Silicon supplementation modulates antioxidant system and osmolyte accumulation to balance salt stress in *Acacia gerrardii* Benth

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**Abstract**

Experiments were conducted to investigate the role of silicon (Si, 2 mM potassium silicate - K$_2$SiO$_3$) in ameliorating the salinity (200 mM NaCl) triggered growth retardation, photosynthetic inhibition and the oxidative damage in Talh trees (*Acacia gerrardii* Benth). Salinity stress reduced length and dry biomass accumulation of root and shoot which were significantly improved by Si supplementation. Application of Si enhanced the synthesis of photosynthetic pigments including chlorophyll a, chlorophyll b, total chlorophylls and carotenoids resulting in greater photosynthetic activity measured in terms of net CO$_2$ assimilation. Stomatal conductance and transpiration rate were declined due to NaCl treatment and supplementation of Si ameliorated the negative impact of NaCl on these attributes and was significantly improved when applied to normal grown plants. Further, lipid peroxidation was more in NaCl stressed plants without Si as compared to those supplemented with Si. Si protected Talh trees from NaCl induced oxidative damage by improving the activity of antioxidant enzymes (SOD, POD, CAT, APX and GR) and the content of ascorbic acid. Accumulation of compatible osmolytes including proline and glycine betaine was increased due to Si supplementation leading to improved growth under saline conditions in addition Si supplementation mitigated the deleterious effects of NaCl on flavonoid content. More importantly Si supplementation prevented excess uptake of Na and also protected the ill effects of excess Na on the uptake and accumulation of K and Ca resulting in significant decline in Na/K ratio. In conclusion, Si mitigates the negative effects of NaCl in *A. gerrardii* by modifying nutrient uptake, osmolyte accumulation and up-regulating antioxidant system.

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**1. Introduction**

The normal growth patterns of plants are often encountered by variety of environmental stresses thereby posing challenge to the sustainable agricultural system. Among the abiotic stresses, high salinity has been considered as a major factor affecting growth and productivity of maximum crop plants all over the globe (Hashem et al., 2015). As per the estimations carried nearly 20% of the irrigated land of the globe is salt affected (Yeo et al., 1999). Salt stress imparts stern restrictions in the metabolic stability of plants by causing ion imbalance reflecting in impeded transport of essential ions and solutes (Ahmad et al., 2016). Among the key physiological and biochemical processes impaired by high salinity are included transpiration, photosynthesis, protein synthesis, etc. (Khan et al., 2014). Apart from the restrictions in the uptake of essential mineral nutrients and the hindrances in key physiological processes greater accumulation of toxic ions leads to excessive generation reactive oxygen species (ROS) thereby leading to oxidative damage to cells (Alqarawi et al., 2014a,b). The key toxic ROS include singlet oxygen, superoxide ions, hydroxyl ion and hydrogen peroxide which can easily target important biomolecules like membrane lipids and proteins, nucleic acids, etc. (Alqarawi et al., 2014a, Hashem et al., 2015). Understanding and developing tolerance to salinity is a complex trait relying on the interrelated primary and secondary response mechanisms. Implementing the usually employed breeding techniques for crop protection against the salinity stands to be a major challenge due to the complexity.
of stress tolerance mechanisms and the lack of proper understandings about them. It is therefore imperative to search and implement the alternatives for improving the salt stress tolerance in plants and the supplementation of mineral elements can be a promising and dynamic approach for overcoming the negative effects of salinity stress in major food crops.

For avoiding the toxic effects of salinity triggered deleterious implication plants have developed several key mechanisms for minimising their ill effects. Among these tolerance mechanisms are included the up-regulation of antioxidant defence system, efficient ion exclusion, accumulation of osmolytes and secondary metabolites (Velarde-Buendia et al., 2012; Ma et al., 2015; Hosseini et al., 2017; Kim et al., 2017). Plant species exhibiting up-regulation of antioxidant system, greater accumulation of osmolytes and quick elimination or compartmentation of toxic concentrations of deleterious ions have better stress adaptability (Ahmad et al., 2016; Hashem et al., 2016). Antioxidant system includes superoxide dismutase, catalase, peroxidases, reductases, ascorbic acid, glutathione, polyphenols, etc. (Kim et al., 2014; Abd_Allah et al., 2017).

