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Research article

Interplaying roles of silicon and proline effectively improve salt and cadmium stress tolerance in *Phaseolus vulgaris* plant

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ABSTRACT

The interplaying defensive roles of silicon (Si) and proline (Pro) in improving growth and yield attributes, physio-biochemical attributes, and antioxidant defense systems in common bean plant grown under saline (NaCl) and/or cadmium (Cd^{2+}) stress were assessed. Seed were sown in plastic pots filled with sand-free ions as a growing medium that watered with a ½-strength Hoagland's nutrient solution. Twenty five days after planting, pots were split into 4 plots; control (no stress), 150 mM NaCl (salt stress), 1.5 mM Cd^{2+} in $CdCl_2$ (Cd^{2+} stress), and 100 mM NaCl + 1.0 mM Cd^{2+} (salt + Cd^{2+} stress). Four treatments; foliar spray with distilled water, 6 mM Si (in $K_2SiO_3.nH_2O$) solution, 6 mM Pro solution, and a combination of Si and Pro were allotted under each of the 4 plots. The experimental layout was a completely randomized design with 15 replicates. Compared to control, NaCl or Cd^{2+} stress significantly ($P \leq 0.05$) reduced plant growth and yield attributes, leaf contents of chlorophylls, carotenoids, N, P, and K^+, K^+/Na^+ ratio, RWC, MSI, *Pn* and *Tr*, while elevated significantly leaf EL, leaf contents of proline, soluble sugar, glutathione, MDA, Na^+, and root, leaf and pod contents of Cd^{2+}. The activities of antioxidant enzymes were also raised. The combined stress (NaCl + Cd^{2+}) was more influential. Addition of Si and/or Pro for common bean plants under NaCl and/or Cd^{2+} stress significantly enhanced all investigated attributes of physiology, morphology, and biochemistry, and further increased the activities of antioxidant enzymes. Supplementation of Si + Pro was the best treatment having more positive influential, especially reducing the Cd^{2+} content in *Phaseolus vulgaris* pods to the limits (0.27 $mg\,kg^{-1}$) for legumes. Therefore, this combined treatment is recommended to use for alleviating environmental stress effects, especially salinity and Cd^{2+} for common bean production.

1. Introduction

Plants are exposed to various types of environmental stresses throughout their life cycle, including salinity and toxic metal ions. These environmental factors restrict plant performance to varying degrees, depending on the severity of the stress (Hayat et al., 2012). Salinity is considered as a lasting menace to crops, where approximately 20% of croplands and 33% of irrigated croplands globally are suffering from high salinity (Heuer, 2010). In arid and semi-arid regions, including Egypt, soil salinization is caused by several factors like insufficiency of irrigation water with poor management and drainage, high evaporation rate-induced hot climate with low rainfall, and proximity to the sea (Rady et al., 2013). Many crops are susceptible to salt stress and unable to tolerate salinity even at low levels (Munns and Tester, 2008). Salinity reduces the plant's ability to benefit from water and causes a decrease in plant growth and production by inhibiting plant metabolism (Munns, 2002). Soil salinity reduces water availability to plant, leading to water shortfall and nutrient imbalance. Nutrient imbalance is caused due to decreased uptake of useful nutrients and increased uptake of specific toxic Na^+ and Cl^- ions that make plants stressful (Marschner, 1995). Accumulation of salts in the leaf apoplasm up to a toxic level leads to turgor loss and dehydration, causing damage of leaf cells. Most physiological processes, such as photosynthesis, are affected by salt stress with chlorophyll pigment reduction and stomata closure associated with CO_2 pressure decrease and Rubisco enzymes suppression (Munns, 2002; Kahrizi et al., 2012).

Heavy metal stress causes reductions in plant growth and production and hazardously affects human health (Buchet et al., 1990; Amari et al., 2017). Cadmium (Cd^{2+}), ranking 7th among the highest toxicants, causes pronounced environmental disruptions to the agricultural

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systems (Kumar, 2013). Phosphate sewage sludge, synthetic fertilizers, manufacturing emissions and mining, and smelting industries are the main sources of Cd^{2+} (Adriano, 1986). Plant absorbs Cd^{2+} from the soil readily and accumulates it in its tissues, disrupting growth and development, photosynthetic rates, nutrient, and water uptake and other physio-biochemical characteristic, and lastly the death of the plant occurs (Di Toppi and Gabbrielli, 1999). In addition, negative modification occurs in structures of organelles linked to the oxidative stress generated by excessive production of reactive species of oxygen (ROS; $^1O_2$, $O_2^{\bullet-}$, H_2O_2, and OH^-) (Yan et al., 2015). These ROS cause considerable damage to chlorophyll, proteins, DNA, and lipids (Schutzendubel and Polle, 2002), and disturb all physio-biochemical processes related functions in plant cells (Apel and Hirt, 2004; Foyer and Noctor, 2005).

Osmoprotectant supplementation for plant can be a feasible strategy to improve stress tolerance (Qirat et al., 2018). Among them, proline (Pro) can be used to reduce unfavorable effects of oxidative stress on plants (Yang et al., 2009). It is a signaling molecule that acts as a regulator of plant growth by triggering cascade signaling processes. It is also a multi-functional amino acid (Yildiz and Terz, 2013). Pro accumulation in plants increases their resistance to stress toxicity (Aslam et al., 2017) by protecting them from acute ROS effects (Singh et al., 2014). Moreover, silicon (Si) can also enhance the plant's ability to withstand various stresses. It helps most plants due to its essential physic-mechanical functions. Although the precipitation of Si on cell walls, it stimulates most physiological pathways (Parveen and Ashraf, 2010), mitigating the effects of salt and Cd^{2+} stresses by promoting stress tolerance in plants (Alzahrani et al., 2018). It improves the activity of photosynthesis, adjusts the nutritional imbalance and reduces the toxicity of the elements (Shi et al., 2016). It also decreases Na^+ uptake and increases K^+/Na^+ ratio to mitigate the effect of ion toxicity in salt-stressed plants (Hajiboland et al., 2016). Si may change translocation and distribution of Cd^{2+} in various parts of plant to assist its healthy growth under Cd^{2+} stress (Zhang et al., 2008).

Common bean (*Phaseolus vulgaris* L.) is one of the most important vegetable legume crops grown for human nutrition due to its high production of seeds as a food legume model (Broughton et al., 2003). It is classified as a sensitive plant to both salt (Mass and Hoffman, 1977) and Cd^{2+} stress (Rady, 2011).

Several previous studies have separately reviewed the Si or Pro and their respective effect on plant growth and production under saline and/or Cd^{2+} stress (Rady et al., 2016; Alzahrani et al., 2018). However, studies using combined Si + Pro to mitigate the effects of saline and Cd^{2+} stress conditions, to our knowledge, have not been reported. Consequently, the main objective of this study was to estimate the protective interplaying effects of the combined Si + Pro supplementation on common bean growth and yield attributes, physio-biochemical attributes, and antioxidant defense systems under the conditions of saline and Cd^{2+} stress. It was hypothesized that the use of integrative Si + Pro can effectively reduce the harmful effects of salt and Cd^{2+} toxicity on the performance of common bean as a sensitive plant to salinity or Cd^{2+} stress by reducing Na^+ content, as well as reducing Cd^{2+} content in edible part (pods) to the limits specified for legumes (Kabata-Pendias and Pendias, 2001).

2. Materials and methods

Phaseolus vulgaris (L.) certified seed (cv. Bronco) were surface sterilized with 0.1% $HgCl_2$ for 1 min. After washing with sterile deionized water and air-drying overnight, 6 seed were planted in each plastic pot (35 cm in diameter, 40 cm depth). After emergence, thinning was performed to maintain 3 uniform seedlings per pot. Plant growing medium used was sterilized ion-free sand. The particle size of the sand was 0.25 mm, obtained through a sieve has 0.25 mm diameter holes. The ions (all anions and cations) were effectively removed from the sand by washing with commercial HCl (30% concentration) for 24 h. Then, the sand was washed with distilled water several times to remove the acid completely. The experiment was carried out during the early summer season; 25th of February 2018. Hoagland's nutrient solution (½-strength) (Hoagland and Arnon, 1938) was used for watering. Without applying any stress or ameliorative treatment, the nutrient solution was added at 100% field capacity once every 3 days for all pots up to 25 days of sowing (DOS).

