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Variations in Sodium Uptake Along Primary Roots of Corn Seedlings[1, 2]

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A. Eshel and Y. Waisel
Department of Botany, Tel Aviv University, Tel Aviv, Israel.

ABSTRACT

Entry of Na^+ into segments of the apical 8-centimeter portion of corn (*Zea mays*) roots was investigated and analyzed for each centimeter segment separately. Influence of temperature in the 0 C to 30 C range was well described by the Arrhenius equation [$U = A \exp(-Ea/RT)$]. Values of A and Ea differed for each segment, tending to lessen with increasing distance from root apex. Time course of Na^+ entry was followed up to 70 minutes. Time relations of the process fit well the expression $U = m[1 - \exp(-nt)]$. Calculated maximal uptake capacity (m) diminished with increasing distance from the apex. The data presented indicate that sodium uptake mechanisms vary qualitatively and quantitatively along corn roots. Thus, the use of entire roots for characterization of uptake mechanisms should be reassessed.

Roots are not uniform organs, and designation of certain root segments as "absorbing zones" was used by plant physiologists since the beginning of the century (*cf.* 22). Attempts to determine the site and size of that root segment through which most uptake of minerals or water occurred were based on sizes of epidermal or cortical cells, abundance of root hairs, and degree of differentiation of tissues such as endodermis and exodermis. In physiological experiments conducted later, differences in uptake of minerals or water were found not only between suberized and young root portions but also along young, unsuberized roots of uniform appearance (16–18, 24, 25). Further investigation into that subject revealed differences in uptake mechanisms among various root portions of increasing age. Passive uptake of minerals was shown to be confined to the first few millimeters behind the root tip, whereas metabolic uptake occurred in older segments of the root (1, 6, 14, 15).

Brouwer (3) found that water conductivity of the apical root segments distinctly differed from that of other segments of the same roots. Conditions which inhibited water uptake through the apical segment enhanced it in the rest of the root.

Distribution of ^{32}P, ^{35}S, or ^{36}Cl along dried pine and wheat roots was investigated with an automatic chromatogram scanner (1, 2, 19, 20). These experiments revealed that patterns of ion distribution were characteristic for plant species, type of ion, and experimental conditions.

Better knowledge of the variations in uptake processes along roots is a basic prerequisite for progress in understanding the physiology of mineral nutrition of plants (20, 21, 26). In the following investigation, sodium uptake mechanisms were studied in the apical 8-cm portion of corn roots.

MATERIALS AND METHODS

Corn seeds (*Zea mays* L. cv. White Horse Tooth) were immersed in 10% (w/v) NaOCl for 1 hr, washed thoroughly in tap water, and soaked in continuously aerated distilled water during the next 24 hr. Seeds were then spread on a plastic sieve between two wet sheets of cheesecloth. The sieve was fitted into a 3-liter beaker which contained 2.5 liters of solution. The solution used was a half-strength Hoagland's nutrient solution containing 3 mM NaCl. Sodium chloride was added to the growth medium, in order to keep it as similar as possible to the treatment solution. The solution was continuously aerated. The beaker was covered with a thin polyethylene sheet to reduce evaporation, and was kept in the darkness at 27 ± 2 C for 6 days. The nutrient solution was changed on the 3rd day. Seeds which failed to germinate after 24 hr were discarded.

The apical 9-cm portions of primary roots were excised, rinsed in distilled water, and immersed for an uptake period in a $^{22}NaCl$ solution. Sodium chloride concentration was 0.3 or 3.0 mM. Temperature of the absorption solution (0 C, 10 C, 20 C, or 30 C) was maintained by a water bath. Unless otherwise stated, uptake period was 10 min. Following uptake, roots were washed for 1 min in cold distilled water (2–5 C) and desorbed for 1 hr in 3 mM NaCl + 5 mM $CaCl_2$ solution at 0 C. Preliminary experiments showed that such a desorption period was adequate for exchanging the adsorbed ^{22}Na off these roots. Such short periods of uptake and desorption were selected in order to avoid complications induced by consequent ion transport and exudation. Thus, data denote entry into the nonexchangeable fraction. Air was bubbled through all solutions continuously. Following the desorption period, roots were rinsed, blotted, and sectioned by either of two methods: (*a*) the apical 8-cm portions were sectioned into forty 2-mm slices. Slices were placed in counting vials containing 5 ml of Bray's scintillation mixture, and assayed for radioactivity by liquid scintillation counting; (*b*) the apical portions were cut into eight 1-cm pieces, which were transferred to plastic vials and assayed by gamma spectroscopy. The proximal 1-cm segment was discarded in both cases because of expected wound effects. Samples of the absorption solution were diluted 1:100 and counted concomitantly. Fresh weight of roots was about 7 mg/cm.

