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Silicon (Si) Supplementation Alleviates NaCl Toxicity in Mung Bean [*Vigna radiata* (L.) Wilczek] Through the Modifications of Physio-biochemical Attributes and Key Antioxidant Enzymes

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Abstract

Mung bean is an important pulse crop. It is highly nutritive but is vulnerable to salinity stress. Therefore, the present study was aimed to investigate the protective effect of silicon (Si) against salt stress-induced damage to mung bean plants. Mung bean plants treated with NaCl (0, 50 and 100 mM) showed considerable declines in length and dry weights of shoots and roots. Chlorophyll-a (chl-a), chl-b, total chl, carotenoids and leaf relative water content (LRWC) decreased under NaCl stress. However, supplementation with Si in the form of sodium silicate (Na_2SiO_3) to NaCl-stressed plants ameliorated the adverse effects of NaCl on growth, biomass, pigment synthesis and leaf relative water content (LRWC). Silicon (Si)-supplemented plants exhibited enhanced chl-fluorescence and gas exchange parameters under normal (non-stress) as well as NaCl stress conditions. Salt-induced decline in the frequency of stomata and number of leaves per plant under salt stress was significantly recovered with Si supplementation. In addition, application of Si increased the levels of proline and glycine betaine in mung bean plants. Furthermore, histochemical staining tests showed that the levels of superoxide radicals and H_2O_2 increased with NaCl treatments, which thereby resulted in increased lipid peroxidation (LPO) and electrolyte leakage. Contrarily, decreased levels of H_2O_2, lipid peroxidation (measured as MDA content), and electrolyte leakage in Si-supplemented plants under NaCl stress indicated the stress mitigating role of Si. The activities of key antioxidant enzymes (SOD, CAT, APX and GR) under NaCl stress showed an increase under the NaCl regime. However, application of Si further boosted the activities of all four antioxidant enzymes in NaCl-stressed plants. The enhanced Na^+ uptake and Na^+/K^+ ratio in mung bean plants accompanied by decreased K^+ and Ca^{2+} uptake under NaCl stress were reversed with Si supplementation thereby resulting in enhanced accumulation of K^+ and Ca^{2+} and decreased Na^+. In conclusion, Si supplementation mitigated the negative effects of NaCl on mung bean plants through modifications in uptake of inorganic nutrients, osmolyte production and the antioxidant defence system.

Keywords Antioxidants · Mung bean · NaCl · Oxidative stress · Silicon · Histochemical staining

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Introduction

Plants are constantly challenged by various abiotic stresses that pose a threat to agricultural systems. In particular, salinity stress is considered to be a major abiotic factor affecting plant growth and productivity all over the world (Ashraf and McNeilly 2004). In view of a projection, about 20% of the irrigated land is affected by salinity and is expanding at an alarming rate (Yeo 1998). Primarily, salt stress disrupts metabolic machinery in plants due to ionic imbalance or disequilibrium, which further affects the transportation of essential solutes. Salinity can also impair physiological and biochemical processes like photosynthesis, protein synthesis, transpiration, and lipid metabolism in plants (Muchate et al. 2016). In plants, higher accumulation of Na^+ and Cl^- ions in plants during saline conditions hinders the uptake of essential nutrients (Agarie et al. 1998; Ahanger and Agarwal 2017). Apart from the above two salt-induced stresses, salt-stressed plants also experience oxidative stress caused by the production of a variety of reactive oxygen species (ROS) like singlet oxygen ($^1O_2$), superoxide ions (O_2^-) and hydrogen peroxide (H_2O_2). The ROS are very deleterious to biomolecules like membrane lipids, proteins, DNA, RNA etc. (Ahmad et al. 2010, 2016; Ahanger and Agarwal 2017). Unfortunately, most of the crops fall under the category of glycophytes which are prone to be affected by salinity. Salt tolerance is a complex trait that involves a network of primary and secondary cellular responses. Improving salt tolerance in plants through breeding is a major challenge due to the complexity and polygenic nature of the salinity tolerance trait. Therefore, it is important to find an alternative approach for enhancing salt tolerance in plants. Silicon supplementation is one of the promising and dynamic approaches to overcome the negative effects of salinity stress on crop plants.

Silicon (Si), the second most abundant element after oxygen in the Earth's crust (Epstein 1999), is beneficial for plants particularly under abiotic (Hattori et al. 2005; Tripathi et al. 2012) and biotic stresses (Epstein 1999; Ma and Yamaji 2006). Plants use Si in the form of silicic acid and is the only nutrient element that is not harmful when accumulated excessively in plants (Ma and Yamaji 2006). In addition, Si strengthens cell walls for improving the mechanical support for monocots and pteridophytes by augmenting suberization, lignification and silicification (He et al. 2013). In particular, biosilicification in plants (deposition of silicic acid within the apoplast) leads to the development of an amorphous silica barrier that provides the primary defence against biotic and abiotic stresses (Guerriero et al. 2016). Improvement of salt tolerance through Si supplementation has also been reported in rice seedlings by Yeo et al. (1999). Generally, Si may improve salinity tolerance by controlling Na^+ and K^+ transport and accumulation, which are the major salt tolerance mechanisms in plants (Zhu and Gong 2013). Previous studies have shown that Si enhanced the growth of barley plants during salinity stress by improving the chlorophyll content and photosynthetic rate of leaf cell organelles (Liang 1998). Zheng et al. (2011) reported that Si supplementation increased the transpiration rate in rice during drought and salt stress. Many studies have revealed that Si increases the activity of key antioxidant defence enzymes including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), as well as glutathione reductase (GR) and the glutathione (GSH) content in salt-stressed plants (Shi et al. 2016; Zhu et al. 2004). Silicon has also been shown to increase the root and whole-plant hydraulic conductance, transpiration, stomatal conductance, and leaf water content under osmotic stress in barley plants (Liu et al. 2014).

Mung bean [*Vigna radiata* (L.) Wilczek] is a prominent member of the *Vigna* genus with great economic and agricultural importance across the globe. Its great importance is due to its high nutritive value; therefore, it is a staple source of protein for millions of people living in developing countries. This crop is very often affected by abiotic stresses. Among them, salinity stress is a major constraint which leads up to 60% crop yield losses (Abd-Alla et al. 1998). Mung bean is generally considered a salt-sensitive crop because high salt concentrations markedly affect seed germination, and reduce shoot and root lengths, photosynthesis, and yield attributes (Saha et al. 2010). In this regard, supplementation of Si may provide an alternative approach to overcome the negative effects of salinity stress on mung bean. In this study, we systematically investigated the effect of Si on the growth, physiology, and antioxidant enzyme activities in mung bean plants under salinity stress.

