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Sodium-induced calcium deficiency in salt-stressed corn

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Abstract. The effect of the Na^+/Ca^{2+} ratio in the root media on salt-stressed corn (*Zea mays* L. cvs DeKalb XL-75 and Pioneer 3906) was determined in greenhouse experiments. Plants grown in a complete nutrient solution salinized with 86.5 mol m^{-3} NaCl exhibited severe Ca^{2+} deficiency symptoms at the four-leaf stage. The symptoms disappeared when part of the NaCl was replaced with 10 mol m^{-3} $CaCl_2$ (Na^+/Ca^{2+} molar ratio = 5.7). Salt stress at an iso-osmotic potential of −0.4 MPa substantially decreased shoot growth at all solution Na^+/Ca^{2+} ratios from 34.6 to 0.26. However, the dry weights of blades at 26 d of age were much less when plants were salinized with NaCl alone, particularly that of DeKalb XL-75 which was more susceptible to Na-induced Ca^{2+} deficiency than was Pioneer 3906. The growth of sheaths was similarily reduced by salt stress at all Na^+/Ca^{2+} ratios. The symptoms of Ca^{2+} deficiency were correlated with low Ca^{2+} concentrations in the leaf tissue. Ca^{2+} concentrations in the developing blades of NaCl-stressed plants were much lower than in control plants. As the Na^+/Ca^{2+} ratio in the solution was decreased, Ca^{2+} levels increased in both the blades and sheaths while Na^+ concentrations greatly decreased. DeKalb XL-75 was much less effective than Pioneer 3906 in restricting the uptake of Na^+. The results clearly indicate that NaCl stress may cause lesions and unique plant responses that are not manifested on agronomic plants grown on saline soils.

Key-words: Zea mays; Gramineae; corn; calcium deficiency; salt stress; Na : Ca ratio.

Introduction

Investigations on the effects of salinity on plants have been increasing rapidly during the past few years. Bibliographies compiled by Francois & Maas (1978, 1985) list 2357 citations for the period 1900 to 1977, and 1001 citations since 1977 that concern plant responses to salinity and boron. Many of these studies involve the artificial salinization of nutrient solution or sand cultures. Unfortunately, there is an increasing tendency of many investigators to salinize these media with a single salt, usually NaCl. The results of these studies are usually purported to describe plant responses to saline conditions. This inference ignores the fundamental distinction between saline and sodic ('alkali') conditions noted 60 years ago (de Sigmond, 1927) and which later led to criteria that distinguish between these very different and unique conditions (USSL staff, 1954). The effects of salinity and sodicity on plants have been reviewed more recently by Bernstein (1975). By definition saline soils contain soluble salts in quantities that adversely affect plant growth but which have a sodium-adsorption-ratio (SAR) of less than 15. SAR = $Na^+/[(Ca^{2+}+Mg^{2+})/2]^{\frac{1}{2}}$ where the ionic concentrations in the soil solution are expressed in meq dm^{-3}. Sodic soils have a SAR greater than 15 and contain sufficient exchangeable sodium to interfere with plant growth. Clearly, the addition of a large quantity of NaCl to base nutrient solutions, which usually contain less than 5 mol m^{-3} $Ca^{2+}+Mg^{2+}$, produces a highly sodic medium. Numerous studies have shown that many agronomic plant species are seriously injured by high Na^+/Ca^{2+} ratios characteristic of sodic conditions (Lagerwerff & Holland, 1960; Bernstein, 1964; Hyder & Greenway, 1965; LaHaye and Epstein, 1971; Nassery, Ogata & Maas, 1979; Imamul Huq & Larher, 1984; Kent & Läuchli, 1985). Highly sodic treatments cause plant lesions that do not usually occur in saline-non-sodic media; the most common symptoms are similar to those caused by a Ca^{2+} deficiency (Kawasaki & Moritsugu, 1979). Some agronomic species, however, are either unaffected or fare better at high Na^+/Ca^{2+} ratios; e.g. rice (Yeo & Flowers, 1985), soybeans (Grattan & Maas, unpublished observations) and spinach (Maas & Grieve, unpublished observations). However, evidence obtained recently by Grieve & Fujiyama (1987) indicates that some cultivars of rice are susceptible to Ca^{2+} deficiency at high Na^+/Ca^{2+} ratios.

