

## ROLE OF SILICON IN MORPHO-PHYSIOLOGICAL, IONIC AND BIOCHEMICAL ACCLIMATION OF MUNGBEAN CHALLENGED WITH SALT STRESS

Ghulam Hassan Abbasi<sup>1,\*</sup>, Amjad Hussain<sup>2</sup>, Muhammad Ali<sup>1</sup>, Moazzam Jamil<sup>1</sup>, M. Anwar-ul-Haq<sup>3</sup>, Zaffar Malik<sup>1</sup>, Shafaqat Ali<sup>4</sup> and Babar Javaid<sup>1</sup>

<sup>1</sup>Department of Soil Science, University College of Agriculture & Environmental Sciences, The Islamia University of Bahawalpur; <sup>2</sup>Director R&D, Higher Education Commission, Islamabad

<sup>3</sup>Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad

<sup>4</sup>Department of Environmental Sciences, Government College University, Faisalabad

\*Corresponding author's e-mail: abbasiuaf@yahoo.com

Silicon (Si) is generally considered as a contributing constituent for plant resistance against abiotic stresses. However, the mechanism underlying the role of Si in alleviating salt stress in mung bean (*Vigna radiata* L.) is still poorly understood. Present study deals with response of mung bean to Si application in relation to growth, gas exchange, ionic and antioxidant attributes under saline condition. Two mung bean varieties Azri-2006 and Anmol were grown for four weeks in half strength Hoagland solution. The growth medium was salinized with 03 levels of salinity (control, 35 mM NaCl L<sup>-1</sup> & 70 mM NaCl L<sup>-1</sup>) and one level of Si (1 mM) alone and in combined form by using NaCl and Na<sub>2</sub>SiO<sub>3</sub> salts, respectively. Salinity stress significantly reduced the plant tissue (roots and shoots) biomass, Relative Water Contents (RWC), Membrane Stability Index (MSI), gas exchange attributes and K/Na ratio and increased the plant tissue concentration of malondialdehyde (MDA). Activities of antioxidant enzymes (SOD, POD, APX and GR) of both varieties were reduced due to salt application and this effect was more prominent at high salt level (70 mM NaCl L<sup>-1</sup>). Addition of Si alleviated salt toxicity is more conspicuous in ANMOL relative to Azri-2006 as demonstrated by increasing RWC, MSI, K/Na ratio and activities of SOD, POD, APX and GR. However, the activity of CAT remains unchanged due to Si application under both saline and non-saline condition in both varieties. Our results depict that Si-mediated alleviation of salt stress is due to enhancement in RWC and photosynthetic capacity, maintaining ions homeostasis and most importantly the increased concentration of antioxidant enzymes SOD, POD, APX and GR in plant tissues.

**Key Words:** Silicon, Salinity, RWC, MSI, K/Na ratio and Antioxidant enzymes.

### INTRODUCTION

Mung bean (*Vigna radiata* L.) is an ecologically prime food grain legume and a best dietary source of protein. It contains 24.5 % proteins, 59.9 % carbohydrates, 1.2 % fat and 4.5% fiber contents along with essential macro nutrients like phosphorus 340 mg 100 g<sup>-1</sup> and calcium 118 mg 100 g<sup>-1</sup> of seeds (Khattak *et al.*, 2008; Aasim *et al.*, 2019). Mung bean cultivation is about 218,000 ha with annual production of 138.46 thousand tons in Pakistan (Economic survey of Pakistan, 2017). It has diverse capability to magnify physical, chemical and biological properties of soil which increases its prominence in achieving sustainable agriculture production in arid region especially stress condition (Ahmed, 2009).

Salinity is the most atrocious environmental aspect restricting the productivity of mung bean in arid and semiarid regions of the world (Saha *et al.*, 2010). Due to rapid salinization, the cultivated area is continuously transforming into saline and there is a fear of 50% land loss up to 2050 globally (Ahanger *et al.*, 2017). Mung bean is generally

considered as salt sensitive crop and salt stress imposes substantial adverse effects on the performance, biochemical changes and physiology of crop and results in reduction in seed germination (Rabie, 2005), fresh and dry biomass (Ahmed, 2009), photosynthesis (Koyro, 2006), influence accumulation of mineral nutrients (Naher and Alam., 2010). Biochemical changes occur when plants are exposed to the harmful effects of reactive oxygen species (ROS) under saline condition which eventually leads to growth arrest and metabolic damage (Qadir *et al.*, 2014). The activities of antioxidant enzymes (CAT, SOD and POD) and non-enzymatic antioxidants (tocopherols, ascorbate and phenolic compounds) have been increased which provide safeguard to the plants from damages caused by salt induced reactive oxygen species (Li *et al.*, 2011).

