Table 1. Joint segregation analysis of loci on chromosome 3.

Cross	Loci	N	Linkage Chi-square	Recomb. Fract.	Std. Error
1	Lap-1 : St	83	27.0	20	±5
A283-19-24	Lap-1 : Acp-3	72	9.8	34	±5
	Lap-1 : Gal-3	78	2.0ns	-	
	St : Acp-3	66	22.0	19	±5
	St : Gal-3	78	1.9ns	-	
	Acp-3 : Gal-3	68	13.0	25	±6
2	St : B	67	5.1	31	±11
C283-562-564	St : Gal-3	68	13.0	25	±6
	B : Gal-3	65	1.1ns	-	
3	Lap-2 : Adh-1	25	8.1	31	±8
A284-290-292	Lap-2 : Gal-3	14	2.2ns	-	
	Adh-1 : Gal-3	14	2.8ns	-	
4	St : Adh-1	51	14.0	22	±6
A284-303-307	St : Acp-3	46	15.0	20	±6
	St : Gal-3	41	8.6	28	±13
	Adh-1 : Acp-3	46	44.0	11	±3
	Adh-1 : Gal-3	41	14.0	19	±7
	Acp-3 : Gal-3	37	10.0	20	±7
5 (11b x J136)	St : Adh-1	59	17.0	21	±6

IDENTIFICATION AND PARTIAL CHARACTERIZATION OF 3 BETA-GALACTOS1DASE ISOZYMES IN PEA LEAVES

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Enzymes catalyzing the hydrolysis of beta-D-galactose units from the non-reducing ends of B-galactosides may be conveniently visualized after electrophoresis on starch gels by flooding the gel with a solution containing 4-methylumbelliferyl galactoside (Sigma) and observing the fluorescence of the 4-methyl umbelliferone product under long-wave ultraviolet light (1). Analysis of pea leaf extracts revealed three zones of beta-galactosidase activity (Fig. 1). One zone migrated anodally on a pH 8.1 gel while the other two migrated cathodally on a pH 6.1 gel (extraction buffers and gel systems were as described in [2]). Sharp, well defined bands of activity were produced by the anodally migrating enzyme, which showed maximum activity at alkaline pH (assay solution: 0.1 M Tris pH 8.5 containing 2 mg 4-methylumbelliferyl-beta-D-galactoside which had been dissolved in 1 ml acetone). The cathodal enzymes exhibited an acid pH optimum (assay solution 0.1 M sodium citrate pH 4.5 containing 2 mg substrate as described above) and formed broader zones of activity which were often very faint (Fig. 1). Young leaves proved to be the most convenient tissue to sample for beta-galactosidase activity; however, seed and root extracts also gave similar phenotypes.

A survey of inbred lines provided by Dr. G. A. Marx and several P.I. accessions revealed polymorphism for all three zones of beta-galactosidase activity. Variants of the anodal enzyme were rare and identified by a different electrophoretic mobility. In contrast, variants in the cathodal regions were relatively common and often exhibited a difference in level of activity (Fig. 1).

In order to determine if the three zones of activity represented products of different loci, appropriate crosses were made and the F_2 populations analyzed for segregation within each zone. Certain of the variants showed so little activity that they were treated as "null" alleles. In these cases the expected ratio was 3:1, for the heterozygotes were difficult to distinguish from the homozygous "active" phenotype and thus were grouped together as "galactosidase plus". For cases in which the mobility of the variants differed, three classes of progeny could be distinguished, and the expected ratio was 1:2:1.

The segregation observed within individual zones is summarized in Table 1. The variants within a zone appeared to be products of different alleles, for normal segregation ratios were obtained in all cases. That each zone of activity represented a distinct locus was demonstrated by joint segregation analysis presented in Table 2. Although the size of the populations listed in Table 2 was too small to eliminate the possibility of loose linkage between loci, the significant level of recombination exhibited between each pair of loci establishes the presence of three distinct beta-galactosidase loci in pea. The loci have been designated Gal-1, Gal-2, and Gal-3 in order of relative mobility of their isozymes in the anodal direction. Thus Gal-1 specifies the anodal forms, Gal-2 the cathodal enzyme closest to the origin, and Gal-3 the most cathodal isozyme.

