

## Protection induced by external $\text{Ca}^{2+}$ application on proline accumulation, ion balance, photosynthetic pigments, protein and ABA concentration of mustard seedlings (*Sinapis alba* L.) under salinity stress

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### ABSTRACT

The effect of external  $\text{Ca}^{2+}$  application of different concentration to salinity-treated (100 mM NaCl) mustard plant (*Sinapis alba* L.) was investigated in relation to ion uptake, proline, chlorophyll a&b, protein and ABA concentrations. Salinity treatment (100 mM NaCl) led to significant decreases in  $\text{Ca}^{2+}$  &  $\text{K}^+$  uptake and Chl. a&b contents, accompanied by significant increases in  $\text{Na}^+$  uptake, proline and ABA concentrations. Salinity treatment also induced the appearance of new protein bands. The most effective concentrations of  $\text{CaCl}_2$  treatment in protecting mustard plants against the adverse effect of NaCl salinity were 5, 10, and 15 mM  $\text{CaCl}_2$ . This could be attributed to the effect of  $\text{CaCl}_2$  in decreasing ABA concentration, the synthesis of new polypeptides and increasing  $\text{K}^+$  and  $\text{Ca}^{2+}$  uptake.

**KEYWORDS:** salinity,  $\text{CaCl}_2$ , proline, protein, ABA.

### INTRODUCTION

Soil salinity affects plant growth and agricultural productivity. For many salt-sensitive plants, glycophytes, which include most crop plants, a major part of growth inhibition is caused by excess  $\text{Na}^+$ . High salinity exerts detrimental effects on plants because of ion toxicity as well as osmotic stress (Binzel & Reuveni 1994).

The importance of  $\text{Ca}^{2+}$  in signaling, growth, and development has long been recognized (Bush 1995; Sanders *et al.* 1999). A variety of external cues, such as drought and salinity, cause transient opening of  $\text{Ca}^{2+}$  channels in the plasma membrane (PM) and/or endomembranes.  $\text{Ca}^{2+}$  fluctuations in microdomains of the cytosol are sensed and decoded to produce specific cellular and physiological responses (Sanders *et al.* 1999), including in abscisic acid-induced closure of stomatal aperture (Ward *et al.* 1995).

High  $\text{Na}^+$  disrupts potassium ( $\text{K}^+$ ) nutrition and, when accumulated in the cytoplasm, inhibits many enzymes. L  uchli (1990) reported that one factor known to be involved in the regulation of  $\text{K}^+/\text{Na}^+$ , selectivity of  $\text{K}^+$  transport during NaCl stress is  $\text{Ca}^{2+}$ . He also found that high external  $\text{Ca}^{2+}$  has been shown to improve plant salt tolerance. A general beneficial effect of external  $\text{Ca}^{2+}$  on plant salt tolerance is often attributed to the suggestion that  $\text{Ca}^{2+}$  is necessary for maintaining plasma membrane integrity and improving  $\text{K}^+/\text{Na}^+$  selectivity of potassium uptake systems (L  uchli 1990).

Kinraide (1998) reported that  $\text{Ca}^{2+}$  in rooting medium is essential for root elongation, even in the absence of added toxicants. In the presence of rhizotoxic levels of  $\text{Al}^{3+}$ ,  $\text{H}^+$ , or  $\text{Na}^+$ , supplementation of the medium with higher levels of  $\text{Ca}^{2+}$  alleviates growth inhibition (Yermiyahu *et al.* 1997). Koryo (1997) discovered that epidermal cytoplasm NaCl stress led to a significant decrease of P, K, Ca and S concentrations accompanied by an increase of Na concentration in root cell of sorghum.

Osmotic stress induced a significant decrease in chlorophyll content and enhanced accumulation of proline (Gibon *et al.* 2000). Proline is known to play an important role as an osmoprotectant in plants subjected to hyperosmotic stresses such as drought and soil salinity (Delauney & Verma 1993). Many studies on proline synthesis and catabolism genes have provided results that are consistent with diverse functions of proline as a source of energy, nitrogen and carbon, and as an osmolyte in response to dehydration (Peng *et al.* 1996; Zhang *et al.* 1997). Accumulation of proline in plants under stress may offer multiple benefits to the

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cell. Hong *et al.* (2000) showed that free radicals are formed during osmotic stress, as measured by an increase in the malondialdehyde production. They also recorded that transgenic plants, which produce more proline, accumulate less malondialdehyde. These radicals can react with many cellular constituents, including DNA, proteins, and lipids, leading to radical chain processes, crosslinks, peroxidation, membrane leakage, and the production of toxic compounds (Davies 1995).

A number of proteins has been identified that typically accumulate in plants in response to any environmental stimulus that has a dehydrative component or is temporally associated with dehydration. This includes drought, low temperature, salinity and seed maturation (Close 1996). Changes in gene expression occur in salt-stressed roots; the accumulation of mRNAs and proteins has been observed in the roots of salt-tolerant barley genotypes (Hurkman *et al.* 1989). Salt-induced changes in polypeptide synthesis are often more pronounced in the roots compared to the shoot (Gulick & Dvorak 1987), a finding that reflects their direct exposure to salt and suggests that they are the primary organ affected in salt exposed plants. Chen & Plant (1999) found that 170mM NaCl treatment resulted in the altered synthesis and accumulation of a number of prominent polypeptides in tomato roots.