Materials and Methods

Plant Materials and Growth Conditions

Healthy seeds of mung bean [*Vigna radiata* (L.) Wilczek] were sterilized for 30 min with 5% (v/v) sodium hypochlorite (NaOCl) solution and then washed and kept in distilled water for 3 h. The seeds were allowed to germinate in Petri dishes lined with Whatman No. 1 filter paper and moistened with half-strength Hoagland's nutrient solution. The germinated seedlings (4 days old; two per pot) were transferred to pots filled with sterilized sand and vermicompost (3:1). Plants were grown in a growth chamber for 3 weeks (25-day-old seedlings) under day/night temperature of 26 °C/16 °C (± 3 °C) and relative humidity of 60–70%.

Modified Hoagland's solution with 50 or 100 mM NaCl was used to irrigate the pots every alternate day for two weeks. The control plants were irrigated with normal Hoagland's nutrient solution. After the NaCl treatment for two weeks (39-day-old seedlings), Si (2 mM) in the form of Na_2SiO_3 was supplemented after every alternate day for two weeks (53-day-old seedlings). The experiments were repeated three times with 5 replicates in a randomized block design. The 53-day-old plants were uprooted and analysed for the following parameters:

Growth and Biomass Yield

Root and shoot lengths were measured for the control and treated plants from three biological replications. Similarly, fresh sample was taken and weighed for fresh weight. The samples were dried in an oven for 24 h at 70 °C and then dry weights measured.

Determination of Photosynthetic Pigments, Chlorophyll Fluorescence and Gas Exchange Parameters

The photosynthetic pigments were estimated by the method of Hiscox and Israelstam (1979). Optical density (OD) was recorded at 480, 510, 645 and 663 nm with a spectrophotometer using DMSO as control. A PAM chlorophyll fluorometer (H. Walz, Effeltrich, Germany) was used to determine the efficiency of PSII (F_v/F_m), quantum yield of PSII (ΦPSII), capture efficiency of PSII (Φ_{exc}), photochemical quenching (qp) and non-photochemical quenching (NPQ) in expanded leaves (Wang et al. 2016). The gas exchange parameters, that is, CO_2 assimilation rate (A), stomatal conductance (g_s) and transpiration rate (E), in expanded leaves of the treated and non-treated plants were determined with an infrared gas analyzer (LCA-4 model, Analytical Development Company, Hoddesdon, England).

Determination of Proline and Glycine Betaine (GB) Contents

Proline content was estimated following Bates et al. (1973) with toluene as control. The Grieve and Grattan (1983) method was employed for the estimation of GB.

Estimation of Leaf Water Content

The leaf relative water content (RWC) was estimated by the method of Smart and Bingham (1974) and calculated using the following formula:

$$\text{RWC (\%)} = \text{FW} - \text{DW}/\text{TW} - \text{DW} \times 100$$

where FW is the fresh weight; DW is the dry weight; TW is the turgid weight

Estimation of Hydrogen Peroxide (H_2O_2) and Lipid Peroxidation [As Malondialdehyde (MDA)]

For the determination of H_2O_2, the procedure of Velikova et al. (2000) was followed. Lipid peroxidation was measured by the method of Heath and Packer (1968).

Determination of Electrolyte Leakage

The method of Dionisio-Sese and Tobita (1998) was followed for the determination of electrolyte leakage. Fresh leaf tissue (0.1 g) was taken in test tubes containing 20 ml of double distilled water and the electrical conductivity (EC_0) was measured. Tubes were kept in water bath for 20 min at 60 °C and for another 10 min at 100 °C and the electric conductivities (EC_1 and EC_2) were recorded at both times were recorded. Calculations were carried using the following formula:

$$\text{Electrolyte leakage(\%)} = \left(\text{EC}_1 - \text{EC}_0\right)/\left(\text{EC}_2 - \text{EC}_0\right) \times 100$$

$$\text{EC}_0 = \text{electrical conductivity was observed at } 0 - \text{time point}$$

$$(\text{EC1}) = \text{electrical conductivity after contents heated at } 60\,^\circ\text{C}$$

$$(\text{EC2}) = \text{electrical conductivity after contents heated at } 100\,^\circ\text{C}$$

In Vivo Visualization of Reactive Oxygen Species (ROS)

Histochemical staining of the superoxide radical (O_2^-) was performed according to the method of Kariola et al. (2006). Leaf and root samples were immersed in 6 mM NBT (nitroblue tetrazolium) solution (prepared in 50 mM sodium phosphate buffer, pH 7.5) and 10 mM sodium azide for 12 h in the dark. The reaction was stopped by soaking the tissue (leaf and root) with lacto-glycerol ethanol (1:1:4 v/v) and boiling in a water bath for 5 min and the cleared leaves and roots were preserved in 50% ethanol and then photographed.

For histochemical staining of hydrogen peroxide (H_2O_2), the method of Thordal-Christensen et al. (1997) was adopted using 3, 30-dimethoxybenzidine (DAB) staining. In brief, the leaves and roots were washed with double distilled water, and then placed in 1% DAB (pH 3.8; Sigma, USA) for 8 h at 25 °C in the light. The stained samples were washed immediately, and then submerged and boiled in 95% ethanol for 10 min twice. Slides were prepared and photographs taken.

Estimation of Activities of Enzymatic Antioxidants

Leaf samples (each 500 mg) were triturated each in Tris–HCl (100 mM, pH 7.5) in the presence of 5 mM

1,4-dithiothreitol (DTT), 10 mM magnesium chloride ($MgCl_2$), 1.0 mM ethylenediamine tetra-acetic acid (EDTA), 5 mM magnesium acetate, 1,5% polyvinyl pyrrolidone (PVP), 1.0 mM phenyl methane sulfonyl fluoride (PMSF) and 1 µg/ml aproptinin. The ground material was centrifuged at 10,000 rpm and the supernatant served as an enzyme source. The extraction buffer for APX was supplemented with 2.0 mM ascorbate. The activities of superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2) were estimated spectrophotometrically following the methods of van Rossum et al. (1997), Luck (1974), Nakano and Asada (1981) and Foyer and Halliwell (1976), respectively. The activities of all enzymes were expressed as Unit mg^{-1} protein.

Determination of Ion Accumulation and Si Uptake

The method of Wolf (1982) was followed for the estimation of Na^+, K^+ and Ca^{2+} from oven-dried plant samples using a flame photometer (Jenway PFP 7). Each dried sample (0.1 g) was ground and digested with 2 ml of H_2SO_4-H_2O_2 mixture, filtered and then diluted with distilled water. The acid mixture (2 ml) containing distilled water was considered as blank. A standard curve of each mineral (10–100 µg ml^{-1}) was used as a reference.

