

The Effect of Some Micronutrients and Heavy Metals on Phosphate Absorption by Maize Root Cortex Segments

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Abstract. The effect of salts (nitrates, chlorides, and sulfates) of microelements, Cd^{2+} , Ni^{2+} , and Co^{2+} and the effect of boric acid and ammonium molybdate on phosphate uptake by maize root cortex segments were tested.

Higher concentration (0.1 mM) of Cu^{2+} salts caused enhancement of phosphate efflux to the extent that efflux was higher than influx.

Inhibitory action on phosphate uptake by maize root cortex segments was exerted by following salts: 0.01 mM Cu^{2+} salts (20–30% inhibition), 0.5 mM ZnSO_4 (9.7%), 0.5 and 0.05 mM ZnCl_2 (34.3% and 20.8%), 0.1 mM salts of Cd^{2+} , Ni^{2+} , Co^{2+} (35–78%).

1 mM FeSO_4 had significant stimulatory effect (92%) on phosphate uptake. Much weaker stimulatory effect was exerted by 1 mM FeCl_3 (14%), 0.05 mM ZnSO_4 (9.6%), 0.005 mM ZnCl_2 and ZnSO_4 (8.4 and 18.5%) and 0.001 mM CdCl_2 and CdSO_4 (20.8 and 12.4%).

All other tested salts — salts of Mn^{2+} (0.1 and 0.01 mM), 0.01 and 0.001 mM salts of Co^{2+} and Ni^{2+} , 0.001 mM salts of Cu^{2+} , 0.001–10 mM boric acid, and 0.001–0.1 mM ammonium molybdate left phosphate uptake unaffected.

Uptake of nutrients in plants is a very complex process, which is controlled by many external and internal factors, as *e.g.* pH, temperature, light, osmotic pressure, internal and external concentration of nutrients *etc.* (LÜTTGE and HIGINBOTHAM 1979, SPANSWICK *et al.* 1980). The uptake of individual nutrients depends on the uptake of other nutrients. All uptake processes interact to maintain electroneutrality, osmotic balance *etc.* (LÜTTGE and HIGINBOTHAM 1979, CLARKSON and HANSON 1980). Moreover, specific interactions of some ions with the uptake systems or processes of other ions may take place.

Thus, there is the well-known essential role of Ca^{2+} ions on the selectivity and rate of uptake of other cations (EPSTEIN 1961) and anions (LEGGETT *et al.* 1965, SKJELBREID and NISSEN 1980). Interaction with micronutrients may also influence uptake rate of other nutrients. Thus, Zn deficiency enhances phosphate accumulation in older leaves (LONERAGAN *et al.* 1982). Recently it was shown that Zn deficiency caused increased phosphate efflux and it is supposed that Zn has a function in maintaining membrane integrity (WELCH *et al.* 1982). Also deficiency of boron was shown to slow down

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phosphate uptake, probably due to its effect on plasmalemma ATPase activity (LOUGHMAN 1983).

Competition of chloride anions with the uptake of other ions was shown (DE WITT *et al.* 1963). Higher doses (10 kg ha^{-1}) of Cu, Zn, Mn, and Fe were shown to be unfavourable to phosphate uptake in rice (DEB *et al.* 1982).

The knowledge of these interactions together with that of mutual interactions of these ions in soil is of great importance for plant nutrition strategy.

We have recently reported on properties of phosphate uptake system in cortex cells of maize roots (MACHÁČKOVÁ *et al.* 1983). This report brings some information on the effect of several micronutrients and heavy metals on phosphate uptake in the experimental material mentioned.

