoretic and staining techniques used have been previously described (Kobrehel and Feillet 1975; Benito and Pérez de la Vega 1979).

Isozyme nomenclature is as follows: endosperm peroxidase isozymes have been numbered 1 to 6 from faster to slower migration. Embryo plus scutellum isozymes have been named a to g also from faster to slower migration. (*S. vavilovii* and JNK showed a faster isozyme, m).

For the estimation of linkage, the progenies with fewer than 30 individuals were not utilized because of their low number. Distance between loci was estimated using maximum likelihood equations developed by Allard (1956).

Results

Embryo plus scutellum showed both anodic and cathodic peroxidases, but since anodic peroxidases were very faint, only cathodic peroxidase isozymes have been utilized in the present work. Figure 1 shows the "theoretical" cathodic peroxidase patterns with all the isozymes observed in every rye line used in this study.

Firstly, it is worth noting the relative high number of heterozygous plants for endosperm peroxidase in the inbred line Pool (P) after 16 generations of selfing (bagging individual spikes); moreover, some of these plants were heterozygous for three and even four loci (Table 1). For the present work only multisegregating plants were chosen.

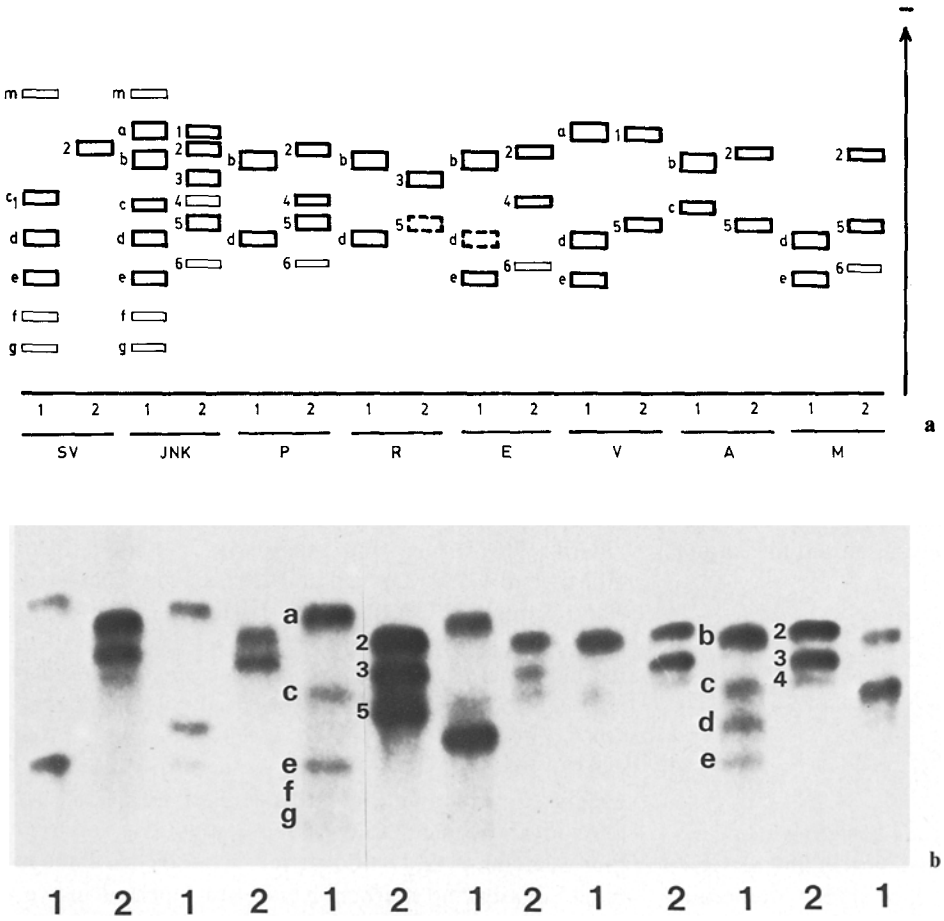

Fig. 1 a and b. **a** Theoretical cathodic peroxidase patterns with all the isozymes observed in every rye line used in this study. *1* embryo plus scutellum, *2* endosperm. (SV) *Secale vavilovii*; (JNK) open pollinated line JNK; (P, R, E, V, A and M) inbred lines Pool, Riodeva, Elymoides, Villarobledo, Anatolicum and Montanum respectively. **b** Different peroxidase patterns of the open pollinated rye line JNK. *1* embryo plus scutellum, *2* endosperm

