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The Differentiation of α- and β-Glucosidase and α- and β-Galactosidase Isoenzymes from Maize and Broad Bean Root Tips Using Disc Electrophoresis in Polyacrylamide Gels

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Abstract. For the separation of α- and β-glucosidase and α- and β-galactosidase isoenzymes from *Zea mays* L. and *Vicia faba* L. root tips the system of disc electrophoresis in polyacrylamide gel developed for basic protein separation proved most suitable. The detection was carried out by a simultaneous azocoupling reaction. In maize α-glucosidase was not detected, β-glucosidase gave 3, α-galactosidase 4, and β-galactosidase 3 zones. In broad bean α- and β-glucosidases were absent, α-galactosidase gave 2 and β-galactosidase 3 zones. α- and β-galactosidase activity zones correspond principially to each other in their position. In maize one zone gives a positive reaction for both β-glucosidase and α- and β-galactosidase.

Within the framework of the biochemical study on cell growth and differentiation the isoenzyme pattern in the meristematic, elongation and maturation zones of root tips has been investigated in our laboratory (HADAČOVÁ and BENEŠ 1977). In this respect we examined glycosidases, not only because they represent important enzymes of the carbohydrate metabolism also utilized in certain cases in industrial processes, but considering especially the fact that the regulation of their synthesis and activity is associated with fundamental physiological phenomena in the cell and organism.

In spite of the fact that for the reasons mentioned considerable attention has been paid to these enzymes, relatively few papers are cited on the identification of glycosidase isoenzymes by disc electrophoresis in polyacrylamide gels (SCANDALIOS 1974, MARKERT 1975). This is true not only of the plant material. Our preliminary experiments revealed that in comparison with our previous studies on hydrolases, this problem would be much more difficult.

The glycosidases in root tips were examined from various points-of-view by a number of authors (ASHFORD 1970, ASHFORD and MCCULLY 1970, 1973, BENEŠ *et al.* 1973, SAVOST'YANOVA *et al.* 1975, ZELENEVA *et al.* 1975, GIERSE and BARZ 1976). The present study was done as an attemp to extend the present knowledge by investigating glycosidase isoenzymes in root tips of maize and broad bean. For this purpose a suitable technique for the electrophoretic separation in polyacrylamide gels, as well as for the detection by means of colour reactions, had to be developed.

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Material and Methods

12 mm long root tips of maize *Zea mays* L. cv. Český bílý koňský zub, which germinated for 2 days, and those of broad bean *Vicia faba* L. cv. Chlumecký, which germinated for 3 days (HADAČOVÁ 1972) were used for the experiments.

The material was homogenized in a mortar at 2 °C using 0.05 M Tris-glycine buffer pH 8.3 when electrophoresis was done according to DAVIS (1964) and in 0.2 M acetate buffer pH 5.0 in the case of the electrophoresis according to REISFELD *et al.* (1962). In both cases ascorbic acid and sucrose were added at final concentrations of 1 and 20 per cent, respectively. The ratio of the root weight to the buffer solution was 1 : 1.

For some experiments the solubilization of bound enzymes was carried out using, on the one hand the application of Triton X-100 into the medium for homogenization (final concentration 1 per cent), or on the other repeated freezing and thawing. In the latter case the plant material along with the homogenization medium in a plastic test tube was cooled in solid CO_2 for 5—10 min, then thawed in a water bath at 40 °C for 5—10 min. The procedure was repeated three times.

The homogenates were centrifuged in a cooled Janetzki K 24 centrifuge for 24 min at 22 000 *g*. The supernatant thus obtained was taken up in 100 or 200 μl amounts by means of a syringe to prevent the contamination of the samples with the material accumulated on its surface in the case of maize.

The separation of isoenzymes was performed by electrophoresis in polyacrylamide gels, partly according to DAVIES (1964), partly using a modified procedure according to REIFSFELD *et al.* (1962): 7.5 per cent gels and half-concentrated buffer.

