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A Kinetic Study of Phosphorus Absorption by Excised Maize Roots from Flowing Solutions with Different Phosphorus Concentrations

RADMILA ČÍŽKOVÁ-MACŮRKOVÁ* and Z. LAŠTŮVKA

Department of Plant Biology, Faculty of Sciences J. E. Purkyně University, Brno**

Dedicated to Academician S. Prát on the occasion of his 80th birthday

Abstract. The effect of two different mechanisms of phosphorus ion transport from the nutrient solution volume to the surface areas of excised maize roots was studied under concentrations ranging from 0.01 mM to 50.0 mM KH_2PO_4. A modified technique of study of kinetic ion absorption was used. In the control series, the roots were placed in absorption solution without flow (the dominant mechanism of ion transport to the roots being diffusion), while in the experimental series the absorption solution was flowing round the roots at a rate of 0.162 cm s^{-1} (the dominant mechanism of ion transport to the roots being mass flow). The rate of phosphorus absorption by the roots from flowing solutions was highly significantly increased at all concentrations of absorption solution except for the 50.0 mM KH_2PO_4 concentration. The increase in phosphorus absorption in the case of 50.0 mM KH_2PO_4 concentration was non-significant due to the fact that the high concentration of phosphorus together with the diffusion of phosphorus ions ensured a sufficient supply of phosphorus to the roots, covering the requirement for their uptake.

The results point to the need for an analysis of environmental factors to be carried out in studying ion absorption kinetics, and reveal the inadequacy of methods usually employed in such investigations, in particular with respect to the homogeneity of the nutrient solution in the whole of its volume and especially round the roots.

Two basic phases can be distinguished from the spatial point of view in the whole process of plant nutrition by phosphorus: (1) the supply of phosphorus anions from soil solution to the root area, transport of anions across the boundary layer of ions adsorbed on the root surface to cell membranes and free spaces of the root up to the sites of absorption, and (2) the anion absorption proper, transition across the cytoplasma membrane to the protoplast, accumulation and transport within the plant. Both phases can be subdivided into more detailed components, and decisive factors engaged in the whole process can be identified. The two phases should be considered as a continuous process which is characterized above all by the following features: the requirement of the plant for the appropriate anion; the rate

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* *Present Address:* Department of Forest Biology, Botanical Institute of the Czechoslovak Academy of Sciences, Poříčí 3b, 603 00 Brno 3, Czechoslovakia.

** *Address:* Kotlářská 2, 611 37 Brno, Czechoslovakia.

of ion absorption; the anion concentration in environment; mechanisms of ion transport from the solution volume to the sites of their absorption, a. o.

A number of authors in their detailed studies point to the effect of phosphorus concentration in outer surrounding on the intensity of its supply to the absorption zone (HANNAPEL *et al.*, 1964, MAHTAB *et al.* 1971, 1972, OLSEN *et al.* 1965, PHILLIPS *et al.* 1968, PHILLIPS and PEASLEE 1970, PLACE *et al.* 1968). Other research workers have examined the influence of phosphorus ion concentration on the mechanisms of their transport through cytoplasma membranes(CARTER and LATHWELL 1967, HAGEN and HOPKINS 1955, NOGGLE and FRIED 1960, PHILLIPS *et al.* 1971). A different mechanism of ion supply to the root area, however, may be of considerable importance for the subsequent processes. HAI and LAUDELOUT (1966) have published a study demonstrating the functional dependence of ion concentration and ion supply mechanisms (diffusion, mass flow). In our previous studies (MACŮRKOVÁ and LAŠTŮVKA 1972, PALÁTOVÁ and LAŠTŮVKA 1974) we have found that the mechanism of ion transport in nutrient solution, particularly that of phosphorus transport, is of considerable importance for subsequent processes of absorption, accumulation and transport, or as the case may be, for other metabolic activities in the plant as well.

Existing literary data are not sufficiently exact in evaluating the effect of transport mechanisms and phosphorus concentration in the outer environment on its absorption by the plants. In the present work this effect has been studied in excised maize roots under the conditions of two dominant mechanisms of ion supply (diffusion and mass flow) and graded phosphorus concentrations in the cultivation medium.

Material and Methods

The method used in our experiments is analogous, except for some modifications, to that described by EPSTEIN and HAGEN (1952), EPSTEIN (1961), HAGEN and HOPKINS (1955), HAGEN *et al.* (1957), and others. We altered that part of the method which concerns the movement of absorption solutions round the roots.

