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HETEROGENEITY AND TISSUE SPECIFICITY OF SOME ENZYMES IN KIDNEY BEAN

It is generally accepted that the ontogenetic development of an organism is, in its general features, genetically predetermined and regulated. When taking into consideration that genetic information finds its realization through the biosynthesis of proteins with coded structure, it becomes clear that the following of changes in protein patterns during the development is of primary importance for understanding the genetic and molecular basis of cellular differentiation and morphogenesis. All other physiological and morphological features and their changes during the ontogenetic development are inevitably derived from changes in protein complement.

Recently developed protein fractionation procedures have provided a new valuable tool for studying qualitative changes in protein complement during the development. Already the first studies of multiple molecular forms of enzymes (Hunter, Markert, 1957; Markert, Møller, 1959) established differences in isoenzyme patterns for separate organs of a single organism. At present, there is a great number of studies demonstrating the tissue and organ specificity of protein complements in animals and showing sequential alteration in their patterns during the development, especially during the insect and amphibian metamorphosis (for further references see Laufer, 1964, and Manwell, 1966).

In plants, the tissue specificity of protein and enzyme complements has also been demonstrated by many investigators. As early as in 1932 Berg found differences in antigenically active proteins of seeds and growing seedling tissues. Since that time, several authors have confirmed serological differences between various parts of the same plant (Kloz *et al.*, 1960; Wright, 1963, etc.). In 1964, Scandalios applied gel electrophoretic technique to study the tissue specificity of enzymes in maize and revealed a large tissue-specific variation among peroxidase, esterase and leucine aminopeptidase isoenzymes. Evans and Alldridge (1965) described differences in peroxidase electrophoretic patterns of different tissues of tomato. Organs of pea seedlings have been reported (Besemer and Clauss, 1968; Macnicol, 1966; Siegel and Galston, 1967) to differ in peroxidase isoenzyme composition. Dvořák and Černohorská (1967) observed variation in peroxidase electrophoretic patterns in roots, hypocotyls and cotyledons of 10-day-old pumpkin seedlings. Barber *et al.* (1967) found differences in protein patterns in various parts of wheat seeds and seedlings. The number and the electrophoretic mobility of malate dehydrogenase isoenzymes was reported (Weimberg, 1968) to differ widely between three separate organs of the ten plant species studied.

Mäkinen (1968) observed variation in electrophoretic patterns of several enzymes in tissues of onion seedlings, while Upadhya and Yee (1968) reported the same for barley seedlings. Characteristic changes in protein and isoenzyme patterns during the seed germination and seedling growth were reported by many authors (Barber, Steward, 1968; Ghetie, Buzila, 1964; Macko *et al.*, 1967; Mäkinen, 1968; Racusen, Foote, 1966; Siegel, Galston, 1967, etc.).

In addition to the above data demonstrating the tissue-specific nature of protein and enzyme complements in plants, several authors (Khavkin *et al.*, 1967; Morris, 1966; Steward *et al.*, 1965) found variation in protein electrophoretic patterns for meristematic, elongation and differentiation zones of maize and pea roots. These data suggest the occurrence of successive qualitative changes in protein complement in the course of cellular differentiation from the meristematic cells in the root tip through the enlargement growth to the differentiation phase. However, some investigators (Besemer, Clauss, 1968; Dudchenko, Sytnik, 1967; Hadačová, Sahulka, 1967) reported that they failed to detect qualitative differences in polyacrylamide gel electrophoretic patterns of soluble proteins from successive root zones of pea, pumpkin and horse bean, indicating the necessity of further studies.

The present communication deals with the results of polyacrylamide gel electrophoretic studies of acid phosphatase, esterase, leucine aminopeptidase, peroxidase and nadi-oxidase isoenzyme systems in seeds and seedling tissues of kidney bean, *Phaseolus vulgaris* L.

Material and Methods

Plant material. Seeds of kidney bean (*Phaseolus vulgaris* L., variety 'Triumph') were allowed to imbibe for 48 hours in aerated distilled water in the dark at about 22° C. Germinating seeds were sown in moist sand and grown under continuous illumination by day-light fluorescent lamps in a humid atmosphere at about 22° C.

