Enhancement of salt tolerance in a black bean variety (*Phaseolus vulgaris* L.) by silicon nutrition

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Abstract

In order to study the effect of silicon nutrition to increase salt tolerance in black bean variety of *Phaseolus vulgaris*, 12-day-old seedlings were treated with NaCl (0 and 50 mM) and NaCl (50 mM) + silicon (0.5 or 3 mM) in the green house for four weeks. The analyzed parameters were fresh and dry weights, total chlorophyll, free proline, relative water content (RWC), electrolyte leakage in the leaves, and the concentrations of Na^+ and K^+ in shoots and roots. Salt stress decreased the values of fresh (39.3%) and dry weight (48.4%), total chlorophyll (20.9%), K^+ (60.6% in roots and 20.3% in the shoots), and RWC (50%). Moreover, the level of free proline (19%), electrolyte leakage (2.4 folds), and Na^+ concentration (4.6 folds in shoots and 3.8 folds in roots) significantly increased. However, silicon (particularly at 3 mM) ameliorated the deleterious effects of NaCl. Compared to exclusively salt-stressed plants, the fresh and dry weights were increased by 20.3% and 66%, respectively. Also, the content of total chlorophyll (21%), RWC (83%), and K^+ (21% in shoots and 90% in roots) augmented, but electrolyte leakage (38%) and the content of Na^+ decreased (39.7% in shoots and 27.8% in roots). Generally, current data suggested that silicon enhanced salt tolerance in black bean plants by reducing the entrance of Na^+ and maintaining the level of K^+ in the salinized tissues. Besides, it improved the water status, membrane integrity, and function of photosynthetic apparatus under salinity.

Keywords: cellular water status; electrolyte leakage; legumes; photosynthetic pigments; salt stress

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Introduction

The black bean (*Phaseolus vulgaris* var. black) is a small, shiny variety of the common bean. The presence of various antioxidants such as flavonols, anthocyanins, and proanthocyanidins or condensed tannins are reported in black beans (Arts and Hollman, 2005; Aparicio-Fernández et al., 2006, 2008). Flavonoids and saponins present in black bean possess a great potential to protect against chronic diseases (Dong et al., 2007; Guajardo-Flores et al., 2013). Moreover, its phenolics inhibit enzymes related to glucose absorption (Mojica et al., 2015).

All varieties of *Phaseolus vulgaris* are more or less sensitive to salt stress and even relatively low levels of NaCl can noticeably reduce their yield (Nosrati and Roshandel, 2018). Therefore, enhancing salt tolerance in black bean would be of interest to increase the productivity and yield of this variety under saline conditions.

Application of silicon nutrition is introduced as an inexpensive and harmless

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strategy to ameliorate salinity impacts on crop plants (Zhu and Gong, 2014). Silicon does not contribute to the normal growth of plants as a necessary element (Hodson et al., 2005). It is also reported that silicon has no effect on the growth of bean plants under normal conditions (Zuccarini, 2008). Nevertheless, there are numerous reports that demonstrate the role of silicon to alleviate injurious effects of biotic (Bélanger et al., 2003; Côté-Beaulieu et al., 2009) and abiotic stresses (Ali et al., 2013; Keller et al., 2015; Ahmed et al., 2016). There are many studies about the beneficial effects of silicon to increase salt tolerance in higher plants such as barley (Liang et al., 2003), cucumber (Zhu et al., 2004), tomato (Romero-Aranda et al., 2006) maize (Moussa, 2006), alfalfa (Wang and Han, 2007), wheat (Tuna et al., 2008), sugarcane (Ashraf et al., 2010), canola (Hashemi et al., 2010), soybean (Lee et al., 2010), purslane (Kafi and Rahimi, 2011), *Spartina densiflora* (Mateos-Naranjo et al., 2013), rice (Shi et al., 2013), *Vicia faba* (Shahzad et al., 2013), and basil (Robatjazi et al., 2020). As suggested, silicon is involved in salinity tolerance through different strategies such as (1) limiting the transpiration by accumulation in leaves (Matoh et al., 1986), (2) confining of Na^+ in the root tissues via complex formation (Ahmad et al., 1992), (3) reducing the entrance of Na^+ by restraining apoplastic pathway in roots (Yeo et al., 1999), (4) increasing the activity of H^+-ATPase (Liang et al., 2005), (5) maintenance of membrane stability and chloroplasts ultrastructure (Liang et al., 2015), (6) alleviation of salt induced osmotic stress (Kafi and Rahimi, 2011; Coskun et al., 2016), and (7) lessening of oxidative stress through stimulation of cellular antioxidant system (Kim et al., 2017; Robatjazi et al., 2020).