Plant Material and its Preparation

Maize seeds (*Zea mays* L. cv. 'Kočovská raná') were placed in tap water for 48 h, then surface sterilized in 3% H_2O_2 for 5 min, and cultured for 5 days on granulated polyethylene covered with filter paper at controlled room temperature of 24 °C, in the dark. Granulated polyethylene was being moistened with 0.6 mM $CaSO_4$ (under the so-called low salt conditions).

The apical segments of maize seedlings were excised, 6 cm long, and put into distilled water. 1 g samples of excised roots were placed in silon 'teabags' of 1 mm^2 mesh size (EPSTEIN *et al.* 1963b.)

Solutions and Experimental Technique

Solutions with concentrations 0.01, 0.1, 1.0, and 50.0 mM KH_2PO_4 containing 0.6 mM $CaSO_4$ were used (EDWARDS 1970, JACOBSON *et al.* 1961). The solutions were labelled ^{32}P isotope; the activity of each absorption solution was 30 μCi l^{-1}. The hydrogen ion concentration of all solutions was adjusted to pH 5.5, the solutions were aerated before the experiment, their temperature was 21 °C, and their volume was chosen so that 1 l of solution was available for every 1 g of roots. The solutions of all concentrations were placed in PVC vessels. In one series, the solutions were still, serving as the control stationary culture. The other series of solutions was kept in vessels permitting a continuous flow of the solution at a rate of 0.162 cm s^{-1}. The solution which flowed out of the vessel was pumped back into the reservoir by PVC pumps.

Techniques of Phosphorus Absorption and Measurements of Activity

At time zero (shortly after weighing the samples), all the bags containing roots were placed simultaneously in the solutions so that the medium was flowing vertically to the bag surface. The samples were collected at 1/2, 1, 2, 4, 8 h intervals in four repetitions. The roots were washed in distilled water for 20 min; see CARTER and LATHWELL (1967), HAGEN *et al.* (1957), PHILLIPS *et al.* (1971). Before measuring their activity, the roots were mineralized by using the concentrated H_2SO_4 (2 ml H_2SO_4 per 1 g of roots). The mineralisate was placed in thin-walled test tubes. The radioactivity of the samples was measured on a Vacutronic scintillations-spectrometer Va-M-120. From the known specific activity of particular phosphorus solution, the total absorption of phosphorus was calculated and expressed in µM P g^{-1} of root fresh weight. The results are given in mean values of the four repetitions. The significance of differences in phosphorus absorption by the roots between the control series (stationary culture) and flowing solution series was evaluated by the *G*-test (FRYER 1957).

TABLE 1

PHOSPHORUS ABSORPTION BY EXCISED MAIZE ROOTS FROM STATIONARY AND FLOWING SOLUTIONS WITH CONCENTRATIONS 0.01, 0.1, 1.0 AND 50.0 mM KH_2PO_4.

Absorption time [h]	Water culture	Phosphorus absorption [µM P g^{-1} root fresh weight]			
		Concentration of KH_2PO_4 absorption solution			
		0.01 mM	0.1 mM	1.0 mM	50.0 mM
1/2	stationary	0.057	0.047	0.323	4.542
	flowing	0.062	0.165 ***	0,382	5.063
1	stationary	0.112	0.332	0.651	6.464
	flowing	0.149***	0.381***	0.935***	7.669*
2	stationary	0.200	0.568	1.174	9.010
	flowing	0.284***	0.728***	1.677**	10.356
4	stationary	0.351	1.003	2.344	13.244
	flowing	0.503***	1.396***	3.287**	14.565
8	stationary	0.817	2.228	4.536	21.370
	flowing	1.093**	2.745***	5.577***	25.368*

Significance of differences in phosphorus absorption between roots from stationary solution [control] and those from flowing solution is designated: $P < 0.05$*, $P < 0.01$**, $P < 0.001$*** ($n = 4$).

Results

The time course of phosphorus absorption is shown in Table 1, the percent rates of increase in phosphorus content in roots absorbing phosphorus from the flowing solution as compared with those in roots absorbing phosphorus from the stationary solution are given in Table 2.

