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Relationship of Cell Sap pH to Organic Acid Change During Ion Uptake[1]

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Summary. Excised roots of barley (*Hordeum vulgare,* var. Campana) were incubated in KCl, K_2SO_4, $CaCl_2$, and NaCl solutions at concentrations of 10^{-5} to 10^{-2} N. Changes in substrate solution pH, cell sap pH, and organic acid content of the roots were related to differences in cation and anion absorption. The pH of expressed sap of roots increased when cations were absorbed in excess of anions and decreased when anions were absorbed in excess of cations. The pH of the cell sap shifted in response to imbalances in cation and anion uptake in salt solutions as dilute as 10^{-5} N. Changes in cell sap pH were detectable within 15 minutes after the roots were placed in 10^{-3} N K_2SO_4. Organic acid changes in the roots were proportional to expressed sap pH changes induced by unbalanced ion uptake. Changes in organic acid content in response to differential cation and anion uptake appear to be associated with the low-salt component of ion uptake.

Several investigators (1, 5, 6, 7, 8, 11, 12) have shown that organic acid content of roots increases when cations are absorbed in excess of anions and decreases when anions are absorbed in excess of cations. In the first report of this phenomenon, Ulrich (11) suggested that whenever cations are absorbed in excess of anions, the root cells tend to compensate for the potential increase in alkalinity through the formation of organic acids. In experiments of 8-hour duration, Ulrich (11, 12) noted slight increases in the pH of cell sap from roots absorbing cations in excess of anions. Hiatt and Hendricks (5) determined the pH of ethanol extracts of roots after the extracts had been dried and resuspended in water. The pH of extracts increased under conditions of excess cation absorption and decreased under conditions of excess anion absorption.

A mechanism was proposed (4) whereby organic acid synthesis and breakdown may be regulated by the pH of the cell sap. The mechanism is based on the influence of pH on equilibria of glycolytic reactions, particularly the reactions catalyzed by glyceraldehyde-3-P dehydrogenase, hexokinase, and P-fructokinase. It was also proposed that carboxylation of P-enol pyruvate to form 4-C organic acids may be causally related to excess cation absorption (5).

Most studies of organic acid changes under conditions of unbalanced ion uptake have been conducted using solutions of near 10^{-2} N concentration. Because multiple uptake mechanisms apparently operate at these concentrations (3), it was of interest to determine the effect of absorption from more dilute solutions on cell sap pH and organic acid changes. The results reported in this paper indicate that organic acid changes are related to the low salt concentration component of ion uptake.

Materials and Methods

Barley seedlings (*Hordeum vulgare,* var. Campana) were dark-grown in continuously aerated 0.2mM $CaSO_4$, essentially as described by Epstein and Hagen (2). Excised roots from 6-day old plants were rinsed several times in 0.2mM $CaSO_4$ and were suspended in approximately 30 times the root volume of 0.2mM $CaSO_4$ for 30 minutes before use.

In most experiments 3.2 g of roots were placed in 2 to 16 liters of the aerated substrate solutions at 23°. At the end of the experiment, the roots were rinsed for 10 minutes in distilled water and blotted dry. Duplicate 0.5 g samples were weighed for inorganic ion analysis and 2 g were weighed for organic acid analysis. In experiments where the pH of the expressed sap was determined, the entire root sample was used for pH determination. Each solution, in addition to the experimental salt under consideration, contained $CaSO_4$ at 0.2mM, added to maintain membrane integrity during the experiments (9).

Changes in pH of the substrate solutions were followed by determining the pH of 5 ml aliquots

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of the solutions periodically and discarding the aliquots. The pH of K_2SO_4 substrate solutions was maintained at pH 5.5 to 5.7 by the addition of 0.1 N KOH. The addition of K^+ as KOH did not exceed the K^+ removed from the solutions by the roots.

Potassium, Cl^-, and Na^+ were extracted by boiling the roots for 10 minutes in each of 3 changes of distilled water (10 ml). The extracts were adjusted to the desired volume by evaporation or dilution. Potassium was determined by flame photometric analysis, Na^+ by atomic absorption and Cl^- by means of a Buchler-Cotlove automatic chloride titrator. Calcium was determined by flame photometric analysis following oxidation of the sample overnight in a muffle furnace at 480°.

Sulfate uptake was determined with $K_2{}^{35}SO_4$. After the absorption period, the roots were rinsed for 20 minutes with several changes of non-radioactive 3×10^{-2} N K_2SO_4. The roots were pressed flat in a planchet and radioactivity was determined by use of a gas flow counter. Specific activity of the $^{35}SO^{2-}{}_4$ in the substrate solution was estimated by drying an aliquot of the solution in a planchet with 1 ml of a 100 mg/ml sucrose solution added to provide self absorption similar to that produced by the roots.

