Influence of the Level of Nitrate Nutrition on Ion Uptake and Assimilation, Organic Acid Accumulation, and Cation-Anion Balance in Whole Tomato Plants

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ABSTRACT

Tomato plants (*Lycopersicon esculentum* L. var. Ailsa Craig) were grown in water culture in nutrient solution in a series of 10 increasing levels of nitrate nutrition. Using whole plant data derived from analytical and yield data of individual plant parts, the fate of anion charge arising from increased NO_3 assimilation was followed in its distribution between organic anion accumulation in the plant and OH^- efflux into the nutrient solution as calculated by excess anion over cation uptake. With increasing NO_3 nutrition the bulk of the anion charge appeared as organic anion accumulation in the plants. OH^- efflux at a maximum accounted for only 20% of the anion charge shift. The major organic anion accumulated in response to nitrate assimilation was malate. The increase in organic anion accumulation was paralleled by an increase in cation concentration (K^+, Ca^{2+}, Mg^{2+}, Na^+). Total inorganic anion levels (NO_3^-, SO_4^{2-}, $H_2PO_4^-$, Cl^-) were relatively constant. The effect of increasing NO_3 nutrition in stimulating organic anion accumulation was much more pronounced in the tops than in the roots.
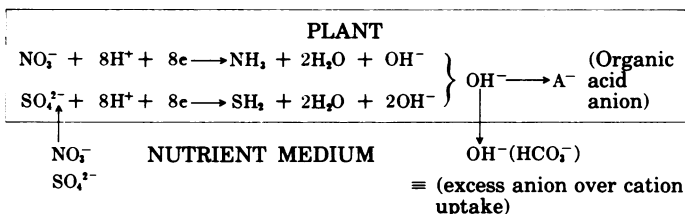
It is suggested that increasing the level of NO_3 nutrition to tomato plants stimulates cation uptake and translocation as counter-ions are required to accompany NO_3^- ions to the upper plant parts, the major site of NO_3 reduction. On NO_3 reduction, the resulting stoichiometric accumulation of organic anions is balanced by the cations originally accompanying NO_3^- ions. Organic anions and cations are largely retained in the upper plant parts. The results suggest that only a small fraction of the total K absorbed by the roots can be translocated downward from the leaves to the roots in the phloem sap. The possible extent of K recirculation is thus low.

In the process of ion uptake by whole plants, electroneutrality is maintained both in the plant and the nutrient medium in which the plant is growing. As plant roots absorb nutrient ions at different rates, electroneutrality is achieved within the plant by the accumulation or degradation of non-volatile organic acids, which at the neutral to slightly acid pH of most cell vacuoles are dissociated and are present mainly as anions (9, 12, 21). In the nutrient medium neutrality is maintained by either H^+ or OH^- excretion from the roots (16, 20). The presence of respiratory CO_2 in the root medium implies that OH^- excretion may appear in the nutrient medium as HCO_3^-.

In the case of plants supplied with non-N-containing salts, it has been concluded from short term experiments that when cations are taken up more rapidly than anions, stoichiometric amounts of non-volatile organic acid anions are accumulated

corresponding to the excess of cations absorbed. Similarly when anion uptake exceeds cation uptake, there is an equivalent decrease in the concentration of organic acid anions. The amount of malate in particular is subject to fluctuation in response to differential rates of cation and anion absorption (9, 12).

When NO_3^- is supplied in the nutrient medium, as is the normal case for plants growing in soil, the situation is different. Anion uptake exceeds cation uptake yet in comparison with other forms of N nutrition, higher concentrations of organic acids are accumulated in NO_3-fed plants (4, 5, 10, 14, 16, 20). Dijkshoorn (7) has suggested that this occurs because in the assimilation of NO_3^- and SO_4^{2-} to form neutral organic N and S compounds, the negative charge from these ions is passed on to form organic anions in the plant or is transferred into the nutrient medium as OH^- in exchange for excess anion over cation uptake. This scheme is shown below:

$$
\begin{array}{l}
\text{PLANT} \\
NO_3^- + 8H^+ + 8e \longrightarrow NH_3 + 2H_2O + OH^- \\
SO_4^{2-} + 8H^+ + 8e \longrightarrow SH_2 + 2H_2O + 2OH^-
\end{array}
\left.\right\} OH^- \longrightarrow A^- \begin{array}{l} \text{(Organic} \\ \text{acid} \\ \text{anion)} \end{array}
$$

$$
\begin{array}{lll}
NO_3^- & \text{NUTRIENT MEDIUM} & OH^-(HCO_3^-) \\
SO_4^{2-} & & \equiv \text{(excess anion over cation} \\
& & \text{uptake)}
\end{array}
$$