Logarithmic transformations of the measured values were

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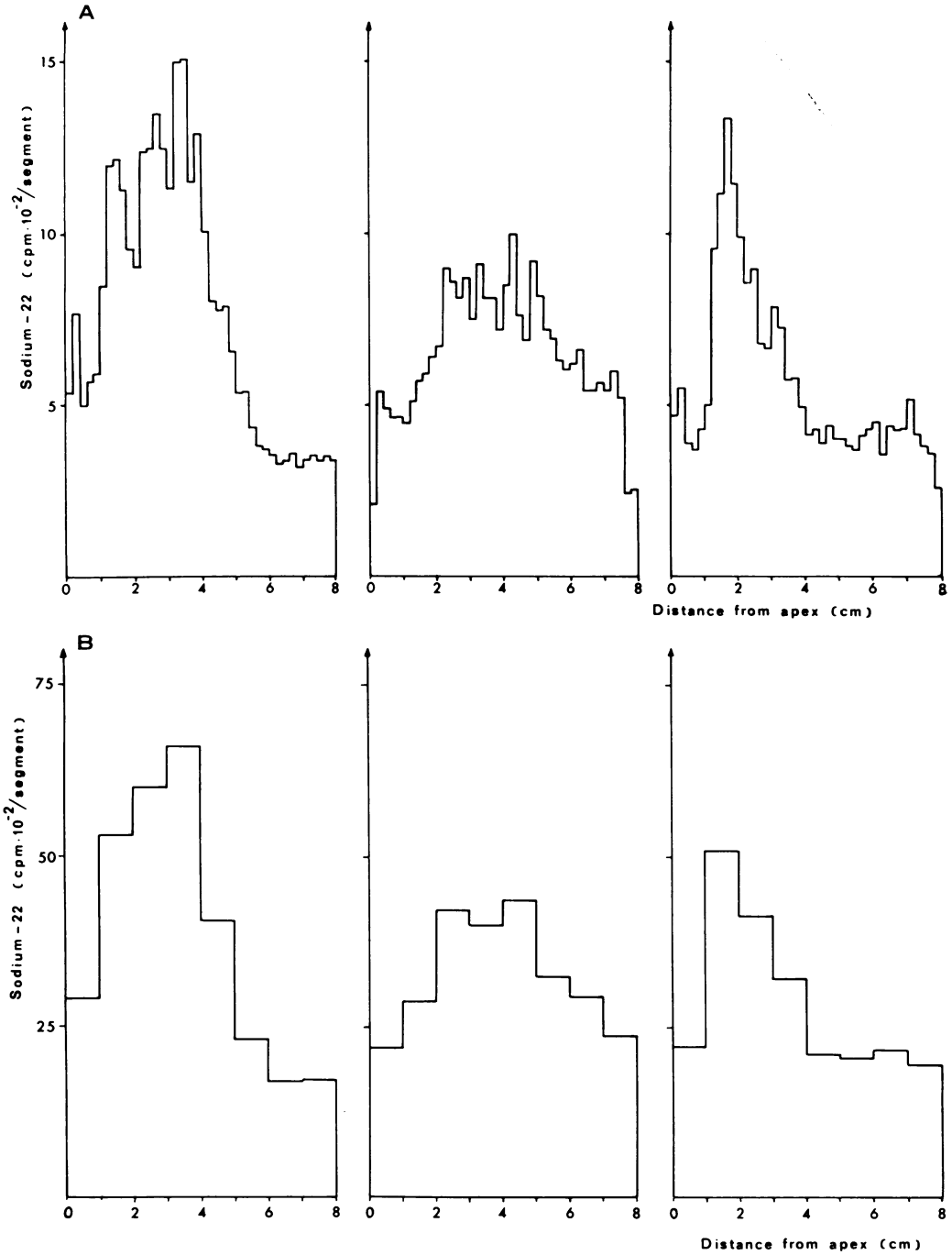