Results obtained from NaCl salinization may not necessarily apply to field-grown crops and, in fact, may lead to misleading and erroneous interpretations about plant responses caused by salinity. Greenway & Munns (1980) stressed this point when they concluded that concurrent increases in Na^+/Ca^{2+} and in total salt concentration not only cause problems in interpretation, but also are irrelevant in an ecological sense. The extreme Na^+/Ca^{2+} ratios used in solution cultures seldom occur in saline agricultural soils. Ayers & Westcot (1985) have compiled the analyses for 250 selected irrigation waters around the world. Of these, only seven are

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both saline (electrical conductivity >2.0 dS m^{-1}) and sodic (SAR >15). Because of either high bicarbonate or high salt levels, four of these waters would be considered unsuitable for irrigation.

Because solution culture experiments are so useful for research on the effects of salt on plants, it is imperative that we determine the appropriate Na^+/Ca^{2+} limits for salinizing these artificial media. This study was designed to assess the effects of various Na^+/Ca^{2+} ratios at constant osmotic potentials in the root media that significantly reduce the vegetative growth of corn.

Materials and methods

Seeds of *Zea mays* L. cvs Pioneer 3906 and DeKalb XL-75 were soaked for 30 h in continuously aerated solutions of 0.5 mol m^{-3} $CaSO_4$ then spread on paper and germinated in the dark. Three-day-old seedlings were placed on cheesecloth supported between two plastic grids with 1.7 cm^2 openings. The seedlings, separated by the grid partitions, were covered with moist vermiculite. The grid assemblies were transferred to the glasshouse and supported over plastic pots containing 28 dm^3 of nutrient solution. The composition of the nutrient solution in mol m^{-3} was: 2.5 $Ca(NO_3)_2$, 3 KNO_3, 1.5 $MgSO_4$, 0.17 KH_2PO_4, 0.05 Fe (as sodium ferric diethylenetriamine pentaacetate), 0.023 H_3BO_3, 0.005 $MnSO_4$, 0.0004 $ZnSO_4$, 0.0002 $CuSO_4$, and 0.0001 H_2MoO_4.

Plants were thinned to 30 seedlings per pot. Seven days after germination the cultures were salinized at a rate calculated to reduce the osmotic potential (OP) of the solutions by 0.1 MPa day^{-1}. Treatments included a non-saline control and six saline treatments (OP $= -0.4$ MPa) with Na^+/Ca^{2+} molar ratios ranging from 0.25 to 34.6. Each treatment was replicated three times. The pH of the solution was maintained between 5.2 and 6.2 with KOH and H_2SO_4. All culture solutions were continuously aerated and were changed four times during the experimental period. The experiment was conducted in January–February 1985 when the average daily maximum and minimum temperatures in the glasshouse were 30 °C and 18 °C, respectively. The mean relative humidity was 35% during the day and 53% at night.

Twenty-six days after germination, the shoots were harvested and examined for injury and symptoms of nutrient stress. The shoots were then weighed, separated into immature blades, mature blades and sheaths (including developing culms), and dried in a forced-air oven at 75–80 °C. The tissues were reweighed, ground in a blender, and stored in glass vials.

The Na, K, Ca and Mg concentrations in the blade tissue were determined by atomic absorption spectral analysis of nitric-perchloric acid digests. Phosphorus was analysed by the molybdovanadate colorometric method (Kitson & Mellon, 1944), and chloride by the coulometric-amperometric titration procedure (Cotlove, 1963).

Results

Visual symptoms

Plants salinized with NaCl only (Na^+/Ca^{2+} molar ratio $= 34.6$) exhibited the characteristic 'bull-whip' Ca^{2+} deficiency symptom (Kawasaki & Moritsugu, 1979); 98% of the DeKalb shoots and 46% of the Pioneer shoots had severely distorted leaves. A close-up view of this symptom is illustrated in Fig. 1. This symptom invariably appeared on the fourth and succeeding leaves. Leaf serrations were also observed but were not as prominent, as Kawasaki & Moritsugu (1979) found on corn plants grown at 1.0 mol m^{-3} Ca^{2+} or less. No injury symptoms were evident in any other treatment, indicating that 12.5 mol m^{-3} Ca^{2+} ($Na^+/Ca^{2+} = 5.7$; SAR $= 19$) was sufficient to overcome the apparent Na^+-induced Ca^{2+} deficiency. Figure 2 illustrates the significant difference in plant response to -0.4 MPa OP when salinized solely with NaCl or with a mixture of NaCl and $CaCl_2$, each contributing -0.2 MPa.