Table 1. Single locus segregation of rye seed peroxidases

Iso-zyme	Plant	+	−	X^2 3:1	X^2 1:3	Plant	+	−	X^2 3:1	X^2 1:1	X^2 9:7
1	JNK-26	82	18	2.61		E × V-1	40	12	0.10		
2	JNK-25	19	8	0.31		Pool-6	109	36	0.01		
	JNK-26	78	22	0.48		E × R-1	31	10	0.01		
	JNK-52	14	4	0.07		E × R-3	20	7	0.01		
	JNK-56	83	33	0.74		E × R-5	54	12	1.64		
	JNK-71	73	34	2.62		E × V-1	35	17	1.64		
	JNK-72	57	19	0.00		P × R-3	18	8	0.46		
	JNK-75	17	4	0.40		P × R-4	9	4	0.23		
	JNK-97	30	14	1.09		P × R-7	18	2	2.40		
	JNK-154	18	6	0.00		R × A-1	34	11	0.01		
		389	144	1.16			328	107	0.04		
3	JNK-26	79	21	0.85		E × R-1	28	13	0.98		
	JNK-52	14	4	0.07		E × R-3	16	11	3.57		
						E × R-5	43	23	3.41		
						P × R-3	19	7	0.05		
						P × R-4	8	5	1.26		
						P × R-7	13	7	1.07		
						R × A-1	32	13	0.36		
		93	25	0.91			159	79	8.52*		
4[a]	JNK-56	25	66	109.63***	0.30	Pool-2	75	52	17.22***	4.16*	0.41
	JNK-69	13	5	0.07	21.41***	Pool-6	112	33	0.38	43.04***	25.97***
	JNK-72	23	53	81.12***	1.12	Pool-7	87	63	23.12***	3.84	0.19
	JNK-75	9	12	11.57**	3.57	Pool-11	77	46	10.08**	7.81*	2.01
	JNK-154	18	6	0.00	32.00***	Pool-12	53	44	21.45***	0.83	0.10
						E × R-1	20	21	15.09**	0.02	0.95
						E × R-3	15	12	5.44*	0.33	0.05
						E × R-5	35	31	10.97***	0.24	0.28
						E × V-1	22	30	29.64***	1.23	4.11
						E × V-2	24	24	16.00***	0.00	0.76
						E × V-3	45	14	0.04	16.29***	9.61**
5	JNK-25	19	8	0.31		Pool-2	91	36	0.76		
	JNK-26	76	24	0.05		Pool-6	107	38	0.11		
	JNK-56	83	33	0.74		Pool-7	113	37	0.01		
	JNK-71	81	25	0.15		Pool-11	96	27	0.61		
						Pool-12	74	23	0.08		
						E × V-1	39	3	0.00		
						E × V-2	38	10	0.44		
						E × V-3	47	12	0.68		
		260	90	0.09			604	197	0.07		
6	JNK-154	17	7	0.22		Pool-2	88	39	2.21		
						Pool-6	103	42	1.21		
						Pool-7	112	38	0.01		
						Pool-11	89	34	0.46		
						Pool-12	71	26	0.17		
							463	179	2.28		
a	JNK-25	18	9	1.00		E × V-4	34	14	0.44		
	JNK-26	62	18	0.27							
	JNK-71	77	24	0.08							
		157	51	0.03							
b	JNK-25	23	4	1.49		E × V-4	32	16	1.77[a]		
	JNK-26	66	14	2.40		P × M-2	39	6	3.26		
	JNK-71	74	27	0.16							
		163	45	1.26							
c						R × A-1	43	11	0.62		

Table 1. (continued)