At this time (25 DOS), pots were split into 4 plots; (1) control (without any of stress treatments), (2) 150 mM NaCl (salt stress), (3) 1.5 mM cadmium (in $CdCl_2$; Cd^{2+} stress), and (4) 100 mM NaCl + 1.0 mM Cd^{2+} (salt + Cd^{2+} stress). Each of the 4 plots was received 4 treatments; (1) foliar spray with distilled water, (2) foliar spray with 6 mM Si (in $K_2SiO_3.nH_2O$) solution, (3) foliar spray with 6 mM Pro solution, and (4) foliar spray with combined solutions of 6 mM Si + 6 mM Pro. All these were composed of 16 different treatments. Our initial studies have shown that different stresses (e.g., 150 mM NaCl, 1.5 mM Cd^{2+}, or 100 mM NaCl + 1.0 mM Cd^{2+}) have significant toxic effects on the growth of common bean seedling. In addition, the concentration of 6 mM Si (in Potassium Silicate; K_2SiO_3, Powder, pH 11.3, Stable Water-Soluble Silicic Acid Potassium Salt; 1312-76-1, Henan Daken Chemical Co., LTD, China) or 6 mM Pro (Pure L-Proline Powder Complies with US Pharmacopeia (USP) Quality Standard, NuSci Brand) has significant positive effects on seedling growth compared to other levels used (data not shown) and, therefore, were selected for the current study. The amount of potassium or chloride found in $K_2SiO_3.nH_2O$ or $CdCl_2$ was calculated and added (as foliar spray or through the nutrient solution, respectively) to plants in other treatments that did not receive $K_2SiO_3.nH_2O$ or $CdCl_2$ to offset the effect of potassium or chloride in all treatments. Foliar applications of distilled water, Si, and Pro were performed three times at 25, 35 and 45 DOS. By using a handheld manual sprayer (model 0417.02.00; Guarany Ind. & Com. Ltd), different solutions were sprayed on the upper leaf surface until run-off (approximately 120 ml per pot), and few drops of Tween-20 were added to the spray solutions as a surfactant. According to treatments, the Cd^{2+} and/or NaCl concentrations (1.5 mM Cd^{2+}, 150 mM NaCl, or 1.0 mM Cd^{2+} + 100 mM NaCl) in the growing medium were maintained and controlled by an inductively coupled plasma atomic emission spectrometry (ICP- AES, IRIS-Advan type, Thermo, USA).

Over the experiment duration, non-stress-exposed plants treated with DW, Si, Pro, or Si + Pro were watered by pure Hoagland's nutrient solution (½-strength). On the other hand, stressed plants sprayed with ameliorants or with distilled water were irrigated by ½-strength Hoagland's nutrient solution contained NaCl salt and/or Cd^{2+} at the specified concentrations. Soil pH was modified back to the control pH (6.2–6.5) using diluted sulfuric acid.

The experimental pots were positioned in a complete randomized block design in a greenhouse with average day/night temperatures of 24 ± 4/14 ± 2 °C, relative humidity of 58–60%, and average day/night length (light/dark photoperiods) of 13/11 h. These averaged conditions were for plant growth throughout the season (February–April). The experiment was composed of 16 treatments, as shown above, and each treatment was repeated with 15 pots. Pots of all treatments were rotated (from place to place) every 2 days to ensure fairness in the distribution of light and sunlight intensity for all plants. All stress (NaCl and Cd^{2+}) and ameliorant (Si and Pro) treatments were terminated 55 DOS and common bean plants were harvested to assess growth, physio-biochemical attributes, nutrients, and antioxidant defense systems. The experiment was continued until the marketable stage of green pods, where green pod yield was estimated.

2.1. Determination of growth characteristics and green yield

Ten common bean plants were cut off from each treatment to estimate plant highest (cm), leaves area (dm^2 $plant^{-1}$), and dry weight (g $plant^{-1}$; shoot system + roots system). At the merchantable green pod stage, samples were reaped from thirty plants (10 pots) from each treatment to estimate number of pod pot^{-1} and green pod yield pot^{-1}.

2.2. Determination of physio-biochemical constituents and antioxidants activities

The method of Fadeels (1962) was used to extract chlorophyll “a”, chlorophyll “b”, and total carotenoids from fresh common bean leaves utilizing pure acetone. As detailed in Bates et al. (1973) method, proline accumulation in common bean leaves was determined. The method of Irigoyen et al. (1992) was utilized to estimate the content of total soluble sugars. According to Griffth (1980) method, glutathione (GSH) content was estimated. Electrolyte leakage (EL), membrane stability index (MSI), relative water content (RWC) and malondialdehyde (MDA) were determined according to the procedures detailed in Weatherly (1950), Rady (2011), Osman and Rady (2014), and Heath and Packer (1968), respectively. By utilizing a portable photosynthesis system (LF6400XTR, LI-COR, USA), leaf net photosynthetic rate (*Pn*) and transpiration rate (*Tr*) were estimated for photosynthetic parameters. At 09:00–11:00 a.m. the appraisals were conducted on the 2nd fully expanded leaf. The dried powdered roots, leaves and pods of common bean were weighed to determine their contents of Cd^{2+}, and measurements were conducted using flame-atomic absorbance spectrometry after digestion of samples with a mixed acid (HNO_3 + $HClO_4$ (3:1, v/v), Shi et al., 2009).

As detailed in Vitoria et al. (2001) method, the enzymes were extracted. Protein concentration of the extract was determined (Bradford, 1976). The activities of catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), and superoxide dismutase (SOD) enzymes were measured according to Chance and Maehly (1955), Thomas et al. (1982), Fielding and Hall (1978), and Sairam et al. (2002), respectively. Additionally, the procedure of Rao et al. (1996) was used to assess the activity of glutathione reductase (GR) after monitoring the oxidation of NADPH for three absorbance times taken at 340 nm, and the activity was expressed as A_{564} min^{-1} mg^{-1} protein.

Digestion of 0.5 g dried leaf was performed using H_2SO_4 acid in the presence of H_2O_2 (Wolf, 1982). Total nitrogen (N) was determined using a micro-Kjeldahl method (Chapman and Pratt, 1982). Total phosphorus (P) was determined calorimetrically using ascorbic acid method (Watanabe and Olsen, 1965). Contents of total Na^+ and K^+ were measured directly using Flame photometer (Lachica et al., 1973).

2.3. Statistical analysis

The data is presented in the form of mean ± standard error (SE). The data was also subjected to one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test to show significant variations among means that were compared at $P \leq 0.05$. The statistical analysis was done by COSTAT computer software (CoHort Software version 6.303, Berkeley, CA, USA).

3. Results

3.1. Effect of Si and Pro on growth and yield of NaCl and Cd^{2+}-stressed plants

Exposing common bean plants to single (150 mM NaCl or 1.5 mM Cd^{2+}) or combined (100 mM NaCl + 1.0 mM Cd^{2+}) stress showed significant decreases in growth and yield components (plant height, leaf area, plant dry weight, number of green pods pot^{-1}, and green pods yield pot^{-1}; Table 1) compared to normal control. Combined (100 mM NaCl + 1.0 mM Cd^{2+}) stress was more toxic to plants, reducing growth and yield components by 64.4, 56.1, 82.7, 73.1, and 82.8%, respectively.

Compared to normal control (DW), foliar application with Si, Pro or Si + Pro significantly ($P \leq 0.05$) increased plant growth and yield components. Foliar spray with Si + Pro was the best treatment producing increases of 17.1, 11.2, 19.3, 18.4, and 26.8%, respectively. The same trend was obtained under NaCl (150 mM) or Cd^{2+} (1.5 mM) stress. Foliar spray with Si + Pro as the best treatment conferred significant increases that were 98.4 or 78.1%, 61.5 or 68.0%, 186.4 or 151.6%, 136.7 or 126.4%, and 204.4 or 191.1%, respectively compared to NaCl- or Cd^{2+}- stressed control. Under combined (100 mM NaCl +1.0 mM Cd^{2+}) stress, Si + Pro treatment significantly ($P \leq 0.05$) exceeded Si or Pro treatment and increased common bean plant height by 79.2%, leaf area by 64.9%, plant dry weight by 166.1%, number of green pods pot^{-1} by 123.2%, and green pods yield pot^{-1} 206.2% compared to the combined-stressed control. The integrative Pro + Si treatment was more effective than individual Pro or Si treatment in either normal or stress conditions. However, all foliar spray treatments were more pronounced under stress conditions than normal condition.

3.2. Effect of Si and Pro on photosynthetic pigments, Pro, soluble sugars, and glutathione contents of NaCl and Cd^{2+}-stressed plants

Common bean plant exposure to single (150 mM NaCl or 1.5 mM Cd^{2+}) or combined (100 mM NaCl + 1.0 mM Cd^{2+}) stress revealed significant decreases in leaf photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) contents, while showed significant elevations in Pro, soluble sugars, and glutathione contents compared to normal control (Table 2). Combined (100 mM NaCl + 1.0 mM Cd^{2+}) stress showed severe toxicity on plants, decreasing chlorophyll *a*, chlorophyll *b*, and carotenoids contents by 39.4, 39.7, and 20.3%, respectively, and increasing Pro, soluble sugars, and glutathione contents by 83.1, 175.0, and 69.5% compared to normal control.

