

## BRIEF COMMUNICATION

**Alleviation of the adverse effects of NaCl on germination of maize grains by calcium**

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The lengths of roots and shoots, fresh and dry matter yield, and the contents of insoluble saccharides and free amino acids were reduced with the rise in NaCl concentration. However, under combination of NaCl with  $\text{Ca}^{2+}$  ions, these parameters generally raised. Contents of soluble saccharides, proline and quaternary ammonium compounds increased with increasing NaCl concentration, but under addition of  $\text{CaCl}_2$  or  $\text{CaSO}_4$ , contents of these compounds were decreased. Low concentrations of NaCl stimulated soluble proteins production, but higher concentrations decreased the content of soluble proteins. Addition of  $\text{Ca}^{2+}$  in the media did not improve the soluble protein production. Insoluble proteins content was increased with the rise of salinity level, but these effects were more pronounced with NaCl and  $\text{CaCl}_2$  or  $\text{CaSO}_4$  than with NaCl only.

Soil salinity is a worldwide problem hampering productivity of several agricultural crops. Many attempts have been made to alleviate the effect of sodium chloride on plant cells. Some of these studies concentrated on the effect of added  $\text{Ca}^{2+}$  in counteracting the inhibitory effect of NaCl salinization on plants (e.g. Hyder and Greenway 1965, Chaudri and Wiebe 1968, Leopold and Willing 1984).

The present investigation was undertaken to study the interactive effect of NaCl salinity and  $\text{Ca}^{2+}$  applied as  $\text{CaCl}_2$  or  $\text{CaSO}_4$  on germination, growth and some metabolic pools of maize plants.

The germination experiments were performed as described by Maftoun and Sepaskhah (1978). Seeds of maize (*Zea mays* L.) were placed on absorbant pads in Petri-dishes to which 25 cm<sup>3</sup> of the nutrient solution with NaCl of different concentration (0.0 (control), 50, 100, 150, 200 mM) and with  $\text{CaCl}_2$  or  $\text{CaSO}_4$  (5mM) solution was added. Seeds were considered to be germinated after the radicle emerged from the testa. After 10 d of germination the length of the roots and of

shoots were measured. The fresh shoots and roots were then dried in an aerated oven at 70 °C during which successive weighing was carried out until a constant dry mass was reached. The amount of saccharides was determined with the anthrone sulphuric acid method (Fales 1951), free amino acids were determined according to Moore and Stein (1948), free proline according to Bates *et al.* (1973), amount of proteins according to Lowry *et al.* (1951), quaternary ammonium compounds (QACs) according to Story and Wyn Jones (1977).

The length of roots and shoots as well as fresh and dry matter, remained more or less unaffected at the 50 mM NaCl, but above this level they decreased sharply (Table 1). The growth of maize was markedly inhibited with the rise of NaCl level. The water content remained more or less unchanged up to the highest salinity level used. However, when the test plants were subjected to salinization and  $\text{Ca}^{2+}$ , length of roots and shoots and fresh and dry matter were higher in comparison with those of plants treated only with the NaCl, especially at the high levels of salinity. The effect of  $\text{Ca}^{2+}$  in promoting salinity tolerance is widely recognized (LaHaye and Epstein 1971, Leopold and Willing 1984 and Kent and L  uchli 1985) and may be related to the essential role of calcium for membrane integrity.

Table 1. Effect of NaCl and  $\text{Ca}^{2+}$  as [ $\text{CaCl}_2$  or  $\text{CaSO}_4$ ] on fresh, dry matter [ $\text{g plant}^{-1}$ ], water content [ $\text{g g}^{-1}(\text{d.m.})$ ] and length of roots and shoots [ $\text{cm plant}^{-1}$ ] of maize seedlings.