Silicon (Si) is among the most abundant element on earth's crust that has been recognised as beneficial for the normal growth of plants for its role in improving vigor and stress tolerance (Ma and Yamaji, 2006). Si interacts with cell wall components in the form of silica (SiO₂) and promotes their strengthening and hence increases the mechanical support to the aerial parts. Silicic acid is the absorbed form of Si and it has been observed that Si accumulates in plants when accumulated in enough concentrations (Ma et al., 2001). The interrelationship among growth-cell wall-Si has been well documented in monocots and pteridophytes (Guerriero et al., 2016). Plants can differ in the potential to accumulate Si thereby resulting in significantly different response in them. It has been estimated that in some grasses accumulated Si can make up to 10% of the total dry mass as compared to others like Arabidopsis which show only 0.1% of the accumulated biomass (Montpetit et al., 2012). Recent research strides have developed some interesting insights about the understanding of Si mediated growth promotion and stress amelioration (Ma and Yamaji, 2006; Deshmukh and Belanger, 2016). Si improves the cell walls by enhancing the silification, suberization and lignifications (Currie and Perry, 2007). More importantly biosilicification within the cell apoplast leads to development of amorphous silica barrier resulting in greater stress withstanding ability (Guerriero et al., 2016). Supplementation or priming of seeds with Si improves stress tolerance of different crop plants including rice, maize and wheat (Tuna et al., 2008; Abdel Latef and Tran, 2016). Abdel Latef and Tran (2016) have demonstrated that Si ameliorates stress and improves growth by enhancing Na/K ratio, a key trait for salt stress tolerance (Mali and Aery, 2008; Mia et al., 2010; Hosseini et al., 2017). Application of Si to rice resulted in significant improvement in the rate of transpiration under water and salt stress (Chen et al., 2011).

Studies have shown that Si enhances the growth of barley plants during salinity stress by improving the chlorophyll content and photosynthetic rate (Liang, 1998), Chen et al. (2011) reported that the Si supplementation increased transpiration rate in rice during drought and salt stress. Yin et al. (2016) have demonstrated that application of Si to Sorghum bicolor enhances the synthesis of key polyamines including putrescine, spermidine and spermine under salt stress thereby imparting greater salt tolerance. Some studies have also reported Si mediated up-regulation of the antioxidant defence system by increasing the activity of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione reductase (GR) and the accumulation of reduced glutathione (Liang et al., 2003a,b; Shekari et al., 2015; Habibi and Hajiboland, 2013). Other growth characteristics including root hydraulic conductivity, transpiration rate, stomatal conductance and relative water content were observed to be greater in Si treated barley plants (Liu et al., 2014; Shi et al. 2016a,b). It has been accepted that crop species exhibiting greater Si uptake get more benefit from the applied Si (Ma and Yamaji, 2015).

Talh trees (Acacia gerrardii Benthi) is an important the most important tree groups in Saudi Arabia because the species is a good source of gums and tannins, besides being used as wood and forage (Hashem et al., 2016) Like most of tree, Talh is also considered as sensitive to stresses like salinity and frequent exposures to salinity result in significant yield losses globally. Therefore immediate need is to develop strategies that can minimise the deleterious effects of excess soil salinity on growth and developmental patterns of Talh trees. Connected to this present study was aimed to investigate the role of Si supplementation in improving the salt tolerance of A. gerrardii focusing on its role in improving tolerance mechanisms including antioxidants and osmolytes.

2. Material and methods

2.1. Experimental design

The seedlings of Acacia gerrardii Benthi. (One month after germination) were provided by AlGhat National Park, Natural resource management, Ministry of Environment, Water and Agriculture, Kingdom of Saudi Arabia. The seedlings were sorted to select the similar in their growth and transplanted into plastic pots (20 cm in diameter, one seedling/pot) filled with five kg of mixture had been composed of autoclaved sand: perlite: peat (1:1:1, v/v/v). The treatments were arranged in completely randomized block design with three replications. The design of pots experiment was a factorial arrangement with the following factors being tested:

1. Control: Talh seedlings with no any treatments.
2. Talh seedlings + Silicon: Talh seedlings treated with Silicon without salt stress.
3. Plants + Salt stress: Plants treated Salt stress (200 mM NaCl) in absence of Silicon.
4. Plants + Silicon + Salt stress: Seeds treated with Silicon under salt stress.

Hogland solution (Hoagland and Arnon, 1950) supplemented with 200 mM sodium chloride NaCl, for salt stress and 2 mM potassium silicate (K₂SiO₃) as silicon treatment, control reference was used for each treatment. The posts were incubated for eight more weeks over transplantation under control conditions (temperature of day/night cycle as 25 C/18 C, 350 µmol photon m⁻² s⁻¹ light intensity provided by fluorescent tubes and 70–75% humidity) in growth chamber of Plant Production Department, College of Food and Agricultural Sciences, King Saud University, Saudi Arabia. At end of pot experiment, the plants were removed from the pots very carefully by washing the root to remove the soil and shoots cut at crown region were collected, the lengths of both root and shoot chickpea plants were measured using meter scale. The third true leaves from the top of each treatment were harvested for determination the different chemical and biochemical parameters. Subsequently, root and shoot samples were oven-dried at 70 °C for three days (72 h) and their dry weight was recorded.