For the determination of Si, the seedlings were washed thoroughly with double distilled water to remove adsorbed culture medium and blotted with paper towels before drying. Oven-dried shoot samples were homogenized by grinding in a stainless steel blender. The ground samples of each treatment (100 mg) were digested in tri-acid mixture (HNO_3, H_2SO_4 and $HClO_4$ in 5:1:1 ratio) at 80 °C until a transparent solution was obtained using a hot plate. After cooling, the digested samples were filtered using Whatman No. 42 filter papers and the filtrate was diluted to 50 ml with double distilled water. Concentrations of Si in the filtrate of the digested samples were estimated using an atomic absorption spectrophotometer (AAnalyst 600; Perkin Elmer, USA). The instrument was calibrated using a standard stock solution of Si.

Analysis of Stomatal Frequency and Number of Leaves Per Plant

For the determination of stomatal frequency of leaf blades, epidermal peels were made and mounted in glycerine jelly, and then the number of stomata was counted in 1 mm^{-2} areas using a digital camera linked to an optical microscope at 10X. The number of leaves was determined manually.

Statistical Analysis

The data for each variable were subjected to analysis of variance. The significance of differences between the control and the treatment mean values were determined by the Duncan's Multiple Range test (DMRT) at $P < 0.05$ significance level. The values presented are the means of three independent experiments.

Results

Restoration of Growth and Biomass Yield by Si Supplementation

Application of 50 and 100 mM NaCl significantly decreased shoot and root length in mung bean plants when compared to the control (Table 1). In contrast, Si supplementation restored the shoot and root length of salt-stressed plants. Although shoot and root dry weights (DW) showed a decline at 100 mM NaCl as compared to that at control, they were improved considerably after Si supplementation (Table 1).

Si Application Reversed the Effect of NaCl on Pigment Contents

Salt (NaCl) treatments reduced the contents of chlorophyll (a and b), total chlorophyll and carotenoids; however, supplementation of Si mitigated the adverse effects of salt stress on pigment content in mung bean plants (Fig. 1).

Supplementation of Si Restored the Chlorophyll Fluorescence

The efficiency of photosystem II (PSII) (Fv/Fm), quantum yield of PSII (ΦPSII), capture efficiency of PSII (Φ_{exc}) and photochemical quenching (qp) of the mung bean plants decreased under NaCl stress. However, application of Si to NaCl-treated plants enhanced these parameters significantly. In contrast, NPQ increased under NaCl stress compared with that of the control plants. Silicon treatment reduced NPQ significantly in salt-stressed mung bean plants (Table 1).

Rehabilitation of Gas Exchange Parameters by Si

Gas exchange parameters, that is, CO_2 assimilation rate (A), transpiration rate (E) and stomatal conductance (g_s), decreased with elevated NaCl stress, and a maximal decrease was observed with 100 mM NaCl stress. The NaCl-treated

Table 1 Si application enhanced the growth and biomass yield, chlorophyll fluorescence and gas exchange parameters in mung bean under NaCl toxicity

Treatments	0	0 + Si	50 mM NaCl	50 mM + Si	100 mM NaCl	100 mM + Si
Shoot length (cm)	52 ± 2.1a	55 ± 2.3a	41 ± 1.6c	47 ± 1.8b	30 ± 1.4d	37 ± 1.5c
Root length	20 ± 0.9a	22 ± 1.2a	12 ± 0.35c	16 ± 0.4b	8 ± 0.15d	10.06 ± 0.3c
Shoot DW (g/plant)	1 ± 0.025a	1.1 ± 0.04a	0.6 ± 0.008c	0.7 ± 0.018b	0.4 ± 0.005d	0.5 ± 0.009c
Root DW (g/plant)	0.6 ± 0.009b	0.7 ± 0.016a	0.3 ± 0.003d	0.4 ± 0.006c	0.2 ± 0.001f	0.3 ± 0.002e
F_v/F_m	0.7 ± 0.015a	0.9 ± 0.018a	0.7 ± 0.018c	0.8 ± 0.007b	0.5 ± 0.004d	0.7 ± 0.009c
ΦPSII	0.8 ± 0.013b	0.8 ± 0.014ab	0.6 ± 0.008c	0.8 ± 0.019a	0.4 ± 0.002e	0.5 ± 0.005d
Φ_{exc}	0.7 ± 0.01b	0.8 ± 0.016a	0.6 ± 0.007c	0.7 ± 0.01b	0.4 ± 0.003e	0.5 ± 0.005d
qp	0.9 ± 0.022a	1 ± 0.026a	0.7 ± 0.015c	0.8 ± 0.019ab	0.6 ± 0.009d	0.7 ± 0.013c
NPQ	0.6 ± 0.005d	0.4 ± 0.003e	0.8 ± 0.017b	0.6 ± 0.008c	0.9 ± 0.024a	0.7 ± 0.015b
A (μmol CO_2 m^{-2} S^{-1})	13 ± 1.12a	15 ± 1.16a	7 ± 0.55c	10 ± 0.84b	4 ± 0.21e	6 ± 0.21d
E (mmol H_2O m^{-2} S^{-1})	1.3 ± 0.035ab	1.4 ± 0.05a	0.7 ± 0.008d	1.1 ± 0.026c	0.5 ± 0.004e	0.7 ± 0.007d
gs (mmol CO_2 m^{-2} S^{-1})	350 ± 5.62b	378 ± 5.75a	170 ± 3.67d	225 ± 3.98c	51 ± 2.06f	130 ± 3.22e

Data presented are the means ± SE ($n = 5$). Different letters next to the number indicate significant difference at $P \leq 0.05$

F_v/F_m—Efficiency of PSII, *ΦPSII* quantum yield of PSII, *qp* photochemical quenching, *NPQ* non-photochemical quenching, *A* CO_2 assimilation, *E* transpiration rate, g_s stomatal conductance

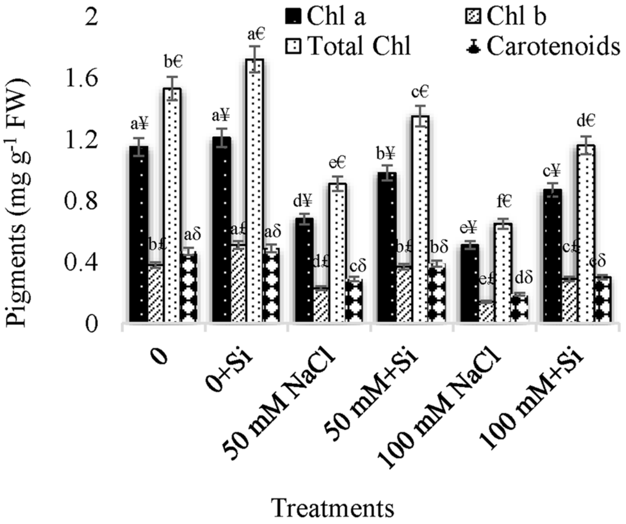

Fig. 1 Silicon supplementation enhanced the concentrations of pigments in mung bean seedlings under NaCl stress. Data presented are the means ± SE ($n = 5$ in each replicate). Different letters indicate significant differences between means within each parameter at $P \leq 0.05$

plants supplied with Si showed enhanced levels of *A*, *E*, and g_s (Table 1).

