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Kinetics of phosphate uptake in excised maize root

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Abstract

The short term uptake of phosphate involving 10 min absorption followed by 5 min desorption, both at 30 °C, in the concentration range 1.0×10^{-9} to 7.5×10^{-2} M KH_2PO_4 by fresh and washed maize (*Zea mays* L. cv. Ganga Safed-2) roots can be described by a single isotherm having five phases (0 and I - IV) with regularly spaced kinetic constants. Almost identical kinetics were observed in both fresh and washed maize roots. The kinetics of phase 0 in the concentration range 1.0×10^{-9} - 3.0×10^{-5} M was sigmoidal in fresh maize roots, however, in washed tissue exhibited 2 phases termed here as 0a and 0b. 0a covered the concentration range 1.0×10^{-9} - 5.0×10^{-6} M and 0b 6.0×10^{-6} - 3.0×10^{-5} M. In the concentration range 1.0×10^{-4} - 7.5×10^{-2} M four distinct phases, termed as I, II, III and IV were evident in both fresh and washed maize roots. Each phase obeyed Michaelis - Menten kinetics. The values of K_m and V_{max} have been estimated for each phase. The uptake isotherm was accompanied by discontinuous transitions.

Key words: kinetic constants, Michaelis-Menten kinetics, single isotherm, *Zea mays*

Introduction

Amongst several metabolic responses evoked by 'washing' in the plant tissues, one of the interesting responses is enhancement of ion accumulation rate (Laties 1969, Leonard and Hanson 1972, Kahl 1973, 1974, Mazliak 1973, Kumar and Pant 1980). Washing-induced ion uptake has been reported in both dormant (Loughman1960) and meristematic tissues (Rains and Floyd 1970, Leonard and Hanson 1972, Jacobson and Young 1975, Kumar and Pant 1980). The molecular nature of the signal generated by washing remains unknown. However, the process leads to

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physiological competence through a stimulation of protein synthesis involving derepression (Van Steveninck 1975).

In plant cells the kinetics of ion uptake agree closely, over limited concentration range, with the classical enzyme kinetics. This is widely taken to indicate that uptake occurs through carriers (Epstein and Hagen 1952). Almost all our knowledge regarding the ion uptake process is based on two main kinetic models, namely, the dual isotherm (Epstein 1966, 1973, 1976) and multiphasic isotherm (Nissen 1974, 1986, 1991, Nandi *et al.* 1987). However, a close examination of these two models reveals that in these models transport across the plasmalemma and tonoplast is not always easy to distinguish.

Phosphate uptake by maize roots was found to correspond to multiphasic isotherm model (Singh and Pant 1982, Nandi *et al.* 1987). However, these authors studied ion uptake kinetics involving 60 min uptake followed by 30 min desorption. This may not present true influx across the plasmalemma over the entire range used (Cram 1969, 1973, Cram and Laties 1971). But it has been argued that quasi-steady overall influx may serve as a useful estimate for influx across plasmalemma from ambient concentration below 10 mM for uptake and wash period shorter than 5 cytoplasmic exchange half times (Nissen 1973a). Short term uptake should, therefore, be useful to study the flux across plasmalemma because the half time of ^{32}P exchange was found to be 26 min for roots of barley (Lefebvre and Clarkson 1984) and 28 min for rhizoid cells of *Chara* (Box *et al.* 1984).

Washing induced ion uptake is an ideal system for the study of uptake mechanism at the molecular level. Moreover, to date kinetics of phosphate uptake have not been studied in washed roots. Therefore, it was decided to investigate phosphate uptake kinetics in maize roots involving 10 min uptake and 5 min desorption in fresh and in 4 h washed roots.

Materials and methods

Primary roots of 3-d-old etiolated maize seedlings (*Zea mays* L. cv. Ganga Safed-2) were used. The caryopses were germinated in glass germinating trays. Each tray contained a 2 cm thick cotton layer wetted with 0.1 mM $CaCl_2$ solution. The trays covered with polythene bags perforated to allow air exchange were placed in a BOD incubator maintained at 29 ± 1 °C. Additional 0.1 mM $CaCl_2$ was added after 2 d to maintain the saturation of cotton pad. For all the experiments 0.5 to 2.5 sections behind the tip of the primary roots of etiolated seedlings were used. Batches of 20 segments were weighed and used within one hour from the time of harvest (Nandi *et al.* 1987).

