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Improving salinity tolerance in chili by exogenous application of calcium and sulphur

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Abstract

Salinity drastically affects the productivity of vegetable crop plants. In this regard, Ca and S application can be helpful to improve the salinity tolerance in crop plants. Thus, an experiment was conducted to examine the improvement of salinity tolerance in chili by foliar application of calcium (Ca) and sulphur (S). The results showed that all the morphological (root/shoot fresh/dry weights and lengths, stem diameter, leaf area, number of leaves, number of flowers and fruits, fruit weight), physiological (photosynthesis rate, stomatal conductance) and biochemical (chlorophyll, root/shoot proline and glycine betain contents) parameters were reduced significantly ($P \leq 0.05$) under saline environment. The foliar application of Ca at 20 or 35 mgL^{-1} and S at 5 or 10g L^{-1}improved the morphological and physiological traits in chille plants subjected to salinity stress. It can be concluded that S at 10gL^{-1} and Ca at 35 mgL^{-1}can be used to enhance the salinity tolerance potential of chille plants.

Keywords: Chili, salinity, stress, foliar application, calcium, sulphur.

Introduction

Chilllis an important vegetable crop all over the globe including Pakistan. It is used both as salad and dried condiments. Chili is gaining a high status due to its high cost and demand in the market. In Pakistan, two of its varieties namely *Capsicum annum* and *Capsicum frutescens* are prominent. These hot types are source of digestive stimulant capsicin (Mehboob *et al*., 1998). It has 1.5% share in the GDP of Pakistan. It is grown on 73.8 thousand hectares with total production of 187.7 thousand tones and average yield of 1.7 tons per hectare (GOP, 2014).

Environmental stresses (abiotic and biotic factors) are the principal cause of crop losses worldwide tumbling average production of most vegetables by more than 50%. The brutality of environmental stress imposed on vegetable crops depends on climatic changes. Moreover, increasing temperatures, flooding, drought and salinity are the major restrictive factors in increasing and sustaining vegetable productivity (Bray *et al*., 2000). There is a dire need to do more scientific research on other vegetables which are affected by drastic effects of environmental extremes. Among abiotic stresses, salinity is a paramount important for crop growth and yield (Capiati *et al*., 2006).

Increased soil salinity has greatly hampered vegetable production particularly in irrigated crop lands which contribute about 40% of the world's food requirements. The production of vegetables including chili is highly sensitive to excessive salinity. High evapotranspiration due to dry and hot weather leads to considerable water loss thus leaving salt around the plant roots which obstructs the plant's capacity to uptake water. Physiological effects of salinity are the preliminary water deficit condition that occurs because of comparatively high solute ratios in the soil, which causes ion toxicity occurring from distorted K^+/Na^+ ratios and direct to enhance the Cl^- and Na^+ that are injurious to crop plants (Yamaguchi and Blumwald, 2005). Plant exposure to salinity is depicted in turgor loss, reduction of growth, wilting, epinasty and curling of leaf, reduced photosynthesis, changes in respiration, loss of cellular integrity, tissue necrosis and finally the plant death (Iqbal *et al*., 2015).

Every living organism requires proper nutrition for its better growth and development. To cope with various environmental stresses and to maintain their production potential. Higher plants have developed a wide range of adaptive mechanisms. It has been suggested by supportive evidences that basic mineral nutrition status of plants play a vital role in rising plant's tolerance to abiotic stresses (Marschner, 1995).

Calcium maintains the structure of cell wall and after the finding of calmodulin, it has become evident that role of calcium is not just restricted as a macronutrient but it is also

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a most important regulator of plant development and metabolism (Poovaiah and Reddy, 2000; Palta, 1990). It has been recommended that Ca is obligatory for recuperation from drought by triggering the membrane plasma enzyme ATPase. Calcium also has a role as calmodulin which manages various metabolic functions of plants and augment plant growth and development under water deficit conditions (Palta, 1990). Calcium plays an important role as calmodulin which controls the plant metabolic activities and enhances plant growth under drought condition (Palta, 1990). The modulation of S metabolism in plants would help in alleviating adverse effects of salinity as its metabolites control wide range of plant processes. The production of S-containing compounds through S metabolism is linked to antioxidant system in plants under salinity stress (Nazar *et al*., 2011; Ashfaque *et al*., 2014). Keeping in view the above facts, the present study was carried out to identify the potential morpho-physiological indicators of salinity stress in chillies and to enhance its salt tolerance potential by exogenous application Ca and S.