FIG. 1. Distribution patterns of ^{22}Na along apical 8-cm portions of corn roots. Uptake from 3 mM $^{22}NaCl$ solution at 30 C in 10-min period, followed by 1-min wash in distilled water and 1-hr desorption in 3 mM NaCl + 5 mM $CaCl_2$ at 0 C. Roots were sectioned at end of experiment, into forty 2-mm slices which were assayed for ^{22}Na separately. A: Data for 2-mm slices; B: same data as in A computed for 1-cm segments.

used for statistical analysis. Standard deviations of such transformations were more uniform than those of the original values and were less dependent on the absolute values of the means. One way analysis of variance was used for analysis of uptake data by each segment under various treatments. In all reported cases, F ratio exceeded 0.0005 level of significance. Equation parameters were estimated by nonlinear regression using a BMDX85 computer program (7). Lack of fit between measured and calculated data was tested by analysis of variance and was found to be insignificant at the 5% level.

RESULTS

In the first set of experiments, roots were sectioned into 2-mm slices. Examples of ^{22}Na distribution patterns along individual roots, as shown by assay of such slices, are presented in Figure 1A. Since it was concluded that major trends of the patterns were not abolished by the use of larger segments (Fig. 1B), such segments were used henceforth, enabling an increase in number of replications. Roots used in these experiments varied in size. Nevertheless, variability of the patterns