Protein extracts were made separately from ungerminated seeds, from seeds germinated for 48-hours in aerated distilled water, and from cotyledons, leaves, stems and roots of individual 9-day-old seedlings. A 500 mg sample of seed or seedling tissue was homogenized by grinding in a prechilled mortar with acid-washed sand and 2.0 ml of cold buffer mixture at a pH of about 7.6—7.8, consisting of 0.25 M sucrose, 0.1 M tris-hydroxymethyl-aminomethane (Tris), 0.06 M ascorbic acid and 0.005 M EDTA. The resulting homogenates were centrifuged at 18.000 *g* for 30 minutes. To the supernatants in small vials, about 5—10 mg/ml of Sephadex *G*-200 were added as an inert protein carrier, and the extracts were stored, frozen at —10°C.

Polyacrylamide gel electrophoresis was performed, adapting apparatus and general procedures of Davis (1964), but omitting the spacer gel and using only a photopolymerized small-pore gel layer of modified composition.

The gel for the anionic system was composed of 10 per cent acrylamide, 0.2 per cent *N,N'*-methylenebisacrylamide, 0.25 M Tris, 0.05 M HCl, 0.1 per cent triethanolamine and 0.5 mg per cent riboflavine-5-phosphate. The upper cathode buffer contained 0.01 M Tris and 0.08 M glycine, whereas the lower anode buffer was 0.1 M Tris-acetate at a pH of about 8.9.

The gel for the cationic system was composed of 10 per cent acrylamide, 0.2 per cent *N,N'*-methylenebisacrylamide, 0.2 M acetic acid, 0.05 M KOH, 0.01 M EDTA and 0.5 mg per cent riboflavine-5-phosphate. The upper anode buffer contained 0.005 M acetic acid and 0.04 M β-alanine, whereas the lower cathode buffer was composed of 0.05 M KOH and 0.2 M acetic acid.

The gels for both the anionic and cationic systems were prepared by photopolymerization of freshly prepared gel solutions in glass tubes placed in a special stand

between two day-light fluorescent lamps at a distance of 2—3 cm from each, for 20 minutes. The protein sample was layered directly on top of the small-pore gel under the upper buffer solution, and electrophoresis was carried out at 2 mA per tube, for 1.5 or 3 hours, until the marker dye (bromphenol blue for the anionic system, methyl green — for the cationic) reached the end of gel. All the electrophoretic runs were made in duplicate, with two parallel gels for a sample simultaneously in each run.

Enzyme Staining. For the demonstration of acid phosphatases and esterases, 1.0 ml aliquots of pre-cooled solution of pararosanilin (basic fuchsin) in 0.6 N hydrochloric acid were diazotized with the addition 0.2 ml of cold 3.5 per cent sodium nitrite. The mixtures were vigorously shaken and left standing in the cold. Just before staining, 10 ml of a solution containing 8.0 g/l sodium hydroxide and 11.6 g/l maleic acid were added for neutralization and buffering at a final pH of about 6, followed by either 0.2 ml 1-naphthyl phosphate solution (10 mg/ml, in ethanol) or 0.2 ml 1-naphthyl acetate (10 mg/ml, in acetone) to localize acid phosphatases or esterases, respectively. The gels were first held in a 0.1 M sodium maleate buffer at pH 5.8 and thereafter incubated in the described reaction mixture for about 20—120 min, until distinct reddish zones developed at the sites of enzyme activity.

Leucine aminopeptidase activity was revealed in a similar way, except by using *N*-leucyl-2-naphthylamine as a substrate and freshly diazotized *o*-dianisidine as a coupler.

Peroxidase isoenzymes were localized by incubating the gels for about 20 min in a mixture of eight volumes of 0.1 M acetate buffer at pH 4.8, with two volumes of 0.005 M substrate (*o*-dianisidine, benzidine) solution in 96 per cent ethanol. The sites of peroxidase activity were visualized by adding to the incubation mixture 0.2 ml 1.5 per cent hydrogen peroxide.

Nadi-oxidase activity was visualized in a freshly made solution consisting of 10 ml 0.1 M phosphate buffer at pH 7.2, 0.2 ml 2 per cent 1-naphthol in ethanol and 0.2 ml freshly made 2 per cent *N,N*-dimethyl-*p*-phenylenediamine in ethanol.

The gels were photographed in a transmitting light for a permanent record. The enzymograms were photographically enlarged to a convenient length, using the buffer front as a marker.

