BIOLOGIA PLANTARUM (PRAHA)

28 (4) : 270—274, 1986

Localization of Acid Phosphatase Activity in Maize Root under Phosphorus Deficiency

MARIE KUMMEROVÁ

Department of Plant Biology, Faculty of Science, University of J. E. Purkyně, Kotlářská 2, 611 37 Brno, Czechoslovakia

Abstract. The effect of phosphorus deficiency on acid phosphatase activity in the apical, middle and basal parts of the root of maize plants was followed. The supernatant obtained by centrifuging the homogenate of plant tissue at $1500 \times g$ was further centrifuged at $18\,000 \times g$, the sediment being marked as fraction II and the supernatant as fraction III. The results obtained document the fact that acid phosphatase activity of the two fractions of all analyzed root segments was higher in plants cultured in nutrient medium without phosphate than in those containing phosphorus in nutrient medium. In most cases this difference was significant to highly significant. The results of experiments proved unambiguously a higher enzymatic activity in all root segments in fraction III than in fraction II. In fraction III the highest acid phosphatase activity was found in the apical part, in fraction II in the basal part of the root.

In different plant species a number of authors (PRICE 1962, BLUM 1965, BIELESKI 1968, 1973, 1974, REID and BIELESKI 1970, CLARK 1975, BESFORD 1980, LACHLAN 1980, BOUTIN *et al.* 1981, KUMMEROVÁ and BUCZEK 1983, PRESS and LEE 1983) have found that phosphorus deficiency in nutrient medium causes an increase in acid phosphatase activity in both root and leaf homogenates.

The increased activity is closely related to the important function phosphorus has in the plant organism (REBEILLE *et al.* 1982). In this connection the problem of its determination as a biochemical assay for judging phosphorus deficiency is more and more often discussed. Conventional methods for determining the needs of plant nutrition include analyses of the mineral component of plant tissue or the root environment, but they cannot explain changes in the plant organism. They are often employed only after visual symptoms of the deficiency have appeared, complementing them only in the quantitative aspect.

A set of problems of no less importance that is being studied at present concerns the choice of the plant organ, tissue and/or subcellular structures whose analysis would best characterize the consequences of the deficiency of the element in question. Thus, EMMERT (1983) in papers dealing with the uptake and translocation of phosphorus in the plant organism, in view of its centripetal transport, divides the root system into individual segments.

Received February 23, 1984; accepted April 22, 1985

With respect to the above problems and continuing our previous papers (KUMMEROVA 1983) we have approached the study of changes in acid phosphatase activity in some subcellular fractions from different isolated segments of maize root.

MATERIAL AND METHODS

Maize plants (*Zea mays* L. cv. CE 170) after germinating on filter paper were placed for 2—3 days on gauze stretched across crystallizing dishes filled with distilled water. Then endosperm was removed and germinating plants were cultured for 5 days in a solution 2.5 mM $CaSO_4$ and 1 mM $MgSO_4$ diluted with distilled water in the 1 : 10 ratio. The plants were then transferred into a solution with phosphate (5 mM KNO_3, 5 mM $Ca(NO_3)_2 . 4 H_2O$, 1 mM KH_2PO_4, 1 mM $MgSO_4 . 7 H_2O$, additions of Fe and microelements) and a solution without phosphate where 1 mM KH_2PO_4 was substituted by 1mM K_2SO_4. In aerated solutions the plants were grown at 28 °C. Acid phosphatase activity was determined separately in the individual root segments (apical segment 0—2 cm, middle segment 2—4 cm from the apex and basal segment) in 48 h intervals.

After homogenization of the individual root segments, washed beforehand with distilled water, the enzyme was extracted in a solution of 0.1 M Na acetate pH 4.5 and 0.25 M saccharose. 10 ml of the extracting solution was used per 1 g of plant tissue.