Isozyme	Plant	+	−	X^2 3:1	X^2 1:3	Plant	+	−	X^2 3:1	X^2 1:1	X^2 9:7
d	JNK-1	123	45	0.28		E × V-4	36	12	0.00		
	JNK-26	63	17	0.60		P × R-3	26	12	0.88		
	JNK-71	82	19	2.06		P × R-4	21	6	0.11		
	JNK-165	49	13	0.54		P × R-7	23	11	0.98		
						R × A-1	39	15	0.22		
		317	94	0.99			145	56	0.88		
e	JNK-1	117	51	2.57		P × M-2	30	15	1.67		
	JNK-56	70	31	1.57							
	JNK-71	79	22	0.56							
	JNK-97	37	7	1.94							
	JNK-154	19	5	0.22							
	JNK-165	45	17	0.19							
		367	133	0.68							
f	JNK-56	77	24	0.08							
	JNK-69	13	5	0.07							
	JNK-72	46	21	1.44							
		136	50	0.35							
g	JNK-69	13	5	0.07							
	JNK-72	46	21	1.44							
		59	26	1.42							

[a] Significant heterogeneity X^2

*, **, *** Significant X^2 at 0.05, 0.01 and 0.001 levels, respectively

The results of single locus segregation are shown in Table 1. Left and right columns correspond to the data observed for the JNK and inbred lines, respectively. The observed data on each plant for each isozyme, were tested for their fit to the expected 3:1 presence-absence segregation by means of the X^2 test. Except for endosperm isozyme 4, the observed data fitted the expected values. Several progenies had less than five expected individuals in their less frequent class, but they have been included in this study due to the good fit to the 3:1 segregation. When the heterogeneity X^2 test of the progenies segregating for each locus was not significant at 5% level, all the individual progeny data were grouped and tested for their fit to expected segregations. Only the total value of isozyme 3 for inbred lines had a significant value because all the progenies had a derivation to a higher number of plants without isozyme than expected. Endosperm isozyme 4 showed a very peculiar result (Table 1); the results for this isozyme are summarized in detail (Table 3).

Table 2 shows the results of two locus segregations. All the progenies, and their summation (since heterogeneity among them was not found) were tested for their fit to the independence hypothesie. If the X^2 was significant the linkage hypothesis was tested. When individuals without both isozymes were not observed the allelism hypothesis was tested; however, among all the possible pairs of isozymes that could be controlled

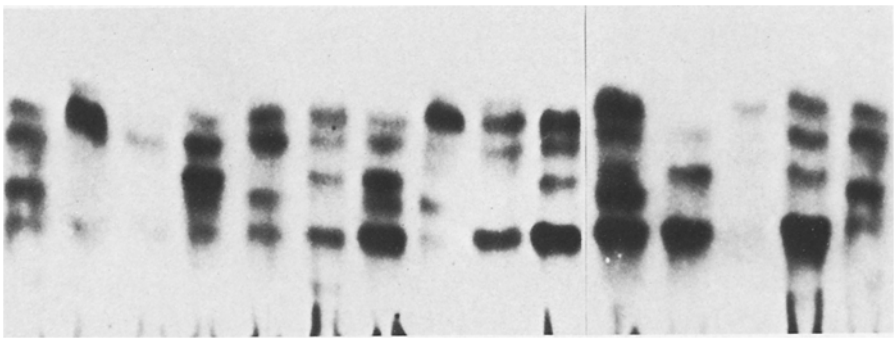

Fig. 2. F_2-like progenies obtained after self-pollination of an heterozygous plant of the open pollinated rye line JNK. Embryo plus scutellum peroxidase isozymes