Washing procedure: The method described earlier (Leonard and Hanson 1972, Kumar and Pant 1980) was used. The tissue was washed in well aerated 0.2 mM $CaCl_2$ solution at 30 °C for various periods of time, *i.e.* 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h and 8 h. The pH of the solution ranged between 5.5 and 6.0 and was adjusted, if needed, with 0.01 M $Ca(OH)_2$. For uniformity 100 cm^3 of washing solution was used for 20 root segments. The washing solution contained 25 μg cm^{-3} chloramphenicol to prevent bacterial contamination.

Uptake procedure: The root segments were allowed to take up phosphate from 100 cm^3 aerated solutions consisting of 0.1 mM $CaCl_2$, and indicated concentration of KH_2PO_4 labeled with carrier free ^{32}P adjusted to pH 6.0 with 0.01 M $Ca(OH)_2$. The uptake period was 10 min and the temperature was 30 °C. After uptake the roots were rinsed for 30 s with 0.1 mM $CaCl_2$ and desorbed with an identical but unlabeled uptake solution for 5 min at 30 °C to remove exchangeable component of absorbed ion (Lefebvre and Clarkson 1984). The roots were then digested to dryness in 5 cm^3 ternary acid mixture (nitric acid:sulfuric acid:perchloric acid 10:1:4, v/v/v) in separate beakers (Nandi *et al.* 1987). 1 cm^3 distilled water was added to each digest and aliquots were taken to determine radioactivity by Cherenkov counting with the help of a liquid scintillation counter (*LKS Wallace - 1217 Rackbeta, Radiations and Isotopic Tracer Laboratory*).

The data obtained were analysed using above mentioned kinetic models. Kinetic parameters V_{max} and K_m were determined by weighted linear least square Lineweaver-Burk plots. For each concentration the experiment was done thrice, each with two replicates. The coefficient of variation for uptake data varied between 0.42 and 18.14 %.

Results

Time course of washing induced phosphate uptake: Washing induced increase in phosphate uptake in maize root could be detected after 1 h washing. It continued for 6 h after which the absorption declined and leveled off (Fig. 1).

Uptake kinetics in fresh roots: Phosphate uptake by excised maize roots in the concentration range 1.0×10^{-9} to 7.5×10^{-2} M could be represented by a single multiphasic isotherm (Figs. 3a,b). Five phases termed here as phase(s) 0, I, II, III and IV were evident with regularly spaced, increasing kinetic constants (Table 1). The

Table 1. Kinetic constants and transition points for the uptake of phosphate by fresh excised maize (*Zea mays* cv. Ganga Safed-2) roots.

Phase number	Concentration range [M]	V_{max} [nmol g^{-1}(f.m.) s^{-1}]	K_m [M]	Transition point [M]
I	1.0×10^{-4} - 1.0×10^{-3}	0.141	5.80×10^{-4}	
II	2.5×10^{-3} - 2.0×10^{-2}	0.456	2.22×10^{-3}	1.0×10^{-3}
III	2.3×10^{-2} - 4.5×10^{-2}	1.761	2.93×10^{-2}	2.0×10^{-2}
IV	5.0×10^{-2} - 7.5×10^{-2}	-	-	4.5×10^{-2}

multiphasic isotherm was constructed solely on the basis of the kinetic constants. Bias can thus be introduced only in the choice of transition points. Phosphate uptake below 3.0 × 10^{-5} M could be accounted for by a single mechanism which showed sigmoidal kinetics and has been termed as phase 0 (Fig. 2a). A close examination of the isotherm in the range from 1.0 × 10^{-4} - 7.5 × 10^{-2} M revealed four phases (Fig. 3a). Each phase conformed to simple hyperbolic type kinetics. The uptake of 1.0 × 10^{-4} - 1.0 × 10^{-3} M phosphate was mediated by phase I, 2.5 × 10^{-3} - 2.0 × 10^{-2} M by phase II, 2.3 × 10^{-2} - 4.5 × 10^{-2} M by phase III, and 5.0 × 10^{-2} - 7.5 × 10^{-2} M by phase IV (Table 1).