Materials and Methods

Chilli seeds were taken from Ayub Agricultural Research Instittue, Faisalabad, Punjab, Pakistan. The seeds were surface sterilized with 5% sodium hypochlorite solution. After surface disinfection the seeds were sown in earthen pots of 12 inch diameter. The pots were filled with fine sand as rooting medium and five seeds per pots were sown. When seedlings attained the height of 4 inches (40 days after germination) then these were transplanted in separate earthen pots (12″x9″) filled with fine sand (1.7 kg pot^{-1}) and three healthy seedlings per pot were maintained. Half strength Hoagland solution was applied as nutrition source. The nutrient solution, 30 mL per liter of distilled water, was applied as per plants requirement by observing the moisture of rooting media.

After two weeks of transplantation, the seedlings were exposed to salinity stress. Salinity level of 5 dS m^{-1} EC using NaCl salt was developed and following treatments were established as Control (without salinity), salinity (5 dS m^{-1} EC), salinity+ Ca1(20 mg L^{-1}), salinity + Ca2 (35 mg L^{-1}), salinity + S1 (5 gL^{-1}),salinity + S2 (10 gL^{-1}).

One week after salinity stress the plants were foliar-sprayed with solution of Ca and S. The plants were treated three times with three days interval with two different doses of Ca and S. Shoot and root length was measured with the help of meter rod from randomly selected plants from each treatment and then the average was calculated (Shahid *et al.*, 2011). Vernier Calliper was used to measure the diameter of the plant stem. Stem diameter was taken from the five randomly selected plants and their average calculated. The number of leaves were calculated from five randomly selected plants in each treatment and averaged. Leaves were obtained from randomly selected plants and leaf area was measured by the help of leaf area meter (CL-01 hansatech instrument kings lynn, UK). After measuring the area of the leaf, average was calculated. The number of flowers were counted from five randomly selected plants of each treatment. Number of fruits were counted from the five randomly selected plants of each treatment. After counting the number of fruits, average data was calculated. Fruits were collected from selected plants and weighed with the help of weighing balance (KERN and Sohn GmbH, Germany).

The fresh weights of root from five randomly selected plants was measured. Afterwards, the roots were kept in oven for 72 hours at 65^{0}C for drying and then the dry weights were taken with the help of an electrical balance.

Photosynthesis rate and stomatal conductance was estimated with the help of portable infra-red gas analyzer (CI-340, CID Bio-Science, USA). Observations were made on third leaf from the top of plant that was fully expanded and the youngest in the morning time from 10:00 a.m. to 1:00 a.m. The adjustments were taken as follows: leaf chamber temperature (25-28^{o}C), leaf surface area (6.2 cm^{2}), ambient CO_2 concentration (371 µmol mol^{-1}), ambient pressure of chamber (97.9 kPa), At leaf surface photosynthetically active radiations (PAR) maximum up to(770 µmol m^{-2} s^{-1}), gas flow rate of leaf chamber (296 mL min^{-1})and flow rate of molar gas in chamber (400 µmol s^{-1}).Chlorophyll contents were measured following the method of Arnon (1949).

The proline contents in shoot and root of chili plant was deliberated by following the method of Bates *et al.* (1973). Shoot and root glycine betaine (GB) contents were determined by the method of Grieve and Grattan (1983).

Flame photometer was used for analyzing the leaf Na^+ contents in digested leaf samples of chillies. A graded series of Na^+ working standard prepared and standard curve was drawn. The values of Na^+ from flame photometer were compared with the standard curve and calculated the original quantities. The experiment was laid out in factorial arrangement under completely randomized design (CRD) with five replications. All the data were analyzed statistically via analysis of variance (ANOVA) and the least significant difference (LSD) test to determine differences between treatments (Steel *et al*., 1997) using the “Statistix 8.1” statistical computer software package.

Results

Shoot and root length

The applied NaCl salinity reduced the shoot length of chillies (Figure 1a) but remained statistically at par with unstressed control. The minimum shoot length (50.8 cm) was observed in plants grown under saline conditions, while 52.4 cm shoot length was recorded in case of control. However, the foliar applied Caat 20 or 35 mg L^{-1} and Sat 5 or 10g L^{-1}) significantly increased shoot length thus conferring increase tolerance to chillies against salinity stress. Among the sprayed plants, the highest shoot length was recorded in plants applied with 35mgL^{-1} Ca and 10gL^{-1} S with the mean value of 83.3 and 92.3 cm, respectively. This showed that foliar application resulted in enhanced shoot length in chillies and thus enhanced the salinity tolerance in chillies (Figure1a). Regarding the root length, again non-significant difference in root length was recorded in plants grown under saline and non-saline conditions and foliar application of Ca and S (Figure 1b) produced significant differences from saline and normal conditions. Under saline conditions, root length was found lower (8.7 cm) compared to control (9.8 cm), among the sprayed plants, the highest root length was recorded in plants supplied with 35 mgL^{-1} Ca and 10gL^{-1} S with the mean value of 14.0 and 17.4 cm, respectively. Whereas, plants sprayed with Ca at 20 mgL^{-1} and S at 5 gL^{-1} had root length of 12.26 and 11.06 cm, respectively.

