Scientia Horticulturae 233 (2018) 61–69

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Integrative application of cyanobacteria and antioxidants improves common bean performance under saline conditions

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ARTICLE INFO

Keywords:
Antioxidative activity
Blue-green algae
Phaseolus vulgaris
Photosynthetic efficiency
Salt stress

ABSTRACT

Cyanobacteria (CB), known as blue-green algae, are useful microscopic organisms that can lead to improve the nutrient uptake, plant growth, and plant tolerance to abiotic stress such as salinity. The impacts of CB, with or without glutathione (GSH) and ascorbic acid (AsA) and their combinations on morphological, physiological, metabolic, and enzymatic activity status in common bean plant grown on salt stressed soil were investigated. All single (CB, AsA or GSH), or combined (CB + AsA, CB + GSH, CB + AsA + GSH or CB + GSH + AsA) applications significantly increased plant length, number and area of leaves, plant fresh and dry weights, yield parameters (green pods weight per plant, dry seed weight per plant and 100-seed weight) and leaf photosynthetic pigments and their photochemical efficiency (*Fv/Fm* and PI) of common bean plants compared to the control in 2015 and 2016 growing seasons. In addition, relative water content, membrane stability index, contents of soluble sugar, proline, AsA, GSH, N, P and K^+ ion contents, and activities of superoxide dismutase, catalase and guaiacol peroxidase were significantly increased with all of the mentioned applications. In contrast, electrolyte leakage and Na^+ ion content was significantly decreased. The best response was obtained with the integrative CB + AsA + GSH and CB + GSH + AsA treatments, with distinguish of the former. Overall, these results suggest that supporting the seed CB application with foliar application of AsA and GSH helped to increase the defense systems of the common bean plant to tolerate the adverse effects of soil salinity.

1. Introduction

Salinity is one of the most important abiotic stresses that cause reduction in plant growth, development, and productivity worldwide, particularly in arid and semi-arid regions, where soil salt content is naturally high and precipitation can be insufficient for leaching (Kusvuran et al., 2016). Growth mediums with high salinity cause many adverse effects on plant growth, which can possibly be due to a low osmotic potential in soil solutions, effects of specific ions (salt stress), imbalance in nutrition, or a combination of such factors. All the factors mentioned have negative effects on plant development at physiological and biochemical levels. During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis, energy and lipid metabolism are affected. Photosynthetic capacity is reduced, due to the osmotic stress and partial closure of stomata (Paul and Lade, 2014). –

Salt stress changes the morphological, physiological, and biochemical responses of plants. There is evidence that high salt concentrations cause an imbalance in cellular ions, resulting in ion toxicity and osmotic stress, leading to the generation of reactive oxygen species (ROS), which cause damage to DNA, lipids, and proteins. Concurrently, ROS cause chlorophyll degradation and membrane lipid peroxidation, decreasing membrane fluidity and selectivity. To prevent the negative effects of ROS, plants have developed various antioxidant enzyme systems including non-enzymatic antioxidants (e.g. ascorbic acid, glutathione and carotenoids) and antioxidative enzymes (e.g. glutathione reductase; GR, superoxide dismutase; SOD and ascorbate peroxidase; APX). The enzyme SOD belongs to a group of enzymes that accelerate the conversion of $O_2^{-}\cdot$ to H_2O_2 (Hodges et al., 1997). While, catalase (CAT) peroxidases detoxify toxic H_2O_2, superoxide is broken down into water and oxygen by catalysis by SOD. The H_2O_2 is then further scavenged by CAT and APX into H_2O and O_2 (Anjum et al., 2012). The APX reduces H_2O_2 using ascorbate as an electron donor in the ascorbate-glutathione cycle. Oxidized ascorbate is then reduced by GSH generated from GSSG catalyzed by GR at the expense of NADPH. Previous studies showed that the level of antioxidative enzymes increases when plants are exposed to oxidative stress including salinity (Zhu et al., 2004; Sevengör et al., 2011; Kusvuran et al., 2016). Plants with high levels of

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https://doi.org/10.1016/j.scienta.2018.01.047
Received 29 April 2017; Received in revised form 9 January 2018; Accepted 16 January 2018

antioxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage (Siringam et al., 2011). The reports suggested that the extent of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity of their antioxidant systems.

Ascorbic acid (AsA) is a small, water-soluble molecule, which acts as a primary substrate in the cyclic pathway for detoxification and neutralization of superoxide radicals and singlet oxygen. Ascorbate functions co-ordinately with glutathione and several enzymatic antioxidants to inactivate superoxide, which is produced by the Mehler reaction and photorespiration (Noctor and Foyer, 1998). Ascorbate is also believed to detoxify singlet oxygen and hydroxyl radicals and AsA is also involved in regulating photosynthetic capacity by controlling stomatal movement (Athar et al., 2008; Dolatabadian et al., 2009). The AsA has also been shown to play multiple roles in plant growth, such as in cell division and wall expansion, and other developmental processes (Smironoff, 1996; Conklin, 2001; Pignocchi and Foyer, 2003).

Glutathione (GSH) is widely used as a marker of oxidative stress in plants, although its part in plant metabolism is a multi-faceted one (Tausz et al., 2004). GSH is the most important non-protein thiol present in plant cells, and the physiological effects of GSH can be divided into two categories: effects on sulphur metabolism and effects on the defense system. In addition, Mullineaux and Rausch (2005) reported that GSH plays an important role in the protection of the cell against oxidative stress. It is involved in the ascorbate-glutathione cycle and the regulation of protein thiol-disulphide redox status of plants in response to abiotic and biotic stress.

Cyanobacteria (CB) are an important component of soil. Some CB can grow successfully on saline soil, whereas most plants cannot. The fertility of soil can be improved by adding CB. They are a group of gram negative photo autotrophic bacteria (Nayak et al., 2001), and are also the most important nitrogen-fixing agents in many agricultural soils (Vargas and Novelo, 2007). They have the ability to secrete growth promoting substances such as hormones, vitamins and amino acids. They can also increase both the water holding capacity through their jelly-like structure and soil biomass after their death and decomposition (Alam et al., 2014). The CB inoculation is known to reduce the content of oxidized matter in soil, provide oxygen to submerged rhizospheres, ameliorate salinity and buffer the pH, solubilize phosphates, increase the efficiency of fertilizer usage of plants, and enhance plant growth (Mandal et al., 1999; Kaushik and Krishna Murti, 1981; Nain et al., 2010). The CB respond to high salinity by restricting the entry of Na ions and preventing cell injury by keeping a low internal Na concentration (Roychoudhury et al., 1985; Jha et al., 1987).

Classifying as a salt-sensitive plant (Maas and Hoffman, 1977), the common bean (*Phaseolus vulgaris* L.) is one of the most important vegetable crops belonging to *Fabaceae*. Food legumes, including beans, are an important component of the agricultural sector in developing countries due to their capacity to produce significant quantities of protein-rich seed for human nutrition.