Under normal condition, although all foliar application treatments (Si, Pro, and Si + Pro) significantly ($P \leq 0.05$) increased chlorophyll *a*, chlorophyll *b*, carotenoids, Pro, soluble sugars, and glutathione contents compared to normal control, integrative treatment (Si + Pro) significantly exceeded the individual treatments. This best treatment (Si + Pro) showed increases of 17.4% for chlorophyll *a* content, 9.0% for chlorophyll *b* content, 2.3% for carotenoids content, 26.9% for Pro content, 35.0% for soluble sugars content, and 20.6% for glutathione content compared to normal control. Under NaCl (150 mM) or Cd^{2+} (1.5 mM) stress, the same trend was reported and foliar spray with Si + Pro conferred the best results, increasing the above attributes by 50.5 or 19.2%, 21.0 or 32.7%, 18.1 or 14.5%, 6.4 or 23.6%, 28.0 or 13.3%, and 12.8 or 9.5%, respectively compared to NaCl- or Cd^{2+}-stressed control. Under combined (100 mM NaCl+1.0 mM Cd^{2+}) stress, all foliar spray treatments significantly increased all above-mentioned attributes compared to the combined-stressed control. Treatment of Si + Pro was the best and significantly ($P \leq 0.05$) exceeded Si or Pro treatment. It increased the above attributes by 16.0, 21.2, 11.3, 11.7, 13.8, and 9.0%, respectively. Generally, the integrative Pro + Si treatment was more effective than individuals in either normal or stress conditions. However, all foliar spray treatments were more pronounced under stress conditions than normal condition for leaf photosynthetic pigments contents.

3.3. Effect of Si and Pro on relative water content (RWC), membrane stability index (MSI), electrolyte leakage (EL), and malondialdehyde (MDA) contents of NaCl and Cd^{2+}-stressed plants

Growing common bean plants with single (150 mM NaCl or 1.5 mM Cd^{2+}) or combined (100 mM NaCl + 1.0 mM Cd^{2+}) stress showed significant decreases in leaf RWC and MSI, while leaf EL and MDA content were significantly increased compared to normal control (Fig. 1). Combined (100 mM NaCl + 1.0 mM Cd^{2+}) stress showed severe negative effects on plants, decreasing leaf RWC and MSI by 46.6 and 45.2%, respectively, while increasing leaf EL and MDA content by 75.3 and 52.1% compared to normal control.

Under normal condition, all foliar application treatments (Si, Pro,

Table 1
Effect of foliar application of silicon (Si; 6 mM) and/or proline (Pro; 6 mM) on growth traits and yield component of bean plants grown under NaCl and/or Cd^{2+} stress.

Stress treatment	Foliar spray	Plant height (cm)	Leaves area plant^{-1} (dm^2)	Plant dry weight (g)	Pods number pot^{-1}	Pods yield pot^{-1} (g)
Control	DW	25.1 ± 0.68 b	15.2 ± 0.20cd	13.5 ± 0.20c	12.5 ± 0.08 d	53.4 ± 1.44 d
	Si	27.9 ± 0.08 a	16.0 ± 0.27 b	15.0 ± 0.32b	14.1 ± 0.26b	63.7 ± 1.69 b
	Pro.	27.5 ± 0.03 a	15.7 ± 0.76bc	14.0 ± 0.26 c	13.2 ± 0.18c	59.3 ± 0.31 c
	Si + Pro.	29.4 ± 0.43 a	16.9 ± 0.40 a	16.1 ± 0.15a	14.8 ± 0.12 a	67.7 ± 0.55a
NaCl (150 mM)	DW	12.2 ± 0.55gf	9.10 ± 0.28 k	4.40 ± 0.30 l	4.9 ± 0.03 n	15.8 ± 0.30l
	Si	22.8 ± 0.43cd	14.4 ± 0.08ef	11.3 ± 0.18e	11.0 ± 0.18 f	43.1 ± 1.31f
	Pro.	21.2 ± 0.35d	14.0 ± 0.15 f	10.3 ± 0.24 f	10.6 ± 0.12g	37.2 ± 0.68 g
	Si + Pro.	24.2 ± 0.41bc	14.7 ± 0.08de	12.6 ± 0.12 d	11.6 ± 0.12e	48.1 ± 1.16 e
Cd^{2+} (1.5 mM)	DW	10.5 ± 0.11 hi	7.74 ± 0.19 l	3.76 ± 0.14m	4.40 ± 0.11°	12.3 ± 0.15 m
	Si	18.2 ± 0.35ef	12.3 ± 0.21 h	8.23 ± 0.20 h	9.23 ± 0.17 i	34.1 ± 0.86 h
	Pro.	17.6 ± 0.23 ef	11.7 ± 0.12 i	7.00 ± 0.15 i	8.26 ± 0.18j	30.1 ± 0.17 i
	Si + Pro.	18.7 ± 0.49e	13.0 ± 0.18g	9.46 ± 0.29 g	9.96 ± 0.19 h	35.8 ± 0.11h
NaCl (100 mM) + Cd^{2+} (1.0 mM)	DW	8.93 ± 0.09 i	6.67 ± 0.22 m	2.33 ± 0.08 n	3.36 ± 0.08p	9.21 ± 0.09 n
	Si	13.8 ± 0.69 g	10.4 ± 0.06j	5.56 ± 0.08 k	6.56 ± 0.16 l	25.4 ± 0.29 j
	Pro.	13.1 ± 0.66 g	9.26 ± 0.30 k	4.90 ± 0.05 l	5.53 ± 0.12m	23.4 ± 0.63 k
	Si + Pro.	16.0 ± 0.30 f	11.0 ± 0.15 j	6.20 ± 0.15 j	7.50 ± 0.15k	28.2 ± 0.73 i

Data are means (n = 9 for plant height, leaves area, plant dry weight, and n = 30 for yield) ± SE. Means were compared at $P \leq 0.05$ by Duncan's Multiple Range Test. Mean pairs followed by different letters are significantly different.
*Control = no stress; normal condition, DW = distilled water, Si = silicon, Pro = proline, and plant dry weight means shoot + roots dry weight.

and Si + Pro) significantly ($P \leq 0.05$) increased common bean leaf RWC and MSI, while significantly reduced leaf EL and MDA content compared to normal control. The integrative (Si + Pro) treatment significantly exceeded the individual treatments. This integrative treatment significantly increased RWC by 10.9% and MSI by 9.2%, and significantly reduced leaf EL by 19.2% and MDA content by 20.7% compared to normal control. Under individual (150 mM NaCl or 1.5 mM Cd^{2+}) stress, the same trend was proved and foliar spray with Si + Pro conferred the best results, increasing leaf RWC by 62.6 or 54.1% and MSI by 45.7 or 47.4%, respectively and decreasing leaf EL by 24.1 or 34.5% and MDA content by 18.8 or 25.8%, respectively compared to NaCl- or Cd^{2+}-stressed control. Under combined (100 mM NaCl + 1.0 mM Cd^{2+}) stress, all foliar spray (Si, Pro or Si + Pro) treatments significantly increased leaf RWC and MSI, while significantly decreased leaf EL and MDA content compared to the combined-stressed control. Treatment of Si + Pro was the best and significantly ($P \leq 0.05$) exceeded Si or Pro treatment, increasing leaf RWC by 32.5% and MSI by 42.1% and reducing leaf EL by 18.7% and MDA content by 14.4%. Generally, the integrative Pro + Si treatment was more effective than individuals in either normal or stress conditions. However, all foliar spray treatments were more pronounced under stress conditions than normal condition.

3.4. Effect of Si and Pro on net photosynthesis (Pn) and transpiration rate (Tr), and Cd^{2+} contents of NaCl and Cd^{2+}-stressed plants

Under single (150 mM NaCl or 1.5 mM Cd^{2+}) or combined (100 mM NaCl + 1.0 mM Cd^{2+}) stress, *Pn* and Tr in common bean plants showed significant decreases, while root, leaf, and pod Cd^{2+} contents showed significant increases compared to normal control (Table 3). Combined (100 mM NaCl + 1.0 mM Cd^{2+}) stress showed severe toxicity effects on plants, decreasing *Pn* and Tr by 65.3 and 70.4%, respectively, while extremely increasing root, leaf, and pod Cd^{2+} contents compared to normal control.