2.2. Determination of photosynthetic pigments and gas exchange parameters

Photosynthetic pigments were estimated in fresh leaves after extraction in dimethyl sulfoxide (DMSO) as described by Hiscox
and Israelstam (1979). Absorbance was determined spectrophotometrically at 480, 510, 645, 663 nm (T80 UV/VIS Spectrometer, PG Instruments Ltd, USA), DMSO was used as blank. The gas exchange parameters including transpiration rate (E), CO₂ assimilation rate (A) and stomatal conductance (gs) were estimated in fully expanded leaves using infrared gas analyzer (LCA-4 model, Analytical Development Company, Hoddesdon, England).

2.3. Determination of lipid peroxidation

Fresh leaves (0.5 g) were macerated in 1% trichloro acetic acid (TCA) and extract was centrifuged for 5 min at 10,000 rpm. To 1.0 mL of supernatant was added 4.0 mL of 0.5% (w/v) thiobarbituric acid and mixture was heated at 95 °C for 30 min. After cooling on ice bath samples were again centrifuged at 5000 rpm for 5 min and absorbance of supernatant was recorded at 532 and 600 nm (Heath and Packer, 1968).

2.4. Determination of proline, glycine betaine and total flavonoids

Method of Bates et al. (1973) was followed for the estimation of proline content. Briefly 0.5 g of tissue was homogenised in 3% sulphosalicylic acid and extract was centrifuged at 3000g for 20 min. 2 mL of supernatant was reacted with equal amount of acetic acid and ninhydrin 1 h at 100 °C. Thereafter samples were cooled on ice and proline was separated using toluene and concentration of proline was determined spectrophotometrically at 520 nm.

Dry plant material (500 mg) was extracted in 20 mL deionized water by shaking overnight at 25 °C. Extract was filtered and mixed with sulphuric acid (2N) and an aliquot of 0.5 mL was mixed with 200 µL of cold KI–I₂ reagent and the resultant solution was centrifuged for 15 min at 10000g. Periodide crystals formed were dissolved using 1.2-dichloroethane and after 2 h absorbance was measured at 365 nm using a spectrophotometer (Beckman 640 D, USA). Calculations were done using standard curve of glycine betaine (Grieve and Grattan 1983).

Flavonoid content was estimated following the method described by Zhishen et al. (1999). Plant samples were extracted in methanol and know volume was reacted with aluminium chloride and the absorbance was recorded at 510 nm. Catechin was solved using 1,2-dichloroethane and after 2 h absorbance was measured at 365 nm using a spectrophotometer (Beckman 640 D, USA). The absorbance was determined spectrophotometrically at 510 nm. Catechin was expressed as mg g⁻¹ FW.

2.5. Assay of antioxidant enzymes and ascorbic acid content

5 gm fresh leaves were homogenized in phosphate buffer (50 mM, pH 7.0) containing 1% soluble polyvinyl pyrrolidone and 1 mM EDTA. After centrifugation at 15,000 rpm for 20 min at 4 °C supernatant was collected and used for the assay of enzyme activity. Protein in the enzyme extract was estimated according to Lowry et al. (1951).

For determination of superoxide dismutase (SOD, EC 1.15.1.1) activity method of Bayer and Fridovich (1987) was adopted and photoreduction of nitroblue tetrazolium (NBT) was measured spectrophotometrically at 560 nm. Activity of SOD was expressed as enzyme unit (EU) mg⁻¹ protein and one unit of SOD was defined as the amount of protein causing 50% decrease of the SOD-inhibitable NBT reduction.

Peroxidase (POD, EC 1.11.1.7) activity was determined by following the method of Kar and Mishra (1976). 5 mL assay mixture contained 125 µM of phosphate buffer (pH 6.8), 50 µM of pyrogallol, 50 µM of H₂O₂ and enzyme extract. The amount of purpurogallin formed was determined by taking the absorbance at 470 nm and activity was expressed as EU mg⁻¹ protein.