A previous publication (15) reported data of ion uptake and assimilation, and organic anion accumulation by whole tomato plants grown at one level of NO_3-N. The organic anion fraction included malate, citrate, oxalate, and pectate. Per plant, the equivalents of organic (N + S) were closely balanced by the total sum of organic anions accumulated plus OH^- excreted into the nutrient medium as calculated from the excess anion over cation uptake. The present paper extends the investigation by considering the effect of varying levels of NO_3 nutrition on this relationship. As the intensity of NO_3 uptake and assimilation may well influence the uptake and distribution of other ions, we also considered the effect of the level of NO_3 nutrition on cation-anion balances in the tops and roots as well as in whole plants.

MATERIALS AND METHODS

Tomato plants (*Lycopersicon esculentum* L. var. Ailsa Craig) were grown in 71 containers in a greenhouse as described previously (16). Each container held two plants which were grown on from the four-leaf stage. Treatments were replicated four times.

Ten different nutrient solutions were prepared in which NO_3 concentrations were varied from a generally accepted low level (0.25 meq NO_3^-/1) to a high level of NO_3 supply (12 meq NO_3^-/1). Details of the nutrient solutions are given in Table I. Cation

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Table I. Composition of the nutrient solutions containing different levels of nitrate (meq/1)

	meq NO₃⁻/1									
	0.25	0.5	0.75	1.0	1.5	2.5	4.0	6.0	8.0	12.0
KH₂PO₄	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
MgSO₄	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	1.5	1.5
Mg(NO₃)₂	-	-	-	-	-	-	-	-	1.5	1.5
Ca(NO₃)₂	0.25	0.5	0.75	1.0	1.5	2.5	4.0	6.0	8.0	8.0
CaSO₄	7.75	7.5	7.25	7.0	6.5	5.5	4.0	2.0	-	-
K₂SO₄	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.0	-
KNO₃	-	-	-	-	-	-	-	-	0.5	2.5

levels were constant in all treatments (Ca^{2+} 8 meq/l, K^+ 4 meq/l, Mg^{2+} 3 meq/l). Other than nitrate, sulfate was the only variable ion. Micronutrients were added as described in an earlier publication (16). The pH values of the solutions were adjusted to 5.5 and maintained at this pH by daily additions either of 0.1 M $Ca(OH)_2$ or 0.1 M H_2SO_4. Every 2 days the nutrient solutions were completely renewed.

After 28 days growth in the nutrient media, the plants were harvested and divided into leaves, petioles, stems, and roots and each replicate weighed. Similar tissues from each treatment were bulked together. Weighed samples of fresh material were freeze-dried. The remaining fresh material was dried at 85 C to constant weight and ground to a fine powder using a microhammer mill.

The oven-dried material was used for the estimation of total N, NO_3-N, total S, SO_4-S, Cl, Pi, Ca, Mg, K, Na, alkalinity of the ash (total organic anions), and oxalate. It was also extracted with boiling water and uronic acids (pectate) estimated in the residues. Malic and citric acids were determined in the freeze-dried tissues. All results were recorded as meq/100 g on a dry weight basis. The methods used were described in earlier publications (14, 16).

Values for cation and anion uptake, as well as organic anion accumulation by whole plants were derived from the weights and analytical data of the individual plant parts. Similar calculations were made in obtaining cation-anion balance data for aerial plant parts (leaves + petioles + stems). OH^- or H^+ excretion was calculated from excess anion over cation, or cation over anion uptake, respectively, by whole plants. Values for assimilated (N + S) by whole plants were calculated from dry matter yield and analytical data of the individual tissues for assimilated N (total N—NO_3-N) and assimilated S (total S—SO_4-S).