Two chloroplastic proteins of (CDSP 32 and CDSP 34) were shown to be substantially synthesized in response to a progressive water deficit in whole *Solanum tuberosum* plants. The thylakoid CDSP 34 protein exhibited enhanced synthesis and substantial accumulation in response to cold and high salinity (Pruvot *et al.* 1995). A significant increase in the leaf abscisic acid content (at least 2.5-fold) was measured in plants subjected to water deficit, high salinity or low temperature. They conclude that ABA probably mediates the increased synthesis of CDSP 34 upon drought, low temperature and high salinity and suggest that another signal, perhaps related to high osmolarity, is involved in the induction of CDSP 32 synthesis. (Pruvot *et al.* 1995)

There is significant evidence that the plant hormone abscisic acid (ABA) acts as the root-to-shoot stress signal. Jeschke *et al.* (1997) reported that increased ABA concentration in the xylem is correlated with reduced leaf conductance and a general inhibition of leaf growth. They also reported that salt stress caused an increase in ABA concentrations of mature *Ricinus* leaves by a factor of 18 at 128 mM NaCl concentration. The present study evaluates the protective role of calcium on the adverse effect of salinity on mustard seedlings.

## **MATERIALS AND METHODS**

Seeds of mustard plants (*Sinapis alba* L.) were grown in plastic pots 15-cm diameter half-filled with sandy soils and irrigated daily with tap water. Two-week old seedlings were irrigated at two-day intervals as follows: tap water (control), 100mM NaCl, 100mM NaCl + 5mM CaCl<sub>2</sub>, 100mM NaCl + 10mM CaCl<sub>2</sub>, 100mM NaCl + 15mM CaCl<sub>2</sub>, 100mM NaCl + 20mM CaCl<sub>2</sub>, 100mM NaCl + 25mM CaCl<sub>2</sub>, 100mM NaCl + 30mM CaCl<sub>2</sub>. Plants were harvested after 2 weeks under this treatment regime. Plants were cut and digested for mineral analyses. Leaves were frozen immediately for chlorophyll analysis.

**Ca, Na, and K analyses:** the wet ashing method described by Westerman (1990) was used for the estimation of Ca, K and Na concentrations. Analysis of Ca was carried out using a Perkin-Elmer 3100 atomic absorption spectrophotometer. Analyses of K and Na were carried out using an Easylyte (Na/K) analyser (Medica corporation, Ma 01730, Bedford, U.S.A).

**Chlorophyll content:** chlorophyll was extracted in 80% acetone and the absorbances at 663 nm and 645 nm were read spectrophotometrically (Withal *et al.* 1971).

**Proline content:** proline was assayed using the method of Bates *et al.* (1973). Plant material was homogenized in 3% aqueous sulphosalicylic acid. The filtrate was added to glacial acetic acid and ninhydrin, then heated in a boiling water bath for 1h. After extraction with toluene, the color of the toluene layer was assessed at 520nm.

**Protein electrophoresis:** plant samples were prepared for electrophoresis by solubilization in equal volumes of SDS buffer (0.0625 M Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 5%

mercaptoethanol and bromophenol blue, 0.001%). Six reference proteins differing in their molecular weights were used as markers and run in parallel with the prepared samples under study. The method used was recommended by King & Laemmli (1971).

**ABA concentration:** frozen seedling tissues were homogenized in a chilled (4C) mortar with 85% methanol at the ratio of 20-ml/g tissue. The homogenate was extracted for ABA as described by Shindy & Smith (1975). Flame ionization detection was used for the identification of ABA using Hewlett Packered Gas Chromatography (5890). Standard error is not tabulated since we use a group of samples in one analysis.

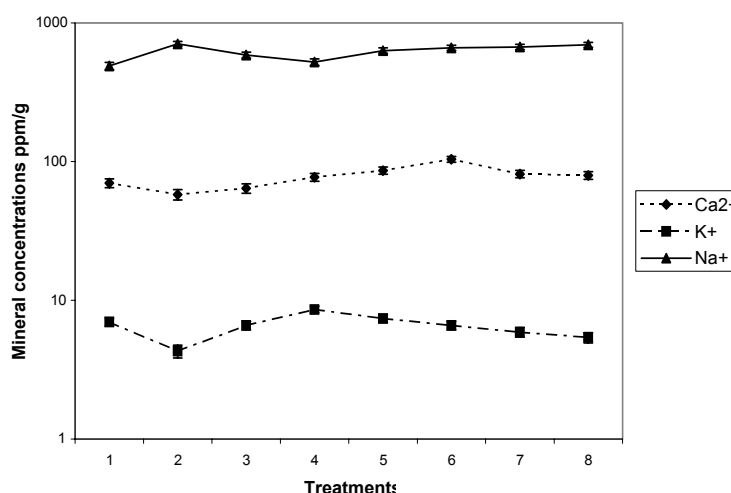
**Statistical analysis:** the data were analyzed using analysis of variance (ANOVA- one way). There were three replicates of every treatment.

## RESULTS

The data in Figure 1 show that 100mM NaCl treatment induced a significant increase in Na uptake and a significant reduction in K uptake by mustard plants. Application of different CaCl<sub>2</sub> concentrations to NaCl treated plants alleviated the effect of salinity on mineral uptake. It was found that 10, 15 and 20 mM CaCl<sub>2</sub> treatments were the most effective concentrations.