| $\text{Ca}^{2+}$          | NaCl [mM] | Fresh mass<br>[ $\text{g plant}^{-1}$ ] | Dry mass<br>[ $\text{g plant}^{-1}$ ] | Water content<br>[ $\text{g g}^{-1}(\text{d.m.})$ ] | Shoot length<br>[ $\text{cm plant}^{-1}$ ] | Root length<br>[ $\text{cm plant}^{-1}$ ] |
|---------------------------|-----------|---|---------------------------------------|---|--|---|
| Control                   | 0         | 0.374 b                                 | 0.049 a                               | 6.659 a   | 4.8 a                                      | 9.5 a                                     |
|                           | 50        | 0.402 Aa                                | 0.049 a                               | 7.217 Aa  | 4.8 Aa                                     | 9.8 a                                     |
|                           | 100       | 0.245 Ac                                | 0.033 Ab                              | 6.472 Aa  | 4.0 Aa                                     | 4.5 Ab                                    |
|                           | 150       | 0.183 Ad                                | 0.025 Ac                              | 6.320 Aa  | 3.5 Aa                                     | 3.3 Abc                                   |
|                           | 200       | 0.160 Ae                                | 0.021 c                               | 6.632 Aa  | 2.8 Aa                                     | 2.0 c                                     |
| $\text{CaCl}_2$<br>[5 mM] | 50        | 0.338 Bb                                | 0.045 a                               | 6.542 Aa  | 5.0 Aa                                     | 9.0 a                                     |
|                           | 100       | 0.260 Bc                                | 0.031 Bb                              | 7.386 Aa  | 4.5 Aa                                     | 6.3 ABb                                   |
|                           | 150       | 0.215 Bd                                | 0.027 Bb                              | 7.000 Aa  | 3.8 Aa                                     | 3.8 Bc                                    |
|                           | 200       | 0.183 Be                                | 0.024 b                               | 6.822 Aa  | 3.2 Aa                                     | 3.3 c                                     |
| $\text{CaSO}_4$<br>[5 mM] | 50        | 0.394 Cb                                | 0.053 a                               | 6.457 Aa  | 5.0 Aa                                     | 10.5 a                                    |
|                           | 100       | 0.403 Bb                                | 0.045 Ba                              | 7.959 Aa  | 5.5 Aa                                     | 7.0 Bb                                    |
|                           | 150       | 0.420 Cc                                | 0.048 Ba                              | 7.889 Aa  | 4.5 Aa                                     | 6.0 Bb                                    |
|                           | 200       | 0.255 Cd                                | 0.032 b                               | 6.990 Aa  | 3.3 Aa                                     | 2.5 c                                     |

Means which are not significantly different are followed by the same letter ( $P = 0.05$ ).

Salinity induced a considerable increase in the content of soluble saccharides. The insoluble saccharides accumulation was stimulated at low NaCl concentration while it was reduced at higher NaCl concentration. The addition of  $\text{Ca}^{2+}$  decrease the accumulation of soluble saccharides and alleviate the depression of insoluble saccharides (Table 2).

Salinity induced a decrease in the soluble proteins content and inclusion of  $\text{Ca}^{2+}$  in the media did not improve soluble protein production. On the other hand,

insoluble proteins were increased with the rise of the salinization level, but these effects were more pronounced by addition of  $\text{Ca}^{2+}$ . These variable changes add more support to the results obtained by (Gates *et al.* 1966, Wilson *et al.* 1970, El-Shourbagy and Missak 1975 and Heikal 1976).

There is a general increasing trend in the concentration of proline in seedlings of experimental plants with increasing salinity levels (Table 2). The pattern of changes in the total free amino acids of the seedlings was opposite to that obtained for changes in proline (Table 2). The opposite trend was exhibited with respect to the production of the other free amino acids by addition of 5 mM  $\text{CaCl}_2$  or  $\text{CaSO}_4$  to the different salinity levels.

Table 2. Effect of NaCl and  $\text{Ca}^{2+}$  on the content of soluble and insoluble saccharides, soluble and insoluble proteins, proline, other free amino acids and quaternary ammonium compounds [ $\text{mg g}^{-1}(\text{d.m.})$ ] of maize seedlings.

