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Genetics of rye phosphatases: evidence of a duplication

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Summary. Genetic analyses were conducted on alkaline phosphatases of the endosperm of dry kernels and leaf acid phosphatases in four open pollinated and one inbred line of cultivated rye (*Secale cereale* L.). A total of seven alkaline phosphatase isozymes were observed occurring at variable frequencies in the different cultivars analyzed. We propose that at least five loci control the alkaline phosphatases of rye endosperm – *Alph-1, Alph-2, Alph-3, Alph-4* and *Alph-5* – all of which have monomeric behaviour. The leaf acid phosphatases are controlled by one locus and have a dimeric quaternary structure. All loci coding for alkaline phosphatase isozymes showed one active, dominant allele and one null, recessive allele, except for the locus *Alph-3* which showed two active, dominant alleles and one null, recessive one. The linkage analyses suggest the existence of two linkage groups for alkaline phosphatases: one of them would contain *Alph-2, Alph-4, Alph-5* and the locus/loci coding isozymes 6 and 7. This linkage group is located in the 7RS chromosome arm. The other group would include *Alph-1* and *Alph-3* loci, being located in the 1RL chromosome arm. Leaf acid phosphatases have been previously located in the 7RL chromosome arm. Our data also support an independent relationship between loci controlling the endosperm alkaline phosphatases and leaf acid phosphatases.

Key words: *Secale cereale* L. – Phosphatases – Genetic control – Chromosomal location – Linkage maps

Introduction

The genetics of rye is poorly developed in comparison with other crop plants, especially such other cereals as maize, barley and wheat.

Recently, several isozyme markers have been located in rye chromosome arms (Tang and Hart 1975; Hart 1979; Chojecki and Gale 1982; Rao and Rao 1980; Bergman and Maan 1973; Salinas and Benito 1984a, b, 1985a, b).

However, linkage maps for isozyme markers have only been reported in rye for esterases (Wehling and Schmidt-Stohn 1984; Wehling et al. 1985), peroxidases (García et al. 1982), 6-phosphogluconate dehydrogenase and glucose phosphate isomerase (Lawrence and Appels 1986), malate dehydrogenase and glutamate oxaloacetate transaminase (Figueiras et al. 1985) and malate dehydrogenase and 6-phosphogluconate dehydrogenase (Figueiras et al. 1985).

The purpose of the present paper is to analyze the genetic control, chromosomal location and the linkage relationships of the phosphatase isozymes of the endosperms and leaves in rye.

Material and methods

The materials used in this work were the following. i) *Triticum aestivum* L. cvs. 'Chinese Spring' (CS) and 'Holdfast' (H) and *Secale cereale* L. cv. 'King II' (KII) and H-KII ditelocentric addition lines, excluding 3RL, 3RS and 2RS, obtained from T. E. Miller (Cambridge). ii) Four open pollinated rye cultivars named 'JNK' (J) from Japan; 'Ailés' (A) from Zaragoza (Spain); 'Elbon' (E) from USDA (U.S.A.) and 'Palencia' (P) from Palencia (Spain). One inbred line of cultivated rye named 'Riodeva' (R) with 20 generations of selfing. iii) Fourteen F2-like progenies obtained after selfpollination of heterozygous plants of the open pollinated rye lines 'JNK' (6), P (1), A (2) and E (5). One F2 of the open pollinated rye lines A and E. iv) Sixteen crosses between different plants of rye A (A×A), 5 crosses between different plants of rye cultivar A and inbred line R (A×R).

The analyses for alkaline phosphatases were carried out with half-endosperms. Each sample (half-endosperm) was crushed and placed in a vial. Sixty µl of 0.1 M sodium acetate, pH 7.2, was added to each sample. After 90 min at 0 °C small pieces (0.5 cm) of filter paper were soaked in the liquid and then inserted into 8% polyacrylamide gels. The other parts of the seed (embryo and scutellum plus half-endosperm) were

allowed to germinate and the biochemical analyses for leaf acid phosphatases were carried out with 12-day-old leaves in 12% starch gels.

Both alkaline phosphatases of the endosperm of dry kernels and leaf acid phosphatases were studied using histidine 0.006 M, pH 7.0 as the gel buffer and Tris-citric acid (0.43 M, pH 7.0) as the electrode buffer. The staining methods used were those described by Shaw and Koen (1968).

