



Full Length Article

Role of Calcium in Increasing Tolerance of *Descurainia sophia* to Salt Stress

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ABSTRACT

The calcium ion has important effects on physiological processes of plants and improves the growth and biochemical parameters of the plant under salt stress. Because of osmotic effect and Na⁺ toxicity, salt stress decrease plant growth and verify plant parameters. In this experiment, we used *Descurainia sophia* plant that is sensitive and has high CaER (Calcium utilization Efficiency Ratio) in salt stress conditions. Plants grown in vermiculite were treated with salts (0, 25, 50 75 & 100 mM NaCl) either with 5 and 10 mM CaCl₂ or without. Results showed that 10 mM CaCl₂ treatment was toxic, but 5 mM CaCl₂ treatment improved most parameters such as the MDA-TBA in salt stress conditions. The MDA-TBA concentration (as the indicator of lipid peroxidation) for 50 mM NaCl treatment was much higher than other salinity treatments. The use of 5 mM CaCl₂ improved the oxidative stress produced by 50 mM NaCl salinity. Based on these results, the threshold for this plant was 50 mM salt. Salt tolerance beyond this threshold was significantly improved in the plants treated first with CaCl₂. To conclude using the plants with high (CaER), can decrease expenses of plants response to salt stress and improve growth efficiency.

Keywords: Salt stress; Calcium; *Descurainia sophia*; Calcium utilization efficiency ratio

INTRODUCTION

The Calcium ion, in optimum concentration, plays crucial roles in different physiological processes of plants and cause increase in plants resistance to stresses such as salt, drought and heavy metal (Rengel, 1992; Watkin Elizabeth *et al.*, 1997). The importance of this cation was studied in natural and stress condition. Caines and Carol (1999a & b) reported that Ca²⁺ can regulate or activate intracellular pathways of eukaryotes. The changes in cytosolic Ca²⁺ mediate the cell processes such as elongation, cell division, transportation, metabolism and secretion (Caines & Carol, 1999a). Although concentration of calcium (Ca²⁺) does not exceed 10% of leaf dry matter component of plants but high concentration of this ion have toxic and inhibitory effects on plants growth in different conditions (Zekri & Parsons, 1990; Mengel & Kirkby, 1987). Low concentration of cytosolic Ca²⁺ regulate plant growth and metabolism (Curtis *et al.*, 1995; Arvin & Kazami-pour, 2002). Ca²⁺ is absorbed via passive transport in apical zone of root but mechanism of transport is un-

known (Beck & Munns, 1985; Tyrman & Skerrett, 1999). It is reported that Ca²⁺ transport is inhibited by other ions such as some monovalent cations. In addition, cations like cadmium interact with Ca²⁺ uptake via Ca²⁺ channels. In addition, Na⁺ interacts with Ca²⁺ in cells, because optimum concentration of Ca²⁺ regulates the Na⁺ influx and membrane transport (Skorzynska-Polit *et al.*, 1998).

This bivalent cation in intracellular space of cell acts as a secondary messenger and improves cell response to stress condition by affecting enzyme stability and activity. It has been reported that high [Ca²⁺]_{cyt} increase proline oxidase enzyme activity in cell (Mengel & Kirkby, 1987; Gallardo *et al.*, 1998). Because Ca²⁺ accumulates within ER and golgi apparatus, concentration of this ion is very low in cytosol in natural condition. In stress condition, accumulated Ca²⁺ is released to the cytosol, (Gennady & Kelvin, 2000).

For more resistance of plants to stress condition, finding an optimum concentration of Ca²⁺ is important. It has been reported that extra cellular Na⁺:Ca²⁺ ratio is high, Na⁺ influx will increase (Rengel, 1992; Caines & Carol, 1999a & b). Extracellular Ca²⁺ act as inhibitor of some ion

channels in cell membrane such as KIRC. This channel was inhibited by high concentration of Ca^{2+} (1-10 mM). The KIRC prevents K^+ and Na^+ influx in natural and salt stress condition respectively (Mengel & Kirkby, 1987; Tyrman & Skerrett, 1999). In the present research, we studied the effect of salinity and Ca^{2+} ion in improving salt stress tolerance during seed germination and mature stages of *D. sophia*.