Under normal condition, foliar application treatment of Si, Pro or Si + Pro significantly ($P \leq 0.05$) increased *Pn* and Tr in common bean plants, while Cd^{2+} content was as in normal control (either traces in plant roots or nil in plant leaves or pods). The integrative (Si + Pro)

Table 2
Effect of foliar application of silicon (Si; 6 mM) and/or proline (Pro; 6 mM) on leaf contents of photosynthetic pigments, free proline, total soluble sugars, and glutathione in bean plants grown under NaCl and/or Cd^{2+} stress.

Stress treatment	Foliar spray	Leaf pigments contents (mg g^{-1})			Proline (µg g^{-1} DW)	Soluble sugars (mg g^{-1} DW)	Glutathione (µg g^{-1} FW)
		Chl.a	Chl.b	Carotenoids			
Control	DW	1.55 ± 0.04cd	0.78 ± .001c	0.345 ± .006b	20.1 ± 0.2l	10.0 ± 0.1k	0.321 ± .003h
	Si	1.64 ± 0.03 b	0.83 ± .003b	0.347 ± .005b	22.8 ± 0.8j	13.1 ± 0.3i	0.352 ± .003h
	Pro	1.62 ± 0.04bc	0.83 ± .006b	0.347 ± .003b	21.5 ± 0.5k	11.2 ± 0.3j	0.333 ± .004h
	Si + Pro	1.82 ± 0.08 a	0.85 ± .004 a	0.353 ± .008 a	25.5 ± 0.4i	13.5 ± 0.4i	0.387 ± .005g
NaCl (150 mM)	DW	1.01 ± 0.03jkl	0.51 ± .004m	0.287 ± .006m	34.5 ± 0.5d	20.7 ± 0.2e	0.475 ± .008de
	Si	1.41 ± 0.03 e	0.74 ± .004e	0.336 ± .005e	36.4 ± 0.6c	25.2 ± 0.6d	0.486 ± .006.d
	Pro	1.31 ± 0.03 f	0.69 ± .006f	0.328 ± .008f	35.1 ± 0.2d	21.4 ± 0.3e	0.485 ± .007d
	Si + Pro	1.52 ± 0.05 d	0.77 ± .003d	0.339 ± .006d	36.7 ± 0.4c	26.5 ± 0.5d	0.536 ± .007c
Cd^{2+} (1.5 mM)	DW	0.99 ± 0.08 kl	0.49 ± .001n	0.283 ± .008 n	27.1 ± 0.1h	17.3 ± 0.4h	0.430 ± .006f
	Si	1.17 ± 0.04gh	0.62 ± .006h	0.320 ± .004h	32.6 ± 0.2f	18.7 ± 0.3g	0.470 ± .005de
	Pro	1.13 ± 0.03 ghi	0.59 ± .002i	0.315 ± .008i	30.4 ± 0.3g	17.7 ± 0.8h	0.454 ± .004ef
	Si + Pro	1.18 ± 0.04 g	0.65 ± .003g	0.324 ± .003g	33.5 ± 0.8e	19.6 ± 0.7f	0.471 ± .009de
NaCl (100 mM) + Cd^{2+} (1.0 mM)	DW	0.94 ± 0.03l	0.47 ± .002°	0.275 ± .001°	36.8 ± 0.6c	27.5 ± 0.6c	0.544 ± .008bc
	Si	1.07 ± 0.01ijk	0.55 ± .003k	0.303 ± .005k	38.9 ± 0.4b	29.5 ± 0.8b	0.585 ± .006a
	Pro.	1.05 ± 0.08 ilk	0.53 ± .001l	0.296 ± .002l	37.1 ± 0.1c	27.9 ± 0.9c	0.571 ± .007 ab
	Si + Pro	1.09 ± 0.06hij	0.57 ± .004j	0.306 ± .003j	41.1 ± 0.9a	31.3 ± 0.8a	0.593 ± .008a

Data are means (n = 3) ± SE. Means were compared at $P \leq 0.05$ by Duncan's Multiple Range Test. Mean pairs followed by different letters are significantly different.
*Control = no stress; normal condition, DW = distilled water, Si = silicon, Pro = proline.

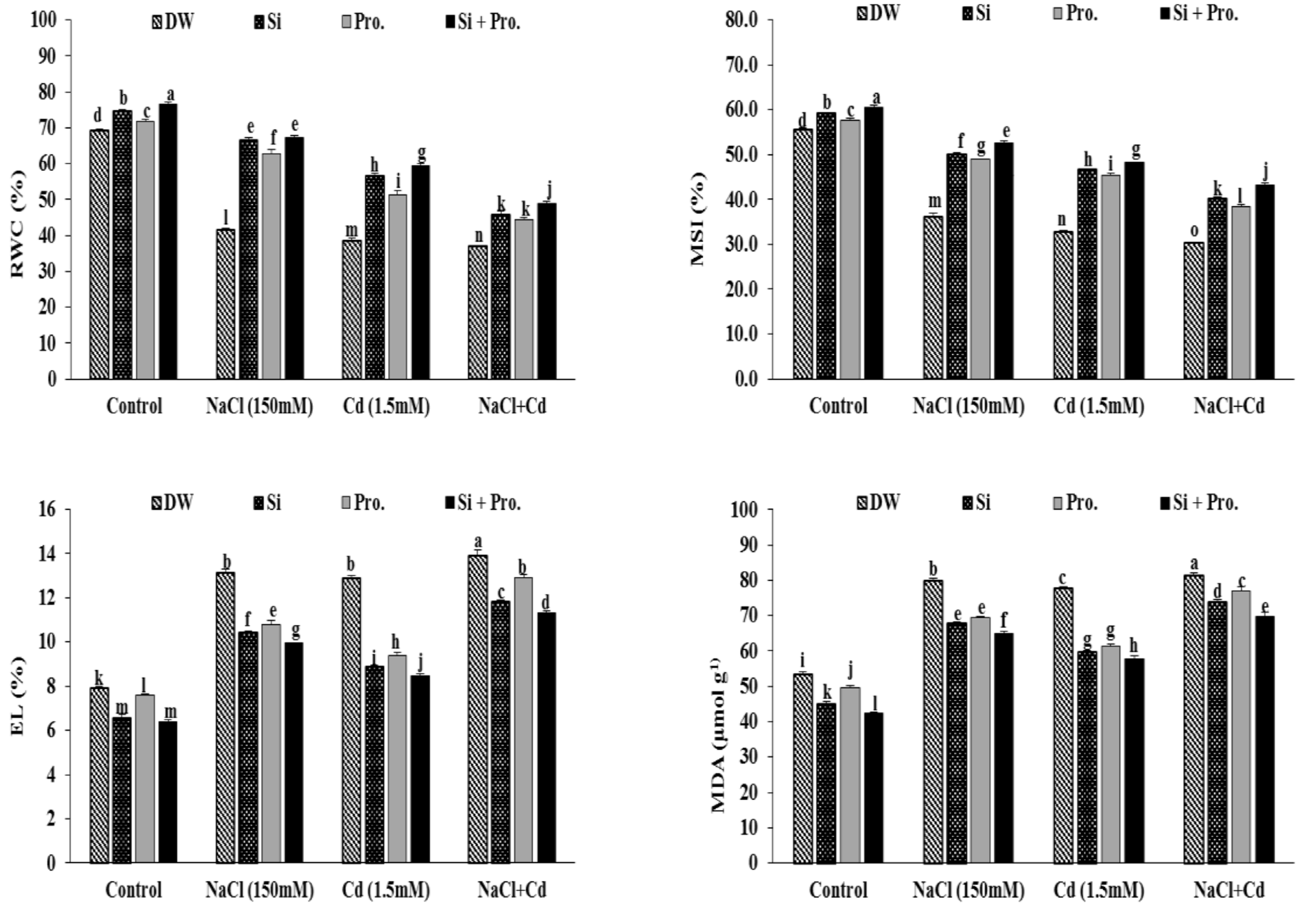

Fig. 1. Effect of foliar application of silicon (Si; 6 mM) and/or proline (Pro; 6 mM) on relative water content (RWC), membrane stability index (MSI), electrolyte leakage (EL), and lipid peroxidation assessed as malondialdehyde (MDA) content of bean leaves grown under NaCl and/or Cd^{2+} stress.

treatment significantly exceeded the individual treatments for only *Pn*. This integrative treatment significantly increased *Pn* by 21.5% and Tr by 4.9% compared to normal control. Under individual (150 mM NaCl or 1.5 mM Cd^{2+}) stress, the same trend was reported and foliar spray with Si + Pro generated the best results, increasing *Pn* by 111.1 or 105.0% and Tr by 121.1 or 101.2%, respectively compared to NaCl- or Cd^{2+}-stressed control. Under Cd^{2+} stress condition, all foliar spray treatments significantly reduced Cd^{2+} contents in roots, leaves, and

Table 3
Effect of foliar application of silicon (Si; 6 mM) and/or proline (Pro; 6 mM) on net photosynthetic rate (*Pn*), transpiration rate (*Tr*) and cadmium (Cd^{2+}) content in roots, leaves and pods of bean plants grown under NaCl and/or Cd^{2+} stress.