Fig. 1: Effect of NaCl (100mM alone) and with different CaCl<sub>2</sub> concentrations on Ca<sup>2+</sup>, K<sup>+</sup> and Na<sup>+</sup> concentrations in *Sinapis alba* L. plants. Data are means ±S.E

1= control, 2= 100 mM NaCl+ 30 mM CaCl<sub>2</sub>, 3= 100 mM NaCl+ 25 mM CaCl<sub>2</sub>, 4= 100 mM NaCl+ 20 mM CaCl<sub>2</sub>, 5= 100 mM NaCl+ 15 mM CaCl<sub>2</sub>, 6= 100 mM NaCl+ 10 mM CaCl<sub>2</sub>, 7= 100 mM NaCl+ 5 mM CaCl<sub>2</sub>, and 8= 100 mM NaCl.



Chlorophyll a and b content were significantly decreased at 100 mM NaCl treatment levels in comparison with control plants. Application of the different CaCl<sub>2</sub> concentrations induced highly significant increases in both chlorophylls in comparison to the salinity treated plants (Table 1). It was also noted that as CaCl<sub>2</sub> concentration was increased the chlorophyll contents also increase.

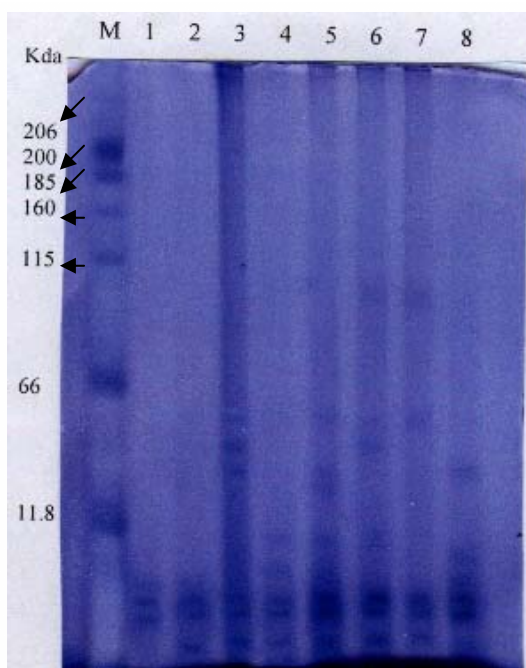
Salinity stress (100 mM NaCl) significantly increased proline content in mustard tissues with nearly about 7-folds. The application of different CaCl<sub>2</sub> concentrations also induced highly significant increases in proline content in comparison with control plants. It was found that CaCl<sub>2</sub> treatment with the concentrations 5 and 10 mM significantly decreased proline content in comparison with the 100 mM NaCl treatment group. (Table 1).

Table 1: Effect of NaCl (100 mM alone) and with different CaCl<sub>2</sub> concentrations on Chl. a & b and proline contents in *Sinapis alba* L. plants

| Treatment                             | Chl. a (µg/g tissue) | Chl. b (µg/g tissue) | Proline (µmol/g tissue) |
|---------------------------------------|----------------------|----------------------|-------------------------|
| Control                               | 440.0                | 262.5                | 11.84                   |
| 100 mM NaCl                           | 260.0                | 155.0                | 79.16                   |
| 100 mM NaCl + 5 mM CaCl <sub>2</sub>  | 282.5                | 182.5                | 73.34                   |
| 100 mM NaCl + 10 mM CaCl <sub>2</sub> | 307.5                | 235.0                | 53.40                   |
| 100 mM NaCl + 15 mM CaCl <sub>2</sub> | 405.0                | 267.5                | 86.02                   |
| 100 mM NaCl + 20 mM CaCl <sub>2</sub> | 430.0                | 180.0                | 92.77                   |
| 100 mM NaCl + 25 mM CaCl <sub>2</sub> | 335.0                | 230.0                | 97.34                   |
| 100 mM NaCl + 30 mM CaCl <sub>2</sub> | 407.0                | 262.5                | 124.25                  |
| F ratio                               | 266.66               | 213.68               | 384.60                  |
| P value (p<0.01)                      | 0.0002               | 0.0001               | 0.0001                  |

Figures 2 illustrates that treatment with 100 mM NaCl induced the appearance of 4 new protein bands in comparison with control plants, their M.Wts. were 81.62, 40.07, 12.15 and 9.50 kDa, respectively. However, the application of 5, 10 and 15 mM CaCl<sub>2</sub> to the salinity treated mustard plants was found to induce the appearance of 4 protein bands with M.Wts equal to 230.90, 229.88, 100.22 and 12.15 kDa, respectively. It was also noted that as CaCl<sub>2</sub> concentration increased, from 5 to 15 mM, the number of protein bands increased. Moreover, the lowest percentage of protein appeared with 20 mM CaCl<sub>2</sub> treatment

Fig. 2: Electrophoretic pattern of protein of mustard plants treated with 100mM NaCl alone and with different concentrations of CaCl<sub>2</sub>. M= markers, 1= control, 2= 100 mM NaCl+ 30 mM CaCl<sub>2</sub>, 3= 100 mM NaCl+ 25 mM CaCl<sub>2</sub>, 4= 100 mM NaCl+ 20 mM CaCl<sub>2</sub>, 5= 100 mM NaCl+ 15 mM CaCl<sub>2</sub>, 6= 100 mM NaCl+ 10 mM CaCl<sub>2</sub>, 7= 100 mM NaCl+ 5 mM CaCl<sub>2</sub>, and 8= 100 mM NaCl.



