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# Influx of $Na^+$, $K^+$, and $Ca^{2+}$ into Roots of Salt-Stressed Cotton Seedlings[1]

## EFFECTS OF SUPPLEMENTAL $Ca^{2+}$

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### ABSTRACT

**High $Na^+$ concentrations may disrupt $K^+$ and $Ca^{2+}$ transport and interfere with growth of many plant species, cotton (*Gossypium hirsutum* L.) included. Elevated $Ca^{2+}$ levels often counteract these consequences of salinity. The effect of supplemental $Ca^{2+}$ on influx of $Ca^{2+}$, $K^+$, and $Na^+$ in roots of intact, salt-stressed cotton seedlings was therefore investigated. Eight-day-old seedlings were exposed to treatments ranging from 0 to 250 millimolar NaCl in the presence of nutrient solutions containing 0.4 or 10 millimolar $Ca^{2+}$. Sodium influx increased proportionally to increasing salinity. At high external $Ca^{2+}$, $Na^+$ influx was less than at low $Ca^{2+}$. Calcium influx was complex and exhibited two different responses to salinity. At low salt concentrations, influx decreased curvilinearly with increasing salt concentration. At 150 to 250 millimolar NaCl, $^{45}Ca^{2+}$ influx increased in proportion to salt concentrations, especially with high $Ca^{2+}$. Potassium influx declined significantly with increasing salinity, but was unaffected by external $Ca^{2+}$. The rate of $K^+$ uptake was dependent upon root weight, although influx was normalized for root weight. We conclude that the protection of root growth from salt stress by supplemental $Ca^{2+}$ is related to improved Ca-status and maintenance of $K^+/Na^+$ selectivity.**

The growth of cotton roots is severely inhibited by high concentrations of NaCl when external $Ca^{2+}$ concentrations are low but adequate for growth under nonsaline conditions. Supplemental $Ca^{2+}$ markedly improves the growth of salt-stressed cotton roots and can actually stimulate growth under certain conditions (8). The maintenance or stimulation of growth by $Ca^{2+}$ is dependent upon an increase in cell length and cell division (19). We hypothesized that transport of $Ca^{2+}$ into the root may be significant in these growth responses.

Maintenance of adequate $K^+$ concentrations and $K^+/Na^+$ ratios in the cell are necessary for normal cellular function under saline conditions (11). Calcium is necessary for the maintenance of adequate $K^+$ transport (10, 21). Supplemental $Ca^{2+}$ mitigated the reduction of K and Ca concentrations in salt-stressed cotton seedlings (18), which suggested that selective ion transport in cotton roots was affected by salinity. Cramer *et al.* (9) found that supplemental $Ca^{2+}$ markedly reduced $K^+$ efflux from salt-stressed cotton roots. Influx of $K^+$ in cotton roots may also be affected by saline conditions. In an earlier study, salt tolerance in cotton has been related to $K^+/Na^+$ selectivity (18).

The interaction of $Na^+$ and $Ca^{2+}$ in salt-stressed plants has been the subject of several previous investigations (12, 20, 23). In general, $Na^+$ is thought to interfere with $Ca^{2+}$ uptake, but this is not always the case. The maintenance of adequate $K^+$ and $Ca^{2+}$ transport and $K^+/Na^+$ selectivity in the root may be related to salt tolerance in cotton. Therefore, the objective of this study was to investigate the short-term influxes of $K^+$, $Na^+$, and $Ca^{2+}$ in cotton roots under salt stress, and to assess the significance of external $Ca^{2+}$ supply in the relationship of these ion fluxes to salt tolerance of cotton.

## MATERIALS AND METHODS

**$^{45}Ca^{2+}$ Influx.** Cotton seeds (*Gossypium hirsutum* L. cv Acala SJ-2) were imbibed in aerated solutions of 0.4 mM $CaCl_2$ for 24 h. Seeds were then planted into germination "sandwiches" (18) and imbibed for 6 d in an aerated 0.1 modified Hoagland solution (18) in 3.7 L plastic containers. The plants were irradiated with GE Plant Gro and Sho light bulbs (400 $\mu$mol $m^{-2}$ $s^{-1}$). The evening before the influx experiment, the seedlings were transferred to plastic grids over identical nutrient solutions. Each grid held 5 seedlings (one replicate) for transfer during the course of the experiment. The seedlings were transferred the evening before so that they would have sufficient time to recover from any possible "transfer shock" before the influx experiment began. Under these growth conditions, the 8-d-old seedlings had one tap root of approximately 6 cm in length with root hairs but no laterals, and the cotyledons were fully developed.

The approach outlined by Cram and Laties (6) was used to estimate ion influx across the plasmalemma. Influx of $^{45}Ca^{2+}$ was measured in roots of whole seedlings (8-d-old) in the light over 10 min at 22°C followed by a 15 min desorption period in ice-cold 10 mM $CaCl_2$. (Chilling temperatures for a period of 1–4 d induced leakage of sugars in cotton roots, but this response was completely mitigated by the presence of 10 mM external $Ca^{2+}$ [3]. Leakage of ions was not investigated in that study. Some leakage may have occurred under our desorption conditions. This would alter the calculated rates of ion uptake, but the relative differences in ion uptake should remain the same. Ion transport to the shoot was found to be negligible for the time period used). Treatment solutions consisted of seven concentrations of NaCl (0–250 mM) and two concentrations of $Ca^{2+}$ (0.4 and 10 mM) in a 0.1 modified Hoagland solution (18). Supplemental $Ca^{2+}$ was added as $CaCl_2$. The specific activity of $^{45}Ca^{2+}$ was 19,900 cpm $\mu$mol$^{-1}$, when counted in a Packard Tri-Carb 300C liquid scintillation system. At the end of the desorption period the roots were placed into glass scintillation vials and dry ashed at 600°C for 12 h. The ash was dissolved in Beckman Ready-Solv EP scintillation cocktail and counted. Each treatment was replicated four times.

