

Kinematics and Dynamics of Sorghum (*Sorghum bicolor* L.) Leaf Development at Various Na/Ca Salinities¹

I. Elongation Growth

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In many salt-sensitive species, elevated concentrations of Ca in the root growth media ameliorate part of the shoot growth reduction caused by NaCl stress. The physiological mechanisms by which Ca exerts protective effects on leaf growth are still not understood. Understanding growth inhibition caused by a stress necessitates locating the leaf expansion region and quantifying the profile of the growth reduction. This will enable comparisons and correlations with spatial gradients of probable physiologically inhibiting factors. In this work we applied the methods of growth kinematics to analyze the effects of elevated Ca concentrations on the spatial and temporal distributions of growth within the intercalary expanding region of salinized sorghum (*Sorghum bicolor* [L.] Moench, cv NK 265) leaves. NaCl (100 mM) caused a decrease in leaf elongation rate by shortening the leaf growing zone by 20%, as well as reducing the peak value of the longitudinal relative elemental growth rate (REG rate). Increasing the Ca concentrations from 1 to 10 mM restored the length of the growing zone of both emerged and unemerged salinized leaves and increased the peak value of the REG rate. The beneficial effects of supplemental Ca were, however, more pronounced in leaves after their appearance above the whorl of encircling older leaf sheaths. Elevated Ca then resulted in a peak value of REG rate higher than in the salinized leaves. The peak value of unemerged leaves was not increased, although it was maintained over a longer distance. The duration of elongation growth associated with a cell during its displacement from the leaf base was longer in salinized than control leaves, despite the fact that the elongation zone was shorter in salinity. Although partially restoring the length of the elongation region, supplemental Ca had no effect on the age of cessation of growth. Elongation of a tissue element, therefore, ceased when a cellular element reached a certain age and not a specific distance from the leaf base.

Many plant species suffer a decline in growth when exposed to NaCl stress. The inhibition of growth in long-term exposures (days) can result from osmotic effects on water availability, reduction in net CO₂ assimilation, specific ion effects, or ion imbalance due to interference with uptake of essential nutrient ions (Läuchli, 1986; Munns and Termaat,

1986). It has previously been demonstrated for sorghum (*Sorghum bicolor* L.) (Bernstein et al., 1993) and other species (Aslam et al., 1986; Lazof et al., 1991) that the primary effects of salt stress are to reduce leaf growth rate, leaf emergence rate, and overall shoot development.

In many, but not all, salt-sensitive species, elevated Ca concentrations in the root media ameliorate part of the growth reduction caused by the stress (Läuchli, 1990, and refs. therein). For example, leaf elongation of salt-stressed barley seedlings was found to improve when the Ca supply was increased from 0.5 to 3 mM (Ward et al., 1986). Elzam and Epstein (1969) found a positive correlation between Ca levels and growth in *Agropyron* sp. exposed to salinity.

The physiological mechanism by which Ca exerts a protective effect on leaf growth under salt stress is still not clearly understood. It was suggested that reduced Ca availability, coupled with high Na/Ca ratios in the expanding tissue, contributed to the growth reduction in barley (Lynch et al., 1988; Rengel, 1992). The experimental evidence available today to support a correlation between low tissue Ca and reduced growth is inconclusive. In barley, no correlation was found between shoot growth and Ca transport or concentration in the shoot as a whole (Cramer et al., 1989). Salinity was shown to inhibit Ca transport from root to shoot, and supplemental Ca was shown to increase Ca concentration in the growing zone and to partially restore leaf growth rate (Lynch and Läuchli, 1985; Ward et al., 1986; Lynch et al., 1988).

A critical issue is to determine the relationships between the spatial distribution of elongation and the concentration profiles of nutrients with and without stress. The concentration of an element in the leaf growing zone could be markedly different from the total leaf concentration; moreover, sharp gradients occur along the growing zone itself (Meiri et al., 1992). Comparison of the spatial distribution of growth inhibition, element concentrations, and deposition rate profiles are, therefore, essential. In a previous study (Bernstein et al., 1993), we applied the methods of growth kinematics (Silk and Erickson, 1979) to test the effects of elevated NaCl on the distribution of growth within developing sorghum leaves. We showed that salinity shortened the leaf growth zone and

Abbreviations: PI, plastochron index; REG rate, relative elemental growth rate.

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reduced the peak value of the REG rate. The extent to which the leaf growth pattern may change under conditions of enhanced Ca supply is not known.