The data in table 2 show that 100 mM NaCl treatment increased ABA concentration as compared to that of control. Application of CaCl<sub>2</sub> with different concentrations induced a significant difference in ABA concentrations. CaCl<sub>2</sub> treatments with concentrations of 5, 10 and 15 mM were found to decrease ABA concentration whereas 20 and 25 mM CaCl<sub>2</sub> treatments increased its concentration as compared with the treatment of 100 mM NaCl only.

Table 2: Effect of 100 mM NaCl only and with different CaCl<sub>2</sub> concentrations on ABA concentration in *Sinapis alba* L. plants

| Treatments                            | ABA concentration (µg/g) |
|---------------------------------------|--------------------------|
| Control                               | 2.9                      |
| 100 mM NaCl                           | 11.2                     |
| 100 mM NaCl + 5 mM CaCl <sub>2</sub>  | 5.6                      |
| 100 mM NaCl + 10 mM CaCl <sub>2</sub> | 5.7                      |
| 100 mM NaCl + 15 mM CaCl <sub>2</sub> | 8.8                      |
| 100 mM NaCl + 20 mM CaCl <sub>2</sub> | 14.0                     |
| 100 mM NaCl + 25 mM CaCl <sub>2</sub> | 12.4                     |
| 100 mM NaCl + 30 mM CaCl <sub>2</sub> | 8.0                      |

## DISCUSSION

Salinity is a major environmental stress that is a substantial constraint to crop production both for dry land and irrigated agriculture (Epstein *et al.* 1980). The problems caused by salt stress arise from the disruption of cellular aqueous and ionic equilibria, so tolerance determinants include effectors that function to restore cellular homeostasis. Several lines of evidence suggest that regulation of intracellular Ca<sup>2+</sup> levels is crucial for adaptation of plants to environmental stress. Kirnaide (1999) defined salinity toxicity as a limitation of growth caused by Ca<sup>2+</sup> displacement at the plasma membrane surface of root cells, caused by the

ionic intoxication of the cytoplasm. In most saline soils,  $\text{Na}^+$  is the major toxic cation. One harmful effect of  $\text{Na}^+$  is that it disrupts  $\text{K}^+$  nutrition (Greenway & Munns 1980).  $\text{K}^+$  is one of the three major components in fertilizers applied to soils and a key factor controlling crop productivity (Glass 1989). Physiological studies have established that at least two mechanisms, the mechanism 1 (high-affinity) and 2 (low-affinity) transport systems mediate  $\text{K}^+$  uptake by plant roots. Because soil solutions often contain  $<1$  mM  $\text{K}^+$ , the high-affinity system is thought to play the predominant role in plant potassium nutrition (Glass 1989). The present study revealed that 100 mM NaCl salinity induced a significant decrease in  $\text{K}^+$  uptake, while the application of different  $\text{CaCl}_2$  concentrations enhanced  $\text{K}^+$  uptake by mustard plants. Koryo (1997) stated that the exclusion of Na from the symplast led to an apparent decrease in cytoplasmic concentrations of such essential elements as Mg, P, S and Ca. Thus it is responsible directly (via energy supply in mitochondria, homeostasis, selectivity of K over Na) or indirectly (via enzyme conformation, cytoplasmic hydration), for ultrastructure degradation.

Adding calcium ( $\text{Ca}^{2+}$ ) to root growth medium enhances salt tolerance in glycophytic plants.  $\text{Ca}^{2+}$  sustains  $\text{K}^+$  transport and  $\text{K}^+$ - $\text{Na}^+$  selectivity in  $\text{Na}^+$ -challenged plants. Also,  $\text{Ca}^{2+}$  increases the selectivity of root  $\text{K}^+$  transport systems (Läuchli 1990). Salt tolerant plants avoid the accumulation of cytoplasmic  $\text{Na}^+$  by a variety of mechanisms.  $\text{Ca}^{2+}$  and some other solutes can alleviate salinity toxicity by reducing  $\text{Na}^+$  uptake by electrostatic displacement of  $\text{Na}^+$  from the plasma membrane surface, or by blocking the channels that allow the entry of  $\text{Na}^+$  into the cells (Roberts & Tester 1997, Kirnaide 1999). This could explain the decrease in  $\text{Na}^+$  uptake by  $\text{CaCl}_2$  treated mustard plants.