The homogenate was centrifuged at $1500 \times g$ (fraction I) for 15 min and the supernatant afterwards at $18\,000 \times g$, also for 15 min. The sediment suspended with the extraction solution, was denoted fraction II and the supernatant fraction III. The whole process was carried out at 0—4 °C.

Acid phosphatase activity was measured in the total volume of 4 ml. Incubation medium consisted of 2 ml 0.1 M Na acetate pH 4.5 and 25 mM KCl; 0.5 ml pNPP (para-nitrophenyl phosphate) and 0.5 ml of the enzymatic preparation from the given fraction. Incubation took place at 37 °C for 30 min, then the reaction was stopped by adding 1 ml cool 20 % TCA. Inorganic phosphorus liberated from pNPP was determined colorimetrically according to FISKE and SUBBAROW (1925).

Acid phosphatase activity was expressed in μmoles of liberated P_i per gram of fresh matter per hour. The results given are average values from three repetitions. They were evaluated statistically by means of the *t*-test.

RESULTS AND DISCUSSION

Despite a number of positive results obtained in studying the relations between phosphorus content in the medium and its uptake, distribution and metabolism we did not quite succeed in clarifying all ways of controlling the above processes.

Interesting in this respect are doubtless the results of BIELESKI (1968) and REBEILLE *et al.* (1982) who divided the cellular content of P_i into the metabolical pool (cytoplasm, plastids, mitochondria) and the non-metabolical one (vacuoles). Plant growth is safeguarded above all by exogenous phosphorus. At its deficiency the growth is limited by the rate of P_i transport across the tonoplast and tissues into the vegetation apex. The growth is stopped when the supply of non-metabolical phosphate is exhausted. Similar

conclusions in studying phosphorus stress has also been arrived at by CLARKSON and SCATTERGOOD (1982).

A multipurpose and irreplaceable function of the phosphorus anion in the plant organism is rendered more significant by its relatively easy redistribution and reutilization.

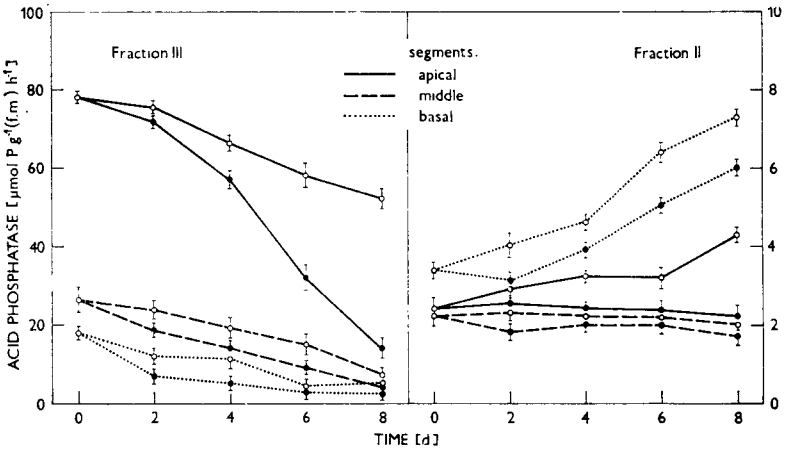

Fig. 1. Acid phosphatase activities (in fractions III and II) in maize roots in the presence (●) or absence (○) of inorganic phosphate in nutrient solution.

From a number of papers quoted in the introductory part of this paper it is evident that the amount of mobile phosphorus in plant organism under phosphorus deficiency is also controlled by the activity of acid phosphatase, an enzyme releasing the phosphate anion hydrolytically from esteric bonds. This fact has been demonstrated in maize plants both in our preceding paper (KUMMEROVA 1983) and in the study of acid phosphatase activity in subcellular structures of the individual segments of the primary root. The graphic representation of the results (Fig. 1) clearly shows that throughout the experiment in both fractions (fractions III and II) acid phosphatase activity in the apical, middle and basal parts of the root was higher in plants cultured in a deficient medium.

