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NaCl stress in rice seedlings: the influence of calcium on root growth

Chuan Chi Lin and Ching Huei Kao[1]

Department of Agronomy, National Taiwan University, Taipei, Taiwan, Republic of China

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Abstract. We investigated the influence of Ca^{2+} on growth inhibition of rice roots induced by NaCl. NaCl concentrations higher than 50 mM significantly inhibited root growth of rice seedlings, but root growth was improved by the addition of $CaCl_2$; Mg^{2+} and K^+ also counteracted the inhibition of root growth induced by NaCl. Addition of $CaCl_2$, $MgCl_2$, or KCl resulted in decreased Na^+ levels. Calcium chloride did not reverse the inhibition caused by mannitol applied at a concentration iso-osmotic with 150 mM NaCl. Pretreatment of rice seeds with $CaCl_2$ counteracted the inhibition of root growth induced by NaCl. Root growth was found to be more sensitive to NaCl when rice seeds were pretreated with EGTA [ethyleneglycol-bis-(β-aminoethylether)-N,N,N'N'-tetraacetic acid)], a calcium chelator. Calcium chloride and EGTA pretreatments decreased and increased Na^+ levels, respectively. Our results suggest that rice seedlings with high internal Ca^{2+} levels are able to counteract the growth inhibition of roots induced by NaCl via a decrease in the root Na^+ levels.

Keywords: Calcium; *Oryza sativa*; Root growth; Salinity.

Abbreviation: EGTA, ethyleneglycol-bis-(β-aminoethylether)-N,N,N'N'-tetraacetic acid.

Introduction

Salinity decreases plant growth and yield of many crops. Several methods, such as reclamation, drainage, high leaching fractions, and soil amendments, have been used to control salinity. An additional approach to the salinity problem is to utilize the genetic potential of many crop species and select more salt-tolerant crops. Selection and breeding for salt tolerance in a particular crop species would be facilitated if we could identify and understand the mechanisms of salt tolerance and toxicity for that species.

Calcium is important in many physiological processes and is believed to act as a second messenger (Helper and Wayne, 1985). It has been shown in several plant systems that the deleterious influences of salinity on plant growth are partially overcome by the addition of $CaCl_2$ (Ben-Hayyim and Kochba, 1983; Cramer et al., 1985; Cramer et al., 1986; Kent and Lauchli, 1985; LaHaye and Epstein, 1971). The ameliorating influence of calcium on salinity stress was not observed in cotton and rice plants (Leidi et al., 1991; Yeo and Flowers, 1985).

In view of the conflicting evidence, we investigated the influence of calcium on salinity-induced root growth inhibition in rice seedlings. Although Na^+ and Cl^- are not the dominant ions in all saline soils (Epstein and Rains, 1987; Maas and Grieve, 1987), most, if not all, research on salinity uses NaCl as the stress agent. In the present investigation we have found that Ca^{2+} is able to counteract the NaCl-induced inhibition of root growth in rice seedlings.

[1] Corresponding author.

Materials and Methods

Rice (*Oryza sativa* L., cv. Tainung 67) seeds were sterilized with 2.5% sodium hypochlorite for 15 min and washed thoroughly with distilled water. These seeds were then germinated in Petri dishes containing distilled water at 37°C in the dark. After a 1-day incubation, uniformly germinated seeds were selected and then transferred to Petri dishes (9.0 cm) containing two sheets of Whatman No. 1 filter paper moistened with 10 ml of distilled water or test solutions. Each Petri dish contained 20 germinated seeds and each treatment was replicated 4 times. The germinated seeds were grown at 27°C in darkness, and 3 ml of distilled water or test solutions was added to each Petri dish on day 3. Root length was measured after 5 day. Harvested roots were dried at 65°C for 2 days, extracted in 1 N HCl at room temperature (Hunt, 1982) and analyzed for Na^+ with a flame photometer.

Results and Discussion

Figure 1 shows the influence of NaCl on root length of rice seedlings. NaCl concentration higher than 50 mM significantly inhibited root growth of rice seedlings. NaCl at 150 mM reduced root growth of about 70%. These results are generally consistent with previous reports by other investigators (Cramer et al., 1988; Gersani et al., 1993; Kent and Lauchli, 1985; Prakash and Prathapasenam, 1988; Snapp and Shennan, 1992).