MATERIALS AND METHODS

Plant growth. *Descurainia sophia* L. plant was chosen from southeastern of Iran (Kerman province). In first experiment, seed germination percentage of *D. sophia* was determined in petriplates. Seeds were surface sterilized with sodium hypochlorite (0.34 M) for 5 min and rinsed with sterile distilled water. Then seeds were pretreated with KNO_3 (0.1 M) and cold temperature (4°C) for 72 h. Pretreated seeds were treated with various concentration of NaCl either with 5 or 10 mM CaCl_2 solutions. The control seeds were, treated with different concentration of NaCl solutions. In each Petri 100 seeds were placed on wet Whatman No.1 paper and all dishes were transferred to germinator with temperature 25°C and humidity of 60-65%. After 7 days, the percentage of germinated seeds was calculated.

In the second experiment, plants were grown in vermiculite pots under control room (dark/light regime: 8/16 h; temperature 23/25°C, humidity 44% & light intensity $240 \mu\text{mol m}^{-2} \text{s}^{-1}$) were treated with salinity treatments (NaCl 0, 25, 50, 75 & 100 mM) either with 5 and 10 mM CaCl_2 or without. Root and shoot tissues of plant were measured for growth parameters.

Measurement of plant growth parameters. After 8 weeks, plants were harvested and placed in ethanol (76%) for 6 min and then samples were dried in oven (80°C) for 48 h. The shoot and root length, fresh weight (FW) and dry (DW) weight were measured in shoot and root.

Tissue determination. The proline content of samples was measured according to Bates *et al.* (1973) method. For colorimetric measurement of chlorophylls a, b and total, the method of Arnon (1949) was used. The reducing sugars were determined by using Somogi-Nelson method (Prado *et al.*, 1952) and total protein content was measured by Lowry method (Lowry *et al.*, 1951).

For lipid peroxidation determination, 0.2 g of the leaf tissue were homogenized in 10 mL of trichloroacetic acid (TCA, 0.1% (w/v)), then centrifuged at 10000 g for 15 min. One mL of supernatant was vortexed with 4 mL of 20% (w/v) TCA containing 0.5% (w/v) 2-thiobarbituric acid (TBA) and the solution was heated for 30 min at 95°C. The samples were cooled on ice for 5 min and centrifuged again for 10 min at 10000 g. Non-specific absorbance of supernatant measured at 600 nm was subtracted from the maximum absorbance at 532 nm for the measurement of MDA-TBA (Malondialdehyde-TBA) complex (Heath & Packer, 1968). For the MDA-TBA complex concentration

calculation, an extinction coefficient (ϵ) of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ was used at 532 nm for MDA.

Statistical analysis. All data were analyzed by SPSS 10.0 software. The mean of each parameter was compared between treatments by one-way analysis of variance (Table I, II & III). Statistical significance was taken as $p < 0.05$.

RESULTS

Results of this study showed that seed germination was significantly higher in 50 and 75 mM NaCl treatments when 5 mM CaCl_2 was used in comparison with same salinity treatments without CaCl_2 . Salt stress also decreased seed germination percentage (5%) severely with 100 mM NaCl treatment and CaCl_2 did not reduce this effect. However, 10 mM CaCl_2 caused decrease in seed germination percentage in all salinity treatments (Fig. 1). The threshold sensitivity of this plant was 75 mM in germination stage and 50 mM salts for vermiculite culture. Salt stress beyond this threshold was significantly improved in the plants treated with CaCl_2 . Length, fresh and dry weight of shoot and root increased when plants were treated with CaCl_2 (Fig. 2). Based on these results, 25 mM NaCl had no toxic effects on seed germination.

In the case of 5 mM CaCl_2 treatment, proline concentration was reduced approximately 3 to 4 fold when compared with those plants not treated with CaCl_2 . Under salt stress conditions, the proline concentration increased significantly. Proline concentration in shoot, increased under 75 mM NaCl salinity (Fig. 3A). The results showed that proline concentration increased when plants were treated with 25, 50 and 75 mM NaCl. Proline did not show a remarkable increase in the plants treated with 5 mM CaCl_2 in comparison with those plants, which were treated with 50 mM NaCl (Fig. 3B).