Leaf area and number of flowers

Salinity significantly reduced the leaf area in chillies (Figure 3a). Minimum leaf area (3.1 cm^2) was observed in saline conditions and4.5cm^2 was found in case of control. The highest leaf area was recorded in plants applied with Ca at 35mgL^{-1} and S at 10g$L^{-1}$was 14.9 and 12.1 cm^2, respectively. The chili plants sprayed with Ca at 20 mgL^{-1} and S at 5 gL had leaf area of 6.7 and 10.1 cm^2, respectively. Salinity also reduced the number of flowers in chili plants as compared to control (Figure 3b). Statistically lower number of flower (49.6) were observed in saline conditions as compared to control (66.2), while the highest number of flowers were recorded in plants treated with Ca at 35mgL^{-1} and S at 10gL^{-1} with the mean value of 155.6 and 189.8, respectively.

Number of fruits and fruit weight

The presence of salinity in the growth medium significantly reduced the number of fruits in chili plants. The minimum (15.2) number of fruits were produced in plants grown under saline conditions. The number of fruits were

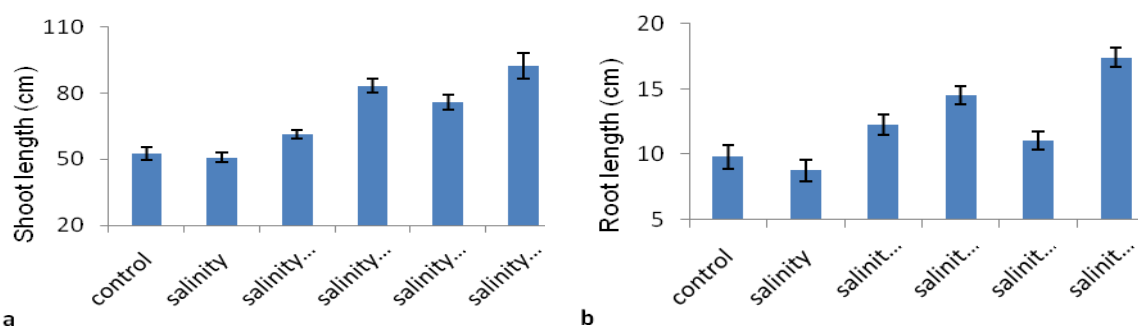

Figure 1: Effect of foliar applied Ca and S on (a) shoot length and (b) root length of chillies under saline conditions
(Each value is a mean ± SE; n = 5 statistically significant at $p \leq 0.05$)

Stem diameter and number of leaves

Stem diameter was reduced significantly under saline condition (5 dS m^{-1}) as compared to unstressed control (Figure 2a). The least stem diameter (7.2 mm) was noted in saline conditions while it was 8.9 mm in control. Among the sprayed plants, the significantly higher stem diameter (13.1 and 14.1mm) was recorded in plants applied with Ca at 35mgL^{-1} and S at 10gL^{-1}. Similarly, salinity reduced the number of leaves in chillies (Figure 2a), minimum number of leaves 106.6 were produced in saline conditions as compared to control (115.8). Among the sprayed plants, the highest number of leaves were recorded in plants applied with Ca at 35mgL^{-1} and S at 10gL^{-1} with the mean value of 338.4 and 376.0 leaves, respectively.

increased and highest in plants treated with Ca 35mgL^{-1} and S at 10g$L^{-1}$with the mean value up to 47.2 and 59.2, respectively (Figure 4a). Salinity reduced the fruit weight of chillies to 61.4 g and 77.6 g in control, while application of Caor Sat 35mgL^{-1}or 10gL^{-1} increased fruit weight with the mean value of 176 g and 244 g, respectively. The plants sprayed with Ca at 20 mgL^{-1} and S at 5 gL^{-1} had fruit weight of 114.6 g and 171.2 g, respectively, (Figure 4b).