Residual apoplastic $Ca^{2+}$ would contribute to the estimated $Ca^{2+}$ influx. Two procedures were used to ascertain the quantity

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of apoplastic $Ca^{2+}$ remaining after the 15 min desorption period. In the first method, roots were treated according to Lynch and Läuchli (23) in an attempt to estimate the residual $^{45}Ca$ remaining in the apoplast after the 15 min desorption period. Roots were excised and placed into cheesecloth tea bags. The roots were treated for 4 h in a 95%:2% (v:v) ethanol:Triton-X solution in order to solubilize the cell membranes and cytosol. The solution was changed every 0.5 h. Following this treatment the roots were rinsed thoroughly in deionized-distilled $H_2O$ and treated in the same manner for $^{45}Ca^{2+}$ influx as the roots of whole seedlings.

A second method, compartmental (efflux) analysis, was tried to estimate the quantity of $Ca^{2+}$ present in the cell wall after the 15 min desorption period. Intact roots of individual whole seedlings, preloaded with $^{45}Ca^{2+}$ for 40 h, were placed into 14 ml centrifuge tubes containing aerated 10 mM $CaCl_2$ at either 4 or 23°C. At each interval, the intact seedling was transferred to a new centrifuge tube containing fresh desorption solution. Each treatment was replicated three times.

**$^{22}Na^+$ and $^{42}K^+$ Influx.** The experimental design was the same as for the $^{45}Ca^{2+}$ influx experiment, except for the following conditions. One mM NaCl (instead of 0 mM NaCl) was present in both the growth solution and the lowest salt treatment. In the solutions of this experiment, $Na^+$ and $K^+$ were double-labeled with $^{22}Na^+$ and $^{42}K^+$. The specific activity of the 1 mM $Na^+$ solution was 39,220 cpm $\mu mol^{-1}$; all other $^{22}Na^+$ solutions had a specific activity of 6,138 cpm $\mu mol^{-1}$. The specific activity of $^{42}K^+$ was 97,500 cpm $\mu mol^{-1}$ and the $K^+$ concentration of the solution was 0.6 mM. Potassium influx was quantified by subtracting the final count rate 1 week later (when the $^{42}K^+$ had decayed to negligible levels) from the initial count rate. The roots were placed in plastic scintillation vials containing Dioxane-Omnifluor (8 g $L^{-1}$ Omnifluor, 30% water [v/v]) scintillation cocktail. The presence of fresh root material in the scintillation cocktail did not cause appreciable quenching. This was verified by using internal standards.

## RESULTS

**$Ca^{2+}$ Influx.** Both methods used to estimate the residual $^{45}Ca$ bound by the cell wall after the 15 min desorption period proved to be problematic with cotton roots. With the first procedure, using the method of Lynch and Läuchli (23), more $^{45}Ca^{2+}$ remained in the tissue when excised roots were treated with ethanol: Triton-X and desorbed (Fig. 1a) than when the roots were simply desorbed without this treatment (Fig. 1b). Apparently, the former treatment failed to solubilize the membranes and cytosolic constituents completely, or $Ca^{2+}$ was bound within the tissue under these conditions.

With the second method of compartmental analysis as an additional tool, differences between $Ca^{2+}$ efflux at 4 or 23°C were not significant (Fig. 2). After 15 min of efflux, 60% of the total $^{45}Ca^{2+}$ that had accumulated in the root remained; after 6 h only 5% remained.

These data can be further analyzed using a nonlinear regression program (2). We used BMDP P3R and fitted the data to either the sum of two (two compartment model) or three (three compartment model) exponential decay functions (Table I). The residual mean square was small for both models indicating a good fit to the data. In addition, the plot of the residuals showed no significant trend away from the mean (data not shown), particularly for the three compartment model, which had a lower residual mean square. When the data were fitted to a four compartment model, the residual mean square increased and the parameters for the fourth compartment had meaningless values (data not shown). Therefore, we rejected this model.

Whether one decides that the best model is the two or the three compartment model, the parameters for the slowly exchanging compartment change very little. This is not true for the more rapidly exchanging compartment(s). In our opinion, the three compartment model provides the better estimates of parameters since this model provided the best fit to the data (lowest residual mean square).

Macklon (24) separated $Ca^{2+}$ efflux from the apoplast of onion roots into three different compartments: superficial, water free space, and Donnan free space. In the symplasm, two other compartments were identified: the cytoplasm and the vacuole. Our estimates for the half time of exchange are of similar magnitude to his estimates of the apoplastic compartments, except for the slowly exchanging compartment. Macklon's estimate for the half-time of exchange for the Donnan free space was about 18 to 19 min. Our estimate for the slowly exchanging compartment varied from 111 to 117 min, whereas Macklon's estimates for the half time of exchange from the cytoplasm and the vacuole were approximately 55 and 800 min, respectively.