The current research was designed to investigate the role of silicon in enhancing salt tolerance in black bean plants. Regarding the possible hypotheses which explain the mechanism of silicon to increase salt tolerance, here, we tested the following:

Hypothesis (1): Silicon nutrition would reduce the entrance of Na^+ from medium into plants tissues.
Hypothesis (2): Silicon nutrition could improve plant water status.

Materials and Methods

Plant material and growth conditions

Seeds of *Phaseolus vulgaris* var. black JAMARAN–4802 were purchased from Yekta Seed Company (Tehran, Iran) and surface-sterilized with 70% ethanol for 2 min. followed by repeated washing with double-distilled water. Then, they were sown in polystyrene boxes with a substrate composed of 50% perlite and 50% fine sand. Experiments (in three replicates with five individuals per each) were conducted in the greenhouse conditions (33 ℃ - 14 L/ 20 ℃ - 10 D and 60-70% of relative humidity) consisting of (a) control (Hoagland solution with no silicon and NaCl added), (b) 50 mM NaCl + 0.5 mM silicon, and (c) 50 mM NaCl + 3 mM silicon. Sodium silicate ($Na_2SiO_3.5H_2O$), purchased from Sigma Company, was used for silicon treatments. The 12-day-old seedlings were subjected to the treatments and the experiments lasted four weeks.

Plant growth

At the end of the experimental period, the 40-day-old plants were harvested to wash thoroughly with tap water and then with distilled water to remove all precipitates from the surface. Fresh and dry weights of the plants were recorded. Dry weights were measured after drying plant parts for 3 days at 70 ℃.

Measurement of total chlorophyll concentration

The content of total chlorophyll (Chl. a + Chl. b) of the leaves was determined according to the method of Lichtenthaler and Buschmann (2001) with 80% acetone as the solvent. The following equations were used for this purpose:

Chl. a ($\mu g\ ml^{-1}$) = 11.64 (A_{663}) − 2.16 (A_{645}), Chl. b ($\mu g\ ml^{-1}$) = 20.97(A_{645}) − 3.94(A_{663})

Chl. Total ($\mu g\ ml^{-1}$) = Chl. a + Chl. b

Table 1
Analysis of variance for some physiological and biochemical traits in 40-day-old plants of black bean treated with NaCl (50 mM) and silicon nutrition (0.5 and 3 mM)

S.O.V	MS										
	df	Fresh weight	Dry weight	Total Chl	EL	Free proline	RWC	K^+ in shoot	K^+ in root	Na^+ in shoot	Na^+ in root
Salt stress	2	12.14*	5.66*	15.6*	64.41*	13.62*	86.2*	92.67*	72.77*	256.12*	201.13*
Salt stress × Silicon	3	22.16*	8.45*	40.3*	54.78*	14.54*	142.5*	152.23*	136.61*	105.87*	121.65*
Error	11	2.36	0.75	3.68	4.82	3.74	3.94	7.29	8.26	4.26	6.44
CV (%)	-	3.53	4.83	5.48	7.66	7.48	8.35	3.84	4.93	5.02	6.46

*: significant at $p<0.05$

where A_{663} and A_{645} represent absorbance values read at 663 and 645 nm wavelengths, respectively.

Assessment of relative water content of leaves (RWC)

The leaf discs (2 cm^2) were weighed instantly to record fresh weight (FW), followed by floating on distilled water for 4 h. The turgid leaf discs were then quickly blotted to remove surface water and weighed to obtain turgid weight (TW). The leaf discs were subsequently oven-dried at about 80 ℃ to obtain a constant weight as dry weight (DW). The RWC was calculated based on Ciulca and Lugojan (2011) as follows:

$$RWC\ (\%) = [FW-DW)/(TW-DW] \times 100$$

Determination of electrolyte leakage percentage

Electrolyte leakage (EL) percentage was used to assess membrane permeability which was measured using an electrical conductivity meter. The procedure used was based on the method of Lutts et al. (1996). Leaf samples were cut into 1 cm segments and placed in individual stoppered vials containing 10 ml of distilled water after three washes with distilled water to remove surface contamination. These samples were incubated at room temperature (25 ℃) on a shaker (100 rpm) for 24 h. Electrical conductivity of bathing solution (EC_1) was read after incubation. Samples were then placed in a thermostatic water bath at 95 ℃ for 15 min and the second reading (EC_2) was carried out after cooling the bathing solutions to room temperature. Electrolyte leakage percentage was calculated as EC_1/EC_2 and expressed as percent.