Silicon (Si) is considered as an important constituent in soil and efficaciously neutralizes the hazardous effects of different stresses like salinity, temperature and various metal stress on plants (Zhu and Gong, 2014). Although, Si is not deliberated as an indispensable plant nutrient but play an important role

in plant growth and development especially under stress condition (Hodson *et al.*, 2005). Si is considered as an important component of cell wall and can act as a resistant material parallel to lignin in some plants like rice and barley (Liang *et al.*, 2007). Many studies have reported ameliorating effect of Si against salt stress on barley (Liang *et al.*, 2003), maize (Moussa, 2006), spinach and tomato (Gunes *et al.*, 2007), wheat (Tuna *et al.*, 2008), grape vine root stock (Liu *et al.*, 2015) and in Cucumber (Zhu *et al.*, 2004). Taking into account the above stated facts, current project was planned to explore the ameliorating role of silicon application on morpho-physiological, ionic and biochemical attributes in mung bean varieties under saline condition.

## MATERIALS AND METHODS

**Seed Material and Growth Conditions:** The seeds of two mung bean varieties were collected from the market. Seeds of both varieties were sown in sand culture and nursery was raised in ambient light and temperature. At two leaf stage, uniform seedlings were transferred to thermopole sheet floating on water in iron tub of 100 liters capacity. Plants were provided complete nutrition by using half strength Hoagland solution (Hoagland and Amon, 1950). NaCl was used to develop three levels of salinity while  $\text{Na}_2\text{SiO}_3$  were used as a source of silicon ( $1\text{mM L}^{-1}$ ). The treatments include control ( $T_1$ ), 35mM NaCl ( $T_2$ ), 70mM NaCl ( $T_3$ ), 1mM Si ( $T_4$ ), 35 mM NaCl+ 1mM Si ( $T_5$ ), 70 mM NaCl+ 1mM Si ( $T_6$ ). Each treatment was consisted of five replicates and arranged according to CRD. Oxygen was supplied to plants through aeration pump. pH of the solution was noted on daily basis and maintained at  $6 \pm 0.5$  with NaOH or HCl as required. Growth, physiological, ionic and biochemical attributes were taken after four weeks of stress.

### Physiological Attributes

**Relative Water Contents:** RWC was measured by adopting the procedure given by Lazcano-Ferrat and Lovatt (1999) after taking fresh, dry and turgid weight of fully expended youngest leaf of plants.

$\text{RWC}\% = (\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{dry weight}) \times 100$

**Membrane Stability Index:** MSI was measured by adopting the method demonstrated by Sairam and Saxena (2000). 100 mg leaf sample was heated at  $40^\circ\text{C}$  in 10 ml water for thirty minutes in water bath and EC ( $C_1$ ) was measured through EC meter. Then same sample was again heated at  $100^\circ\text{C}$  for ten minutes in water bath and EC was observed as  $C_2$ . The value of MSI was measure by using the following formula

$\text{MSI}\% = [1 - (C_1 / C_2)] \times 100$

**Biochemical Attributes:** 0.5 g tissue sample was homogenized in 10 ml of an extracting buffer (50 mM phosphate, pH 7.8, 1 mM EDTA, 1 g polyvinylpyrrolidone (PVP) and 0.5 % Triton X-100). The prepared homogenate was centrifuge at 12,000 rpm for 20 minutes and the

supernatant used for the analysis of SOD, POD, APX, CAT and GR. All operations were carried out at  $0-4^\circ\text{C}$  (Nahar *et al.*, 2015). MDA contents were measured by using the method of Zhou *et al.* (2004). Leaf material (1.0 g) was homogenized in 0.1 % trichloroacetic acid (TCA) and mixed with thiobarbituric acid (TBA) and heated at  $100^\circ\text{C}$  for 20 minutes. The absorbance was measured at 532 and 600 nm.