Our results from compartmental analysis are difficult to interpret. Macklon (24) found distinct differences between $Ca^{2+}$ efflux from untreated onion roots and that from roots after freezing. The slowest component of exchange—the vacuole—was almost entirely eliminated by freezing. This suggested that $Ca^{2+}$ efflux from a metabolically active compartment of the root could be measured. Spanswick and Williams (29), however, found that $Ca^{2+}$ efflux from isolated cell walls of *Nitella* was identical with that from the intact cell. Calcium efflux in our study was unaffected by temperature, suggesting that the $Ca^{2+}$ exchange we detected was physical and not metabolically active. Chilling temperatures would be expected to cause an increase in the free cytosolic $Ca^{2+}$ concentration by disrupting various internal compartments that sequester $Ca^{2+}$ away from the cytosol. The rise in free cytosolic Ca;$2^+$ concentration would be expected to increase $Ca^{2+}$ efflux from the root. Our data suggest that either $Ca^{2+}$ is located entirely in the apoplast, which seems highly improbable, or that the apoplast acts as a rate limiting diffusion barrier for $Ca^{2+}$ efflux from the cell, or that most $Ca^{2+}$ inside the cells is bound. Calcium may exit the cell from a cytosolic compartment, but the rate of this process may not be detectable with this method because the rate of transport across the plasmalemma, out of the cell, is masked by the diffusion limitations of the cell wall.

Better methods need to be developed to estimate the extent that cell-wall adsorption of $Ca^{2+}$ contributes to $Ca^{2+}$ influx in cotton roots. Therefore, the $Ca^{2+}$ influx data (Fig. 1b) are presented without indication of the amount of residual $^{45}Ca^{2+}$ that may be bound by the cell wall after the 15 min desorption period and are not meant to represent influx across the plasmalemma only. Increasing NaCl concentrations up to 100 mM diminished $Ca^{2+}$ influx in both $Ca^{2+}$ treatments (Fig. 1b). Above this concentration of NaCl, $Ca^{2+}$ influx increased markedly at the high, and slightly at the low external $Ca^{2+}$ concentration. At 250 mM NaCl, $Ca^{2+}$ influx for the high $Ca^{2+}$ treatment (10 mM) was the same as that of its control at 1 mM NaCl, but for the low $Ca^{2+}$ treatment (0.4 mM), $Ca^{2+}$ influx at the highest salt concentration was only 26% of that of the control.

**$Na^+$ Influx.** Sodium influx rate increased roughly in proportion to the external NaCl concentration (Fig. 3). Supplemental $Ca^{2+}$ (10 mM) significantly decreased $Na^+$ influx over the entire salt concentration range, even at 1 mM NaCl where $Na^+$ influx for the high $Ca^{2+}$ treatment was only 52% of the low $Ca^{2+}$ treatment (not apparent in Fig. 3).

**$K^+$ Influx.** In contrast to $Na^+$ influx, $K^+$ influx varied greatly between treatment replications, although both were determined with the same roots. Examination of the data indicated that $K^+$ influx depended upon root weight, although influx was normalized for root weight. Figure 4 depicts the relationship between $K^+$ influx and root weight, irrespective of salt treatment. Potassium influx ($\mu mol$ g fresh weight$^{-1}$ $h^{-1}$) increased with increasing