Assessment of free proline content

Free proline was extracted from 0.5 g fresh shoot samples in 3% (w:v) aqueous sulphosalicylic acid and estimated by ninhydrin reagent (Bates et al., 1973). The absorbance of the fraction with toluene aspired from the liquid phase was read at 520 nm. Proline concentration was determined from a calibration curve and was expressed as μmol proline g^{-1} FW.

Measurement of Na^+ and K^+ concentrations

Dried material of separated roots and shoots were ground to a fine powder for ion analysis. Ground samples were ashed at 550 ℃ for 5 hours and digested with 6 N HCl. Na^+ and K^+ were determined with a flame photometer.

Statistical Analysis

Data were analyzed using SPSS software. Statistically different groups were determined by Duncan's test ($p<0.05$). The graphs were prepared via Excel program.

Results

The results of variance analysis of the physiological and biochemical traits of black bean plants under study are shown in Table 1. According to Table 1, salinity affected the studied traits significantly ($p<0.05$). In addition, a significant interaction was obtained between silicon and salinity ($p<0.05$).

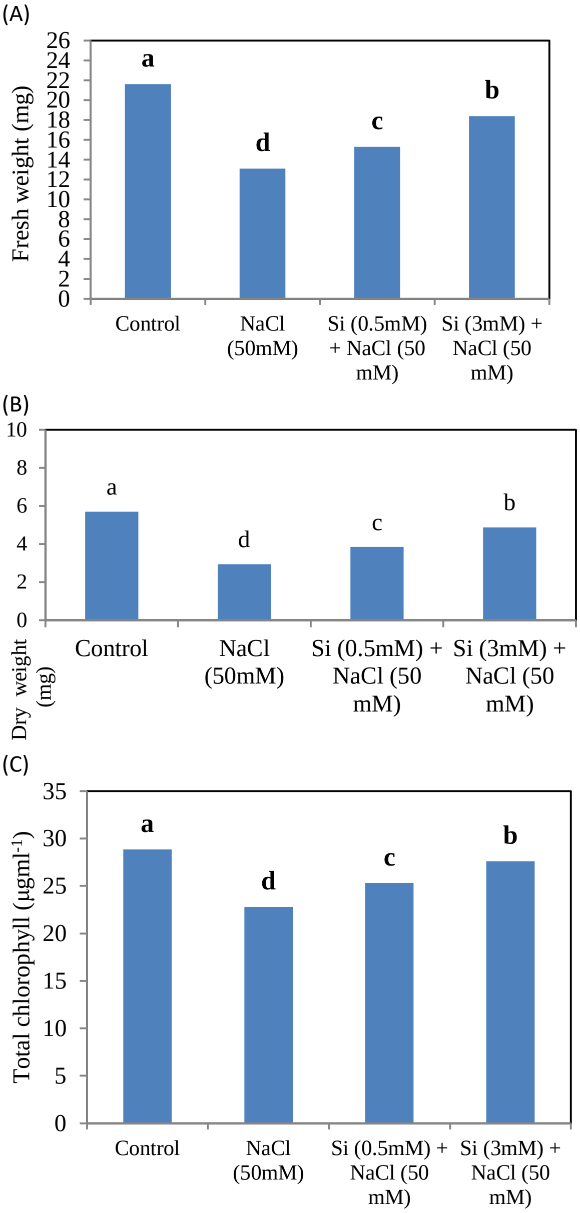

Fig. I. The effect of NaCl (50 mM) and silicon nutrition (0.5, 3 mM) on 40-day-old black bean plants; (A) total fresh weight, (B) total dry weight, (C) total chlorophyll content; means with the same letter are not significantly different at $p<0.05$. Each column represents three replicates containing 10 plantlets.

