

ALCOHOL DEHYDROGENASE EXPRESSION IN THE PEA

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Alcohol dehydrogenase (ADH) isozymes have been intensively investigated in several crop species (1, 2). In every case the enzyme has proven to be dimeric, and in most cases two or more loci code for ADH subunits. The ADH isozyme system has proven especially interesting to study because the individual loci often exhibit tissue specificity and can be induced by anaerobic conditions (3). We report here our observations on the ADH system in pea.

Tissues were examined for ADH expression by crushing small samples in Tris malate pH 8.5 extraction buffer and subjecting this extract to starch gel electrophoresis on Tris/borate buffer system at pH 8.1 (4). After electrophoresis an anodal slice was stained for ADH activity using a standard assay: 0.1 M Tris/HCl pH 8.0, 2% ethanol, 0.4 mM NAD, 0.3 mM MTT and 0.1 mM PMS.

Dry and imbibed seeds express a single major band of ADH activity. Seeds that have been imbibed and submerged or otherwise placed in an anaerobic environment exhibit three ADH activity bands. These results suggest a two-locus isozyme system, one of which is normally expressed in cotyledon tissue and the other induced by anaerobic conditions. Such a system has been described in several species including chickpea (5). ADH activity could not be detected in well aerated root and leaflet tissue, but a three-banded phenotype was observed in root extracts after the roots had been immersed in water for 12 or more hours. Developing testa and cotyledon extracts also gave a three-banded ADH banding pattern when subjected to electrophoretic analysis. ADH activity can also be induced in leaf tissue by anaerobic conditions.

A genetic test of the two-locus model was made possible by the discovery of a relatively rare polymorphism in the ADH phenotype. The variant lines showed a triplet of bands in anaerobic tissue, but the two more anodal bands were faster migrating than their counterparts in the more common phenotype (Fig. 1).

Hybrid plants were produced from a cross between a line with the normal slow-migrating form and a line exhibiting the fast variant. Under anaerobic conditions, root tissue from these hybrids expressed the six-banded ADH phenotype predicted by the two-locus model. This finding confirmed the heterodimeric nature of the intermediate band in each of the triplets expressed in the parental lines because a third band was not formed between these two bands. Self-pollination of the hybrid plants gave an F₂ population which contained both parental ADH phenotypes as well as that of the hybrid (Table 1). The relative number of plants expressing each of these phenotypes corresponded to that expected for segregation of two alleles at a single locus, Adh-1. Most anodal bands of the parental phenotypes may be designated ADH-1aa and ADH-1bb because they are the homodimeric combinations of the subunits produced by the a and b alleles of Adh-1. The ADH band observed in the hybrid at a position intermediate between these homodimeric bands is the intra-genic heterodimer, consisting of a subunit from the 1a allele and a sub-unit from the 1b allele. The middle band in each parental ADH triplet must be an intergenic heterodimer, consisting of a subunit of ADH-1 and a subunit of ADH-2. In both parents the slowest migrating band is the homodimer of ADH-2 subunits. Adh-1 is the locus expressed in dry seed tissue while Adh-2 appears to be induced only under anaerobic conditions.

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3. Freeling, M. 1973. Mol. Genet. 127:215-227.
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Table 1. Segregation of allelic forms at Adh-1.

Cross	N	Phenotype			χ^2 (1:2:1)
		Slow	Heterozygous	Fast	
168 x 344	51	11	26	14	0.4
11b x J136	60	20	24	16	2.9

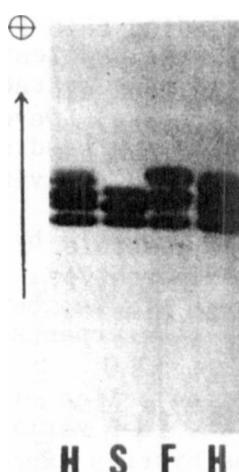


Fig. 1. Alcohol dehydrogenase phenotypes observed in extracts from pea roots submersed in water for 12 hours. The slow phenotype, marked "S", is that expressed in most of the pea lines we have examined. The "F" phenotype has been found in only a few lines, including 168 and J136. The "H" phenotype, shown in both outer lanes, is that observed in F1 plants produced from a cross between a line exhibiting the F phenotype and one possessing an S phenotype. The H phenotype was scored as the heterozygous pattern. Anode is at top of figure. Migration was in the direction of the arrow.