Silicon Treatment Enhanced Proline, Glycine Betaine and RWC

Proline and glycine betaine contents increased in the mung bean plants under both treatments of NaCl stress (Fig. 2a, b). Interestingly, exogenous application of Si showed a significant increase in proline and glycine betaine contents (Fig. 2a, b). Relative water content (RWC) in the salt-stressed plants decreased markedly; however, Si supplementation enhanced the RWC in these plants (Fig. 2c).

Application of Si Decreased H_2O_2, MDA and Electrolyte Leakage

Salt (NaCl) stress caused a marked increase in H_2O_2 and MDA contents compared with that in the control plants (Fig. 3a, c). However, application of Si together with NaCl showed less accumulation of H_2O_2 and MDA compared to that in the NaCl-treated plants alone. Electrolyte leakage also increased under NaCl stress; however, Si-treated salt-stressed plants showed less increase in electrolyte leakage when compared to that in the NaCl-treated plants alone (Fig. 3c).

Localization of H_2O_2 and O_2 in the Leaves of Mung Bean Seedlings

Histochemical estimation of H_2O_2 in the roots and leaves of mung bean plants treated with NaCl stress showed high intensity brown spots compared with those in the control plants, thus showing greater accumulation of H_2O_2 due to NaCl stress (Fig. 4). In contrast, application of Si along with NaCl lowered the intensity and number of brown spots in the roots and leaves of the mung bean seedlings, suggesting a protective role of Si against NaCl-induced oxidative stress (Fig. 4).

The histochemical staining used for detection of O_2^- in the roots and leaves of the NaCl-stressed mung bean plants showed intense dark-blue-coloured patches (Fig. 4),

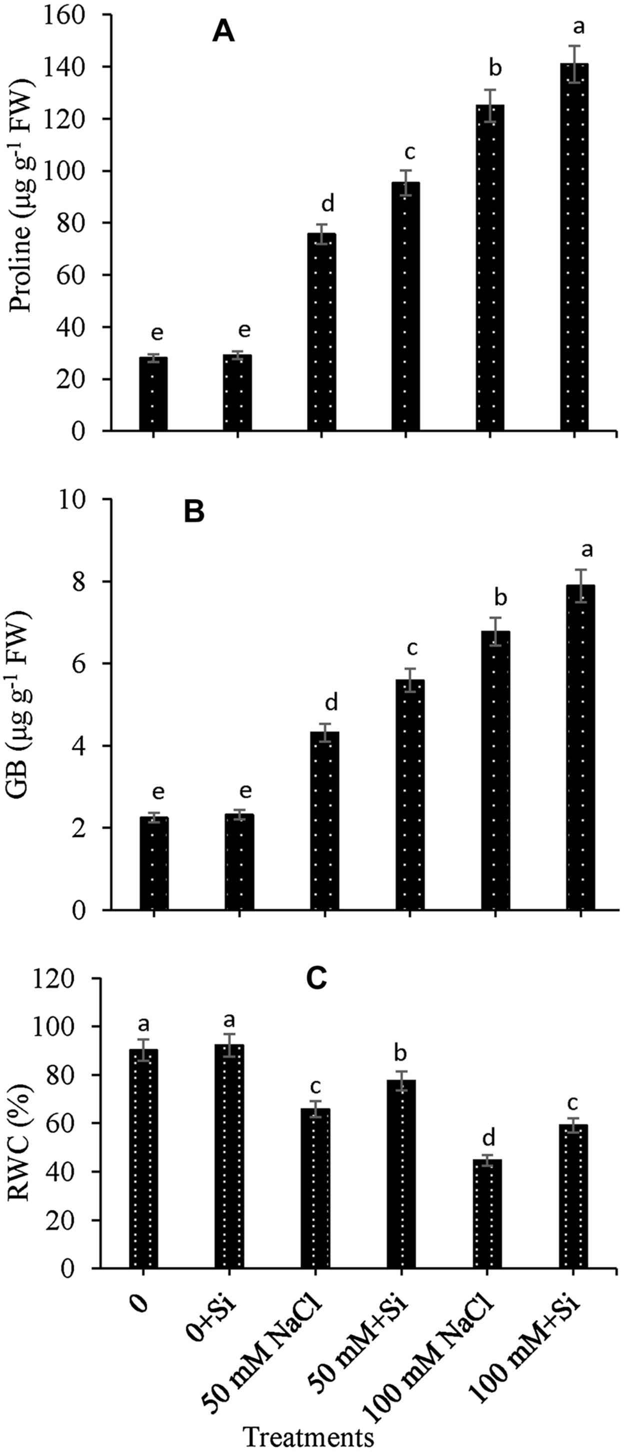

Fig. 2 Silicon application enhances the concentration of proline (**a**), glycine betaine (GB) (**b**), relative water content (RWC) (**c**) in mung bean seedlings under NaCl toxicity. Data presented are the means ± SE ($n=5$ in each replicate). Different letters indicate significant differences between means within each parameter at $P \leq 0.05$