The influence of $CaCl_2$ on NaCl-inhibited growth of roots is shown in Figure 2. Root growth was significantly improved by $CaCl_2$ in NaCl treatment. Additional experiments were conducted to see if Mg^{2+} or K^+ can also reduce growth inhibition by NaCl. Magnesium chloride and KCl were substituted for the $CaCl_2$ in these experiments. Root growth in the NaCl medium was found also to be enhanced by the addition of $MgCl_2$ or KCl (Figure 3). Magnesium cloride is even more effective than $CaCl_2$ in counteracting growth inhibition by NaCl. This is an interesting finding, since a hypothesis that KCl application can also reduce the deleterious influences of salinity (Ben-Hayyim et al., 1987) has not been proven because no response (Bar-Tal, 1991; Kent and Lauchli, 1985) or even negative response to potassium nutrition of saline-affected plants (Lauter et al., 1988) was reported. Kent and Lauchli (1985) also demonstrated that the growth of cotton root in saline medium was not enhanced by the addition of $MgSO_4$, Since the experiments were done by adding $CaCl_2$ (or KCl or $MgCl_2$) and NaCl simultaneously, the beneficial influence of Ca^{2+}, K^+ or Mg^{2+} ion on root growth of rice seedlings was most likely due to competition between Ca^{2+}, K^+ or Mg^{2+} and Na^+ leading to a reduced level of internal Na^+. As indicated in Figure 4, this is indeed the case.

The primary. action of salt is thought to take place in roots (Meizner et al., 1991; Munns and Termaat, 1986). The primary influence on root growth is osmosis and/or specific ion effects. To determine whether supplemental Ca^{2+} alleviates the inhibition of root growth by the toxic or the osmotic nature of Na^+, the influence of calcium and man-

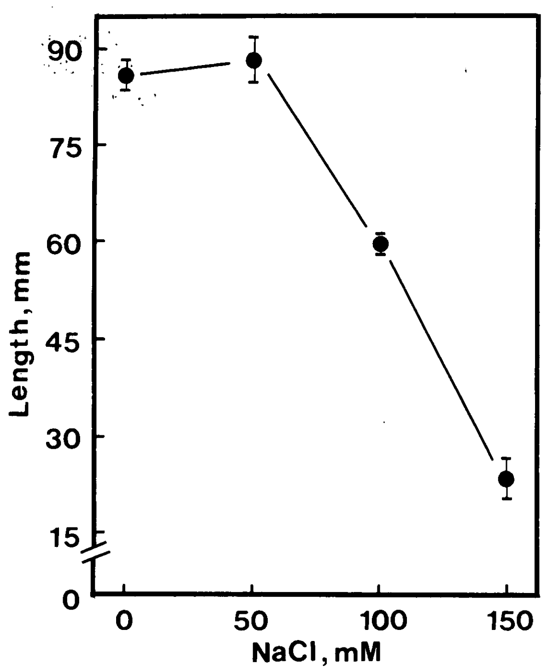

Figure 1. Influence of NaCl on root length of rice seedlings. Bars represent standard errors. Only those standard errors larger than symbol size are shown.

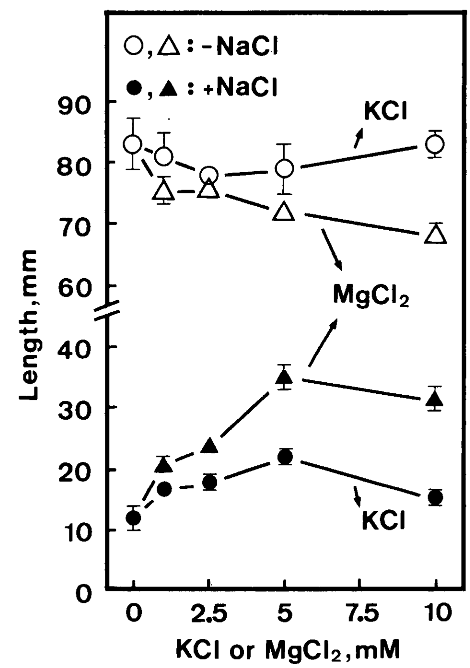

Figure 3. Influence of NaCl, KCl, and $MgCl_2$ on root length of rice seedlings. Bars represent standard errors.

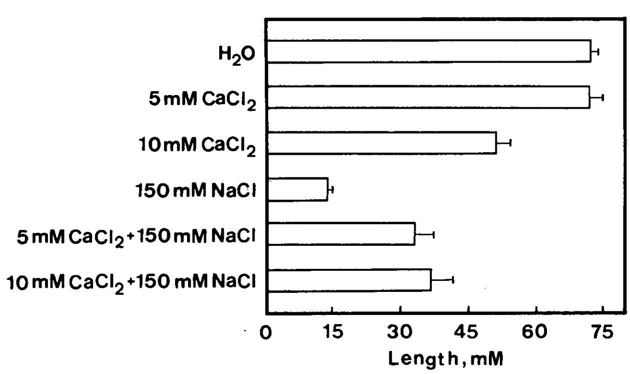

Figure 2. Influence of NaCl and $CaCl_2$ on root length of rice seedlings. Bars represent standard errors.