Results

Fig. 1 presents a photograph of enzymograms of anodically moving acid phosphatases (*A*), esterases (*B*) and leucine aminopeptidases (*C*) from the seeds and different vegetative organs of bean seedlings. For convenience, separate enzyme bands have been designated by the distances of migration from the origin to the anode given in arbitrary units.

Acid phosphatase enzymograms for ungerminated seeds and for seeds germinated in aerated distilled water for up to 48 hours revealed, on the whole, identical patterns consisting of at least four active fractions of low electrophoretic mobility. In addition, some enzymatic activity remained at the site of sample application. The sixth diffuse zone seen in these two enzymograms at the migration distance of about 3.5 to 3.8 was not due to the enzyme activity, but was caused by the precipitation of a nonactive protein during the incubation in the reaction mixture for acid phosphatases and was also developed in the controls without any added substrate.

Enzymograms for cotyledons from 9-day-old seedlings revealed the presence of highly active phosphatases, but two major zones of activity were too diffuse to distinguish the exact number of fractions from which they were composed. However, the general appearance of the enzyme pattern for cotyledons was clearly distinct from the pattern for ungermi-

nated and germinating seeds. In addition, one fast-moving acid phosphatase fraction, never seen in seed enzymograms, was present in cotyledons. Bean leaf, stem and root had similar acid phosphatase enzymograms consisting of two broad zones of diffuse appearance; they also revealed a much weaker fast-moving band identical, in electrophoretic mobility, with a fraction in the cotyledons. However, this band appeared only when longer reaction times were used, and for this reason it cannot be distinguished in the enzymograms presented in the figure.

The results show that acid phosphatase patterns of vegetative organs of young bean seedlings were clearly different from those for ungerminated or germinating seeds. At the same time, the patterns for different vegetative organs (leaves, stem, roots and cotyledons) were essentially similar, except for the presence of some additional activity in the cotyledons.

Esterase enzymograms (Fig. 1*B*) of bean seed and seedling tissues revealed the presence of a series of fractions, which, with the exception of one band, were of close electrophoretic mobility and fused together as a diffuse broad staining without allowing to compare the isoenzyme patterns exactly. Evidently, the polyacrylamide gel electrophoretic technique applied here is not suitable for fractionating bean esterases sufficiently. The presence of separate bands could be distinguished in the underdeveloped enzymograms only.

Leucine aminopeptidase patterns (Fig. 1*C*) for ungerminated and germinating seeds were identical, showing the presence of two bands at about 4.8 and 6.8. Some enzymatic activity remained at the site of sample application. A weak diffuse staining at about 3.5 was due to the precipitation of a nonactive protein in the reaction mixture and was also seen in the controls without substrate.

During the seedling development, the band at 4.8 disappeared, and the enzymograms for cotyledons of 9-day-old bean seedlings showed the presence of only a faster-moving leucine aminopeptidase band at 6.8. This band also appeared after longer reaction time in enzymograms for leaves, but it was never observed in enzymograms for the stem and root, which showed no band with leucine aminopeptidase activity. These results suggest the gradual disappearance of proteolytic leucine aminopeptidase activity in bean cotyledons and in vegetative organs in the course of seedling development.

Fig. 2 presents enzymograms of anodically and cathodically moving bean peroxidases and anodical nadi-oxidases.

Peroxidase enzymograms for ungerminated and germinating seeds in the anionic electrophoresis system (Fig. 2*A*) showed only a weak diffuse staining in the region from 1.0 to 2.0 and no distinct peroxidase fraction present. Band at about 4.0, and partially the band at the site of sample application were due to the precipitation of nonactive proteins in the reaction mixture for peroxidase, and they can be seen in the control as well. Cotyledons from 9-day-old bean seedlings showed the presence of six electrophoretically distinct and highly active anodical peroxidases (at 2.0, 2.3, 3.9, 4.1, 4.9 and 5.8), in addition to a diffuse broad zone of staining, extending from the site of sample application to about 1.5. Enzymograms from the leaf and stem revealed the presence of only four major anodical peroxidase fractions. Several fractions found to be present in cotyledons were absent from the leaf and stem tissue. In enzymograms for roots, up to eight electrophoretically distinct fractions could be distinguished. Four bands with the migration distances at about 0.1, 0.6, 2.3 and 5.8 were found to be in common to all the four bean seedling

organs studied. A couple of fast-moving bands at 6.9 and 7.1 were specific for roots only, since they were absent in enzymograms for the other three organs. Bands at 2.0 and 3.9 seem to be specific for cotyledons only, while those at about 4.1 and 4.9 were found both in cotyledons and roots, but were clearly absent in the leaf and stem tissue. In all, up to 10 electrophoretically distinct anodical peroxidase isoenzymes were demonstrated in the bean seedling, but no single organ possessed all the enzyme fractions.