For estimation of catalase (CAT, EC 1.11.1.6) activity method described by Luck (1974) was followed and change in absorbance was recorded at 240 nm for 2 min. The activity of CAT was calculated using the extinction co-efficient of 36 × 10³ M⁻¹ cm⁻¹ and expressed as EU mg⁻¹ protein.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined by following the method of Nakano and Asada (1981). Briefly assay mixture contained 1 mL of potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM EDTA, 0.1 mM H₂O₂ and 0.1 mL of enzyme extract. The decrease in absorbance was recorded at 290 nm and activity was expressed as EU mg⁻¹ protein.

Glutathione reductase (GR, EC 1.6.4.2) activity was assayed according to Carlberg and Mannervik (1985) and decrease in absorbance was read at 340 nm for 2 min. Activity was calculated using the extinction coefficient of 6.2 mM⁻¹ cm⁻¹ and expressed as EU mg⁻¹ protein.

For estimation of ascorbate 0.8 g fresh leaf tissue was extracted in 5% ice-cold meta-phosphoric acid containing 1 mM EDTA. Homogenate was centrifuged at 10,000g for 20 min and the supernatant was used for ascorbate analysis (Huang et al. 2005).

2.6. Estimation of ions

Oven dried leaf samples were acid digested and Na⁺, K⁺ and Ca²⁺ were estimated according to the method of Wolf (1982) using a flame photometer (Jenway Flame Photometer, Bibby Scientific Ltd-Stone-Staffs-St15 0SA–UK).

2.7. Statistical analysis

Data presented is mean of three replicates and significant differences between the control and treatments was determined following Duncan’s multiple range test (DMRT) at P < .05 significance level.

3. Results

Plant growth parameters measured in the present study include length and dry weight of root and shoot as affected by NaCl and the application of Si (Table 1). NaCl stress resulted in impeded growth by declining the root and shoot length by 43.73 and 21.96% over control, however Si (2 mM potassium silicate, K₂SiO₃) supplemented improved the root (11.85%) and shoot (19.51%) length significantly and when applied to NaCl (NaCl + Si) stressed plants it mitigated the negative effects considerably (Table 1). Dry weight increased by 12.57 and 20.70% in root and shoot respectively due to Si supplementation and such promotory effect of Si was maintained when applied to NaCl stressed plants (Table 1).

Application of 200 mM NaCl to Talh seedlings reduced the chlorophyll contents and the photosynthetic parameters like stomatal conductance, transpiration rate and CO₂ assimilation considerably (Tables 2 and 3). Relative to control, NaCl stressed plants exhibited a decline of 64.91, 68.72, 54.20 and 31.32% in chlorophyll a, chlorophyll b, total chlorophylls and carotenoids which was ameliorated by 49.40, 54.48, 38.36 and 18.63% due to Si supplementation. Under normal conditions Si improved chlorophyll a (15.69%), chlorophyll b (28.43%), total chlorophylls (17.37%) and carotenoids (11.82%) over control (Table 2). Photosynthetic parameters like transpiration rate, stomatal conductance and CO₂ assimilation increased in Si supplemented Talh seedlings as compared to control as well as NaCl stressed plants (Table 3). In NaCl stressed plants transpiration rate, stomatal conductance and CO₂ assimilation rate declined by 75.80, 50.85 and 46.97% respectively over control plants and Talh seedlings treated with NaCl + Si (2 mM potassium silicate, K₂SiO₃) exhibited a reduction of only 33.34, 26.34 and 15.40% in transpiration rate, stomatal conductance and CO₂ assimilation reflecting in the amelioration of the negative
effects of NaCl stress (Table 3). Under normal growth conditions Si (2 mM potassium silicate, K2SiO3) application enhanced all these parameters resulting in increased water use efficiency (WUE) by 20.91% over control plants (Table 3).

200 mM NaCl stress increased lipid peroxidation (measured as MDA content) by 20.90% over control, however Si application under control conditions reduced rate of lipid peroxidation by 28.10% (Table 4). Supplementation of Si to NaCl stressed plants ameliorated the stress effects by causing 36.58% reduction over the NaCl stressed plants (Table 4).

For helping Talh seedlings to avert the ill effects of high salinity Si application increased the accumulation of compatible solutes for providing greater protection to them. Under controlled growth conditions Si supplementation resulted in 13.01 and 8.30% increase in proline and glycine betaine. Proline and glycine betaine increased by 1.75 and 1.61 fold respectively with 200 mM NaCl stress, on the other hand treatment with NaCl + Si elevated the proline (1.99 fold) and glycine betaine (1.82 fold) than control plants (Table 4). Interestingly, exogenous application of Si increased the content of total flavonoids by 17.12% which was reduced by NaCl treatment by 40.27% over control (Table 4). Supplementation of Si (2 mM potassium silicate, K2SiO3) to NaCl (NaCl + Si) stressed Talh seedlings mitigated the negative impact of salinity on total flavonoids by causing an improvement of 31.33% over NaCl stressed plants (Table 4).