This work was designed to investigate the impacts of seed inoculation with CB in addition to foliar application of AsA or GSH, and their integrations on the salt tolerance of *Phaseolus vulgaris*. To examine this purpose, the antioxidantive enzymatic (SOD, GPOX, and CAT) and non-enzymatic (AsA, GSH, and proline) activities, which are among the main antioxidative defences in plants, were assessed.

2. Material and methods

2.1. Experimental conditions and treatments

The seeds of the common bean (*Phaseolus vulgaris* L., cv. Bronco) were obtained from the Agricultural Research Center, Giza, Egypt. Two pot experiments were conducted in two successive summer seasons (2015 and 2016) at the Experimental Farm of the Faculty of Agriculture in Fayoum, Egypt. Seeds were inoculated with cyanobacteria (CB) before being sowed on 25 February of each season in plastic pots (50 cm in diameter, 50 cm in depth) containing saline soil. The soil analyses were carried out according to Black et al. (1965) and Jackson (1967). The soil characteristics are shown in Table 1. Based on the determined EC values (7.35–7.42 dS m^{-1}), the soil is classed as being saline according to Dahnke and Whitney (1988).

Table 1
Physical and chemical properties of the experimental saline soil.

Parameter	2015	2016
Clay	51.25	50.50
Silt	31.50	32.50
Sand	17.25	17.0
Soil texture	Clay	
pH	7.82	7.86
EC (dS m^{-1})	7.35	7.42
Organic matter%	0.81	0.79
CEC* ($cmol_c\ kg^{-1}$)	5.68	5.56
Field capacity (%)	32.9	32.2
Available water (%)	30.1	29.7
Available N (mg kg^{-1} soil)	12.1	11.8
Available P (mg kg^{-1} soil)	147.4	140.9
Available K (mg kg^{-1} soil)	12.5	11.9
Available Fe (mg kg^{-1} soil)	119.8	116.7
Available Mn (mg kg^{-1} soil)	30.5	28.9
Available Zn (mg kg^{-1} soil)	9.7	10.2

* CEC; cation exchange capacity.

The recommended mineral fertilization program for common bean in newly-reclaimed saline soils is a total of 450, 450 and 225 kg ha^{-1}, calcium superphosphate (15.5% P_2O_5), ammonium nitrate (33.5% N), and potassium sulphate (48% K_2O), respectively. This means that each pot (12 kg soil) was received 0.2, 0.2 and 0.1 g of these fertilizers, respectively. Soil in each pot was saturated with water and left until reached a soil water holding capacity. Seeds were then sown at a rate of 5 seeds per pot, and after full emergence seedlings were thinned to 3 per pot. Pots were irrigated with an equal volume of tap water day after day or once every 3 days according to the climate to maintain optimum soil moisture for plants. Using of pesticides was avoided so as not to interfere with the treatments effects, and weeds were collected manually in case of their emergence.

A preliminary study was performed using small plastic pots (0.75 kg soil) to select the best concentration of ascorbic acid (AsA) and glutathione (GSH) to use in the main study. The concentration of 1.0 mM from AsA and 0.75 mM from GSH was generated the best vegetative growth under the tested saline soil (data not shown). Each experiment was consisted of 7 treatments: control, 1.0 mM AsA foliar spray, 0.75 mM GSH foliar spray, inoculation of seeds with CB + AsA foliar spray, CB + GSH foliar spray, CB + AsA foliar spray first time + GSH foliar spray second time, and CB + GSH foliar spray first time + AsA foliar spray second time.

The AsA and GSH, at the amounts mentioned before, were sprayed on the foliage of plants to run off at 25 and 35 days after sowing for each or as a sequenced application of AsA then GSH or GSH then AsA. To ensure optimum penetration into leaf tissues, 0.1% (v/v) Tween-20 solution was added to the spraying solution as a wetting agent. The experiments were arranged in a randomized block design with one level of each of AsA (1.0 mM) and GSH (0.75 mM), applied singly or in combination with CB, with 20 replications/pots (3 plants pot^{-1}) per treatment.

2.2. Measurements of growth and yield characteristics

Forty five days after sowing, 9 plants were randomly chosen from each treatment and their growth characteristics: plant length, number of leaves per plant, leaf area per plant, plant fresh weight and plant dry weight were measured. Green pod characteristics were assessed from

several pod collections 2-d intervals beginning from reaching the pods to be marketable. Dry seed yield characteristics were measured at the end of experiment after air-drying the pods and extraction of seeds.

2.3. Physiological and biochemical measurements

Relative water content (RWC%), membrane stability index (MSI%), and electrolyte leakage (EL%) of leaf tissues were assessed in leaf discs with excluding the midrib following the methods of Hayat et al. (2007), Premchandra et al. (1990) with a modification of Rady (2011), and Sullivan and Ross (1979), respectively.

Photosynthetic efficiency (chlorophyll fluorescence) was measured on a corresponding time in two different sunny days using a portable fluorometer (Handy PEA, Hansatech Instruments Ltd, Kings Lynn, UK) with a corresponding leaf on each selected plant. Maximum quantum yield of PS II F_v/F_m was calculated using the formulae; $F_v/F_m = (F_m - F_0)/F_m$ (Maxwell and Johnson, 2000), and performance index of photosynthesis based on the equal absorption (PI_{ABS}) was calculated as reported by Clark et al. (2000).

Total chlorophylls and total carotenoids contents (mg g^{-1} FW) were estimated adopting the procedure given by Arnon (1949). Leaf discs (0.2 g) of 45-day-old plants were homogenized with 50 ml 80% acetone. After centrifugation at 15,000 × g for 10 min, optical densities of the extract were measured at 663, 645 and 470 nm using a UV160A UV Visible Recording Spectrometer, Shimadzu, Japan.

Total soluble sugar content was assessed after homogenization of 0.2 g leaves with 5 ml 70% ethanol with 5 ml 96% ethanol. The extract was centrifuged at 3500 × g for 10 min and the supernatant was collected and stored at 4 °C (Irigoyen et al., 1992). Freshly prepared anthrone (3 ml) was added to 0.1 ml supernatant. After incubation in hot water bath for 10 min, absorbance was recorded at 625 nm with a Bausch and Lomb-2000 Spectronic Spectrophotometer.

Proline content in 0.5 g leaves was measured after extraction in 10 ml of 3% sulphosalicylic acid (Bates et al., 1973). After centrifugation at 10,000 × g for min, 2 ml of the supernatant was added into test tubes and 2 ml of freshly prepared acid-ninhydrin solution was also added. Incubation was done in a water bath at 90 °C for 30 min, and then the reaction was terminated in an ice-bath. Using 5 ml of toluene, extraction of toluene phase in the dark at room temperature was performed. After separation of the toluene phase, it was read at 520 nm using a UV-160A UV Visible Recording Spectrometer, Shimadzu, Japan.