Endosperms of 'Chinese Spring' were used as the control in all the gels.

Results

The analyses of alkaline phosphatases of the endosperm of four open pollinated rye cultivars have revealed the existence of seven different isozymes (Fig. 1 A). A high number of different alkaline phosphatase patterns were observed and it was possible to find patterns in individuals that ranged from 0 to 5 isozymes (Fig. 1 A).

The frequency of the different isozymes observed in the four rye cultivars are shown in Table 1. Isozymes 2 and 5 are present in all cultivars in a high frequency, while isozymes 1 and 3 show a low frequency. The study of the leaf acid phosphatases revealed the existence of three different patterns in all the rye cultivars (Fig. 1 G).

The results of single locus segregation for leaf acid phosphatases (Table 2) indicated that they are controlled by one locus with two active alleles and have a dimeric behaviour.

The results of single locus segregation for endosperm alkaline phosphatases (Table 1) showed that all

Table 1. Frequency of the different isozymes in the four rye cultivars analyzed

Cultivar	Total plants	No. and %[a] of plants showing each isozyme							Total isozymes
		1	2	3	4	5	6	7	
'JNK'	104	0 (0.00)	73 (70.19)	4 (3.85)	3 (2.88)	100 (96.15)	4 (3.85)	49 (47.12)	233
'Elbon'	104	4 (3.85)	31 (29.81)	8 (7.69)	60 (57.69)	75 (72.12)	27 (25.96)	31 (29.81)	236
'Ailés'	100	1 (1.00)	61 (61.00)	12 (12.00)	27 (27.00)	91 (91.00)	19 (19.00)	28 (28.00)	239
'Palencia'	119	5 (4.20)	66 (55.46)	5 (4.20)	30 (25.21)	99 (83.19)	8 (6.72)	14 (11.76)	227
Total	423	10 (2.34)	231 (54.10)	29 (6.79)	120 (28.10)	365 (85.48)	58 (13.58)	122 (28.57)	935

[a] Each percentage ( ) was calculated as: $\frac{\text{no. of plants showing the isozyme}}{\text{total of plants}}$

Table 2. Single locus segregations of alkaline and acid phosphatases of rye endosperm

Alkaline isozymes	Plants analyzed	Parental phenotype	+	−	X^2	Segregation
1	63	1×−	28	35	0.78	1:1
2	791	2	601	190	0.40	3:1
2	425	2×−	200	225	1.47	1:1
2	109	2	100	9	0.74	15:1
2	237	2×−	87	147	0.01	3:5
3	210	3	158	52	0.01	3:1
3	613	3×−	298	315	0.47	1:1
4	559	4	417	142	0.05	3:1
4	427	4×−	208	219	0.28	1:1
5	1,057	5	810	247	1.50	3:1
5	93	5×−	47	46	0.01	1:1
5	105	−	33	72	2.31	1:3
5	63	−	6	57	3.51	3:13
			11	12	22	
Acid phosphatase	308	11×12	153	155	0.01	1:1
	81	12×12	18	41	22	0.41	1:2:1