Antioxidant system was up-regulated by the application of Si resulting in greater protection of Talh seedlings against NaCl stress. The activity of ROS scavenging enzymes such as SOD, POD, CAT,

Table 1
Effect of salt stress (200 mM NaCl) on length and dry weight of shoot and root of A. gerrardii with and without silicon (2 mM potassium silicate, K2SiO3) supplementation. Data presented is mean of three replicates and mean values designated by different letters are significantly different at P < .05.

| Treatments | Morphological criteria |
|------------|------------------------|
|            | Root length (cm)       | Root DW (g) | Shoot length (cm) | Shoot DW (g) |
| Control    | 32.7b                  | 1.46b       | 13.2bc            | 0.563b       |
| Si         | 37.1a                  | 1.67a       | 16.4a             | 0.710a       |
| NaCl       | 18.4d                  | 0.94d       | 10.3d             | 0.340d       |
| NaCl + Si  | 25.8c                  | 1.23c       | 12.8c             | 0.450c       |
| LSD at 0.05| 3.56                   | 0.17        | 0.32              | 0.08         |

Table 2
Effect of salt stress (200 mM NaCl) on chlorophyll and carotenoids (mg g⁻¹ FW) of A. gerrardii with and without silicon (2 mM potassium silicate, K2SiO3) supplementation. Data presented is mean of three replicates and mean values designated by different letters are significantly different at P < .05.

| Treatments | Photosynthetic pigments (mg/g FW) |
|------------|-----------------------------------|
|            | Chlorophyll a | Chlorophyll b | Total pigments | Carotenoid |
| Control    | 0.972b        | 0.438b        | 2.14b          | 0.731b     |
| Si         | 1.153a        | 0.612a        | 2.59a          | 0.829a     |
| NaCl       | 0.341d        | 0.137d        | 0.98d          | 0.502d     |
| NaCl + Si  | 0.674c        | 0.301c        | 1.59c          | 0.617c     |
| LSD at 0.05| 0.14         | 0.07          | 0.25           | 0.02       |

Table 3
Effect of salt stress (200 mM NaCl) on transpiration rate (E, mmol H2O m⁻² s⁻¹), stomatal conductance (gs, mmol CO2 m⁻² s⁻¹), CO2 assimilation (A, l mol CO2 m⁻² s⁻¹), and water use efficiency (WUE, A/E) of A. gerrardii with and without silicon (2 mM potassium silicate, K2SiO3) supplementation. Data presented is mean of three replicates and mean values designated by different letters are significantly different at P < .05.

| Treatments | Gas exchange parameters |
|------------|-------------------------|
|            | Transpiration rate (E) | Stomatal conductance (gs) | CO2 assimilation (A) | Water use efficiency (WUE) |
| Control    | 2.153b                  | 187.2b                  | 11.84b              | 5.502                   |
| Si         | 2.876a                  | 254.3a                  | 20.01a              | 6.957                   |
| NaCl       | 0.521d                  | 92.01d                  | 6.28d               | 12.05                   |
| NaCl + Si  | 1.435c                  | 137.9c                  | 10.02c              | 9.683                   |
| LSD at 0.05| 0.57                   | 28.1                    | 1.04                | 0.28                    |

Table 4
Effect of salt stress (200 mM NaCl) on lipid peroxidation (nM MDA g⁻¹ FW), proline (µg g⁻¹ fresh weight), glycine betaine (µM g⁻¹ fresh weight) and total flavonoids (mg catechin/ g fresh weight) of A. gerrardii with and without silicon (2 mM potassium silicate K2SiO3) supplementation. Data presented is mean of three replicates and mean values designated by different letters are significantly different at P < .05.

| Treatments | MDA (µmol g FW) | Proline (µg g FW) | Glycine Betaine (µmol g FW) | Total flavonoid (mg catechin g⁻¹ extract) |
|------------|-----------------|-------------------|-----------------------------|------------------------------------------|
| Control    | 14.98b          | 32.02d            | 2.341d                      | 12.44b                                   |
| Si         | 10.77d          | 36.81c            | 2.533c                      | 15.01a                                   |
| NaCl       | 18.94a          | 56.13b            | 3.792b                      | 7.43d                                    |
| NaCl + Si  | 12.01c          | 63.89a            | 4.267a                      | 10.82c                                   |
| LSD at 0.05| 1.26            | 2.93              | 0.08                        | 2.04                                     |