1. Vallejos, E. E. 1983. In S. D. Taksley and T. J. Orton (eds.). Isozymes in Plant Genetics and Breeding, Part. A. Elsevier, Amsterdam, pp. 469-516.

Table 1. Segregation of allozymes at Gal-1, Gal-2, and Gal-3.

Locus	Cross	N	Number of progeny with designated phenotype[1/] S/2	Het	F/+	Expected ratio	X^2
Gal-1	239x19L	17	2	9	6	1:2:1	1.9
Gal-2	239x19L	17	6	5	6	1:2:1	2.9
	236x327	37	9		28	1:3	<0.1
	288x236	39	10		29	1:3	<0.1
Gal-3	239x19L	20	9		11	1:3	4.3
	234x248	44	11	21	12	1:2:1	0.1
	234x257	105	32		73	1:3	1.7
	254x237	80	21		59	1:3	0.1

[1]Phenotype designations: S/- = slow or no activity; het = heterozygous; F/+ = fast or activity present.

Table 2. Joint segregation at Gal-1, Gal-2, and Gal-3 in the cross 239 x 19L.

Loci	N	Number progeny with designated phenotype[1/]								
		SS	SH	SF	HS	HH	HF	FS	FH	FF
Gal-2:Gal-2	16	1	0	1	3	4	3	0	1	3
Gal-3:Gal-1	16	0	3	4				2	6	1
Gal-3:Gal-2	17	2	2	2				4	3	4

[1] Phenotype designations: S=slow or no activity; H=heterozygous; F=fast or activity present.

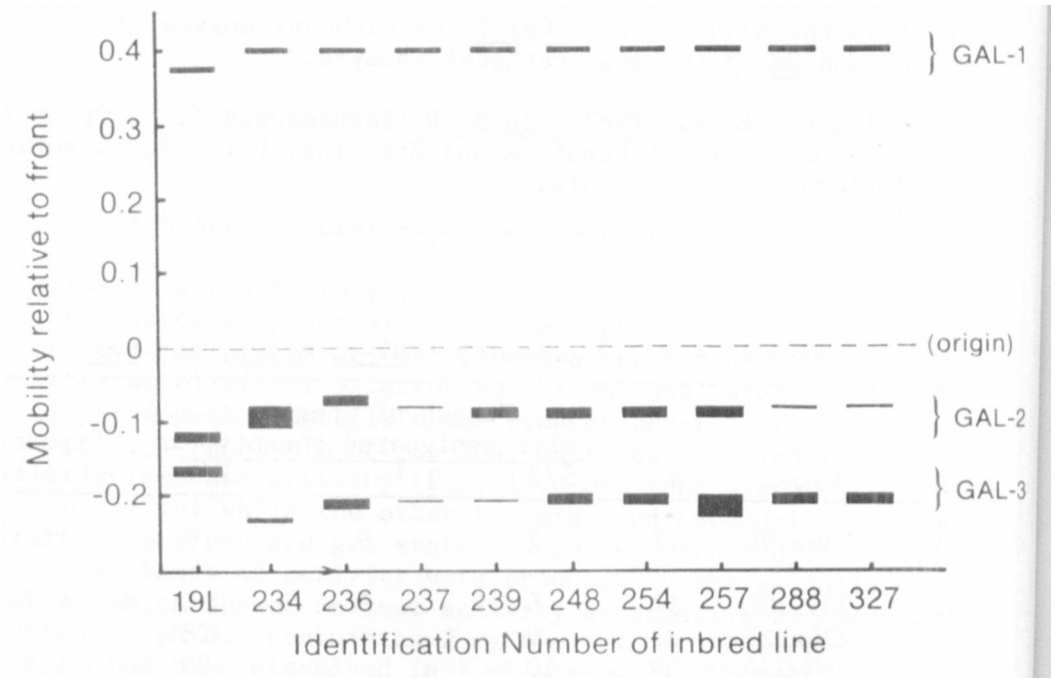

Fig. 1. Zymogram of beta-galactosidase phenotypes observed after electrophoresis on horizontal starch gels. Pea lines mentioned in text are designated in the figure by their final 3-digit code. Width of band in the cathodal region reflects relative intensity of respective isozyme band.