Stress treatment	Foliar spray	*Pn* (μmol Co_2 m^{-2} s^{-1})	Tr (mmol H_2O m^{-2} s^{-1})	Cd^{2+} content (mg kg^{-1} dry weight)		
				Root	Leaf	Pod
control	DW	12.1 ± 0.29cd	6.73 ± 0.11 ab	Trace	ND	ND
	Si	13.4 ± 0.66b	6.95 ± 0.14 a	Trace	ND	ND
	Pro.	12.6 ± 0.12 c	6.88 ± 0.13a	Trace	ND	ND
	Si + Pro.	14.7 ± 0.12 a	7.06 ± 0.09 a	Trace	ND	ND
NaCl (150 mM)	DW	5.40 ± 0.11 kl	2.94 ± 0.02 i	Trace	ND	ND
	Si	10.7 ± 0.15 e	6.09 ± 0.07c	Trace	ND	ND
	Pro.	10.2 ± 0.20 ef	5.63 ± 0.10 d	Trace	ND	ND
	Si + Pro	11.4 ± 0.34d	6.50 ± 0.08b	Trace	ND	ND
Cd (1.5 mM)	DW	4.76 ± 0.08lm	2.59 ± 0.03 j	35.2 ± 1.3 b	22.1 ± 1.10 b	16.5 ± 0.06 b
	Si	9.30 ± 0.05 g	4.88 ± 0.08 f	7.43 ± 0.61e	5.43 ± 0.19 e	Trace
	Pro.	8.20 ± 0.35 h	4.60 ± 0.08 f	13.2 ± 0.92 d	8.53 ± 0.21d	1.93 ± 0.05 e
	Si + Pro.	9.76 ± 0.08 fg	5.21 ± 0.05 e	3.30 ± 0.14 f	3.23 ± 0.08f	Trace
NaCl + Cd	DW	4.20 ± 0.05m	1.99 ± 0.02 k	56.1 ± 1.5a	34.1 ± 1.13 a	22.4 ± 0.01 a
	Si	6.76 ± 0.08 j	3.90 ± 0.04gh	13.8 ± 0.81d	12.6 ± 0.36c	3.06 ± 0.02 d
	Pro.	6.03 ± 0.18 k	3.66 ± 0.03h	18.2 ± 0.96c	13.8 ± 0.41 c	6.23 ± 0.04 c
	Si + Pro.	7.50 ± 0.15 i	4.06 ± 0.05g	8.20 ± 0.66e	5.07 ± 0.18d	0.27 ± 0.00 f

Data are means (n = 3) ± SE. Means were compared at $P \leq 0.05$ by Duncan's Multiple Range Test. Mean pairs followed by different letters are significantly different.
*Control = no stress; normal condition, DW = distilled water, Si = silicon, Pro = proline.
*ND = not detected, Trace = amount less than one.

pods of common bean plants compared to Cd^{2+}-stressed control. Integrative Si + Pro treatment was the best decreasing Cd^{2+} content by 90.6% in roots, 85.4% in leaves, and 99.9% in pods compared to Cd^{2+}-stressed control. Under combined (100 mM NaCl + 1.0 mM Cd^{2+}) stress, all foliar spray (Si, Pro or Si + Pro) treatments significantly increased *Pn* and *Tr*, while significantly decreased root, leaf, and pod contents of Cd^{2+} compared to the combined-stressed control. Among all foliar spray treatments, integrative Si + Pro was the best, increasing *Pn* by 78.6% and Tr by 104.0%, and reducing root Cd^{2+} content by 85.4%, leaf Cd^{2+} content by 85.1%, and pod Cd^{2+} content by 98.8%. Foliar application of Pro and/or Si significantly ameliorated the toxicity caused by NaCl and/or Cd^{2+} stress and significantly reduced the content of Cd^{2+} in different plant organs at varying degrees compared to the stressed control. The interplay between Si and Pro (Si + Pro treatment) extremely reduced Cd^{2+} content ($0.27\,g\,kg^{-1}$) up to the limits for legumes under both stresses. Generally, the integrative Pro + Si treatment was more effective than individuals in either normal or stress conditions. However, all foliar spray treatments were more pronounced under stress conditions than normal condition.

3.5. Effect of Si and Pro on antioxidant enzymes activities of NaCl and Cd^{2+}-stressed plants

Single (150 mM NaCl or 1.5 mM Cd^{2+}) or combined (100 mM NaCl + 1.0 mM Cd^{2+}) stress caused significant increases in common bean leaf activities of CAT, POX, APX, SOD and GR compared to normal control (Fig. 2). Combined (100 mM NaCl + 1.0 mM Cd^{2+}) stress showed highest activities of antioxidant enzymes by 77.9, 322.9, 116.9, 90.1, and 168.5%, respectively compared to normal control.

Under normal condition, foliar application of Si, Pro or Si + Pro significantly ($P \leq 0.05$) increased leaf enzymatic (CAT, POX, APX, SOD, and GR) activities compared to normal control. However, exposing to NaCl and/or Cd^{2+} showed further increases in the activities of these antioxidant enzymes compared to unstressed or stressed control. Moreover, application of Pro and/or Si led to further elevations in all enzymes activities. Uppermost activities of these enzymes were noticed in the plants exposed to the combined treatment of NaCl + Cd^{2+} + Pro + Si. The integrative (Si + Pro) treatment significantly increased CAT, POX, APX, SOD and GR activities by 18.2, 67.5, 43.5, 15.0, and 36.1%, respectively, under normal condition, compared to normal control. This integrative treatment also increased the activities of these enzymes by 11.4 or 13.4%, 20.1 or 21.6%, 9.2 or 20.4%, 19.6 or 17.3%, and 27.3 or 19.4% under NaCl or Cd^{2+} stress, respectively compared to NaCl- or Cd^{2+}-stressed control. Under combined (100 mM NaCl + 1.0 mM Cd^{2+}) stress, all foliar spray (Si, Pro or Si + Pro) treatments significantly increased the activities of these enzymatic antioxidants compared to the combined-stressed control. Treatment of Si + Pro was the best and significantly ($P \leq 0.05$) exceeded Si or Pro treatment, increasing enzymatic activities by 10.2, 38.2, 4.6, 7.1, and 5.6% compared to the combined-stressed control.

3.6. Effect of Si and Pro on nutrients contents and K^+/Na^+ ratio of NaCl and Cd^{2+}-stressed plants

Under single (150 mM NaCl or 1.5 mM Cd^{2+}) or combined (100 mM NaCl + 1.0 mM Cd^{2+}) stress, N, P and K^+ contents, and K^+/Na^+ ratio in common bean plants showed significant decreases, while Na^+ content showed significant increase compared to normal control (Table 4). Combined (100 mM NaCl + 1.0 mM Cd^{2+}) stress showed severe toxic effects on plants, decreasing N, P and K^+ contents, and K^+/Na^+ ratio by 47.1, 48.4, 49.4 and 73.1%, respectively, while extremely increasing Na^+ content by 147.7% compared to normal control.

Under normal condition, all foliar application treatments (Si, Pro, and Si + Pro) significantly ($P \leq 0.05$) increased leaf contents of N, P, K^+, and ratio of K^+/Na^+, while significantly decreased leaf content of Na^+ compared to normal control. The integrative (Si + Pro) treatment significantly exceeded both individual treatments. This integrative treatment significantly increased N, P and K^+ contents, and K^+/Na^+ ratio by 11.6, 19.4, 8.7, and 37.1%, and significantly decreased Na^+ content by 20.6% compared to normal control. Under individual (150 mM NaCl or 1.5 mM Cd^{2+}) stress, the same trend was proved and foliar spray with Si + Pro conferred the best results, increasing leaf N content by 40.3 or 19.8%, P content by 38.1 or 26.3%, K^+ content by 43.1 or 18.4%, and K^+/Na^+ ratio by 154.9 or 86.0%, and decreasing Na^+ content by 43.7 or 36.2% compared to NaCl- or Cd^{2+}-stressed control. Under combined (100 mM NaCl + 1.0 mM Cd^{2+}) stress, all foliar spray (Si, Pro or Si + Pro) treatments significantly increased leaf contents of N, P, K^+, and ratio of K^+/Na^+, while significantly decreased leaf content of Na^+ compared to the combined-stressed control. Foliar spray with integrative Si + Pro was the best treatment that significantly ($P \leq 0.05$) exceeded Si or Pro treatment, increasing leaf N content by 20.2%, P content by 43.8%, K^+ content by 8.5%, and K^+/Na^+ ratio by 60.6%, and reducing leaf content of Na^+ by 32.1% compared to the combined-stressed control. Generally, the integrative Pro + Si treatment was more effective than individuals in either normal or stress conditions. However, all foliar spray treatments were more pronounced under stress conditions than normal condition.