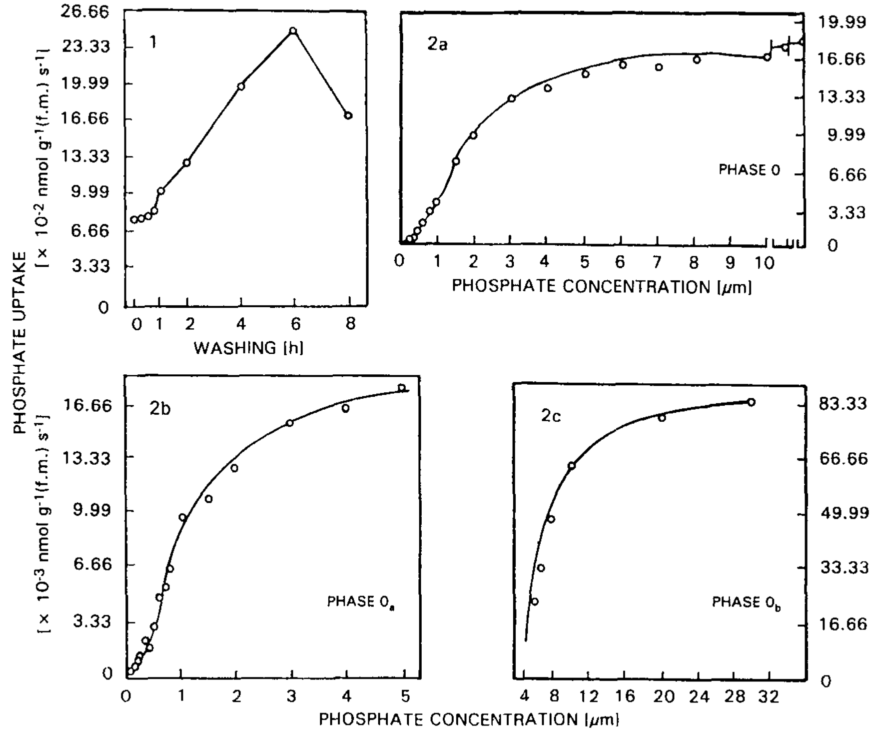


Fig. 1. The effect of washing period on phosphate uptake by excised maize (*Zea mays* L. cv. Ganga Safed-2) roots.

Fig. 2. Phosphate uptake rate by excised maize roots as a function of phosphate concentration over a range of (*a*) 1.0 × 10^{-9} to 3.0 × 10^{-5} M in fresh roots (phase 0), (*b*) 1.0 × 10^{-9} to 5.0 × 10^{-6} M in 4 h washed roots (phase 0a), (*c*) 6.0 × 10^{-6} to 3.0 × 10^{-5} M in 4 h washed roots (phase 0b).

Uptake kinetics in washed roots: Phosphate uptake by 4 h washed excised maize roots in the concentration range 1.0 × 10^{-9} - 7.5 × 10^{-2} M could be represented by a single multiphasic isotherm (Fig. 3a,b). Six phase(s) 0a, 0b, I, II, III, IV were evident with regularly spaced, increasing kinetic constants. Phosphate uptake below 5.0 × 10^{-6} M could be accounted for by a single mechanism which showed sigmoidal kinetics and has been termed as phase 0a (Fig. 2b). A close examination of uptake isotherm in the range from 6.0×10^{-6} to 7.5×10^{-2} M KH_2PO_4 revealed five phases (Fig. 3b). Each phase conformed to simple hyperbolic type kinetics.

The uptake in the range 6.0×10^{-6} to 3.0×10^{-5} M phosphate was mediated by a system which shows hyperbolic kinetics. Since it falls in the concentration range covered by sigmoidal uptake kinetics in fresh roots, it has been termed here as phase 0b (Fig 2c). Likewise, phosphate uptake in 1.0×10^{-4} - 1.0×10^{-3} M was mediated by phase I, 2.5×10^{-3} - 1.8×10^{-2} M by phase II, in 2.0×10^{-2} - 4.0×10^{-2} M by phase III and in 4.5×10^{-2} - 7.5×10^{-2} M by phase IV (Table 2).