RESULTS

The influence of the level of NO_3 supply on dry matter yield production is shown in Table II. As expected, increasing the level of NO_3 nutrition from N deficiency levels to high levels of N supply exerted a very marked influence. Yields in the 12 meq NO_3^-/l treatment were about 2½ times greater than in the lowest NO_3 treatment. Plants supplied with 1.5 meq NO_3^-/l or less showed N deficiency symptoms. Table II also shows the influence of the level of NO_3 nutrition in whole tomato plants on the amount and fate of negative charge resulting from the assimilation of NO_3^- and SO_4^{2-}. Increasing the level of NO_3^- in the nutrient medium from 0.25 meq/l to 12 meq/l produced a dramatic effect raising the total assimilated (N + S) values by over 100%. This largely resulted from the increase in the levels of assimilated N. The values of assimilated (N + S) were paralleled at each NO_3 level by almost equivalent values for the totals of organic anions plus OH^- excretion. The regression of (organic anions + OH^- excretion) on assimilated (N + S) were highly significant ($r = 0.99$, $b = 0.95$, SE = ± 0.046, $P < 0.01$).

The increase in assimilated (N + S) values resulting from enhanced NO_3 nutrition was largely reflected in an increase in organic anion accumulation. The fraction of charge directed toward OH^- excretion was low, the maximum level of 20% of

the negative charge shift occurring in the 4 meq/l NO_3 treatment. At the two lowest levels of NO_3 nutrition, an excess uptake of cations over anions occurred indicating H^+ exudation from the roots.

Figure 1 shows the accumulation of the major organic acids in whole tomato plants in relation to increasing levels of NO_3 nutrition. The stimulating effect on malate accumulation is particularly marked. The concentrations of the other organic acids are raised to a lesser degree by increasing the level of NO_3 nutrition.

Organic anion accumulation, cation uptake, and inorganic anion accumulation data by whole plants are presented in Figure 2. The increase in organic acid anion accumulation from the N deficiency threshold level of 1.5 meq NO_3/l to the 12 meq NO_3/l treatment is paralleled by an increase in cation uptake. This is reflected in the fairly constant level of inorganic anions making up the balance between cation and organic anions above the N deficiency threshold. At lower levels of NO_3 nutrition, organic acid anion accumulation is low and inorganic anions are thus quantitatively more important in balancing inorganic cations.

The uptake and proportions of cations in relation to NO_3^- level are shown in Table III. Although cation uptake is considerably stimulated by NO_3 nutrition there is no major effect on individual cations and the proportions of the four cations do not vary greatly.

Table II. Influence of the level of nitrate nutrition on organic anion accumulation, OH^- excretion and total levels of assimilated (N+S) by whole tomato plants (meq/100 g dry weight).

Figures in parentheses indicate the % OH^- excretion of the total charge (organic anions + OH^- excreted)

Level of NO₃⁻ (meq/1)	Dry matter yields/g 10 plants	Organic anion accumulation	OH⁻ Excreted (Excess anion over cation uptake*)	Total Organic Anions Accumulated + OH⁻ excreted	Total Assimilated (N-S)
0.25	76.9	116	-21(-)	95	116
0.50	97.7	125	-3(-)	122	125
0.75	129.8	127	9(7)	136	138
1.00	147.4	129	23(15)	152	159
1.50	160.3	159	24(13)	183	190
2.50	191.2	169	27(14)	196	201
4.00	194.1	203	52(20)	255	250
6.00	213.7	245	18(7)	263	265
8.00	198.4	246	5(2)	251	272
12.00	202.6	251	4(2)	255	252

* Excess anion over cation uptake
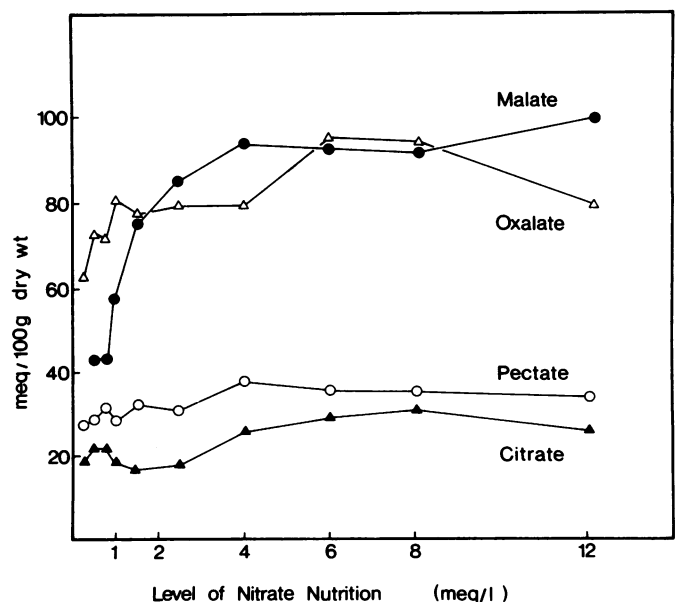 = (N+S P-Cl) - (K+Ca+Mg+Na)