4. Discussion

Plants suffer greatly when they grow under stress conditions, especially under more than one stress such as salinity and Cd^{2+}. This plant growing condition leads to inhibition of plant growth and yield restriction. In the current study, common bean plants suffered from defects and disorders in all physiological, biochemical and morphological characters due to salt and/or Cd^{2+} stress (Tables 1–4; Figs. 1 and 2). These stress-induced disorders in metabolic processes cause decreases in meristematic activity and cell elongation that have been linked with high rate of respiration because of the high energy requirements. As a result, plant growth and yield attributes have been restricted under abiotic stress (Abdul Qados, 2015). Tsai et al. (2004) and Yan et al. (2015) have reported that plant responds to stress by generation of ROS like H_2O_2, $^1O_2$, $O_2^{\bullet-}$, OH^-. Excessive generation of ROS establishes oxidative stress in plants (Cheeseman, 2007). This oxidative stress harms chlorophylls and consequently photosynthesis process, gas exchange, membrane functions, and protein structure. Halliwell and Gutteridge (2007) have reported that plants adopt complex antioxidant system to repair and alleviate ROS-caused damages. Plants advance the strength of the antioxidant system by integration of enzymatic and non-enzymatic antioxidants. However, in most cases, the adopted endogenous antioxidants (enzymatic + non-enzymatic) are not enough; therefore, antioxidants should be used exogenously to help stressful plants to cope effectively with stress (Rady et al., 2019). In this study, the antioxidant systems in plants have many enzymatic (SOD, CAT, POX, APX, and GR) and non-enzymatic (proline, carotenoids, glutathione, and soluble sugars) compounds that developed by the interplay between Si and Pro (exogenously applied) to help stressful common bean plants to cope effectively with different stresses.

Common bean plants stressed by NaCl and/or Cd^{2+} had reduced growth and yield (Table 1), photosynthetic pigments (Table 2), RWC and MSI (Fig. 1), net photosynthesis and respiration rate (Table 3), while they had elevated enzymatic (Fig. 2) and non-enzymatic (Table 2) antioxidant activities and Cd^{2+} contents (Table 3) compared to unstressed plants. This result is often attributed to that NaCl and Cd^{2+} are considered as inhibiting factors for normal physiological process, causing limitations of plant growth and productivity (Rady, 2011). Choudhury and Panda (2004) have reported that these inhibiting factors suppress the proton pump that is in charge of the physiological processes mechanisms. However, in this study, foliar application of Si and/or Pro for NaCl- and/or Cd^{2+}-stressed bean plants led to positive changes of all biochemical, physiological and morphological characteristics, and the highest consequences were found with Si in

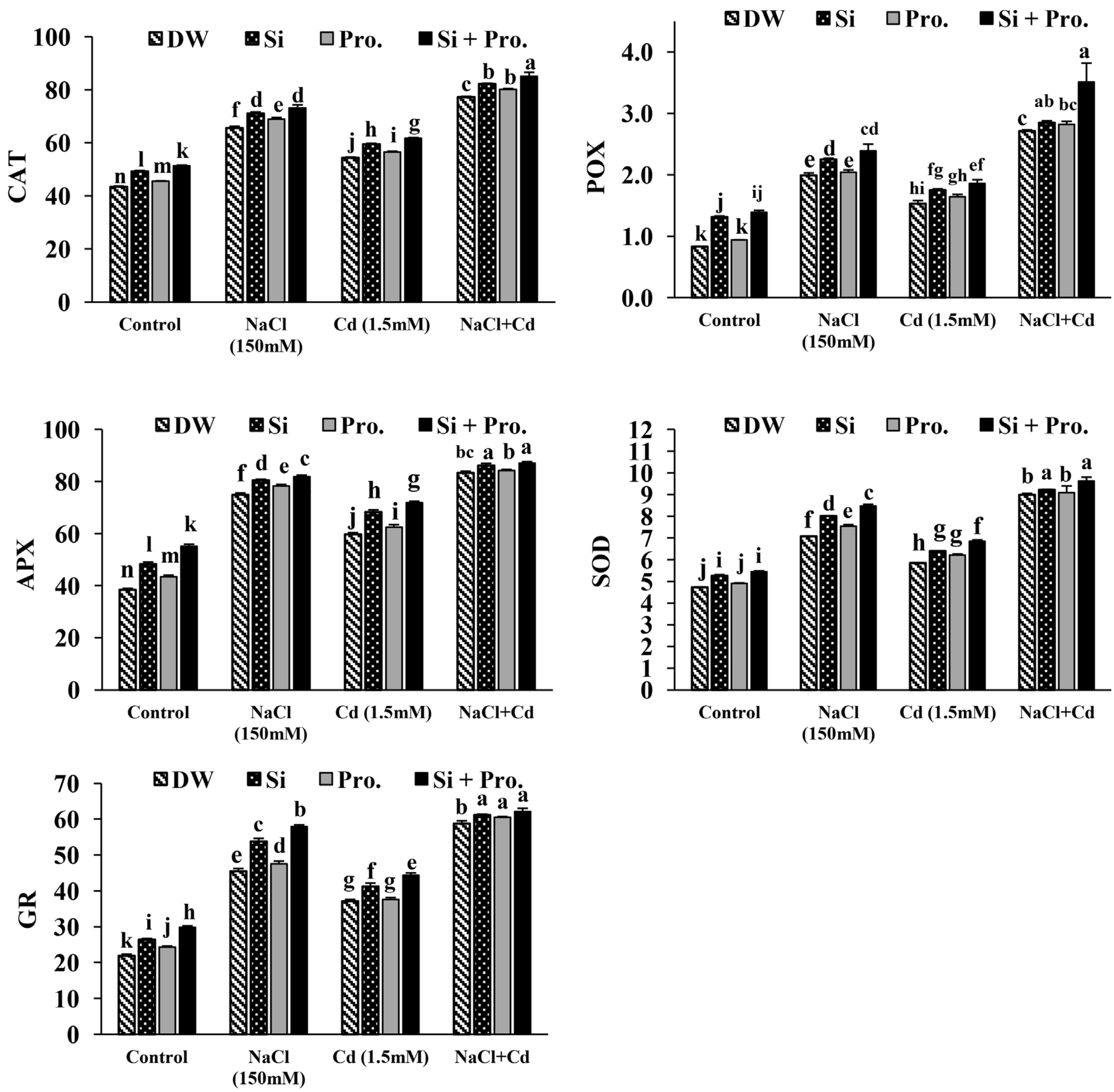

Fig. 2. Effect of foliar application of silicon (Si; 6 mM) and/or proline (Pro; 6 mM) on the activities (expressed as A_{564} min^{-1} g^{-1} protein) of catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), superoxide dismutase (SOD), and glutathione reductase (GR) in bean leaves grown under NaCl and/or Cd^{2+} stress.

interplaying with Pro (Tables 1–4; Figs. 1 and 2). Previous studies have indicated that addition of Si significantly improves growth parameters under stress conditions (Merwad et al., 2018) due to that Si has useful effects on abiotic stress tolerance in several plants, enhancing their growth and yields (Ouzounidou et al., 2016). Alzahrani et al. (2018) have reported that exogenous Si increases dry weight of bean plants under different stress conditions due to its ability to adjust cell wall metabolism by enhancing tissue extensibility and improving cell physio-biochemical processes activities. Increasing leaf rigidity by making it rougher in texture is another probable mechanism of Si (Ouzounidou et al., 2016). On the other hand, other previous studies have reported that Pro improves growth and maintains nutrient balance in stressed bean plants by boosting the N, P, and K^+ uptake under stress conditions (Ali et al., 2008; Vicente et al., 2016). Accumulation of Pro is a widespread physiological reaction in several abiotic stressed plants and is, therefore, an indicator of stress resistance. Pro protects plants from the negative effects of stress by stabilizing the mitochondrial electron transport complex II, membranes, proteins, and enzymes such as Rubisco (McNeil et al., 1999; Vicente et al., 2016).

