OBSERVATIONS ON THE RELATIVE ELECTROPHORETIC MOBILITY OF PISUM SEED AMYLASES

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Two procedures, called here Method A and Method B, were used to detect variation in electrophoretic patterns of pea seed amylases (2,3). In Method A proteins were separated in 9% polyacrylamide gels, in a discontinuous buffer system according to Davis (1); following electrophoresis gels were incubated for 5 hrs in 1% solution of soluble starch in 0.2 M acetate buffer, pH 5.3, and then stained with I_2-KI solution(2). In Method B the electrode buffer was 0.125 M Tris-H_3B0_3, pH 8.9, instead of Tris—glycine, pH 8.5, and hydrolyzed starch was incorporated into gels instead of being put into the incubation mixture (3). In both procedures, electrophoresis was continued until after the dye marker (bromophenol blue) had reached the anodic edge of a gel.

Zymograms obtained with Method A showed two anodic variant zones of enzyme activity; the faster moving bands, forming variant zone Amy-1, were well defined while the slower bands of Amy-2 variant zone were very faint, scarcely discernible (2). In Method B separation and detection of amylase-variants were significantly improved but Amy-1 variants were not revealed; marked destaining near the start of migration was at first not attributed to the Amy-1 variant zone (3).

In further investigations with the use of Method B different commercial products of hydrolyzed and soluble starch were tried. Results obtained with the hydrolyzed starch and with the soluble starch "Analar" from BDH were as reported earlier. However, zymograms obtained with the use of a soluble starch purchased from POCh, Gliwice, Poland, showed slow migrating bands forming an additional variant zone. The additional zone seemed to correspond to the Amy-1 zone on zymograms obtained with the use of Method A (Fig. 1). It was therefore assumed that under conditions of Method A and Method B variants forming Amy-1 and Amy-2 zones migrate in a reverse order. This was confirmed by eluting the Amy-1 and Amy-2 variant bands from a gel obtained with Method A, followed by re-electrophoresis under conditions of Method B (Fig. 2). It should be mentioned, however, that in the case of the Amy-2 variant, besides the characteristic fast moving band two additional slow moving bands could be seen. These additional bands are probably artifacts, possibly aggregations.

The order of electrophoretic mobilities of the respective Amy-1 variants in gels obtained with Method A and Method B seems to be similar (see Fig. 1). However, on screening a wider plant material differences may be found.

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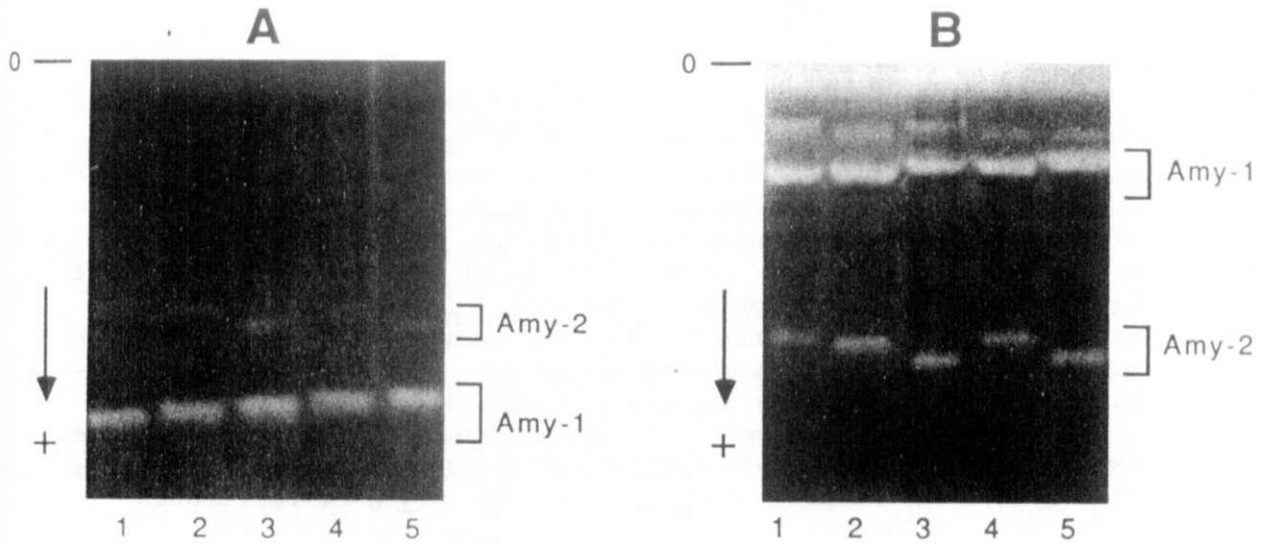

Fig. 1. Zymograms of seed amylases from several Pisum lines, obtained under conditions described as Method A (A) and Method B (B) (see text).
1-5 -- extracts from the following lines:
P. sativum W 110, P. elatius W 226, P. sativum W 809, P. sativum W 1201, P. sativum W 1998.

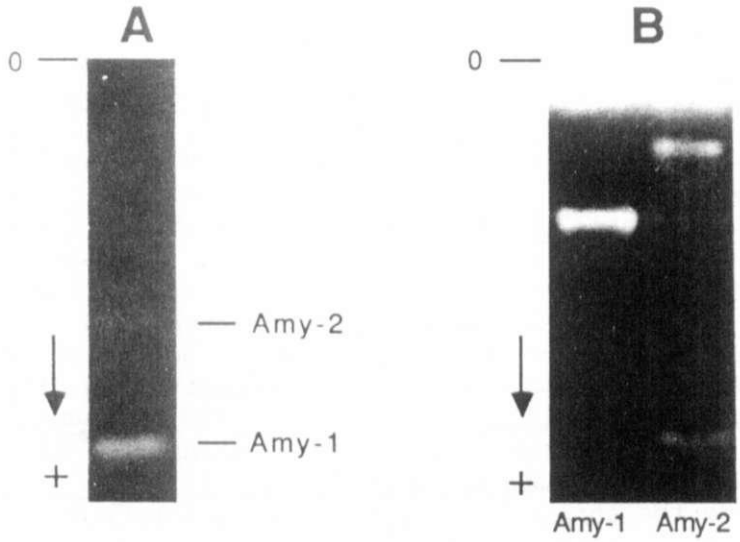

Fig. 2. Amy-1 and Amy-2 variants from Pisum sativum W 110 separated using Method A (A), individually eluted from unstained gel fragments, and subjected to re-electrophoresis using Method B (B).

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