Ascorbic acid was extracted by 6% (w/v) trichloroacetic acid and determined using 2% (w/v) dinitrophenylhydrazine and 10% (w/v) thiourea in 70% (v/v) ethanol according to the method of Mukherjee and Choudhuri (1983).

The GSH content was determined in 50 mg fresh fully-expanded leaf tissue (Griffth, 1980). After homogenization in 2 ml of 2% (v/v) metaphosphoric acid and centrifuged at 17,000 × g for 10 min, the supernatant (0.9 ml) was neutralized by adding 0.6 ml of 10% (w/v) sodium citrate. Three replicate determinations were made. Each 1.0 ml assay was consisted of 700 µl of 0.3 mM NADPH, 100 µl of 6 mM 5,5′-dithio-bis-2-nitrobenzoic acid, 100 µl distilled water and 100 µl of extract, and was stabilized at 25 °C for 3–4 min. Ten µl of 50 Units ml^{-1} GSH reductase was added and the absorbance was recorded at 412 nm. The GSH content was calculated from a standard curve and expressed on a DW basis.

Nitrogen content was colorimetrically determined by using the Orange-G dye according to the method of Hafez and Mikkelsen (1981). Phosphorus (P) content was determined colorimetrically using a chlorostannus molybdo-phosphoric blue colour method in a sulphuric acid system (Jackson, 1967). Sodium (Na^+) and potassium (K^+) ions contents were estimated using a Perkin-Elmer Model 52-A Flame Photometer (Page et al., 1982). Mineral contents were expressed in mg g^{-1} DW leaf tissue.

2.4. Determination of antioxidative enzyme activities

Leaves of bean plants were excised, rapidly weighed (0.5 g fresh weight), and ground with a pestle in an ice-cold mortar with 3 ml of 0.1 mM potassium phosphate buffer. The homogenates were centrifuged at 15,000 × g for 20 min. The supernatant was used for the determination of protein content and antioxidative enzyme activities. Protein content was estimated according to Lowry et al. (1951). Superoxide dismutase, catalase and guaiacol peroxidase activities were determined according to the methods of Kono (1978), Aebi (1984), and Putter (1974), respectively.

2.5. Statistical analysis

The data obtained from both experimental seasons were analyzed by one-way analysis of variance (ANOVA). When significant differences were observed among treatments, a means comparison test was performed using Tukey's test ($P \leq 0.05$). SPSS Version 10.0 for Windows pocket program was used for these analyses.

3. Results

Enhancement values in growth parameters of the bean plants grown on a saline soil by using bio-fertilization and antioxidants are presented in Table 2. All single (CB, AsA or GSH), and combined (CB + AsA, CB + GSH, CB + AsA + GSH or CB + GSH + AsA) applications significantly increased growth traits (plant length, number of leaves, leaf area, and plant fresh and dry weights) of common bean plant compared to the control in both seasons (2015 and 2016). Compared to the control, the above mentioned traits were increased by 8–18%, 3–17%, 5–24%, 12–33%, and 14–34%, respectively. Also, the integrative CB + AsA + GSH and CB + GSH + AsA treatments had stimulated growth characteristics that were significantly highest compared to the all other treatments. According to the results, under salinity bio-fertilizer (CB) and antioxidants treatments illustrated that maximum growth and yield parameters of the common bean were mostly observed for CB combined with AsA and GSH application (Table 3). Although the integrative CB + AsA + GSH and CB + GSH + AsA treatments were observed to show insignificant differences for growth characteristics, the integrative CB + AsA + GSH treatment significantly exceeded the integrative CB + GSH + AsA treatment, and consequently all other treatments for bean yield ant its components. This integrative application exceeded the control treatment by 77–82%, 23–24% and 20–21% for green pods weight per plant, dry seed weight per plant and 100-seed weight. With distinguish of the integrative treatment of CB + AsA + GSH, the CB + AsA + GSH and CB + GSH + AsA integrative treatments were most effective in alleviating the adverse effects of salinity and effectively raised yield parameters of the common bean.

The RWC significantly increased due to the different treatments applied to plants under stress conditions. At 7.4 dS m^{-1}, maximum improvement in RWC was observed with the combinations of CB + AsA + GSH and CB + GSH + AsA, where the increase in RWC compared with its control was 16%. By using the bio-fertilizer (CB), EL was significantly decreased. This reduction was maximized in integrative CB + AsA + GSH and CB + GSH + AsA treatments (7.52-7.57%). On average, the highest EL value (%) was in the control groups (12%). However, when the CB was used, EL was decreased by 7.5-10.7%. Treatments by CB, AsA, GSH, and their different combinations on the plants had caused a significant improvement in the MSI as shown in Table 4. According to the results, the highest MSI was established in integrative CB + AsA + GSH and CB + GSH + AsA treatments (79.8-80.6%). In spite of this, the MSI was by 67.0-67.6% in control plants (Table 4).

Supplemental addition of bio-fertilizers (CB) to the salt-stressed common bean plants induced significant increase in total chlorophyll

Table 2
Effect of seed inoculation with cyanobacteria (CB), and foliar spray with ascorbic acid (AsA; 1.0 mM) and glutathione (GSH; 0.75 mM) on growth characteristics of common bean (*Phaseolus vulgaris* L., cv. Bronco) plants grown under saline soil conditions.