In the present study, our aim was to evaluate whether Ca plays a role in the tolerance of sorghum leaves by affecting the concentration profiles of nutritional or toxic elements in the growing regions. In this paper we concentrate on the effects of various Na/Ca treatments on the spatial distribution of growth along the leaf. This information will be combined with data concerning concentration profiles and deposition rates of different elements to determine their role in limiting/restoring growth. Similar approaches to the study of environmental variation have been applied to leaves exposed to different irradiances (Schnyder and Nelson, 1989) and nitrogen levels (Volenc and Nelson, 1984) and to roots under water stress (Sharp et al., 1988, 1990) and salinity stress (Zhong and Läuchli, 1993) and at various temperatures (Pahlavanian and Silk, 1988).

MATERIALS AND METHODS

Plant Material and Growth Conditions

Sorghum plants (*Sorghum bicolor* L. Moench, cv Northrup King 265) were grown in solution culture in a growth chamber. Seeds were washed in 0.5% NaOCl for 15 min, rinsed for 30 min, and germinated on a stainless steel grid over a one-tenth-concentration, aerated, modified Hoagland solution, as described previously (Bernstein et al., 1993). The germination container was placed in the dark at 27°C in a growth chamber. After 3 d the seedlings were exposed to a 15-h photoperiod and were kept in that state for an additional 3 d. Seedlings chosen for uniformity were then transferred to 4-L containers containing one-half or one-fourth-strength modified, aerated Hoagland solution (two seedlings per container), except that Fe was supplied as 50 μM Fe-EDTA and 20 μM $\text{Fe}(\text{NH}_4\text{SO}_4)_2$ and the Na level was elevated to 1 mM.

The pH was adjusted daily to 5.7 with the addition of KOH (200 mol m^{-3}), and the solutions were changed every other day. Average growth chamber humidity throughout the growth period was 65% during the day and 75% at night. Light intensity was 750 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. A temperature of 28°C was maintained at the base of the plant, where the leaf elongation zone is located.

Na and Ca Treatments

Four different combinations of NaCl and CaCl_2 concentrations were applied in each experiment. The four treatments used were: (a) control, (b) salt, (c) control plus supplement of high Ca, and (d) salt plus supplement of high Ca. NaCl concentrations were 1 mM in the control treatments and 100 mM in the salinity treatments. The Ca concentration was 10 mM in the high-Ca treatments and 1 mM in the lower-Ca treatments. In one experiment, the effect of a more concentrated nutrient solution was tested. Half-concentration, modified Hoagland solution (containing 2 mM Ca) was then used as the basic nutrient solution; the low-Ca treatments were, therefore, subjected to 2 mM Ca. Quarter-concentration modified Hoagland solution was used in all experiments unless stated otherwise. A summary of the different treatments is

given in Table I. Ca concentration was elevated from 1 or 2 mM in the control treatment to 10 mM in the high-Ca treatments by the addition of CaCl_2 .

The supplemental Ca was added on d 7 after the beginning of germination, 24 h before commencement of salinization. Salinization was initiated on d 8 after germination, at which time leaf 4 has not yet emerged from the whorl of enclosing older leaf sheaths and leaf 6, if visible as a primordium, was smaller than 2 mm. NaCl was increased to 25 mM on the 1st d of salinization and later by daily increments of 25 mM d^{-1} until the desired concentration of 100 mM was reached. The salt was added exactly 1 h after the transition to the dark period, and the solution pH was adjusted immediately thereafter.

Leaf Elongation Measurements and PI Calculation

A nondestructive method was used to analyze leaf growth rate and overall shoot development. The length of every visible leaf was measured daily with a ruler as previously described (Bernstein et al., 1993). The leaf growth rate was calculated from the daily leaf length measurements as the slope of the linear regression through the data in the linear growth phase.

The PI, an index of plant developmental age, was calculated for each day of measurement using method 1 of Erickson and Michelini (1957). A reference length of 15 mm was used. On each day, the first leaf exceeding that reference length was identified (leaf n). The extent to which that leaf length exceeded the reference length was calculated as a fraction of the difference between its length and the length of the next younger leaf (leaf $n + 1$), according to Equation 1 of Erickson and Michelini:

$$\text{PI} = n + \frac{\log(\text{length}_n) - \log(\text{length}_{\text{ref}})}{\log(\text{length}_n) - \log(\text{length}_{n+1})}$$

where n is the leaf just exceeding the reference length, $n + 1$ is the next younger leaf, and ref is the reference length. Leaves were numbered so that later emerging (younger) leaves had higher numbers.