MATERIAL AND METHODS

Cortex layers (5–100 mm behind the tip) were isolated from roots of 8 days old maize plants (*Zea mays* L.-hybrid CE-240 from Breeding Station in Čejč) grown 4 days on diluted Knop's solution and 2 days on 0.2 mM CaSO_4 , as recently described in detail (MACHÁČKOVÁ *et al.* 1983). Cortex layers were cut into 1 mm segments, which were washed (augmented — VAN STEVENINCK 1975) for 3 h in aerated 0.2 mM CaSO_4 at room temperature and then used for absorption experiments. Aliquots (0.5 g) of washed segments were transferred into 7 ml 1 mM KH_2PO_4 in 0.2 mM CaSO_4 (pH 5.0) with or without addition of various salts. Cations (with exception of Mn^{2+} , Zn^{2+} , Fe^{2+} , and Fe^{3+}) were tested in the form of three salts: chloride, nitrate, and sulfate. Incubation proceeded on a temperature-controlled water bath at $25 \pm 0.5^\circ\text{C}$ for 4–5 h under continuous shaking. At the end of the incubation period an aliquot of absorption medium was withdrawn and kept on ice till the phosphorus content determination. The segments were filtered off and washed for 10 min in 10 ml cold (4°C) 0.2 mM CaSO_4 to remove free space and surface-sorbed phosphate (CRAM 1973). Aliquots of washing media were also analyzed for phosphorus content and these values subtracted from those of absorption.

Phosphorus content was determined on a Technicon autoanalyzer using colorimetric method of MURPHY and RILEY (1962). All measurements were carried out in triplicate. All results are given in % of the control value: the average SE was 7.8%. Differences lower than 16.3% were not statistically significant (*t*-test, $n = 3$, $P = 0.05$).

RESULTS

The effects of salts of some micronutrients and heavy metals on phosphate absorption by maize root cortex segments are shown in Table 1. It was impossible to study the effect of Mn^{2+} salts in higher concentration (1 mM) as $\text{Mn}_3(\text{PO}_4)_2$ precipitated. Lower concentrations of Mn^{2+} salts (0.1 and 0.01 mM) had no effect. All three salts of Cu^{2+} in 0.1 mM concentration caused enhanced efflux of phosphate ions so that efflux of phosphate exceeded its influx. 0.01 mM Cu^{2+} salts were inhibitory to phosphate uptake (10.8–35.3%) and still lower concentration (0.001 mM) of Cu^{2+} salts did not affect phosphate uptake.

Salts of Zn^{2+} differed in their effect. Both ZnSO_4 and especially ZnCl_2 were weakly inhibitory in 0.5 mM concentration (9.7 and 34.3%). But

Table 1

The effect of salts of some micronutrients and heavy metals on phosphate uptake from 1 mM KH_2PO_4 in 0.2 mM CaSO_4 (pH 5.0) by maize root cortex segments. Absorption proceeded at 25 °C for 5 h. Results are given in % of control value. Mean S.E. was 7.8%. Differences lower than 16.3% are not statistically significant (*t*-test, *n* = 3, *P* = 0.05).

Cation	Concentration [mM]	Anion of tested salt		
		Cl^-	NO_3^-	SO_4^{2-}
Mn^{2+}	1	precip.	—	precip.
	0.1	102.1	—	98.7
	0.1	0	0	0
Cu^{2+}	0.01	64.7	74.7	89.2
	0.001	94.1	95.3	100.9
Zn^{2+}	0.5	65.7	—	90.3
	0.05	79.1	—	109.6
	0.005	108.4	—	118.5
Fe^{2+}	1.0	—	—	192.0
	0.1	—	—	121.3
	0.01	—	—	107.8
Fe^{3+}	1.0	114.3	—	—
	0.1	109.8	—	—
	0.01	106.2	—	—
Cd^{2+}	0.1	64.9	48.6	60.7
	0.01	105.1	87.3	94.6
	0.001	120.8	101.2	112.4
Co^{2+}	0.1	37.6	65.0	21.3
	0.01	101.2	95.6	84.2
	0.001	104.9	102.3	99.8
Ni^{2+}	0.1	37.5	28.3	66.2
	0.01	80.9	83.0	89.7
	0.001	102.3	101.6	107.3

0.05 mM ZnCl_2 remained inhibitory (20.9%) while ZnSO_4 in this concentration was slightly stimulatory (9.6%). In 0.005 mM concentration both zinc salts are weakly stimulatory, but only in the case of ZnSO_4 is this effect statistically significant (18.5%).

FeSO_4 is the only salt of those tested which had significant stimulatory effect on phosphate absorption by maize root cortex segments. 1 mM FeSO_4 stimulated phosphate uptake by 92%. Not so FeCl_3 — its stimulatory effect was much lower (14%) — *i.e.* statistically not significant.