**Gas Exchange Attributes:** Measurements of photosynthetic rate, stomatal conductance, transpiration rate and internal carbon dioxide ( $\text{CO}_2$ ) concentration were made by taking fully expended youngest leaf from each treatment by using Infrared Gas Analyzer (LCA-4 ADC, Hoddesdon, UK).

**Ionic Attributes:** Sodium ( $\text{Na}^+$ ) and potassium ( $\text{K}^+$ ) concentration were determined by taking dried root and shoot material and digested with hydrogen peroxide and sulphuric acid according to the method explained by Wolf (1982) by using Flame Photometer.

**Statistical Analysis:** Data reported in this study were analyzed by using statistical package Statistics 8.1 (Statistics, IL USA). The bar in the graph show the average values of 5 replicates and error bars are the standard deviations. The means were compared by least significant difference (LSD) test to quantify and evaluate the source of variation at 5% probability (Steel *et al.*, 2007).

## RESULTS

**Interactive effect of salt stress and silicon application on growth attributes:** The data regarding growth attributes of mung bean varieties under saline and non-saline condition (Fig. 1) depict that salt stress caused reduction in root and shoot length, fresh weight and dry weight of both mung bean varieties used in the experiment. However, this reduction was maximum when NaCl was applied at  $70\text{mM L}^{-1}$  relative to low stress level ( $35\text{mM L}^{-1}\text{NaCl}$ ). Moreover, the impact of salinity differed significantly among both varieties which were concluded by more reduction in growth attributes of variety AZRI-2006 compared to ANMOL. Addition of Si in the growth medium markedly changed the growth behavior of both mung bean varieties and prominent effect of Si application under saline condition was observed at low stress level ( $35\text{mM L}^{-1}\text{NaCl}$ ). Additionally, the response of mung bean variety ANMOL towards Si application was better than AZRI-2006.

**Interactive effect of salt stress and silicon application on physiological attributes:** Exalted salt application proved deleterious for RWC and MSI in mung bean varieties as depicted in Fig. 2. Under high salt level, 60 % reduction in plant RWC and MSI was observed which was partially recovered under low and high salt stress with the addition of Si in the growth medium but this recovery was more prominent at low stress ( $35\text{mM L}^{-1}\text{NaCl}$ ) compared to high stress ( $70\text{mM L}^{-1}\text{NaCl}$ ). Both varieties also showed the significant variation in MDA contents under saline and non-

saline condition. The mung bean variety AZRI-2006 showed more accumulation of MDA contents, lower RWC and MSI as compared to ANMOL which depicts its more sensitivity towards salinity.

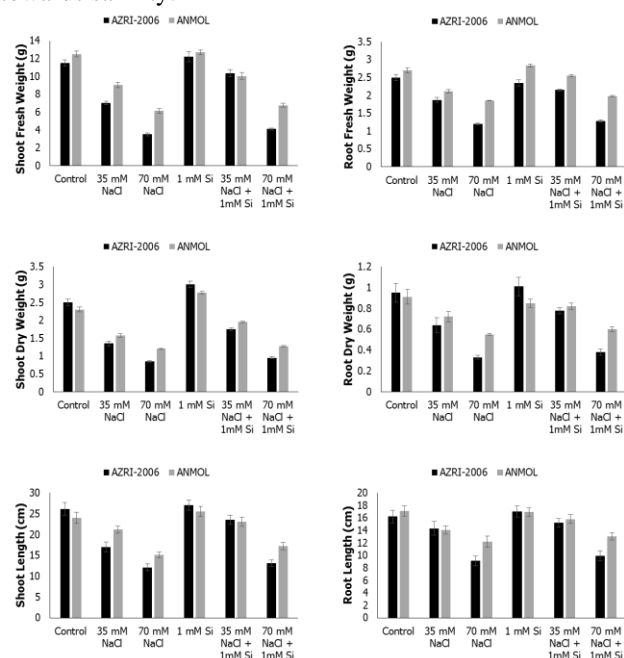


Figure 1. Effect of silicon on growth attributes of two mung bean seedlings under salinity stress. The bars in the graph show the average values of 5

replicates and the error bars are the standard deviations.