Table 2. Two-locus segregation for the detection of linkage

Iso-zymes	Plant	++	+−	−+	−−	X^2 9:3:3:1	X^2 link-age	X^2 1:2:1	Dis-tance	Plant	++	+−	−+	−−	X^2 9:3:3:1	X^2 link-age	X^2 1:2:1	Dis-tance
1 – 2	JNK-26	60	22	18	0	7.09		4.32		E × V-1	28	12	7	5	2.29			
1 – 3	JNK-26	66	16	13	5	4.11												
1 – 5	JNK-26	65	17	11	7	4.82				E × V-1	30	10	9	3	0.10			
2 – 3	JNK-26	64	14	15	7	3.11				E × R-1	18	13	10	0	8.03*			
										E × R-5	31	23	12	0	14.27**			
										R × A-1	21	13	11	0	6.79			
											70	49	33	0	27.77***	22.65***	4.31	1.00 ± 8.11
2 – 5	JNK-26	55	23	21	0	5.67				Pool-6	71	38	36	0	17.59***			
	JNK-56	50	33	33	0	22.45***				E × V-1	22	13	17	0	11.52*			
	JNK-71	48	25	34	0	20.05***												
		153	81	88	1	42.09***	41.22***		10.35 ± 5.49		93	51	53	0	27.51***	27.05***	0.65	1.00 ± 7.12
2 – 6										Pool-6	67	42	36	0	22.50***	21.08***	1.33	1.00 ± 8.30
3 – 5	JNK-26	55	24	21	0	8.02*	7.12**		1.00 ± 10.00									
5 – 6										Pool-2	88	3	0	36	145.00***			
										Pool-6	103	4	0	38	65.90***			
										Pool-7	112	1	0	37	144.73***			
										Pool-11	89	7	0	27	88.40***			
										Pool-12	71	3	0	23	83.18***			
											463	18	0	161	600.31***	597.47***		2.79 ± 0.69
a – b	JNK-26	48	14	18	0	5.87		3.60		E × V-4	18	16	14	0	14.22***	12.01***	3.16	
	JNK-71	50	27	24	0	11.91**	11.67**	0.19										
		98	41	42	0	14.83**	13.99***	1.25										
a – d	JNK-26	49	13	14	4	0.89				E × V-4	29	5	7	7	7.70			
	JNK-71	64	13	18	6	2.83												
		113	26	32	10	3.35												
a – e	JNK-71	61	16	18	6	0.83												
b – d	JNK-26	55	11	8	6	6.75				E × V-4	23	9	13	3	2.37			
	JNK-71	60	14	22	5	2.23												
		115	25	30	11	4,53												
b – e	JNK-71	61	13	18	9	3.36				P × M-2	27	12	3	3	5.13			
d – e	JNK-1	86	37	31	14	2.89												
	JNK-71	67	15	12	7	5.26												
	JNK-165	36	13	9	4	0.79												
		189	65	52	25	2.71												
e – f	JNK-56	53	17	24	7	1.88												
f – g	JNK-72	42	4	4	17	51.37***	48.49***		11.53 ± 4.22									
1 – a	JNK-26	53	14	8	4	5.01												
1 – b	JNK-26	55	12	10	2	6.36												

Table 2. (continued)

Isozymes	Plant	++	+−	−+	−−	X^2 9:3:3:1	X^2 linkage	X^2 1:2:1	Distance	Plant	++	+−	−+	−−	X^2 9:3:3:1	X^2 linkage	X^2 1:2:1	Distance
1 – d	JNK-26	52	15	10	2	4.60												
2 – a	JNK-26	46	13	15	5	0.28												
	JNK-71	51	16	26	8	4.13												
		97	29	41	13	2.54												
2 – b	JNK-26	48	11	17	3	2.35												
	JNK-71	52	15	22	12	6.85												
		100	26	39	15	3.86												
2 – c										R × A-1	27	7	8	2	0.61			
2 – d	JNK-26	44	15	18	2	2.44				R × A-1	25	9	6	4	1.25			
	JNK-71	53	14	29	5	7.16												
		97	29	47	7	7.65												
2 – e	JNK-56	48	22	21	9	3.25												
	JNK-71	52	15	27	7	4.73												
	JNK-97	27	3	10	4	4.48												
		127	40	58	20	6.22												
2 – f	JNK-56	57	13	19	11	5.39												
	JNK-72	32	16	13	2	2.89												
		89	29	32	13	1.00												
2 – g	JNK-72	33	15	12	3	1.25												
3 – a	JNK-26	49	14	12	4	1.22												
3 – b	JNK-26	51	21	14	2	3.29												
3 – c										R × A-1	23	8	12	1	2.95			
3 – d	JNK-26	49	14	13	3	1.49				R × A-1	21	10	10	3	1.35			
5 – a	JNK-26	47	14	14	4	0.41												
	JNK-71	62	18	15	6	1.35												
		109	32	29	10	1.49												
5 – b	JNK-26	50	11	15	3	2.44												
	JNK-71	57	23	13	4	1.92												
		107	34	28	7	2.89												
5 – d	JNK-26	48	13	14	4	0.73												
	JNK-71	69	11	13	8	8.25*												
		117	24	27	12	6.69												
5 – e	JNK-56	48	21	21	10	4.00												
	JNK-71	64	16	15	6	2.20												
		112	37	36	16	1.04												
5 – f	JNK-56	51	18	25	6	2.61												