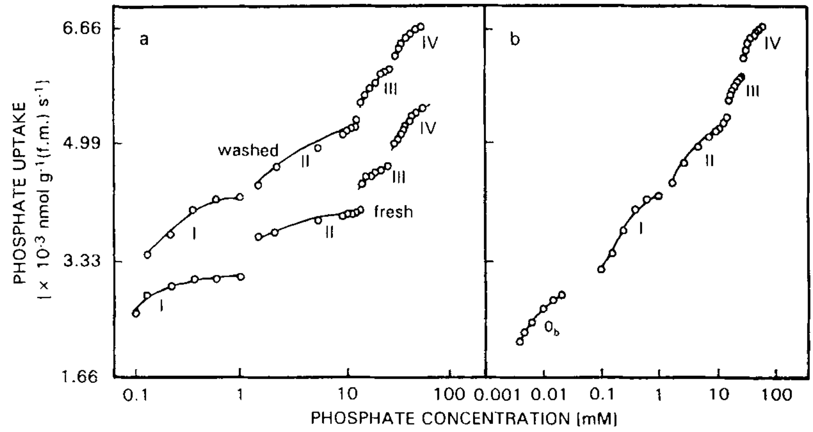
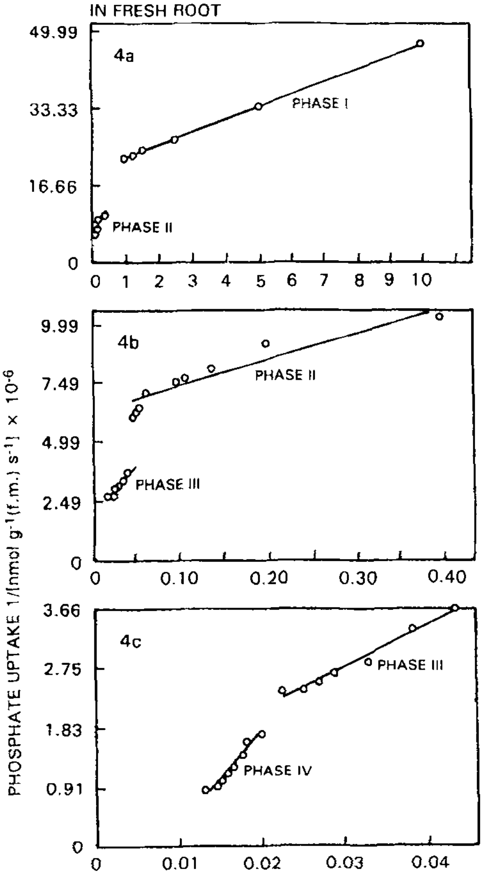


Fig. 3. Phosphate uptake rate by excised fresh and 4 h washed maize roots as a function of phosphate concentration over a range of (*a*) 1.0×10^{-4} to 7.5×10^{-2} M in fresh and washed roots, (*b*) 6.0×10^{-6} to 7.5×10^{-2} M in washed roots.

Table 2. Kinetic constants and transition points for the uptake of phosphate by washed excised maize (*Zea mays* cv. Ganga Safed-2) roots.

Phase number	Concentration range [M]	V_{max} [nmol g^{-1}(f.m.) s^{-1}]	K_m [M]	Transition point [M]
0b	6.0×10^{-6} - 3.0×10^{-5}	0.202	2.63×10^{-5}	
I	1.0×10^{-4} - 1.0×10^{-3}	0.828	6.00×10^{-4}	3.0×10^{-5}
II	2.5×10^{-3} - 1.8×10^{-2}	3.638	1.30×10^{-3}	1.0×10^{-3}
III	2.0×10^{-2} - 4.0×10^{-2}	8.735	5.60×10^{-2}	1.8×10^{-2}
IV	4.5×10^{-2} - 7.5×10^{-2}	-	-	4.0×10^{-2}