Salts of Cd^{2+} , Ni^{2+} , and Co^{2+} were inhibitory in higher concentration (0.1 mM). Lower concentrations were without effect or weakly stimulatory: 0.001 mM CdSO_4 and CdCl_2 (12.4 and 20.8%). Of Ni^{2+} and Cd^{2+} salts the nitrates and of Co^{2+} salts the sulfate were the most effective ones.

Boric acid (0.001–10 mM) and ammonium molybdate (0.001–0.1 mM) had no influence on phosphate uptake.

DISCUSSION

Discussing the results we have to be aware of the fact that the absorption experiments lasted for 5 h and that we have to take into account the possible metabolic effects of ions and salts under study.

The inhibitory effect of higher concentrations of salts of Cu^{2+} , Zn^{2+} , and heavy metals Cd^{2+} , Co^{2+} , and Ni^{2+} is probably due to their metabolic effect.

It is known *e.g.* that copper has a negative influence on malate content and activity of some malate-linked enzymes (VELTRUP 1981). Phosphate uptake in maize root cortex tissue is a metabolic-dependent process (MACHÁČKOVÁ *et al.* 1983) and any disturbance in metabolism, especially energetic metabolism, would result in a decrease of uptake rate. The effect of divalent ions on transport processes might also be the cause of their effect on membrane potential (MARRÉ *et al.* 1982).

The significant stimulatory effect of Fe^{2+} ions is in good accordance with the reversing effect of Fe^{2+} (FeEDTA) on phosphate-induced chlorosis in maize leaves (VEJSADOVÁ and LAŠTŮVKA 1982). This phenomenon is thought to be the consequence of phosphate precipitation with iron in the root tissue resulting in iron deficiency in the tops. It is possible that this precipitation may increase the concentration gradient for phosphate uptake. In contrast to Fe^{2+} ions only weak (not significant) stimulation was found for Fe^{3+} ions. This may be the result of phosphate inhibition of Fe^{3+} reduction, which is a prerequisite for absorption (OLSEN and BROWN 1980).

It is evident that different salts of the same cation may exert different effects on phosphate uptake. Thus, 0.05 mM ZnCl_2 acts as an inhibitor while the same concentration of ZnSO_4 acts slightly as a stimulator. In other cases some salts proved to be more inhibitory than others (in the case of Ni and Cd nitrates and in the case of Co sulfate were the most effective ones). These results show that interaction of both cation and anion is decisive for the entire action of the salt. Recently BEHL and JESCHKE (1979) stated that the duration of K^+ transport inhibition by abscisic acid depends on the accompanying anion. The cause of this phenomenon is not exactly known.

To interpret our results on interaction of phosphate uptake with some other ions and salts for plant nutrition purposes, interactions of these ions in individual types of soil must be taken into account.

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REFERENCES

- BEHL, R., JESCHKE, W. D.: On the action of abscisic acid on transport, accumulation and uptake of K^+ and Na^+ in excised barley plants; effects of accompanying anions. — *Z. Pflanzenphysiol.* **95** : 335—353, 1979.
- CLARKSON, D. T., HANSON, J. B.: The mineral nutrition of higher plants. — *Annu. Rev. Plant Physiol.* **31** : 239—298, 1980.
- CRAM, W. J.: Chloride fluxes in cells of the isolated root cortex of *Zea mays*. — *Aust. J. biol. Sci.* **26** : 757—779, 1973.
- DEB, D. L., DAS, S. K., SACHDEV, P.: Study of phosphorus-micronutrient cation relationship in rice by splitroot technique. — *J. nucl. agr. Biol.* **11** : 40—42, 1982.
- EPSTEIN, E.: The essential role of calcium in selective cation transport by plant cells. — *Plant Physiol.* **36** : 437—444, 1961.
- LEGGETT, J. E., GALLOWAY, R. A., GAUGH, H. G.: Calcium activation of orthophosphate absorption by barley roots. — *Plant Physiol.* **40** : 897—902, 1965.
- LONERAGAN, J. F., GRUNES, D. L., WELCH, R. M., ADUAYI, E. A., TENGAT, A., LAZAR, V. A., CARY, E. E.: P accumulation and toxicity in leaves in relation to Zn supply. — *Soil Sci. Soc. Amer. J.* **46** : 345—352, 1982.
- LOUGHMAN, B. C.: Movement of phosphate across membranes. — Poster presentation on the Symposium on Membrane Transport in Plants, Praha 1983.
- LÜTTGE, U., HIGINBOTHAM, N.: Transport in Plants. — Springer Verlag, New York—Heidelberg—Berlin 1979.