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4 MDA: Malondialdehyde.
APX and GR increased by 3.87, 4.78, 8.65, 9.57 and 8.20% due to Si (2 mM potassium silicate, K₂SiO₃) supplementation over control plants. Though activity of SOD, POD, CAT, APX and GR was stimulated by 7.75, 11.25, 11.07, 18.75 and 15.95% respectively due to NaCl stress, supplementation of Si further increased the activities of SOD (1.4%), POD (5.8%), CAT (5.88%), APX (6.35%) and GR (10.47%) over NaCl stressed plants (Fig. 1A–E). In order to investigate further the Si triggered growth promotion in seedlings (Talh) content of ascorbic acid (AsA) were determined and results revealed that Si supplemented Talh seedlings exhibited an increase of 8.38% over control and NaCl stressed plants showed 20.23% reduction (Fig. 1F).

Uptake and accumulation of Na⁺, K⁺, Ca⁺ and Na⁺/K⁺ ratio displayed a considerably variable picture with Si supplementation in both shoot and root tissues (Table 5). NaCl stressed plants analysed after NaCl and NaCl + Si treatments showed a considerable difference in accumulation of key element ions like K and Ca. Treatment of 200 mM NaCl declined uptake of K and Ca by 32.64, 59.87% and 46.74, 4.08% in shoot and root respectively over control plants. According to observed results, Na⁺ content increased by 38.85% in shoot and 69.08% in root causing an increase of 58.81 and 83.53% in Na/K ratio of shoot and root respectively. However supplementation of Si, relative to control, improved the uptake of K and Ca with significant reduction in the accumulation of Na in shoot (23.59%) and root (10.65%) resulting in significant decline in Na/K ratio (Table 5). However, application of Si (2 mM potassium silicate, K₂SiO₃) together with NaCl showed less accumulation of Na⁺ and hence Na/K over NaCl stressed plants (Table 5).

4. Discussion

Increased salinity in the soils has resulted in the conversion of fertile productive soils into the unproductive waste lands. Restricted growth and development growing on such soils mainly results due to the cellular sodium accumulation and the resulting
osmotic and ionic stress (Hashem et al., 2015). Supplementing Si can be an important and promising strategy for improving salt stress tolerance in crop plants like Talh seedlings. Several studies have demonstrated the potentiality of Si application in mitigating the salinity triggered negative effects on important crop plants (Zhu and Gong, 2014; Wang et al., 2015). In addition of its role in stress mitigation Si has been reported to improve the positive impact of plant growth promoting hormones (Khan et al., 2014) and Si might have improved the concentration of Rubisco in addition of its obvious effect on the lipid peroxidation. Earlier amelioration of salt stress induced decline in the chlorophyll pigments due to Si supplementation has been reported in Zea mays (Parveen and Ashraf, 2010) and Brassica napus (Nezami and Bybordi, 2011). Si application increases synthesis of pigments resulting in improved yield in Anethum graveolens (Shekari et al., 2015). Application of Si improves the positive impact of plant growth promoting rhizobacteria on the chlorophyll synthesis of mung bean under salinity stress (Mahmood et al., 2016). Significant improvement in photosynthetic attributes (transpiration rate, stomatal conductance and CO₂ assimilation) due to Si application may be due to the reduced accumulation of toxic Na⁺ ions within chloroplast. Similar to our findings improved photosynthetic rate (measured in terms of CO₂ assimilation), greater stomatal conductance, transpiration rate and the water use efficiency due to Si application has been observed in tomato and okra subjected to salt stress (Abbas et al., 2015). Exogenous Si protects the PSII by improving the chlorophyll fluorescence hence reflecting on the greater photosynthetic efficiency in pistachio (Habibi and Hajiboland, 2013), Oryza sativa (Mahdieh et al., 2015) and wheat (Mahgsoudi et al., 2015). Greater CO₂ assimilation in Si treated Talh seedlings resulting from greater stomatal conductance and transpiration rate proves Si to be a promising element in enhancing its yield potential under salinity as well as normal growth conditions.