Measurement of Spatial Distribution of Growth

Data concerning the spatial distribution of growth along the elongation zone of selected leaves were obtained by marking the leaf growth zone with pin pricks and evaluating a short-term displacement of the marks (Schnyder et al., 1987). Leaves were marked as previously described (Bernstein et al., 1993). In short, a series of 13 fine insect-mounting pins (size 00; Hamilton Bell Co., Montvale, NJ) spaced 3 mm apart

Table I. Treatment conditions

Treatment	NaCl	Ca
	<i>mM</i>	<i>mM</i>
Control	1	1 (or 2)
Salt	100	1 (or 2)
Control + Ca	1	10
Salt + Ca	100	10

and mounted on a piece of Plexiglas were used. The pins were longitudinally aligned with the center of the stem and inserted through the sheaths of older outer leaves and the growing region of the young enclosed leaves and then removed. The plants were pricked at least 3 h after initiation of the photoperiod. Growth was allowed to continue for 6 h before growing leaves were carefully freed from older enclosing leaves, and the final positions of the pin marks were measured under a dissecting microscope. Data concerning the spatial distribution within the elongation zone of leaves were collected from five to eight plants. Data from a specific leaf were included in the analysis only if the first prick hole was placed less than 0.6 mm above the base of the leaf. Unlike the nondestructive daily leaf measurements that average growth throughout the photoperiod, the marking experiments evaluate day growth. Because rates of elongation are faster at night (Schnyder and Nelson, 1988), calculated rates from marking data are expected to be lower than the averaged daily measurements. Each experiment was performed twice.

The effect of the pin pricks on the overall leaf growth was evaluated by measuring overall leaf elongation during the 6 h immediately following the pricking. Marking affected the leaf growth similarly in all treatments, and it caused a 34% reduction in the rate of leaf elongation. Schnyder et al. (1987), working with fescue leaves and using similar marking technique, showed that although the overall leaf growth rate was slower for several hours after marking, the spatial distribution of the REG rate was not affected. The REG rate profiles that were obtained from cell length data also corresponded well with those obtained from marking experiments (Schnyder et al., 1990).

Growth Analysis

The predominant growth of a monocotyledonous leaf is in one direction, from the intercalary meristem at the leaf base. Growth of such a unidirectionally growing organ can be characterized spatially by the distribution of growth velocity and REG rate (Erickson and Sax, 1956) and by growth trajectories (Silk and Erickson, 1979; Gander, 1983).

Growth velocity is the displacement rate of a tissue element from the leaf base. It was calculated as the distance that the prick hole shifted from the leaf base, per unit time (mm h^{-1}). Growth velocity increases with distance from the leaf base, because progressively more tissue is expanding. Past the end of the growth zone, the growth velocity no longer increases with position. If leaf growth is steady, then the velocity curve does not change in time.

REG rate is the relative growth rate of a leaf segment in one dimension. It is the change that occurs in a length of a leaf segment per unit length. REG rate was calculated using a two-point forward difference formula to estimate the derivative of the growth velocity curve with respect to position along the leaf.

$$\text{REG rate} = \partial v / \partial z \approx \frac{v(z_2) - v(z_1)}{z_2 - z_1}$$

where v is growth velocity, $v(z_i)$ is velocity at point z_i , and z

is the original distance from the leaf base. REG rate is, therefore, presented in units of $(\text{mm mm}^{-1} \text{h}^{-1}) = \text{h}^{-1}$.

A growth trajectory is a plot of a particle position versus time as it is displaced through the growth zone of the leaf (Silk and Wagner, 1980). The growth trajectories were constructed from the displacement velocity data by numerical integration during the period of leaf expansion where the growth was demonstrated to be time invariant (Bernstein et al., 1993). The temporal patterns of REG rate were obtained by combining the spatial distribution of the REG rates with the growth trajectories.

RESULTS

Shoot and Leaf Growth

Salinity (100 mM NaCl) substantially reduced leaf growth and shoot development. This reduction was partially prevented when elevated concentrations of Ca were supplied to the root medium. Figure 1 shows the results of nondestructive daily leaf length measurements. During the rapid phase of leaf growth, leaf elongation is linear with time. Elevation of Ca concentration from 1 to 10 mM partially prevented the shortening of the rapid phase of growth that occurred under saline conditions. The development of control shoots is shown not to differ when plants are grown at 1 or 10 mM Ca (analyzed on the basis of emerged leaf length).