Enzymograms of cathodically moving peroxidases (Fig. 2*B*) showed the presence, in four seedling organs, of up to four zones of enzymatic activity. Some of the zones were broad and diffuse, indicating that they may consist of several closely spaced fractions. Enzymograms for the cotyledons showed the presence of an additional organ-specific fraction at about 3.7—4.1, which was never seen in enzymograms for the other three seedling organs. The remaining three cathodic peroxidase zones were common in all the four organs, but differed in relative staining intensity and in zone broadness, which were characteristically different for each organ. Ungerminated as well as germinating bean seeds showed the presence of no distinct cathodic peroxidase fraction, except for a very slight staining at about 6.2.

The above results clearly demonstrate the formation, in bean seedling tissues, of many electrophoretically distinct peroxidase fractions, which were not found in nongerminated and germinating seeds. The presence of organ-specific peroxidase isoenzymes in some tissues of bean seedlings was observed, in addition to those isoenzymes which were common to all seedling organs.

From four to eight anodically moving bands of nadi-oxidase activity (Fig. 2*C*) could be observed in gels with the use of histochemical indophenole reaction. The patterns were characteristically distinct for each organ. No nadi-oxidase activity was found in enzymograms from seeds. Comparison of the nadi-oxidase enzymograms with those for anodically moving peroxidases (Fig. 2*A*) revealed general similarity between peroxidase and nadi-oxidase patterns for each seedling organ in the electrophoretic mobility of individual bands. The same was found for cathodically moving peroxidases and nadi-oxidases. Differences between peroxidase and nadi-oxidase patterns were mainly in the relative staining intensity of some fractions.

It is important to note that the nadi-oxidase enzymograms were obtained without adding any hydrogen peroxide to the reaction mixture, indicating that the formation of blue indophenole staining was due to the oxidase activity and was not caused by the peroxidase reaction. At the same time, we failed to detect any polyphenol oxidase activity in gels when incubating them in ordinary reaction mixtures for this enzyme containing 0.005 M 3,4-dihydroxyphenylalanine or 0.01 M catechol and 0.01 M glycine in 0.1 M phosphate buffer at pH 7.2.

These data seem to indicate that bean seedling peroxidase isoenzymes also possess the nadi-oxidase activity, the second one not being due to polyphenol oxidase activity.

Discussion

The results of our studies revealed distinct qualitative differences between the storage tissue of bean seed and the growing seedling tissue with respect to their enzyme complements. The polyacrylamide gel electrophoregrams showed the absence, in seedling tissues, of several enzyme fractions found in seeds, and, reversely, the presence of many other fractions, not found in the seed tissue.