With all the concentrations examined, the absorption of phosphorus by excised roots of maize from the flowing absorption solutions was higher than that from the stationary solutions having coresponding concentrations. The differences in phosphorus absorption between stationary and flow cultures were significant at all concentrations except for the 50.0 mM KH_2PO_4 concentration, and this was so nearly at all the intervals of evaluation.

TABLE 2

PERCENT INCREASES IN PHOSPHORUS ABSORPTION BY EXCISED MAIZE ROOTS FROM FLOWING SOLUTIONS AS COMPARED WITH PHOSPHORUS ABSORPTION FROM STATIONARY SOLUTIONS (= 100%) WITH CONCENTRATIONS 0.01, 0.1, 1.0 AND 50.0 mM KH_2PO_4.

Absorption time [h]	Phosphorus absorption [%]			
	concentration of KH_2PO_4 absorption solution			
	0.01 mM	0.1 mM	1.0 mM	50.0 mM
1/2	8.77	12.24	18.27	11.47
1	33.04	14.76	43.63	19.75
2	42.00	28.17	42.84	14.94
4	43.30	39.18	40.23	9.97
8	33.76	23.20	22.95	18.71

Significant differences between the control and experimental (flow) series were observed already after 1/2 to 1 h absorption at low concentrations of absorption solutions. It follows from the percent expression of differences in phosphorus absorption (Table 2) that the differences tend to lessen already after 8 h of absorption.

Discussion

The rate of absorption of a certain ion by the plant is a function of ion concentration in the nutrient solution near the absorption surface of the root, of the area of the absorption root surface, and of the conductivity of the absorption root surface for a given ion (NIELSEN 1972). At the same time, however, the concentration of given ions on the root surface at particular intervals is a function of the rate of transport from the whole solution volume to the root surface and the rate of their absorption by the root. Whenever the ion absorption rate exceeds the rate at which the ions are conveyed to the roots, the ion concentration on the root surface appears lower than that in the whole volume of nutrient solution (BARBER 1962, LEWIS and QUIRK 1967, *etc.*). A concentration gradient is formed round the absorption surface of roots, and it may well be assumed that, under this condition, the real ion absorption rate will be lower than when the rate of ion transport from the whole solution volume to the roots, and further up to the sites of ion absorption, exceeds the absorption rate, thus not being a limiting factor in the whole process. The rate of ion transport to the root surface is primarily dependent upon the amount of ions per volume unit of medium (BRIGGS *et al.* 1961, FRIED and SHAPIRO 1961, JESCHKE and SIMONIS 1965, TANADA 1964), as well as on the rate at which the ions are transported to the roots (HAI and LAUDELOUT 1966). At a given ion concentration in nutrient medium, the rate of ion transport to the roots can, to a degree, be influenced by the choice of one of the different mechanisms determining the rate of ion movement, *i.e.*, by ion diffusion or mass flow. It may be theoretically assumed that when diffusion does not ensure the absorption rate in a given concentration, more ions will be carried to the roots by the flow of solution plus ions, and the absorption rate will increase. Mass flow, however, loses its force when the ion transport through diffusion provides the roots, at a higher ion concentration in the medium, with an amount of ions large enough to wholly satisfy the requirements of the plant.

When comparing the results of the present work with the above assumption, we find them to be in agreement. In the whole range of concentrations, the rate of phosphorus absorption by excised maize roots in flowing medium becomes increased, the increase being quite significant at the concentrations 0.01 mM to 1.0 mM KH_2PO_4. Our assumption is proved correct also by the fact that the differences in absorption rate between the two series are non-significant in the case of the 50.0 mM KH_2PO_4 concentration. The results suggest that at low phosphorus concentrations, the rate of ion transport to the root zone may be a factor limiting the absorption rate, especially if diffusion is the dominant mechanism of ion movement. The continuous supply of phosphorus ions through flowing solution to the absorption surface of the roots substantially narrowed the width of the dilute layer, thus speeding up the transition of ions to the cell membranes and free root spaces, and finally to the sites of their absorption. In our opinion, however, it could not essentially change or affect the mechanism of ion movement across the cell membranes and free spaces to the sites of absorption. The marked decline in mass flow effectiveness at the 50.0 mM KH_2PO_4 concentration may be accounted for by the fact that the diffusion of ions to the roots at such concentration was considerably high and the mass flow did not offer more ions during an equal time unit. It may be, however, that in both series the absorption rate was limited by the transition of ions across the very root structures lying between the surface and absorption site, even though some publications report the opposite (FRIED and SHAPIRO 1961, WELCH and EPSTEIN 1969). It should be taken into account that in this region ion transitions across ion barriers, by one cell membrane at least, are involved, but rather than these, the whole system of extrafascicular space, ion movement through free spaces, and complex adsorption phenomena on lignocellulose surfaces are concerned here (ULRICH and OBERLÄNDER 1964).