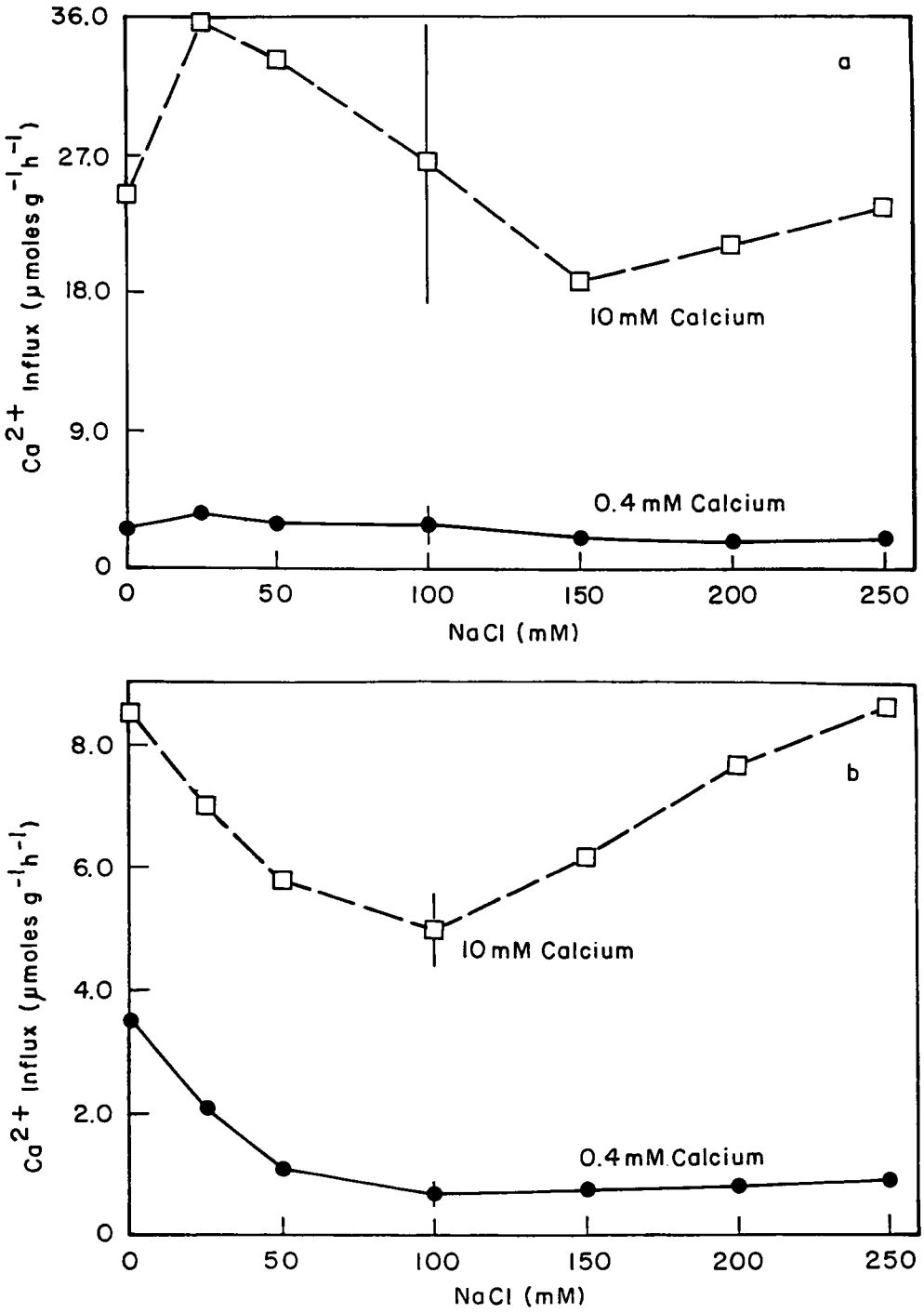


FIG. 1. The effect of increasing NaCl concentrations on $^{45}Ca^{2+}$ influx into cotton roots at two different $Ca^{2+}$ concentrations. (a), Excised roots treated with 95% ethanol:2% Triton-X); (b), whole seedlings without 95% ethanol:2% Triton-X. The error bars represent 95% confidence intervals for each $Ca^{2+}$ treatment.

root weight. The data were divided into two classes of NaCl treatments (low and high) and plotted in the same manner as in Figure 4 (Fig. 5). The high salt range (considered as a separate class) appeared to reduce $K^+$ influx as compared to the low salt range. These data were further analyzed by multiple linear regression to determine the effects of root weight, $Ca^{2+}$, and $Na^+$ on $K^+$ influx (Table II). This accounted for most of the variability in root weight ($R^2 = 0.88$), and permitted a statistical evaluation of the effect of $Ca^{2+}$ and $Na^+$ on $K^+$ influx. Sodium significantly reduced $K^+$ influx, but $Ca^{2+}$ had no significant effect. The $Na^+$ effect notwithstanding, root weight was the dominant factor governing $K^+$ influx.

## DISCUSSION

The transport of $Ca^{2+}$ into the root and shoot is complex. Calcium can enter the xylem via an apoplastic or symplastic pathway (5). Calcium influx across the plasmalemma appears to be passive (25). Free cytosolic $Ca^{2+}$ concentrations are thought to be kept low and highly buffered in plants by precipitation or binding, intracellular compartmentation, and active efflux mediated by a $Ca^{2+}$-ATPase (25). Enhanced plasmalemma permeability brought about by the ethanol:Triton-X treatment (resulting in an increased $Ca^{2+}$ influx), along with precipitation and sequestration of the accumulating $Ca^{2+}$, may explain why the inferred $Ca^{2+}$ influx in this treatment was greater than that without the treatment (Fig. 1).

Calcium influx with increasing salinity in cotton roots appears to reflect at least two different phenomena. It declined at low, but increased at higher salt concentrations (Fig. 1). The measured $Ca^{2+}$ influx may include some $^{45}Ca$-binding to the cell wall or transport to the xylem through the apoplast. Calcium in the