Treatments	Parameters				
	Plant length	Number of leaves plant^{-1}	Leaf area plant^{-1}(dm^2)	Plant fresh weight (g)	Plant dry weight (g)
2015					
Control	41.3 ± 2.8 c	6.90 ± 0.42 c	8.87 ± 0.33 c	41.1 ± 3.0 c	6.32 ± 0.38 d
AsA	44.8 ± 3.1 b	7.16 ± 0.45 bc	9.75 ± 0.37 b	47.2 ± 3.6 b	7.23 ± 0.44 c
GSH	45.1 ± 3.2 b	7.12 ± 0.44 bc	9.80 ± 0.38 b	47.2 ± 3.6 b	7.29 ± 0.44 c
CB + AsA	47.2 ± 3.5 ab	7.42 ± 0.48 ab	10.57 ± 0.43ab	50.9 ± 4.4 ab	7.56 ± 0.51 bc
CB + GSH	47.3 ± 3.5 ab	7.41 ± 0.48 ab	10.56 ± 0.43ab	51.1 ± 4.6 ab	7.48 ± 0.50 bc
CB + AsA + GSH	48.5 ± 3.7 a	7.80 ± 0.49 a	10.91 ± 0.45 a	55.0 ± 4.9 a	8.51 ± 0.53 a
CB + GSH + AsA	48.0 ± 3.6 a	7.72 ± 0.48 a	10.90 ± 0.44 a	54.7 ± 4.7 a	8.44 ± 0.52 a
2016					
Control	42.1 ± 2.6 c	6.77 ± 0.39 c	9.04 ± 0.33 c	42.8 ± 2.9 d	6.45 ± 0.36 c
AsA	45.1 ± 2.8 b	7.17 ± 0.42 b	9.50 ± 0.36 bc	47.9 ± 3.4 c	7.58 ± 0.41 b
GSH	45.0 ± 2.8 b	7.19 ± 0.42 b	9.54 ± 0.36 bc	48.4 ± 3.6 c	7.56 ± 0.41 b
CB + AsA	47.0 ± 3.0 ab	7.32 ± 0.44 ab	9.96 ± 0.40 b	52.5 ± 4.2 b	8.14 ± 0.43 ab
CB + GSH	47.2 ± 3.0 ab	7.36 ± 0.44 ab	9.94 ± 0.40 b	52.2 ± 4.2 b	8.13 ± 0.43 ab
CB + AsA + GSH	49.9 ± 3.4 a	7.90 ± 0.48 a	11.23 ± 0.46 a	57.1 ± 4.8 a	8.65 ± 0.47 a
CB + GSH + AsA	49.2 ± 3.3 a	7.83 ± 0.48 a	11.20 ± 0.45 a	56.8 ± 4.7 a	8.62 ± 0.47 a

Mean values (n = 9) followed by different letters within the same column are significantly different ($P \leq 0.05$) based on Tukey's test.

and carotenoid contents (Table 5). The magnitude of response was most pronounced with integrative CB + AsA + GSH and CB + GSH + AsA treatments (1.85-1.96 mg g^{-1} fresh weight in total chlorophyll and 0.39-0.46 mg g^{-1} fresh weight in total carotenoid contents). Total chlorophyll and carotenoid contents with these applications increased by 28–76% and 30–45% compared to control plants, respectively. The photochemical efficiency (*Fv/Fm*) ratio ranged between 68.5 and 81.1 and the lowest range was observed to be in control plants during the two years. The *Fv/Fm* values of the CB bio-fertilized plants were higher than those of the non-bio-fertilized control plants growing under the same stress conditions. The difference between PI values of plants treated with bio-fertilizers was found to be statistically significant. In fact, PI values were determined to have an average of 60.7 in control plants, 65.9, 66, 69.4, 66, 72.7, and 72.3 in CB, AsA, GSH, CB + AsA + GSH, and CB + AsA + GSH applications, respectively. Compared to control plants, the highest increase was observed in integrative CB + AsA + GSH and CB + GSH + AsA treatments (19.8 and 19.1%, respectively).

It is evident from Table 6 that the CB bio-fertilizer treatments had a significant effect on total soluble sugar, proline, AsA, and GSH contents of the common bean during the two seasons. Thus, all CB bio-fertilizer treatments, individually or combined, proved to result in significant increases in the mean values of total soluble sugar, proline, AsA and GSH contents compared with the control treatments. The maximum mean values were obtained in integrative CB + AsA + GSH and CB + GSH + AsA applications. Whereas, the least mean values were obtained from control treatments (average 3.65 mg g^{-1} DW, 275 µg g^{-1} DW, 7.29 µmol AsA g^{-1} DW, 1.04 µmol GSH g^{-1} DW, respectively).

The antioxidative enzymes; superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPOX) exhibited an increasing trend in response to CB and CB with AsA, GSH combinations (Table 7). Especially, CB with AsA and GSH combination treatments caused a significant increase in the activities of all enzymes. The highest increase in the activities of these enzymes was recorded in the plants exposed to integrative CB + AsA + GSH and CB + GSH + AsA treatments. With these treatments, SOD, CAT and GPOX activities increased by 89–144%, 104–248%, and 96–130%, respectively, compared to the control plants.

In this study, the effects of different CB bio-fertilizer with antioxidants on accumulation of ions in the common bean were investigated (Table 8). A significant increase in N, P, and K^+ ion contents was recorded as a result of all applications compared to the control. In contrast, Na^+ ion accumulation was reduced. The integrative

Table 3
Effect of seed inoculation with cyanobacteria (CB), and foliar spray with ascorbic acid (AsA; 1.0 mM) and glutathione (GSH; 0.75 mM) on green pod and dry seed yields of common bean (*Phaseolus vulgaris* L., cv. Bronco) plants grown under saline soil conditions.

Treatments	Parameters				
	Average pod weight (g)	Pods No. plant^{-1}	Pods weight plant^{-1} (g)	Dry seed weight plant^{-1} (g)	100-seed weight (g)
2015					
Control	2.08 ± 0.15 c	14.3 ± 1.2 d	29.7 ± 1.8 d	10.0 ± 0.7 c	15.7 ± 1.1 c
AsA	2.31 ± 0.19 b	18.0 ± 1.4 c	41.6 ± 2.3 c	11.1 ± 0.8 b	17.4 ± 1.2 b
GSH	2.29 ± 0.18 b	17.8 ± 1.4 c	40.8 ± 2.2 c	11.1 ± 0.8 b	17.3 ± 1.2 b
CB + AsA	2.32 ± 0.18 b	17.9 ± 1.4 c	41.2 ± 2.3 c	11.2 ± 0.8 b	17.4 ± 1.2 b
CB + GSH	2.30 ± 0.20 b	19.4 ± 1.6 b	46.6 ± 2.5 b	11.2 ± 0.9 ab	17.5 ± 1.3 ab
CB + AsA + GSH	2.52 ± 0.21 a	21.3 ± 1.8 a	52.5 ± 3.0 a	12.3 ± 0.9 a	18.9 ± 1.4 a
CB + GSH + AsA	2.45 ± 0.20 a	20.7 ± 1.7 a	48.2 ± 2.9 c	11.4 ± 0.9 b	17.6 ± 1.3 b
2016					
Control	2.11 ± 0.14 c	14.6 ± 1.1 d	30.8 ± 2.0 d	10.2 ± 0.6 c	16.2 ± 1.2 c
AsA	2.36 ± 0.18 b	18.3 ± 1.4 c	43.2 ± 2.9 c	11.2 ± 0.7 b	17.8 ± 1.4 b
GSH	2.34 ± 0.18 b	18.2 ± 1.4 c	42.6 ± 2.8 c	11.1 ± 0.7 b	17.7 ± 1.4 b
CB + AsA	2.36 ± 0.18 b	18.4 ± 1.5 c	43.4 ± 2.9 c	11.4 ± 0.7 b	18.1 ± 1.4 b
CB + GSH	2.46 ± 0.19 ab	20.0 ± 1.7 b	49.2 ± 3.3 b	11.4 ± 0.7 b	18.2 ± 1.5 b
CB + AsA + GSH	2.59 ± 0.20 a	21.8 ± 1.8 a	56.0 ± 3.7 a	12.6 ± 0.8 a	19.6 ± 1.5 a
CB + GSH + AsA	2.54 ± 0.20 a	21.4 ± 1.7 a	51.8 ± 3.6 b	11.6 ± 0.8 b	18.4 ± 1.5 b

Mean values (n = 18) followed by different letters within the same column are significantly different ($P \leq 0.05$) based on Tukey's test.