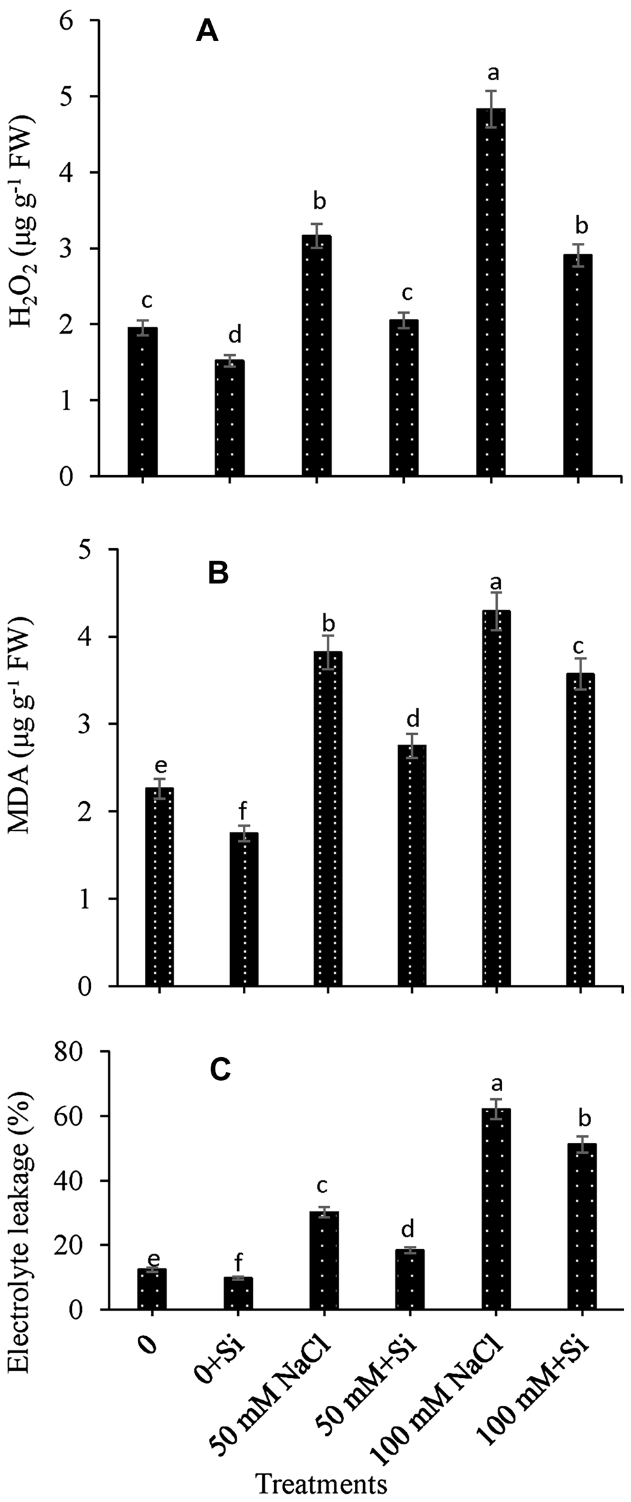

Fig. 3 Silicon supplementation reduces the accumulation of hydrogen peroxide (H_2O_2) (**a**), malondialdehyde (MDA) (**b**) and electrolyte leakage (**c**) in mung bean seedlings under NaCl toxicity. Data presented are the means ± SE ($n=5$ in each replicate). Different letters indicate significant differences between means within each parameter at $P \leq 0.05$

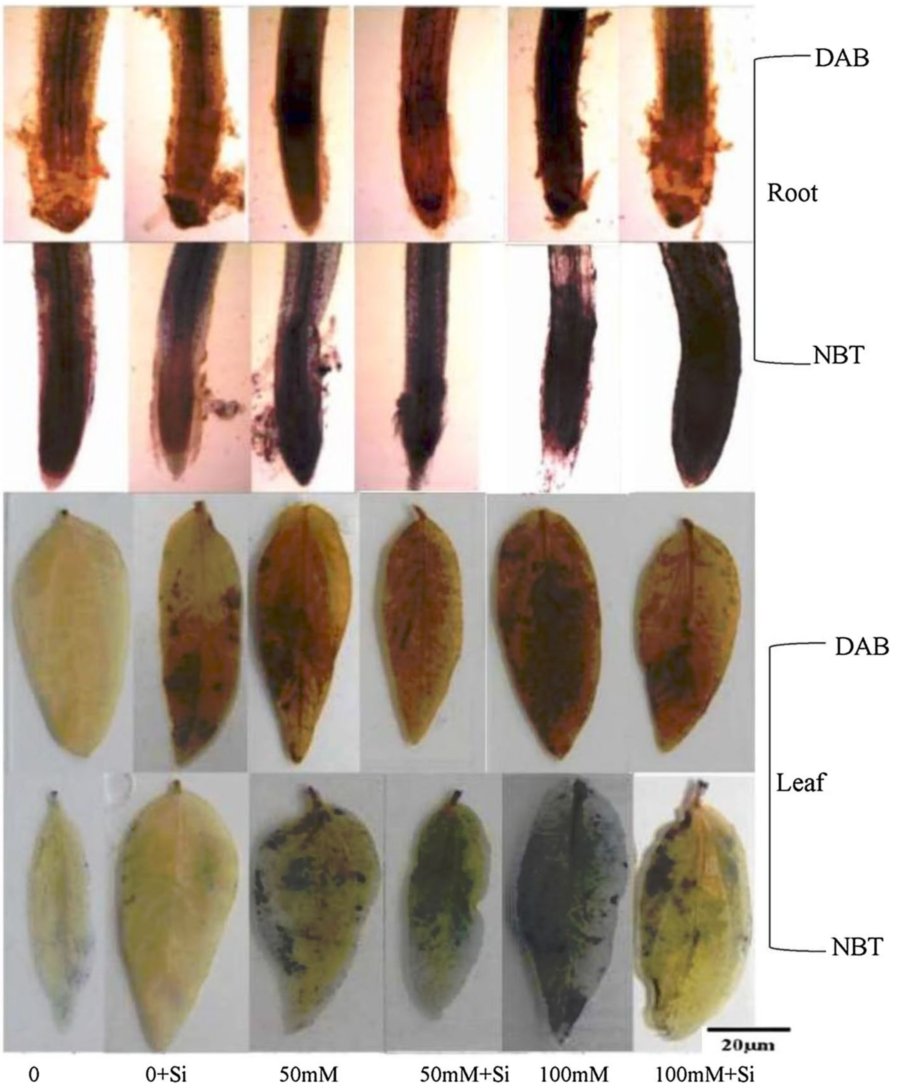

Fig. 4 In vivo localization of H_2O_2 and superoxide anion (O_2^-) in the roots and leaves of mung bean seedlings with five samples per replicate, using DAB and NBT staining. Dark-stained patches represent O_2^- and H_2O_2 produced in cells

suggesting that NaCl treatments appreciably enhanced O_2^- generation in the roots and leaves. However, Si supplementation significantly reduced the intensity and number of formazan spots in the roots and leaves of mung bean plants under NaCl treatments (Fig. 4). These results suggested that Si suppressed NaCl-induced O_2^- generation in the mung bean leaves.

Enhancement in the Activities of Enzymatic Antioxidants by Si Supplementation

Plants treated with 50 and 100 mM NaCl showed increased activities of SOD, CAT, APX and GR compared with those in the control plants (Fig. 5a, d). Supplementation of Si together with NaCl further increased the activities of SOD, CAT and APX, and levels of GR significantly.