Rios et al. (2017) have elucidated that salt stress reduces activity of cell physiology including photosynthesis due to salt-induced osmotic stress that causes nutritional imbalance, toxicity, and oxidative stress. Chlorophyllase enzyme activity enhanced by stress degrades chlorophylls leading to a considerable decrease in photosynthetic pigments (Reddy and Vora, 1986). However, in this study, Si and/or Pro improved chlorophylls contents and photosynthetic process in bean plants under salinity and/or Cd^{2+} stress (Tables 2 and 3). As Fadzilla and Burdon (1997) reported, Si increases leaf turgidity by holding more horizontally, leading to delay leaf aging and rising chlorophyll content and activity of ribulose-bisphosphate carboxylase. It also increases leaf blade erectness to preserve higher leaf water potential and water use efficiency (WUE), and smooth light penetration in favor of higher photosynthesis (Gong et al., 2003). This is in addition to that Si inhibits destruction of chlorophyll and increases leaf area, which leads to more

Table 4
Effect of foliar application of silicon (Si; 6 mM) and/or proline (Pro; 6 mM) on leaf contents of nutrients and K^+/Na^+ ratio of bean plants grown under NaCl and/or Cd^{2+} stress.

Stress treatment	Foliar spray	N (%)	P (%)	K^+ (%)	Na^+ (%)	K^+/Na^+ ratio
Control	DW	2.25 ± 0.02d	0.31 ± 0.004c	2.63 ± 0.13 abc	1.07 ± 0.02j	2.45 ± 0.21c
	Si	2.42 ± 0.02b	0.35 ± 0.003b	2.81 ± 0.08 ab	0.90 ± 0.01l	3.12 ± 0.03a
	Pro	2.32 ± 0.08c	0.34 ± 0.002b	2.78 ± 0.01 ab	0.98 ± 0.03k	2.83 ± 0.13b
	Si + Pro	2.51 ± 0.01a	0.37 ± 0.005a	2.86 ± 0.11a	0.85 ± 0.02l	3.36 ± 0.08a
NaCl (150 mM)	DW	1.49 ± 0.03gh	0.21 ± 0.006h	1.81 ± 0.04ef	2.54 ± 0.02b	0.71 ± 0.03j
	Si	2.01 ± 0.01f	0.28 ± 0.002de	2.56 ± 0.04bc	1.45 ± 0.01h	1.76 ± 0.08d
	Pro	1.95 ± 0.02f	0.27 ± 0.001e	2.41 ± 0.23c	1.52 ± 0.01 fg	1.58 ± 0.01de
	Si + Pro	2.09 ± 0.04e	0.29 ± 0.002cd	2.59 ± 0.02bc	1.43 ± 0.01h	1.81 ± 0.02d
Cd^{2+} (1.5 mM)	DW	1.26 ± 0.08l	0.19 ± 0.003i	1.79 ± 0.05ef	2.07 ± 0.03c	0.86 ± 0.02ij
	Si	1.46 ± 0.01 ghi	0.24 ± 0.001f	2.02 ± 0.08de	1.46 ± 0.01gh	1.38 ± 0.02ef
	Pro	1.41 ± 0.06ijk	0.23 ± 0.002 fg	1.94 ± 0.02def	1.56 ± 0.03f	1.24 ± 0.03 fg
	Si + Pro	1.51 ± 0.05g	0.24 ± 0.004f	2.12 ± 0.01d	1.32 ± 0.01i	1.60 ± 0.02e
NaCl (100 mM) + Cd^{2+} (1.0 mM)	DW	1.19 ± 0.03m	0.16 ± 0.01j	1.76 ± 0.03f	2.65 ± 0.02a	0.66 ± 0.01j
	Si	1.41 ± 0.07jk	0.22 ± 0.003gh	1.85 ± 0.01ef	1.85 ± 0.02e	1.0 ± 0.08 ghi
	Pro	1.36 ± 0.09k	0.22 ± 0.01gh	1.83 ± 0.05ef	1.91 ± 0.08d	0.96 ± 0.08hi
	Si + Pro	1.43 ± 0.03hij	0.23 ± 0.002 fg	1.91 ± 0.06def	1.80 ± 0.01e	1.06 ± 0.07gh

Data are means (n = 3) ± SE. Means were compared at $P \leq 0.05$ by Duncan's Multiple Range Test. Mean pairs followed by different letters are significantly different.
*Control = no stress; normal condition, DW = distilled water, Si = silicon, Pro = proline.

light available for fulfill photosynthesis process (Agarie et al., 1998). In addition, Ouzounidou et al. (2016) have reported that exogenous Si helps enhancement of plant tolerance to water deficit stress. This result due to that Si contributes to maintain water for increasing plant leaf content of chlorophyll and carotenoids, helping to pave the way for constant supply of assimilates to growing tissues. Si also improves water efficiency due to that it possesses a significant role in improving photosynthesis under various abiotic stress conditions (Alzahrani et al., 2018). Photosynthesis process amelioration might be linked to the alleviation of stress-induced damages. Increased power of antioxidant defenses by Si addition alleviates oxidative damage of photosynthesis enzymes (Ming et al., 2012).

The present study indicates that osmoprotectants and antioxidants (i.e. Pro, soluble sugar and glutathione; GSH) increased significantly under NaCl and/or Cd^{2+} stress (Table 2). As mentioned by Rios et al. (2017), growing plants under stress conditions creates and accumulates various osmolytes/osmoprotectants as compatible solutes in plants as a mechanism to protect themselves against negative stress effects, maintaining water in cells for normal physio-biochemical processes. Under stress conditions, accumulation of Pro and soluble sugars (as osmoprotectants) along with GSH (as antioxidant) safeguards plant cells by the balance between cytosol and vacuole osmotic strengths and osmotic strength of outer environment (Gadallah, 1999). Moreover, accumulation of Pro is considered as an osmotic stress response because it is a paramount osmolyte, contributing to osmotic modification in plant cells (Zhang et al., 2017). According to this study, the interplay between Si and Pro led to further increases in the contents of Pro, sugars, and GSH in bean plants compared to the increased contents obtained from individual Pro or Si application. In a previous report (Kavi Kishor et al., 2005), cellular Pro is piled from around 5% of the amino acid pool in the standard conditions, and accumulated up to 20–80% under stress conditions due to raised synthesis and reduced degradation of Pro in several plant species. Exogenous Pro mitigates the stress of NaCl-salinity and/or Cd^{2+} by detoxification of excessive ROS generated under these stresses (Rady, 2011; Liang et al., 2013). Siripornadulsil et al. (2002) have reported that among Pro mechanisms, it reacts directly with OH^- radicals or it physically quenches singlet oxygen; $^1O_2$ declining ROS damages (low levels of malondialdehyde; MDA) along with high levels of cellular GSH. This case maintains cell membranes for health physio-biochemical processes in plant cells. The elevated level of GSH facilitates creation of phytochelatin and sequestration of Cd^{2+} ions by conjugation with a phytochelatin in the vacuole (Hasanuzzaman et al., 2017). Pro has an effective role in maintenance of membrane safety and subcellular structures, stabilization of proteins, and safeguard of cellular functions by scavenging of ROS under stress conditions (Kavi Kishor et al., 2005). On the other hand, exogenous Si may enhance plant tolerance to NaCl and/or Cd^{2+} stress by elevating osmolyte contents and adjustment of osmotic potential (Zhang et al., 2017). The current study reports more effective tolerance to stress by interplay between Pro and Si that confers more contents of osmolytes and antioxidative protectants against stress (Table 2–4; Figs. 1 and 2). Among these protectants, Pro and soluble sugar accumulations are the major solutes that contribute to osmotic modification in glycophytic plants exposed to stress (Rady, 2011).

Based on our results, NaCl and/or Cd^{2+} stress resulted in a decrease in RWC (Fig. 1) and an increase in EL (Fig. 1). However, Si and/or Pro foliar application alleviated the water stress caused by NaCl and Cd^{2+} stresses and provided plants with increased RWC and decreased inorganic ions that leaked from plant cells. This positive result was obtained due to the positive interplaying effects of Si and Pro that protects cell membranes from stress damages by boosting the membrane stability in stressed-plants. Elevation of EL was obtained in this study in parallel line with the increase of membrane lipid peroxidation (measured as MDA) under NaCl and/or Cd^{2+} stress (Fig. 1). This unfavorable result was positively modified by the interplay between Si and Pro that conferred better membrane status than individual Si or Pro. Where lipid peroxidation is considered as an indicator of stress effect, Si minimizes the end-product of lipid peroxidation (i.e. content of MDA) shown in plant tissue. Therefore, Si may aid to preserve the safety of cell membranes and depress their permeability, which is agreed with the results of Coskun et al. (2016). In this study, addition of Si reduces leaf cell plasma membrane permeability in terms of increase of MSI and decrease of EL that further supported by the interplay between Si and Pro. This favorable result may be due to the effective increases in enzymatic and non-enzymatic antioxidants activities, which are key defense mechanisms against various environmental stresses. In previous reports, Agarie et al. (1998) have concluded that Si improves the stability of cell membranes of plants growing under stress conditions, indicating that Si prohibits the deterioration in structural and functional cell membranes in stressed plants. Therefore, the enhancement of plant growth and yield as obtained in the current study (Table 1). Liu et al. (2014) have also reported that Si elevates water absorption and transport in sorghum plants grown under stress conditions by increment of hydraulic conductance of roots, which imputed to Si-mediated up-regulation of the transcription of some aquaporin genes.