Plant growth

The effect of the Na^+-induced Ca^{2+} deficiency was also apparent in the shoot weights (Table 1). The dry weight of blades was significantly less for plants stressed with NaCl than for those stressed with mixed NaCl and $CaCl_2$. As indicated by the extent of the deficiency symptoms, the blade weight of DeKalb was reduced much more than Pioneer. Although all sheath weights were reduced by salt stress, there were no significant differences among the various Na^+/Ca^{2+} ratios for either cultivar.

Mineral composition

Figure 3 shows the Ca^{2+} concentration in the mature and immature blades and sheaths of both cultivars as a function of the Na^+/Ca^{2+} ratio in the root medium. Ca^{2+} concentrations in all three shoot parts were significantly decreased by salinization with 86.5 mol m^{-3} NaCl, but the concentration in the immature blades was the most seriously affected. The addition of 10 mol m^{-3} Ca^{2+} ($Na^+/Ca^{2+} = 5.7$) increased the Ca^{2+} concentration of the immature blade up to that of the control and increased that of the mature blade and sheath above the control. Ca^{2+} concentrations in all three tissues continued to increase with further decreases in the Na^+/Ca^{2+} ratio, but they remained two to three times higher in the sheaths and mature blades than in the immature blades.

Sodium ion (Na^+) concentrations decreased in all three tissues with decreasing Na^+/Ca^{2+} ratios, but the largest decrease occurred with the first addition

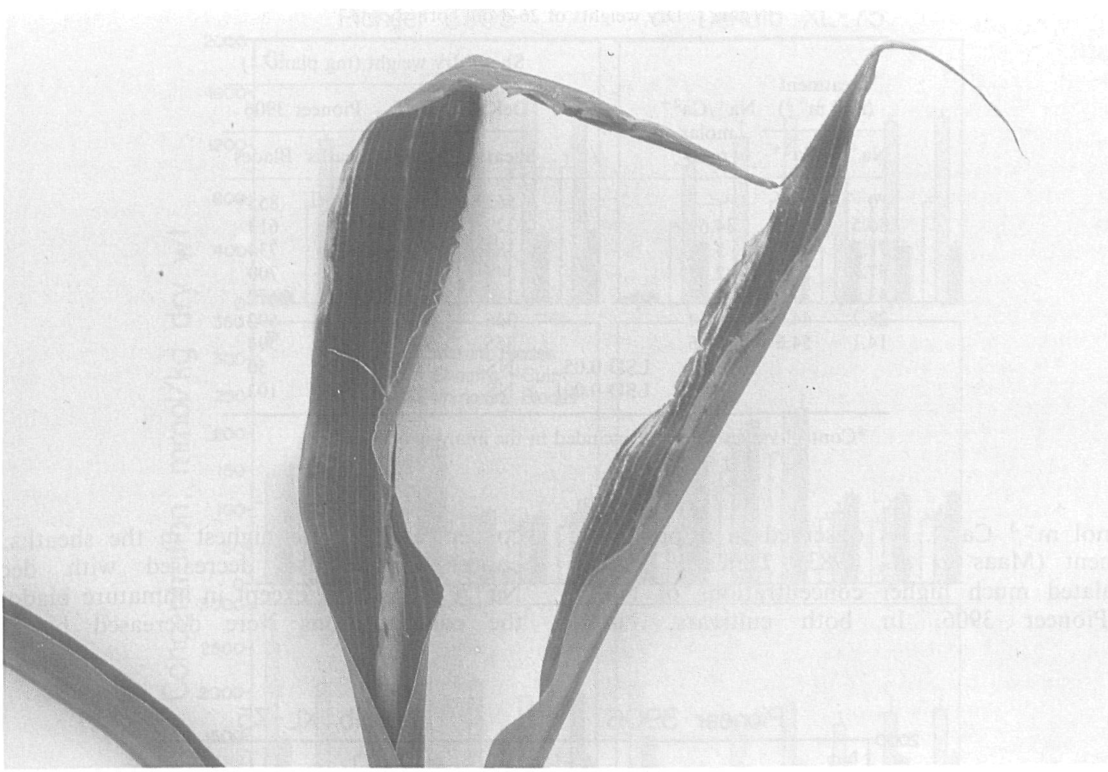

Figure 1. Calcium (Ca^{2+}) deficiency symptoms on a DeKalb XL-75 corn plant grown in complete nutrient solution salinized with 86.5 mol m^{-3} NaCl.