Si Promoted the Accumulation of Essential Mineral Elements in Mung Bean Plants Under NaCl Stress

Leaf Na^+ content and Na^+/K^+ ratio increased with 100 mM NaCl stress compared with those in the control plants (Table 2). However, application of Si together with NaCl showed less accumulation of Na^+ and Na^+/K^+ ratio in the leaves with respect to that in the control plants. Leaf K^+ and Ca^{2+} accumulation decreased under 100 mM NaCl stress. However, addition of Si to the NaCl-treated plants improved the accumulation of K^+ and Ca^{2+} in the leaves.

Addition of Si to the growth medium significantly lowered Na^+ accumulation in the shoots as compared to that in the NaCl-treated plants alone. The results further demonstrated that addition of Si significantly enhanced Si accumulation in the mung bean seedlings (Table 2). It was observed that under

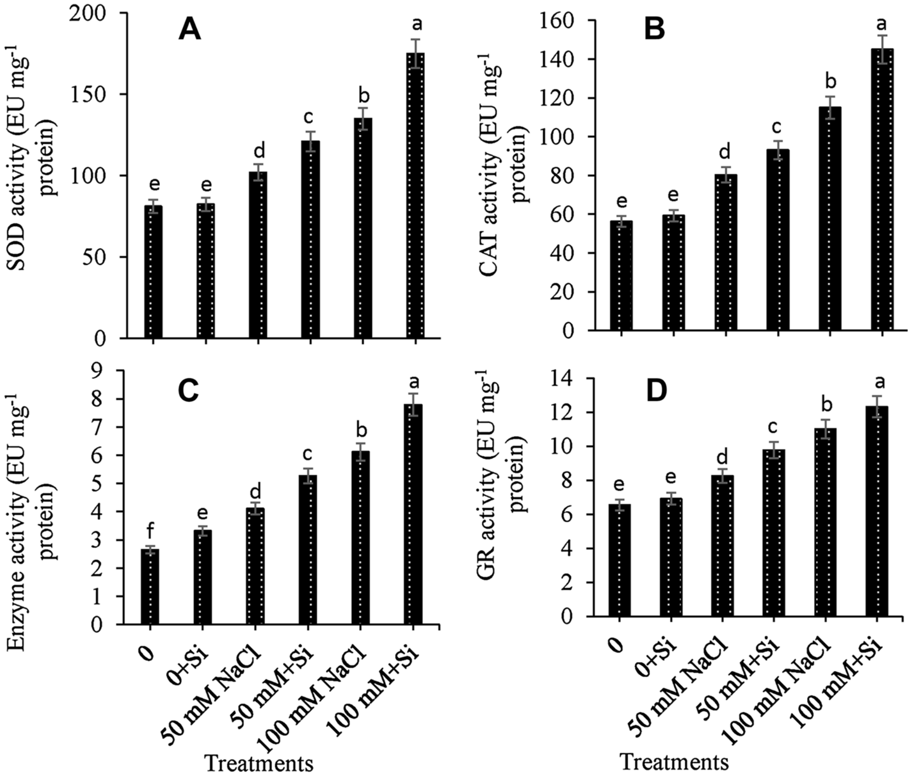

Fig. 5 Supplementation of Si boosts the activities of SOD (**a**), CAT (**b**), APX (**c**) and GR (**d**) in mung bean seedlings under NaCl toxicity. Data presented are the means ± SE (*n* = 5 in each replicate). Different letters indicate significant differences between means within each parameter at $P \leq 0.05$

Table 2 Si enhanced the uptake of K^+ and Ca^+ and decreased the uptake of Na^+ and ratio of Na^+/K^+ in mung bean under NaCl stress

Treatments	0	0 + Si	50 mM NaCl	50 mM + Si	100 mM NaCl	100 mM + Si
Si (mg kg^{-1} DW)	0.27 ± 0.01d	1.4 ± 0.048a	0.28 ± 0.001d	0.70 ± 0.009b	0.22 ± 0.007e	0.43 ± 0.006c
Na^+ (mg g^{-1} DW)	5.2 ± 0.32c	5.1 ± 0.3c	26.4 ± 2.4ab	20.6 ± 1.4b	35.2 ± 3a	28.8 ± 2.7ab
K^+ (mg g^{-1} DW)	26.2 ± 1.7b	38.1 ± 3.6a	20.1 ± 1.4c	30 ± 2.6ab	11 ± 0.5d	20 ± 1.3c
Na^+/K^+ ratio	0.42 ± 0.006e	0.34 ± 0.003f	1.3 ± 0.042c	0.70 ± 0.009d	3.3 ± 0.2a	1.5 ± 0.06b
Ca^{2+} (mg g^{-1} DW)	4.2 ± 0.49a	4.9 ± 0.57a	2.4 ± 0.1c	3.6 ± 0.22ab	1.7 ± 0.071d	2.6 ± 0.14c

Data presented are the means ± SE (*n* = 5). Different letters next to the number indicate significant difference at $P \leq 0.05$

NaCl stress, Si supplementation enhanced accumulation of Si three times more than that in the control plants (Table 2).

Si Application Improved Number of Leaves and Stomatal Frequency

Salt (NaCl) stress significantly decreased the frequency of stomata and number of leaves per plant. However, application of Si together with NaCl prevented a decrease in frequency of stomata as it was greater than that in the NaCl-treated plants alone (Fig. 6a, b).

Discussion

Salinity is the most common adverse environmental stress that limits mung bean growth and development mainly due to cellular sodium toxicity and osmotic stress. Application of Si to plants is one of the promising strategies to improve salt tolerance in mung bean plants. Several previous studies have reported the potential role of Si in alleviating salt-induced adverse effects in different crops (Liu et al. 2014; Wang et al. 2015; Zhu and Gong 2013; Ghassemi-Golezani