From the methodological point of view, it is obvious that the diluting of ion concentrations on the root surfaces due to a competition among neighbouring roots for ions (NEWMAN and ANDREWS 1973) may considerably affect the rate of their absorption. We tried to eliminate this competition by placing the roots in bags so as to prevent their overlapping and touching one another, and to make the solution, or solution flow, pass round the whole absorption surface of the roots.

The results obtained from our experiments permit us to draw certain more general methodological conclusions. Numerous works studying the kinetics of absorption of different elements (EPSTEIN and HAGEN 1952, EPSTEIN *et al.* 1963a, b, HAGEN and HOPKINS 1955, *etc.*) report that aeration of the absorption solution is fully sufficient for levelling out its concentration in the whole solution volume, including the area round the roots. Depending on whether the roots are clustered in one place or placed at the opposite side of the aeration tube, the solution may not be renewed sufficiently in the very critical region round the root with all the due consequences for the ion absorption. We assume that a determination of ion absorption kinetics should include not only a deduction of equations expressing the rates and changes of the intensity of the active uptake of given ions, but also a definition of environmental factors involved in the active uptake of ions, including the rate of their transport from the whole solution volume up to the sites of their absorption.

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RADMILA ČÍŽKOVÁ-MACŮRKOVÁ, Z. LAŠTŮVKA (Brno): **Kinetická studie absorpce fosforu odřezanými kořeny kukuřice z proudících roztoků o různé koncentraci fosforu.** — Biol. Plant. **17** : 385—391, 1975.

Byl sledován efekt dvou rozdílných mechanismů transportu iontů fosforu z objemu živného roztoku k povrchům odřezaných kořenů kukuřice v koncentračním rozpětí 0,01 mM až 50,0 mM KH_2PO_4. Byla použita modifikovaná metodika studia kinetiky absorpce iontů. V kontrolní sérii byly kořeny umístěny v absorpčním roztoku bez pohybu (dominantním mechanismem transportu iontů ke kořenům je difuse), v pokusné sérii proudil absorpční roztok kolem kořenů rychlostí 0,162 cm s^{-1} (dominantním mechanismem transportu iontů ke kořenům je mass flow). Míra absorpce fosforu kořeny z proudícího roztoku byla vysoce průkazně zvýšena při všech koncentracích absorpčních roztoků mimo koncentraci 50,0 mM KH_2PO_4. Zvýšení absorpce fosforu při koncentraci 50,0 mM KH_2PO_4 bylo neprůkazné proto, poněvadž vysoká koncentrace fosforu spolu s difusí fosforečných iontů zabezpečovala dostatečnou dodávku fosforu ke kořenům, pokrývající požadavek jejich příjmu.

Výsledky ukazují na nutnost analýzy faktorů vnějšího prostředí při studiu kinetik absorpce iontů a na neadekvátnost obvykle používaných metodik při těchto studiích, především z hlediska homogenity živného roztoku v celém objemu a zvláště kolem kořenů.

BOOK REVIEW

CUSHING, D. H.: **The Productivity of the Sea.** — Oxford Biology Readers. Oxford University Press, London 1975. Price 16 p.

The brief review of the oceanic production biology by D. H. Cushing makes a very good introduction into the topic for students as well as research workers of other fields. However, it is popular enough to give some information to the wide public. A short description and selected pictures of marine phyto- and zooplankters remain on the traditional level of such articles. What clearly exceeds this niveau are the introduction into quantitative production studies, summary of the influence of the main environmental factors, and, particularly, the outlines of a simple model of the pelagic ecosystem. I admit that simplifications were necessary, but to call counting of cells and photosynthetic measurements together "estimating algal quantities" seems to be a bit misleading. Among the factors, the penetration of light and water movements, both vertical and horizontal, are explained splendidly and very well depicted. Relatively scarce is the information on nutrient effect and dynamics. The concise part on marine fisheries reveals the professional.

I recommend the paper to everybody who needs or wants a quick information about sea productivity.

P. JAVORNICKÝ (*Praha*)