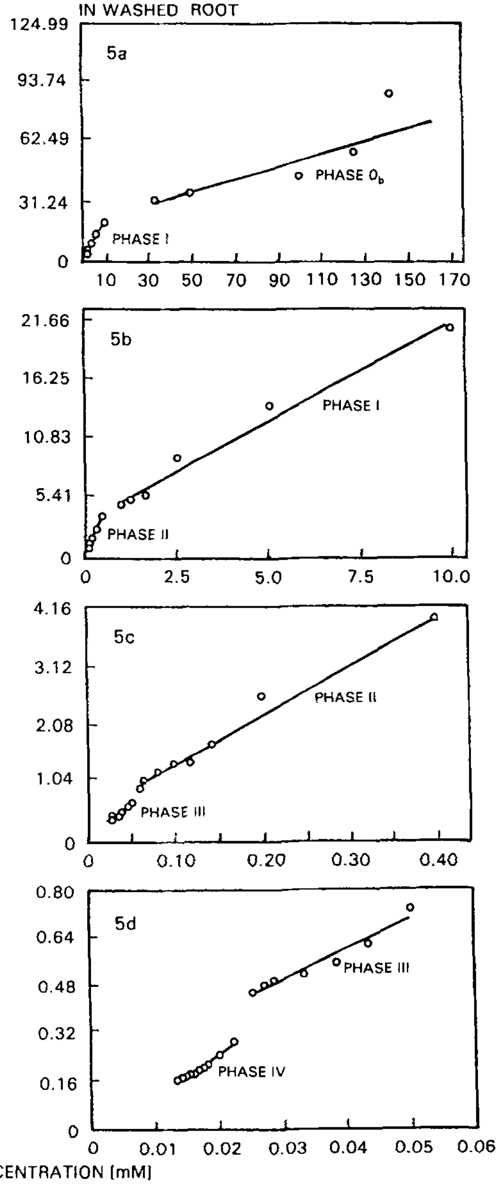


Fig. 4. Double reciprocal plots of data for phosphate uptake by excised fresh maize roots. The data for the different phases have been paired to emphasize the non-continuity of phases in the uptake isotherm.(*a*) Phases I and II (1.0 x 10^{-4} - 2.0 x 10^{-2} M), (*b*) phases II and III (2.5 x 10^{-3} - 4.5 x 10^{-2} M), (*c*) phases III and IV (2.3 x 10^{-2} - 7.5 x 10^{-2} M).

Fig. 5. Double reciprocal plots of data for phosphate uptake by excised 4 h washed maize roots. (*a*) Phases 0b and I (6.0 x 10^{-6} - 1.0 x 10^{-3} M), (*b*) phases I and II (1.0 x 10^{-4} - 1.8 x 10^{-2} M), (*c*) phases II and III (2.5 x 10^{-3} - 4.0 x 10^{-2} M), (d) phases III and IV (2.0 x 10^{-2} - 7.5 x 10^{-2} M).

Discussion

The development of increased phosphate uptake capacity in washed roots required *ca.* 45 min lag period (Fig. 1). Earlier workers have reported a similar lag period (Leonard and Hanson 1972). This induction has been reported to be dependent on respiration, RNA and protein synthesis (Click and Hackett 1963, Leonard and Hanson 1972, Kumar and Pant 1980). The maximum capacity for phosphate uptake was found after 6 h washing (Fig. 1). Hence 4 h washing ought to be adequate to study the effect of washing on the kinetics of phosphate uptake in maize roots.

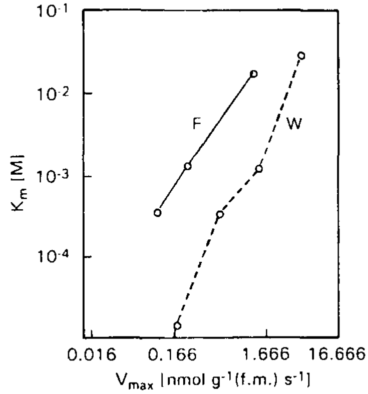


Fig. 6. Plots of K_m *vs* V_{max} for multiphasic phosphate uptake by excised fresh and 4 h washed maize roots.

The isotherm of phosphate uptake involving 10 min uptake by maize roots was found to be nearly identical with the isotherm for the phosphate uptake in 60 min at 30 °C followed by desorption of 30 min at 2 - 3 °C (Singh and Pant 1982, Nandi *et al* 1987). Apparently the rate limiting step for phosphate uptake is at the plamalemma and 60 min uptake studied earlier also reflects the steady state uptake. The kinetics of phosphate uptake by fresh maize roots in the range 1.0×10^{-9} - 3.0×10^{-5} were sigmoidal in nature even with 10 min uptake period (Fig. 2a). This confirms the presence of sigmoidal kinetics for phosphate uptake in this range reported earlier (Singh and Pant 1982, Nandi and Pant 1984). However, in case of washed roots the sigmoidal kinetics were evident only between 1.0×10^{-9} - 5.0×10^{-6} M KH_2PO_4 (Fig. 2b). In keeping with the nomenclature used earlier (Nandi *et al.* 1987) it has been termed as phase 0a. As opposed to the fresh roots the kinetics of uptake between 6.0×10^{-6} - 3.0×10^{-5} M KH_2PO_4 were hyperbolic in nature and because this range is within the range of phase 0 reported earlier this phase has been termed as 0b (Fig. 2c) (Nandi *et al.* 1987). No further attempt was, however, made to characterize phase 0a and uptake in this range may require further characterization.