Table 4
Effect of seed inoculation with cyanobacteria (CB), and foliar spray with ascorbic acid (AsA; 1.0 mM) and glutathione (GSH; 0.75 mM) on leaf relative water content (RWC%), electrolyte leakage (EL%) and membrane stability index (MSI%) of common bean (*Phaseolus vulgaris* L., cv. Bronco) plants grown under saline soil conditions.

Treatments	Parameters		
	RWC (%)	EL (%)	MSI (%)
2015			
Control	68.5 ± 2.4 c	12.66 ± 0.47 a	67.0 ± 3.4 c
AsA	75.2 ± 3.1 b	10.65 ± 0.32 b	74.7 ± 4.1 b
GSH	75.7 ± 3.2 b	10.52 ± 0.31 b	74.4 ± 4.0 b
CB + AsA	77.1 ± 3.8 ab	9.42 ± 0.26 b	77.1 ± 4.8 ab
CB + GSH	77.2 ± 3.9 ab	9.40 ± 0.26 c	77.5 ± 4.9 ab
CB + AsA + GSH	80.0 ± 4.2 a	7.54 ± 0.21 d	80.0 ± 5.3 a
CB + GSH + AsA	79.9 ± 4.2 a	7.58 ± 0.22 d	79.8 ± 5.2 a
2016			
Control	69.4 ± 2.3 c	12.73 ± 0.43 a	67.6 ± 3.1 c
AsA	75.3 ± 3.0 b	10.40 ± 0.32 b	75.5 ± 3.9 b
GSH	75.7 ± 3.0 b	10.37 ± 0.32 b	75.3 ± 3.8 b
CB + AsA	77.3 ± 3.2 ab	9.29 ± 0.28 c	77.4 ± 4.4 ab
CB + GSH	77.2 ± 3.2 ab	9.41 ± 0.28 c	77.4 ± 4.4 ab
CB + AsA + GSH	81.1 ± 3.9 a	7.50 ± 0.20 d	80.6 ± 4.9 a
CB + GSH + AsA	80.8 ± 3.9 a	7.56 ± 0.20 d	80.3 ± 4.8 a

Mean values (n = 9) followed by different letters within the same column are significantly different ($P \leq 0.05$) based on Tukey's test.

CB + AsA + GSH and CB + GSH + AsA treatments showed an increase in N (24–31%), P (37–50%), and K^+ (29–36%) ion contents as compared to control plants. In addition to these results, Na^+ ion content was determined to be 0.68% and 0.66% in 2015 and 2016 studies, however these values were reduced by 0.32–0.51% with bio-fertilizers. Especially, with integrative CB + AsA + GSH and CB + GSH + AsA treatments, Na^+ content was decreased by 47–51% compared to the control plants.

4. Discussion

As expected, common bean plants that received CB bio-fertilizer and antioxidants produced higher amounts of fresh and dry matter compared to the control plants under salt stress. Increase in fresh and dry weights was a result of the increase in plant height, number of leaves, and leaf area, and may also be due to an increase in photosynthetic efficiency (chlorophylls, carotenoids, Fv/Fm, and PI) that supported by the increased contents and activities of non-enzymatic and enzymatic antioxidants during the treatments (Tables 2–8). The positive effect of CB bio-fertilizer could be attributed to its effects on supplying plants with their requirements of various nutrients as well as their effect on lowering soil pH; which could facilitate the availability of soil nutrients and their effects on the plant physiological processes such as photosynthetic activity as well as the utilization of carbohydrates. CB, therefore, improves the soil chemical characteristics as well as enhances the physical and biological characters which in turn favour root development (Khalil and El-Noemani, 2015). Mahajan and Tuteja (2005) and Tawfik (2008) indicated that high salt accumulation in the soil generates a low water potential zone in the soil making it increasingly difficult for the plant to acquire both water as well as nutrients. Thus, salt stress results in water deficiency in the plant and takes the form of physiological drought. Relative water content (RWC) is a key marker for salt stress studies. RWC measurement is a general method used to determine leaf water balance in plants during water deficient periods and estimates the percentage of water present in a leaf as a fraction of the total volumetric water that the leaf can hold at full turgor. When RWC can be maintained in cells and tissues, it allows continuation of metabolic activity by osmotic adjustments and other traits of adaptation to salinity and/or drought (Slabbert and Krüger, 2014). The use of different types of CB bio-fertilizer resulted in significant increase in RWC% compared with the control. This result may be due to the associated increase in the hydraulic nature of branch root junctions which facilitate the radial flow of water, or due to changes in root morphology such as root dry weight and root branching (Ordookhani et al., 2011; Khalil and El-Noemani, 2015). The CB bio-fertilizer also modifies the membrane structure/stability under stress conditions. Cell membranes are one of the first targets of many undesirable environmental factors and it is generally accepted that the maintenance of their integrity and stability is one of the major components for achieving high and acceptable yields (Namvar et al., 2013; Namvar and Khandan, 2015). Membrane stability index (MSI) was significantly affected by CB in this study (Table 4). Therefore, the plants pre-treated with CB bio-fertilizers had a higher MSI. The highest MSI and RWC values and the lowest electrolyte leakage (EL) values were observed in the integrative CB + AsA + GSH application followed by the integrative CB + GSH + AsA one.

The favourable effects of the combinations of CB + AsA + GSH and CB + GSH + AsA can be explained based on the beneficial effects of CB bio-fertilizer on improvement of soil physical, chemical and biological characteristics. In addition, the beneficial effects of the low molecular weight antioxidants; AsA and GSH that form an important part of the

Table 5
Effect of seed inoculation with cyanobacteria (CB), and foliar spray with ascorbic acid (AsA; 1.0 mM) and glutathione (GSH; 0.75 mM) on leaf photosynthetic pigments (mg g^{-1} fresh weight) and chlorophyll fluorescence of common bean (*Phaseolus vulgaris* L., cv. Bronco) plants grown under saline soil conditions.