Figure 2. Effect of iso-osmotic solutions (OP = −0.4 MPa) of 86.5 mol m^{-3} NaCl and mixed 71.3 mol m^{-3} NaCl + 10.1 mol m^{-3} $CaCl_2$ on 26-d-old corn plants. Labels on salinized pots indicate concentrations of added salts. The nutrient solution in all pots contained 2.5 mol m^{-3} $Ca(NO_3)_2$.

Table 1. Dry weights of 26-d-old corn shoots

Treatment ($mol\ m^{-3}$) Na^+	Ca^{2+}	Na^+/Ca^{2+} molar ratio		Shoot dry weight ($mg\ plant^{-1}$) DeKalb XL-75 Sheaths	DeKalb XL-75 Blades	Pioneer 3906 Sheaths	Pioneer 3906 Blades
0	2.5	—		562*	980*	571*	855*
86.5	2.5	34.6		329	379	359	613
71.3	12.5	5.7		384	657	377	734
57.1	22.2	2.57		348	551	383	700
43.1	34.0	1.27		344	573	394	679
28.2	44.0	0.64		346	622	399	692
14.1	54.6	0.26		355	602	365	594
			LSD 0.05	NS	72	NS	50
			LSD 0.001	NS	148	NS	103

*Control values were not included in the analysis of variance.

of 10 mol m^{-3} Ca^{2+}. As observed in a previous experiment (Maas *et al.*, 1983), DeKalb XL-75 accumulated much higher concentrations of Na^+ than Pioneer 3906. In both cultivars, Na^+ concentrations were highest in the sheaths. Mg^{2+} concentrations also decreased with decreasing Na^+/Ca^{2+} ratios, except in immature blades where the concentrations were decreased by the salt