In the concentration range between 1.0×10^{-4} - 7.5×10^{-2} M in fresh and 6.0×10^{-6} - 7.5×10^{-2} M in washed roots a single multiphasic mechanism was found to mediate phosphate uptake in maize roots (Fig. 3). This is similar to the earlier findings (Singh and Pant 1982, Nandi *et al.* 1987). Four distinct and discontinuous phases termed phase(s) I, II, III and IV were evident both in fresh as well as in washed roots (Fig. 3 - 5). Thus the kinetics of phosphate uptake were similar to those reported earlier for several other ions (Nissen 1971, 1973b, 1987, 1991, Singh and Pant 1982, Nandi *et al.* 1987).

The demonstration by kinetic means of separate sites of uptake and transition (Vange 1974a, b, Nissen 1980, Haverstein and Nissen, 1981) and the demonstration that 'jump' can be produced by the use of analogues which interact only with transition sites, confirm that multiphasic kinetics are indeed discontinuous (Figs. 3 - 5). The transition points in the isotherm for short term phosphate uptake both for fresh and washed root (Tables 1 and 2) are strikingly similar to those reported earlier for phosphate uptake by fresh maize root in which uptake period was 1 h (Singh and Pant 1982, Nandi *et al.* 1987). In phases I - III a good fit of K_m and V_{max} in plot of V_{max} as a function of K_m (Fig. 6) also indicates that the phosphate uptake in maize root is multiphasic (Nissen 1991). In phase IV, however, both in fresh and washed roots, the values of K_m and V_{max} were found to be meaningless. A close look of uptake kinetics in this ambient KH_2PO_4 concentration would reveal that the uptake kinetics are not exactly linear (Figs. 4c and 5d) either, but may reflect a high contribution of diffusion in the uptake.

The kinetics of phosphate uptake in fresh as well as in washed maize roots further strengthen the view that a single multiphasic mechanism is located in the plasmalemma (Nissen 1974, 1991). The phosphate uptake data could not be interpreted in terms of dual isotherms with their attendant parallel operation (Epstein 1972, 1973, 1976) and operation in series (Laties 1969). Evidently washing response in terms of augmented ion uptake is located in plasmalemma and may not relate to the tonoplast suggested by Hanson *et al.* (1973).

References

Box, R., Boxer, M., Boxer, D.: Compartmentation of PO_4^{3-} uptake and ^{32}P efflux in the rhizoid cells of *Chara hispida* L. - Biochem. Physiol. Pflanz. **180**: 551-555, 1985.

Click, R.E., Hackett, D.P.: The role of protein and nucleic acid synthesis in the development of respiration in potato tuber slices. - Proc. nat. Acad. Sci. USA **50**: 243-250, 1963.

Cram, W.J.: Short term influx as a measure of influx across plasmalemma. - Plant Physiol. **44**: 1013-1015, 1969.

Cram, W.J.: Chloride fluxes in cells of isolated root cortex of *Zea mays*. - Aust. J. biol. Sci. **26**: 757-779, 1973.

Cram, W.J., Laties, G.G.: The use of short term quasi-steady influx in estimating plasmalemma and tonoplast influx in barley root cells at various external and internal chloride concentration. - Aust. J. biol. Sci. **24**: 633-646, 1971.

Epstein, E.: Dual pattern of ion absorption by plant cells and by plants. - Nature **212**: 1324-1327, 1966.

Epstein, E.: Mineral Nutrition of Plants: Principles and Perspectives. - John Wiley & Sons, New York 1972.

Epstein, E.: Mechanism of ion transport through plant cell membranes. - Int. Rev. Cytol. **34**: 123-168, 1973.

Epstein, E.: Kinetics of ion transport and the carrier concept. - In: Lüttge, U., Pitman, M.G. (ed.): Encyclopedia of Plant Physiology. New Series. Vol. 2. Transport in Plants II. Part B. Tissue and Organs. Pp. 70-74. Springer-Verlag, Berlin - Heidelberg - New York 1976.

Epstein, E., Hagen, C.E.: A kinetic study of the absorption of alkali cations by barley. - Plant Physiol. **27**: 456-474, 1952.