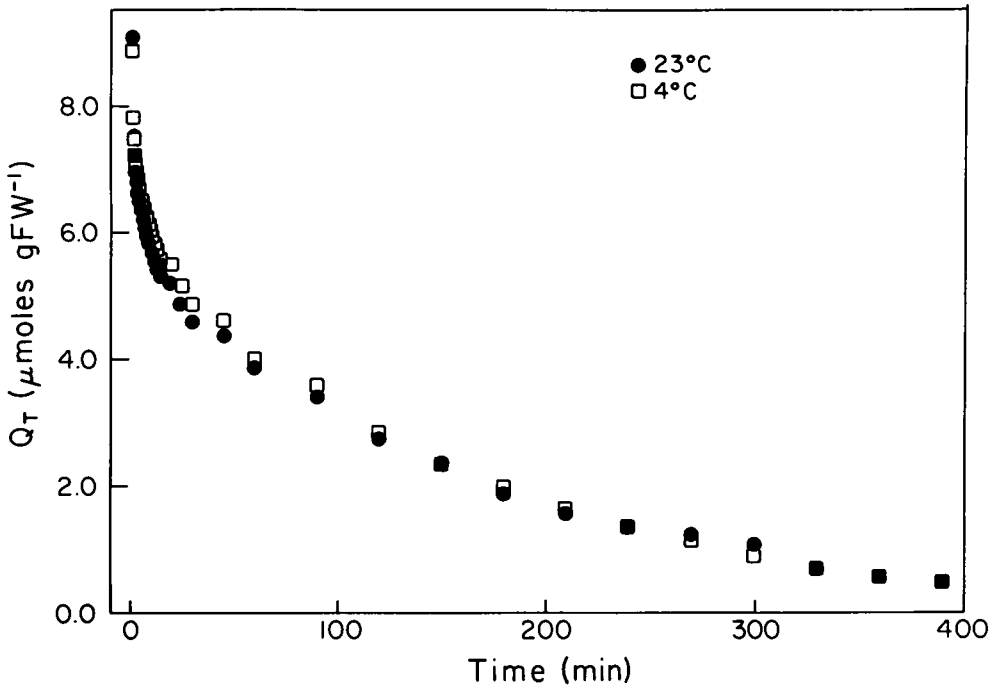


FIG. 2. Calcium ($^{45}Ca$) efflux from intact roots of 8-d-old cotton seedlings given as the Ca concentration ($Q_T$) of the root *versus* time. Efflux proceeded in aerated 10 mM $CaCl_2$ solutions at either 23°C (●) or 4°C (□). Each data point represents the mean of three replicate samples (one plant per replicate). Each plant was transferred to a fresh solution at each time interval. The plants were grown in a 0.1 modified Hoagland solution and preloaded with 0.4 mM $^{45}Ca$ for 40 h before the initiation of the experiment.

Table I. *Compartmental Analysis of $Ca^{2+}$ Efflux at 23 or 4°C*

The data were fitted to either the sum of two (two compartment model) or three (three compartment model) exponential decay functions using the program BMDP P3R, a nonlinear regression. $Q_i$ (μmol g fresh wt$^{-1}$) is the quantity of $Ca^{2+}$ in the ith compartment in series; $k_i$ (min$^{-1}$) is the rate constant for exchange from that compartment; $t_{h_i}$ (min) is the half-time for exchange which is equal to 0.693/k.

| Parameter | Two Compartment Model | | Three Compartment Model | |
|---|---|---|---|---|
| | 23°C | 4°C | 23°C | 4°C |
| $Q_1$ | 2.56 | 2.18 | 1.74 | 1.26 |
| $k_1$ | 0.505 | 0.345 | 3.19 | 2.42 |
| $t_{h_1}$ | 1.37 | 2.01 | 0.217 | 0.286 |
| $Q_2$ | 5.98 | 6.21 | 1.74 | 1.64 |
| $k_2$ | 0.00646 | 0.00656 | 0.140 | 0.150 |
| $t_{h_2}$ | 107 | 106 | 4.95 | 4.62 |
| $Q_3$ | | | 5.57 | 5.95 |
| $k_3$ | | | 0.00592 | 0.00624 |
| $t_{h_3}$ | | | 117 | 111 |
| Residual mean square | 0.0451 | 0.0242 | 0.00471 | 0.00398 |

apoplast makes up a major portion of the Ca content of the root (12). With increasing salinity, the $Ca^{2+}$ influx decreases in much the same manner as the activity of $Ca^{2+}$ in the external solution (7). The diminished $Ca^{2+}$ influx may be the result of decreasing $Ca^{2+}$ activity in solution, but may also reflect an enhanced exchange with $Na^+$ in the apoplast (7). The increased $Ca^{2+}$ influx at high NaCl concentrations, however, cannot be accounted for by these factors. The $Ca^{2+}$ activity in solution further decreases over this NaCl concentration range and exchange displacement of $Ca^{2+}$ by $Na^+$ in the cell wall would be expected to diminish rather than enhance $Ca^{2+}$ binding in the cell wall. The increase in $Ca^{2+}$ influx may be due to greater plasmalemma permeability. Since free cytosolic $Ca^{2+}$ concentrations are low (on the order of $10^{-7}$ to $10^{-6}$ M), passive $Ca^{2+}$ transport would tend to proceed inward. This explanation is supported by the finding of $Na^+$ displacement of $Ca^{2+}$ from the plasmalemma of cotton root hairs and $K^+$ ($^{86}Rb$) leakage in cotton roots, which increased over the same range of NaCl concentrations as $^{45}Ca^{2+}$ influx (9). At high NaCl concentrations, the increase in $Ca^{2+}$ influx was greater at high than at low external $Ca^{2+}$ concentrations. This may be related to a higher $Ca^{2+}$ concentration gradient across the plasmalemma or saturation of the putative $Ca^{2+}$-efflux pumps. In addition, the mechanisms of uptake may be different at these two $Ca^{2+}$ concentrations.

Lynch and Läuchli (23) observed a transient increase in short-term $Ca^{2+}$ influx in barley induced by 30 mM NaCl. Over longer time intervals, $Ca^{2+}$ influx was unaffected by this NaCl concentration. We observed a decrease in short-term $Ca^{2+}$ influx into cotton roots over the same range of NaCl concentrations. Thus, this response to $Na^+$ may vary, depending on the genotype.