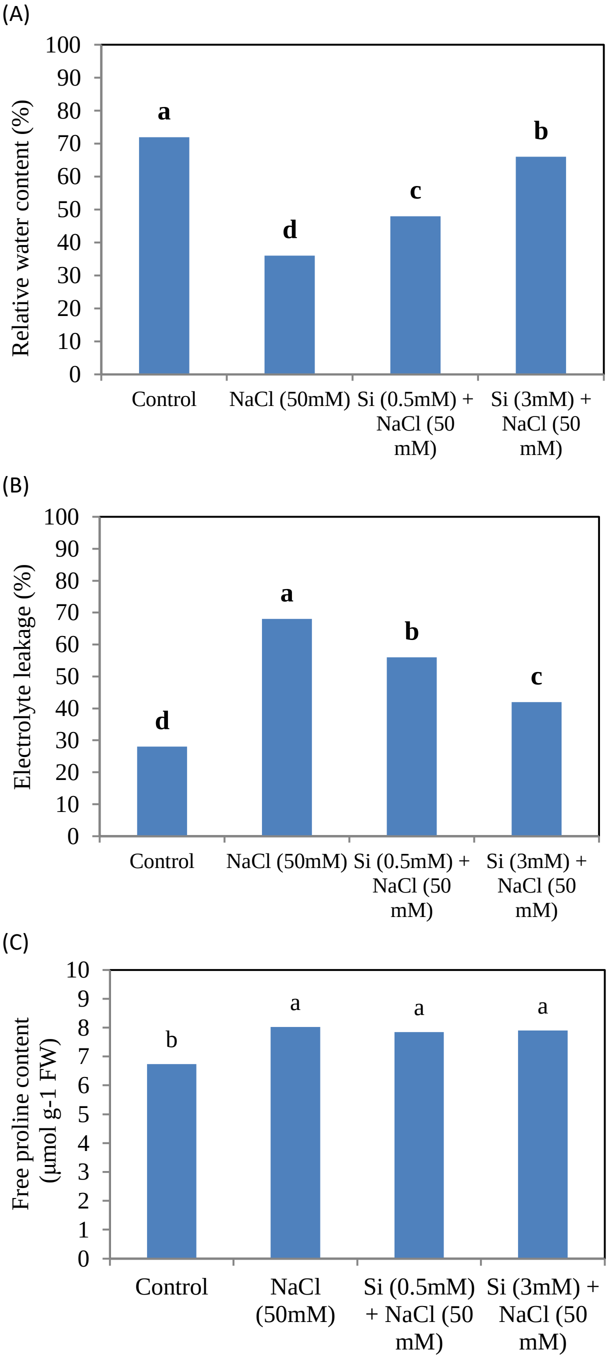

Fig II. The effect of NaCl (50 mM) and silicon nutrition (0.5, 3 mM) on 40-day-old black bean plants; (A) relative water content; (B) electrolyte leakage; (C) free proline content.; means with the same letter are not significantly different at $p<0.05$. Each column represents three replicates containing 10 plantlets.

Salinity treatment (50 mM NaCl) significantly reduced the fresh and dry weight in black bean plants ($p<0.05$) (Fig. I. A, B). The extent of reduction was 39.5% for fresh weight and 48.4% for dry weight of the salinized plants compared to control. Under salt stress, silicon nutrition significantly increased the mentioned parameters ($p<0.05$). The positive effect of silicon was higher at 3 mM, as the values of fresh and dry weight increased by 20.3% and 66%, respectively in comparison to the exclusively salt stress ones.

The level of total chlorophyll content significantly diminished (by 20.9%) in salt stressed black bean plants compared to control ($p<0.05$)