Studies on the total protein of shoot indicated that concentration of protein increased by using of 25 and 50 mM NaCl, but protein did not increase in 75 mM NaCl treatment. In root tissue, total protein content decreased significantly in 25 mM NaCl treated plants. The 50 mM NaCl treatment with 5 mM CaCl_2 caused decrease in root protein content. However, shoot total protein did not change in those plants treated with solution containing 50 mM NaCl and 5 mM CaCl_2 salts. Maximum total protein content was observed in 25 mM NaCl treatment (Fig. 3C).

Chlorophyll a (Chl a) total (Chl T) content increased significantly in 25 mM NaCl treated plants compared than control. However, increase in Chl b was not obvious. At 75 and 100 mM salinity treatments, decline in Chl T and Chl a content was observed. The 50 mM NaCl solution containing 5 mM CaCl_2 had no effect on Chl T and Chl b content but increase in Chl a was significant when calcium were added (Fig. 3E). MDA-TBA content at 75 mM NaCl treatments was much higher than other treatments. Use of 5 mM CaCl_2 improved the oxidative stress created by 50 mM NaCl salinity (Fig. 3D).

Fig. 1. Comparison between mean of seed germination (%) in control and salinity treated plants. Each data represent mean of three replicates ±SEM

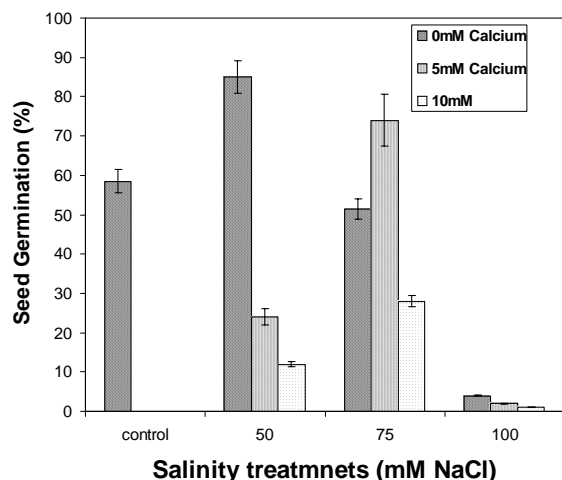


Table I. Mean squares from analysis of variance of seed germination percentage for plants at different salt and calcium concentrations (0, 25, 50, 75, 100 mM NaCl & 5, 10 mM CaCl₂)

Dependent Variable: Seed Germination

Source	SS	df	MS	F	Sig.
Among pots	5651.172 ^a	17		8.004	.025
Experimental error	38.428	12	3.818	488.852	.066
TIMAR	5651.172	11	7018.886	8.004	.012
Sampling error	117713.3	94	513.324		
Total	577388.2	141			
Corrected Total	173864.4	141			