Among the well accepted tolerance mechanisms initiated for averting the deleterious effects of stresses the accumulation of compatible osmolytes marks a key position. In the present study Si supplementation increased the accumulation of proline and glycine betaine keeping the major plant metabolic pathways protected from the salinity stress by maintaining the tissue water content (Sayed and Gadallah, 2014; Mali and Aery, 2008). Accumulation of proline and glycine betaine protects photosynthetic machinery and ameliorates the oxidative damage during abiotic stresses (Hashem et al., 2015; Ahmad et al., 2016; Abd Allah et al., 2015b, 2017). Results of present study depicting the increase in proline and glycine betaine due to Si supplementation support the findings of Torabi et al. (2015) and Abdel Latef and Tran (2016). Reports describing the impact of Si on osmolyte accumulation are rare, however are contradictory. Contrary to results of Liu et al. (2014) and Mauad et al. (2016) who have observed reduction in osmolyte accumulation due to Si supplementation in rice and sorghum plants our results support stimulatory role of Si supplementation in osmolyte accumulation. Crop species displaying

### Table 5

| Treatments | Na | K | Na/K | Ca | K/Ca |
|------------|----|---|------|----|------|
| **Shoot**  |    |   |      |    |      |
| Control    | 26.36c | 18.01b | 1.463 | 3.14b | 5.722 |
| Si         | 20.14d | 23.73a | 0.848 | 3.87a | 6.129 |
| NaCl       | 43.11a | 12.13d | 3.552 | 1.26d | 9.608 |
| NaCl + Si  | 32.87b | 16.78c | 1.958 | 2.01c | 8.345 |
| LSD at 0.05| 3.78 | 1.46 | 0.21 | 0.08 | 0.24 |
| **Root**   |    |   |      |    |      |
| Control    | 21.02d | 12.60b | 1.668 | 1.89b | 6.660 |
| Si         | 18.78c | 17.34a | 1.083 | 2.30a | 7.539 |
| NaCl       | 67.99d | 6.71c | 10.13 | 1.00d | 6.664 |
| NaCl + Si  | 34.77b | 10.88c | 3.195 | 1.33c | 8.133 |
| LSD at 0.05| 1.92 | 3.04 | 0.04 | 0.08 | 0.01 |
greater potential to accumulate sufficient quantities of osmolytes thrive well under stressful conditions as compared to low accumulating ones (Liang et al., 2003a,b; Wang et al., 2015; Abd_Allah et al., 2015a,b, Hashem et al., 2016). Greater accumulation of osmolytes including proline and glycine betaine in Talh seedlings under salt stress maintained the osmotic balance and hence protecting the cellular and enzyme structures. Greater accumulation of proline and glycine betaine in soybean as a result of Si supplementation may have improved the tissue water content and neutralised toxic ROS hence protecting the photosynthetic system from salt stress triggered negative effects (Mali and Aery, 2008). In addition osmolyte accumulation in Si supplemented plants may be due to its direct effect on the metabolism and catabolism of the key osmotic constituents.

The common and most deleterious outcome of high salt stress is damage to the structural and functional integrity of cell membrane. Excessive peroxidation of membrane lipids due to generation of toxic free radicals lead to the loss of their structural integrity. Increased lipid peroxidation observed in Talh seedlings subjected to salt stress corroborate with the findings of Giannakoula and Ilias (2013), Abd_Allah et al. (2015b) and Hashem et al. (2016). Stress triggered damage to membranes due to generation of toxic radicals result into leakage of important cellular constituents including electrolytes, ions, etc. Talh seedlings supplemented with Si exhibited reduced damage to membranes as compared to salt stressed ones and similar results have been observed by Zhu et al. (2004) in Cucumis sativus under salinity stress and Abdel Latef and Tran (2016) in maize under alkaline stress. Possible mechanism underlying the reduced lipid peroxidation in Si treated Talh seedlings may be due to the up-regulation of antioxidants and osmolytes (Abdel Latef and Tran, 2016). Si may have contributed to membrane protection by increasing the concentrations of polyunsaturated fatty acids which is often altered by high salinity (Alqarawi et al., 2014a).