| $\text{Ca}^{2+}$          | NaCl<br>[mM] | Saccharides |            | Proteins  |           | Proline  | Amino<br>acids | QACs    |
|---------------------------|--------------|-------------|------------|-----------|-----------|----------|----------------|---------|
|                           |              | soluble     | insoluble  | soluble   | insoluble |          |                |         |
| Control                   | 0            | 59.54 a     | 266.04 a   | 89.39 a   | 53.03 a   | 4.27 a   | 6.50 a         | 2.17 a  |
|                           | 50           | 74.42 Ab    | 367.44 Ab  | 98.49 Ab  | 43.93 Ab  | 7.82 Ab  | 8.33 ab        | 2.89 Aa |
|                           | 100          | 81.16 c     | 314.19 Ac  | 93.94 Ac  | 39.39 Ac  | 9.73 Ab  | 7.33 ab        | 2.39 Aa |
|                           | 150          | 91.86 Ad    | 225.35 d   | 77.27 Ad  | 80.31 Ac  | 10.46 Ac | 7.00 b         | 2.44 Aa |
|                           | 200          | 93.02 Ae    | 213.96 Ad  | 75.76 Ad  | 96.97 Ac  | 12.36 Ad | 4.08 BC        | 2.11 Aa |
| $\text{CaCl}_2$<br>[5 mM] | 50           | 92.33 Bb    | 303.02 Bb  | 84.24 ABa | 218.79 Bb | 18.18 Bb | 7.67           | 2.86 Aa |
|                           | 100          | 78.14 b     | 247.44 Abc | 83.33 Ba  | 189.40 Bc | 12.91 Bc | 7.67           | 2.33 Aa |
|                           | 150          | 74.72 Bb    | 239.53 bc  | 69.70 Bb  | 160.60 Bc | 8.73 Bd  | 7.50           | 2.13 Aa |
|                           | 200          | 70.93 Bc    | 242.56 Bc  | 65.15 Ab  | 146.97 Bd | 6.36 Be  | 8.50 A         | 1.83 Aa |
| $\text{CaSO}_4$<br>[5 mM] | 50           | 76.28 Ba    | 335.35 Ca  | 90.91 Bab | 103.03 Ca | 11.46 Cb | 5.92           | 2.83 Aa |
|                           | 100          | 78.49 a     | 328.49 Ba  | 86.36 Bb  | 104.55 Ca | 6.64 Cb  | 5.33           | 2.32 Aa |
|                           | 150          | 80.00 Ba    | 313.72 a   | 84.85 Cc  | 103.03 Cb | 5.55 Cc  | 6.83           | 2.16 Aa |
|                           | 200          | 77.44 Bb    | 314.42 Cb  | 78.79 Bd  | 90.91 Bc  | 3.27 Cc  | 7.33 A         | 2.06 Aa |

Means which are not significantly different are followed by the same letter ( $P = 0.05$ ).

Quaternary ammonium compounds (QACs) were increased at low salinization levels, while at high salinization levels they were decreased (Table 2). Addition of  $\text{CaCl}_2$  or  $\text{CaSO}_4$  had no effect on the concentration of QACs.

Seedling growth of maize subjected to lower and moderate salinizations were found to be associated with more or less unchanged values of water content. This behaviour of the water content was found to be linked with a pronounced accumulation of soluble saccharides, amino acids, proline and quaternary ammonium compounds (QACs) of maize plants, which might play an important role in increasing the internal osmotic pressure. This conclusion is in accordance with Hellebust (1976) and Flower *et al.* (1977), working with some halotolerant plants, and Munns *et al.* (1979) and Drossopoulos *et al.* (1987), with glycophyte plants. The addition of  $\text{Ca}^{2+}$  reduced accumulation of soluble saccharides, QACs and proline contents, which is in agreement with the results obtained by Imamul-Huq and Larher

(1984). The addition of  $\text{Ca}^{2+}$  seems to counteract the toxic effect of  $\text{Na}^+$ . Accumulation and transport of the low molecular mass organic compounds considered to be either compatible solutes or stress markers (Hsiao 1973, Stewart and Larher 1980, Imamul-Huq and Larher 1983) were also modified by the presence of  $\text{Ca}^{2+}$ .