Photosynthesis rate and stomatal conductance

Summative salinity level of 5 dS m^{-1} reduced the photosynthesis rate (11.2 µmol CO_2 $m^{-2}s^{-1}$) in chillies as compared to control (14.2 µmol CO_2 $m^{-2}s^{-1}$) even then

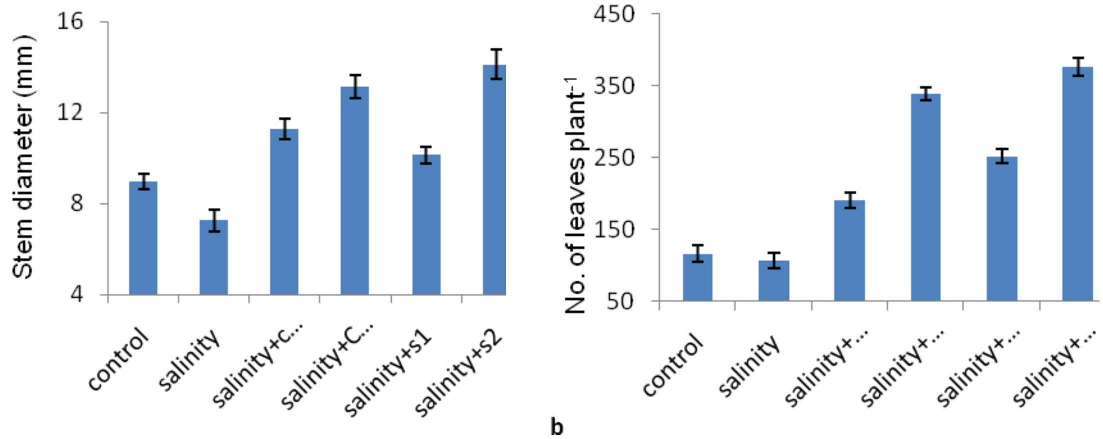

Figure 2: Effect of foliar applied Ca and S on (a) stem diameter and (b) number of leaves of chillies under saline conditions

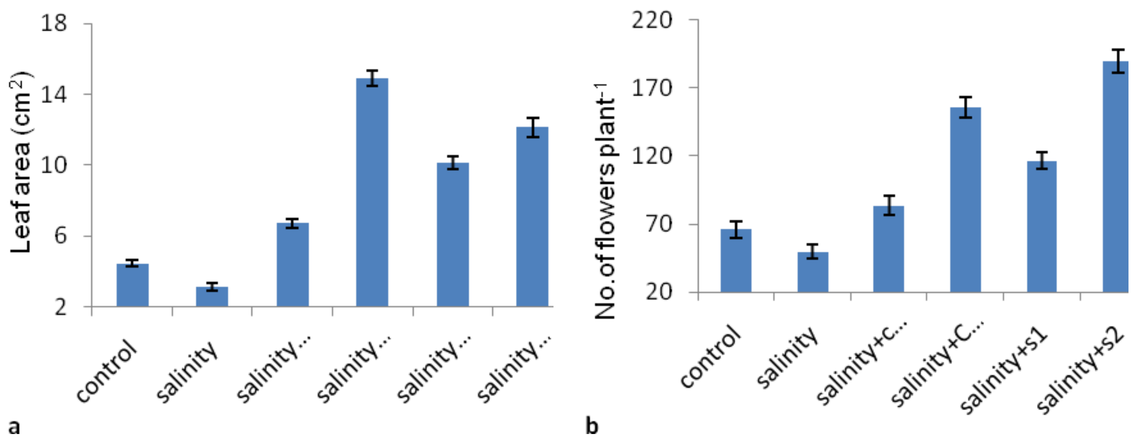

Figure 3: Effect of foliar applied Ca and S on (a) leaf area and (b) no. of flowers of chillies under saline conditions