Exposure to 100 mM NaCl caused a reduction in leaf growth rate and final length achieved by the individual leaves and reduced the plastochron age of the plants (Fig. 2). Increased Ca supply partially restored the growth rate and length of

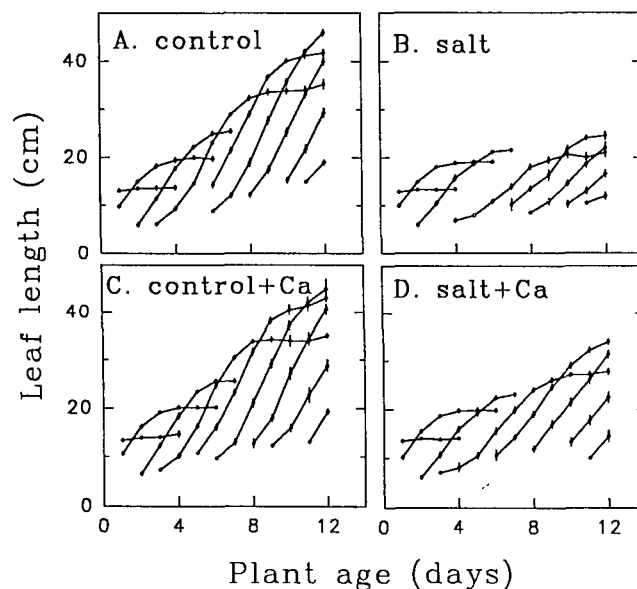


Figure 1. Na/Ca interaction in leaf development. Leaf length was measured daily in plants grown at 1 mM NaCl and 1 mM CaCl_2 (control; A), 100 mM NaCl and 1 mM CaCl_2 (salt; B), 1 mM NaCl and 10 mM CaCl_2 (control plus Ca; C), and 100 mM NaCl and 10 mM CaCl_2 (salt plus Ca; D). Data are means \pm SD ($n = 8$). Each trace represents data from one leaf.

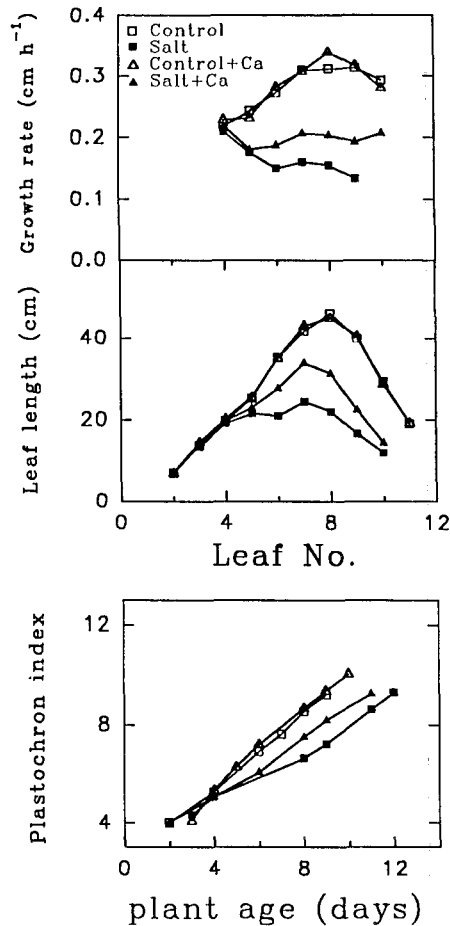


Figure 2. Na/Ca effects on leaf growth and shoot development. Data were evaluated from nondestructive daily leaf measurements. Leaf growth rate, leaf length, and PI of plants grown at different Na/Ca regimens. Treatments were as described for Figure 1.

the salinized leaves and increased the PI to values closer to the controls.

Effect of Supplemental Ca on the Spatial Distribution of Growth

We first evaluated the effect of elevated Ca concentrations on the spatial distribution of growth within the control leaves. The elongation of leaves was affected differently at different stages of development. The growth of young, rapidly elongating, unemerged leaves was substantially reduced by the elevated Ca treatment (Fig. 3A). The peak value of the REG rate of these leaves was greatly suppressed. The length of the elongation region was not affected. During later stages of leaf elongation, after the leaf had emerged from the protective whorl of older sheaths, growth was less sensitive to the elevated Ca treatment. Leaf growth was always reduced to some extent by the supplemental Ca, but this reduction was not always statistically significant and was smaller than for the younger leaves (Fig. 3B). This experiment was repeated four times with similar results. The effects of Na/Ca interactions on the spatial distribution of growth was evaluated

separately on leaves before and after their appearance above the whorl of older leaf sheaths.