Treatments	Parameters			
	Total chlorophylls	Total carotenoids	Fv/Fm	PI
2015				
Control	1.05 ± 0.03 c	0.30 ± 0.01 d	68.5 ± 1.0 c	60.2 ± 1.0 c
AsA	1.39 ± 0.04 b	0.33 ± 0.01 c	72.2 ± 1.2 bc	65.5 ± 1.2 b
GSH	1.40 ± 0.04 b	0.33 ± 0.01 c	72.7 ± 1.2 bc	65.4 ± 1.2 b
CB + AsA	1.68 ± 0.05 ab	0.36 ± 0.02 b	75.1 ± 1.3 b	68.9 ± 1.3 ab
CB + GSH	1.67 ± 0.05 ab	0.36 ± 0.02 b	75.2 ± 1.3 b	69.1 ± 1.3 ab
CB + AsA + GSH	1.87 ± 0.06 a	0.40 ± 0.03 a	80.0 ± 1.5 a	72.4 ± 1.4 a
CB + GSH + AsA	1.85 ± 0.06 a	0.39 ± 0.03 a	79.9 ± 1.4 a	71.8 ± 1.4 a
2016				
Control	1.53 ± 0.03 c	0.31 ± 0.02 d	69.4 ± 0.9 c	61.2 ± 1.0 c
AsA	1.77 ± 0.04 b	0.35 ± 0.03 c	74.5 ± 1.2 b	66.3 ± 1.2 b
GSH	1.76 ± 0.04 b	0.36 ± 0.03 c	74.7 ± 1.2 b	66.1 ± 1.2 b
CB + AsA	1.84 ± 0.04 ab	0.41 ± 0.03 b	76.3 ± 1.4 ab	69.9 ± 1.3 ab
CB + GSH	1.85 ± 0.04 ab	0.41 ± 0.03 b	76.0 ± 1.3 ab	70.0 ± 1.3 ab
CB + AsA + GSH	1.99 ± 0.05 a	0.46 ± 0.03 a	81.1 ± 1.6 a	73.0 ± 1.4 a
CB + GSH + AsA	1.96 ± 0.05 a	0.45 ± 0.03 a	80.6 ± 1.6 a	72.8 ± 1.4 a

Mean values (n = 9) followed by different letters within the same column are significantly different ($P \leq 0.05$) based on Tukey's test.

Table 6
Effect of seed inoculation with cyanobacteria (CB), and foliar spray with ascorbic acid (AsA; 1.0 mM) and glutathione (GSH; 0.75 mM) on the shoot concentrations of total soluble sugars, free proline, ascorbic acid (AsA), glutathione (GSH) of common bean (*Phaseolus vulgaris* L., cv. Bronco) plants grown under saline soil conditions.

Treatments	Parameters			
	Soluble sugars (mg g^{-1} DW)	Free proline (μg g^{-1} DW)	AsA (μmol AsA g^{-1} DW)	GSH (μmol GSH g^{-1} DW)
2015				
Control	3.54 ± 0.12 c	264 ± 5 d	1.25 ± 0.01 d	1.02 ± 0.01 d
AsA	4.05 ± 0.17 b	347 ± 7 c	3.22 ± 0.03 b	1.28 ± 0.02 d
GSH	4.07 ± 0.17 b	343 ± 7 c	2.46 ± 0.02 c	2.27 ± 0.03 c
CB + AsA	4.16 ± 0.18 b	376 ± 8 b	3.57 ± 0.04 b	2.00 ± 0.02 c
CB + GSH	4.13 ± 0.18 b	373 ± 8 b	2.61 ± 0.03 c	3.80 ± 0.04 b
CB + AsA + GSH	4.58 ± 0.22 a	464 ± 10 a	4.58 ± 0.05 a	4.38 ± 0.06 a
CB + GSH + AsA	4.50 ± 0.22 a	452 ± 10 a	4.47 ± 0.05 a	4.32 ± 0.06 a
2016				
Control	3.75 ± 0.09 d	286 ± 5 d	1.33 ± 0.02 e	1.06 ± 0.01 e
AsA	4.41 ± 0.13 c	357 ± 7 c	3.54 ± 0.04 c	2.13 ± 0.02 d
GSH	4.44 ± 0.13 c	360 ± 7 c	2.81 ± 0.03 d	3.24 ± 0.04 c
CB + AsA	4.72 ± 0.16 b	403 ± 9 b	4.35 ± 0.05 b	2.17 ± 0.02 d
CB + GSH	4.70 ± 0.16 b	405 ± 9 b	2.84 ± 0.03 d	4.10 ± 0.05 b
CB + AsA + GSH	5.08 ± 0.18 a	465 ± 11 a	5.83 ± 0.06 a	4.61 ± 0.06 a
CB + GSH + AsA	4.98 ± 0.18 a	457 ± 11 a	5.64 ± 0.06 a	4.52 ± 0.06 a

Mean values (n = 9) followed by different letters within the same column are significantly different ($P \leq 0.05$) based on Tukey's test.

abiotic stress response in plant cells. The AsA and GSH have been reported to alleviate and repair the damage initiated by reactive oxygen species (ROS) and enable plants to develop a complex antioxidant defence systems to increase the cellular defence strategy against salt stress induced oxidative stress (Wutipraditkul et al., 2015; Rady and Hemida, 2016; Rady et al., 2016). The application of AsA and GSH as integration treatment with CB beginning by AsA generated better results, particularly for bean yield (Table 3) than the integrative CB + GSH + AsA application that began with GSH. This result attributed perhaps to that AsA is an important antioxidant that reacts not only with H_2O_2 but also with $O_2^{-}\cdot$, OH− and lipid hydroperoxidases. It has been also implicated in several types of biological activities in plants such as an enzyme co-factor, an antioxidant, and a donor/acceptor in electron transport at the plasma membrane or in the chloroplasts, all of which are related to oxidative stress resistance (Conklin, 2001). In chloroplasts, the so-called "Halliwell-Asada" pathway indicates that ascorbate peroxidase uses AsA and oxidizes it to monodehydroascorbate (MDA) that may give rise to dehydroascorbate (DHA). Both MDA and DHA will then be reduced to regenerate the ascorbate pool. This type of scavenging is thought to occur near PSI to minimize the risk of escape and reaction of ROS with each other (Foyer and Noctor, 2000). The AsA is also known to function as the "terminal antioxidant" because the redox potential of AsA/MDA pair (280 mv) is lower than that of most of the bioradicals (Scandalios et al., 1997). The biosynthesis of AsA from hexose phosphate and its involvement in protection against photo-oxidative stress suggest that there may be links between photosynthesis and the AsA pool size (Reddy et al., 2004).

