LINKAGE BETWEEN Dia-1 AND LOCI ON CHROMOSOME 3

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Enzymes catalyzing the reaction:

NADH + acceptor = NAD + reduced acceptor

can be visualized after horizontal starch gel electrophoresis by using an assay consisting of 100 ml 0.1 M Tris HC1 pH 8.5, 40 mg NADH, 40 mg MTT and 1 mg 2,6 dichlorophenol indophenol. At least four NADH diaphorases (DIA) isozymes can be resolved in pea leaf extracts, and we have found variation in the most anodal isozyme DIA-1 and the most intensely staining isozyme DIA-3 (Fig. 1). The DIA-1 polymorphism is best resolved using the pH 6.5 histidine buffer system of Cardy et al. (1), whereas the DIA-3 variation is more clearly observed on a Tris borate-EDTA system (2). In a survey of a wide sample of Pisum germplasm, we identified at least 2 common variants for DIA-1 the more anodal of which we designated "a" and the other "b". We demonstrate here that the variation in DIA-1 phenotype shows monogenic inheritance, being encoded by a locus that exhibits linkage with markers near M on chromosome 3.

Marker lines fixed for DIA-la were crossed with lines fixed for DIA-lb, and the resulting hybrids selfed to form F2 progenies. Segregation for DIA-1 phenotype was observed in each of the four F2 progenies analyzed (Table 1). In three of the four progenies the DIA-1 variants behaved as codominant alleles at a single segregating locus, which we designated Dia-1. The fourth progeny derived from the cross A73-91 x PI 179449 gave all three of the expected phenotypes but the relative number of these was significantly different from the expected 1:2:1 ratio. Joint segregation analysis of the loci segregation in these progenies indicated linkage between Dia-1 and loci near M and chromosome 3 (Table II).

Previous results indicate that Aat-c is about 15 map units from M and Lap-2 (3). Comparative map distances and the lack of linkage between Dia-1 and Acp-3 or St (results not presented) suggest that Dia-1 is located about midway between Lap-2 and Aat-p on the distal side of M from the centromere. The availability of two common alleles at Dia-1 should make this locus very useful in further mapping studies.

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2. Shaw, C. R. and R. Prasad. 1970. Biochem. Genet. 4:297-320.
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Table 1. Phenotypic segregation and chi square analysis for DIA-1 in four F2 populations

Cross	N	No. of progeny with designated phenotype a	ab	b	X^2 1:2:1
(1) Slow x B77-291	31	9	15	7	0.29
(2) PI179449 x A578-235	30	8	17	5	1.13
(3) JI2018 x B77-291	22	7	12	3	0.32
(4) A73-91 x PI179449	17	13	3	1	14.73**

**Significant at P<0.01.

Table 2. Joint segregation analysis of Dia-1 with other loci on chromosome 3

Cross	Locus	No. progeny with designated phenotype[1] a/a	a/h	a/b	h/a	h/h	h/b	b/a	b/h	b/b	X^2 (1:2:1)	Recomb. Fract.	S.E.
1	M	9_2^2	---	0	12	---	3	1	---	6_2^2	15.2	13	6
2	Aat-c	8^2	0	0_2	1	14	2	0_2	2	3^2	32.2	9	4
3	Lap-2	0_2	2	3^2	2	9	0	5^2	1	0_2	34.4	9	4
4	Aat-c	12^2	1	0	0	2	1	0	0	1^2	----[3]	--[3]	-

[1] Phenotypic designations: a = allozyme a, b = allozyme b, h = both allozymes present.
[2] Parental phenotypes.
[3] Not calculated because of distorted segregation ratios (see Table I).

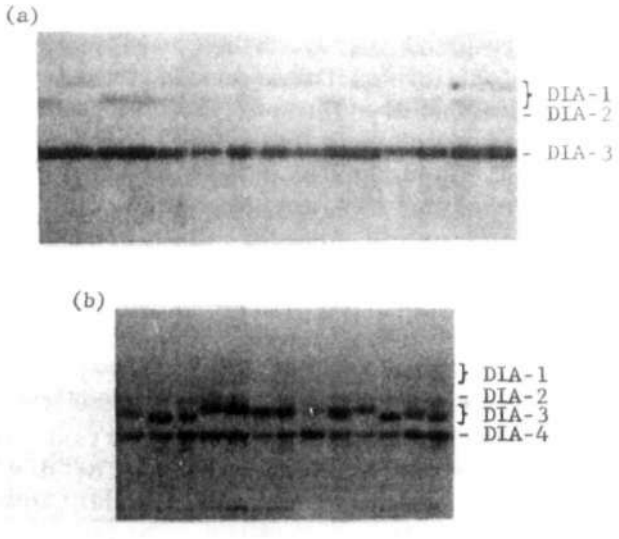

Fig. 1. (a) Diaphorase phenotypes on histidine gel, pH 6.5.
(b) Diaphorase phenotypes on tris-borate-EDTA gel, pH 8.0.
Anode is at top of each photograph.

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