Na/Ca Interaction Effects on the Leaf Growth Fields

The beneficial effects of elevated Ca concentrations under saline conditions were evident in rapidly growing leaves before, as well as after, their emergence from the enclosing whorl of older leaf sheaths. The spatial distribution of growth velocity and REG rate of leaf 8 when still enclosed within the protected sheaths of older leaves is shown in Figure 4. Growth velocity is known to increase with distance from the leaf base, because progressively more tissue is expanding. The end of the growth zone can, therefore, be estimated as the point at which the growth velocity no longer increases with position. It can also be identified as the location where the REG rate curve becomes zero. Saline stress reduced the length of the leaf growth zone and lowered the peak value of the REG rate by 72%. Supplemental Ca lessened the adverse effects of NaCl. It fully restored the length of the growth zone and improved the longitudinal REG rate. The peak value of the REG rate at high NaCl was not increased by the elevated Ca concentration but was maintained over a longer distance. The overall leaf elongation rate, as indicated by the maximal value of the growth velocity, was, therefore, higher (Fig. 4B).

Later in development, when leaf 8 was visible above the whorl of older leaf sheaths, salinity lowered the maximal value of the growth velocity by 64% (Fig. 5). When supplemental Ca was added, this reduction was only 46%. As in early stages of leaf elongation, elevated Ca restored the length of the elongation zone. The REG rate was lowered by salinity and was partially restored by increased Ca concentration. Unlike early in development, after the leaf was visible, the peak value of the REG rate also was partially restored with supplemental Ca (Fig. 5).

Growth Trajectories and Temporal Patterns of REG Rates

If the spatial distribution of growth is steady, then a growth trajectory (i.e. plot of a mark position versus time as it is displaced from the leaf base through the growth zone) can

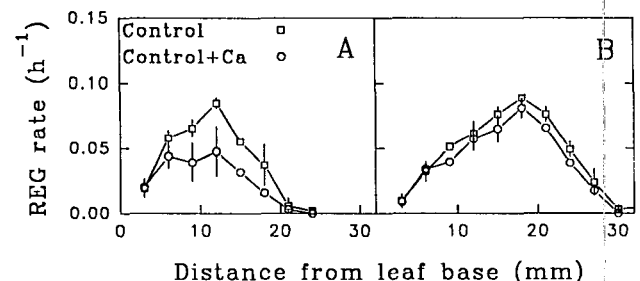


Figure 3. Effect of supplemental Ca on the spatial distribution of elongation along leaves, expressed as REG rate, during two stages of leaf growth. A, Early stage of rapid leaf elongation, when the leaf is still enclosed in older leaf sheaths. B, After the leaf has emerged from the enclosing whorl. Data were evaluated from prick hole-marking experiments for leaf 8 on 14- and 16-d-old plants. Data are means \pm SD ($n = 5-8$).

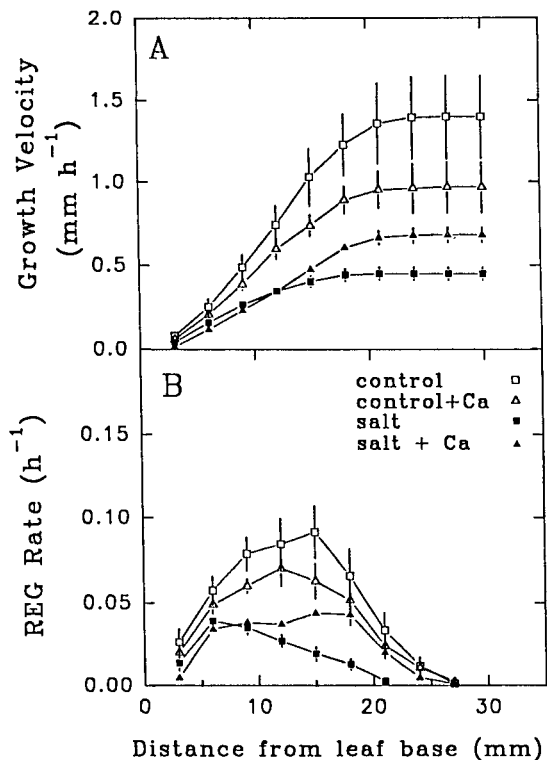


Figure 4. Spatial distribution of growth along a sorghum leaf before its emergence from the whorl of older leaf sheaths: effects of salinity and Ca. A, Displacement velocity (rate of displacement from the leaf base). B, REG rate. Plants grown at four different Na/Ca combinations (treatments as for Fig. 1). Data were evaluated from prick hole-marking experiments of leaf 8 during rapid elongation. Data are means \pm SD ($n = 5-8$).