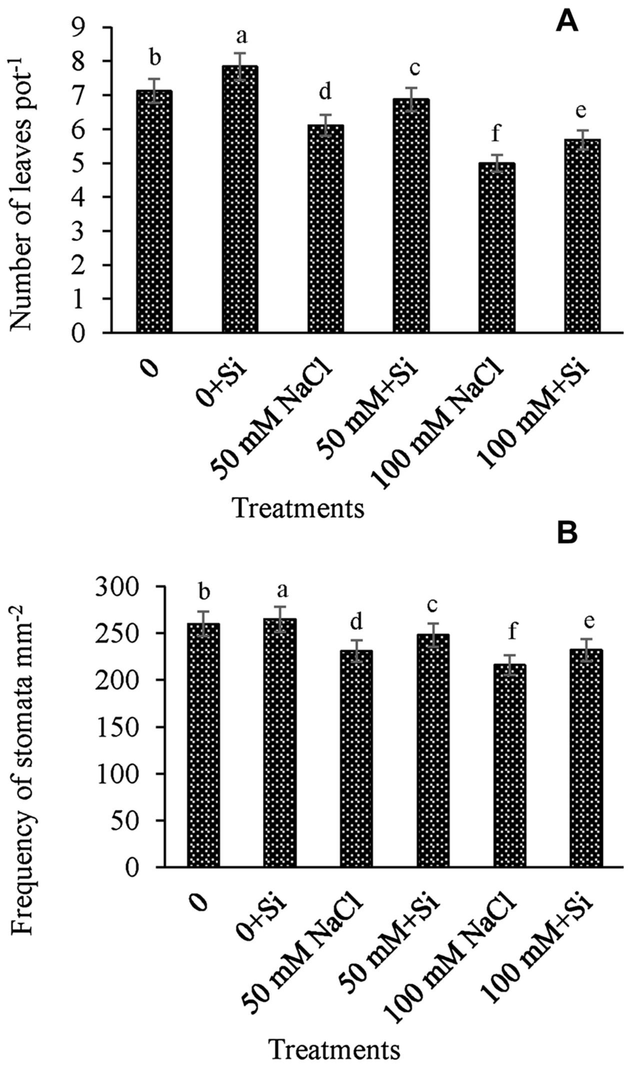

Fig. 6 Impact of Si application on number of leaves pot^{-1} (**a**) and frequency of stomata mm^{-2} (**b**) in mung bean seedlings under NaCl toxicity. Data are means ± SE. ($n=5$ in each replicate). Different letters indicate significant differences between means within each parameter at $P \leq 0.05$

et al. 2015). Although there are numerous reports on Si-induced improvement in salt tolerance of many crop plants, this is the first report on mung bean.

In the present study, supplementation of Si resulted in a significant increase in growth and biomass yield in mung bean plants grown under high saline conditions. These findings are in line with some previous reports, indicating the potential role of Si in alleviating the detrimental effects of salt stress on different plant species such as wheat (Tahir et al. 2012) and rice (Ming et al. 2011).

Salinity stress can also hamper the photosynthesis process in plants by decreasing the concentration of chlorophyll pigments, which further reduces photosynthetic rate thereby leading to poor plant growth. In addition, decrease in chlorophyll concentration due to salinity stress has been attributed to severe damage to chloroplast membranes, which may enhance membrane permeability or loss of membrane integrity (Tuna et al. 2007). Here, salt stress decreased the concentrations of both chlorophyll and carotenoids in mung bean plants, so our results are in agreement with the earlier findings observed in *Cicer arietinum* (Rasool et al. 2013); *Brassica juncea* (Ahmad et al. 2015), and *Solanum lycopersicum* (Al-aghabary et al. 2005). In this work, Si-supplemented plants exhibited increased levels of photosynthetic pigments under normal as well as salinity-stressed conditions. Previous reports have also shown that Si improved the chlorophyll content and photosynthetic activity in most crops (Mahmood et al. 2016; Parveen and Ashraf 2010). Improvement in net photosynthetic rate by the application of Si is attributed to decreased translocation of Na^+ from root to shoot in *Phaseolus vulgaris* seedlings under NaCl stress (Savvas et al. 2009). Abbas et al. (2015) also reported that application of Si improved photosynthetic rate, stomatal conductance, transpiration rate, and water use efficiency in tomato and okra plants exposed to salt stress. Therefore, Si-treated mung bean plants with improved physiological parameters further suggest the potential role of Si in alleviating harmful effects of salinity stress.

In plants, chlorophyll fluorescence parameters are frequently considered as important tools to determine the impact of various environmental stresses on photosynthesis machinery (Ghassemi-Golezani and Lotfi 2015). Generally, abiotic stresses including salinity stress decrease *F*v/*F*m, ΦPSII, Φ_{exc} and qp in different plants (Maghsoudi et al. 2015). The present study showed a decline in *F*v/*F*m under NaCl stress, which may have been due to reduced efficiency of energy transfer from antennae to the reaction centres (Ghassemi-Golezani and Lotfi 2015; Rosenqvist and van Kooten 2003). Decrease in *F*v/*F*m signifies critical destruction of PSII that might decrease photosynthetic rate which in turn can reduce plant growth and yield (Rosenqvist and van Kooten 2003). Decrease in *F*v/*F*m ratio is believed to hamper the activity of PSII, thereby increasing non-photochemical quenching due to decrease in utilization of light energy (Maghsoudi et al. 2015). Application of Si enhanced the *F*v/*Fm*, ΦPSII, Φexc and qp and decreased NPQ. Maghsoudi et al. (2015) also reported enhanced *F*v/*F*m, ΦPSII, Φexc and qp, and decreased NPQ in Si-supplemented water-stressed wheat plants. The role of Si in maintaining the chlorophyll fluorescence is also reported in different plants (Zheng et al. 2011; Habibi and Hajiboland 2013).

Gas exchange parameters are generally considered as indicators of salinity tolerance in plants. The application of NaCl to mung bean plants resulted in a strong reduction in primary gas exchange parameters such as CO_2 assimilation rate (*A*), transpiration rate (*E*) and stomatal conductance (g_s), respectively. Our results are in agreement with the findings of Parveen and Ashraf (2010), Wang et al. (2015) and Mahmood et al. (2016) who also observed a marked decrease in these gas exchange attributes in different plants under saline stress. Different abiotic stresses have been reported to decrease the gas exchange parameters such as zinc toxicity in maize (de Sousa Paula et al. 2015), and drought stress in pistachio (Habibi and Hajiboland 2013) and wheat (Maghsoudi et al. 2016). Salt (NaCl) stress imposes osmotic stress due to which plants close their stomata so as to lower down the transpiration rate, which ultimately prevents water loss from the plant body. Closure of stomata also decreases the CO_2 fixation, which results in reduced photosynthetic rate. The detrimental effects of salt stress on gas exchange parameters in mung bean plants were significantly mitigated by Si supplementation. Wang et al. (2015) also reported that Si enhanced the CO_2 assimilation rate, transpiration rate, and stomatal conductance in cucumber plants. Silicon also enhanced stomatal conductance that in turn enhanced gas exchange and CO_2 uptake that ultimately improved photosynthetic efficiency (Parveen and Ashraf 2010). Silicon-induced enhancement in transpiration was also observed by Matoh et al. (1986). According to Liang (1998), silicic acid (mono- or polymerized) forms hydrogen bonds between water and hydrated silica on epidermal cells, thereby preventing water loss. Ali et al. (2012) reported Si enhancement in stomatal conductance in rice plants under NaCl stress, indicating reduced uptake of Na^+ by silicate through decreased transpiration rate. Rios et al. (2017) proposed that Si mediates up-regulation of expression of several aquaporin genes involved in enhancing Si entry into the cell, thereby affecting root hydraulics and water uptake.