Hanson, J.B., Leonard, R.T., Mollenhauer, H.M.: Increased electron density of tonoplast membranes in washed corn root tissue. - Plant Physiol. **52**: 292-300, 1973.

Havarstein, L.S., Nissen, P.: Stimulation of plasmalemma adenosine triphosphatase from oat roots by inorganic and organic cations: Concentration dependence, selectivity and sites. - Plant Physiol. **68**: 597-604, 1981.

Jacobson, L., Young, L.C.T.: The effect of aging on ion uptake by existed roots. - Physiol. Plant. **35**: 243-248, 1975.

Kahl, G.: Genetic and metabolic regulatation in different plant storage tissue cells. - Bot. Rev. **39**: 274-299, 1973.

Kahl, G.: Metabolism in plant storage slices. - Bot. Rev. **40**: 263-314, 1974.

Kumar, A., Pant, R.C.: Washing induced phospholipid synthesis in corn roots. - Indian J. exp. Biol. **18**: 192-196, 1980.

Laties, G.G.: Dual mechanism of salt uptake in relation to compartmentation and long-distance transport. - Annu. Rev. Plant Physiol. **20**: 89-116, 1969.

Lefebvre, D.D., Clarkson, D.T.: Compartmental analysis of phosphate in barley roots in intact barley seedlings. - Can. J. Bot. **62**: 1076-1080, 1984.

Leonard, R.T., Hanson, J.B.: Induction and development of increased ion absorption in corn root tissues. - Plant Physiol. **49** : 430-435, 1972.

Loughman, B.L.: Uptake and utilization of phosphate associated with respiratory changes in potato tuber slices. - Plant Physiol. **35**: 418-424, 1960.

Mazliak, P.: Lipid metabolism in plants. - Annu. Rev. Plant Physiol. **24**: 287-310, 1973.

Nandi, S.K., Pant, R.C.: Temperature effect on kinetics of phosphate uptake by excised corn (*Zea mays* L. var. Ganga-5) root. - Indian J. exp. Biol. **22**: 564-567, 1984.

Nandi, S.K., Pant, R.C., Nissen, P.: Multiphasic uptake of corn roots. - Plant Cell Environ. **10**: 463-474, 1987.

Nissen, P.: Uptake of sulfate by root and leaf slices of barley: mediated by single, multiphasic mechanism. - Physiol. Plant. **24**: 315-324, 1971.

Nissen, P.: Kinetics of ion uptake in higher plants. - Physiol. Plant. **28**: 113-120, 1973a.

Nissen, P.: Multiphasic ion uptake in roots. - In: Anderson, W.P. (ed.): Ion Transport in Plants. Pp. 539-553. Academic Press, London 1973b.

Nissen, P.: Uptake mechanism: inorganic and organic. - Annu. Rev. Plant Physiol. **25**: 53-79, 1974.

Nissen, P.: Multiphasic uptake of potassium by barley roots of low and high potassium content: separate sites for uptake and transitions. - Physiol. Plant. **48**: 193-200, 1980.

Nissen, P.: Nutrient uptake by plants. Effect of external ion concentration. - Acta Hort. **176**: 21-28, 1986.

Nissen, P.: Multiphasic uptake mechanism in plants. - Plant Cell Environ. **10**: 475-485, 1987.

Nissen, P.: Multiphasic uptake mechanisms in plants. - Int. Rev. Cytol. **26**: 89-134, 1991.

Rains, D.W., Floyd, R.A.: Influence of calcium on sodium and potassium absorption by fresh and aged bean stem slices. - Plant Physiol. **45**: 93-98, 1970.

Singh, S.P., Pant, R.C.: Kinetics of phosphate uptake in excised corn (*Zea mays* L. var. Ganga-5) root. - Indian J. exp. Biol. **20**: 324-326, 1982.

Vange, M.S., Holmern, K. Nissen, P.: Multiphasic uptake of sulfate by barley roots I. Effect of analogues, phosphate, and pH. - Physiol. Plant. **31**: 292-301, 1974a.

Vange, M.S., Holmern, K., Nissen, P.: Uptake of sulfate by barley roots. Separate sites for uptake and phase transition. - In: Zimmermann, U., Dainty, J. (ed.): Membrane Transport in Plants. Pp. 329-333. Springer-Verlag, Berlin 1974.

Van Steveninck, R.F.M.: The "washing" or "aging" phenomenon in plant tissues. - Annu. Rev. Plant Physiol. **26**: 237-258, 1975.