Some plants avoid serious growth reduction, or damage to the photosynthetic system, by compartmentalization of sodium in special organs, while recirculation of sodium through xylem and phloem has been reported for bean (Jacoby 1979) and *Lupinus albus* (Munns 1988). To remove sodium from the cytoplasm, special transporters on the plasma membrane (Rausch *et al.* 1996) efficiently transfer sodium into glands or extracellular compartments (Barkla and Pantoja 1996). In the case of sodium recirculation, uptake from the xylem into pith cells of the stem occurs, followed by sodium efflux directed towards the phloem vessel (Jeschke *et al.* 1997). Yoshiyuki *et al.* (2000) studied the effects of salinity on the characteristics of  $\text{Ca}^{2+}$  binding to the outer surface of plasma membrane (PM) of protoplasts isolated from two types of tobacco (*Nicotiana tabacum* L., cv. Bright Yellow) cultured cells that were adapted (tolerant) and unadapted (sensitive) to 50 mM NaCl stress. They showed that  $\text{Na}^+$  induced an appreciably higher degree of reduction in the amount of  $\text{Ca}^{2+}$  bound to PM compared with  $\text{K}^+$  with increasing concentration from 0.1 to 30 mM. Their results suggest that, under these physiological conditions, the PM of salt-sensitive NaCl-unadapted cells has an appreciable amount of PM-bound  $\text{Ca}^{2+}$  that is desorbed much easier by  $\text{Na}^+$  than  $\text{K}^+$ , whereas PM of salt-tolerant NaCl-adapted cells has the PM-bound  $\text{Ca}^{2+}$  that can be equally desorbed by  $\text{Na}^+$  and  $\text{K}^+$ . Increases in cytosolic  $\text{Ca}^{2+}$  regulate several ion transporters that are essential in the control of stomatal aperture (MacRobbie 1997; McAinsh *et al.* 1997). The plasma membrane proton pump of guard cells, which hyperpolarizes the plasma membrane and thus provides the driving force for  $\text{K}^+$  and  $\text{Cl}^-$  uptake, is inhibited by increased cytosolic  $\text{Ca}^{2+}$  (Kinoshita *et al.* 1995). The inward  $\text{K}^+$  channels in the plasma membrane of guard cells, which are responsible for  $\text{K}^+$  influx, are also inhibited by elevated cytosolic  $\text{Ca}^{2+}$  (Schroeder & Hagiwara 1989). Consistent with these electrophysiological explanations, exogenous application of  $\text{Ca}^{2+}$  inhibits opening of closed stomata and stimulates closure of open stomata (De Silva *et al.* 1985; Schwartz 1985; Schwartz *et al.* 1988). Such  $\text{Ca}^{2+}$  application is known to increase cytosolic  $\text{Ca}^{2+}$  levels (Gilroy *et al.* 1991). In addition, a variety of stimuli such as ABA,  $\text{CO}_2$ , and oxidative stress can rapidly induce increases in cytosolic  $\text{Ca}^{2+}$  concentrations in guard cells (McAinsh *et al.* 1997).

It was found, in the present study that 100 mM NaCl significantly reduced chlorophyll a&b content, while application of different  $\text{CaCl}_2$  concentrations restored Chl. a&b content

nearly to control level. Suleyman *et al.* (2000) found that osmotic stress due to 1.0 M sorbitol, inactivated the oxygen-evolving machinery of PSII and disrupted the electron-transport activity of PSI in intact cells. The effect on PSII was greater than that on PSI. Osmotic stress due to 1.0 M sorbitol decreased the cytoplasmic space by about 50%. Shrinkage may have increased the concentrations of major intracellular ions such as  $K^+$  and  $Cl^-$ , and led to the gradual inactivation of PSI and PSII.

Suleyman *et al.* (2000) found that when extracellular osmotic pressure increases, the water in the intracellular space leaves the cell through the water channels and the cytoplasmic concentration of  $K^+$  ions increases. This increase leads, in turn, to an increase in the concentration of  $K^+$  ions in the intra-thylakoid space and, as a result, the oxygen-evolving machinery is partially inactivated by dissociation of the extrinsic proteins. A similar mechanism can be postulated for the osmotic-stress-induced inactivation of PSI. An increase in the intra-thylakoid concentration of  $K^+$  ions results in the dissociation of plastocyanin or Cyt  $c_{553}$  from the PSI complex, which causes partial inactivation of the PSI-mediated transport of electrons. This lead to the oxidation of chlorophylls and so explain the reduction of chlorophyll content as a result of 100 mM NaCl treated mustard. When the cell is released from the osmotic stress, water enters through the water channels and the cytoplasmic concentration of  $K^+$  ions decreases. The intra-thylakoid concentration of  $K^+$  ions then also decreases and, as a result, the renewed binding of the extrinsic proteins to these complexes restores the integrity of PSII and PSI.

Salinity treatment (100 mM NaCl) and the different  $CaCl_2$  concentrations resulted in significant accumulation in proline content in mustard plant. Proline accumulation in response to stress is widely reported, and may play a role in stress adaptation within the cell (Gilbert *et al.* 1998). Evidence for the transport of proline to the root tip, where it accumulates during stress, has been reported. The rapid accumulation of amino acids during salinity stress suggests that these compounds may be acting as sinks for excess N in relation to the decreased growth occurring during the imposed stress. (Dubay & Pessarakli 1995). They also play a role in osmotic adjustment, and serve as available sources of carbon and nitrogen.

Accumulation of proline in plants under stress is a result of the reciprocal regulation of two pathways: increased expression of proline synthetic enzymes (P5CS and P5CR) and repressed activity of Pro degradation (Delauney & Verma 1993; Peng *et al.* 1996). This leads to a "proline cycle," the homeostasis of which depends on the physiological state of the tissue (Verma 1999). Plants may have evolved a mechanism to coordinate synthesis, catabolism, and transport activities for the accumulation of proline (Hong *et al.* 2000).

Several explanations for the accumulation of free amino acids and amides under stress have been suggested. These include stimulated *de novo* synthesis, inhibited degradation of amino acids, impaired protein synthesis, and/or enhanced protein degradation (Ranieri *et al.* 1989; Gilbert *et al.* 1998).

Proteins have been predicted to play a role in the amelioration of osmotic stress. (Chen & Plant 1999). For salinity stress tolerance in plants, the vacuolar type  $H^+$ -ATPase (V-ATPase) is of prime importance in energizing sodium sequestration into the central vacuole and it is known to respond to salt stress with increased expression and enzyme activity. (DeWald *et al.* 2001).

