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A Kinetic Study of Phosphorus Absorption by Excised Maize Roots from Flowing Solutions Influenced by 2,4 Dinitrophenol and Viscosity of Solution

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Abstract. The effect of 2,4 dinitrophenol and increased viscosity of the absorption solution on the absorption of phosphorus by excised roots of maize plants was investigated. The concentration of the solution was 0.1 mM KH_2PO_4, the activity of ^{32}P was 52 μCi l^{-1}. The temperature of the absorption solution was 26 °C, pH 5.5, aeration prior to the experiment. There was 1 l of solution for every 1 g of roots. Two basic variants were used for comparison: with non-flowing solution and with solution flow (circulation) of 0.162 cm s^{-1}, respectively. In all cases, 2,4 dinitrophenol reduced the rate of phosphorus absorption by the roots regardless of the mechanism of phosphorus supply to the roots (diffusion, mass flow). If it is proved that 2,4 dinitrophenol inhibits the active uptake of phosphorus, then the uptake of phosphorus by the roots increased under the influence of mass flow will be active, *i.e.*, connected with energy metabolism.

Raising the viscosity of the absorption solution 3.3 times over that of water by means of potato starch substantially reduced the absorption of the phosphorus transported to the absorption zone by diffusion and practically did not affect the rate of absorption, or the amount of anions transported to the absorption area by mass flow.

By comparing the rates of phosphorus absorption by the roots of maize plants from the flowing culture and those from the non-flowing medium we have found (ČÍŽKOVÁ-MACŮRKOVÁ and LAŠTŮVKA 1975) that the absorption rate increases significantly when the solution is continuously flowing. We assume that a rise in absorption can be obtained by supplying phosphorus anions to the active surfaces more intensively and by thinning the layer of diluted solution around the roots. Both processes, that of phosphorus anion supply to the roots as well as that of increasing the absorption rate, are evidently related to each other, though they may be caused by other factors.

The experiments described in this paper were intended first of all to get a knowledge of the movement of phosphorus anions outside the roots while the viscosity of the solution varies, and further to study the process of

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absorption itself with the aim of discovering whether active or passive uptake is involved.

Material and Methods

The experiments were carried out in a way similar to that described in the foregoing study (ČÍŽKOVÁ-MACŮRKOVÁ and LAŠTŮVKA 1975), the technique having been extended so as to fit the aims set by the present work. Only this part is presented here.

Samples of phosphorus absorption by excised roots of maize were collected at given intervals from control solutions containing 0.1 mM KH_2PO_4 with labelled ^{32}P, then from the same solution with the addition of 10^{-5} M 2,4 dinitrophenol, and lastly from the same solution additionally containing 1% of potato starch. In all the three cases two variants were chosen, the solution being stationary in one variant and flowing at the defined rate in the other. The volume of solution was always identical, *i.e.*, 1 l per 1 g of roots. The activity of solutions was 52 μCi l^{-1}. All solutions had pH adjusted to 5.5 and were aerated before the experiment proper. The temperature of solutions was 26 °C. The viscosity of the starch solution was 3.3 times the viscosity of water (measured by a Rheo-viscosimeter after Höppler). The roots for the 2,4 dinitrophenol solution were put, one hour before dipping them in the active solution, in a 2,4 dinitrophenol solution of the same concentration; so, just at the beginning, the roots had a decreased metabolic activity.

At given time intervals the roots were removed from the particular solutions and washed in distilled water for 20 min. Before measuring the ^{32}P activity, the roots were mineralised in the wet way by using concentrated H_2SO_4.

Results

It is clear from Table 1 that the 2,4 dinitrophenol applied to the absorption solution inhibited phosphorus absorption by excised roots in all cases. The differences in absorption of phosphorus by roots from stationary and flowing solutions are inessential and statistically not significant.

The application of 1% of starch to the absorption solution brought about clearly significant differences in phosphorus absorption: the flowing solution, even with high viscosity, supplied the roots with phosphorus better (and to the same degree when compared with the control solution without starch), in other words, it brought a greater amount of phosphorus anions to the absorption zone than was the case with the stationary, non-flowing solution. The differences in absorption of phosphorus by roots from the stationary and from the flowing solutions are statistically highly significant.

In all cases the flowing control solution increased the rate of phosphorus absorption in given experimental conditions (see also Table 2).