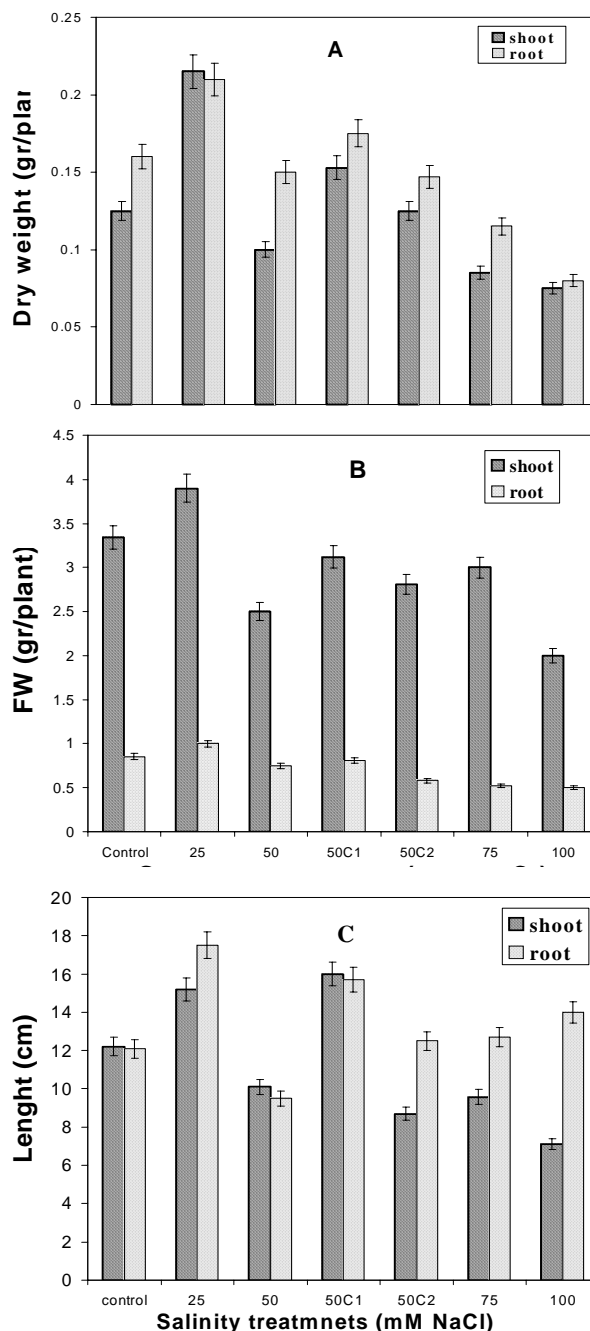
a. R Squared= .323(Adjusted R Squared= .287)

DISCUSSION

Calcium is required to maintain the structural integrity of α-amylase. Removal of Ca²⁺ leads to decreased thermostability and/or decreased enzymatic activity or increased susceptibility to proteolytic degradation (Saboury & Karbassi, 2000). There was a set of 17 binding sites for Ca²⁺ binding on enzyme with weak positive binding. The binding of Ca²⁺ to enzyme causes a greater stability of enzyme against surfactant and thermal denaturation. Moreover, the binding of calcium prevents from the spontaneous decrease in biological activity of α-amylase (Saboury & Karbassi, 2000). Data indicated that α-amylase activity was improved in NaCl media containing Ca²⁺ as inferred from seed germination percentage, which was much higher (Fig. 1).

The optimum concentration of calcium for maximum seed germination in salt stress condition (50 mM) was determined to be 5 mM CaCl₂, this concentration of calcium increased seed germination up to 25% more than that where no-Ca²⁺ was added. Twenty five mM NaCl treatment had no

Fig. 2. Comparison between mean of growth parameters in control and salinity treated plants. 50C1 and 50C2 represent plants which were treated with either 5 or 10mM Calcium respectively. A; Dry weight (g plant⁻¹), B; Fresh weight (g plant⁻¹), C; length (cm). Each data represent mean of three replicates ±SEM



toxic effect on seed germination (Fig. 1). The dry weight of plant increased when 5 mM CaCl₂ with 50 mM NaCl was applied (Fig. 2A). Thus, we concluded that calcium use efficiency of *D. sophia* in 50 mM NaCl was remarkable. Use of 50 mM NaCl with 5 mM CaCl₂ treatment alleviated the

Table II. Mean squares from analysis of variance of root and shoot growth parameters for plants at different salt and calcium concentrations (0, 25, 50, 75, 100 mM NaCl & 5, 10 mM CaCl₂)

A. Dependent variable: shoot fresh weight

Source	SS	df	MS	F	Sig.
Among pots	4213.012 ^a	17		6.916	.048
Experimental error	7.030	12	.580	1200.640	.054
TIMAR	4211012	11	382.92	6.916	.000
Sampling error	19.104	94	.232		
Total	1124.311	141			
Corrected Total	162.117	141			

C. Dependent variable: shoot dry weight

Source	SS	df	MS	F	Sig.
Among pots	3.049 ^a	17		32.348	0.00
Experimental error	.123	12	.01	7560.823	.000
TIMAR	4.554	11	.41	32.348	.000
Sampling error	6.08 E-02	94	.06		
Total	3.267	141			
Corrected Total	.165	141			

a. R Squared= .632 (Adjusted R Squared= .612)