Abdelhamid et al. (2013) indicated that carotenoids are involved in protecting the photosynthetic apparatus against photo-inhibitory damage by singlet oxygen ($^1O_2$) which is produced by the excited triplet state of chlorophyll. Carotenoids directly deactivate singlet oxygen and also quench the excited triplet state of chlorophyll, thus indirectly reducing the formation of $^1O_2$ species. Stomatal closure due to osmotic stress or salt induced damage of photosynthetic apparatus caused reduction in total chlorophyll content of mung bean leaves (Mohammed, 2007; Tawfik, 2008). Plants treated with integrative CB with AsA and GSH gave the highest amount of photosynthetic pigments than others (Table 5). Ordog (1999) showed that CB can induce a plant growth substance that improves the content of leaf chlorophyll. Similarly, various responses of chlorophyll content to salt stress were found in

Table 7
Effect of seed inoculation with cyanobacteria (CB), and foliar spray with ascorbic acid (AsA; 1.0 mM) and glutathione (GSH; 0.75 mM) on leaf enzymatic protein and the activities of enzymatic antioxidants (superoxide dismutase; SOD, catalase; CAT and guaiacol peroxidase; GPOX) of common bean (*Phaseolus vulgaris* L., cv. Bronco) plants grown under saline soil conditions.

Treatments	Parameters			
	Protein (mmol g^{-1} FW)	SOD (μmol mg^{-1} protein)	CAT (μmol mg^{-1} protein)	GPOX (μmol mg^{-1} protein)
2015				
Control	0.92 ± 0.04 d	0.86 ± 0.02 d	0.54 ± 0.02 d	0.42 ± 0.02 d
AsA	1.00 ± 0.04 c	1.34 ± 0.03 c	0.98 ± 0.04 c	0.59 ± 0.03 c
GSH	1.01 ± 0.04 c	1.39 ± 0.03 c	0.97 ± 0.04 c	0.60 ± 0.03 c
CB + AsA	1.19 ± 0.05 b	1.71 ± 0.05 b	1.44 ± 0.06 b	0.71 ± 0.03 b
CB + GSH	1.17 ± 0.05 b	1.75 ± 0.05 b	1.48 ± 0.06 b	0.74 ± 0.03 b
CB + AsA + GSH	1.37 ± 0.06 a	2.10 ± 0.07 a	1.88 ± 0.07 a	0.97 ± 0.04 a
CB + GSH + AsA	1.34 ± 0.06 a	2.08 ± 0.07 a	1.83 ± 0.07 a	0.96 ± 0.04 a
2016				
Control	1.05 ± 0.09 d	1.16 ± 0.06 d	1.03 ± 0.04 d	0.66 ± 0.02 d
AsA	1.41 ± 0.13 c	1.57 ± 0.08 c	1.44 ± 0.06 c	0.83 ± 0.03 c
GSH	1.37 ± 0.13 c	1.65 ± 0.09 c	1.41 ± 0.05 c	0.84 ± 0.03 c
CB + AsA	1.60 ± 0.16 b	1.93 ± 0.10 b	1.75 ± 0.07 b	1.07 ± 0.03 b
CB + GSH	1.60 ± 0.16 b	1.95 ± 0.10 b	1.74 ± 0.07 b	1.10 ± 0.04 b
CB + AsA + GSH	1.88 ± 0.18 a	2.25 ± 0.12 a	2.13 ± 0.09 a	1.31 ± 0.05 a
CB + GSH + AsA	1.80 ± 0.18 a	2.20 ± 0.12 a	2.11 ± 0.09 a	1.29 ± 0.05 a

Mean values (n = 9) followed by different letters within the same column are significantly different ($P \leq 0.05$) based on Tukey's test.

Table 8
Effect of seed inoculation with cyanobacteria (CB), and foliar spray with ascorbic acid (AsA; 1.0 mM) and glutathione (GSH; 0.75 mM) on the contents of macro-nutrients (N, P, K and Ca) and sodium (Na) of common bean (*Phaseolus vulgaris* L., cv. Bronco) plants grown under saline soil conditions.

Treatments	Parameters			
	N (%)	P (%)	K (%)	Na (%)
2015				
Control	2.51 ± 0.06 d	0.27 ± 0.01 d	2.71 ± 0.06 d	0.68 ± 0.02 a
AsA	2.74 ± 0.08 c	0.30 ± 0.02 c	3.00 ± 0.08 c	0.51 ± 0.02 b
GSH	2.76 ± 0.08 c	0.31 ± 0.02bc	3.01 ± 0.08 c	0.50 ± 0.02 b
CB + AsA	2.95 ± 0.08 b	0.33 ± 0.02 b	3.22 ± 0.09 b	0.49 ± 0.02 b
CB + GSH	3.01 ± 0.08 b	0.34 ± 0.02 b	3.26 ± 0.09 b	0.43 ± 0.01 c
CB + AsA + GSH	3.28 ± 0.10 a	0.37 ± 0.03 a	3.57 ± 0.11 a	0.36 ± 0.01 d
CB + GSH + AsA	3.21 ± 0.10 a	0.37 ± 0.03 a	3.50 ± 0.10 a	0.37 ± 0.01 d
2016				
Control	2.58 ± 0.06 c	0.26 ± 0.01 d	2.76 ± 0.05 d	0.66 ± 0.03 a
AsA	2.95 ± 0.07 b	0.33 ± 0.02 c	3.20 ± 0.07 c	0.47 ± 0.02 b
GSH	2.97 ± 0.07 b	0.32 ± 0.02 c	3.19 ± 0.07 c	0.48 ± 0.02 b
CB + AsA	3.06 ± 0.08 ab	0.35 ± 0.02 b	3.44 ± 0.09 b	0.40 ± 0.02 c
CB + GSH	3.04 ± 0.08 ab	0.36 ± 0.03 b	3.45 ± 0.09 b	0.40 ± 0.02 c
CB + AsA + GSH	3.21 ± 0.10 a	0.39 ± 0.03 a	3.74 ± 0.12 a	0.32 ± 0.01 d
CB + GSH + AsA	3.19 ± 0.10 a	0.38 ± 0.03 a	3.71 ± 0.11 a	0.33 ± 0.01 d

Mean values (n = 9) followed by different letters within the same column are significantly different ($P \leq 0.05$) based on Tukey's test.

some CB. In Synechocystis sp. PCC 6803, under 342 mM NaCl concentration, the chlorophyll content increased, while under 684 or 1026 mM it sharply decreased (Schubert et al., 1993; Sudhir and Murthy, 2004). In our study, CB or CB with GSH or/and AsA combinations had increased total chlorophyll and carotenoid contents during both growing seasons. In CB, various salt stress conditions stimulate the rate of respiration and PS1 activity and impair the rate of photosynthesis (Sudhir and Murthy, 2004). The overall PI represents a single multiparametric expression that combines all three independent functional steps of photosynthesis; the density of the reaction centres in the chlorophyll pool, trapped excitation energy, and its conversion to electron transport (Gururani et al., 2013). The PI and *Fv/Fm* values shown in Table 5 clearly indicate that the photosynthetic performance of CB and plants treated with the other combinations under stress conditions significantly increased, indicating the positive effect of the CB bio-fertilizer on photosynthetic machinery of the plants. Common bean plants treated with integrative CB + AsA + GSH and CB + GSH + AsA showed a higher overall PI and *Fv/Fm* under stress conditions compared to them of plants treated only with the CB bio-fertilizer.