*, **, *** Significant X^2 at 0.05, 0.01 and 0.001 levels, respectively

Table 3. Endosperm isozyme 4 segregation

Plant	+	−	X^2 3:1	X^2 1:1	X^2 9:7	X^2 1:3
Pool-2	75	52	17.22***	4.16*	0.41	
Pool-7	87	63	23.12***	3.84*	0.19	
Pool-11	77	46	10.08***	7.81*	2.01	
Pool-12	53	44	21.45***	0.83	0.10	
E × R-1	20	21	15.09***	0.02	0.95	
E × R-3	15	12	5.44*	0.33	0.05	
E × R-5	35	31	10.97***	0.24	0.28	
E × V-1	22	30	29.64***	1.23	4.11	
E × V-2	24	24	16.00***	0.00	0.74	
	408	323	143.51***	9.88**	0.06	370.25***
Pool-6	112	33	0.38	43.04***	25.97***	
E × V-3	45	14	0.04	16.29***	9.61**	
	157	47	0.42	59.31***	35.56***	293.75***
JNK-56	25	66	109.63***	18.47***		0.30
JNK-72	23	53	81.12***	11.84***		1.12
JNK-75	9	12	11.57***	0.43		3.57
	57	131	200.17***	29.13***	51.37***	2.84
JNK-69	13	5	0.07	3.56		21.41***
JNK-154	18	6	0.00	6.00*		32.00***
	31	11	0.03	9.52**	5.26*	53.36***

*, **, *** Significant X^2 at 0.05, 0.01 and 0.001 levels

by alleles of the same locus, only isozymes a and b are alleles. Other combinations could be rejected because in some crosses one of the isozymes was segregating presence-absence while the other was always present. For instance, isozymes 2 and 6 cannot be alleles since in Pool 2, 7, 11 and 12 progenies, isozyme 2 is always present but isozyme 6 is segregating. The same is true for isozymes 3 and 5 in JNK-56. When the double recessive individuals were not observed the arbitrary distance (theoretical distance 0 Morgans) of 1 Morgan and its standard error (Allard 1956) was accepted. The allelic condition of a and b is also supported by the fact that among 200 embryos of the JNK population of *S. cereale* none of them simultaneously lacked both isozymes.

Endosperm isozyme 4 segregations are showed in Table 3. Some progenies of both open-pollinated and inbred lines fitted a 3:1 presence-absence segregation, three progenies of the open-pollinated JNK line fitted a 1:3 presence-absence segregation, and a group from inbred plants showed a 9:7 and 1:1 segregation in most of their progenies, in spite of which the total value had a significant X^2 value for the 1:1 segregation. None of the plants in this study fitted the epistatic 13:3 segregation. All the groups in Table 3 had a non-significant homogeneity X^2 value. Due to this particular behaviour of isozyme 4, we have calculated separately the linkage relationships between this isozyme and each other isozymes (Table 4). The independence hypotheses tested were 3:3:1:1, 3:9:1:3, 9:3:3:1 and 27:21:9:7 (not shown in Table 4) when the isozyme 4 segregated 1:1, 1:3, 3:1 and 9:7 respectively. Isozyme 4 was shown to be linked to all the other endosperm isozymes, except isozyme 1, and to be independent of embryo isozymes. Pool 2, 7 and 11 plants were tested for the independence hypothesis for both the epistatic 9:7 (see Table 3) and the 1:1 segregation, in spite of the significant X^2 value for this latter segregation; in both cases the independence hypothesis could be rejected because of the highly significant X^2 values obtained (between 19.74 and 43.23, $P < 0.001$ for 27:21:9:7; and between 24.49 and 52.80, $P < 0.001$ for 3:3:1:1). Since no proper linkage relationships could be established, because of either the epistasis or the significant X^2 value of the single locus segregation, the data have been not included in Table 4.

Therefore, the results obtained for rye seed peroxidase isozymes can be summarized as follows: i) Except embryo isozymes a and b, which are controlled by active alleles of the same locus, all the peroxidase loci have one active and dominant allele coding for one isozyme and one null and recessive allele. ii) Except the locus for isozyme 1, all the loci controlling endosperm peroxidases seem to be linked. iii) Embryo plus scutellum peroxidase loci behave independently among themselves. iv) Endosperm loci behave independently in relation to embryo plus scutellum loci.