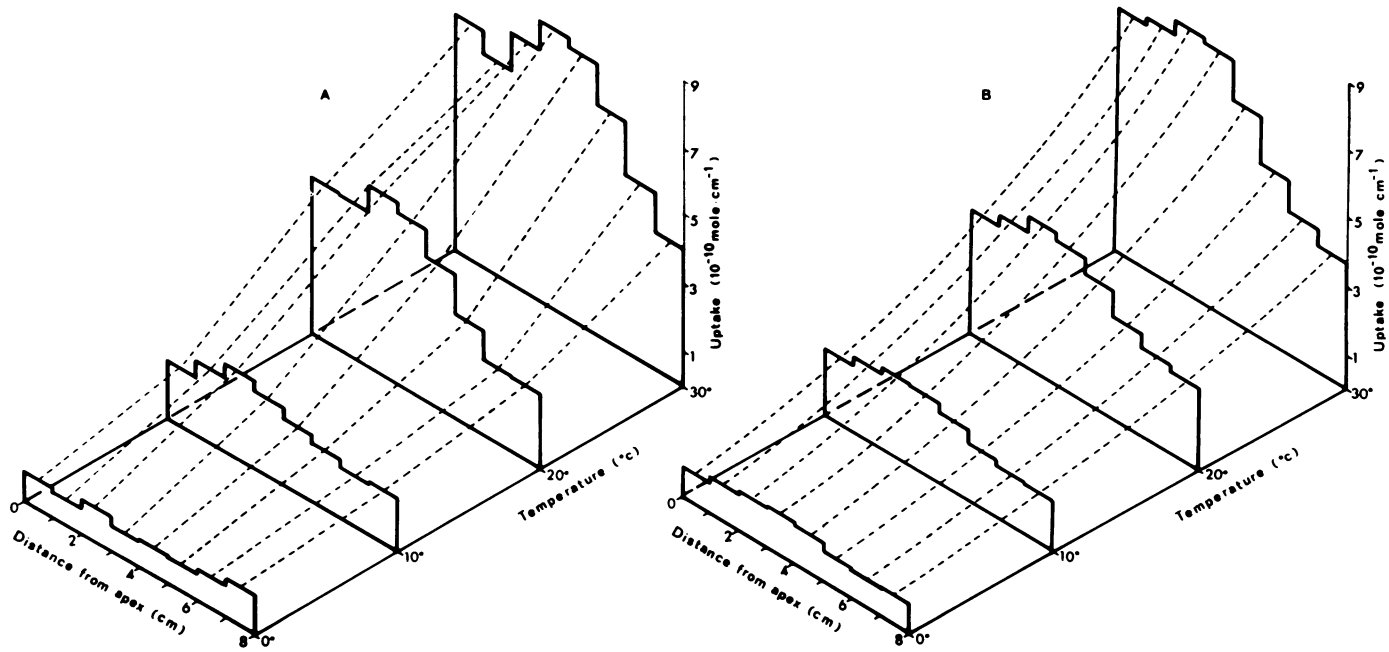

FIG. 2. Effects of temperature on Na^+ uptake by different segments of the apical eight centimeters of corn roots. Uptake from 3 mM $^{22}NaCl$ solution in 10-min periods, followed by 1-min wash in distilled water and 1-hr desorption in 3 mM NaCl + 5 mM $CaCl_2$ solution at 0 C. Roots were sectioned at end of experiment and segments assayed for ^{22}Na separately. A: Means of measurement; B: uptake computed by the Arrhenius equation [$U = A \exp(-Ea/RT)$] using A and Ea values listed in Table I.

of uptake could not be correlated with morphological characteristics of the tissue, such as total root length, root diameter, number or diameter of xylem vessels, or ratio of radii of cortex and stele. Consequently, all the roots examined were treated as belonging to one population with inherent variability.

Temperature treatments during uptake period were 0 C, 10 C, 20 C, or 30 C. During subsequent stages of all experiments temperature was kept low (0 C) to reduce metabolic activity. Effects of temperature on rates of sodium influx to the eight segments can be seen from the results plotted in Figure 2A. While at low temperature (0 C) influx to all segments was similar, different rates were obtained for various segments at higher temperatures. Differences among segments were positively correlated with temperature. The dependence of uptake rate (U) on the absolute temperature (T) can be well described by the Arrhenius equation: $U = A \exp(-Ea/RT)$ where R is the gas constant. For biological processes this equation holds true only in a limited range of temperatures. The pre-exponential factor (A) and activation energy (Ea) for uptake of the eight apical 1 cm root segments are listed in Table I. Gradual decrease of A and Ea was observed from the apical segment to the 8th cm. While similar values of A and Ea were obtained in the apical segments at external concentrations of 0.3 and 3 mM, such values were far lower for the distal segments in the 3 mM treatment. The uptake rates which were calculated using the constants listed in Table I were plotted in Figure 2B. The trends shown in Figure 2, A and B, are very similar in the range investigated.

Time course of sodium influx was followed up to 70 min. Data for uptake from a 3 mM NaCl solution by the eight segments are plotted in Figure 3A. Dependence of sodium uptake (U) on time (t) was described by the equation $U = m[1-\exp(-nt)]$. The constant (m) is the maximal quantity which can be absorbed by the segment in these experimental conditions, while (n) is a coefficient inversely proportional to the time needed to reach (m). These constants for the eight segments are presented in Table II, and the calculated curves of uptake *versus* time are plotted in Figure 3B.

Table I. *Values of Pre-exponential Factor and Activation Energy of Na^+ Uptake Calculated by the Arrhenius Equation*

Experimental conditions are the same as in Figure 2.

Distance from Apex	NaCl Concn			
	0.3 mM		3.0 mM	
	A	Ea	A	Ea
cm	*10^{-19} mole/cm·10 min*	*cal/mole*	*10^{10} mole/cm·10 min*	*cal/mole*
0–1	182.6	11268	199.2	11036
1–2	167.1	10312	210.0	11598
2–3	160.7	9898	213.1	11764
3–4	163.2	10054	186.4	10310
4–5	152.6	9516	154.3	8566
5–6	142.0	9018	121.6	6762
6–7	136.0	8762	100.1	5624
7–8	116.9	7684	75.0	4132

DISCUSSION

Differences in uptake capacity of the various segments could be interpreted either on quantitative or on qualitative bases. By quantitative differences it is meant that uptake is executed by the same mechanism in all segments, but differences in uptake capacity occur along roots.