By dividing the root into apical, middle and basal segments we wanted to express the possible relation of acid phosphatase activity to certain parts of the root under conditions of phosphorus deficiency. Some authors using cytochemical methods and electron microscopy have proved increased acid phosphatase activity in places with intense metabolic activity, such as the regions of differentiation of blossom primordia (ANDERSET and GREPPIN 1975), in the palisade and spongy parenchyme of leaves cultured in deficient medium (BESFORD and SYRED 1979), or in cells of the forming xyleme of the primary root (LUX 1982).

From the graphic (Fig. 1) representation of the results a several times higher acid phosphatase activity is evident in cytosol fraction III of the apical segment of the primary root of maize in comparison with the middle and

basal segments. This finding can be understood in view of the fact that in this segment intense cellular division and differentiation of plant tissues take place. The assumption that the phosphate anion is necessary for the synthesis of phosphorylated forms of proteins has been confirmed by determining a greatly increased acid phosphatase activity in this cytosol fraction (fraction III).

The results obtained are in accordance with the conclusions reported by BOUTIN *et al.* (1981) who speak of increased activity of surface phosphatases of roots, due above all to the growth of new roots with higher enzyme activity under the conditions of phosphate deficiency.

In studying the relation of enzyme activity in the particular fraction (fraction II) of the individual root segments of maize to phosphorus deficiency in the nutrient solution, its many times lower activity was found in comparison with acid phosphatase activity in the cytosol fraction (Fig. 1).

The values of activities expressed in the amount of liberated ortophosphate from pNPP in fraction II from the beginning of plant culture remained either on the same level or increased (particularly in the basal root segment).

We are aware that after bringing about phosphorus deficiency not only reutilization but also distribution into overground organs in the experimental plants must have taken place. The above results give some basic data for physiological interpretation, but more general conclusions can be drawn only after the results of further experiments are considered.

REFERENCES

ANDERSET, G., GREPPIN, H.: Mise en évidence de deux types d'activité phosphatatique acide dans le meristème. — Saussurea **6** : 259—269, 1975.

BESFORD, R. T.: A rapid tissue test for diagnosing phosphorus deficiency in the tomato plant. — Ann. Bot. **45** : 225—227, 1980.

BESFORD, R. T., SYRED, A. D.: Effect of phosphorus nutrition on the cellular distribution of acid phosphatase in the *Lycopersicon esculentum* L., — Ann. Bot. **43** : 431—435, 1979.

BIELESKI, R. L.: Effect of phosphorus deficiency on levels of phosphorus compounds in *Spirodela*. — Plant Physiol. **43** : 1309—1316, 1968.

BIELESKI, R. L.: Phosphate pools, phosphate transport and phosphate availability. — Annu. Rev. Plant Physiol. **24** : 225—252, 1973.

BIELESKI, R. L.: Development of an externally-located alkaline phosphatase as a response to phosphorus deficiency. — In: BIELESKI, R. L., FERGUSON, A. R., CRESSWELL, M. M. (ed.): Mechanisms of Regulation of Plant Growth. Pp. 165—170. Bull. 12 Roy, Soc. New Zeeland, Wellington 1974.

BLUM, J. J.: Observations on the acid phosphatase of *Euglena gracilis*. — J. Cell Biol. **24** : 223 to 234, 1965.

BOUTIN, J. P., PROVOT, M., ROUX, L.: Effect of cycloheximide and renewal of phosphorus supply on surface acid phosphatase activity on phosphorus deficient tomato roots. — Physiol. Plant. **51** : 353—360, 1981.

CLARK, R. B.: Characterization of phosphatase of intact maize roots. — J. agr. Food Chem. **23** : 458—460, 1975.

CLARKSON, D. T., SCATTERGOOD, CH. B.: Growth and phosphate transport in barley and tomato plants during the development of, and recovery from, phosphate-stress. — J. exp. Bot. **33** : 865—875, 1982.

