

LINKAGE DETERMINATIONS FOR SEVERAL ISOZYMIC LOCI IN PISUM

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A number of isozyme systems have been described in Pisum (1,2,5,6,9), but only one locus, that specifying a leucine aminopeptidase, has been mapped on the pea genome (1). We have initiated a program aimed at producing an isozyme linkage map for the entire Pisum genome. This report presents data confirming the position of the gene coding Lap-1 and describes approximate map positions of two other isozyme loci.

The parents and three F<sub>2</sub> progenies were grown in the greenhouse. The two parental lines for progeny C282-231 contained the following markers: Female (WL 1466): A, D, I, b am-2. P1, R

Male: a, i, mo, En, b, er., z, r

In addition, the female parent exhibited a Lap-1 (fast), 6pgd-2 (fast) phenotype while the male possessed the "slow" allele at both of these loci. For progenies C282-232 and C282-233 the parents possessed markers: Female: A, D<sup>o</sup>, I, k, st, f, b, le, fs, wlo, t1, R

Male: a, i, k, gp, cov., te, Fs, p, R

Progeny C282-232 segregated for allozymes at Acp-1 and Acp-3, in addition to the other known markers.

Isozyme phenotypes were determined by starch gel electrophoresis, performed as described by Gottlieb (4). Two buffer systems were used in the preparation of the gels, a tris-citrate/lithium borate system at pH 8.1 (7) and an N-(3-aminopropyl)-morpholine/citric acid buffer at pH 6.1 (3). Assay mixtures were slight modifications of those described in Shaw and Prasad (8). Linkage between loci was analyzed by comparing observed and expected joint segregation ratios in pairwise combinations.

Tight linkage was observed between the b locus on chromosome 3 and Lap-1 (Table 1), thus corroborating a previous report (1). Our data gave a recombinant frequency between the two loci of about 4%. A second isozyme locus, Acp-3, can also be placed on chromosome 3- Acp-3 was found to give 9% recombinants with respect to st and 32% with b, while 24% recombination was observed between st and b (Table 1). Thus, the linkage order appears to be Acp-3—st—b.

A second acid phosphatase locus, Acp-1, assorted independently of Acp-3, but showed non-random joint segregation ratios with two markers, gp and te on chromosome 5. Recombination between gp and Acp-1 was 7% and that between te and Acp-1 was 25% (Table 1). The estimated recombination between the two marker loci was 23%, suggesting that Acp-1 is located distal to gp, i.e. toward cp. The relative mobilities of the acid phosphatase isozymes are shown in Fig. 1.

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Table 1. Joint segregation analysis of isozyme and marker loci in the  $F_2$  of two crosses.

Segregating loci	Number of plants with phenotype						$\chi^2$	Estimated % recomb.
	+/fast	+/het	+/slow	-/fast	-/het	-/slow		
<u>b/Lap-1</u>	3	32	23*	15*	2	0	48	4
<u>b/Acp-3</u>	5*	12	1	2	4	4*	7	27
<u>st/Acp-3</u>	13*	20	2	0	2	8*	23	4
<u>gd/Acp-1</u>	12*	36	1	0	4	6*	25	7 $9.5 \pm 4\%$
<u>te/Acp-1</u>	9*	29	2	2	10	5*	12	25 $31.8 \pm 7.2$
"F" / <u>6Dgd-2</u> <sup>1/</sup>	17*	36	2	1	2	18*	59	5 $7.7 \pm 3.2$
Expected ratio	3	6	3	1	2	1		
<u>st/b</u>	28	--	7	3	--	4		29
<u>gd/te</u>	40	--	11	3	--	7		23 $27.7 \pm 7$

\*Parental phenotypes

<sup>1/</sup>"F"=seeds having anthocynin spots

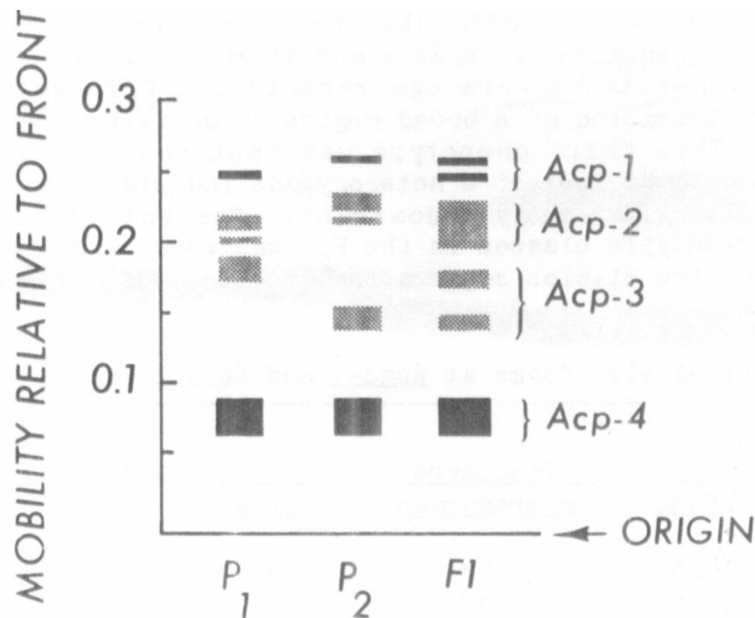


Fig. 1. Acid phosphatase phenotypes after electrophoresis of raw leaf extracts as described in text. Migration is toward the anode at top of figure. Variation was not observed in the most intensely staining acid phosphatase band (Acp-4). The parental genotypes for Acp-1, Acp-2, and Acp-3 were for P1: fast/fast/slow and for P2: slow/slow/fast.