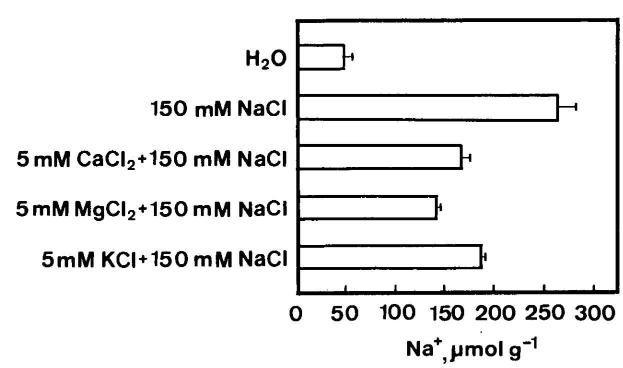

Figure 4. Influence of NaCl, $CaCl_2$, $MgCl_2$, and KCl on Na^+ level in roots of rice seedlings. Bars represent standard errors.

nitol on root growth of rice seedlings was examined. The results are presented in Figure 5. At a concentration iso-osmotic with 150 mM NaCl, mannitol inhibited root growth, but $CaCl_2$ did not reverse the root growth inhibition, suggesting that the ameliorative effect of calcium was not mediated through osmosis but by alleviation of the toxicity of Na^+.

To confirm the beneficial influence of Ca^{2+} on root growth of rice seedlings in NaCl medium, we raised rice seedlings under conditions of Ca^{2+} availability. Rice seeds were soaked in distilled water, $CaCl_2$, or EGTA for 24 h in darkness. Germinated seeds were washed thoroughly with distilled water and then transferred to distilled water or NaCl for 5 days. Seedlings raised in the presence of additional Ca^{2+} and EGTA were expected to contain high and low internal Ca^{2+} levels, respectively, compared with those raised in distilled water. As indicated in Figure 6, root growth in the NaCl medium was enhanced by the pre-treatment with $CaCl_2$. This suggests that rice seedlings with high internal Ca^{2+} are able to counteract the growth inhibition induced by NaCl. If this conclusion is correct, root growth inhibition of seedlings raised in the presence of EGTA would be expected to be more sensitive to NaCl than that of seedlings raised in distilled water. As indicated in Figure 7, this is indeed the case. We tested whether pretreatment with $CaCl_2$ and EGTA resulted in changes of the Na^+ levels. Our results indicated that the pretreatments resulted in decreased and increased Na^+ levels, respectively (Figure 8).

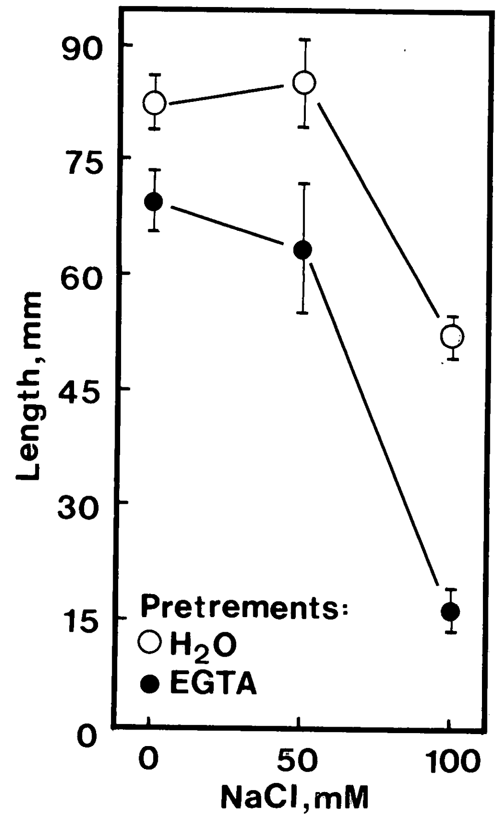

Figure 7. Influence of EGTA pretreatment on root growth of rice seedlings grown in the presence or absence of NaCl. Seeds were presoaked in 10 mM EGTA for 24 h in darkness. Bars represent standard errors.