Measurement of symplasmic $Ca^{2+}$ is difficult in plants. Our inability to separate efflux from the apoplast and the symplasm limits the interpretations that can be made from our $Ca^{2+}$ influx data, raising questions about those data for which this complication was not considered. Because of these questions, and the recent recognition that $Ca^{2+}$ acts as a second messenger (12, 14, 28), better techniques that discriminate apoplastic from symplasmic $Ca^{2+}$ will have to be developed.

The reduction of $Na^+$ influx by high $Ca^{2+}$ concentrations (Fig. 3) is in agreement with other findings (10, 15, 20, 26, 27). This does not necessarily contradict the findings of Kent and Läuchli (18), who found that the tissue concentrations of Na in cotton seedlings were unaffected by external $Ca^{2+}$ concentrations at 200 mM NaCl. Their study presented results of long-term $Na^+$ uptake in roots (8 d), whereas the present study focuses on short-term $Na^+$ influx (10 min). Also, we did not investigate the possible contribution of $Na^+$ efflux, which is another component of net uptake.

Sodium influx increased proportionally to NaCl concentration without evidence of saturation. Kochian and Lucas (16) have described in detail the uptake isotherm for $K^+$ in corn roots. They find two components to the isotherm: a saturable component at low concentrations and a nonsaturable component dominating at high concentrations. They provide evidence that the nonsaturable component may represent $K^+$ channels (17). Kochian and Lucas (16) have used $K^+$ concentrations as high as 40 mM. In this study, we have extended the concentration range to 250 mM NaCl, providing evidence for a nonsaturable component

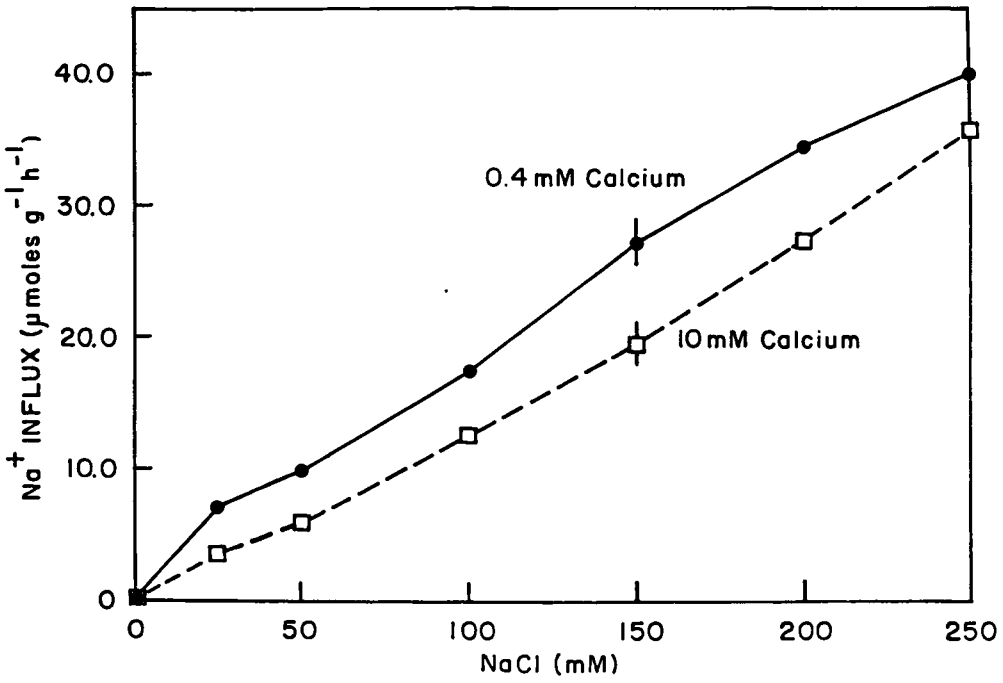


FIG. 3. The effect of increasing NaCl concentrations on $^{22}Na^+$ influx into cotton roots at two different $Ca^{2+}$ concentrations. The error bars represent 95% confidence intervals for each $Ca^{2+}$ treatment.