**Interactive effect of salt stress and silicon application on gas exchange attributes:** Silicon and salt stress application on gas exchange attributes of two mung bean seedlings are depicted in Fig.3. Relative to control, NaCl addition caused remarkable reduction in photosynthetic rate, transpiration rate and stomatal conductance at both level of salinity in both mung bean varieties. However, salinity had no impact on internal  $\text{CO}_2$  concentration in both varieties. Furthermore, the higher reduction in all gas exchange attributes except internal  $\text{CO}_2$  concentration was exhibited by mung bean variety AZRI-2006 especially at higher salt regime (70 mM  $\text{L}^{-1}$  NaCl) relative to ANMOL. Silicon nutrition induced ameliorating effect against salt stress and a marked increase in photosynthetic rate, transpiration rate and stomatal conductance was noted under Si application at low level of salt stress (35 mM  $\text{L}^{-1}$  NaCl). Si application under saline and non saline conditions showed no significant impact on internal  $\text{CO}_2$  concentration in both varieties. Mung bean variety ANMOL performed significantly better and showed improved values of photosynthetic, transpiration rate and stomatal conductance which depicts its more tolerance towards salinity.

**Interactive effect of salt stress and silicon application on ionic attributes:** Increased salt concentration in the growth medium caused notable increase in  $\text{Na}^+$  concentration (Fig. 4) and exhibited remarkable reduction in  $\text{K}^+$  concentration in root and shoot of under examined mung bean varieties. Under

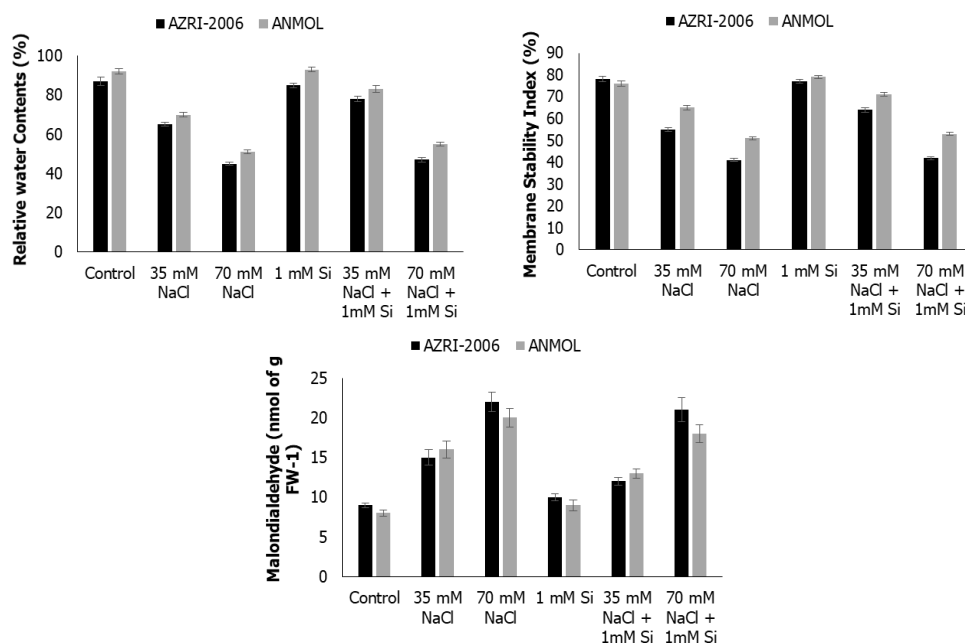
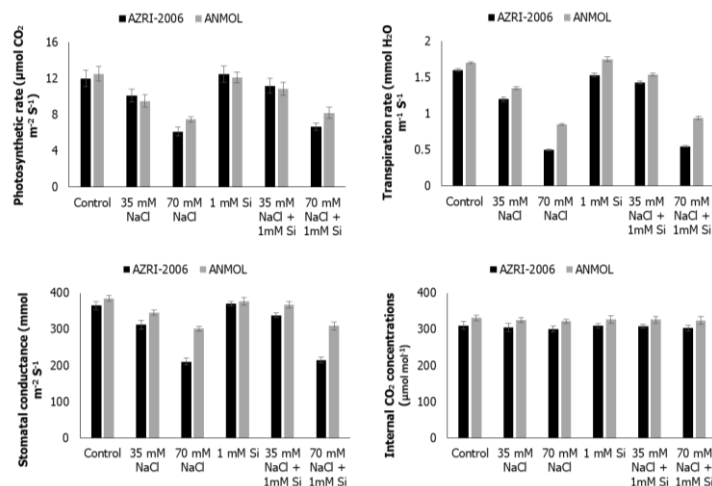
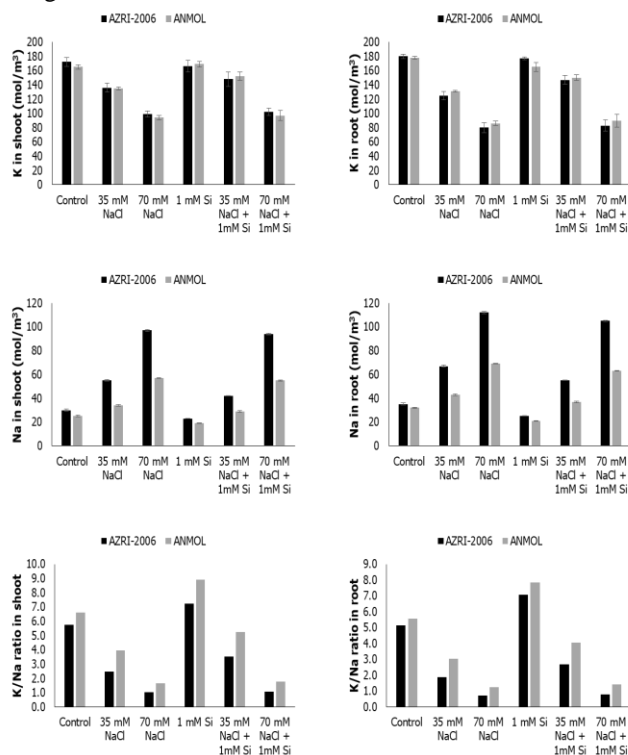