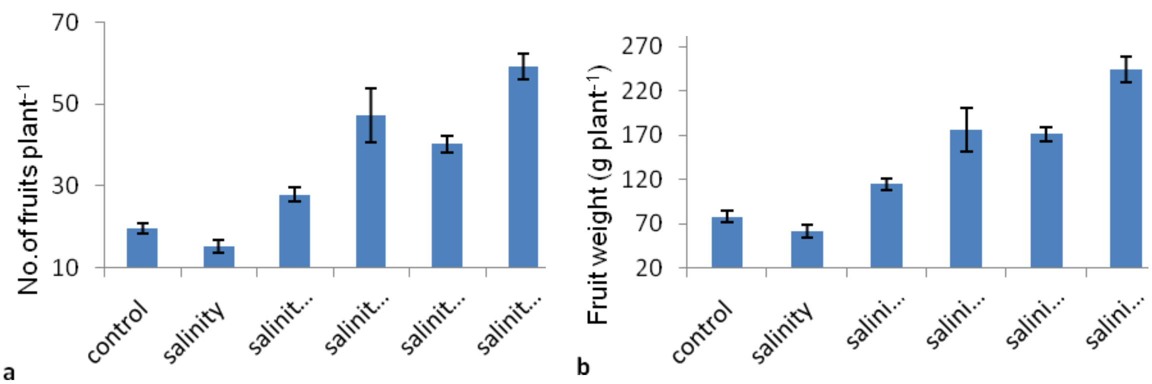

Figure 4: Effect of foliar applied Ca and S on (a) number of fruits and (b) fruit weight of chillies under saline conditions

(Each value is a mean ± SE; n = 5 statistically significant at $p \leq 0.05$).

remained statistically similar to control (Figure 5a). Foliar application of Ca and S increased rate of photosynthesis in chili plant and the highest photosynthesis rate was recorded in plants treated with Ca at 35mgL^{-1} and S at 10 gL^{-1} with the mean value of 26.8 and 29.0 µmol CO_2 m^{-2}s^{-1}, respectively. However, stomatal conductance in chillies reduced significantly under saline environment as compated to control (Figure 5b). Stomatal comductance up to 24.8mmol m^{-2} S^{-1} under salinity and 32.9 mmol m^{-2}s^{-1} in case of control was observed. Following the foliar application of Ca and S the highest stomatal conductance was recorded in plants applied with 35mgL^{-1} Ca and 10gL^{-1} S with the mean value of 51.4 and 56.5mmol m^{-2}s^{-1}, respectively (Figure 5b).

Shoot and root proline contents

Salinity caused a significant increase in proline contents in shoot of chili plants over control (Figure 7a). Salinity elicited the greater shoot proline content up to 1.14µmol g^{-1} as compared to control (0.81µmol g^{-1}). Exogenous foliar application of Ca at 20 or 35 mgL^{-1} and S at 5 or 10gL^{-1} markedly enhanced the proline contents in shoots thus conferring increase in tolerance of chillies against salinity stress. The highest proline production was recorded in plants supplied with higher doses of Ca at 35mgL^{-1} and Sat 10gL^{-1} with the mean value of 1.48 and 1.47 µmol g^{-1}, respectively. Chili plants sprayed with lower concentrations of Ca at 20 mgL^{-1} and S at 5 gL^{-1} produced

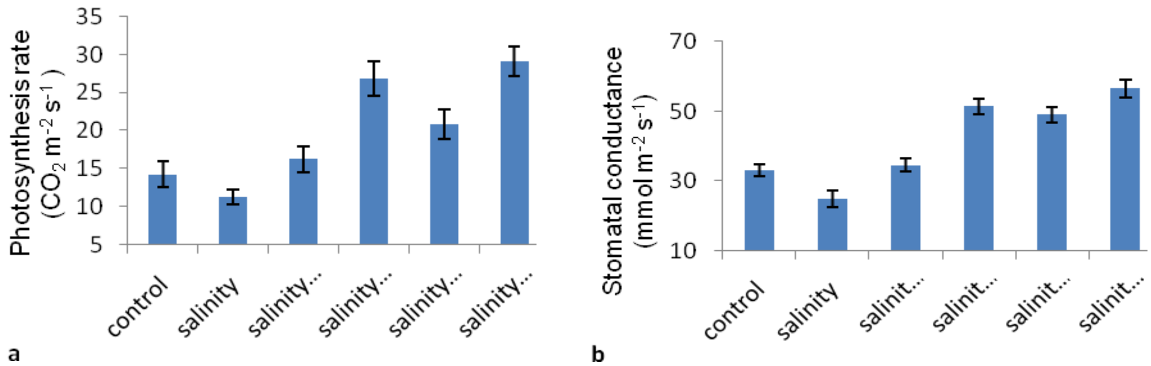

Figure 5: Effect of foliar applied Ca and S on (a) photosynthesis rate and (b) stomatal conductance of chillies under saline conditions (Each value is a mean ± SE; n = 5 statistically significant at $p \leq 0.05$).