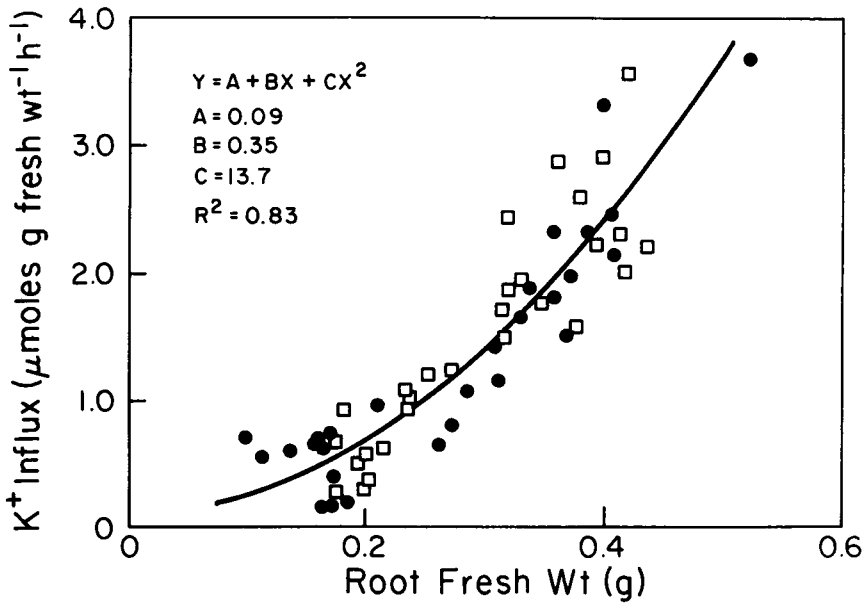


FIG. 4. The relationship between root weight and $^{42}K^+$ influx ($\mu$mols g fresh $weight^{-1}$ $h^{-1}$) into cotton roots. (●), 0.4 mM $Ca^{2+}$; (□), 10 mM $Ca^{2+}$. Each point represents one replicate (five roots per replicate). The line represents the regression for all data points.

for a different cation in a different plant species. There is, however, evidence for a saturable, high-concentration $Na^+$ transport mechanism in barley (26, 27).

Jacoby and Hanson (15) have investigated the nature of the $Ca^{2+}$ inhibition of $Na^+$ influx. They found that $Ca^{2+}$ inhibited two components of $Na^+$ influx, one of which was considered an energy-linked carrier and the other a channel. In our study, there was no effect of $Ca^{2+}$ on the slopes of the two concentration isotherms in the range of 25 to 250 mM NaCl. This suggests that in cotton, $Ca^{2+}$ has no effect on the nonsaturable component (Na channels?), but may have an effect on a saturable component that would be operative at lower concentrations. Indeed, Rains and Epstein (26) found that the addition of $Ca^{2+}$ increased the $K_m$ for $Na^+$ influx and decreased the $V_{max}$, thus, reducing $Na^+$ influx markedly in the concentration range found for the saturable component (mechanism 1). In this earlier study, the influence of $Ca^{2+}$ on $Na^+$ influx was investigated either in the presence or the absence of $Ca^{2+}$. In our study, the comparison was made in the presence of two different concentrations of $Ca^{2+}$. In light of the detrimental effects of the lack of $Ca^{2+}$ on membrane properties, more research on the effect of $Ca^{2+}$ on $Na^+$ influx seems warranted.

Increasing concentrations of NaCl significantly inhibited $K^+$ influx. This effect has also been observed in barley roots (22, 27). Epstein (10) found that $Ca^{2+}$ was important for the maintenance of $K^+$ influx and $K^+/Na^+$ selectivity in barley roots. Potassium influx in salt-stressed roots, however, was unaffected by the $Ca^{2+}$ concentrations (0.4 and 10 mM) used in this study (Table II). This does not mean that $Ca^{2+}$ is not important for $K^+$ uptake in cotton; a $Ca^{2+}$ effect may become apparent at $Ca^{2+}$ concentrations below 0.4 mM. (In Epstein's study [10], 0.5 mM $Ca^{2+}$ fully reversed the inhibitory effect of $Na^+$.)

In a long-term study of salt-stressed cotton seedlings (18), tissue concentrations (net flux) of $K^+$ were greater at high than that at low external $Ca^{2+}$ concentrations. The inhibition of $K^+$ influx by NaCl in the present study cannot account for these

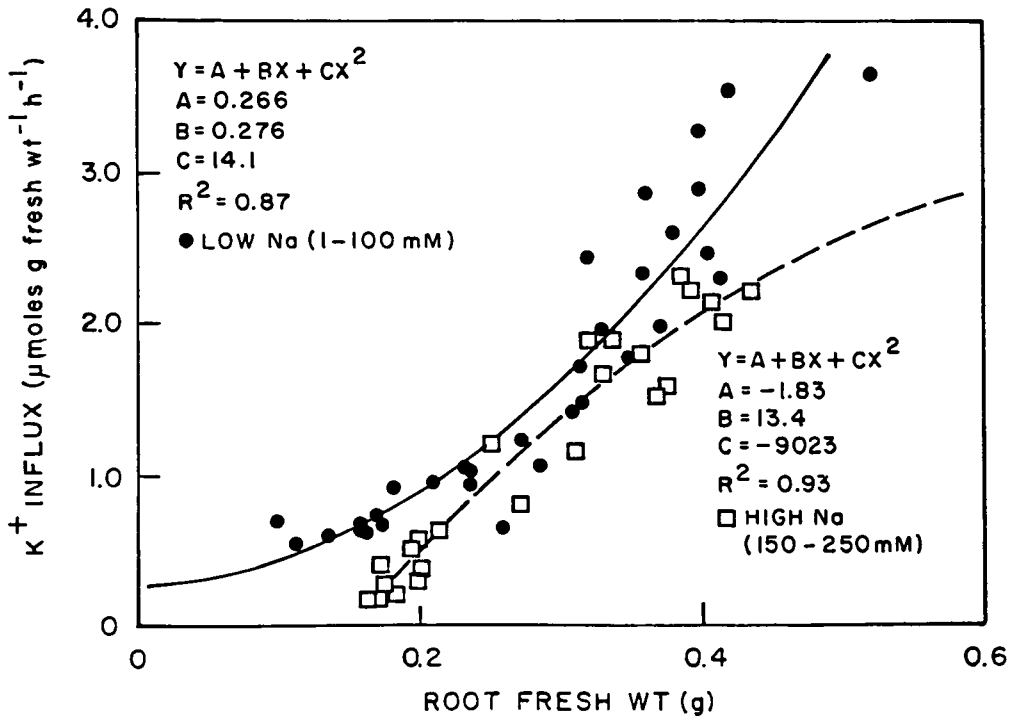


FIG. 5. The relationship between root weight and $^{42}K^+$ influx into cotton roots separated into two classes of NaCl concentration treatments (0–100 mM equals low NaCl; 150–250 mM equals high NaCl).