Salt altered the accumulation of several polypeptides, all of which were previously observed in water-deficit-stressed roots, indicating that their synthesis was the result of the osmotic component of the salt stress (Jin *et al.* 2000). They concluded that ABA is required for their synthesis. Jae-Ung & Youngsook (2001) found that ABA, a signal for stomatal closure, induces rapid depolymerization of cortical actin filaments and the slower formation of a new type of actin that is randomly oriented throughout the cell. This change in actin organization has been suggested to be important in signaling pathways involved in stomatal closing movement, since actin antagonists interfere with normal stomatal closing responses to ABA. They also found that treatment of guard cells with  $CaCl_2$  induced changes in actin

organization similar to those induced by ABA. These results indicated that  $\text{Ca}^{2+}$  acts as a signal mediator in actin reorganization during guard cell response to ABA. Also, they suggested that protein kinase(s) and phosphatase(s) participate in actin remodeling in guard cells during ABA-induced stomatal closure.

Salt stress stimulated synthesis in roots and xylem transport of ABA was well related to stomatal reactions. This may be explained by the fact that roots are directly exposed to the salt. ABA in roots stimulates ion accumulation in vacuoles of barley roots, which may be necessary for adaptation to saline conditions (Jeschke *et al.* 1997). Changes in the cytosolic free  $\text{Ca}^{2+}$  concentration play a central role in the transduction of external signals through the cytosol of plant cells. A wide array of physiological events such as salt stress, low temperature, heat shock, and touch and exposure to hormones, red light, or fungal elicitors has been shown to be accompanied by transient changes in the concentration of the cytosolic free  $\text{Ca}^{2+}$  (Bush 1995; Sanders *et al.* 1999). This universal  $\text{Ca}^{2+}$  signal is thought to be encoded by differences in the temporal and spatial distribution of  $\text{Ca}^{2+}$  in the cell, as well as by the amplitude and frequency of the changes in the cytosolic  $\text{Ca}^{2+}$  concentration (Trewavas 1999). In response to a stimulus,  $\text{Ca}^{2+}$  enters from the extracellular space and/or is released from intracellular stores (in plants mainly the vacuole and/or ER) by differently activated  $\text{Ca}^{2+}$  channels. It is vital to the cell that excess  $\text{Ca}^{2+}$  is removed from the cytosol following a  $\text{Ca}^{2+}$  signal to bring the cell back to a resting state. Two different systems are believed to be involved in pumping  $\text{Ca}^{2+}$  from the cytosol over the plasma membrane (PM) or back into intracellular stores: P-type  $\text{Ca}^{2+}$  pumping ATPases and a  $\text{Ca}^{2+}/\text{H}^+$  antiporter. The former has been shown to be present in the ER and the vacuolar membranes as well as in the PM, whereas the antiporter is present in the vacuolar membrane only (Askerlund 1996; Evans & Williams 1998). The ATPases have a high affinity for  $\text{Ca}^{2+}$  and are therefore able to bring the concentration of  $\text{Ca}^{2+}$  back to submicromolar levels, while the antiporters have a lower affinity for  $\text{Ca}^{2+}$  but a higher capacity and are thought to function under conditions of high cytosolic  $\text{Ca}^{2+}$ .  $\text{Ca}^{2+}$  appears to alleviate the effects of rhizotoxic cations by multiple mechanisms. First, through the electrostatic displacement of toxicant from plasma membrane surfaces and secondly by the restoration of toxicant displaced  $\text{Ca}^{2+}$  at plasma membrane surfaces. This mechanism is unlikely to be as important for  $\text{Na}^+$  toxicity than for  $\text{Al}^{3+}$  and  $\text{H}^+$  toxicants. Thirdly, a class of interactions between  $\text{Ca}^{2+}$  and toxicants is highly specific and may reflect in part the  $\text{Ca}^{2+}$  blockade of plasma membrane channels that admit toxicants. Nakamura *et al.* (1990) concluded that  $\text{Ca}^{2+}$  prevents the leakage of intracellular  $\text{K}^+$  and thereby supports the elongation of roots under salt stress.

In Conclusion, this study suggests that application of external  $\text{Ca}^{2+}$  alleviates the salinity effects on mustard plants through the synthesis of new polypeptides, decreasing the level of ABA and increasing uptake of  $\text{K}^+$  and  $\text{Ca}^{2+}$  and accumulation of proline.

## REFERENCES

- Askerlund P (1996) Modulation of an intracellular calmodulin-stimulated  $\text{Ca}^{2+}$ -pumping ATPase in cauliflower by trypsin: the use of calcium green-5N to measure  $\text{Ca}^{2+}$  transport in membrane vesicles. *Plant Physiol* 110: 913-922
- Barkla BJ & Pantoja O (1996) Physiology of ion transport across the tonoplast of higher plants. *Ann. Rev. plant. Physiol. Mol. Biol.* 47:159-184.
- Bates LS, Waldren RP & Teare ID (1973) Rapid determination of free proline for water-stress studied. *Plant & Soil* 39: 205-207.
- Bush DS (1995) Calcium regulation in plant cells and its role in signaling. *Ann. Rev. Plant Physiol. Mol. Biol.* 46: 95-122
- Chen CSC & Plant AL (1999) Salt-induced protein synthesis in tomato roots: the role of ABA. *J. Exp. Bot.* 50(334):677-687.
- Close TJ (1996) Dehydrins: Emergence of a biochemical role of a family of plant dehydration proteins *Physiologia Plantarum* 97 (4): 795-803
- Davies KJ (1995) Oxidative stress: the paradox of aerobic life. *Biochem. Soc. Symp.* 61: 1-31
- De Silva DLR, Hetherington AM & Mansfield TA (1985) Synergism between calcium ions and abscisic acid in preventing stomatal opening. *New Phytol.* 100: 473-482

