ELECTRQPHORETICAL INVESTIGATIONS ON SEED ALBUMINS OP Pisum sativum: IDENTIFICATION OF SEVERAL ENZYMES

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Seed albumins, the water soluble fraction of pea seeds, are composed predominantly of enzymes. There is comparatively little enzyme activity in dormant seed because, in this status, metabolism is reduced. Nevertheless, enzymes must be available as soon as germination begins to enhance respiration and to initiate glycolysis. This is why several enzymes should be detectable already in dry seeds.

Total seed protein was extracted from dry seeds by a 0.2 M salt solution. A globulin-free albumin solution was obtained after dialysing this extract against running tap water. Separation of albumins was carried out by flat-gel electrophoresis. Five identical assays were separated on the same gel. Each time 50 ul of the albumin solution (1 mg/ml) was placed on the gel's surface and each time one of the 5 assays was stained with Coomassie Blue. As Fig. 1 (first column) shows, 33 bands can be detected in the pherogram. The two distinct bands at position R_p 0.28 and Rp 0.35 are characteristic for the Pisum-albumin pattern. A group of distinct slow running bands is also evident (upper third of pherogram) as well as a number of fast running bands which are not so pronounced (lower part).

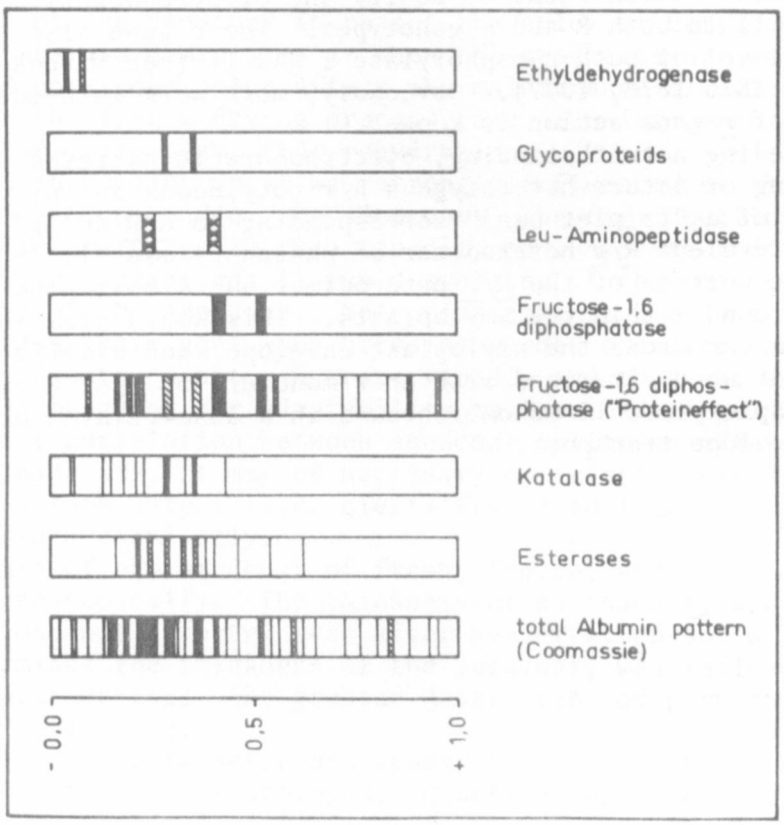

Fig. 1. Gel-electrophoretic distribution pattern of seed albumins of Pisum sativum and of several enzymes.

By applying specific methods, single enzymes can be detected within the albumin pattern. So far, the following enzymes were examined:

Dehydrogenase	
Ethyldehydrogenase	+
Oxidases	
Tyrosinase	-
Peroxidase	-
Catalase	+
Hydrolases	
Esterases (unspecific)	+
Lipase	+
Leu-aminopeptidase	+
Amylase	-
α-Glucosidase - 1	+
Alkaline phosphatase	-
Acid phosphatase	-
Fructose-1,6 diphosphatase	+
Glycoproteins	+

In contrast to the findings of other investigators, amylase and acid phosphatase could not be determined under our conditions. Fig. 1 presents the distribution of some of the above-mentioned enzymes on the gel. The enzymes show a multiple banding pattern. The dark blue bands with Rp 0.28 and R_p 0.35 are the position of several substances (esterases, catalase, and glycoproteins), thus being a collection of proteins with similar or identical electrophoretic properties.

The determination of fructose-1,6 diphosphatase was complicated by the "protein effect". The "protein effect" was described by Searcy et al. (1965) in connection with starch-iodine reaction in human serum protein. Zimniak-Przybylska and Przybylska (1976) found the same effect during their investigations on amylase, and Jacobsen (1980) during investigations with rlbonuclease, Thus it seems reasonable that the "protein effect" is not exclusively restricted to starch-iodine reaction, as was proposed by Zimniak-Przybylska and Przybylska, but that it is connected with the method of "negative staining" which is the common feature of all three methods (determination of amylase, ribonuclease, and fructose-1,6 diphosphatase).

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Zimniak-Przybylska, Z. and J. Przybylska. 1976. Genetica Polonica 17:133