E. Dependent variable: shoot length

Source	SS	df	MS	F	Sig.
Among pots	1875.061 ^a	17		5.611	.040
Experimental error	175.764	12	11.265	425.577	.065
TIMAR	1875.061	11	170.543	5.811	.045
Sampling error	87.061	94	1.030		
Total	26364.977	141			
Corrected Total	8182.122	141			

a. R Squared= .229 (Adjusted R Squared= .188)

Table III. Mean squares from analysis of variance of root and shoot Biochemical parameters for plants at different salt and calcium concentrations (0, 25, 50, 75, 100 mM NaCl & 5, 10 mM CaCl₂)

A. Dependent variable: shoot sugar

Source	SS	df	MS	F	Sig.
Among pots	6980.973 ^a	17		473.988	.000
Experimental error	17.218	12	1.420	98847.28	.000
TIMAR	6980.973	11	625.215	473.988	.000
Sampling error	274.012	94	2.620		
Total	195204.8	141			
Corrected Total	7154.985	141			

a. R Squared= .962 (Adjusted R Squared= .960)

C. Dependent variable: shoot total protein

Source	SS	df	MS	F	Sig.
Among pots	45271.971 ^a	17		1098.016	.000
Experimental error	143.103	12	11.910	277663.6	.043
TIMAR	45271.971	11	41150.245	1096.016	.000
Sampling error	778.229	94	8.270		
Total	1555373	141			
Corrected Total	46050.200	141			

a. R Squared= .993 (Adjusted R Squared= .982)

E. Dependent variable: shoot proline

Source	SS	df	MS	F	Sig.
Among pots	29787.326 ^a	17		103.832	.000
Experimental error	162.793	12	13.543	4671.751	.056
TIMAR	42977.326	11	3907.584	103.832	.012
Sampling error	547.368	94	5.820		
Total	2094451	141			
Corrected Total	352014.7	141			

a. R Squared= .846 (Adjusted R Squared= .838)

G. Dependent variable: lipid peroxidation

Source	SS	df	MS	F	Sig.
Among pots	143.543 ^a	17		41.590	.000
Experimental error	10.543	12	.854	4363.879	.052
TIMAR	265.765	11	24.090	41.590	.000
Sampling error	4.950	94	4.776E-02		
Total	167.209	141			
Corrected Total	15.856	141			

a. R Squared= .698 (Adjusted R Squared= .671)

B. Dependent variable: root fresh weight

Source	SS	df	MS	F	Sig.
Among pots	4811.083 ^a	17		31.997	
Experimental error	8.655	12	.722	1423.587	
TIMAR	3544.978	11	322.27	31.997	
Sampling error	5.088	94	.06		
Total	59.803	141			
Corrected Total	13.740	141			

D. Dependent variable: root dry weight

Source	SS	df	MS	F	Sig.
Among pots	2.966 ^a	17		.738	.658
Experimental error	.349	12	.03	6.662	.011
TIMAR	2.968	11	.269	.738	.658
Sampling error	.810	94	.503		
Total	62.952	141			
Corrected Total	78.879	141			

a. R Squared= .038 (Adjusted R Squared= .013)

F. Dependent variable: root length

Source	SS	df	MS	F	Sig.
Among pots	2148.724 ^a	17		166.203	.003
Experimental error	15.361	12	1.280	9688.731	.065
TIMAR	2148.724	11	192.255	166.203	.000
Sampling error	249.022	94	2.950		
Total	18535.991	141			
Corrected Total	2392.745	141			

a. R Squared= .698 (Adjusted R Squared= .893)

B. Dependent variable: root sugar

Source	SS	df	MS	F	Sig.
Among pots	15423.430 ^a	17		67.583	.032
Experimental error	463.754	12	39.580	141333.3	.056
TIMAR	12845.00	11	91.255	67.583	.001
Sampling error	128.520	94	1.375		
Total	13575.567	141			
Corrected Total	593.274	141			

a. R Squared= .782 (Adjusted R Squared= .770)