Table 4. Two-locus segregation for detection of linkage between isozyme 4 and other isozymes

Isozymes	Plant					X^2 indepen.	X^2 linkage	Distance
		3++	3+−	1−+	1−−			
1−4	E×V-1	19	21	3	9	2.97		
		3++	3+−	1−+	1−−			
2−4	E×V-1	22	13	0	17	25.95***	23.08***	
	E×R-1	19	12	1	9	7.85*	7.82**	
	E×R-5	35	19	0	12	15.53**	13.66***	
		76	44	1	38	44.92***	44.74***	2.94 ± 2.65
		9++	3+−	3−+	1−−			
	Pool-6	99	10	13	23	43.40***	43.01***	17.77 ± 3.59
		3++	9+−	1−+	3−−			
	JNK-56	24	41	0	25	15.10**	14.50***	
	JNK-72	22	35	1	18	9.57*	8.45**	
		46	76	1	43	24.19***	22.35***	13.79 ± 7.58
		3++	3+−	1−+	1−−			
3−4	E×R-1	7	21	13	0	23.85***	22.84***	
	E×R-5	12	31	23	0	39.60***	35.95***	
		19	52	36	0	66.28***	61.94***	1.00 ± 1.91
		3++	3+−	1−+	1−−			
5−4	Pool-12	30	44	23	0	24.60***	24.42***	
	E×V-1	11	28	11	2	13.64**	12.41***	
	E×V-2	14	24	10	0	11.55**	11.11***	
		55	96	44	2	46.48***	47.18***	4.72 ± 3.00
		9++	3+−	3−+	1−−			
	[a]Pool-6	74	33	38	0	15.30**	14.80***	1.00 ± 8.30
	E×V-3	35	12	10	2	1.05		
		3++	9+−	1−+	3−−			
	JNK-56	3	59	22	7	65.50***	62.91***	10.84 ± 3.49
		3++	3+−	1−+	1−−			
6−4	Pool-12	27	44	26	0	31.20***	30.93***	1.00 ± 2.00
		9++	3+−	3−+	1−−			
	Pool-6	71	32	41	1	18.29***	16.70***	15.84 ± 8.00
		3++	9+−	1−+	3−−			
e−4	JNK-56	17	41	7	18	1.84		
f−4	JNK-56	21	46	3	13	3.25		
	JNK-72	13	32	4	14	0.86		
		34	78	7	27	2.31		
g−4	JNK-72	13	32	4	14	0.86		

[a] Significant heterogeneity X^2
*, **, *** Significant X^2 at 0.05, 0.01 and 0.001 levels

Discussion

Our results agree with the fact that peroxidases in almost all plants species are monogenic. We propose that at least 13 loci control the peroxidases of *Secale cereale* seeds. The following nomenclature is proposed to describe the genetic system of these cathodal peroxidases:

CPX-M		CPX-M^n?
CPX-A	CPX-A^b	CPX-A^n
CPX-C		CPX-C^n
CPX-D		CPX-D^n
CPX-E		CPX-E^n
CPX-F		CPX-F^n
CPX-G		CPX-G^n
CPX-1		CPX-1^n
CPX-2		CPX-2^n
CPX-3		CPX-3^n
CPX-4		CPX-4^n
CPX-5		CPX-5^n
CPX-6		CPX-6^n

In the above the alleles with the superscript n indicate the null alleles. CPX-M^n is the only null not demonstrated by segregation, but its existence is supposed because it is not present in inbred lines.

The linkage analyses showed that five loci of the endosperm (2–6) must be linked (Tables 2, 4). Recently Benito and Pérez de la Vega (1979) and Benito et al. (1980) reported that hexaploid wheat endosperm peroxidase loci are not linked, while some embryo peroxidase loci are linked. Peroxidase mapping was not definitive because i) different lines probably have different recombination frequencies, ii) only some progenies are segregating simultaneously for several of the above mentioned loci, and iii) some distances are fictitious because the error of estimation is very high. However, two tentative maps are show for endosperm linkage group, using the more suitable progenies (Fig. 3). The location of the chromosomes carrying peroxidase genes and the completion of linkage maps involving peroxidase and other enzymatic loci is now under way in our laboratory.