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- Delauney AJ & Verma DPS (1993) Proline biosynthesis and osmoregulation in plants. *Plant J* 4: 215-223
- DeWald DB, Torabinejad J, Jones CA, Shope JC, Cangelosi AR, Thompson J E, Prestwich GD & Hama H (2001) Rapid Accumulation of Phosphatidylinositol 4,5-Bisphosphate and Inositol 1,4,5-Trisphosphate Correlates with Calcium Mobilization in Salt-Stressed Arabidopsis. *Plant Physiol.* 126: 759-769
- Dubay R & Pessaraki M (1995) Physiological mechanisms of nitrogen absorption and assimilation in plants under stressful conditions. In: Handbook of Plant and Crop Physiology. New York: Marcel Dekker, Inc., p; 605-625.
- Enami I, Kikuchi S, Fukuda T, Ohta H & Shen JR (1998) Binding and functional properties of four extrinsic proteins of photosystem II from a red alga *Cyanidium caldarium*, as studied by release-reconstitution experiments. *Biochemistry* 37: 2787-2793
- Evans DE & Williams LE (1998) P-type calcium ATPases in higher plants: biochemical, molecular and functional properties. *Biochim Biophys Acta* 1376: 1-25
- Gibon Y, Sulpice R & Larher F (2000) Proline accumulation in canola leaf discs subjected to osmotic stress is related to the loss of chlorophylls and to the decrease of mitochondrial activity. *Physiologia Plantarum* 110 (4): 469-476.
- Gilbert AG, Gadush MV, Wilson C & Madore MA (1998) Amino acid accumulation in sink and source tissues of *Coleus blumei* Benth. During salinity stress. *J. Exp. Bot.* 49(318): 107-114.
- Gilroy S, Fricker MD, Read ND & Trewavas AJ (1991) Role of calcium in signal transduction of *Commelina* guard cells. *Plant Cell* 3: 333-344
- Glass ADM (1989) Plant Nutrition: An Introduction to Current Concepts. Jones & Bartlett, Boston, MA.
- Greenway H & Munns R (1980) Mechanism of salt tolerance in nonhalophytes. *Annu. Rev. Plant Physiol.* 31: 149-190
- Gulick PJ & Dvorak J (1987) Gene induction and repression by salt treatment in roots of the salinity-sensitive Chinese Spring wheat and the salinity-tolerant Chinese Spring x *Elytrigia elongata* amphiploid. *PNAS.* 84:99-103.
- Hong Z, Lakkineni K, Zhang Z & Verma DPS (2000) Removal of Feedback Inhibition of <sup>1</sup>-Pyrroline-5-Carboxylate Synthetase Results in Increased Proline Accumulation and Protection of Plants from Osmotic Stress. *Plant Physiol.* 122: 1129-1136
- Hurkman WJ, Tanaka CK & Fornari CS (1989) A comparison of the effects of salt on polypeptides and translatable mRNAs in the roots of a salt-tolerant and a salt-sensitive cultivar of barley. *Plant Physiol.* 90:1444-1456.
- Jacoby B (1979) Sodium recirculation and loss from *Phaseolus vulgaris* L. *Annals of Botany* 43: 1709-1716.
- Jae-Ung H & Youngsook L (2001) Abscisic acid-induced actin reorganization in guard cells of dayflower is mediated by cytosolic calcium levels and by protein kinase and protein phosphatase activities. *Plant Physiol.* 125: 2120-2128
- Jeschke WD, Peuke AD, Pate JS & Hartung W (1997) Transport, synthesis and catabolism of abscisic acid (ABA) in intact plants of castor bean (*Ricinus communis* L.) under phosphate deficiency and moderate salinity. *J. Exp. Bot.* (48)314: 1737-1747.
- Jin S, Chen CCS & Plant AL (2000) Regulation by ABA of osmotic-stress-induced changes in protein synthesis in tomato roots. *Plant Cell & Environment* 23 (1): 51-60.
- King J & Laemmli UK (1971) Polypeptide of the tail fibres of bacteriophage T4. *J. Mol. Biol.* 62:465-473.
- Kinoshita T, Nishimura M & Shimazaki K (1995) Cytosolic concentration of Ca<sup>2+</sup> regulates the plasma membrane H<sup>+</sup>-ATPase in guard cells of faba bean. *Plant Cell* 7: 1333-1342
- Kinraide TB (1999) Interactions among Ca<sup>2+</sup>, Na<sup>+</sup> and K<sup>+</sup> in salinity toxicity: quantitative resolution of multiple toxic and ameliorative effects. *J. Exp. Bot.* 50(338): 1495-1505.
- Kinraide TB (1998) Three mechanisms for the calcium alleviation of mineral toxicities. *Plant Physiol.* (118): 513-520
- Koryo H (1997) Ultrastructure and physiological changes in root cells of sorghum plants (*Sorghum bicolor* X S. sudanesis cv. Sweet Sioux) induced by NaCl. *J. Exp. Bot.* 48: 693-706.
- Läuchli A (1990) In Calcium in Plant Growth and Development. Leonard RT & Hepler PK (Eds). The American Society of Plant Physiologists Symposium Series (American Society of Plant Physiologists. Rockville, MD), Vol. 4, pp. 26-35.
- Lynch J, Polito VS & Lauchli A (1989) Salinity stress increases cytoplasmic Ca activity in maize root protoplasts. *Plant Physiol.* 90: 1271-1274
- MacRobbie EAC (1997) Signalling in guard cells and regulation of ion channel activity. *J. Exp. Bot.* 48: 515-528
- McAinsh MR, Brownlee C & Hetherington AM (1997) Calcium ions as second messengers in guard cell signal transduction. *Physiol. Plant.* 100: 16-29
- Munns R (1988) Effect of high external NaCl concentration on ion transport within the shoot of *Lupinus albus*. 1. Ion in xylem sap. *Plant cell & Environment* 11: 263-2289.
- Nakamura Y, Tanaka K, Ohta E & Sakata M (1990) Protective effect of external Ca<sup>2+</sup> on elongation and the intracellular concentration of K<sup>+</sup> in intact mung bean roots under high NaCl stress. *Plant & cell Physiology.* 31: 815-821.