D. Dependent variable: root total protein

Source	SS	df	MS	F	Sig.
Among pots	14708.599 ^a	17		1080.313	.000
Experimental error	236.192	12	19.660	138076.6	.082
TIMAR	15708.599	11	1428.526	1080.313	.014
Sampling error	256.986	94	2.702		
Total	259762.9	141			
Corrected Total	14965.584	141			

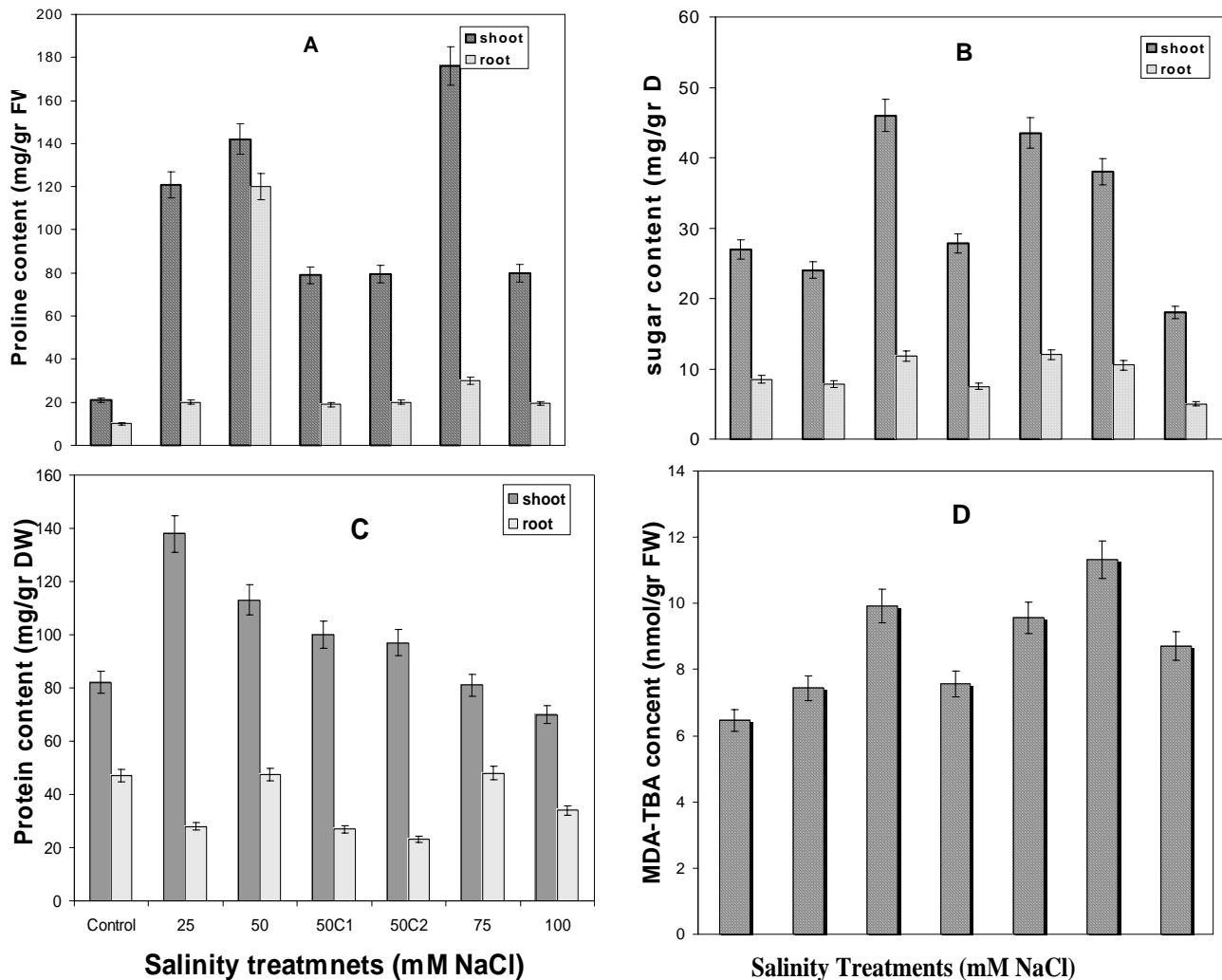
a. R Squared= .993 (Adjusted R Squared= .982)