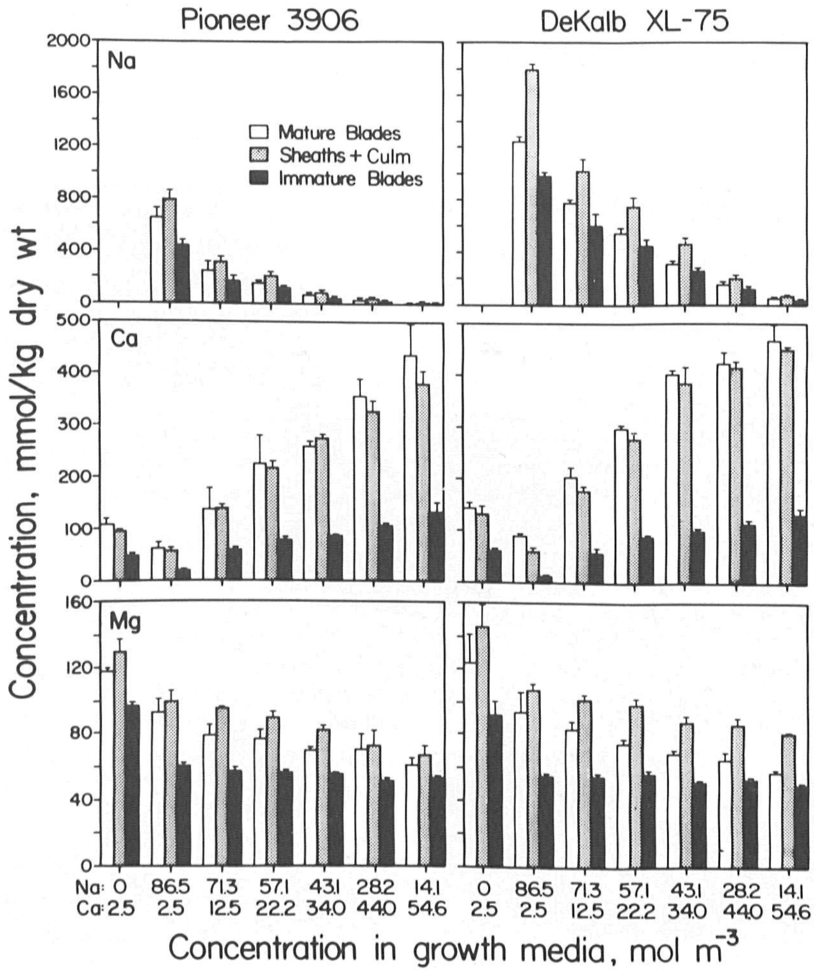

Figure 3. Sodium, calcium, and magnesium concentrations in shoot tissue of 26-d-old corn plants as a function of Na^+/Ca^{2+} ratios in the growth media. Values are the means of three replications; error bars = SD.

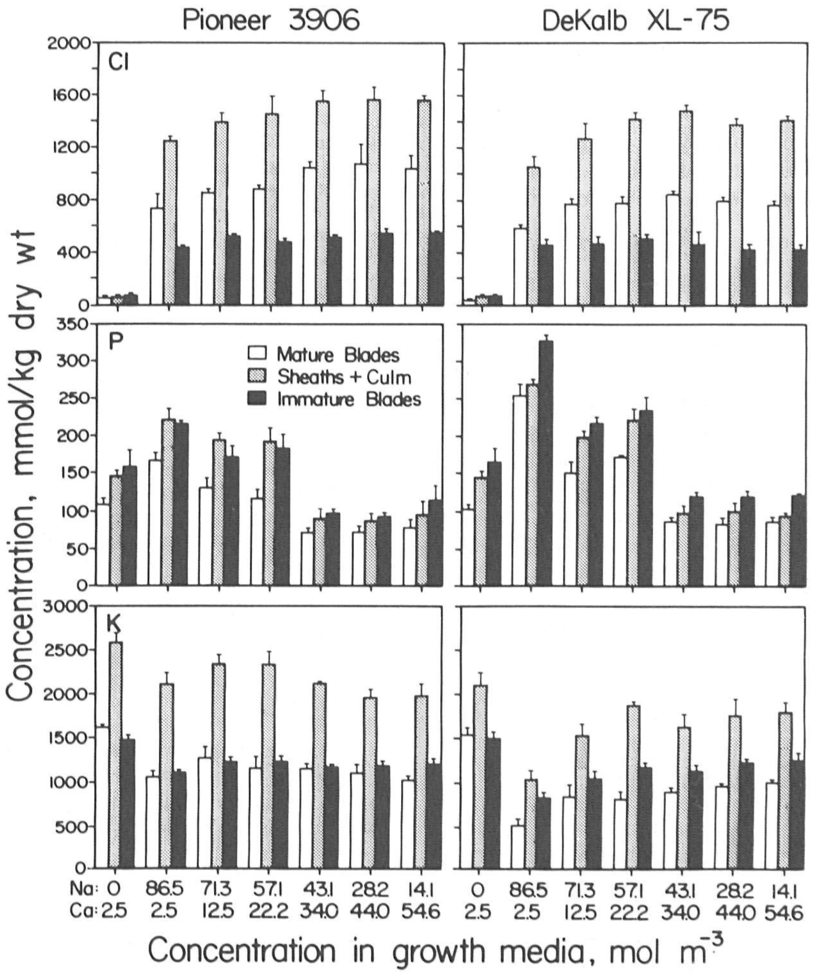

Figure 4. Potassium, phosphorus, and chloride concentrations in shoot tissue of 26-d-old corn plants as a function of Na^+/Ca^{2+} ratios in the growth media. Values are the means of three replications; error bars = SD.

treatment but remained fairly constant at all ratios.

The effect of the Na^+/Ca^{2+} ratio on K^+, P, and Cl^- concentrations are given in Fig. 4. Potassium concentrations in the shoot tissues decreased when plants were salinized; however, in DeKalb XL-75 the decrease was much greater when they were salinized only with NaCl. Although K^+ concentrations were similar for both cultivars under control conditions, Pioneer 3906 accumulated higher K^+ concentrations than DeKalb XL-75 when the plants were salinized. The sheaths accumulated significantly higher K^+ concentrations than the blades. Phosphate concentrations increased markedly in all tissues in the NaCl treatment and then generally decreased with decreasing Na^+/Ca^{2+} ratios. Chloride was readily accumulated by the corn shoots when salinized and its concentration in the tissue generally increased as the solution Na^+/Ca^{2+} ratio increased to about 1. In both cultivars the sheaths accumulated the highest Cl^- concentrations; the immature blades accumulated the lowest.