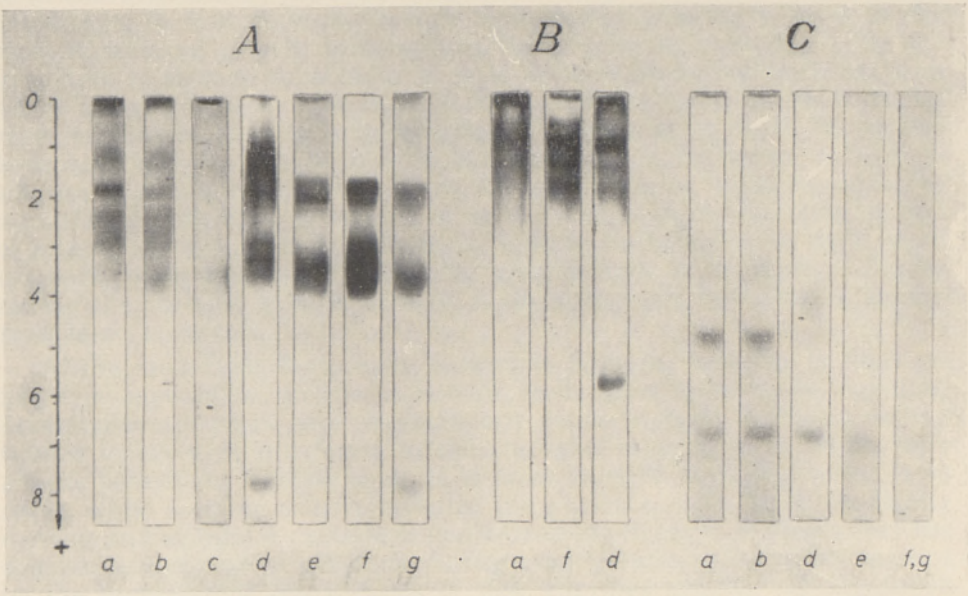

Fig. 1. Polyacrylamide gel electrophoretic patterns of kidney bean acid phosphatases (*A*), esterases (*B*), and leucine aminopeptidases (*C*). Enzymograms: *a* — ungerminated seeds, *b* — the seeds imbibed in aerated water for 48 hours, *c* — the control for seed without added substrate, *d* — cotyledons, *e* — leaf, *f* — stem, *g* — roots.

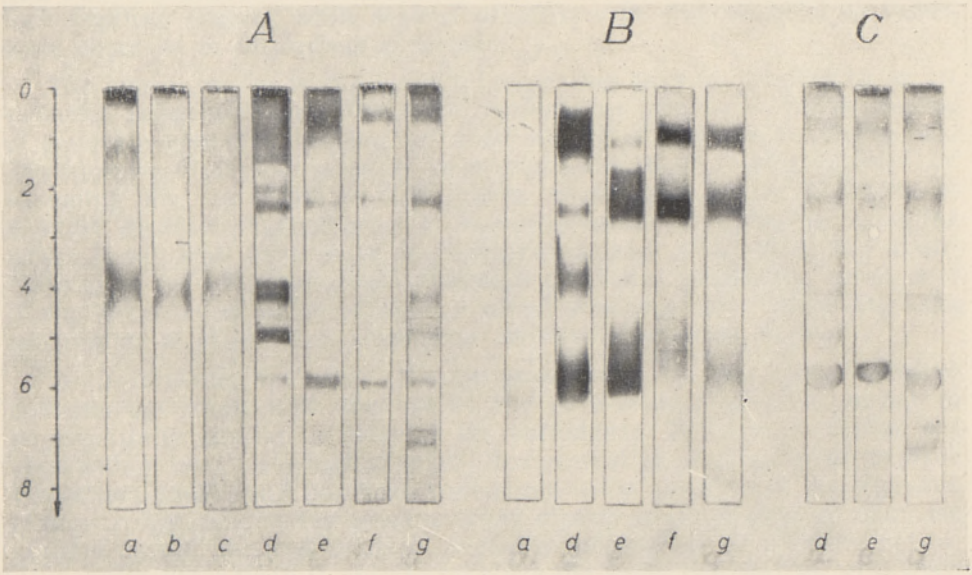

Fig. 2. Polyacrylamide gel electrophoretic patterns of anodical peroxidases (*A*), cathodical peroxidases (*B*), and anodical nadi-oxidases (*C*). Designations see under Fig. 1.

Germination of legume seeds is known (Danielson, 1951, and others) to be characterized by the hydrolysis of reserve proteins and other storage substances to low-molecular weight compounds which are used in the biosynthetic and growth processes in the developing seedling. The degradation of storage substances is catalyzed by various hydrolytic enzymes which must be present in the seed tissue. Indeed, our electrophoretic studies revealed the presence, in ungerminated and germinating seeds, of several hydrolytic enzymes — acid phosphatases, esterases and leucine aminopeptidases, in multiple molecular forms. Of these, leucine aminopeptidase as a carrier of proteolytic activity is of special importance in the degradation of reserve proteins. Two isoenzymes of leucine aminopeptidase were found in seeds, while one of them or both disappeared in vegetative tissues during the seedling growth without synthesizing any new leucine aminopeptidase isoenzyme.