Figure 2. Effect of silicon on relative water contents (RWC), membrane stability index (MSI) and malondialdehyde (MDA) of two mung bean seedlings under salinity stress. The bars in the graph show the average values of 5 replicates and the error bars are the standard deviations.



**Figure 3.** Effect of silicon on gas exchange attributes of two mung bean seedlings under salinity stress. The bars in the graph show the average values of 5 replicates and the error bars are the standard deviations.

both level of salinity, highest  $\text{Na}^+$  concentration in root and shoot was noted in mung bean variety AZRI-2006 while least was remarked in mung bean variety ANMOL. The trend was opposite in case of  $\text{K}^+$  uptake by displaying least  $\text{K}^+$  concentration under highest salt level (70mM NaCl) while the maximum  $\text{K}^+$  concentration was observed at control in both mung bean varieties.



**Figure 4.** Effect of silicon on ionic attributes of two mung bean seedlings under salinity stress. The bars in the graph show the average values of 5

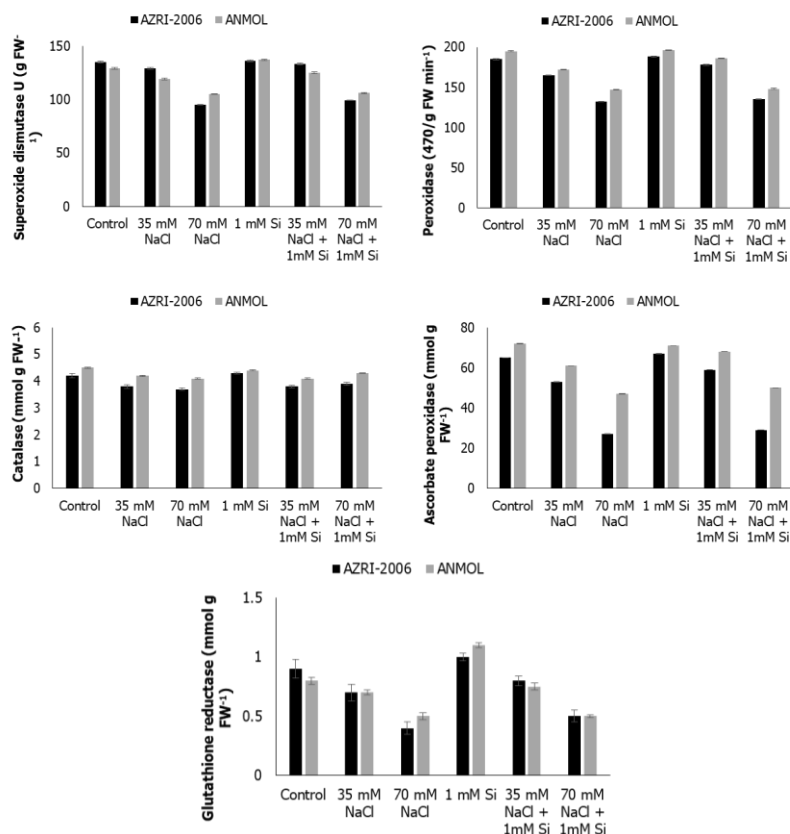
replicates and the error bars are the standard deviations.