Results reported herein show that Si and/or Pro foliar treatment led to minimized contents of Cd^{2+} up to near limits in bean plant organs (roots, leaves, and pods) grown under Cd^{2+} stress. The interplay between Si and Pro (Si + Pro treatment) was more effective in this concern than individual (Si or Pro) applications under both stresses (Table 3). Interplay between Si and Pro reduced the content of Cd^{2+} up to 0.27 mg kg^{-1} in pods (the plant edible part), where Cd^{2+} limit in legumes is assessed to be 0.28 mg kg^{-1} (Kabata-Pendias and Pendias, 2001). In a report, Wang et al. (2016) have shown that Si minimizes Cd^{2+} ion uptake and restricts their translocation from roots to the upper parts and thus the rise of plant tolerance to Cd^{2+} stress.

As reported by Schutzendubel and Polle (2002), it is well known that antioxidant systems are the main controllers of ROS levels in plant cells. Subsequently, as in the current study, it is expected that the antioxidant enzyme activity will increase in salt- and/or Cd^{2+}-stressed bean plants. In addition, Si and/or Pro addition to bean plants led to further improvements of enzymatic activities (Fig. 2) to scavenge and control various ROS types in participation with non-enzymatic antioxidants (Table 2). Activities of CAT, POX, APX, SOD, and GR, in this study, were elevated against oxidative stress generated by NaCl and/or Cd^{2+} stress. These raised enzymatic activities were progressed and reached their maximum activity by the interplay between Si and Pro to minimize cellular ROS under stress. The $O_2^{\cdot-}$ that is emerged in plant tissues due to stress is converted by SOD to H_2O_2, which is as a strong oxidant by its generation from SOD canalization. It is prohibited by the ascorbate (AsA)–glutathione (GSH) cycle. Another serious reactive and toxic oxidant is the OH^-. Without distinction, it can react with all macromolecules. By combining their actions, CAT and SOD can prohibit or decrease formation of OH^- (Kusvuran et al., 2016). Gong et al. (2003) have reported that Si increases SOD and CAT activities. In another study under Cd^{2+} stress, CAT and SOD activities are significantly elevated in wheat leaves with Si application (Alzahrani et al., 2018). Peroxidases (POXs) can adjust ROS levels because of their function in scavenging and consuming H_2O_2 during physiological pathways. POXs have a high affinity to H_2O_2 compared to CAT, however, POXs may also participate to generate H_2O_2 by oxidation of various molecules including NADPH (Ranieri et al., 2005). In addition, Li et al. (2016) have reported that 6 mM Si increases POX activity in salt-stressed *Glycyrrhiza uralensis* seedlings. Also, Si leads to reduce ROS production and increase ROS scavenging by enzymatic and non-enzymatic antioxidants (Rios et al., 2017). In the present study, POX activity was increased under both NaCl and Cd^{2+} stresses. Therefore, at a cellular level, Si can mitigate stress-induced oxidative stress through using Si effectively in metabolic pathways that scavenge ROS, enhancing the safety and integrity of cell membranes. In addition, common bean tolerance to salt and Cd^{2+} stresses is improved with the increased activities of enzymatic and non-enzymatic antioxidant systems by foliar addition of Si and/or Pro. The interplay between Si and Pro conferred more effective antioxidant defense systems against stresses under study than those established with individual Si or Pro application, improving bean growth, yield, photosynthetic efficiency, and nutrient homeostasis (Tables 1–4; Figs. 1 and 2).

The increased RWC and MSI in Si- and/or Pro-treated bean plants grown under NaCl and/or Cd^{2+} stress effectively administered considerable ameliorations that occurred due to the safety protection of cell membranes and the prevention of plasma membrane damage under both stresses. These results are agreed with those obtained by Ouzounidou et al. (2016). Soluble sugar, proline and GSH contents along with chlorophylls contents and photosynthetic attributes were remarkably enhanced in foliar sprayed bean plants compared to the controls. These increases reached highest values under the interplay between Si and Pro, which also conferred highest activities of enzymatic antioxidants (CAT, POX, SOD, APX, and GR). These improved enzymatic activities increase the plant's ability to withstand the stress of NaCl and/or Cd^{2+} due to the further rise in their defense machineries. All improved attributes of physio-biochemistry and antioxidant defense systems help to restore the reduced contents of N, P, and K^+, increasing the ratio of K^+/Na^+ and reducing the accumulation of Na^+ ions in plant tissues under Cd^+ and/or NaCl stress with the ameliorating effects of Si and Pro, especially when applied in interplaying status (Table 4). Si and Pro prevented the predominance of the osmotic pressure of soil solution induced by stresses, and consequently increased the ability of plant roots to absorb water and nutrients. This increased nutrient absorption, especially K^+ acted to antagonize the toxicity of Na^+ ions through increasing the ratio of K^+/Na^+. As indicated in a previous study (Rios et al., 2017), one of the essential mechanisms of salt tolerance is the maintenance of low intracellular Na^+ levels, and the mode of action of this mechanism is the rise of Na^+ efflux and/or the decrease of Na^+ influx. Concentration of Na^+ in plant cells is considered as an indicator of salt tolerance, and low levels of Na^+ in plant cells is considered as an indicator of lower Na^+ uptake by plant roots (Tahir et al., 2006). In the present study, the imbalance of K^+ in cell cytosols under stress was positively modified by Si and/or Pro application increasing and maintaining high cytosol K^+/Na^+ ratio to represent basic mechanism in plants in favor of salt stress tolerance. This positive result is previously reported in the study of Meloni and Martınez (2009). Ali et al. (2008) have reported that exogenous Pro increases N, P, and K^+ uptake under stress condition. On the other hand, Si may play an important role in alleviating salinity stress by affect K^+ and Na^+ levels in plant cells (Ashraf et al., 2010).

In the present study, Si in interplaying with Pro allowed an effective strategy to mitigate the injuries in bean plants that are grown under salt and Cd^{2+} stresses due to the strong decrease in Na^+ and Cd^{2+} uptake and/or in their translocation to the upper plant parts, effectively increasing K^+ content, and thus K^+/Na^+ ratio. This finding indicates that the interplay between Si and Pro was more pronounced in limiting the uptake and translocation of Cd^{2+}, and in increasing the absorption, selectivity, and translocation of K^+ to the upper parts of common bean plants.

5. Conclusion

The interplaying between Si and Pro plays an important role in increasing tolerance to NaCl-salt and Cd^{2+} stresses in common bean plants. This Si + Pro interplaying extremely reduced Cd^{2+} content in *Phaseolus vulgaris* pods (the edible part) to the limits (0.28 mg kg^{-1}) for legumes. Si and Pro act as signal molecules and induce stress tolerance in bean plants by improving the activities of antioxidant defense systems; enzymatic and non-enzymatic. Interplaying between Si and Pro regulates Pro, sugars, and GSH biosynthesis and significantly increased the activities of antioxidant enzymes (SOD, CAT, POX, APX, and GR) under study for plant adaptation to environmental stressors through increasing water content of plant tissues maintaining cell membranes stability and integrity against increased toxic ions; Na^+ and Cd^{2+}. The regulatory interplaying between Si and Pro can be manipulated for adjusting common bean plants to change the environment for developing sustainable agriculture. However, further studies in this concern are needed to track the signaling pathways of Si and Pro actions and the biosynthesis of Pro in response to the environmental stressors.

Author contributions

Conceived and designed the experiments: ASER and ESMD. Performed the experiments: ASER, MFAEM, and ESMD. Analyzed the data: MMR, MFAEM, and ESMD. Contributed reagents/materials/analysis tools: MMR, ASER, MFAEM, and ESMD. Wrote the paper: MMR, MFAEM, and ESMD. Revised the paper: MMR, ESMD, MFAEM, and ESMD. All authors read and approved the final manuscript.

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