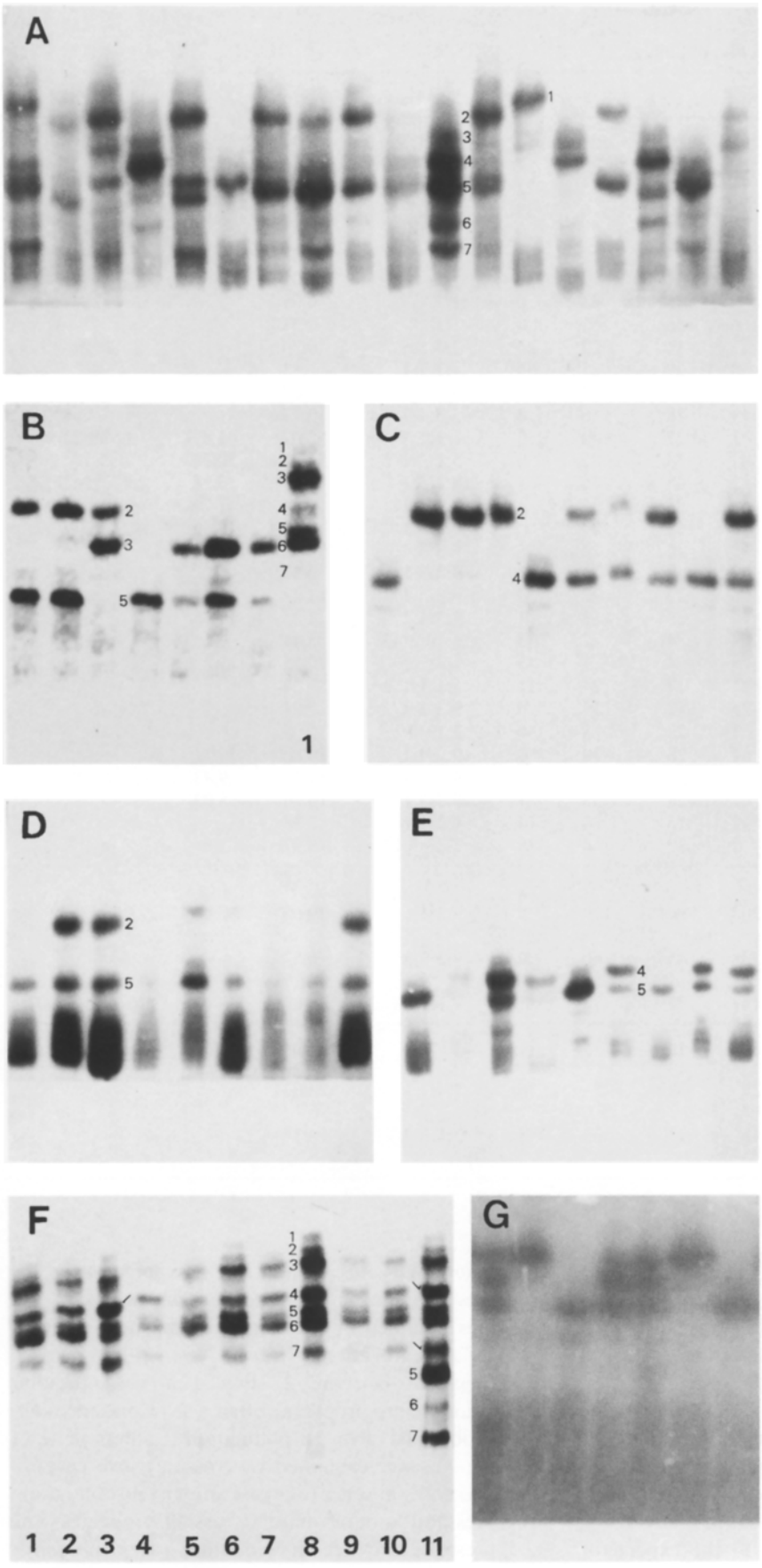

Fig. 1. **A** Different alkaline phosphatase patterns of the endosperms of rye cvs. 'Ailés' and 'King II'. **B** Segregating progeny for isozymes 2, 3 and 5. 1: CS. **C** Segregating progeny for isozymes 2 and 4. **D** Segregating progeny for isozymes 2 and 5. **E** Segregating progeny for isozymes 4 and 5. **F** Zymogram phenotypes of different ditelocentric wheat-rye addition lines 'Holdfast'–'King II': 1) 'Holdfast' or 'Chinese Spring', 2) 1RS, 3) 1RL, 4) 2RL, 5) 4RS, 6) 4RL, 7) 5RS, 8) 5RL, 1 . . . 7 indicate wheat isozymes, 9) 6RS, 10) 6RL, 11) 7RS, 5, 6 and 7 indicate rye isozymes. Arrows, in 8 and 11, indicate wheat isozymes showing greater relative staining intensities. **G** Different leaf acid phosphatase patterns of rye cultivars used in this study. From left to right: 12, 11, 22, 12, 12, 11, 22