Total organic acids were determined by the procedure described by Hiatt and Hendricks (5). The pH of cell sap was determined after macerating the roots with mortar and pestle and straining the mixture through cheesecloth.

Results

Three g of roots were incubated for 6 hours in aerated solutions of K_2SO_4, $CaCl_2$, and KCl. Organic acid content, K^+, Ca^{2+}, and Cl^- uptake were determined. Sulfate uptake was determined in a parallel experiment. The results (table I) indicate that at all salt concentrations, changes in organic acid content were proportional to the difference between cation and anion uptake. Increase in organic acid content of roots in K_2SO_4 was approximately equivalent to excess cation uptake. Although organic acid content of roots in $CaCl_2$ decreased markedly, the decrease in organic acid content was not equivalent to excess anion uptake.

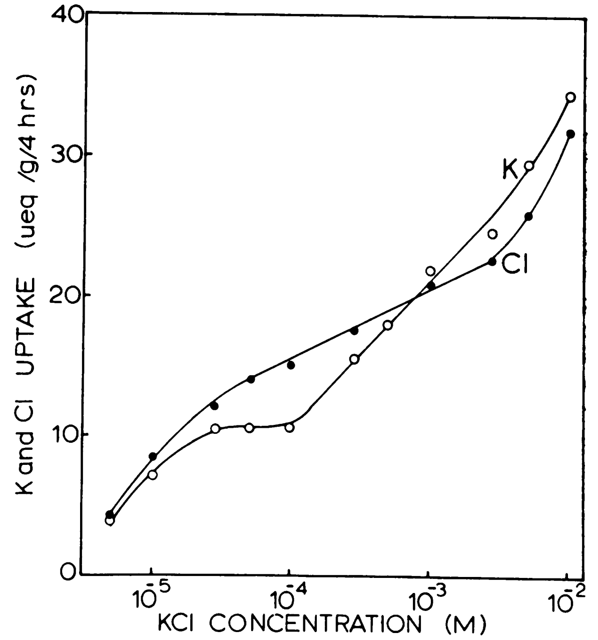

FIG. 1. Effect of KCl concentration of K^+ and Cl^- uptake of barley roots.

Table I. *Effect of Concentration of K_2SO_4, $CaCl_2$, and KCl on Cation Uptake, Anion Uptake, and Organic Acid Change of Barley Roots*

Roots were incubated for 6 hours in the indicated solutions. Initial levels were: K content = 18 µeq/g; Cl content = 4 µeq/g; organic acid content = 28.4 µeq/g.

Salt	Concentration	Substrate volume	Cation uptake	Anion uptake	Organic acid change
	N	Liters	µeq/g	µeq/g	µeq/g
K_2SO_4	10^{-5}	8	9	<1	8.7
	10^{-4}	4	12	<1	12.2
	10^{-3}	4	17	<1	15.1
	10^{-2}	4	22	2.1	18.0
	5×10^{-2}	4	25	6.4	20.6
$CaCl_2$	3×10^{-5}	8	<1	14	−9.0
	10^{-4}	8	<1	14	−10.7
	10^{-3}	4	<1	15	−9.7
KCl	5×10^{-5}	8	16	19	−2.9
	10^{-4}	4	14	20	−4.6
	5×10^{-4}	2	23	26	−0.4
	10^{-3}	2	28	29	−0.2
	5×10^{-3}	2	39	36	0.8
	10^{-2}	2	43	39	1.2

Table II. *K, Cl, and Organic Acid Content of Roots after 24 Hours in 10^{-3} N Salt*

Salt	K Content	K Change	Cl Content	Cl Change	Organic acids Content	Organic acids Change
	μeq/g		μeq/g		μeq/g	
Initial roots	15	...	4	...	21	...
K_2SO_4	48	33	3	−1	53	32
$CaCl_2$	14	−1	31	27	8	−13
KCl	71	56	61	57	19	−2

These roots consistently absorbed Cl^- in greater quantities than K^+ from KCl solutions of 5×10^{-4} N or less (fig 1). In KCl solutions of higher concentrations than 10^{-3} N, K^+ was absorbed in excess of Cl^-. At low concentrations, organic acid decrease was proportional to excess anion absorption, and at high KCl concentration, the organic acid increase was proportional to excess cation uptake (table I). The lower level of accumulation of K^+ from 10^{-4} N KCl as compared to 5×10^{-5} N KCl (table I) is of interest. This phenomenon has been observed in many of our experiments and is not a result of depletion of K^+ from the substrate solution. A possible explanation for this phenomenon is included in the discussion section.

