PEA 6-PHOSPHOGLUCONATE DEHYDROGENASE ISOZYMES

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Two isozymes of 6-phosphogluconate dehydrogenase (6PGD) have been identified in spinach (1), radish (2), castor bean (4) and Senecio sylvaticus (5). In each of these species one of the isozymes is found in the cytosol while the second is localized in the plastid (chloroplast, leucoplast, etc.). In this report I present evidence that two isozymes of 6PGD are also present in pea leaves, that one of these is in the cytosol and the other in the chloroplast, and that the two isozymes are specified by distinct genes.

Starch gel electrophoresis was performed as described in the accompanying article (8) using the pH 6.1 buffer system. Chloroplast and cytosolic fractions were obtained as described previously (6). The assay for 6PGD was modified from Shaw and Prasad (3). Two zones of 6PGD activity were observed after electrophoresis of extract from a number of inbred lines (Fig. 1). In every line examined only one band of activity within Group A and one within Group B were observed (see Fig. 1). The more anodal set (Group A) was present in whole leaf and chloroplast extracts but absent from soaked pollen extracts, indicating a plastid localization. The slower migrating bands (Group B) were observed in whole leaf and soaked pollen but not in the chloroplast pellet and are believed to be contained in the cytosol. Mitochondrial, peroxisomal and vacuolar compartments were not investigated.

The variability present between lines permitted genetic analysis of the isozymes by classical inheritance studies. An appropriate F2 progeny was tested for segregation of forms A and A' and forms B and B'. Within each set three phenotypes were observed in the F2; two were parental and the third consisted of a broad region of activity covering both parental zones. This third phenotype was that seen in all F_1 plants and is considered to be that of a heterozygous individual because allelic forms of isozymes are generaly codominant. For both sets the ratio of the three phenotypic classes in the F2 was very close to the 1:2:1 ratio expected for two alleles segregating at one locus (Table 1).

Table 1. Segregation of allelic forms at 6pgd-1 and 6pgd-2

Locus	Phenotype			Chi-square (1:2:1)
	Fast	Heterozygous	Slow	
6pgd-1 (Group A)				
C282-231	36	72	30	0.8
C282-232-233	16	30	16	0.1
6pgd-2 (Group B)				
C282-231	33	68	37	0.3

These results demonstrate that the 6PGD's in each subcellular compartment are coded by genes located on nuclear DNA. In order to determine whether the two sets of 6PGD's are coded by the same or by distinct nuclear genes their joint segregation was investigated (Table 2). The analysis indicates that the chloroplast and cytosolic isozymes are specified by distinct genes, 6pgd-1 and 6pgd-2 respectively, and are only distantly linked, if linked at all. These findings represent the first demonstration of the genetic basis of plastid and cytosolic 6PGD's. The results parallel those for three other chloroplast/cytosolic isozyme pairs which have been investigated at the genetic level (7).

Table 2. Joint segregation of 6pgd-1 and 6pgd-2

	Phenotype* (6PGD-1, 6PGD-2)								
	F,F	F,H	F,S	H,F	H,H	H,S	S,F	S,H	S,S
Observed	13	18	5	15	33	24	5	17	8
Expected	1	2	1	2	4	2	1	2	1

Linkage X^2 = 7.3 (not significant at P = 0.05)

*F = fast, H = heterozygous, S = slow

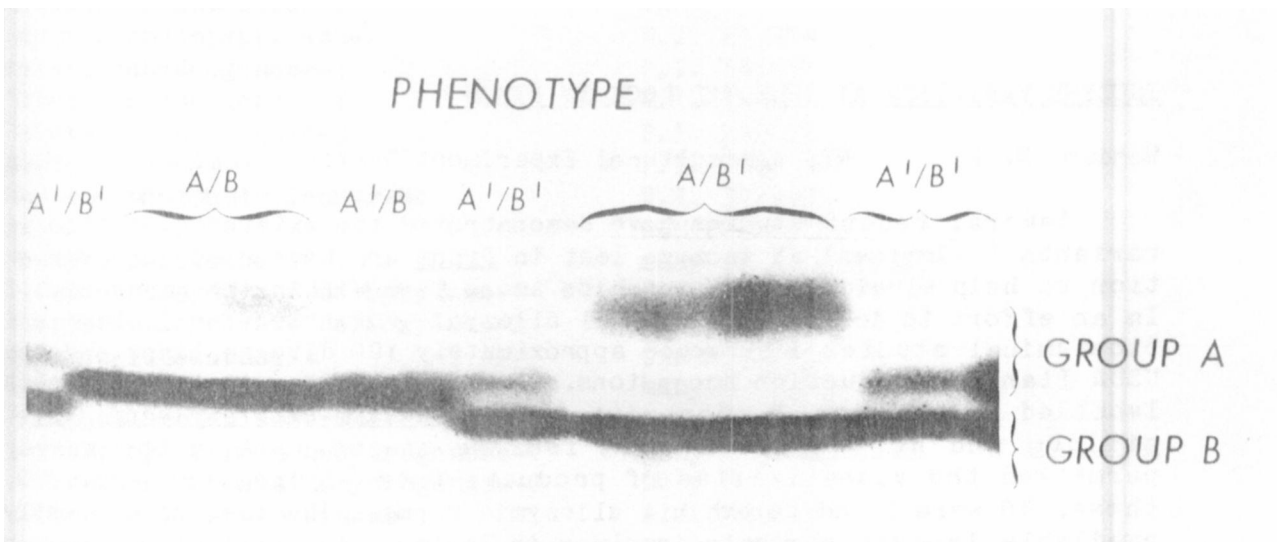

Fig. 1. Electrophoretic patterns of 6PGD isozymes in homozygous breeding lines. Direction of migration is toward the anode at the top of the photograph. Group A consists of the two more anodal bands labeled A and A'. Group B includes the intense bands designated B and B'.

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ISOZYME VARIATION AT SELECTED LOCI IN PISUM

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Several recent studies have demonstrated the existence of allelic variants (allozymes) at isozyme loci in Pisum and have used such variation to help elucidate relationships among taxa within the genus (1,2). In an effort to identify additional allozymic variation for linkage and biochemical studies I surveyed approximately 100 diverse lines and ten USDA Plant Introduction accessions. The latter group included samples labelled P. elatius. P. jomardii. and P. sativum ssp. syriacum. abyssinicum and hortense. The 43 isozyme stains used in the survey permitted the visualization of products from 77 distinct loci. Of these, 40 were found to exhibit allozymic forms. The list of currently available isozyme variants is given in Table 1 along with the lines in which the rarer variants were discovered. This author would appreciate information regarding polymorphism at additional isozyme loci not included in Table 1.

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