At the same time, the present study has provided evidence demonstrating the appearance, in the course of seed germination and seedling development, of many new enzymatically active proteins in bean seedling tissues which were not found in the storage tissue of seeds. Thus, only a weak peroxidase fraction could be demonstrated in protein extracts from seeds, while seedling tissues revealed the presence of up to 10 anodically moving and at least 4 cathodical peroxidase isoenzymes. It shows that the degradation of reserve proteins during seed germination is accompanied by the induction of biosynthesis of new enzymes specific to vegetative organs.

Depending on the occurrence in different seedling tissues, two main types of isoenzymes could be distinguished. Some isoenzymes could be found in all or in several seedling tissues, while others were restricted to one particular organ, only. Thus, four anodical peroxidase isoenzymes and three cathodical peroxidase zones were found to be common to all the four seedling organs, while several organ-specific peroxidase isoenzymes were observed in cotyledons and roots.

The appearance of organ-specific isoenzymes may be interpreted as an evidence suggesting the differential activity, in various seedling tissues, of separate genes controlling the biosynthesis of individual isoenzymes. The absence of certain isoenzymes in some tissues may be explained by assuming the repression of the genes controlling their biosynthesis. Another possible explanation may be the presence of a specific inhibitor which forms with the enzyme an inactive complex which does not dissociate upon gel electrophoresis. Although the latter possibility seems less likely, the present study offers no direct evidence for explaining the mechanism of differential isoenzyme activity in separate tissues.

An interesting feature to be discussed here is that bean oxidase patterns developed with the use of the nadi-oxidase method proved to be essentially similar to those of the peroxidase. The Nadi reaction has been widely used for a histochemical demonstration of cytochrome oxidase (Burstone, 1962), and has been considered to be specific for this enzyme. However, the data of the present study clearly indicate that, in the case of plant tissues, the nadi-oxidase method is not specific for cytochrome oxidase, since peroxidase is able to catalyze the same reaction as well, even without any added hydrogen peroxide. Furthermore, the data show that bean peroxidase is able to exhibit oxidase activity towards certain substrates, e. g., towards *N,N*-dimethyl-*p*-phenylenediamine, while no *o*-diphenoloxidase activity towards catechol as a substrate could be detected in the gels upon electrophoresis. This finding extends the obser-

vations of several other investigators (Ivanova, Rubin, 1962; Kenten, Mann, 1953; Mazelis, 1962, and others) about the possible oxidase function of plant peroxidases.

Summary

Isoenzyme composition of acid phosphatase, esterase, leucine aminopeptidase, peroxidase, and nadi-oxidase in seeds and seedling tissues of kidney bean, *Phaseolus vulgaris* L., has been studied by means of polyacrylamide gel electrophoresis.

Distinct qualitative differences between the seed and seedling tissues in the electrophoretic patterns of enzymes were demonstrated. Two isoenzymes of leucine aminopeptidase were found in seeds, while one of them or both disappeared in the seedling tissues. Conversely, many new fractions of other enzymes not found in seeds appeared in the seedlings. Only a weak peroxidase fraction could be demonstrated in seeds, while the seedlings revealed the presence of up to 10 anodical and at least 4 cathodical peroxidase isoenzymes. Some of the isoenzymes were common in all or several seedling tissues, while others were restricted to one particular organ only.

Acid phosphatase patterns of seedling tissues were different from those of seeds. The patterns for different seedling organs were essentially similar, except for the presence of some additional activity in the cotyledons.

Nadi-oxidase and peroxidase patterns proved to be essentially similar for each tissue. It is concluded that bean seedling peroxidase is able to oxidize *N,N*-dimethyl-*p*-phenylenediamine without any added hydrogen peroxide. No *o*-diphenoloxidase activity towards catechol could be demonstrated.

REFERENCES

Barber J. T., Steward F. C., 1968. The proteins of *Tulipa* and their relation to morphogenesis. Developm. Biol. **17** (3) : 326—349.

Barber J. T., Wood H. L., Steward F. C., 1967. The separation of the proteins of wheat by acrylamide gel electrophoresis, with special reference to mottled and unmottled wheat. Canad. J. Bot. **45** (1) : 5—19.

Berg H., 1932. Über serologische Organspezifität bei Pflanzen. Ber. Dtsch. Bot. Ges. **50** : (93)—(99).