F. Dependent variable: root proline

Source	SS	df	MS	F	Sig.
Among pots	112455.5 ^a	17		180.587	.021
Experimental error	2480.638	12	206.230	3127.960	.063
TIMAR	112455.5	11	10223.540	180.587	.000
Sampling error	153.853	94	1.624		
Total	352185.4	141			
Corrected Total	124209.4	141			

a. R Squared= .905 (Adjusted R Squared= .900)

Fig. 3. Comparison between mean of Biochemical parameters in control and salinity treated plants. 50C1 and 50C2 represent plants which were treated with either 5 or 10 mm Calcium respectively. A; proline content (mg.gr⁻¹ FW), B; reducible sugars content (mg.gr⁻¹ DW), C; protein concentration (mg.gr⁻¹ DW), D; MDA-TBA content (nmol.gr⁻¹ FW), E; chlorophyll content (mg.gr⁻¹ FW). Each data represent mean of three replicates ±SEM



dry weight loss (increased up to 61.5%) in the plants treated with 50 mM NaCl. At 100 mM NaCl treatment conditions, calcium did not improve the dry weight loss (Fig. 2A). Stem and leaf dry weight of NaCl stressed bean plants increased as the CaSO₄ concentration of the external solution was increased from 1 to 10 mM (Al-Rawahy *et al.*, 1992). Furthermore similar result have been reported on *Leucouena leucophala* and in *Citrus aurantium*, (Caines & Carol, 1999a; Poostini & Salmani, 2000). The results of CaCl₂ amelioration of NaCl inhibited root growth is in agreement with others studies on cotton, maize barley and tomato (Caines & Carol, 1999b).

Increase in free proline content at 25 to 75 mM NaCl treatments was noted (Fig. 3A) as a general response to salinity stress (Girija *et al.*, 2002). Proline may contribute to osmotic adjustment at cellular level. Proline act as enzyme protectant and stabilizes the structure of macromolecules

and organelles (Gennady & Kelvin, 2000). Increase in proline content under NaCl stress in this experiment was probably due to breakdown of proline-rich protein or *de-novo* synthesis of proline (Girija *et al.*, 2002). Addition of CaCl₂ to the salt-stressed seedlings caused decreased proline content (Fig. 3A). The cause of proline decrease could be due to an increase in proline oxidase activity. The 100 mM salinity treatment is a toxic concentration for salt sensitive plant and causes injury, which in turn inhibited metabolism and proline synthesis (Girija *et al.*, 2002).

The content of chlorophyll is related with increased growth. In *D. sophia* plants treated with 5 mM Ca²⁺, we observed a remarkable increase in Chl a and Chl T compared with control (Fig. 3E). Chloroplast membrane structure was protected and photosynthetic proteins were activated in calcium treated plants and plants grew well in this condition (Yeo *et al.*, 1991).

The MDA-TBA content was high in NaCl treatments when compared with control (Fig. 3D). In 50 mM NaCl with 5 mM Ca²⁺ treatment, we observed a remarkable decrease in MDA-TBA content when compared with control (Fig. 3E). Various oxidants cause Ca²⁺ influx into the cytoplasm and consequently into both the mitochondria and nucleus. It is reported that oxidants cause a rapid increase in Ca²⁺ concentration in cytoplasm (Blokhin *et al.*, 2003). The Ca²⁺ can be used as a signal molecule to respond to oxidant stimuli. It can activate and suppress proteins and gene transcription (Gennady & Kelvin, 2000).

CONCLUSION

Optimum concentration (5 mM) of calcium was able to improve salinity injury effects and cause increased plant resistance to salt stress and possibly is applicable in agricultural in saline soil.