## References

- Bates, L.S., Waldren, R.P., Teare, I.D.: Rapid determination of free proline for water stress studies. - *Plant Soil* **39**: 205-207, 1973.
- Chaudri, I.I., Wiebe, H.H.: Influence of calcium pretreatment on wheat germination in saline media. - *Plant Soil* **27**: 208-216, 1968.
- Drossopoulos, J.B., Karamanos, A.J., Niavis, C.A.: Changes in ethanol soluble saccharides during the development of two wheat cultivars subjected to different degrees of water stress. - *Ann. Bot.* **59**: 173-180, 1987.
- El-Shourbagy, M.N., Missak, N.L.: Effect of growing season and salinity on growth, mineral composition and seed-lipid characteristics of some *Ricinus communis* L. varieties. - *Flora* **164**: 51-71, 1975.
- Epstein, E., Kingsbury, R.W., Norlyn, J.D., Rush, D.W.: Production of food crops and other biomass by sea-water culture - In: Hollaender, A. (ed.): *The Biosaline Concept: an Approach to Utilization of Underexploited Resources*. Pp. 77-99. Plenum Press, New York 1979.
- Fales, F.W.: The assimilation and degradation of saccharides by yeast cells. - *J. biol. Chem.* **193**: 113-124, 1951.
- Flowers, T.J., Troke, P.P., Yeo, A.R.: The mechanism of salt tolerance in halophytes. - *Annu. Rev. Plant Physiol.* **28**: 89-93, 1977.
- Gates, C.T., Haydock, K.P., Claringbold, P.J.: Response to salinity in *Glycine*. Differences in cultivars of *Glycine javanica* in dry weight, nitrogen and water content. - *Aust. J. exp. Agr. anim. Husb.* **6**: 374-379, 1966.
- Heikal, M.M.: Physiological studies on salinity. 5 - Effect of salinity on photosynthetic pigments and nitrogen content and on growth of wheat and radish plants. - *Bull. Sci. Assiut Univ.* **5**: 243-256, 1976.
- Hellebust, J.A.: Osmoregulation. - *Annu. Rev. Plant Physiol.* **27**: 485-505, 1976.
- Hsiao, T.C.: Plant response to water stress. - *Annu. Rev. Plant Physiol.* **24**: 519-570, 1973.
- Hyder, S.Z., Greenway, H.: Effect of  $\text{Ca}^{2+}$  on plant sensitivity to high NaCl concentrations. - *Plant Soil* **23**: 258-260, 1965.
- Imamul-Huq, S.M., Larher, F.: Osmoregulation in higher plants: Effect of NaCl salinity on non-nodulated *Phaseolus aureus* L. II. Changes in organic solute. - *New Phytol.* **93**: 209-216, 1983.
- Imamul-Huq, S.M., Larher, F.: Osmoregulation in higher plants: Effects of maintaining a constant Na:Ca ratio on the growth, ion balance and organic solute status of NaCl stressed cowpea (*Vigna sinensis* L.). - *Z. Pflanzenphysiol.* **113**: 163-176, 1984.
- Kent, L., Läuchli, A.: Germination and seedling growth of cotton: salinity-calcium interactions. - *Plant Cell Environ.* **8**: 155-159, 1985.
- LaHaye, P.A., Epstein, E.: Calcium and salt toleration by bean plants. - *Physiol. Plant.* **25**: 213-218, 1971.
- Leopold, A.C., Willing, R.P.: Evidence for toxicity effects of salt on membranes. - In: Staples, R.C., Toennissen G.H. (ed.): *Salinity Tolerance in Plants, Strategies for Crop Improvement*. Pp. 67-91. John Wiley & Sons, New York - Chichester - Brisbane - Toronto - Singapore 1984.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the folin phenol reagent. - *J. biol. Chem.* **193**: 291-297, 1951.

- Moore, S., Stein, W.: Photometric Ninhydrin Method for Use in the Chromatography of Amino Acids. - Laboratories of the Rockefeller Institute for Medical Research, New York 1948.
- Munns, R., Brady, C.I., Barlow, E.W.R.: Solutes accumulation in the apex and leaves of wheat during water stress. - Aust. J. Plant Physiol. 6: 379-389, 1979.
- Stewart, G.R., Larher, F.: Accumulation of amino acids and related compounds in relation to environmental stress. - In: Stumpf, P.K., Conn, E.E. (ed.): The Biochemistry of Plants. Vol. 5. Amino Acids and Derivatives. Pp. 609-635. Academic Press, London 1980.
- Storey, R., Wyn Jones, R.G.: Quaternary ammonium compounds in plants in relation to salt resistance. - Phytochemistry 14: 447-453, 1977.
- Wilson, J.R., Haydock, K.P., Robins, M.F.: Response to salinity in *Glycine*. 5: Changes in the chemical composition of three Australian species of *G. wightii* (*G. javanica*) over a range of salinity stresses. - Aust. J. exp. Agr. anim. Husb. 10: 156-165, 1970.