Table 3. Two locus segregations for the detection of linkage

Isozyme	Plant	Phenotype	++	+−	−+	−−	Segregation	X^2 ind.	X^2 lin.	Distance
1–2	A×A-2	12× −−	8	20	20	15	1:1:1:1	6.14		
1–3	A×A-2	13× −−	0	28	28	7	1:1:1:1	38.90	37.34	11.11±3.95
1–4	A×A-2	14× −−	18	10	13	22	1:1:1:1	5.38		
1–5	A×A-2	1− × −−	0	28	6	29	3:13:3:13	6.58		
2–3	A1 ⊗	23	44	23	27	0	2:1:1	0.72		
	A×A-2	23× −−	15	13	13	22	1:1:1:1	3.48		
	A×A-3	23× −−	0	12	20	0	1:1	2.00		
	A×A-4	23× −−	0	25	23	0	1:1	0.08		
	A×A-5	23× −−	0	56	62	0	1:1	0.31		
	A×A-7		12	11	23	24	3:3:5:5	0.70		
	A×A-10	2− × −3	32	29	25	36	1:1:1:1	2.13		
2–4	A×E-1 ⊗	24× −−	68	20	24	9	9:3:3.1	0.67		
	A×A-1	24×2−	19	28	6	8	3:3:1:1	2.17		
	A×A-2	24× −−	1	27	30	5	1:1:1:1	42.07	41.87	9.52±3.69
	A×A-6	24× −4	41	38	85	0	3:1:3:1	51.25	50.74	
2–5	J2 ⊗	25	112	48	56	4	9:3:3:1	14.41	13.58	23.30±6.20
	J3 ⊗	25	68	33	24	1	9:3:3:1	9.84	7.79	19.80±8.50
	J4 ⊗	25	28	15	20	0	9:3:3:1	12.04	10.46	
	J6 ⊗	25	43	16	22	2	9:3:3:1	4.93		
	P1 ⊗	25	29	7	11	1	9:3:3.1	2.37		
	A×E-1 ⊗	25	66	22	27	6	9:3:3:1	1.22		
	A×A-1	25×2−	19	28	13	1	3:3:1:1	11.35	11.06	
	A×A-2	2− × −−	4	24	2	33	3:13:3:13	5.44		
	A×A-3	2− × −5	8	4	7	13	1:1:1:1	5.25		
3–4	A×A-2	34× −−	11	17	24	11	1:1:1:1:1	7.28		
3–5	E3 ⊗	35	25	12	15	0	9:3:3:1	7.21		
	A×A-2	3− × −−	1	27	5	30	3:13:3:13	5.05		
4–5	A×E-1 ⊗	45	64	28	29	0	9:3:3:1	10.80	10.51	
	E-1 ⊗	45	36	23	15	0	9:3:3:1	11.47	9.13	
	E-2 ⊗	45	47	26	33	1	9:3:3.1	10.76	8.03	
	A×A-1	−5×4−	12	15	20	14	1:1:1.1	2.28		
	A×A-2	4− × −−	2	29	4	28	3:13:3:13	3.87		
2–5	Total (J2 ⊗, J3 ⊗, J4⊗)		208	96	100	5	9:3:1	30.60	30.48	21.89±0.09
2–5	Total (J6 ⊗, P1 ⊗, A×E-1 ⊗)		138	45	60	9	9:3:3:1	6.53		
4–5	Total (E1 ⊗, E2 ⊗, A×E-1 ⊗)		147	77	77	1	9.3:3.1	34.67	34.47	10.98±0.0007
			12,+	12,−	11,+	11,−				
3-Acid Phos	A×A-10	3× − (12×11)[a]	29	32	25	36	1:1:1.1	2.06		
5-Acid Phos	A×A-10	5×5 (12×11)[a]	48	13	53	8	3:1:3:1	5.03		

[a] Genotype for leaf acid phosphatase

phosphatase loci have one active, dominant allele coding for one isozyme and one null, recessive allele, except for 2b and 3 isozymes. Progenies segregating for isozymes 6 and 7 could not be obtained.

The observed data on each plant for each isozyme were tested for their fit to the expected 3 : 1 or 1 : 1 presence–absence segregation by means of the X^2 test. When the heterogeneity X^2 test applied to the different progenies segregating for a given locus was not significant at the 5% level, all the individual progeny data were grouped and tested for their fit to the expected segregation. Isozymes showed a 3 : 1 segregation in the progenies obtained by self-pollination, while in the progenies obtained by crossing, the isozymes showed a 3 : 1 or 1 : 1 segregation.

Endosperm isozyme 2 showed a very peculiar segregation: some progenies fitted a 3 : 1 presence–absence (obtained by self-pollination), others a 1 : 1 presence–absence (obtained by crossing); two cases of 15 : 1 presence–absence (one case among self-pollination progenies and another among crossing progenies) and 3 : 5 presence–absence in two other cases (progenies

obtained by crossing). Also, endosperm isozyme 5 showed a very peculiar segregation: 1 : 3 presence–absence in one case (progeny obtained by selfing) and 3 : 13 presence–absence (obtained by crossing) in another.