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- Nishiyama Y, Los DA, Hayashi H & Murata N (1997) Thermal protection of the oxygen-evolving machinery by PsbU, an extrinsic protein of photosystem II in *Synechococcus* sp. PCC 7002. *Plant Physiol.* 115: 1473-1480
- Peng Z, Lu Q & Verma DPS (1996) Reciprocal regulation of 1-Pyrroline-5-carboxylate synthetase and proline dehydrogenase genes control levels during and after osmotic stress in plants. *Mol. Gen.* 253: 334-341
- Pruvot G, Massimino J, Peltier G & Rey P (1995) Effects of low temperature, high salinity and exogenous ABA on the synthesis of two chloroplastic drought-induced proteins in *Solanum tuberosum*. *Physiologia Plantarum* 97 (1): 123-131.
- Ranieri A, Bernardi R, Lanse P & Soldatini G (1989) Changes in free amino acid content and protein pattern of maize seedlings under water stress. *J. Exp. Bot.* 29: 351-357.
- Rausch T, Kirsch M, Low R, Lehr A, Viereck R & Zhigang A (1996) Salt stress responses of higher plants: the role of proton pumps and Na<sup>+</sup>/H<sup>+</sup> antiporters. *J. Plant Physiol.* 148: 425-433.
- Roberts SK & Tester M (1997) A patch clamp study of Na<sup>+</sup> transport in maize roots. *J. Exp. Bot.* 48: 431-440.
- Sanders D, Brownlee C, Harper JF (1999) Communicating with calcium. *Plant Cell* 11: 691-706
- Schroeder JI & Hagiwara S (1989) Cytosolic calcium regulates ion channels in the plasma membrane of *Vicia faba* guard cells. *Nature* 338: 427-430
- Schwartz A (1985) Role of Ca<sup>2+</sup> and EGTA on stomatal movements in *Commelina communis* L. *Plant Physiol* 79: 1003-1005
- Schwartz A, Ilan N, Grantz DA (1988) Calcium effects on stomatal movements in *Commelina communis*. *Plant Physiol.* 87: 583-587
- Shen J-R, Qian M, Inoue Y & Burnap RL (1998) Functional characterization of *Synechocystis* sp. PCC 6803 psbU and psbV mutants reveals important roles of cytochrome c-550 in cyanobacterial oxygen evolution. *Biochemistry* 37: 1551-1558
- Shindy WW & Smith OE (1975) Identification of plant hormones from cotton ovules. *Plant Physiol.* 55: 550-554.
- Suleyman IA, Atsushi Sakamoto, Yoshitaka Nishiyama & Norio Murata S (2000) Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp. *Plant Physiol.* 123: 1047-1056
- Trewavas A (1999) Le calcium, c'est la vie: calcium makes waves. *Plant Physiol.* 120: 1-6
- Verma DP (1999) Osmotic stress tolerance in plants: role of proline and sulfur metabolisms. Shinozaki K & Yamaguchi-Shinozaki K (Eds). Molecular responses to cold, drought, heat and salt stress in higher plants. RG Landes Company, Austin, TX, pp 153-168
- Ward JM, Pei Z & Schroeder JI (1995) Roles of ion channels in initiation of signal transduction in higher plants. *Plant Cell* 7: 833-844
- Westerman RL (1990) Soil testing and plant analysis. Soil Sci. of America, Inc. Medson, Wisconsin, U.S.A.
- Witham FH, Blaydes DF & Devlin RM (1971) Experiments in Plant Physiology Van Nostrand New York p. 245.
- Yermiyahu U, Rytwo G, Brauer DK & Kinraide TB (1997) Binding and electrostatic attraction of lanthanum (La<sup>3+</sup>) and aluminum (Al<sup>3+</sup>) to wheat root plasma membranes. *J. Membr. Biol.* 159: 239-252.
- Zhang CS, Lu Q & Verma DPS (1997) Characterization of 1-Pyrroline-5-carboxylate synthetase gene promoter in transgenic *Arabidopsis thaliana* subjected to water stress. *Plant Sci.* 129: 81-89

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