Discussion

The results of this study in which the Na^+/Ca^{2+} ratio was varied over a wide range demonstrate that the Ca^{2+} levels commonly used in, and which are adequate for, non-saline nutrient cultures are insufficient for corn when salinized with NaCl alone. Two different cultivars of corn grew normally in modified, half-strength Hoagland's solution ($Ca^{2+} = 2.5$ mol m^{-3}) but became severely Ca^{2+}-deficient when salinized with NaCl at -0.4 MPa OP. This deficiency was eliminated at a Na^+/Ca^{2+} molar ratio of 5.7. Chemical analyses of the young and old blades and the sheaths revealed extremely low Ca^{2+} concentrations in the young developing leaves that exhibited Ca^{2+} deficiency symptoms. Since the symptoms did not appear on the first three leaves, it is possible that the seed provided ample Ca^{2+} at this stage of growth. On the other hand, Ca^{2+} may be accumulated preferentially by the sheaths and older leaves at the expense of the young leaves as suggested by Loneragan & Snowball (1969). DeKalb XL-75 appeared less efficient than Pioneer 3906 at accumulating sufficient Ca^{2+} for normal development of the young leaves. As shown previously by Bernstein (1964) and Kawasaki & Moritsugu (1979), corn is highly susceptible to Ca^{2+} deficiency. We have found that sorghum, barley, and wheat are also susceptible to Na^+-induced Ca^{2+} deficiency (Grieve & Maas, unpublished observations).

Yeo & Flowers (1984) suggested that NaCl salinization of rice is a simple treatment which does not introduce more complex nutrient deficiency problems. However, our results indicate that for several gramineous species, including some rice cultivars (Grieve & Fujiyama, 1987), the effects of salinization with NaCl alone may also be complex considering its effect on Ca^{2+} nutrition. The resulting high Na^+/Ca^{2+} ratio impairs the selective permeability of membranes, causes nutritional imbalance and induces serious Ca^{2+} deficiencies. Although not all plant species appear to be as susceptible to Ca^{2+} deficiency as the gramineous species mentioned above, many others, including the salt-tolerant cotton (Kent & Laüchli, 1985), are likely to experience changes in K^+/Na^+ selectivity and membrane integrity at high Na^+/Ca^{2+} ratios. Cramer, Läuchli & Polito (1985) recently obtained evidence that Na^+ displaced Ca^{2+} from membranes of cotton root hairs. Both the loss of Ca^{2+} and the resultant increase in membrane permeability were mitigated upon the addition of 10 mol m^{-3} Ca^{2+}. The protective effect of Ca^{2+} against Na^+ injury of carrot cell membranes has also been documented (Nieman & Willis, 1971).

Although the essentiality of Ca^{2+} for preserving structural and functional integrity of plant membranes is widely recognized (Hanson, 1984), the importance of maintaining an adequate Ca^{2+}/Na^+ ratio in saline treatments is often overlooked. A multitude of papers have appeared recently (see Francois & Maas, 1985) that report plant responses to NaCl without acknowledging that NaCl causes unique nutritional effects that are not apparent in saline soils. Perhaps these and other recent results (Kent & Läuchli, 1985; Lynch & Läuchli, 1985; Cramer *et al.*, 1985) will reinforce the admonition of Greenway & Munns (1980) that salinization with NaCl may cause effects that are confounded by concurrent increases in the Na^+/Ca^{2+} ratio.

References

Ayers, R.S. & Westcot, D.W. (1985) Water quality for agriculture. *FAO Irrigation and Drainage Paper 29 (Rev 1)*. Food and Agriculture Organization of the United Nations, Rome, Italy, 174 pp.

Bernstein, L. (1964) Effects of salinity on mineral composition and growth of plants. *Plant Analysis and Fertilizer Problems*, **4**, 25–45.

Bernstein, L. (1975) Effects of salinity and sodicity on plant growth. *Annual Review of Phytopathology*, **13**, 295–312.

Cotlove, E. (1963) Determination of true chloride content of biological fluids and tissues. II. Analysis by simple nonisotopic methods. *Analytical Chemistry*, **35**, 101–105.