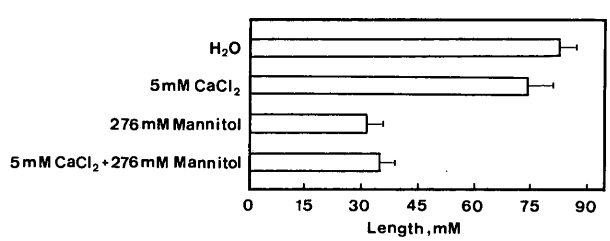

Figure 5. The influence of mannitol and $CaCl_2$ on root length of rice seedlings. Mannitol concentration of 276 mM was iso-osmotic with 150 mM NaCl. Bars represent standard errors.

It has been shown that increased salinity reduced the amount of Ca^{2+} bound to plasma membranes of salinized roots and protoplasts and greatly reduced Ca^{2+} concentration in root apical meristems (Cramer et al., 1985; Lazof and Lauchli, 1991; Lynch et al., 1987). Future study should examine the influence of NaCl on the calcium status in roots.

Seedling vigor, particular root growth, is very important for establishing a strong stand, and rapid root growth from highly saline parts of the soil are essential to good stand establishment, and consequently, yield. A potential application of the beneficial influence of Ca^{2+} is the addition of

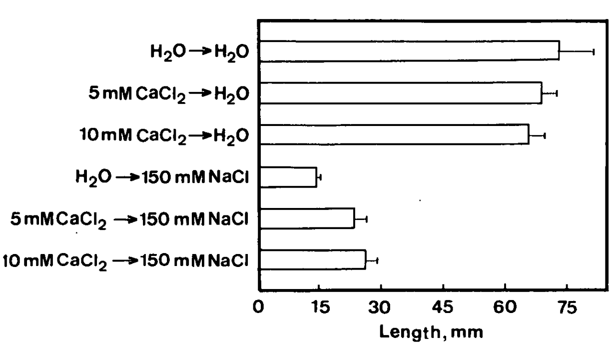

Figure 6. Influence of $CaCl_2$ pretreatment on NaCl-inhibited root growth of rice seedlings. Seeds were presoaked in $CaCl_2$ for 24 h in darkness. Bars represent standard errors.

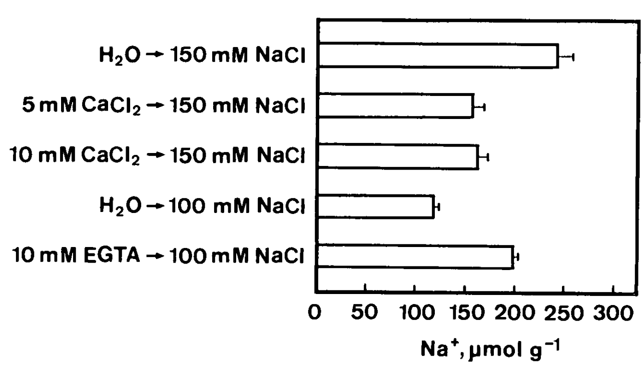

Figure 8. Influence of $CaCl_2$ and EGTA pretreatment on Na^+ level in roots of rice seedlings. Seeds were presoaked in $CaCl_2$ and EGTA for 24 h in darkness. Bars represent standard errors.

$CaCl_2$ or some other calcium salt to saline soils which are not dominated by calcium. The possibility that rice seeds with a high calcium level can be used to select NaCl-tolerant rice plants should also be examined.

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水稻幼苗之氯化鈉逆境：鈣離子對根生長之效應

林傳琦 高景輝

國立台灣大學農藝學系

本研究係以台農 67 號水稻幼苗爲材料，探討鈣離子對氯化鈉所抑制水稻幼苗根生長之影響。氯化鈉濃度高於 50 mM 時，顯著的抑制根的生長。氯化鈣與氯化鈉同時處理可顯著降低氯化鈉所抑制的根生長。鎂離子與鉀離子亦可降低氯化鈉所抑制的根生長。氯化鈣、氯化鎂或氯化鉀與氯化鈉同時處理，均可降低根內鈉離子之含量。種子經氯化鈣前處理後，可降低氯化鈉所抑制的根生長。然而，當種子先以鈣的箝合劑 EGTA [ethyleneglycol-bis-(β -aminoethylether)-N,N,N'N'- tetraacetic acid)] 處理，則根對氯化鈉的敏感度增加。氯化鈣與 EGTA 前處理可分別降低或增加根內鈉離子之含量。我們的結果顯示，水稻幼苗如含有較高的鈣離子濃度，則可經由鈉離子含量的降低，而降低氯化鈉所抑制之根生長。

關鍵詞：鈣；水稻；根生長；鹽害。