Table 3 shows the results of two locus segregations. All the progenies and their summations (since heterogeneity among them was not found) were tested for their fit to the independence hypothesis. If the X^2 was significant the linkage hypothesis was tested. When the individuals without both isozymes (––) were not observed, the allelism hypothesis was tested. The genetic distance was not calculated when individuals (––) were not observed in progenies obtained either by selfing or crossing, if the 3 : 1 segregations were expected.

In several cases, isozyme 2 is linked to isozyme 4 and also to isozyme 5, while in other cases it behaves independently of both isozymes. These results and those obtained from single locus segregation analyses (such as 15 : 1 presence–absence for isozyme 2) mentioned above, support the hypothesis that isozyme 2 would be the result of two different and not linked loci that code for two isozymes with the same electrophoretic mobility (2a and 2b isozymes). One of these loci would be linked to isozymes 4 and 5 (locus *Alph-2* coding 2a isozyme) and the other would be linked to isozyme 1 (locus *Alph-3* coding 2b isozyme).

Among all the possible pairs of isozymes that could be controlled by alleles of the same locus, only isozymes 2b and 3 could be alleles of a locus also showing a null allele. Other combinations could be rejected because in some selfings and crosses, one of the isozymes was segregating presence–absence while the other was always present. For instance, isozymes 4 and 2 cannot be alleles since in E4⊗, isozyme 4 is always present while isozyme 2 is segregating. The same is true for isozymes 2 and 5 (E5⊗, J1⊗), 5 and 3 (A×A-4, A×A-8) and 5 and 4 (A×A-9). Moreover, when the double recessive individuals (––) were observed in progenies obtained by self-pollination it is possible to reject the allelism hypothesis (Table 4).

Therefore, there would be two linkage groups: one of them including the locus for isozyme 1 and the locus for isozymes 2b and 3 (*Alph-1* and *Alph-3* loci, respectively), while the other group would include the loci for isozymes 2a, 4 and 5 (*Alph-2, Alph-4* and *Alph-5*, respectively). All of these isozymes have a monomeric behaviour.

The endosperm of euploid 'Holdfast' wheat showed an alkaline pattern composed by seven well-stained isozymes labelled 1 to 7. Rye cultivar 'King II' revealed different phenotypes (Fig. 1 A). Isozymes 2 and 4 of 'King II' rye showed the same migration as isozymes 4 and 7 of 'Holdfast' wheat, respectively. The electrophoresed endosperms of the wheat-rye ditelocentric lines H-K II-1RL and H-K II-7RS exhibited all the isozymes of wheat. Wheat isozyme 4 showed a greater staining intensity than that of the euploid 'Holdfast' in both lines, while wheat isozyme 7 only stained more intensively in the H-K II-7RS line. This line also carried rye isozymes 5, 6 and 7 in addition to the wheat endosperm pattern. New isozymes having intermediate migrations between wheat and rye isozymes were not observed (Fig. 1 F).

Table 4. Progenies in which one of the isozymes is segregating while the other is always present

Plant	Isozymes	
	Present in all individuals of the progeny	Segregation
J1⊗	2	5
E4⊗	4	2
A×A-4	5	2 and 3
E5⊗	5	2
A×R-1	5	2
A×A-8	5	3
A×R-2	5	4
A×R-3	5	4
A×R-5	5	4
A×R-6	5	4
A×A-9	5	4

Discussion

The endosperm alkaline phosphatases of rye have proven to be highly variable as can be deduced from the analyses among and within the rye cultivars. The isozyme patterns and the variability observed for acid phosphatases in rye by Jaaska (1983) are very similar to that observed in the present research. We have found that it is possible to stain the rye endosperm phosphatases at both alkaline and acid pH and observe the same patterns. Therefore, these isozymes could probably be active in a wide range of pH.

We propose that at least five loci control the alkaline phosphatases of rye endosperm and one locus controls the leaf acid phosphatases. The following nomenclature is proposed to describe the genetic system of the alkaline phosphatases of rye endosperm: *Alph-1, Alph-2, Alph-3, Alph-4* and *Alph-5* loci, that control the isozymes 1, 2a and 3, 4 and 5, respectively. All these loci showed an active allele and one null allele, except locus *Alph-3* that presented two active alleles (coding 2b and 3 isozymes) and one null allele.