Table II shows the K^+, Cl^- and organic acid content of roots after 24 hours in 10^{-3} N concentrations of K_2SO_4, KCl, and $CaCl_2$. After 24 hours, the roots have approached equilibrium with the substrate solution. As in the experiment of 4-hour duration, K^+ uptake from 10^{-3} N K_2SO_4 was accompanied by an equivalent increase in organic acid content of the roots. In the 24-hour experiment, Cl^- uptake from 10^{-3} N $CaCl_2$ was approximately twice the decrease in organic acid content.

If organic acid synthesis and decarboxylation are regulated by cell pH, there should be a rapid response of cell pH to unbalanced ion uptake. Four g of roots were placed in each of 6 flasks of aerated solutions containing 10^{-3} N K_2SO_4 and 2×10^{-4} M $CaSO_4$. At 30-minute intervals, the substrate solutions were adjusted to pH 5.6 with KOH to counter the hydrogen ions released from the roots. At the indicated time intervals (table III) the roots were removed, and the pH of the expressed sap was determined. The experiment was repeated and the results were virtually identical.

Barley roots absorb K^+ at the rate of approximately 4 meq/g/hr and SO^{2-}_4 absorption is negligible from solutions of 10^{-3} N K_2SO_4. There was a definite increase in pH of expressed cell sap within 15 minutes (table III). The cell sap pH continued to increase during the first 2 hours but did not change between 2 hours and 6 hours. The method used gave the average cell sap pH of the whole root; however, during short interval studies, it is unlikely that all root cells are accumulating ions. Furthermore, ion accumulation into the vacuole would be expected to lag behind accumulation into the cytoplasm. Therefore, during early stages of ion accumulation, the change in pH within the cytoplasm of cortical cells is probably considerably greater than the values recorded in table III.

Table III. *pH Change of Expressed Root Sap with Time of Incubation in 10^{-3} N K_2SO_4*

Incubation period	Expressed Sap
	pH
Initial roots	5.48
15 min	5.51
30 "	5.54
1 hr	5.56
2 "	5.59
4 "	5.59
6 "	5.59

Roots were incubated for 2 hours in K_2SO_4, $CaCl_2$ and KCl at various concentrations, and the pH of the expressed sap was determined (table IV). The shifts in pH of expressed sap corresponded to differences in cation and anion uptake, as indicated by changes in pH of the substrate solution and the data in table I. The pH of cell sap responded to differences in cation and anion uptake even at salt concentrations of 10^{-5} N. With KCl the largest decrease in pH occurred in the 10^{-4} N solution, the concentration resulting in the greatest excess of anion uptake (table I).

The pH of expressed sap of roots in K_2SO_4 increased with each increase in salt concentration. Expressed sap of roots in $CaCl_2$ decreased, but little additional response resulted from increasing $CaCl_2$ concentrations to levels higher than 10^{-4} N. Likewise, increasing $CaCl_2$ concentrations to 5×10^{-2}N resulted in little increased Cl^- absorption over absorption from 10^{-4} N $CaCl_2$.

Na^+ uptake, Cl^- uptake, and changes in cell pH of roots incubated 4 hours in NaCl solutions are shown in table V. Roots in NaCl responded in a manner similar to roots in KCl, the cell sap pH decreasing with excess Cl uptake and increasing with excess Na uptake.

Discussion

At least 2 mechanisms are involved in the absorption of ions by plant roots (3). The high-salt-concentration mechanism contributes negligibly to ion uptake from solution of concentrations less than 10^{-3} M. The low-salt-concentration mechanism

Table IV. *Effect of Concentration of K_2SO_4, $CaCl_2$, and KCl on pH of Substrate Solution and Expressed Cell Sap*
Expressed sap pH of initial roots = 5.45. Incubation period = 2 hrs.

Salt	Concentration	Substrate Initial	Substrate Final	Expressed sap
	N	pH	pH	pH
K_2SO_4	10^{-5}	5.70	*	5.49
	10^{-4}	5.70	*	5.52
	10^{-3}	5.70	*	5.54
	10^{-2}	5.70	*	5.56
$CaCl_2$	10^{-4}	5.63	5.78	5.13
	10^{-3}	5.65	5.82	5.07
	10^{-2}	5.68	5.90	5.08
KCl	10^{-5}	5.70	5.77	5.38
	10^{-4}	5.70	6.02	5.21
	10^{-3}	5.70	5.86	5.27
	10^{-2}	5.70	5.63	5.47

* Final pH levels of K_2SO_4 solutions are not given because pH was maintained at 5.5 to 5.7 with KOH.