Differences in uptake along various root segments are considered quantitative only when responses of such segments to various environmental factors remain constant. However, when the responses of the segments to temperature and ion concentration vary, differences in uptake are regarded as

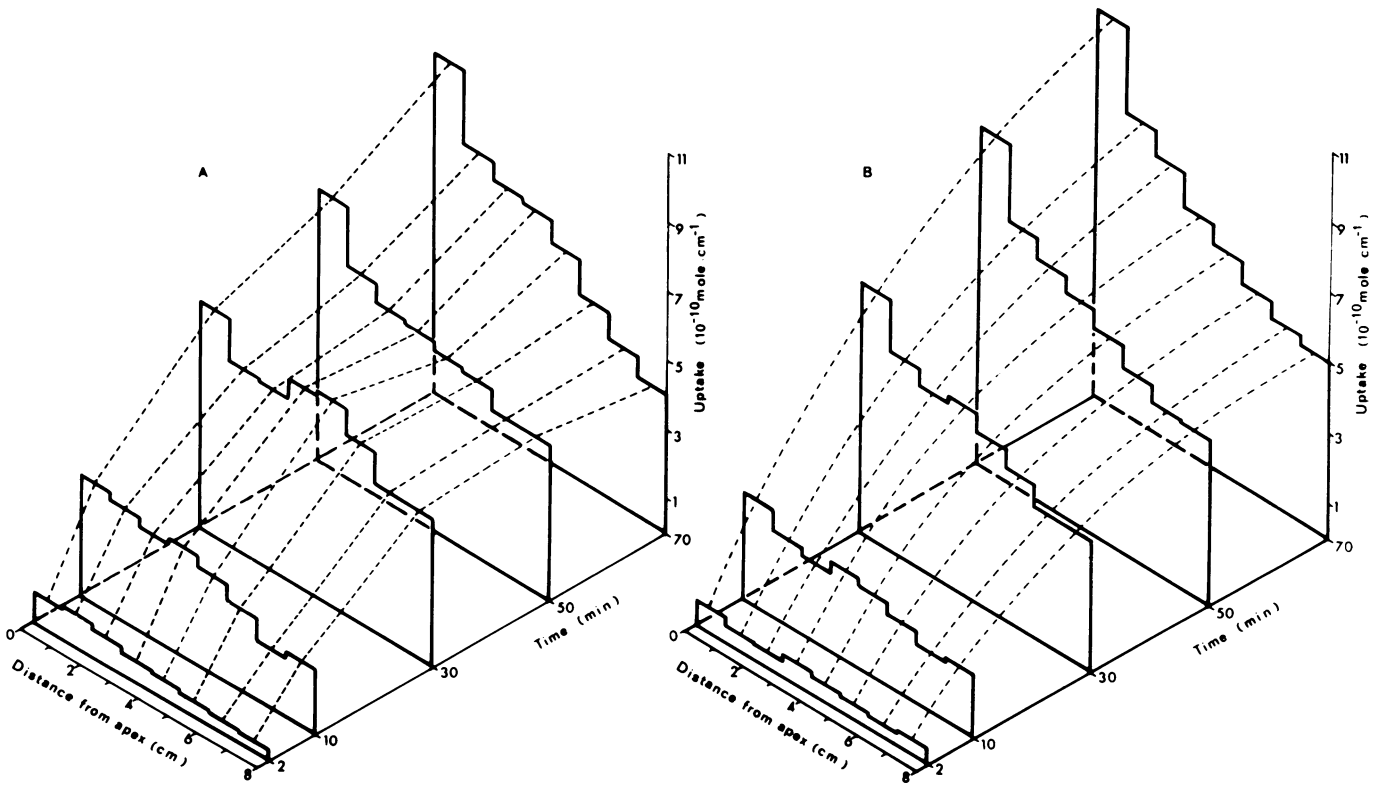

FIG. 3. Time course of Na^+ uptake by different segments of the apical eight centimeters of corn roots. Uptake from 3 mM $^{22}NaCl$ solution at 30 C, followed by 1-min wash in distilled water and 1-hr desorption in 3 mM NaCl + 5 mM $CaCl_2$ solution at 0 C. Roots were sectioned at the end of the experiment, and segments were assayed for ^{22}Na separately. A: Means of measurements; B: uptake computed by the equation $U = m\,[1\text{-}\exp(-nt)]$ using m and n values listed in Table II.

Table II. *Values of the Constants m and n for Time Course of Na^+ Uptake*

Experimental conditions are the same as in Figure 3. These constants are calculated by the equation $U = m[1 - \exp(-nt)]$.