Discussion

Making a definition on the active ion transport across the membrane of a plant cell and drawing an exact experimental and theoretical distinction between passive and active transport are rather problematic tasks. Active ion uptake has been ascertained by many research workers, in addition to other effects, on the basis of inhibition of this uptake by metabolic inhibitors, particularly 2,4 dinitrophenol. In our experiments, the content of phosphorus in the roots absorbing phosphorus from the stationary solution and from the solution flowing round the roots decreased, due to the application of 2,4 dinitrophenol, nearly to the same level (Tables 1, 2). In fact, the absorption of phosphorus was limited by the application of 2,4 dinitrophenol irrespective of the amount of anions coming from the solution. Thus 2,4 dinitrophenol inhibited the same process in both variants (stationary culture and flowing culture). The increased amount of phosphorus in the roots absorbing phosphorus from the flowing solution (ČÍŽKOVÁ-MACŮRKOVÁ and LAŠTŮVKA 1975)

TABLE 1
Phosphorus absorption by excised maize roots from stationary and flowing solutions with concentration 0.1 mM KH_2PO_4 influenced by 2,4 dinitrophenol and viscosity of absorption solution

Absorption time [h]	Water culture	Phosphorus absorption [µmol P g^{-1} root fresh weight]		
		absorption solution 0.1 mM KH_2PO_4		
		control solution	10^{-5}M 2,4 dinitrophenol	1% potato starch
1	stationary	0.399	0.119	0.376
	flowing	0.428	0.121	0.431^{+}
2	stationary	0.938	0.241	0.732
	flowing	0.946	0.223	0.936^{+}
4	stationary	1.687	0.284	1.396
	flowing	1.817^{++}	0.286	1.862^{+++}
8	stationary	2.951	0.381	2.553
	flowing	3.588^{+++}	0.433	3.794^{+++}
16	stationary	5.750	0.530	4.137
	flowing	7.307^{+++}	0.551	7.362^{+++}

Significance of differences in phosphorus absorption between roots from stationary solution and those from flowing solution is designated: $P < 0.05^{+}$, $P < 0.01^{++}$, $P < 0.001^{+++}$ ($n = 4$).

TABLE 2

Percent increases in phosphorus absorption by excised maize roots from flowing solutions as compared with phosphorus absorption from stationary solutions (= 100%) with concentration 0.1 mM KH_2PO_4 influenced by 2,4 dinitrophenol and viscosity of absorption solution

Absorption time [h]	Phosphorus absorption [%]		
	absorption solution 0.1 mM KH_2PO_4		
	control solution	10^{-5}M 2,4 dinitrophenol	1% potato starch
1	7.27	1.68	14.63
2	0.85	—7.47	27.87
4	7.71	0.70	42.65
8	21.59	13.65	48.61
16	27.08	3.96	75.41

was transported to the root cells by the same mechanism as was the phosphorus from the non-flowing solution. Moreover, an active uptake can be claimed to occur, or such uptake as is inhibited by 2,4 dinitrophenol of the concentration applied. It is possible to draw this conclusion about the active phosphorus uptake on the basis of the facts reported in the literature, namely, that 2,4 dinitrophenol inhibits the active absorption of phosphorus by plants (HAI and LAUDELOUT 1966, KURSANOV and VYSKREBENTSEVA 1960, STENLID 1959, a.o.). The effect of 2,4 dinitrophenol on phosphorus absorption consists in the inhibition of the oxidative phosphorylation, hence, on the reduction of the level of P compounds rich in energy (BOÏKO 1965, SALYAJEV 1965). Besides that, 2,4 dinitrophenol inhibits the uptake of phosphorus also competitively (HOPKINS 1956). 2,4 dinitrophenol can cause disturbances in the whole protoplasma structure and a loss of functions of the semi-permeable membranes (LUNDEGÅRDH 1960, MICHALÍK 1971, a.o.), however, has proved that in spite of the volume of free root spaces being increased, the increase in the volume of free space did not stimulate the phosphorus uptake, but, on the contrary, the phosphorus uptake was significantly inhibited.

The transport of ions through a system of carriers is determined primarily by the specific properties of the ions, by the concentration of carriers, the rate of carrier turnover and the relative proportions of the carriers (VOSE 1963). We assume that the continuous supply of phosphorus ions to the absorption surfaces of roots and the flowing solutions did not influence any of the aforementioned properties of the reaction sites; a higher amount of anions supplied, however, facilitated their better saturation, presumably up to the limit to which the absorption of phosphorus could be ensured by the capacity of the mechanisms of the active phosphorus uptake. We believe that in natural conditions in which a high concentration gradient can be developed in root surfaces, a low concentration of ions in the zone of their absorption by the roots is not sufficient for the saturation of the carriers. Hence it follows that concentrations of outer solution which are considered to be saturating for plant uptake on the basis of examination of ion uptake by methods of stationary water cultures, do not accord with the real situation and, therefore, neither can the statements on ion uptake based on measuring the concentrations of the whole solution volume be true. The limit concentration of ions characterized as the concentration at which neither a pure uptake nor a pure emission of ions by the plant occurs (SCHEFFER and KAUFMANN 1961), is thus in fact much lower than the concentration of the whole volume of the medium measured by current methods, because it is the concentration of ions near the active sites of their uptake that is of decisive importance for both uptake and emission.