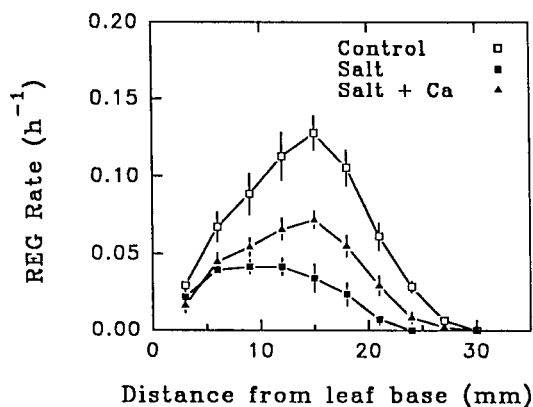


Figure 5. Spatial distribution of REG rate along a rapidly growing sorghum leaf while visible above the whorl of older leaf sheaths: effects of salinity and Ca. Plants grown at 1 mM NaCl and 1 mM CaCl₂ (control), 100 mM NaCl and 1 mM CaCl₂ (salt), or 100 mM NaCl and 10 mM CaCl₂ (salt plus Ca). Data were evaluated from prick hole-marking experiments of leaf 8 when just appearing above the whorl. Data are means \pm SD ($n = 5-8$).

be calculated from data on the spatial distribution of growth velocity obtained from the marking experiments. Under our experimental conditions, growth is steady to a first approximation when the leaves are growing rapidly, after their appearance above the whorl of older leaf sheaths (Bernstein et al., 1993). Calculated growth trajectories for such leaves are presented in Figure 6A. Relative to the control, salinity shifted the growth trajectory to the right. This indicates that in the control leaves a cellular particle moves away from the leaf base through the growth zone faster than in the salinized leaves. When elevated concentrations of Ca were applied to the salinity treatment, the rate of particle movement was faster, i.e. the trajectory was shifted to the left. Displacement from 6 to 30 mm required 53 h in the salinity treatment and only 40 h in the salinity plus elevated Ca treatment.

We combined the growth trajectories with the spatial distribution of REG rate to describe the temporal patterns of REG rate of a material element initially located 3 mm from the leaf base (Fig. 6B). Because the REG rate in all Na/Ca treatments was similar at locations apical to 3 mm, tissue elements would have required similar times to be displaced through this region regardless of the salinity treatment, as reasoned by Sharp et al. (1988). The analysis revealed that the duration of growth associated with a material element

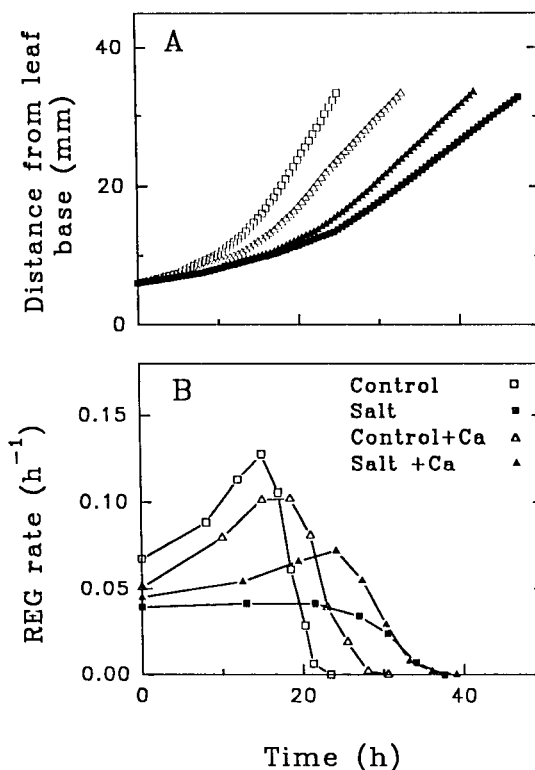


Figure 6. Growth trajectories and temporal patterns of REG rates at different Na/Ca treatments. A, Growth trajectories of a material element initially located 6 mm from the leaf base, estimated by numerical integration of the velocity field. B, Temporal patterns of REG rate of a material element located initially 3 mm from the leaf base, generated by combining the spatial distribution of the REG rate (from pin prick displacement measurements) with the growth trajectories.

during its displacement is longer in salinized than control leaves in spite of their shorter growth zone. It is interesting that elevated Ca concentrations did not change the length of time that a salinized tissue element elongates, in spite of the changes in the length of the growth zone and magnitude of elongation.