Table V. *Effect of Concentration of NaCl on Na Uptake, Cl Uptake, Substrate Solution pH, and Expressed Sap pH*
The incubation period was 4 hours.

Concentration	Na uptake	Cl uptake	Substrate solution	Expressed sap
N	µeq/g	µeq/g	pH	pH
10^{-5}	5	10	5.74	5.25
10^{-4}	15	18	5.75	5.40
10^{-3}	23	20	5.53	5.52
10^{-2}	42	33	5.48	5.58
Initial levels	2.5	3.5	5.61	5.45

approaches maximum rate at salt concentration of approximately 10^{-4} M and is usually considered to operate at maximum rates at salt concentrations higher than 10^{-3} M. At salt concentrations exceeding 10^{-3} M both mechanisms of uptake operate. The concentration of organic acids in barley roots responds to imbalances in cation and anion uptake at all salt concentrations (table I). Therefore, if this phenomenon is confined to either of the uptake mechanisms, it would be associated with the low-salt-concentration component of ion uptake. This is in conflict with the views of Torii and Laties (10), who state that the high-salt-concentration component of ion uptake is responsible for accommodating organic acid changes. According to table I and the data reported by Epstein et al. (3), K^+ and Rb^+ uptake from solutions of concentrations less than 10^{-4} N are virtually independent of the rate of uptake of the associated anion. Roots absorb K^+ from 10^{-5} to 10^{-4} N SO^{2-}_4 salts and Cl^- salts at approximately equal rates, even though SO^{2-}_4 absorption is negligible. At these concentrations, ion uptake is limited to the low-salt mechanism. If changes in organic acids were associated with the high-salt mechanism only, the observed response of organic acid levels to unbalanced ion uptake (table I) would not be expected.

The response of expressed sap pH to excess cation uptake is immediate (table III) and confirms the report of Ulrich (11, 12) that the pH of cell sap tends to increase when cations are absorbed in excess of anions. Furthermore, there is a marked decrease in cell sap pH when anions are absorbed in excess of cations (table IV). The pH change is proportional to the magnitude of ion uptake imbalance and is sensitive to small differences in ion uptake. A mechanism by which cell pH may control organic acid levels in roots was discussed in a previous paper (4).

At the pH levels maintained in these experiments (5.6–6.0), Cl^- uptake exceeded K^+ uptake from KCl solutions of below 10^{-3} N (fig 1). Replication of studies of K^+ and Cl^- uptake versus concentration was excellent except for points near 10^{-4} N KCl. In experiments of longer than 4-hour duration, K^+ uptake from 10^{-4} N KCl was frequently observed to be equal to or less than K^+ uptake from 3×10^{-5} or 5×10^{-5} N KCl. A leveling off of the uptake versus concentration curve is consistent with the saturation of an uptake mechanism. The perplexing decrease in K^+ uptake from 10^{-4} N KCl compared with 5×10^{-5} N KCl (table I) was not observed in short term experiments (3) and might be explained on the basis of cell pH shift and organic acid change. When the KCl concentration of the substrate solution is increased from 10^{-5} to 10^{-4} N, the rate of increase of Cl^- uptake exceeds the rate of increase of K^+ uptake (fig 1). This induces a decrease in cell sap pH, causing a decrease in the organic acid content of the roots. The K^+ initially neutralized by the organic acids being decarboxylated would be released as a free ion in the cytoplasm. The freed K^+ might become associated with the incoming Cl^-. Thus, electrical neutrality would be maintained in the cell without additional uptake of K^+ or the synthesis of an organic cation. The phenomenon does not result from loss of K^+ from the roots; rather, it is due to a reduction in K^+ uptake. Hydrogen ions accompanying the incoming Cl^- would be utilized in the reverse operation of the glycolytic pathway (4).

Initial rates of Cl^- uptake from solutions of low $CaCl_2$ and KCl concentration (10^{-5} to 5×10^{-5} N) are nearly the same. However, Cl^- uptake rates from these concentrations of $CaCl_2$ decrease rapidly with time and are much less than uptake from KCl after 4 hours. It seems logical that Cl^- uptake from $CaCl_2$ might be limited by the availability of endogenous cations initially associated with organic acids. The cations initially associated with organic acids in the cytoplasm must not be the only source of cations available to balance incoming Cl^-, however, because net Cl^- uptake from $CaCl_2$ may exceed the quantity of organic acids originally present in the tissue (table II).

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