Plants accumulate compatible organic osmolytes such as proline and glycine betaine that protect plants from osmotic stress and oxidative stress during abiotic stresses (Ahmad et al. 2010, 2016; Nasir Khan et al. 2009). In this study, NaCl-treated plants exhibited a significant accumulation of proline and glycine betaine in the mung bean plants. Similar reports were also observed in other crops including *Brassica juncea* (Khan et al. 2012), *Linum usitatissimum* (Nasir Khan et al. 2009), *Morus alba* (Ahmad et al. 2013), and *Cicer arietinum* (Ahmad et al. 2016). Our results show that Si supplementation increased accumulation of both proline and glycine betaine in the mung bean plants exposed to salt stress. However, our results are not in agreement with those observed in sorghum plants where Si supplementation caused reduction in osmolytes during osmotic stress (Liu et al. 2014). Generally, plants with increased levels of osmolytes grow better under saline conditions because of osmotic adjustment, and also by maintaining cellular and enzyme structures. In plants, decrease in relative leaf water content is a characteristic feature of plants under osmotic stress (Fahad et al. 2014). Relative water content was found to decrease in mung bean plants under NaCl treatments. In contrast, Si supplementation restored RWC in mung bean plants under salinity stress. Silicon was reported to increase RWC under salinity stress in tomato (Haghighi and Pessarakli 2013) and wheat plants (Tahir et al. 2012).

In mung bean plants under salinity stress, increased H_2O_2 and MDA contents led to enhanced electrolyte leakage. Accumulation of H_2O_2 and MDA was also reported in many plants when they were exposed to salinity stress (Ahmad et al. 2013; Ashraf et al. 2010). However, Si treatment decreased the H_2O_2 and MDA levels in mung bean plants subjected to salt stress, suggesting that Si can act as an effective antioxidant (Kim et al. 2013). Our results are in agreement with some previous reports where application of Si decreased the accumulation of H_2O_2 and MDA through the combined action of antioxidants and osmolytes which get accumulated after Si treatment (Moussa 2006). Zhu et al. (2004) have also reported that Si application reduced electrolyte leakage because of reduced H_2O_2 and lipid peroxidation in cucumber plants under salinity stress.

In plants, salinity stress leads to excess ROS accumulation, which alters the balance between ROS production and scavenging, resulting in cellular oxidative damage. Plants have evolved an efficient defence mechanism of scavenging ROS, which includes both enzymatic, such as SOD, CAT and APX, and non-enzymatic antioxidants such as AsA and GSH, to protect against cellular damage and keep the beneficial implications of ROS-mediated signal transduction operating at a normal tune (Shi et al. 2014). Increase in the activities of antioxidant enzymes (SOD, CAT, APX and GR) in the mung bean plants under NaCl stress corroborates with the findings of Rasool et al. (2013) in chickpea seedlings. Similar findings showing increased antioxidant enzymes have also been reported in other plants like, *Solanum lycopersicum* (Manai et al. 2014), *Morus alba* (Ahmad et al. 2013), *C. aeritinum* (Rasool et al. 2013; Ahmad et al. 2016), *Brassica juncea* (Ahmad et al. 2015) and *Triticum aestivum* L (Ahanger and Agarwal 2017). Antioxidants have been reported to quench the ROS and protect the cells from oxidative damage induced by salinity stress (Ahmad et al. 2015, 2016). Recently, Si has been shown to play an important role in ROS detoxification by promoting the activities of antioxidant enzymes in tomato plants under salt stress (Muneer and Jeong 2015). Si supplementation proves beneficial to plants under salt stress after continued application for quite some time through the significant enhancement in the production of GSH thereby coupling directly with enzymatic components for quick elimination of ROS and hence greater membrane protection (Liang et al. 2003).

Plants have evolved a specific strategy such as ion-uptake regulation, vacuolar compartmentation and ion exclusion to survive when they face cellular salt-induced ion imbalance (Blumwald 2000). In addition, salt tolerance in plants is generally related to low uptake/accumulation of Na^+, and this has been used as a potential selection criterion in breeding for salt tolerance (Munns et al. 2006). Accumulation of Na^+ under NaCl stress in the present study is analogous to the results of Kao et al. (2006) in soybean. When the Na^+ concentration is high, K^+ uptake by the plant is generally inhibited thereby resulting in an enhanced Na^+/K^+ ratio in the tissues (Ahmad et al. 2015). Silicon has been reported to stabilize the biological membranes as well as to lower the concentration of Na^+ and elevate those of K^+ and Ca^{2+} as has been earlier observed in cucumber (Khoshgoftarmanesh et al. 2013). How Si is able to do this is still unexplored and thus further work is to be done to uncover the underlying mechanism. According to Shi et al. (2016), Si supplementation reduces the oxidative stress and membrane damage, and leads to enhanced root hydraulic conductance. Application of Si adjusts the osmotic potential of cells through accumulation of osmolytes like proline, glycine betaine, sugars, inorganic ions, etc. (Liu et al. 2014). Silicon accumulation in plants greatly varies from plant to plant, because of the architecture of Si transporter system and prevalent environmental conditions. In the present study, we found that mung bean plants accumulated significant amounts of Si under salt stress as well depicted its requirement for alleviation of NaCl toxicity. Thus, it could be inferred that mung bean is a Si accumulator. Similarly, Tuna et al. (2007) have shown that Si accumulation increased significantly in the roots and leaves of wheat plants after Si supplementation.

Conclusion

In conclusion, the present study shows that NaCl stress significantly decreased the growth and biomass yield of mung bean plants mainly by altering morphological, physiological and biochemical processes. However, application of Si alleviated these stress-induced detrimental effects by modulating important metabolic processes such as photosynthetic rate, antioxidant enzyme metabolism (SOD, CAT, APX and GR) and production of osmolytes. In addition, Si supplementation decreased Na^+ uptake, and accumulation of H_2O_2 and MDA as well as electrolyte leakage. Further studies are required to investigate the molecular mechanisms of Si-mediated salt tolerance in mung bean plants which can ultimately lead to the development of salt-tolerant cultivars of this crop.

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Author Contributions PA, MAA and MNA designed the experimental work. PA and LW carried out the statistical analysis. PA, MA, MAA and SA wrote and revised the manuscript. All the authors have read the manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interest.

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