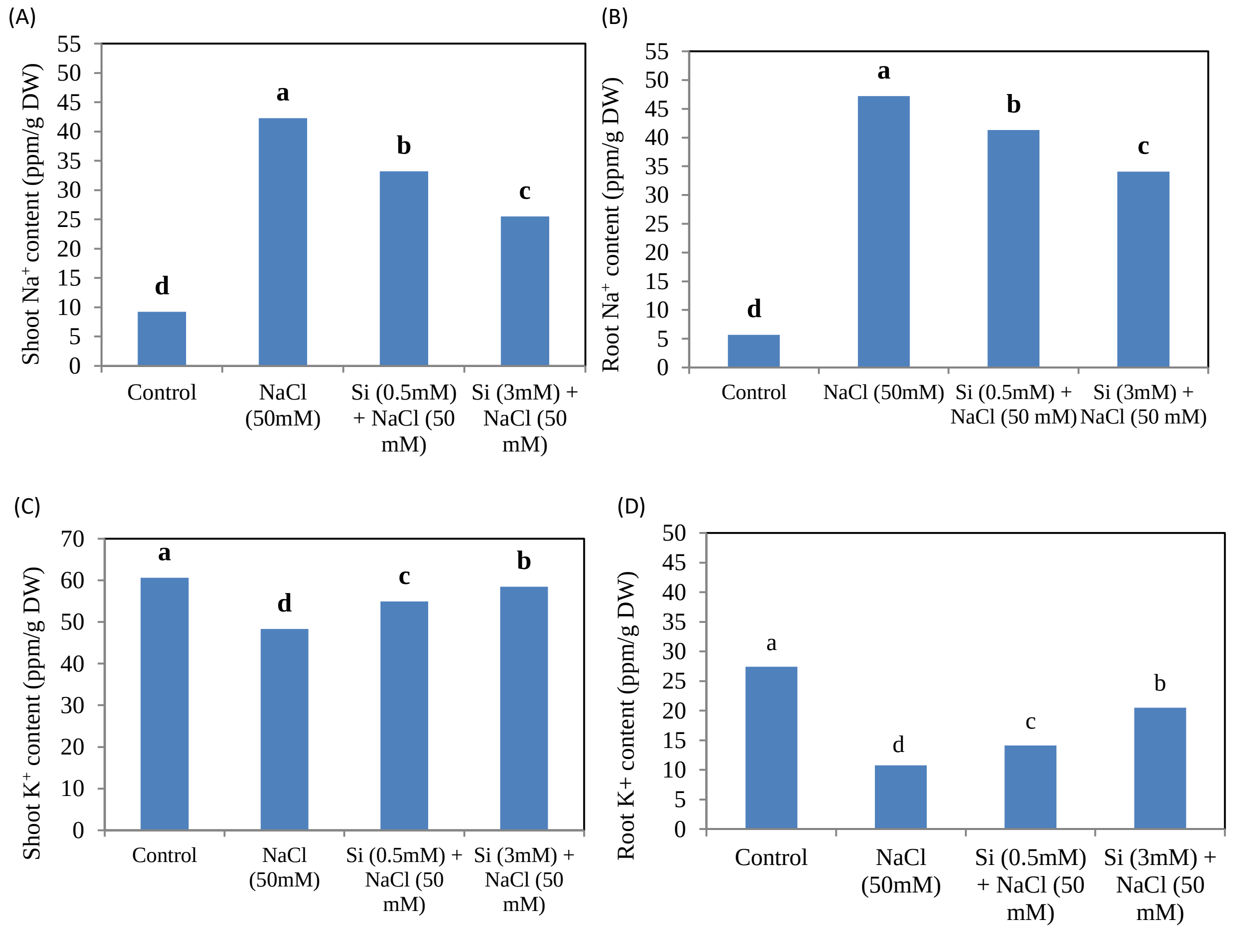

Fig. III. The effect of NaCl (50 mM) and silicon nutrition (0.5, 3 mM) on 40-day-old black bean plants; (A) shoot sodium concentration; (B) root sodium concentration; (C) shoot potassium concentration; (D) root potassium concentration; means with the same letter are not significantly different at $p<0.05$. Each column represents three replicates containing 10 plantlets.

(Fig. I. C). Silicon nutrition (particularly at 3 mM) markedly restored chlorophyll content under salt stress (Fig. I. C). The concentration of total chlorophyll increased by 11% and 21% at 0.5 and 3 mM silicon nutrition, respectively compared to salinity alone.

Relative water content (RWC) in the leaves of black bean plants significantly decreased (by 50%) under saline condition ($p<0.05$) (Fig. II. A). Silicon supplement (0.5 and 3 mM) significantly recovered RWC level under salinity ($p<0.05$). At the best case (3 mM silicon), the increment percentage of RWC was 83.3% compared to exclusively salt stress.

Salinity treatment significantly impaired membrane stability through increasing electrolyte leakage in the cells of black bean leaves ($p<0.05$) (Fig. II-B). In this case, the amount of electrolyte leakage increased by 2.4 folds in the salt-stressed leaves. Addition of silicon (particularly 3 mM) to the irrigation water could relatively protect cellular membrane stability in the salinized plants ($p<0.05$) (Fig. II. B). The level of electrolyte leakage reduced by 17.7 and 38.2% at 0.5 and 3 mM silicon compared to salinity treatment alone.