Effect of Nutrient Concentration on Spatial Distribution of Growth

Interestingly, growth conditions can alter the length of the basal leaf segment, which is unaffected by salinity. In plants grown at one-half-concentration Hoagland solution the length of the unaffected segment was 6 to 9 mm (data not shown). This is consistent with the findings of Bernstein et al. (1993). The unaffected zone was shorter in leaves grown at one-fourth concentration solution (Figs. 3 and 4); a statistically significant reduction in rate of growth is then already observable 6 mm from the leaf base.

The difference in growth distribution along the basal 6 to 9 mm is not caused by the higher concentration (2 mM) of Ca in one-half-strength Hoagland solution, because elevating Ca concentration of one-fourth-strength Hoagland solution to 10 mM did not restore the REG rate of the salinized tissue located 6 to 9 mm from leaf base to the control values (data not shown).

In both one-half- and one-fourth-strength Hoagland solutions the length of the growth zone was restored by elevated Ca in emerged leaves but only partially restored in unemerged leaves. The beneficial effects of elevated Ca concentrations were more pronounced in plants grown in one-fourth than in one-half-concentration Hoagland solution. We consider that concentrations of specific elements other than Ca, the faster growth of plants in one-fourth-strength Hoagland solution, or total osmotic potential of the solution may have induced such a difference. Possibly, the plant osmoregulates better or supplies higher levels of nutrients to the growth zone, therefore partially preventing the inhibitory effects of salinity.

DISCUSSION

The beneficial effects of elevated Ca concentrations under saline conditions are evident in sorghum plants. Salinity reduces the leaf growth rate and shortens the period of rapid leaf elongation, thereby producing shorter leaves. Supplemental Ca prevents part of the reduction in leaf length by partially restoring both growth rate and duration of the rapid elongation period (Figs. 1 and 2).

The analysis of short-term displacement of marks throughout the growth zone under conditions of varied Na/Ca concentrations allowed us to investigate the basis for the changes in leaf growth rate. NaCl stress imposes a decrease in leaf elongation rate by shortening the leaf growing zone as well as reducing the peak value of the longitudinal REG rate (Figs. 4 and 5). These results are consistent with a previous report (Bernstein et al., 1993). Supplemental Ca restored the length of the growing zone of both unemerged and emerged leaves and increased the magnitude of the REG rate. The beneficial effects of supplemental Ca are more pronounced in salinized

leaves after their appearance above the whorl of older leaf sheaths. The peak value of the REG rate is increased with Ca in the somewhat older leaves. The peak value of the REG rate in young, unemerged leaves was not increased by supplemental Ca, although it was maintained over a longer distance.

During the first phase of leaf elongation, when grass leaves are still enclosed in the whorl of older leaf sheaths, transpiration rates and, therefore, xylem transport are reduced. Such slowly transpiring leaves are particularly sensitive to scarcity of Ca (which is relatively phloem immobile) (Marschner, 1986). Collier and Huntington (1983) reported that young, enclosed, nontranspiring leaves of lettuce are deficient in Ca. The problem is expected to become more severe under saline conditions. Slowly transpiring organs such as enclosed leaves are dependent on nocturnal pressure to supply Ca via the xylem. Under saline conditions, the solute potential in the external medium is low, and root pressure is reduced. The nocturnal supply of Ca to the enclosed leaves is, therefore, reduced. Lazof and Lauchli (1991) showed that salinization reduced the total Ca concentration of young, enclosed lettuce leaves. If indeed Ca concentration in the leaf growth zone is limiting growth under saline conditions, then unemerged leaves are expected to be more sensitive to the stress than older, rapidly transpiring leaves. Elevated Ca concentration in the root media may result in higher Ca concentration and higher growth rates, especially in young leaves. Under our experimental conditions this may explain the larger inhibition of growth observed in salinized unemerged versus emerged leaves (Figs. 4 and 5; Bernstein et al., 1993) and the partial prevention of such inhibition under conditions of elevated Ca.