Distance from Apex	m	$n \cdot 10^2$
cm	*10^{-10} mole*	*per min*
0–1	132.6	2.61
1–2	100.6	2.81
2–3	89.3	3.06
3–4	72.3	4.52
4–5	66.4	4.53
5–6	60.6	4.29
6–7	57.9	3.59
7–8	54.5	4.04

qualitative. Qualitative differences imply existence of different uptake mechanisms at various sites along the root.

Occurrence of different uptake mechanisms in various segments within the first centimeter of corn roots was clearly demonstrated (9–12). Ion accumulation was passive in the apical 1.8-mm portion, but metabolic beyond that portion. Differences in uptake were related in these studies to degree of cell vacuolation with metabolic uptake occurring only in vacuolated root segments. Torii and Laties (27) also noted differences in uptake mechanisms between cells at different stages of differentiation. They reported that ion uptake by nonvacuolated root tips (0–2 mm) exhibited characteristics of the so-called system 1 only. Proximal segments (2–15 mm from apex), containing vacuolated cells, exhibited characteristics of both system 1 and 2.

Like other cell maturation processes, vacuolation is gradual and can be reflected by changes of various anatomical parameters. Brown and Broadbent (4) described changes of cell anatomy along the apical centimeter of roots. Measurements of dry weight per cell, cell volume and protein N per cell volume all indicated that vacuolation was terminated only at the 5th mm of the roots examined. Attempts to correlate variations in ion uptake to variations of such anatomical parameters were not always successful. For example, potassium uptake could not be directly correlated either with cell number or with protein-N content per segment (*cf.* 5). On the other hand, Grasmanis and Barley (8) showed that nitrate and ammonium uptake could be well correlated with tissue nitrogen content. Poor correlations were found also with surface area, dry weight, or fresh weight of root segments.

Sodium uptake capacity varied most distinctly in the 4th to 8th cm segments of roots used in our experiments. In these segments no apparent changes of cell size, structure or number could be seen. It was thus assumed that the variations in uptake capacity were of physiological nature. This assumption was further supported by the fact that changes in metabolic activity, *e.g.*, by varying the ambient temperature, induced instantaneous changes in uptake patterns. No distinct differences in sodium influx were found among segments at 0 C, when metabolic uptake was mostly inhibited. Activation of metabolism induced by rise of temperature caused an increase in influx rates in all segments. However, entry into the 4 apical cm was enhanced much more than into older parts of the roots. In the 4th to 8th cm segments, a gradual decrease in the effect of temperature was observed. Changes in uptake patterns, induced by temperature were also observed by Bowen (1).

Enhancement of the rate of a process by temperature is de-

termined by its activation energy. In processes if ion uptake, activation energy reflects characteristics of the mechanism involved in passage of selective cell barriers into the so-called nonexchangeable fraction. Differences in activation energy values among root segments indicate differences in the reactions involved, *i.e.*, in uptake mechanisms. Existence of various uptake mechanisms in different segments along roots were proposed by Hanson and Kahn (13) who made a kinetic analysis of potassium uptake in segments of the apical 35 mm of roots. Using the Michaelis-Menten equation, different values of Km and V_{max} were found for each segment, indicating diversity of uptake mechanisms.

Summarizing all the available information, it is thus evident that average values of various uptake parameters, obtained for long excised root sections, are meaningless for characterizing ion uptake on the mechanism level. When a metabolic uptake mechanism is studied, only the root segment in which uptake is executed by this mechanism should be used. Inclusion of other root portions only impairs the information that can be obtained for metabolic dependence of ion uptake. Furthermore, not only does uptake capacity of one ion vary along roots, but various ions have different uptake patterns, with maxima located at different sites (19, 23). Thus, any investigation of mutual effects between ions (competition) must consider differences between their uptake patterns.

A full description of ion uptake by roots should take into account all the variables involved. Therefore, mineral uptake should be described as a multidimensional space, within which uptake changes as a function of several environmental and plant parameters. Such a description can be achieved by a mathematical expression with constants of physiological meaning, which will define relationships of uptake to all parameters together. Unfortunately, such an expression is not yet available. Attempts must be made to present experimental data in a way which will readily fit into such a scheme in the future.

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