REFERENCES

- Al-Rawahy, S.A., J.L. Stroehlein and M. Pessarakli, 1992. Dry matter yield and nitrogen-15, Na, Cl and K content of tomatoes under sodium chloride stress. *J. Plant Nutrition*, 15: 341–58
- Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.*, 24: 1
- Arvin, M.J. and N. Kazami-pour, 2002. Effects of salinity and drought stresses on growth and chemical and biochemical composition of 4 Onion (*Allium cepa*) Cultivas. Science and technology. *Agri. Resour.*, 5: 41–52
- Bates L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39:205–7
- Beck, D.P. and D.N. Munns, 1985. Effect of calcium on the phosphorous nutrition of *Rhizobium meliloti*. *Soil Sci. America J.*, 49: 334–7
- Blokhina, O.B., E. Virolinen and K.V. Fagerstedt, 2003. Antioxidant, oxidative damage and oxygen deprivation stress. *Ann. Rev. Bot.*, 91: 179–94
- Caines, A.M. and S. Carol, 1999a. Growth and nutrient composition of Ca²⁺ use efficient and Ca²⁺ use inefficient genotype of tomato. *Plant Physiol. Biochem.*, 37: 559–67
- Caines, A.M. and S. Carol, 1999b. Interactive effects of Ca²⁺ and NaCl salinity on the growth of two tomato genotypes differing in Ca²⁺ use efficiency. *Plant Physiol. Biochem.*, 37: 569–76
- Curtis, W.R., P. Wang and Humphrey, 1995. A. Role of calcium and differentiation in enhanced sesquiterpene elicitation from calcium alginate-immobilized plant tissue. *Enzyme Microb. Technol.*, 17: 554–7
- Gallardo, M., G.M. Carmen and A. Matilla, 1998. Involvement of calcium in ACC-oxidase activity from *Cicerarietinum* seed embryo nic axes. *Phytochemistry*, 50: 373–6
- Gennady, E. and D.J. Kelvin, 2000. Calcium and oxidative stress: From cell signaling to cell death. *Mol. Immunol.*, 38: 713–21
- Girija, C., B.N Smith. and P.M. Swamy, 2002. Interactive effects of sodium chloride and calcium chloride on the accumulation of proline and glycinebetaine in peanut (*Arachis hypogaea* L.). *Environm. Exp. Bot.*, 47:1–10
- Heath, R.L. and Packer, 1968. Photoperoxidation in isolated chloroplast and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.*, 125: 189–98
- Lowry, O.H., N.J. Roseberough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin-phenol reagent. *J. Biol. Chem.*, 32: 27–39
- Mengel, K. and E.A. Kirkby, 1987. *Principles of Plant Nutrition*. 4th edition. Kluwer Academic Publishers, Netherlands
- Poostini, K.S. and Z. Salmani, 2000. Effect of salinity on production and retransporting of dry mass in 2 triticum cultivars. *J. Agric. Sci. Iran*, 29: 11–6
- Prado, F.E., C. Boero, M. Gallardo and J.A. Gonzalez, 2000. Effects of NaCl on germination and soluble sugar of plants. *Bot. Bull. Acad. Sin.*, 12: 27–34
- Rengel, Z., 1992. The role of calcium in salt toxicity. *Plant Cell Environ.*, 15: 625–32
- Saboury, A.A. and F. Karbassi, 2000. Thermodynamic studies on the interaction of calcium ions with alpha-amylase. *Thermochim. Acta*, 362: 121–9
- Skorzynska-Polit, E., A. Tukendorf, E. Selstam and T. Baszynski, 1998. Calcium modified Cd effects on runner bean plants. *Environ. Exp. Bot.*, 40: 275–86
- Tyrman, S.D. and I.M. Skerrett, 1999. Root ion channels and salinity. *Sci. Hort.*, 78: 175–235
- Watkin Elizabeth, L.J., O.W. Graham and A.R. Glen, 1997. Calcium and acid stress interact to affect the growth of *Rhizobium leguminosarum* bv. *Soil Biol. Biochem.*, 29: 1427–32
- Yeo, A.R., K.S. Lee, P. Lizard, P.J. Boursier and T.J. Flowers, 1991. Short and long term effects of salinity on leaf growth in rice (*Oryza sativa* L.). *J. Exp. Bot.*, 42: 881–9
- Zekri, M. and L.R. Parsons, 1990. Calcium influences growth and leaf mineral concentration of citrus under saline condition. *Hortscience*, 25:784–6

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