One of the most striking results observed is that almost all, if not all, the seed peroxidase loci have null alleles. Certainly, the objection that null alleles detected after electrophoresis could not be inactive alleles in vivo can be argued; but, while the presence of nulls has been reported repeatedly in peroxidases (Clegg and Allard 1973; Smit 1972, in *Avena*; Rick and Fobes 1975, in *Lycopersicon;* Pérez de la Vega 1980, in *Secale* leaves; Benito et al. 1980, in *Triticum*) no other isozymatic system shows this behaviour, except for esterases (Allard and Kahler 1971, in *Hordeum;* Selander and Yang 1969, in *Mus musculus*); so that Rick and Fobes (1975) stated, "A literature survey thus gives the impression that nulls tend to be infrequent in natural populations". Therefore, nulls seem to be peculiar to some isozymatic systems, not the result of experimental manipulation. Our own experience with seed peroxidases from wheat and rye indicates that peroxidases are relatively thermostable enzymes and that they resist the delipidization of seeds, previous to their extraction, showing activity after electrophoresis (unpublished). Benito et al. (1980) reported that among several cultivars of hexaploid wheat each cultivar was homozygous for null alleles in 3 or 4 different seed peroxidase loci. They stated that this abundance of nulls could be due to homoeology, because several loci could be fixed for null while other homoeologous isozymes, with the same or similar function, are coded by alleles in homoeologous loci. However, this statement is not valid for rye because this pecies is not an allopoliploid. Therefore, the high number of nulls for peroxidases could be related to the inspecificity of peroxidases (Liu 1975; Saunders et al. 1964). If any isozyme could carry out most, if not all, the metabolic steps where a peroxidase is needed, then, one or several loci could be fixed for null alleles without lethality (an indirect evidence of this is that *S. vavilovii* has only one isozyme in its endosperm). The fact that the other reported isozymatic system similar to the rye seed peroxidase system is the esterase system in *Hordeum* (Allard and Kahler 1971) supports the last statement, since the inspecificity of esterases has also been argued.

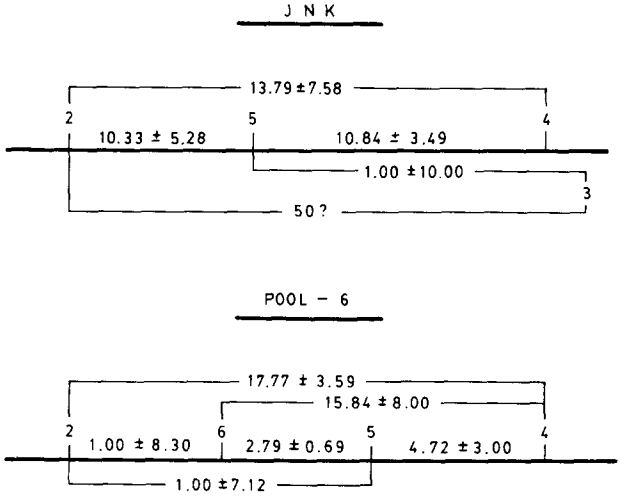

Fig. 3. Tentative linkage maps for endosperm peroxidase isozymes

Another result which needs a separate discussion is the peculiar mode of segregation of endosperm peroxidase 4, because some progenies of both open-pollinated JNK line and inbred lines fitted the expected 3:1 presence-absence segregation, but other progenies were significantly different from this segregation, fitting a 1:3, 9:7 and/or 1:1 segregation. The 9:7 segregation could be due to an epistatic effect, where a recessive gene prevents the expression of isozyme 4 (Table 5); the 1:3 segregation could be due to another epistatic effect, where a dominant gene prevents the expression of isozyme 4 (Table 5). Therefore three different loci should be postulated in relation to isozyme 4, one structural locus and two controlling loci. Another alternative hypothesis that could explain these results is a dosage effect, since the endosperm is a triploid tissue (Table 5). In JNK plants segregating 1:3 only endosperm CPX-4 CPX-4 CPX-4 would show the presence of isozyme 4. In the F_2 from inbred lines with a 1:1 segregation, endosperms CPX-4 CPX-4 CPX-4 and CPX-4 CPX-4 CPX-4^n would show the isozyme (Table 5). This last hypothesis is supported by two experimental results. i) Isozyme 4 is always fainter in JNK than in inbred lines. This could be because three of the active alleles are needed in JNK, and only two in inbred lines. ii) When the inbred lines ♀ E×R ♂ are crossed (CPX-4 CPX-4×CPX-4^n CPX-4^n) the F_1 endo-