Antioxidants work for neutralisation of ROS for strengthening the protection against the stresses including high salinity (Velarde-Buendia et al., 2012; Alqarawi et al., 2014b; Abd_Allah et al. 2015a,b). In the present study the investigated antioxidants including SOD, POD, CAT, APX and GR exhibited up-regulation in their activity after supplementation of Si under normal and NaCl stress conditions. Under salinity stress increased activity of antioxidant activity has been observed in several crop species like chickpea (Engambardieva et al., 2017), Brassica juncea (Ahmad et al., 2015), Panicum turgidum (Hashem et al., 2015), Acacia gerrardii (Hashem et al., 2016) and Triticum aestivum L. (Saqib et al., 2008). However existing research reports depicting the involvement of Si in activation of antioxidant defence system are quite few and in the present study greater antioxidant activity helped Talh seedlings in counteracting the oxidative damage to membranes significantly. Up-regulated antioxidant system arbitrates the oxidative stress and the signalling benefits of ROS hence keeping their beneficial role in normal jingle (Abdel Latef and Tran, 2016). Our results of increased activity of antioxidant enzymes by Si supplementation support the findings of Habibi and Hajiboland (2013) for Pistacia vera, Shekari et al. (2015) for Anethum graveolens and Abdel Latef and Tran (2016) for maize. Increased activity of CAT, APX and POD prevent the formation of more toxic hydroxyl radical. Si triggered enhancement in the activity of GR and APX prevent damage to photosynthetic apparatus by maintaining the optimal concentration of NADP for keeping the uniformity in electron flow and hence the generation of toxic superoxide radical is prevented (Abd_Allah et al., 2015b). The most important benefit of Si-mediated antioxidant system activation observed in the present study was the prevention of lipid peroxidation to considerable extent. Quenching excess ROS rapidly via activation of antioxidant system in Si supplemented plants optimised photosynthetic efficiency by preventing damage to PSI1 from toxic radicals like superoxide (Habibi and Hajiboland, 2013). Further studies investigating the expression pattern of antioxidant enzymes due to Si treatment can boost understanding about Si induced salinity tolerance.

Our earlier results have also revealed the antagonistic relation of excess NaCl concentration with important mineral ions like K, Ca, Mg, etc. (Hashem et al., 2015, 2016; Alqarawi et al., 2014b). Though there are indigenous mechanisms like selective ion uptake and sequestration of toxic ions into less sensitive spaces to prevent the negative effects of these ions. However Si supplementation significantly reduced the uptake of toxic Na ions into the upper aerial parts of Talh thereby preventing its deleterious impact on the key mineral elements like K and Ca. It has been advocated from times that greater salinity tolerance is directly related to low accumulation of Na+ ions and integration of this trait for developing and breeding new crop varieties is being extensively worked out (Munns et al., 2006). Na directly competes with K at the plasma membrane level resulting in its restricted uptake however Si supplementation prevented Na uptake protecting K ion channels from the negative regulation induced by Na (Hashem et al., 2015; Ahmad et al., 2015). Si induced greater uptake of K and Ca resulted significant decline in the ratio of Na/K thereby protecting the cellular functioning. Greater K/Na ratio is considered as key requirement for several processes including enzyme activation and Ca mediated signalling (Ahmad et al., 2015). Potassium and calcium individually as well as conjuncturally regulate several functions including protein synthesis, enzyme activity, photosynthetic efficiency, water use efficiency and the mitigation of oxidative damage (Hosseini et al., 2017; Abd_Allah et al., 2017). Improved Si uptake leads to membrane stabilization and increases K+ and Ca2+ uptake concomitant with decrease in Na ion (Khoshogfamarnesh et al., 2014). In maize Abdel Latef and Tran (2016) have demonstrated that priming with Si enhances the uptake of K. Similarly, Mali and Aery (2008) and Shekari et al. (2015) have demonstrated greater K uptake in Si supplemented wheat and Anethum graveolens. In addition to this reasons underlying the increased mineral uptake in Si supplemented Talh seedlings may include enhanced hydraulic conductivity (Shi et al., 2016a,b) and the significant accumulation of osmolytes leading to maintenance of osmotic potential (Liu et al., 2014). Greater uptake of K and declined uptake of Na in Si treated plants occurs due to increased activity of H-ATPase (Liang et al., 2003a,b). Further reduced Na uptake may be attributed to the significant deposition of silicate in the exodermis and endodermis of roots causing obstruction in the NaCl absorption (Wang et al., 2015).

5. Conclusions

Conclusively it can be said that supplementation of Si protected Talh seedlings from the ill effects of NaCl stress by causing significant improvement in the growth and biomass accumulation. Stress triggered reduction in chlorophyll pigments and the photosynthetic efficiency was mitigated by Si application. Si mediated growth promotion under normal and salt stress conditions was supported by the modulation in the activity of antioxidant enzymes including SOD, POD, CAT, APX and GR and the synthesis of ascorbic acid, proline and glycine betaine leading to reduced lipid peroxidation in them. Besides causing a significant improvement in the uptake of mineral elements Si prevented excess accumulation of Na ions suggesting multitude usage of Si. Future investigations are required to develop Si responsive crop cultivars for improved salt tolerance. Based on the physiological results, we have to propose a model explain the roles of silicon supplementation to balance salt stress in Acacia gerrardii Benth. (Fig. 2).
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