SHORT COMMUNICATIONS

GENETICALLY CONTROLLED ELECTROPHORETIC VARIANTS OF A STORAGE PROTEIN IN *PISUM SATIVUM**

By M. J. Hynes†

A number of electrophoretic variants of plant enzymes have been described and the genetic control of these variants determined (e.g. Beckman, Scandalios, and Brewbaker 1964; Schwartz 1964; Scandalios 1965). However, little work has been done on structural and storage proteins of plants. Varietal differences have been observed in the electrophoretic patterns of wheat storage proteins (Graham and Morton 1963) and two forms of arachin, a storage protein of the peanut, have been described (Tombs 1964), though no genetic studies of these differences have been made. This communication describes the detection and the partial characterization of variants of proteins extracted from the cotyledons of *Pisum sativum* seeds and some preliminary breeding tests to determine their genetic control.

The testa and embryo were removed from dry seeds and the cotyledons ground in a mortar to a flour. Crude preparations were made by extracting 0·5–1·0 g of flour with 2–4 ml of 0·2M NaCl (pH 7·0). Salt-soluble proteins (globulins) were precipitated by dialysis against distilled water and redissolved in a small volume of 0·2M NaCl. Purer preparations of globulins were made according to the methods of Danielsson (1949, 1950, 1952).

Starch gel electrophoresis of extracts was carried out in horizontal water-cooled gel trays (29 by 13·5 cm by 4 mm) with sample slots 11 cm from the cathode end of the gel. The starch concentration was 12% and gels contained 7M urea and 0·076M Tris–citrate buffer (pH 8·6) (Wake and Baldwin 1961). The electrolyte used was 0·1M lithium hydroxide–0·38M boric acid (pH 8·6) (Ferguson and Wallace 1961). Voltages applied were 20–25 V per centimetre of gel length and the front was run 6–8 cm past the sample slots. Gels were sliced horizontally and stained with amido black or nigrosin.

The gels of both crude sodium chloride and globulin extracts showed consistent resolution of the zones in which the variants were detected (Fig. 1). In addition a number of darkly staining, slow-moving zones were observed in all gels and it is likely that these resulted from association of breakdown products caused by the action of urea on the proteins (Grant and Lawrence 1964). The fastest-moving zones (Fig. 1) were not clearly resolved, and it could not be determined whether any consistent variation occurred.

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† Department of Genetics, University of Adelaide; present address: School of Biological Sciences, Flinders University, Bedford Park, S.A. 5042.

Figure 1 shows the three variant types (designated A, B, and C). Initially the C type was detected in the commercial cultivar Yorkshire Hero and the cultivar Mummy, while the B type was found in three lines held by the Department of Genetics for teaching purposes (derived from the cultivars Witham Wonder and French Sugar) and in the commercial cultivar Greenfeast. Subsequently, in a survey of 22 varieties obtained commercially and from the Department of Agronomy, University of Adelaide, three varieties were found to show the C type, three the A type, and the remainder the B type.

The same variant was found consistently in different extracts of the same variety irrespective of year of harvest of the seeds. Extracts of progeny seeds from crosses between varieties of identical type yielded the same type as the parents. Extracts of seeds from crosses between B and C types yielded the BC pattern shown in Figure 1, and this was obtained independently of the direction of the cross.

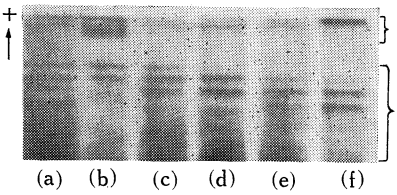

Fig. 1.—Part of starch gel electrophoretic pattern (12% starch in 0·076M Tris–citrate buffer plus 7M urea) of crude extracts of globulins of pea seeds showing the following variants: (*a*) AC type; (*b*) A type; (*c*) AB type; (*d*) B type; (*e*) BC type; (*f*) C type.

Individual seeds from the progeny resulting from the selfing of BC plants were extracted with 0·2M NaCl and scored. This yielded 15 B types, 20 BC types, and 11 C types consistent with a 1 : 2 : 1 segregation ($\chi^2_{(2)} = 1{\cdot}478, P > 0{\cdot}3$). BC plants were crossed to a variety showing the A type, and in the progeny 13 AB and 7 AC types (Fig. 1) were scored, consistent with a 1 : 1 ratio ($\chi^2_{(1)} = 1{\cdot}8, P > 0{\cdot}1$). These results indicate that the types are under the control of three allelic genes.

From Figure 1 it is seen that the homozygous types consist of a doublet of two intensely staining bands and both of these are subject to electrophoretic shifts. The difference in mobilities between the B and C types is equal to the difference between the A and B types, which may indicate equal charge alterations. Fainter bands—one moving ahead and one behind the doublet—were visible in some extracts, but were not clearly resolved in all cases (see the C type in Fig. 1). The BC and AB types each have three bands, the more intense one in the middle apparently resulting from the overlap of the upper and lower bands of the relevant homozygous patterns, while the AC type has four. It is evident that these heterozygous types arise from the additive patterns of the two parental types, i.e. no interaction products are formed in the heterozygotes. A similar situation has been observed to occur in esterase variants in *Peromyscus*, and these results were interpreted as indicating that alterations in a common subunit led to equal electrophoretic shifts in both bands of a pair (Shaw 1964). Thus the patterns observed here may be most readily explained if the bands of the doublet have a single polypeptide chain in common, and amino acid substitutions in this chain give rise to the variants.

As noted above, the variant bands occurred in both crude and purified globulin extracts. The storage globulins of pea cotyledons consist of two protein fractions, vicilin (soluble at pH 4·7) and legumin (isoelectrically precipitated at pH 4·7)

(Danielsson 1949, 1950, 1952). A solution of globulins in 0·2M NaCl (unbuffered) was dialysed against 0·2M NaCl buffered to pH 4·7 with citrate–phosphate buffer for 24 hr, and a partial separation of protein insoluble and soluble at pH 4·7 effected. After concentration by freeze-drying, electrophoresis of the fractions was carried out as before. In both a B- and a C-type variety it was found that the variant proteins occurred in the fraction soluble at pH 4·7 (vicilin), while the fastest-moving zones and much of the slow-moving material occurred in the fraction insoluble at pH 4·7. Thus it is indicated that the variant protein occurs in the vicilin fraction. Grant and Lawrence (1964) have found that acrylamide gel electrophoresis of vicilin, which had been separated from legumin by DEAE-cellulose column chromatography in the presence of urea at pH 8·5, yields two main protein bands—as found in this work. Hence these results suggest that urea dissociates vicilin into two major fragments, each containing a common subunit. *N*-terminal amino acid studies on vicilin of *Pisum sativum* have shown that two moles of aspartic acid and one of serine occur per molecular weight of 186,000. Smaller amounts of glutamic acid also occur. (Vaintraub and Goffman 1961). In the closely related legume *Vicia sativa*, vicilin has been shown to have glutamic acid, lysine, and serine in the ratios 2 : 1 : 1 as the principle *N*-terminal amino acids (Vaintraub, Shutov, and Klimenko 1962). These results are therefore not inconsistent with the suggestion that two identical subunits occur in the vicilin molecule.

More detailed work on the chemistry of the storage proteins is required to characterize more fully the nature of these differences. It is suggested that the finding of electrophoretic variants in the storage proteins may aid in elaborating the polypeptide chain composition of these proteins and the way in which these chains are assembled.

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