The loci *Alph-2* and *Alph-3* could be duplicated loci since both present active alleles that code for two isozymes with the same migration (2a and 2b isozymes

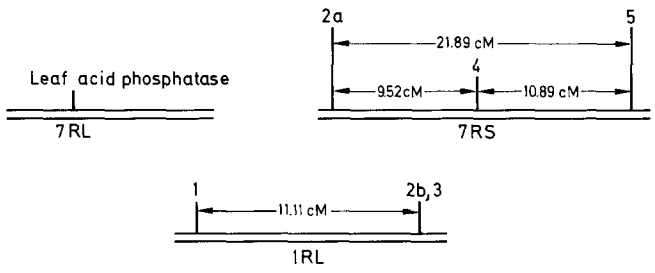

Fig. 2. Chromosomal location and linkage relationships for leaf acid and endosperm alkaline phosphatases of rye

overlap). The 15 : 1 segregation observed for isozyme 2 could support this hypothesis. Moreover, in three cases (JR ⊗ , J3 ⊗ and J4 ⊗) isozymes 2 and 5 are linked, while in three other cases (J6 ⊗ , P1 ⊗ and A × E-1 ⊗) isozymes 2 and 5 are not linked. These results could be explained if two independent loci exist which carry information for two isozymes with the same mobility. On the other hand, chromosomal location data indicate that two different chromosome arms are related to isozyme 2.

Rye isozyme 2 and wheat isozyme 4 have the same electrophoretic mobility. Wheat isozyme 4 presented a greater staining intensity than that of euploid 'Holdfast' in both 7RS and 1RL ditelocentric addition lines. This fact suggests that each one of these lines would have wheat isozymes 4 and also one of the two rye isozyme 2 (2a or 2b). So, these data would also support the 15 : 1 segregation observed for rye isozyme 2.

The linkage analyses showed the existence of two linkage groups for alkaline phosphatases (Fig. 2). One of them would contain *Alph-2, Alph-4* and *Alph-5* loci, located on the 7RS chromosome arm. The other group would include *Alph-1* and *Alph-3* loci, located on the 1RL chromosome arm.

Segregating progenies for isozymes 6 and 7 could not be obtained, as was previously mentioned, so single locus segregation and linkage analyses were not carried out. However, isozymes 6 and 7 are related to the 7RS chromosome arm. Therefore, this chromosome arm carries at least four different loci: *Alph-2, Alph-4, Alph-5* and the locus/loci coding for isozymes 6 and 7.

On the other hand, leaf acid phosphatases have been linked to the 7RL chromosome arm (Salinas and Benito 1984a). As can be deduced from our data (Table 3), the locus controlling leaf acid phosphatases appears to be linked to none of the two alkaline linkage groups. Therefore, linkage analyses agree with the chromosomal location data.

These results point out the possible existence of a remarkable series of tightly linked loci. Similar results have been found for rye esterases by Wehling and Schmidt-Stohn (1984).

The peculiar mode of segregation of endosperm alkaline phosphatase 2 and 5 is worthy of attention: some progenies fitted the 1 : 3, 3 : 5 and 3 : 13 presence–absence expected segregations. Similar results have been found for peroxidases in rye (García et al. 1982), or in *Oryza* (Shahi et al. 1969). These authors postulated the existence of genes regulating the expression of peroxidases and also a dosage effect in the endosperm. Our results are also similar to those reported in *Oryza* by Shahi et al. (1969), who also found a peroxidase isozyme segregating 3 : 1, 1 : 1 and 1 : 3 (or 3 : 13). They attributed these results to an epistatic effect with a controlling gene.

The behaviour of endosperm alkaline phosphatases is very similar to that of the peroxidases: they are monomeric enzymes and under monogenic control. The presence of null alleles and the possible existence of controlling genes is also likely.

The alkaline phosphatases of rye endosperm, endosperm and leaf peroxidases and also esterase enzyme systems share some features: a high variability, a low substrate specificity, many null alleles, and they are controlled by tightly linked loci.

The future development of complete linkage maps for isozymatic genes would provide a useful tool for further genetic studies.

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