Cramer, G.R., Läuchli, A. & Polito, V.S. (1985) Displacement of Ca^{2+} by Na^+ from the plasmalemma of root cells. *Plant Physiology*, **79**, 207–211.

François, L.E. & Maas, E.V. (1978) Plant responses to salinity: an indexed bibliography. USDA-ARS, *Agricultural Reviews and Manuals*, W-6.

François, L.E. & Maas, E.V. (1985) Plant responses to salinity: a supplement to an indexed bibliography. USDA, *Agricultural Research Service*, ARS-24.

Greenway, H. & Munns, R. (1980) Mechanisms of salt tolerance in non-halophytes. *Annual Review of Plant Physiology*, **31**, 149–190.

Grieve, C.M. & Fujiyama, H. (1987) The response of two rice cultivars to external Na/Ca ratio. *Plant and Soil*, (in press).

Hanson, J.B. (1984) The functions of calcium in plant nutrition. In *Advances in Plant Nutrition*, (eds P. B. Tinker and A. Läuchli). Vol. 1, pp. 159–208. Praeger Publishers, New York.

Hyder, S.Z. & Greenway, H. (1965) Effects of Ca on plant sensitivity to high NaCl concentrations. *Plant and Soil* **23**, 258–260.

Imanul Huq, S.M. & Larher, F. (1984) Osmoregulation in higher plants: effect of maintaining a constant Na:Ca ratio on the growth, ion balance and organic solute status of NaCl stressed cowpea (*Vigna sinensis* L.). *Zeitschrift für Pflanzenphysiologie*, **113**, 163–176.

Kawasaki, T. & Moritsugu, M. (1979) A characteristic symptom of calcium deficiency in maize and sorghum. *Communications in Soil Science and Analysis*, **10**, 41–56.

Kent, L.M. & Läuchli, A. (1985) Germination and seedling growth of cotton: salinity-calcium interactions. *Plant, Cell and Environment*, **8**, 155–159.

Kitson, R.E. & Mellon, M.G. (1944) Colorimetric determination of phosphorus as molybdovanado-phosphoric acid. *Industrial and Engineering Chemistry, Analytical Edition*, **16**, 379–383.

Lagerwerff, J.V. & Holland, J.P. (1960) Growth and mineral content of carrots and beans as related to varying osmotic and ionic-composition effects in saline-sodic sand cultures. *Agronomy Journal*, **52**, 603–608.

LaHaye, P.A. & Epstein, E. (1971) Calcium and salt toleration by bean plants. *Physiologia Plantarum*, **25**, 213–218.

Loneragan, J.F. & Snowball, K. (1969) Calcium requirements of plants. *Australian Journal of Agricultural Research*, **20**, 465–478.

Lynch, J. & Läuchli, A. (1985) Salt stress disturbs the calcium nutrition of barley (*Hordeum vulgare* L.). *New Phytologist*, **99**, 345–354.

Maas, E.V., Hoffman, G.J., Chaba, G.D., Poss, J.A. & Shannon, M.C. (1983) Salt sensitivity of corn at various growth stages. *Irrigation Science*, **4**, 45–57.

Nassery, H., Ogata, G. & Maas, E.V. (1979) Sensitivity of sesame to various salts. *Agronomy Journal*, **71**, 595–597.

Nieman, R.H. & Willis, C. (1971) Correlation between the suppression of glucose and phosphate uptake and the release of protein from viable carrot root cells treated with monovalent cations. *Plant Physiology*, **48**, 287–293.

de Sigmond, A.A.J. (1927) The classification of alkali and salty soils. *Proceedings of the First International Congress of Soil Science*, **1**, 330–334.

United States Salinity Laboratory Staff (1954) Diagnosis and improvement of saline and alkali soils. *U.S. Department of Agriculture*, Handbook 60. 160 pp.

Yeo, A.R. & Flowers, T.J. (1984) Mechanisms of salinity resistance in rice and their role as physiological criteria in plant breeding. In *Salinity Tolerance in Plants: Strategies from Crop Tolerance*, (eds R. C. Staples and G. H. Toenniessin), pp. 151–170. Wiley, New York.

Yeo, A.R. & Flowers, T.J. (1985) The absence of an effect of the Na/Ca ratio on sodium chloride uptake by rice (*Oryza sativa* L.). *New Phytologist*, **99**, 81–90.