FIG. 1. Influence of level of nitrate nutrition on accumulation of organic acids by whole tomato plant (meq/100 g dry wt).
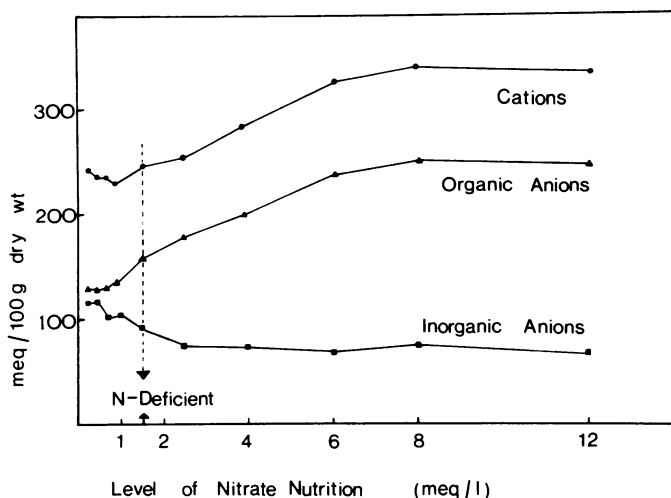
FIG. 2. Influence of the level of nitrate nutrition on cation uptake, organic anion accumulation, and inorganic content of whole tomato plants (meq/100 g dry wt).

Table III.   Influence of the level of nitrate nutrition on cation uptake by whole tomato plants (meq/100 g dry weight).

Figures in parentheses indicate the % uptake of each cation of the total cation uptake for each treatment.

Level of NO₃⁻ (meq/l)	K	Ca	Mg	Na	Total
0.5	108 (44)	92 (39)	40 (16)	3 (1)	243
1.5	130 (51)	85 (33)	37 (14)	4 (2)	256
4.0	146 (52)	95 (33)	36 (12)	5 (2)	282
6.0	162 (49)	122 (37)	39 (12)	8 (2)	331
8.0	159 (47)	134 (39)	44 (13)	5 (1)	342

The distribution of the major ionic constituents and cation-anion balances of the aerial plant parts and roots in relation to the level of NO_3^- nutrition is shown in Table IV. The following conclusions may be drawn from the data:

a. Regardless of the level of NO_3^- nutrition there is a close balance in both the tops and roots, between the total cations (K^+, Ca^{2+}, Mg^{2+}, Na^+) and total anions (NO_3^-, $H_2PO_4^-$, SO_4^{2-}, Cl^- and organic acid anions). The total charge varies from about 220 to 350 meq/100 g dry weight.

b. In the tops the total ionic charge increases by approximately 50% by increasing the level of NO_3 nutrition from 0.5 meq NO_3^-/l to 8.0 meq NO_3^-/l. The anion increase resulted from organic acid anions. All inorganic anions except NO_3^- are depressed by increased NO_3 nutrition. The weights of aerial plant parts were much in excess of root weights in all treatments. Ion balance data of the tops are thus similar to those for entire plants (Table III and Fig. 2).

c. In the roots, in contrast to the tops, increasing levels of NO_3 nutrition did not bring about an increase in total ionic concentrations. Indeed, at the lowest level of NO_3 supply they were somewhat higher. Despite the relatively constant total cation levels, K^+ content was depressed in the higher NO_3 treatments. Concentrations of organic anions were increased by enhanced NO_3 nutrition but the effect was less marked than in the tops. All inorganic anions with the exception of NO_3^- were depressed. In the two low N treatments there was a marked accumulation of $H_2PO_4^-$ and Cl^-.

DISCUSSION

Increasing the level of NO_3 nutrition stimulated dry matter production (Table II). The enhanced growth was accompanied by an increase in organic anion concentration in the plants and a parallel increase in cation content (Fig. 2). These effects must have resulted as a direct consequence of raising the NO_3 concentration in the nutrient medium, for the cation nutrient levels were all the same and the compensating ion was sulfate. This ion is known to have little influence on the uptake of other nutrient ions.

By using whole plant data it was possible to follow quantitatively the fate of anion charge arising from the enhanced NO_3 assimilation in its distribution between organic anion accumulation in the plant, and OH^- efflux into the nutrient medium in exchange for excess anion over cation uptake (Table II). With increasing NO_3 nutrition the bulk of additional anion charge could be accounted for by an increase in organic anion accumulation.