For isoenzyme detection the following substrates (mostly products of Lachema Brno, Czechoslovakia) were available: 6-bromo-2-naphthyl-α-D-glucoside, 6-bromo-2-naphthyl-β-D-glucoside, 6-bromo-2-naphthyl-α-D-galactoside, 6-bromo-2-naphthyl-β-D-galactoside, 1-naphthyl-β-D-glucoside, 1-naphthyl-β-D-galactoside. They were used as 0.3 per cent stock solutions in dimethylformamide. For azocoupling Fast blue RR salt (Lachema Brno, Czechoslovakia, and G. T. Gurr, London, England) was employed. The medium for simultaneous coupling consisted of 20 ml buffer pH 6.5 (prepared from 0.1 M citric acid and 0.2 M Na_2HPO_4), 1 ml stock substrate solution and 20 mg diazonium salt. The same buffer was used in post coupling for the preparation of the medium with substrate and the medium with diazonium salt, the concentration of ingredients being the same as with simultaneous coupling (BENEŠ *et al.* 1973). For each experiment fresh media were prepared. Simultaneous azocoupling took 4 h, in post coupling the gels were 3 h in the medium with substrate and 3 h in that with diazonium salt, all at room temperature. After detection the gels were stored in 7 per cent acetic acid. The post coupling served in the study on the effect of pH on the activity of individual isoenzymes. 0.2 M phosphate buffer was used for the preparation of the medium with diazonium salt, as well as for that with substrate. The acidic substrate medium was prepared of 0.2 M NaH_2PO_4 (pH 4.4), the basic medium of 0.2 M Na_2HPO_4 (pH 8.9). The increased buffer concentration was necessary to prevent greater changes in pH after the transfer of gels into media (without further washing). The pH of buffers and of media before and

after incubation was checked on a pH meter (with a glass electrode), differences fluctuating within tenths of pH from the given values being considered acceptable.

The gels were evaluated both visually and densitometrically on an adapted Zeiss ERI 65 densitometer, considering their quality (position and number of zones) and quantity (width and colour intensity of zones), as described earlier (HADAČOVÁ and BENEŠ 1977).

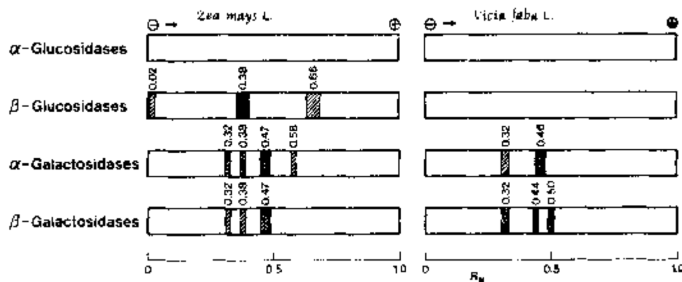

Fig. 1. The diagram of the distribution of isoenzymes of α- and β-glucosidase and α- and β-galactosidase in maize and broad bean roots. Hatched area: weak activity; cross-hatched area: medium activity; black area: strong activity.

Results

The electrophoretic procedure according to DAVIS (1964) did not prove suitable for the enzymes examined. A considerable portion of proteins with glucosidase and galactosidase activity remained on the start. If any zones appeared, they were diffuse and their number fluctuated. The results were not reproducible, especially with the broad bean. For this reason we recommend a modified procedure according to REISFELD *et al.* (1962) as a standard technique.

Concerning detection no substantial differences were obtained when using corresponding substrates on the basis of 1-naphthol and 6-bromo-2-naphthol. No greater discrepancies appeared between the simultaneous procedure and post coupling either. 6-Bromo-2-naphthyl-glycosides in simultaneous coupling were used in standard detection.

Fig. 1 presents the results obtained by means of the standard technique recommended. With broad bean no isoenzymes of α- and β-glucosidase were detected; α-galactosidase exhibits 2 zones, β-galactosidase 3, 2 of which correspond to α-galactosidase in their position. In maize the reaction for α-glucosidase was negative; β-galactosidase gives 3 zones, α-galactosidase 4, β-galactosidase 3 which correspond to 3 zones of α-galactosidase concerning their position. In broad bean and maize the zones of α- and β-galactosidase thus principally correspond to each other. It is remarkable that in maize one zone of β-glucosidase is conformable with one zone of α- and β-galactosidase.

On using Triton X-100 and repeated freezing-thawing no new isoenzymes appeared. Only the mobility and activity of the isoenzymes already revealed were slightly affected.

Both in maize and broad bean the dependence was studied of isoenzyme activity on pH (post coupling). No qualitative differences were found in comparison with the standard procedure. The individual isoenzymes did not give any striking response to pH changes, either. The total activity, *i.e.* the intensity of colouring of β-glucosidase zones increases with increasing pH, whereas the activity of galactosidases decreases in alkaline media.

Discussion

This paper aimed at finding suitable techniques for the plant material investigated concerning the separation of glycosidase isoenzymes by means of electrophoresis in polyacrylamide gels and their detection. As was shown the detection by colour reaction caused no problem, but it was difficult to develop a suitable separation system. The system according to DAVIS (1964) currently used gave no reliable results in the separation of glycosidases from root tips as referred to also by KOMP and HESS (1977). On the other hand, the electrophoretic procedure according to REISFELD *et al.* (1962), developed for the separation of basic proteins, proved to be suitable. This fact might give evidence in favour of the basic character of molecules of the enzymes (isoenzymes) investigated. A more detailed analysis of our results in this respect is beyond the scope of this paper. The electrophoretic system according to DAVIS (1964) involves the use of Tris-buffers. It is well-known that certain glycosidases are inhibited by these buffers (MURRAY and BANDURSKI 1975). No sufficient data are available to correlate the isoenzyme number with that of subunits of the enzyme given (see *e.g.* PRIDHAM and DEY 1974).