Total chlorophyll contents and leaf Na^+ concentration

The results indicated that salinity significantly reduced the total chlorophyll content over control (Figure 6a). Under saline and control conditions, the cholorphyll contents were found 1.01 and 1.49 mg g^{-1}leaf fresh weight, respectively. Increase in total chlorophyll content was recorded in plants treated with 35mgL^{-1} Ca and 10gL^{-1} S with the mean value of 1.91and 2.16 mg g^{-1}leaf fresh weight, respectively, under saline conditions. Highly significant ($P \leq 0.05$) differences were recorded in leaf Na^+ content in chillies under normal, saline condition and plants foliar sprayed with Ca and S (Figure 6b). Under salinity, significantly high Na^+ content in chili leaves (0.96 µmol g^{-1}) as compared to control (0.58 µmol g^{-1}) as well as plant foliar supplied Ca or S. Following the foliar application of Ca at 20 and 35 mgL^{-1} and S at 5 and 10gL^{-1}, the lowest leaf Na^+ content was recorded in plants treated with 35mgL^{-1} Ca and 10gL^{-1} S with the mean values of 0.55 and 0.40 µmol g^{-1}, respectively.

1.19 and 1.26 µmol g^{-1}proline, respectively (Figure 7a). Salt-stress enhanced the root proline contents by 0.47 µmol g^{-1} as compared to control 0.3 µmol g^{-1}, however, both Caat 20 or 35 mgL^{-1} and Sat 5 or 10gL^{-1} foliar application further increased the proline production in the roots of salt-stressed chili plants. The highest proline contents about 1.26 and 1.32 µmol g^{-1} were recorded in plants treated with Ca at 35mgL^{-1} and S at 10gL^{-1}, respectively (Figure 7b).

Shoot and root glycine betaine

Salinity increased the shoot glycine betaine (GB) production with respect to the control. Exogenous application of Caat 20 or 35 mgL^{-1} and Sat 5 or 10 gL^{-1}further increased the level of GB in shoot (Figure 8a). Among the treated plants, the highest shoot GB production was recorded in plants applied with 35mgL^{-1} Ca and 10gL^{-1} S with the values of 2.03 and 2.38 µmol g^{-1}, respectively. Salt stress enhanced root GB by 1.01 µmol g^{-1} as compared to control (0.83 µmol g^{-1}). Whereas the foliar sprayed plants, the highest GB production was recorded in plants

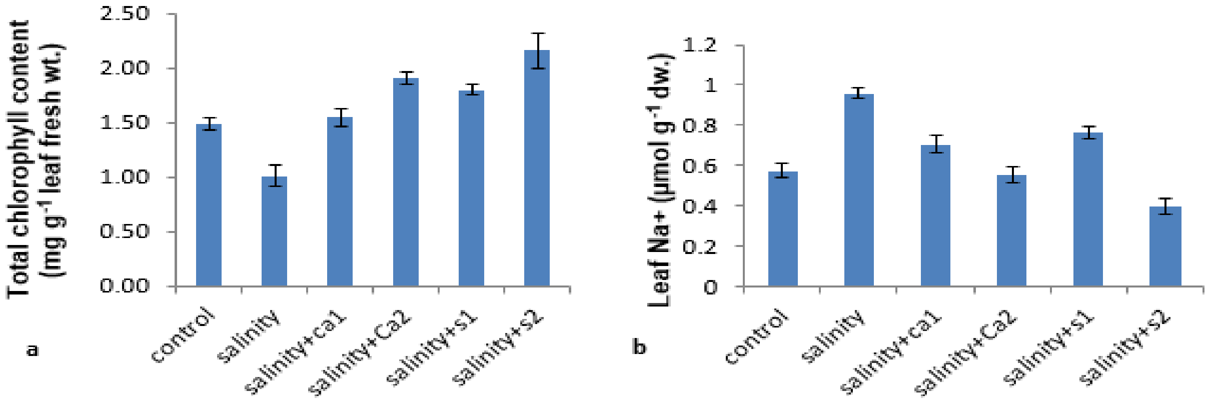

Figure 6: Effect of foliar applied Ca and S on (a) chlorophyll content and (b) leaf Na^+ of chillies under saline conditions (Each value is a mean ± SE; n = 5 statistically significant at $p \leq 0.05$)