Besemer J., Clauss H., 1968. «Disc-Electrophorese» von löslichen Pflanzenproteinen. Z. Naturforsch. **23** b (5) : 707—716.

Burstone M. S., 1962. Enzyme Histochemistry and Its Application in the Study of Neoplasms. Acad. Press. New York and London.

Danielson G. E., 1951. The breakdown of the high-molecular reserve proteins of peas during germination. Acta Chem. Scand. **5** : 541—554.

Davis B. J., 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N. Y. Acad. Sci. **121** (2) : 404—427.

Dvořák M., Černohorská J., 1967. Peroxidases of different parts of the pumpkin plant (*Cucurbita pepo* L.). Biol. Plant. **9** (4) : 308—316.

Dudchenko L. G., Sytnik K. M., 1967. On qualitative composition of proteins of growth zones of the root. C. r. Acad. Sci. Ukrainan S.S.R. Ser. B (11) : 1024—1027 (in Ukrainian, English summary).

Evans J., Alldridge N. A., 1965. The distribution of peroxidases in extreme dwarf and normal tomato (*Lycopersicon esculentum* Mill.). Phytochemistry **4** (3) : 499—503.

Ghetie V., Buzila L., 1964. The modification of antigenic properties of tissues during seed germination and development of horse bean plants (*Vicia faba*). Biol. Plant. **6** (3) : 202—208.

Hadačová V., Sahulka J., 1967. Electrophoretic investigation of protein in different root zones of *Vicia faba* L. Biol. Plant. **9** (5) : 396—400.

Hunter R. L., Markert C. L., 1957. Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. Science **125** (3261) : 1294—1295.
Ivanova T. M., Rubin B. A., 1962. On the nature of phenoloxidase action of peroxidase. Biochem. (U.S.S.R.) **27** (4) : 622—630 (in Russian, English summary).
Kenten R. H., Mann P. J. G., 1953. The oxidation of certain dicarboxylic acids by peroxidase systems in the presence of manganese. Biochem. J. **53** : 498—505.
Khavkin E. E., Varakina N. N., Peschkova A. A., 1967. Isolation and fractionation of soluble proteins from the root growth zones of maize seedlings In "Growth and Cellular Differentiation of Plants". Publishing House "Nauka", Moscow: 44—55 (in Russian).
Kloz J., Turková V., Klozová E., 1960. Serological investigation of taxonic specificity of proteins in various plant organs in some taxons of the family *Viciaceae*. Biol. Plant. **2** (2) : 126—140.
Laufer H., 1964. Macromolecular patterns in development and evolution. In "Taxonomic Biochemistry and Serology". Ed. by Ch. A. Leone. The Roland Press Company, New York, 171—189.
Macko V., Honold G. R., Stahman M. A., 1967. Soluble proteins and multiple enzyme forms in early growth of wheat. Phytochemistry **6** (4) : 465—471.
Macnicol P. K., 1966. Peroxidases of the Alaska pea (*Pisum sativum* L.). Enzymic properties and distribution within the plant. Arch. Biochem. Biophys. **117** (2) : : 347—356.
Manwell C., 1966. Metamorphosis and gene action. Electrophoresis of dehydrogenases, esterases, phosphatases, hemoglobins and other soluble proteins of tadpole and adult bullfrogs. Comp. Biochem. and Physiol. **17** (3) : 805—823.
Markert C. L., Møller F., 1959. Multiple forms of enzymes: tissue, ontogenetic, and species specific patterns. Proc. Nat. Acad. Sci. U.S.A. **45** (5) : 753—763.
Mazelis M., 1962. The pyridoxal phosphate-dependent oxidative decarboxylation of methionine by peroxidase. I. Characteristics and properties of the reaction. J. Biol. Chem. **237** (1) : 104—108.
Morris R. O., 1966. Changes in pea root proteins associated with tissue differentiation. The effect of 2,4-dichlorophenoxyacetic acid. Biochim. Biophys. Acta **127** (1) : 273—276.
Mäkinen Y., 1968. Isoenzyme polymorphism in flowering plants. VI. Variation of isoenzyme patterns in onion seedlings. Physiol. Plant. **21** (4) : 858—865.
Racusen D., Foote M., 1966. Peroxidase isoenzymes in bean leaves by preparative disc electrophoresis. Canad. J. Bot. **44** (12) : 1633—1638.
Scandalios J. G., 1964. Tissue-specific isoenzyme variations in maize. J. Heredity **55** (6) : 281—285.
Siegel B. Z., Galston A. W., 1967. The isoperoxidases of *Pisum sativum*. Plant Physiol. **42** (2) : 221—226.
Steward F. C., Lyndon R. F., Barber J. T., 1965. Acrylamide gel electrophoresis of soluble plant proteins: a study on pea seedlings in relation to development. Amer. J. Bot. **52** (2) : 155—164.
Upadhya M. D., Yee J., 1968. Isoenzyme polymorphism in flowering plants. VII. Isoenzyme variations in tissues of barley seedling. Phytochemistry **7** (6) : 937—944.
Weimberg R., 1968. An electrophoretic analysis of the isoenzymes of malate dehydrogenase in several different plants. Plant Physiol. **43** (4) : 622—628.
Wright S. T. C., 1963. Cellular differentiation at the molecular level with special reference to proteins. Symposia Soc. Exptl Biol. **17** : 18—38.