Plants have a high demand for nitrogen and developing meristematic tissues provide a very strong sink for the metabolically controlled process of NO_3^- absorption by roots (19). For ionic balance regulation within the plant, the site of NO_3 reduction is important. If it occurs in the tops, the upward translocation of cations is quantitatively more important because every NO_3^- ion reduced must be accompanied by an inorganic cation. This cation provides the counter-ion for the organic anion produced after nitrate reduction. When NO_3^- is reduced in the roots, meristematic tissues in the upper plant parts are supplied with N mainly in the form of uncharged amino compounds. The charge from NO_3^- reduction may appear in the nutrient medium as OH^- efflux in exchange for NO_3^- uptake, or in the roots as organic acid anions. Organic acid anions as such are present in xylem sap in only low concentrations and are therefore of little importance in cation translocation (22).

For tomato plants fed with NO_3-N the upper plant parts provide the major site of NO_3 reduction. Nitrate is quantitatively by far the most dominant anion in the xylem sap and is balanced by an equivalent amount of K^+, Ca^{2+}, and Mg^{2+} (Armstrong and Kirkby, unpublished data). When the level of NO_3 nutrition is raised the rate of uptake and translocation of NO_3^- and cations must also be increased. We suggest that on reduction of NO_3^- in the upper plant parts, organic anions are accumulated following the negative charge shift and these are retained in the tops to balance cation charge originally present with NO_3^- in the xylem sap (Table II). The charge transfer from NO_3 assimilation is thus largely directed toward organic anion accumulation and not to OH^- efflux (Table II). This explanation would account for the stimulating effect of NO_3 nutrition on cation uptake observed in this experiment and also by other authors (3, 11).

As NO_3 reduction in tomato plants occurs mainly in the tops, increasing the level of NO_3 supply has the most effect in stimulating organic anion accumulation and cation contents in the upper plant parts (Table IV). At lower levels of NO_3 nutrition it is probable that a higher proportion of the supplied NO_3 is reduced in the roots (23). This should also accentuate differences in ionic balance between tops and roots caused by increasing the level of NO_3 nutrition.

If the concepts outlined above are correct, it would be expected that under conditions of NO_3 stress in tomato, the translocation of cations to the tops might be restricted. This is consist-

Table IV.   Influence of the level of nitrate nutrition on the yields and cation-anion balances in the tops and roots of tomato plants (meq/100 g dry weight).

NO₃ level	Plant part	Yield/g 10 plants	CATIONS					ANIONS					
			K	Ca	Mg	Na	Total	NO₃	H₂PO₄	Cl	SO₄	Org. Anions	Total
0.5	Tops	85.9	99	102	36	3	240	1	25	27	47	128	228
	Roots	11.8	178	22	64	3	267	1	58	49	26	105	239
1.5	Tops	142.0	126	94	37	4	261	1	19	23	38	165	246
	Roots	18.3	164	22	36	4	226	6	42	29	25	118	220
4.0	Tops	175.2	146	102	35	5	288	8	19	19	31	211	288
	Roots	18.9	151	24	49	8	232	19	36	19	17	136	227
8.0	Tops	180.7	162	143	44	5	354	15	20	9	22	256	322
	Roots	17.7	131	40	52	6	229	22	25	9	18	148	222

ent with the higher contents of K^+ found in the roots of plants supplied with low levels of nitrate (Table IV). It also indicates that under NO_3 stress, K^+ translocation is more affected than K^+ uptake.

Malate is the major organic acid anion accumulated in response to increasing levels of NO_3 assimilation (Fig. 2). It is well established that malic acid synthesis can result from the carboxylation of PEP to OAA which is then reduced to malate by the enzyme malic dehydrogenase. According to Hiatt (8, 9), the oxidation of glyceraldehyde-3-P to 3-P-glyceric acid, a precursor of PEP, is markedly favored by an increase in pH. In ion uptake studies by roots he has proposed that malate synthesis is induced in this way. Jacoby and Laties (13) have also suggested that an increase in pH may raise the HCO_3^- level in the cytoplasm and thus induce carboxylation by PEP carboxylase. The basic feature of both of these postulated mechanisms is that an increase in cellular pH induces malate accumulation. Nitrate reduction is also accompanied by an alkaline pH shift in the plant (7, 16) and it is this effect which stimulates malate accumulation.