- MACHÁČKOVÁ, I., KRÁL, J., ZMRHAL, Z.: Characterization of phosphate absorption in maize root cortex segments. — *Biol. Plant.* **25** : 366–372, 1983.
- MARRÉ, M. T., ROMANI, G., COCUCCI, M., MOLONEY, M. M., MARRÉ, E.: Divalent cation influx, depolarization of the transmembrane electric potential and proton extrusion in maize root segments. — In: MARMÉ, D., MARRÉ, E., HERTÉL, R. (ed.): *Plasmalemma and Tonoplast: Their Function in Plant Cell*. Pp. 31–36. Elsevier Biomed. Press, Amsterdam 1982.
- MURPHY, J., RILEY, J. P.: A modified single solution method for the determination of phosphate in natural waters. — *Anal. Chim. Acta* **27** : 31–36, 1962.
- OLSEN, R. A., BROWN, J. C.: Factors related to iron uptake by dicotyledonous and monocotyledonous plants. II. The reduction of Fe^{3+} as influenced by roots and inhibitors. — *J. Plant Nutr.* **2** : 647–660, 1980.
- SKJELBREID, E., NISSEN, P.: Effect of calcium on sulfate uptake by barley roots. — *Physiol. Plant.* **49** : 383–387, 1980.
- SPANSWICK, R. M., LUCAS, W. J., DAINITY, J. (ed.): *Plant Membrane Transport: Current Conceptual Issues*. Elsevier/North-Holland Biomed. Press, Amsterdam–New York–Oxford 1980.
- VAN STEVENINCK, R. F. M.: The “washing” or “ageing” phenomenon in plant tissues. — *Annu. Rev. Plant Physiol.* **26** : 237–258, 1975.
- VEJSADOVÁ, H., LAŠTŮVKA, Z.: The importance of FeEDTA for reversibility of phosphate-induced chlorosis in maize (*Zea mays* L.). — *Biol. Plant.* **24** : 401–406, 1982.
- VELTRUP, M.: The influence of copper on the metabolism of malic acid in intact barley roots. — *J. Plant Nutr.* **4** : 217–230, 1981.
- WELCH, R. M., WEBB, M. J., LONERAGAN, J. F.: Zinc in membrane function and its role in phosphorus toxicity. — *Plant Nutrition 1981. Proc. IX. Int. Plant Nutr. Coll.* Pp. 710–715. Univ. England, Warwick 1982.
- WITT, C. T. DE, DIKSHORN, W., NOGGLE, J. C.: Ionic balance and growth of plants. — *Verslagen landbouw. Onderzoek (Wageningen)* 1963 : 1–68, 1963.

BOOK REVIEW

ZIMMERMANN, M. H.: *XYLEM STRUCTURE AND THE ASCENT OF SAP*. — Springer-Verlag, Berlin—Heidelberg—New York—Tokyo 1983. 143 pp. 64 Figs. Cloth DM 54,—; approx. US \$ 22.30.

The evolution of dry land plants depended upon the development of water-conducting systems which enabled a sufficient rate of water transport in the soil-plant-atmosphere continuum. Therefore the monograph providing an integrated description of sap ascent very appropriately inaugurates The Springer Series in Wood Science. (A wide scope of this series is proposed: from the structure, chemistry and physical properties of wood, its formation and biodegradation to the utilization of forest biomass.)

The first two chapters are mostly anatomical, the following four mostly physiological, the last one phytopathological. It is very important that through the whole book the accent is laid on the close connection between structure and function.

The first chapter deals with the structure of the main conducting units: tracheids and vessels, and the second with the vessel network in the stems of different plant types. The mechanism of water transport in xylem based on cohesion theory is the main item of the third chapter. The fourth chapter considers velocities of water transport in individual plant parts and also in relation to transpiration of leaves, and the fifth the radial water movement in stem and the adaptation of xylem to different environments. The sixth chapter deals with the ontogeny of conducting tissues and the function of xylem during the year. The xylem blockage due to diseases is the subject of chapter 7.

The name of the author is a sufficient guarantee of the high professional standard of this book. In addition, the information is well presented and readily comprehensible due to readable text and very descriptive figures.

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