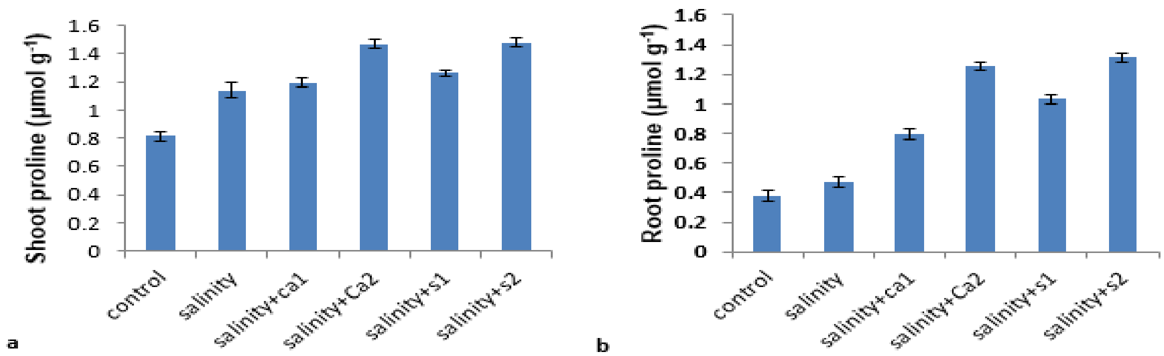

Figure 7: Effect of foliar applied Ca and S on (a) shoot proline and (b) root proline of chillies under saline conditions (Each value is a mean ± SE; n = 5 statistically significant at $p \leq 0.05$).

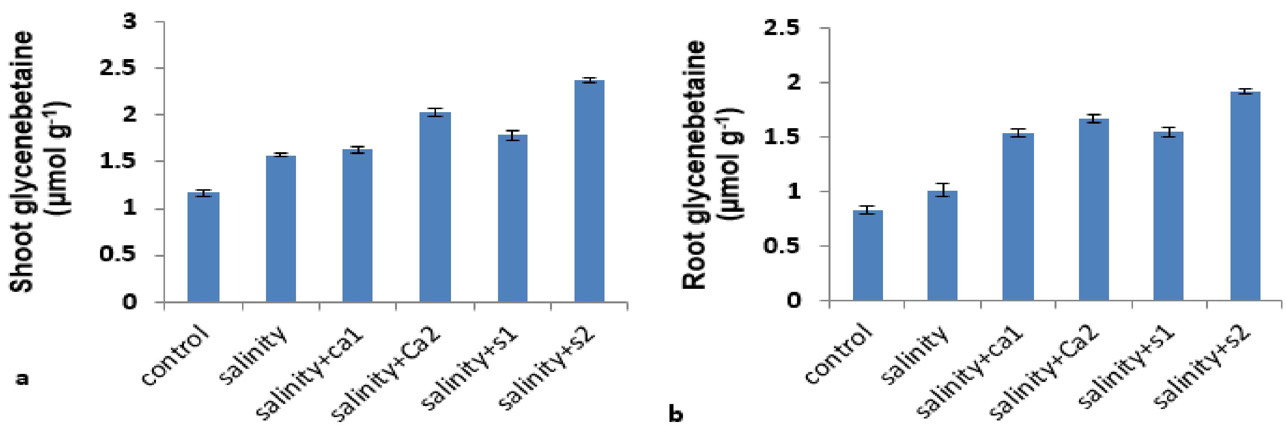

Figure 8: Effect of foliar applied Ca and S on (a) shoot glycine betaine and (b) root glycine betaine of chillies under saline conditions (Each value is a mean ± SE; n = 5 statistically significant at $p \leq 0.05$).

with Ca at 35mgL^{-1} and S at 10gL^{-1} with the values of 1.67 and 1.92 µmol g^{-1}, respectively. The plants sprayed with Ca at 20 mgL^{-1} and S at 5 gL^{-1} produced 1.54 and 1.55 µmol g^{-1} root GB, respectively (Figure 8b).

Discussion

In the present study, a considerable variation in morphological, physiological and biochemical traits of chili plants was noted under saline conditions (5 dS m^{-1} EC). Therefore, need to supply higher quantities of the required minerals and nutrient to plants increases many folds under stress environment. But under saline environment, the availability of nutrients decreases due to binding with soil particles and reduced availability of water in root zone. These plant nutrients not only contribute in growth and development of plants but also take part in tolerance mechanisms against various kinds of abiotic stresses including salinity. This is being widely accepted that nutrients play an important role in inducing resistance in plants against various abiotic stresses (Shahid *et al*., 2015).