The importance of the ion transport by diffusion or convection from the whole volume of the solution to the sites of ion absorption in the root has further been proved by examining the phosphorus absorption from a solution with increased viscosity (Tables 1, 2). We started from the well-known fact that the ion diffusion is inversely proportionate to the viscosity of the solution (OLSEN and KEMPER 1968), which made us presume that, under the conditions of a more difficult ion diffusion, it would be possible to detect their transport more clearly by convection. The uptake of phosphorus by excised roots of maize from the absorption solution with increased viscosity which was not flowing was markedly lower than the phosphorus uptake from the absorption solution with water viscosity. When the absorption solutions were flowing, the absorptions of phosphorus by excised maize roots from the solution with water viscosity and from that with 3.3 times higher viscosity were balanced. This indicates that even though the flow of the nutrient solution was always identical, as to the ratio of the root weight to the volume of the solution in litres, as with the stationary culture at any moment it supplied a greater amount of phosphorus anions to the area round the roots, and hereby also to the free space of the root and to the closest proximity of absorption mechanisms. Even though this increased phosphorus absorption (with subsequent higher accumulation) is made possible by mechanisms operating at low as well as high concentrations of phosphorus in the solution, it may have considerable consequences in the metabolism of the plant (MACŮRKOVÁ and LAŠTŮVKA 1972, PALÁTOVÁ and LAŠTŮVKA 1974).

It should be noted that an application of chemicals enhancing the viscosity of experimental solutions, generally employed in studies of the physiological processes in plants, such as polyethylene glycol and mannitol (KAUFMANN and ECKARD 1971, KOLEK and PALOVČÍKOVÁ 1972, RESNIK 1970, RESNIK and FLOWERS 1971, a.o.) was technically impracticable for us. That is why we used potato starch to increase the viscosity of the absorption solution. We are well aware of possible secondary effects of starch, but we hold the view, that our experiments were of short duration and that the mutual comparison prevents possible errors.

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BOOK REVIEW

RENSING, L., HARDELAND, R., RUNGE, M., GALLING, G.: **Allgemeine Biologie. Eine Einführung für Biologen und Mediziner.** — Uni-Taschenbücher 417. Verlag Eugen Ulmer, Stuttgart 1975. 411 S. 190 Abb. 23,80 DM.

Dieses Taschenbuch ist eine besonders für Studenten bestimmte Einführung in die Welt der Biologie: in die Organisation und das Funktionieren von biologischen Systemen. Das Buch ist der Komplexität der biologischen Systeme entsprechend gegliedert (Zelle, Organismus, Ökosystem) und besonders betont werden die hier auftretenden molekularen, physiologischen und ökologischen Mechanismen. Das Buch behandelt zuerst die Zelle, deren Aufbau und chemische Zusammensetzung und die sich darin abspielenden biochemischen, biophysikalischen, enzymatischen und energetischen Vorgänge. Es informiert weiter über Genetik, Entwicklung und Evolution, über die Prinzipien der vielzelligen Organisation an Beispielen aus dem Pflanzen- und Tierreich und oft am Beispiel des Menschens. Der abschliessende Teil des Buches ist der Ökologie und dem Verhalten der Tiere gewidmet. Den Eigenschaften der Pflanzen sind einige Abschnitte des Kapitels Die Zelle gewidmet, wie z.B. den Plastiden, der Photosynthese, weiter des Kapitels Entwicklung (Meristeme, Wachstum, Wirkung von Wuchstoffen, vegetative und sexuelle Phase der Pflanzenentwicklung usw.) und schliesslich das 6 .Kapitel ,,Der Organismus der Pflanzen". Hier werden der Bau der höheren Pflanze (Blatt, Sprossachse, Wurzel) und die wichtigsten Vorgänge wie Photosynthese, Atmung, Wasser- und Mineralstoffhaushalt behandelt. Da Pflanzen in sehr viel direkterer Weise als Tiere von ihrer Umwelt abhängig sind, werden die Beziehungen Standort — Organismus im 8. Kapitel (Ökologie) am Beispiel der höheren Pflanzen beschrieben. Das Taschenbuch ist sehr übersichtlich und klar abgefasst, ist durch 190 meist zweifarbige Abbildungen illustriert und bringt ein kurzes Verzeichnis weiterführender Literatur. Sehr nützlich finde ich die graphisch betonten Zusammenfassungen am Ende einzelner Kapitel oder sogar einzelner Abschnitte eines Kapitels. Ein kurzes Sachverzeichnis ermöglicht das rasche Auffinden des Gesuchten. Dieses Taschenbuch in flexiblem Kunststoffeinband ist zwar vorallem für Studenten der Biologie und Medizin bestimmt, wird jedoch auch vielen anderen als Nachschlagbuch und zur schnellen Orientierung in biologischen Fragen dienen.

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