ELECTROPHORETIC ANALYSIS OF PISUM SEED AMYLASES[1/]

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Zymograms of pea seed amylases were reported to display two anodic variant zones of enzyme activity (2); bands forming the faster moving zone Amy-1 were well-defined while those in the slower moving zone Amy-2 were faint. In 108 accessions, representing different Pisum forms, six single-banded phenotypes in each zone were distinguished (2).

Separation and detection of Amy-2 variants have since been improved. In a modified technique, electrophoretic separation i3 performed in a discontinuous buffer system; resolving and stacking polyacrylamide gels are prepared according to Davis (1) and 0.125 M tris-borate buffer, pH 8.9 (3) is used as an electrode buffer. Starch (0.3%) is incorporated into gels. After electrophoresis, gels are incubated in 0.2 M acetate buffer, pH 5.3, for 5 hrs and then stained with I_2-KI solution. Under the above experimental conditions zone Amy-1 is not revealed.

The 108 Pisum accessions previously examined have now been reanalyzed with this modified technique. The comparative electrophoretic analysis was performed in slab gels and the distinction of Amy-2 variants was based on the observed differences in migration distance. In total, eleven Amy-2 variants could be distinguished. It should be stressed, however, that differences in electrophoretic mobility among some of the successive variants are so small that the bands could not be resolved if a mixture of the respective extracts were subjected to electrophoresis. The distinguished Amy-2 variants (phenotypes) are shown in Fig. 1. Variants designated now as $2c_1$, $2c_2$, and $2c_3$ were not separated in the previous investigation and were classified as variant 2c. Similarly, variants $2e_1$, $2e_2$, $2e_3$, and $2e_4$ were previously classified as variant 2e.

The modified technique has revealed an additional polymorphism of the Amy-2 zone in ecotypes el.atius, humile, and sativum. The most commonly occurring Amy-2 phenotypes were variants $2c_2$, and $2e_3$. Variant $2c_2$ was found in 40 Pisum forms classified as P. humile, P. sativum, P. abyssinicum. and P. fulvum. Variant $2e_3$ was observed in 32 P. sativum accessions and in P. elatius. W 805. "Variants 2a, 2b, $2c_3$, and 2d were found in single accessions indicated in Fig. 1. The distribution of Amy-2 phenotypes seems to show no correlation to this taxonomic scheme.

1. Davis, B. J. 1964. Ann. New York Acad. Sci. 121:404-427.
2. Przybylska, J., S, Blixt, H. Parzysz, Z. Zimniak-Przybylska. 1982. Genetica Polonica 23:103-121.
3. Stegemann, S., H. Francksen, and V. Kacko. 1973. Z. Naturforsch. 28:722-732.

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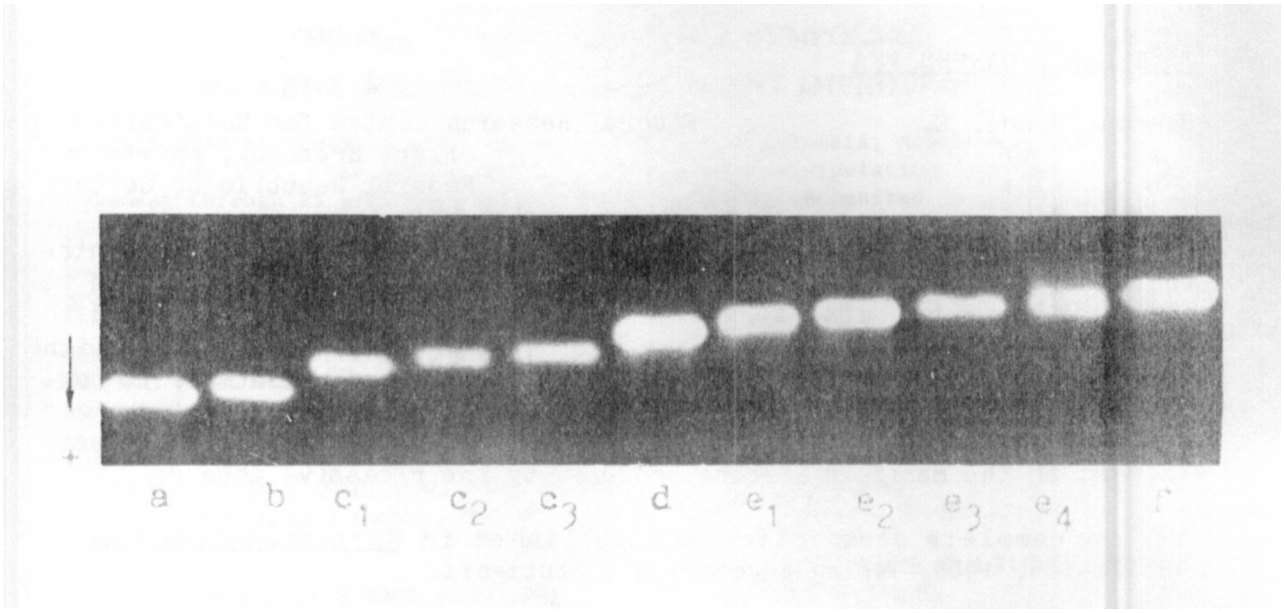

Fig. 1. Amy-2 phenotypes in pea seeds. The phenotypes shown are produced by the following accessions: a - The accession of P. fulvum obtained from the Hebrew University of Jerusalem; b - Gat. 2 55, P. elatius; c_1 - W 809, P. sativum; c_2 - W 1 998, P. sativum; c_3 - W 1951, P. sativum; d - JI 224a, P. fulvum; e_1 - W226, P. elatius: e_2 - W 1447, P. elatius: e_3 - W 1201, *P*. sativum: e_4 - W 1968, P. sativum: f - W 1647, P. sativum.

Editor's Note: In order to maintain a consistent system of nomenclature for the amylase variants observed, the authors have used subscripts to designate further variation within mobility classes (a-e) originally reported in Przybylska, et al. Genetica Polonica 23:103, 1982. This somewhat unconventional format may lead to very cumbersome allelic designations should additional cryptic variation be revealed in further studies. We respectfully suggest that after the investigators have completed their analysis of amylase phenotypes and the genetic basis for the observed variation has been determined, the allelic designations be modified to a more conventional and convenient form. The rules for genetic symbols (PNL 9:67-70) do not explicitly deal with this problem, but since more and more biochemical markers are being reported, it is important that we develop a consistent system which is acceptable to most, if not all, Pisum geneticists.