EMMERT, F. H.: Comparison of centripetal passage of phosphorus across roots versus accumulation in individual cells, with respect to pH and ion supply. — J. Plant Nutrition **6** : 267—274, 1983.

FISKE, C. H., SUBBAROW, Y.: The colorimetric determination of phosphorus. — J. biol. Chem. **66** : 375—400, 1925.

KUMMEROVA, M.: The effect of phosphorus on activity of acid phosphatase in maize (*Zea mays* L.) — Scripta Fac. Sci. nat. Univ. Purk. Brun. **13** : 343—348, 1983.

KUMMEROVA, M., BUCZEK, J.: Nitrate reductase and acid phosphatase activities as affected by inorganic phosphate in corn roots. — Acta Soc. Bot. Pol. **52** : 77—86, 1983.
LACHLAN, K. D.: Acid phosphatase activity in intact roots and phosphorus nutrition in plants. I. Assay conditions and phosphatase activity. — Aust. J. agr. Res. **31** : 429—440, 1980.
LUX, A.: Ontogenesis of xylem cells in barley roots. II. Ultrastructural study of the acid phosphatase. — Acta Fac. Rer. nat. Univ. Comen., Physiol. Plant. **18** : 43—49, 1982.
PRESS, M. C., LEE, J. A.: Acid phosphatase activity in *Sphagnum* species in relation to phosphate nutrition. — New Phytol. **93** : 567—573, 1983.
PRICE, C. A.: Repression of acid phosphatase synthesis in *Euglena gracilis*. — Science **135** : 46, 1962.
REBEILLE, F., BLIGNY, R., DOUCE, R.: Regulation of P_i uptake by *Acer pseudoplatanus* cells. — Arch. Biochem. Biophys. **219** : 371—378, 1982.
REID, M. S., BIELESKI, R. L.: Changes in phosphatase activity in phosphorus-deficient *Spirodela*. — Planta **94** : 273—281, 1970.

BOOK REVIEW

HOPPE, W., LOHMANN, W., MARKL, H., ZIEGLER, H. (ed.): BIOPHYSICS. — Springer Verlag, Berlin—Heidelberg—New York—Tokyo 1983. 940 pp. Cloth DM 168,—; approx. US $ 65.20.

The first English edition of Biophysics based on the second German edition has been now offered to a wider, more international audience. The editors of this volume have chosen for such interdisciplinary field as biophysics the form of a multi-author book. If the book consists of a collection of articles by experts in each speciality, there is a good chance that the contributions are up to date and at the same time present the essential points in each special field. This idea has been amply confirmed by this book.

It is impossible to do biophysics without having a certain background knowledge in biology, physics, physical chemistry, chemistry and biochemistry. It is useful and less time-consuming to present a selection of supplementary knowledge in concentrated form, together with the subject matter specific to biophysics. The reader will thus find in this book introductions to such subjects as the structure and functions of the cell, the chemical structure of biogenic macromolecules and even theoretical chemistry. The comprehensive publication consists of 17 chapters which deal with different biophysical problems such as the structure of cell, the chemical structure and structure determination of biologically important macromolecules, molecular interactions between biomolecules and mechanism of energy transfer. Other chapters inform on a subject of radiation biophysics and isotope methods. Several chapters are devoted to enzymes, biological function of nucleic acid, photobiophysics and neurobiophysics. Special attention is given to the subject of biomembranes. Cybernetics in biology, a field which is just developing, is also noticed. The chapter dealing with the evolution summarizes current ideas on this topic.

The description of methods takes up a large portion of this book. It has been attempted to acquaint the reader with the methods in some detail. The book presents manifold aspects of biophysics and the perspective of their many interconnections in a great depth. The publication is useful not only to those who seek an introductory overview of biophysical problems and research results, but also to experienced scientists, who wish to obtain a glimpse of the state of research in biophysical problems which lie outside their own immediate field.

HELENA BENEŠOVÁ (*Praha*)