Table 5. Tentative hypotheses of the endosperm isozyme 4 segregation

Dosage hypothesis to explain the 1:1 and 1:3 segregations		
Spermatic cell	Secondary nucleus	
	CPX-4 CPX-4	CPX-4^n CPX-4^n
CPX-4	CPX-4 CPX-4 CPX-4	CPX-4 CPX-4^n CPX-4^n
CPX-4^n	CPX-4 CPX-4 CPX-4^n	CPX-4^n CPX-4^n CPX-4^n

Epistatic hypothesis to explain 9:7 (right) and 1:3 (left) segregations				
H_1 h_1 CPX-4 CPX-4^n Phenotypes		Parental genotypes selfing	H_2 h_2 CPX-4 CPX-4	
H_1 CPX-4 H_1 CPX-4^n	9	Isozyme 4 present	h_2 CPX-4	1
h_1 CPX-4 h_1 CPX-4^n	7	Isozyme 4 absent	H_2 CPX-4	3

H_1, h_1 and H_2, h_2 represent hypothetical genes controlling isozyme 4 expression

sperm (CPX-4 CPX-4 CPX-4^n) shows isozyme 4, while when ♀ R×E ♂ are crossed the F_1 endosperm (CPX-4 CPX-4^n CPX-4^n) does not show the isozyme 4 (Fig. 4). If the dosage hypothesis is true, our interpretation of the 9:7 segregation is that in exceptional gels and/or endosperm it is possible to detect the isozyme 4 of CPX-4 CPX-4^n CPX-4^n genotypes, thus causing the deviation of segregation from the expected 1:1, both in individual plants and in the total. Since in JNK and inbred lines some progenies segregated 3:1 the existence of a gene controlling the dosage effect could be postulated. A third hypothesis would be to include the dosage effect and the epistatic effect. Therefore, any stated hypothesis explaining isozyme 4 segregation postulates the existence of controlling gene(s) (Rieger et al. 1976) governing the expression of this isozyme, in addition to the structural gene. The real existence of such gene(s) and the discrimination between the epistatic and dosage hypotheses needs further experimental work. The possibility of genes regulating the expression of peroxidases have previously been reported by Pai et al. (1973) and Shahi et al. (1969) in *Oryza,* and Gale and Spencer (1974, 1977) and May et al. (1973) in *Triticum.* Our results are very similar to the results reported in *Oryza* by Shahi et al. (1969) who also found that a peroxidase isozyme segregated 3:1, 1:1, 3:5 and 1:3 (or 3:13). They attribute these results to an epistatic effect with a controlling gene.

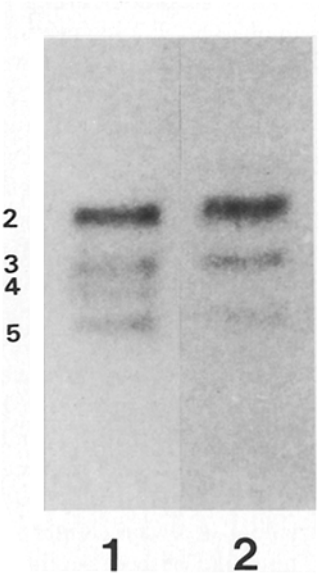

Fig. 4. Endosperm peroxidase isozymes. *1* F_1 endosperm ♀ E×R ♂, isozyme 4 present; *2* F_1 endosperm ♀ R×E ♂, isozyme 4 absent

Rye seed peroxidases fit the general features of higher plant peroxidases i.e., monomeric enzymes under monogenic control, presence of null alleles, and the possible existence of controlling genes.

Although peroxidases do not fulfill all the advantageous characteristics of typical isozymatic markers as listed by Tanksley and Rick (1980), they are suitable markers for genetics studies, their principal advantage being their high variability among plants and lines. The future development of more and complete linkage maps for isozymatic genes would provide a useful tool for further genetic studies, especially for the study of chromosomal mutations affecting linkage groups such as translocations.

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