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AEDOA ENSÜÜMIDE HETEROGEENSUSEST JA KOESPETSIIFILISUSEST

Resümee

Polüakrüülamiidgeelelektroforeesi abil uuriti aedoa *Phaseolus vulgaris* L. seemne ja tõusmete happelise fosfataasi, esteraasi, leutsiinaminopeptidaasi, peroksüdaasi ja nadioksüdaasi isofermentset koostist ja selle organispetsiifilisust.

Seemne ensümogrammil täheldati kahte leutsiinaminopeptidaasi fraktsiooni, kuna tõusmete erinevates organites ilmnes vaid üks neist või puudusid mõlemad. Tõusmetel

ilmnesid paljud uued fraktsioonid, mis ei esinenud seemnel. Nii oli seemne peroksüdaasne aktiivsus madal, tõusmete ensümogrammidel leiti aga kuni 10 anoodset ja vähemalt 4 katoodset peroksüdaasi isoensüümi. Mõned isoensüümid olid ühised paljudele kudedele, teised omased vaid tealud taimeorganitele.

Tõusmete erinevate organite happelise fosfataasi ensümogrammid, välja arvatud mõned täiendavad fraktsioonid idulehtede puhul, olid üldiselt sarnased, erinesid aga seemne ensümogrammidest. Tõusmete peroksüdaasi ensümogrammid sarnanesid vastavate organite nadi-oksüdaasi ensümogrammidega. Seega oksüdeerib oatõusmete peroksüdaas *N,N*-dimetüül-*p*-fenüleendiamiini ka ilma vesinikülihapendi lisamiseta. *o*-difenooloksüdaasset aktiivsust pürokatehhiini suhtes ei täheldatud.

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Saabus toimetusse
3. II 1969

ВИЛЬВЕ ЯАСКА, ВЕЛЛО ЯАСКА

МНОЖЕСТВЕННОСТЬ И ТКАНЕВАЯ СПЕЦИФИЧНОСТЬ НЕКОТОРЫХ ФЕРМЕНТОВ У ФАСОЛИ

Резюме

Методом электрофореза в полиакриламидном геле показаны определенные качественные различия в изоферментном составе кислой фосфатазы, эстеразы, лейцинаминопептидазы, пероксидазы и нади-оксидазы семян и проростков фасоли *Phaseolus vulgaris* L. В семенах определены две фракции изоферментов лейцинаминопептидазы, из которых одна или обе отсутствовали в проростках. Пероксидазная активность в семенах, напротив, была незначительной, тогда как в тканях проростков обнаружено до 10 анодных и не менее 4 катодных фракций изоферментов пероксидазы. Многие фракции изоферментов были общими для всех тканей проростков, а некоторые свойственны только определенным органам.

Энзимограммы кислой фосфатазы у различных органов проростков были в большой мере сходными и отличались от таковых у семян.

Энзимограммы нади-оксидазы и пероксидазы у соответствующих тканей в большой мере сходны. Пероксидаза проростков фасоли способна окислять *N,N*-диметил-*n*-фенилендиамин и без добавления перекиси водорода. *o*-Дифенолоксидазной активности относительно пирокатехина не обнаружено.

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