The level of free proline significantly increased in the shoots of black bean plants under saline condition ($p<0.05$) (Fig. II. C). The percentage of increment of free proline content was +19% in the salinized shoots compared to control. The amount of this parameter was also higher under the treatment of silicon nutrition + salinity in comparison to control. However, there were no significant differences between the treatments of 0.5 mM and 3 mM silicon or between the silicon-treated and exclusively salt-stressed plants.

As shown in Fig. III, sodium concentrations in the shoots and roots were noticeably higher in the black bean plants in the presence of NaCl (alone). The accumulation of Na^+ was much greater in the salinized roots compared to their shoots. The Na^+ level in the salinized shoots and roots were approximately 460 and 828% more than control. Silicon nutrition significantly decreased the concentration of sodium in the stressed shoots and roots of black bean plants but these levels were still much higher than control. Under salinity, the inhibitory effect of silicon supplement on the entrance of Na^+ into the plants tissues was much more obvious at 3 mM than that at 0.5 mM (approximately more than two folds). Furthermore, the role of silicon nutrition (at 3 mM) to lessen the level of Na^+ in the tissues was stronger in shoots (resulting in a reduction of 39.7% in the level of Na^+) than that in roots (a reduction of 27.8%) compared to the exclusively salt-stressed plants.

Under saline condition, potassium concentration was significantly reduced in tissues of black bean plants ($p<0.05$). The percent of reduction of K^+ content in roots (60.6%) was larger than that in shoots (20.3%). However, the concentration of potassium increased under salinity when silicon was added to the medium. Under the best condition (3 mM silicon), the level of K^+ increased in salinized roots (+90%) and shoots (+21%) compared to exclusively salt-stress group. Results showed the positive effect of silicon to preserve the content of K^+ in the tissues was more efficient in roots compared to that in shoots.

Discussion

Salt stress harmfully affects normal growth of plants via establishment of osmotic stress and ion toxicity (Munns and Tester, 2008). In the present study, application of silicon significantly ameliorated the salt-induced reduction of biomass in black bean plants. This result is in agreement with the previous reports (see Rizwan et al., 2015). As suggested, different mechanisms could be involved to improve plant biomass by silicon nutrition under salinity. One theory is that the utilization of silicon can recover water status and water-use efficiency in plants (Coskun et al., 2016). This point is important because the primarily reduction of plant growth under salinity is due to osmotic effect of NaCl (Munns et al., 2002). Current data showed that fresh weight and RWC of black bean plants were reduced under salinity whereas silicon supplement significantly improved these parameters. It is already reported (for example: in sorghum) that the root and whole-plant hydraulic conductance, transpiration, stomatal conductance, and leaf water content are increased by silicon under osmotic stress. One view indicates that the role of silicon to enhance root hydraulic conductance is associated with increased expression and activity of plasma-membrane intrinsic protein (PIP) aquaporins, which are mediated in water transport. However, the associated mechanisms are remained to be detected. Plants' ability to preserve their whole water content is a strategy to increase salt tolerance in the tissues through dilution of Na^+ concentration in the cells. Neuman and Figueiredo (2002) have shown that high-molecular weight silicon complexes can be transported into vacuoles through an endocytotic process causing higher degree of osmotic adjustment and so a higher capacity of tissue to retain water. It is documented that silicon is actively absorbed through the roots in the form of uncharged monosilicic acid, and is then passively transported by the transpiration stream, irreversibly precipitating as SiO_2-nH_2O in cell walls and cell lumens of all plant tissues (Richmond and Sussman, 2003). SiO_2-nH_2O deposition could help to keep water, to dilute salts and to protect tissues from physiological drought. Thus, another ameliorative effect of silicon could be related to the hydrophilic nature of silicon. Furthermore, the binding of silicon with cell-wall hemicelluloses results in improved structural stability (He et al., 2015), which is obviously valuable under water deficit. As well-documented, silicon can strengthen plant cell walls and contributes to cell mechanical support through enhancing suberization, lignification, and silicification (Guerriero et al., 2016).

Moreover, it is suggested that silicon can adjust the osmotic potential of cells via increased osmolytes accumulation, e.g. proline (Sonobe et al., 2010) and consequently affects water status. However, the result of the present study did not confirm this point since the concentration of free proline did not change in the silicon treated plants in comparison to the exclusively salt-stressed ones.