Table II. *Multiple Linear Regression Analysis of the Effects of Root Weight, and Concentrations of $Na^+$ and $Ca^{2+}$ in the Medium on $^{42}K^+$ Influx*

$Y = a + b_1$ (root wt) $+ b_2$ ($Na^+$) $+ b_3$ ($Ca^{2+}$)
Dependent variable: $^{42}K$ influx ($\mu$moles g fresh $wt^{-1}$ $h^{-1}$)

| Source | SS | MS | *df* | *F* | PR > *F*[a] |
|---|---|---|---|---|---|
| Regression | 40.0928 | 13.3643 | 3.0000 | 126.1982 | $7.41 \times 10^{-24}$ |
| Residual | 5.5067 | 0.1059 | 52.0000 | | |
| Total | 45.5995 | | | | |
| $R^2$: | | 0.8792 | | | |
| Root of Residual MS: | | 0.3254 | | | |

| Parameter | Estimate | Standard Error | *t* | PR > \|t\|[b] |
|---|---|---|---|---|
| Intercept | −0.6372 | | | |
| $b_1$ for root wt (g) | 8.3216 | 0.4435 | 18.7655 | $8.25 \times 10^{-25}$ |
| $b_2$ for $Na^+$ (mM) | −0.0028 | 0.0005 | −5.5040 | $1.15 \times 10^{-6}$ |
| $b_3$ for $Ca^{2+}$ (mM) | 0.0019 | 0.0091 | 0.2100 | 0.8345 |
| Mean of dependent variable: | | | | 1.4253 |
| Standard deviation of dependent variable: | | | | 0.9105 |

[a] Probability that a random value of *F* will be greater than that observed. [b] Probability that a random value of *t* will be greater than the absolute value of *t* observed.

differences; therefore, they are probably due to the reduction in $K^+$ efflux brought about by the high $Ca^{2+}$ treatment (9). This points out that interpretations of uptake mechanisms of salt-stressed roots using solution depletion techniques (30) should be made with caution, since the theory for determination of the kinetic parameters using this method assumes that efflux remains constant (4).

Potassium influx, though normalized for root weight ($\mu$moles g fresh $weight^{-1}$ $h^{-1}$), was nevertheless dependent upon root weight (Fig. 4; Table II). To our knowledge, this phenomenon has not been described before, but HE Joham (personal communication) stated earlier that $K^+$ uptake in cotton roots was dependent upon the age of the seedlings. Indeed, Hanson and Kahn (13) found that $K^+$ influx increased as the cells matured along the root. They found that $V_{max}$ increased and the $K_m$ decreased as the cells matured. In their experiments, potassium influx was also shown to increase if it was expressed on a per mg protein basis. Our data seem to support this view, since differences in root weight may reflect differences in root age caused by variation in onset of seed germination. On the other hand, other factors may be involved, such as variations in seed carbohydrate reserves or seedling vigor. This effect was specific for $K^+$; it was not observed for the $^{22}Na^+$ influx investigated in the same seedlings. This would suggest that $K^+$ and $Na^+$ influx occur by different mechanisms (26). Any increase in $K^+$ influx relative to $Na^+$ influx, *i.e.* improved $K^+/Na^+$ selectivity, appears to be related to salt tolerance in cotton (18). This effect may in part explain why the seedling stage in cotton is the most salt-sensitive (1); it merits further consideration.

Root growth in cotton is stimulated under saline conditions by 10 mM $Ca^{2+}$ (8). This stimulation appears to be caused not only by the effects of the $Ca^{2+}/Na^+$ ratio in the external solution on cell elongation, but also by the high $Ca^{2+}$ treatment promoting or maintaining cell division (19). The increase in $Ca^{2+}$ influx

with supplemental $Ca^{2+}$ may be responsible for the increase in cell division, since the cytosolic $Ca^{2+}$ concentration is thought to play a role in cell division (12, 14, 28). In addition, $K^+/Na^+$ selectivity may play a role, since metabolism is adversely affected by low $K^+/Na^+$ ratios (11). This becomes all the more significant for dividing cells, because they are largely nonvacuolate, and therefore lack the compartment in which $Na^+$ could be sequestered without damage to the cytoplasm.

**Conclusion.** Influx of $K^+$ and $Ca^{2+}$ in cotton roots was inhibited by high NaCl treatments at low $Ca^{2+}$ concentration. The protection of root growth from salt stress by supplemental $Ca^{2+}$ (8, 19) is related to improved Ca-status and maintenance of $K^+/Na^+$ selectivity. These protective $Ca^{2+}$ effects may be most significant in the root tip where the cells are largely nonvacuolate and cell division occurs.

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