Accumulation of sugars, proline, AsA, and GSH under salt stress conditions protects the cells by balancing the osmotic strength of the cytosol with that of the vacuole and external environment (Greenway and Munns, 1980). Moreover, proline has a dual role in improving salt stress tolerance as it is able to act in a similar way to the peroxidase enzyme and scavenge reactive oxygen species (Zhu, 2001). Therefore, proline accumulation is an important physiological index for the response of a plant to salt stress (Abdelhamid et al., 2013). Our results (Table 6) exhibit that bio-fertilizer application increased the accumulation of proline in the leaves of common bean plants. Higher contents of proline under salt stress are favourable to plants, as proline participates in the osmotic potential of leaves and thus in osmotic adjustment (Abdelhamid et al., 2013). Also in this study, total soluble sugar, ascorbic acid and glutathione contents were increased with bio-fertilizers. The accumulation of organic solutes especially sugars are the main solutes involved in osmotic adjustment in glycophytic plants submitted to osmotic and saline stress (El-Bassiouny et al., 2015). Our results showed that common bean plants in the presence of CB and the other applications led to marked increase in soluble sugars when compared with control plants cultivated without CB. This increase was significant in integrative CB + AsA + GSH and CB + GSH + AsA treatments. The effects of glutathione on accumulation of soluble sugars can probably be attributed to the protective effects of glutathione on photosynthetic systems. GSH, also, plays a protective role in salinity tolerance by maintenance of the redox status (Chaparzadeh et al., 2004).

Protein accumulation under salt stress may provide a storage form of nitrogen that is reutilized when the stress is over and may play a role in osmotic adjustments (Ashraf and Harris, 2004). Treatment with CB singly or in integration with AsA and GSH significantly increased protein content of common bean plant. Karthikeyan et al. (2009) observed that CB cultures were capable of enhancing plant growth and that they increased the presence of extracellular proteins in the range of 32–82 μg ml^{-1} and an array of amino acids.

Salt stress leads to oxidative damage in plants by inducing the production of reactive oxygen species (ROS). Superoxide radicals that emerge as a result of stress in plant tissues are transformed into H_2O_2 by the SOD enzyme (Dixit et al., 2001; Mittova et al., 2002). The accumulation of H_2O_2, which results from the canalization reaction of the SOD enzyme and is a powerful oxidant, is prevented by the ascorbate-glutathione cycle. SOD enzyme activity, which is responsible for diminishing the oxidative stress-derived oxygen species, increased linearly with prolonged stress conditions. CAT enzyme plays a role in the elimination of oxidative stress-derived reactive oxygen derivatives such as H_2O_2, by converting it into water and molecular oxygen (Dionisio-Sese and Tobita, 1998). In the presented study, it has been shown that using bio-fertilizers resulted in a similar reaction to salt stress and that the CAT enzyme activity increased with CB and CB with GSH or/and AsA combinations under a salt medium. APX enzymes, which are a part of the defense mechanism of plants against salt, drought, and chilling stress, are generally effective in the reduction of H_2O_2 to water in chloroplasts and mitochondria, thereby detoxifying them (Scandalios et al., 1997; Shalata et al., 2001). APX functions in the reduction of H_2O_2 to water in the ascorbate-glutathione cycle. During this reaction, the ascorbate is oxidized to monodehydroascorbate (MDHA). MDHA is converted into ascorbate by MDHA reductase enzyme activity. Together with this reaction, 2 molecules of MDHA are converted into MDHA and dehydroascorbate (DHA) in an unproportional non-enzymatic sideway reaction. This study, in which the response to bio-fertilizer applications in salinity mediums were studied, investigators showed an increase in APX enzyme activities. These increases were statistically significant, especially in the integrative CB + AsA + GSH and CB + GSH + AsA treatments. El-Bassiouny et al. (2015) indicated that CB probably increased the levels of antioxidant substances and enhanced the activities of some antioxidant enzymes among them SOD, CAT, and POX in wheat plants under salt stress condition. Singh et al. (2002) reported that they have developed a number of mechanisms by which CB defend

themselves against environmental stressors. Also these resources emphasized that among them the production of photo-protective compounds such as mycosporine-like amino acids (MAAs) and scytonemin, enzymes such as SOD, CAT and POX, repair of DNA damage, and synthesis of shock proteins were important.

Salt stress caused disruption of ionic equilibrium, such as the influx of Na^+, which results in the dissipation of the membrane potential and facilitates the uptake of Cl^- down the chemical gradient. Na^+ is toxic to cell metabolism and has a deleterious effect on the functioning of some enzymes. High concentration of Na^+ causes osmotic imbalance, membrane disorganization, reduction in growth inhibition of cell division and expansion. In addition, high Na^+ levels also lead to reduction in photosynthesis and production of ROS (Yeo, 1998; Tawfik, 2008). Singh and Dhar (2010) indicated that in CB, accumulation of internal osmotica in the form of inorganic ions and prevention of intracellular Na^+ accumulation by the curtailment of Na^+ influx, and by efficient active efflux mechanisms or metabolic adjustments have been investigated in depth. The Na^+ extrusion in CB is driven by a Na^+/H^+ antiporter, which is energized by enhanced activity of cytochrome oxidase. The inhibition of Na^+ influx appears to be a major mechanism for the survival of CB against salt stress, and the synthesis of salt-stress proteins have been found to occur in CB. As a result of this study, biofertilizers caused the inhibition of toxic Na^+ accumulation and ensured ionic equilibrium with increased N, P, and K^+ accumulation. So, there was significant recovery in bean growth at salinity conditions. Rady et al. (2013) indicated that the maintenance of ionic balance during salinity stress is a prerequisite to protecting the plant against the build-up of toxic ions, with K^+ accumulating and Na^+ reaching the minimum content in bean leaves. The influence of N on plant growth and development is often connected with the process of photosynthesis (Ivanova and Vassilev, 2003; Hasaneen et al., 2009). So in this study, increase of N accumulation with CB enhanced growth and yield parameters. Moreover, Sharma et al. (2012) indicated that CB have the ability to fix atmospheric Na and possess some soil phosphate, solubilizing the insoluble phosphate through excreting organic acids that solve the common problem of P chemical fixation in all types of soil.

5. Conclusions

The application of CB bio-fertilizer to an environment of high salinity condition appeared to be beneficial to growth and development as well as to the physiological processes of the common bean. The integrative CB + AsA + GSH application was most effective compared to using CB singly or in combination with AsA or GSH. This effective integrative CB + AsA + GSH treatment had enhanced the level of physio-biochemical and antioxidant defense systems and increased salt stress tolerance in common bean plants. Therefore, the integrative CB + AsA + GSH treatment has been concluded to be a useful strategy for enhancing the growth and increasing the yield of the common bean plants when grown under salt affected soils.

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