The enhanced uptake of  $\text{Na}^+$  ions and declining  $\text{K}^+$  ions concentration with enhancing salinity caused reduction in  $\text{K}^+/\text{Na}^+$  ratio. Silicon application mitigated the adverse effect of salinity and significantly decreased the  $\text{Na}^+$  while increase the  $\text{K}^+$  concentration in root and shoot at low and high salinity in under examined mung bean varieties. The maximum potassium concentration under both salt stress (35 and 70  $\text{mM L}^{-1}\text{NaCl}$ ) resulted in retaining highest  $\text{K}^+/\text{Na}^+$  ratio in mung bean variety ANMOL, showing better growth under salt stress environment relative to AZRI-2006.

**Interactive effect of salt stress and silicon application on biochemical attributes:** Effect of salt stress and silicon on biochemical attributes in seedlings of both mung bean varieties is illustrated in Fig. 5. It is evident from the results that increase salinization reduced the activity of SOD, POD, APX and GR as compared to control. The maximum values were observed under sole application of Si (1mM Si) while least values were observed at maximum level of salt stress (70  $\text{mM L}^{-1}\text{NaCl}$ ). However, Si nutrition lead to the increase in the activities of these enzymes in salt treated plants. Furthermore, Si application did not convey any substantial changes in Catalase (CAT) concentration both under saline and non-saline condition. Mung bean variety AZRI-2006 showed inferior response while mung bean variety ANMOL showed superior response towards Si application under saline and non-saline condition which resulted in improved antioxidant enzymes activities.

## DISCUSSION

Increased salinity in the growth medium results in decline of plant growth attributes, physiological characteristics, biochemical and gas exchange attributes as well as ionic



**Figure 5.** Effect of silicon on biochemical attributes of two mung bean seedlings under salinity stress. The bars in the graph show the average values of 5 replicates and the error bars are the standard deviations.

concentration which ultimately disturb the yield of crop plants while Si application significantly ameliorates the hazardous effect of salt stress. The decline in plant growth attributes is due to less uptake of important nutrients, disturbance in plant photosynthetic activities and production of ROS (Thapar *et al.*, 2008). High accumulation of  $\text{Na}^+$  ions in plants drastically affects the plant physiological attributes like photosynthetic rate, transpiration rate and stomata opening and closing (Khan *et al.*, 2014). Reduction in photosynthesis and hindrance in uptake of essential nutrients results in scarcity of important metabolites under certain stresses (Abbasi *et al.*, 2015). Salt stress rigorously impaired the new cells synthesis by damaging cell membrane in beans (Munns, 2005).

Silicon plays multifunctional role in improving plant physiology when applied under stress and non-stress condition. Up regulation of antioxidant defense system, reduction of  $\text{Na}^+$  influx, osmotic adjustment, alleviation of oxidative stress and enhanced photosynthesis are some major ramifications of Si in improving salt tolerance (Gong *et al.*, 2005). Si normalizes the high concentration of  $\text{Na}^+$  ions in cytoplasm by changing efflux movement of  $\text{Na}^+$  ions in roots of salt affected mung bean plant (Shabala, 2009). Moreover, enlargement in growth traits of plants is due to role of silicon

in improving scavenging ability of mung bean plant against reactive oxygen species (ROS), stabilizing photosynthetic apparatus and prevented membrane from being oxidized (Gunes *et al.*, 2007).