Concerning the identity of the enzymes examined, it is based only on the hydrolysis of synthetic naphtholic substrates. Inhibition tests (LEVVY and SNAITH 1972, LEGLER 1973) were not carried out. The enzymological identification of individual glycosidases still represents the subject of special studies (group specificity: specific disaccharidases, see *e.g.* LOJDA *et al.* 1973).

The use of colour reaction of various types for glycosidase detection which are available in contemporaneous histochemistry (PEARSE 1972), proved unnecessary. The azocoupling reactions were preferred because of an easy availability of the reagents.

We have already mentioned the fact that glycosidases — if one may generalize — participate in a series of important physiological processes on both cell and organism level.

It is well-known that glycosidases represent classical enzymes of the studies on induction and repression. The genetics of glycosidases were studied in many systems (see *e.g.* RICHMOND 1968, PIRAS and PONTIS 1972). Certain studies point out a "programmed" character of the synthesis or activity of glycosidases in the course of ontogenesis and differentiation (see *e.g.* PAIGEN *et al.* 1976). Certain glycosidases exhibit a detoxication function (FISHMAN 1970). Considerable attention has been paid to the intracellular localization of glycosidases in connection with lysosomal conception (TOUSTER 1973).

As for plant material, or root tips, the papers have already been referred to which deal with the localization of glycosidases in these subjects. It is probable that the mechanisms are responsible for the localization of enzymes in individual tissue complexes which are analogical to those participating in

the induction or "programming". As for the "programming" of glycosidases, the study of their dynamics in the course of germination (PRIDHAM and DEY 1974) should be mentioned. The processes of conjugation and hydrolysis, participating in the metabolism of carbohydrates and phenolic substances in general, evidently represent an analogy to detoxication mechanisms. In this way the level of growth substances in plants is regulated, as well (SEMBDNER 1974). Recently, glycosidases have been investigated mostly in connection with their localization on the cell surface. This involves both lignification and growth of cell walls. Considering the latter case, the loosening of the cellulose net of cell walls is the necessary prerequisite for the volume growth. It thus appears that glycosidases play an important role here and that they participate in the effect of auxin on this process (EVANS 1974, JOHNSON *et al.* 1974, MURRAY and BANDURSKI 1975). In connection with root function their ability to excrete glycosidases (GÖRING 1966) should be remembered, and, on the contrary, the participation of these enzymes in sugar uptake (GÖRING 1970).

All facts referred to indicate the possibility of a multilateral utilization of the methods of separation and detection of glycosidase isoenzymes described in the present paper.

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BOOK REVIEW

HILLEL, D.: **Computer Simulation of Soil-Water Dynamics: A Compendium of Recent Work.** — International Development Research Centre, Ottawa 1977. 214 pp. $ 10.00, in English.

The real world, or any system within it, is too complex for our limited intellect to comprehend or to define in its entirety. Mathematical modeling and simulation techniques, relying on the use of high-speed computers, can help us to reduce this hopeless complexity to manageable, orderly proportions. In the process of designing, operating and validating simulation models enable us to gain insight into the dynamics of the complex natural system and develop criteria for predicting its future behaviour under varying conditions.

This readable monograph describes a number of models of soil — plant — water relations. The first two chapters describe models of the evaporating processes (the isothermal evaporation and the non-isothermal evaporation, including the effect of fluctuating evaporativity, the effect of soil energy balance, the rate of hysteresis *etc.*). The model study of the profile moisture dynamics in relation to the soil texture and the hydraulic properties is the subject of the third chapter. The next chapter deals with the hydrological balance of fields with variable slope and textural composition. The most complicated model is the last one devoted to the water uptake by plant roots and concurrent movement of water and salt in the soil profile. For the readers who are not familiar with computer programming the introduction describing principles of the mathematical modeling is also very useful.

It is necessary to highly appreciate the critical approach of the author to computer simulation. He clearly shows not only its advantages but also its disadvantages. He emphasizes that simulation is not a substitute for experimentation but it can help to economize experimentation by guiding it to where it is most needed.

The text is illustrated with many figures and listing of programs written in CSMP language.

The book is a valuable contribution towards the endeavour to enhance the efficiency of the soil and water management without damaging the environment.

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