Under nonsaline conditions high Ca concentration inhibited leaf elongation. The reduction in REG rate of unemerged leaves was large in comparison to more mature, emerged leaves (Fig. 3). Because Ca was increased from 1 to 10 mM by the addition of CaCl₂, the observed decrease in the peak value of the REG rate could be a result of decreased osmotic potential in the root medium or even a specific Cl effect. Under saline conditions, this inhibitory effect may be masked by the greater beneficial effects of the supplemental Ca. It may, however, be responsible for the lack of increase in the peak value of the REG rate of leaves before their emergence (such an increase occurs in emerged leaves only) (Figs. 4B and 5).

The duration of elongation growth associated with an element during its displacement from the leaf base was longer in salinized than control leaves (Fig. 6). Despite the fact that the elongation zone was shortened under salinity, it took a cellular element 15 h longer to be displaced beyond 6 mm to the distal edge of the growth zone. A salinized elongating cell is, therefore, exposed to factors acting in the elongating tissue longer than an elongating nonsalinized cell. It is interesting that the cessation of elongation growth in salinized leaves occurred in tissue of the same age, regardless of the applied Ca concentration. The transition point between longitudinal growth and cessation of growth of a cell under salinity was not determined by location but by tissue age, i.e. growth ceased when a cellular element reached a certain age and not a specific distance from the leaf base. In light of the observations that salinized leaf elongation rate, peak value

of REG rate, and length of the growth zone all increased with elevated concentrations of Ca but the time of termination of growth was not affected, we consider that the beneficial effects of Ca on leaf growth did not counteract directly the influences of NaCl but improved growth via another mechanism. It may also be that preservation of the duration of elongation is coincidental (an integrated result of the change in spatial and temporal distributions of REG rate and length of the elongation zone).

Temporal aspects of growth were studied in maize roots at low water potentials (Sharp et al., 1988) and in cotton roots at different NaCl/CaCl₂ ratios (Zhong and Läuchli, 1993). Water stress reduced the peak of REG rate but not the age of cessation of growth. As in our salinized sorghum leaves, NaCl increased the duration of cellular element elongation in the salt-stressed cotton root. But in roots, unlike leaves, Ca shortened the duration of elongation in both salinized and control treatments.

Although no experimental evidence is currently available showing a positive correlation between reduced Ca concentration in the growing region and inhibition of growth, NaCl salinity is known to induce Ca deficiency symptoms in several plant species (Grieve and Maas, 1988, and refs. therein). Furthermore, the decrease in symptoms of saline stress under elevated Ca supply was correlated with increased Ca concentrations in the developing leaves (Maas and Grieve, 1987; Grieve and Maas, 1988). The effect of salt stress on Ca nutrition was studied in barley. Salinity lowered shoot Ca concentration, especially in young leaves, and depleted Ca from the elongation region (Lynch and Läuchli, 1985; Lynch et al., 1988). It was, therefore, suggested that reduced Ca availability in the leaf growth region and high Na/Ca ratios in that region contributed to the inhibition of growth. Total shoot Ca concentration was found not to be harmful for growth under saline conditions. An important issue is, of course, that Ca concentrations of the leaf elongation zone can markedly differ from total shoot concentrations (Cramer et al., 1989); conclusions regarding growth, therefore, can only be drawn from analysis of the growing region.

Salinity was suggested to inhibit transport of Ca from root to shoot by interfering with the release of Ca to the xylem (Lynch and Läuchli, 1985). In a later report, Wolf et al. (1990) demonstrated that concentrations of Ca in the xylem were unaffected by the presence of salt and suggested instead that the reduced Ca content in the shoot of salt-stressed barley was due to a reduced transpiration rate in the presence of the salt. Ward et al. (1986) found that salinity inhibited NO₃⁻ uptake and that Ca partially restored growth and NO₃⁻ uptake to about the same extent. Cramer et al. (1989) posed the question whether NO₃⁻ uptake and assimilation drive shoot growth or, alternatively, whether the inhibition of shoot growth is affecting NO₃⁻ uptake.

The differences presented between Ca effects on growth of emerged and unemerged leaves, coupled with our knowledge of lowered transpiration rates of unemerged leaves, indirectly suggest that the improved growth of salinized leaves under conditions of elevated Ca supply is due to increasing levels of the nutrient that reach the developing leaf.

This study provides the basis for investigation of beneficial

physiological mechanisms of Ca amelioration of NaCl stress symptoms. It enables comparisons and correlations of local levels of growth inhibition, growth restoration by elevated Ca concentrations, and distribution of factors that are suspected to be of importance. In a forthcoming paper in this series we correlate spatial distributions of growth and patterns of inorganic solute concentrations and deposition rates within growing leaves under varied concentrations of Na and Ca.

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