The nitrate reduction process takes place in two steps:

$$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O \tag{1}$$

$$NO_2^- + 6H^+ + 6e^- \rightarrow NH_3 + H_2O + OH^- \tag{2}$$
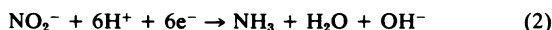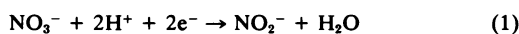
In green cells, the first step from NO_3^- to NO_2^- is generally held to take place in the cytoplasm whereas the reduction of NO_2^- occurs in the chloroplasts. The conversion of NO_3^- to NO_2^- does not involve a charge transfer. It is only when NO_2^- is reduced by nitrite reductase utilizing electrons from photoreduced ferredoxin that charge shift takes place. Attempting to trace the charge transfer from NO_2^- to organic acid anions is somewhat speculative. It may be supposed, however, that there is an NO_2^-/OH^- exchange on the outer thylakoid membrane which induces malate synthesis in the stroma of the chloroplast or in the cytoplasm. For root cells charge transfer is even more a matter of speculation. Even the electron source for the reduction is unknown.

The close agreement obtained in cation-anion balances (Table IV) indicates that the major cations and anions have been taken into account. This confirms earlier work where the inorganic cations (K^+, Ca^{2+}, Mg^{2+}, and Na^+) were found to be largely balanced by the organic acid anions together with NO_3^-, $H_2PO_4^-$, Cl^-, and SO_4^{2-} (15, 17). This balance infers that in the plant different cations compete for the bulk of anions charge and vice versa. This probably explains the fact that when NO_3 nutrition was inadequate an increase in concentration of all of the inorganic anions occurred to make up for the anion deficiency in both roots and tops (Table IV). Similarly, at higher levels of NO_3 nutrition the stimulation in cation uptake appeared to be largely nonspecific (Table III).

There is current interest in the possibility of K^+ recirculation in plants and the results obtained for tomato in this investigation are pertinent to this study. Ben-Zioni *et al.* (1, 2), following the work of Dijkshoorn (6), have proposed the scheme outlined in Figure 3. Potassium and NO_3^- are the major ions translocated together from the root to the shoot in the xylem. It is suggested that on reduction of NO_3^- in the tops, malate is formed and some of the K-malate is then translocated in the phloem to the roots where it is decarboxylated. The charge from malate originating in the tops is thus transferred to the nutrient medium as HCO_3^-. For every HCO_3^- excreted, a NO_3^- ion can be taken from the nutrient solution to balance the K^+ already in the root and the cycle can be repeated.

In our results for tomato, OH^- excretion represents a small fraction of the total charge from NO_3^- and SO_4^{2-} reduction (Table II). This finding would indicate a low possible extent of K recycling as envisaged in the scheme shown in Figure 3. This is supported by the observation that the level of malate in the
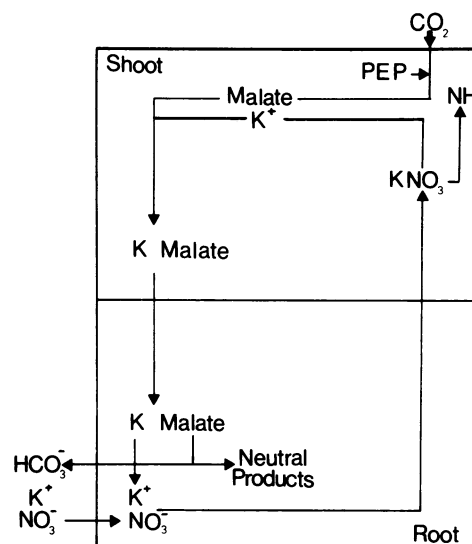

FIG. 3. Ben-Zioni *et al.*-Dijkshoorn model describing the movement of K^+, NO_3^-, and malate between shoot and root.

phloem sap can be considerably lower than that of K^+ (18). The recent evidence of Mengel and Haeder (18) also suggests that K^+ translocated in the phloem appears to be balanced in part by amino acids which at the high pH of the phloem sap (about pH 8) are negatively charged. However, if NO_3^- reduction takes place in the root, which may be the predominant site under low levels of NO_3 nutrition, HCO_3^- excretion could occur without involving the translocation of K^+ and an organic anion from the tops. These ideas are receiving current attention in our laboratory. A modified Ben-Zioni *et al.*-Dijkshoorn K-recirculation scheme has been presented in another publication by Kirkby (15).

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