In the present trial, Ca and S, two important plant nutrients required for proper growth and development were applied to the chillies plants grown under saline environments. Chili plants grown in saline soils were treated with foliar applied Ca at 20or 35 mgL^{-1} and S at 5 or10 $gL^{-1}$to help the plants in coping with saline stress. Plant's reduced response to salinity impacts might be due to the fact that Caplays an important role in cell wall structure and essential plant metabolisms (Poovaiah and Reddy, 2000). It also takes part in healing injured plant tissue and promote recovery from injury and adjustments to stresses (Palta, 1990). Similarly**,** S played an important role in combating salinity stress by maintaining redox state of cell by enhanced S metabolism in plant cells. It is generally assumed that S is essential nutrient for plants under abiotic stress due to its role in metabolic compounds such as amino acids like cysteine, methionine, sulfolipids and proteins (Nocito *et al*., 2007; Khan *et al*., 2013). The S containing compounds are linked to antioxidant system in plants which play an important role in alleviating salinity stress (Nazar *et al*., 2011; Ashfaque *et al*., 2014).

In the present study, it was found that root and shoot lengths, photosynthesis rate, stomatal conductance, chlorophyll content, stem diameter, leaf area and number of leaves, number of flowers and fruits, fruit weight, root and shoot weights were markedly reduced except root and shoot proline and GB contents, under saline stress. This can be linked to the previous findings that salinity reduced both nutrient uptakes by the roots and also limited mineral transport from the roots to the shoots due to reduced transpiration rates and damaged membrane permeability (Alam, 1999). The presence of extreme ratios of Na^+/K^+, Na^+/Ca^{2+}, ions under salinity stress also create the toxic and imbalanced ionic environment which reduce plant growth (Grattan and Grieve, 1999) and finally cause plant cell death (Bhardwaj and Yadav, 2012).

Salinity leads to the accelerated production of ROS (Asada, 2006) which destroy the cellular membranes and inhibit the photosynthetic carbon fixation (Apel and Hirt, 2004). These previous findings also supported the decline in photosynthesis rate in present study under saline conditions (Naz *et al*., 2015). Reduction in photosynthesis in present experiments was also supported by previous researchers. The stomatal closure under saline conditions resulted in low photosynthesis rate (Bota *et al*., 2004). It has been shown that the decline in photosynthesis under stress is primarily due to CO_2 deficiency, as the photochemical efficiency could be brought back to normal after a fast transition of leaves to an environment enriched in CO_2 (Meyer and Genty, 1998; Naz *et al*., 2015).

It was observed that the two foliar sprays i.e. Caat 20 or 35 mgL^{-1} and S at 5 or 10gL^{-1} enhanced all the morphological and physiological traits studied, which indicates the improvement in salinity tolerance potential of tested chili plants. The application of S at 10gL^{-1} S followed by Ca at 35 mgL^{-1}can significantly ($P \leq 0.05$) increase the salinity tolerance in chili plants. Role of Ca in conferring tolerance against abiotic stresses has been linked to ATPase activation which pumps back the nutrients that were lost during cell damage. The Ca also plays a role as calmodulin which controls the plant metabolic activities and enhances the plant growth under stressed condition (Palta, 1990).

Improvement in all the morphological and physiological traits studied due to exogenous application of Ca is due to involvement of Ca in various important metabolic activities like membrane structure and stomatal function, cell division and cell wall synthesis, direct or signaling roles in systems involved in plant defense and repair of damage from biotic and abiotic stress and rates of respiratory metabolism and translocation (McLaughlin and Wimmer, 1999; Rahman *et al.*, 2014). Similar results were reported by He and Cramer (1992) who depicted that Cais the only ion for which the change in concentration is highly correlated with the relative salt resistance of *Brassica* species. The distribution of Ca^{2+} in the shoots decreased greatly in the salt sensitive plants under salt stress suggesting that the ability of plants to retain Ca^{2+} is associated with their resistance to salinity (Nocito *et al*., 2007; Khan *et al*., 2013).

Sulfur-containing metabolites, amino acids (cysteine and methionine), vitamins (biotin and thiamine), thioredoxin system, glutathione lipoic acid and glucosinolates have potential to promote or modify physiological and molecular processes under salinity stress in plants. Thus, modulation of S metabolites production could alter physiological and molecular mechanisms to provide tolerance against salinity (Khan *et al*., 2014), hence, in the present study, S palyed an important role in conferring salt-tolerance in chillies.

Conclusion

The results showed that all the morphological, biochemical and physiological attributes varied to a considerable extent under salinity stresses. While the accumulation of compatible osmolytes i.e. proline and GB was increased in response to saline stress. It was observed that the two foliar sprays i.e. Caat 20 or 35 mgL^{-1} and Sat 5 or $10gL^{-1}$ enhanced all the morphological and physiological traits. Thus, it can be concluded that foliar application of both nutrients may significantly ($P \leq 0.05$) enhance the salinity tolerance in chilli plants by improving the growth and physiological metabolisms.

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