Current data showed salinity induced electrolyte leakage of the cellular membranes in the leaves of black bean plants, but silicon nutrition partially recovered it. Salinized plants usually experience a water deficit that leads to oxidative stress which is overproduction of reactive oxygen species such as H_2O_2, •OH, superoxide anion and singlet oxygen. These toxic radicals disrupt normal metabolism and damage plant cell membranes (Gill and Tuteja, 2010). As a consequence, the electrolyte leakage of cellular membranes occurs. The beneficial effect of silicon on decreasing electrolyte leakage is shown in barley (Liang et al., 2003) and wheat (Tuna et al., 2008). It is suggested that silicon ameliorates cellular membrane damage through reducing oxidative stress under salinity (Robatjazi et al., 2020).

Under salinity, high salt concentration changes the balance of ions in plants. For instance, the competition between Na^+ and K^+ uptake perturbs the K^+ /Na^+ ratio. Under normal physiological conditions, the protoplasm maintains a high K^+ /Na^+ ratio. However, the similarity of K^+ and Na^+ radii makes it difficult for plants to discriminate between them, so the normally high K^+ /Na^+ ratio is altered upon the influx of Na^+ through K^+ pathways (Blumwald, 2000). Salinity treatment usually results in an increase in Na^+ and Cl^- accumulation and a decrease in certain other cations like K^+ and Ca^{2+} (Wang and Han, 2007). High levels of Na^+ are injurious to plant cells, e.g. to cellular metabolism, and leads to reduced plant growth and also overproduction of reactive oxygen species. Under salt stress, plants must expend additional cellular resources to maintain a high concentration of cytosolic K^+ and low concentration of Na^+. In the present work, salt stress increased Na^+ accumulation in the roots (around 8.3 folds) and shoots (approximately 4.6 folds) of salinized black bean plants. However, the increase of Na^+ in the salinized roots was greater compared to the salinized shoots. As indicated, Na^+ exclusion from actively photosynthetic organs is an important strategy to increase salt tolerance in glycophytes (Munns et al., 2002). Additionally, data showed that silicon nutrition decreased Na^+ buildup in the salinized tissues. According to the current results, silicon markedly reduced Na^+ accumulation in the roots and shoots of black bean plants compared to exclusively salt-stress group. This is consistent with the hypothesis indicating silicon restricts salt stress via partial blockage of apoplastic transport of Na^+ which is mainly responsible for Na^+ entry to the roots and then shoots (Romero-Aranda et al., 2006). Research has detected that silicon deposition in the cell walls of exodermis and endodermis of roots reduces the transport of Na^+ to the central tissues of root and shoots (Gong et al., 2006). Another study showed that silicon decreased Na^+ transport to the shoots through complexes formation with Na^+ (Ahmad et al., 1992).

In the current work, the reduction of K^+ level in the salinized roots was greater in comparison to the salinized shoots. In other words, the beneficial effect of silicon on the maintenance of K^+ was more evident in the salinized shoots in comparison to that in roots. This finding may suggest that roots serve as a source to provide K^+ for the salinized shoots. As reported, a greater ability to preserve cytoplasmic K^+ and then less Na^+ accumulation would bring about a better salt tolerance in crop plants. In the same line; studies have showed that silicon enhances K^+/Na^+ ratio against the toxic effects of sodium (reviewed by Zhu and Gong, 2014).

Photosynthesis, as one of the most fundamental biochemical processes to increase plant biomass, can be simply injured by salt stress. The buildup of Na^+ in plant tissue damages chloroplast ultrastructure and reduces the levels of photosynthetic pigments. As shown in the current work and in agreement with the previous reports (Tuna et al., 2008; Mateos-Naranjo et al., 2013; Yin et al., 2014), silicon addition can improve photosynthetic apparatus (for instance, increasing the level of chlorophylls and carotenoids) under saline condition. As suggested, the promising effect of silicon on the photochemical apparatus and photosynthetic pigments is partly attributed to the silicon-mediated decrease in Na^+ uptake and increase in K^+ uptake in plants tissues under salt stress. Silicon application has additional benefits on gas exchange characteristics, water potential, and reduction in oxidative stress in plants which all may positively affect the level of photosynthetic pigments.

Conclusion

Overall, current data showed that silicon nutrition, particularly at 3 mM, could enhance salt tolerance in salt sensitive black bean plants. Accordingly, silicon supplement improved the growth of salinized plants through reducing the entrance of Na^+ from medium into the root tissues and then increasing K^+/Na^+ ratio, predominantly in shoots. Furthermore, silicon nutrition significantly improved membrane integrity and water status in salinized black bean plants.

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