Salt stress disturbs plants water relation and membrane stability in plants as depicted in current project. The increasing concentration of  $\text{Na}^+$  in soil significantly reduced the RWC and MSI which results in reduction of water uptake by plants and membrane damage (Zengin and Munzuroglu, 2005). Membrane stability index decreased with increasing salts concentration which depreciate membrane reliability and membrane solemnity especially in salt sensitive plants (Abbasi *et al.*, 2015). Si application significantly improved the plants water contents by enhancing the ability of plant root to absorb more water from growth medium and regulates the water potential (Rizwan *et al.*, 2015). The perfection in osmotic adjustment results in the improvement of hormones in cytoplasm which results in regulating transpiration rate and stomatal conductance of plants (Balaknina and Borkowska, 2013). Si act as ROS scavenger against  $\text{H}_2\text{O}_2$  and  $\text{O}_2$  and enhance antioxidant machinery and ensure balanced ROS equilibrium at cellular level which enhance membrane stability and permeability (Ali *et al.*, 2014).

Salinity leads to nutritional imbalance in plants due to its effects on nutrient availability, competitive uptake and transport within the plant (Zeng *et al.*, 2009). Potassium retaining capacity of plant cell is a major factor to check salt tolerance. The major cause of less potassium uptake under saline condition is nutrient imbalance and competition with other ions like  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{Ca}^{2+}$  and  $\text{Mn}^{2+}$  etc for up take from growth medium (Saqib *et al.*, 2012). Higher  $\text{K}^+/\text{Na}^+$  ratio is a key factor that helps the salt-tolerant genotypes to perform well under salinity by regulating protein synthesis, photosynthesis, enzymes activity, osmotic adjustment and retraining turgor pressure of cell (Abbasi *et al.*, 2012). Exogenously applied silicon plays an influential role in reducing  $\text{Na}^+$  toxicity by increasing  $\text{K}^+$  accumulation and improving  $\text{K}^+/\text{Na}^+$  ratio and decreased NaCl induced membrane depolarization (Shabala *et al.*, 2007).

Results revealed that imposition of salt stress in the growth medium remarkably reduced the stomatal conductance, transpiration rate and photosynthesis of mung bean plants. This might be due to reduce in water availability to the plant under saline environment (Warren, 2006). Results depict that application of Si under saline and non-saline condition has no significant impact on internal  $\text{CO}_2$  concentration which may be due to possible variation in stress duration and intensity, growth stage and among species. Silicon nutrition in the growth medium may increase salt tolerance by enhancing root water uptake via active accumulation of soluble sugars, amino acids and by adjusting osmotic potential (Rizwan *et al.*, 2015).

Salt stress altered the amount and activities of antioxidant enzymes in scavenging ROS (Liu *et al.*, 2014) by efficient destruction caused by  $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$  in chloroplast and cytoplasm causes cellular damage (Hernandez *et al.*, 2000). Our results suggest that ameliorate activities of antioxidant enzymes influenced by addition of Si might protect plant tissues from membrane oxidative damage and by regulating ROS scavenging enzymes under salt stress (Liang *et al.*, 2003, Wang *et al.*, 2009). Salt stress and Si application have no significant impact as revealed in present project which might be due to variation in plant species, stress intensity, duration of stress exposure and growth stage of crop plants (Abbasi *et al.*, 2015).

**Conclusion:** Salt stress created ionic and osmotic stress which results in disrupted growth, physiological, gas exchange, ionic and biochemical processes. When exposed to salt stress, mung bean varieties showed high cellular  $\text{Na}^+$  contents, imbalance in the mineral nutrition, oxidative damage and growth inhibition. Silicon application played multifunctional roles in imparting salinity tolerance in mung bean seedlings. Mung bean variety ANMOL showed high plant biomass,  $\text{K}^+/\text{Na}^+$  ratio along with improved morpho-physiological and gas exchange attributes under saline and non saline treatments as compared to mung bean variety

AZRI-2006. Despite the non-essentiality of Si for crop species, the data described in the present study show new dimensions of the beneficial effects of Si on plants grown under saline environment.

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