

Mitigation of Salinity Stress Effects on Growth, Physio-Chemical Parameters and Yield of Snapbean (*Phaseolus vulgaris* L.) by Exogenous Application of Glycine Betaine

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Abstract. Pots experiment was carried out during season 2017 at greenhouse of the Agric. Bot. Dep., Fac. of Agric., Zagazig Univ., Egypt to evaluate the effect of glycine betaine (GB) application under salinity stress (50 and 100 mM NaCl) on growth, physio-chemical analysis and yield of snap bean cv. Bronco. A complete randomized blocks design was used in this search with three replications. Growth parameters, chlorophyll content and green pod yield were significantly decreased with subjecting plants to NaCl. However foliar application of GB detoxified the stress generated by NaCl and significantly improved the above mentioned parameters. Salinity stress increased the electrolyte leakage (EL) and decreased membrane stability index (MSI) and relative water content (RWC). While foliar application of GB was improved MSI and RWC and minimized EL. Proline content and antioxidant enzymes significantly increased in the response to NaCl stress as well as GB application.

1. Introduction

Salinity stress is one of major environmental problems that can effect directly or indirectly on crop production. Many of crop plants is sensitively to salt stress and unable to tolerate low level of salinity. Irrigation with low quality water is one of main reason which accumulate dissolved salts in the soil which inhibit plant growth and physiological process by inhibition metabolism and root-shoot length. Salinity reduce the ability of plants to advantage of water and cause reduction in plant growth and yield by inhibition plant metabolism [1]. Plant metabolism may changes with salinity through osmotic stress and toxicity of ions, while salinity inhibition plant growth by high production of reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydroxyl radical (OH^\cdot) and hydrogen peroxide (H_2O_2) [2]. ROS defined as byproduct...of ordinary cellular metabolism and important for reaction of enzymatic of inter- and intra cellular signaling when plants exposed to salinity stress [3]. Plants grown under saline condition basically stressed by three ways; depress water potential in soil which caused water deficit, phytotoxicity of Na^+ and Cl^- ions and imbalance of nutrient by reduction in uptake and/or shoot transport [4]. Plant physiology affected by salt stress, both at whole plant as well as cellular levels, through ionic and osmotic stress. It obstructs water relations of plant which resulting in an osmotic stress or in drought physiology [1,5,6]. Accumulations of salt in the leaf apoplast up to toxic cause turgor loss and dehydration which lead to death of tissues and cells. Photosynthesis is the most processes harshly affected by salt stress [7], which lead to reduction in chlorophyll pigment [5] stomata closure, thereby, diminishing the CO_2 pressure [8] and inhibition of rubisco [9].

Glycine betaine (GB) is an osmoprotectant which produced in plant to defending abiotic stress condition. GB may protect function of membrane for high concentration of Na^+ and Cl^- [10]. In response to stress, plants synthesized endogenously some of osmolytes such as proline, soluble sugars, polyols and amino acids and glycine betaine (GB) to ameliorate the adverse effect caused by salinity [11]. This osmotic adjustment maintains sub-cellular structures and reduces the oxidative damages caused by ROS under high salinities [12]. GB endogenous biosynthesis is reported to be stress-inducible, and the level of biosynthesized and accumulated GB depends mainly on the degree of salt tolerance [13]. In this concern, plants unable to accumulate GB can tolerate salt stress by exogenous supplements of GB [14], because GB maintains osmotic regulation in cells of salt-stressed

plants [15] and supports different transporters for normal functioning. In addition, application GB may enhance tolerance of plant through its role in ion balance and Na^+ / K^+ discrimination under salt stress condition [16]. The natural accumulation of GB is not enough to improve osmotic stress caused by abiotic stress such as salinity stress [13]. Exogenous application of GB may be ameliorate the bad effect of salt stress in low GB accumulation in maize plants [17]. Positive results of foliar application of GB on growth of plants grown under salt stress condition have been investigated in *Phaseolus vulgaris* [18] and wheat [19].

Snap bean (*Phaseolus vulgaris* L.) is a standout amongst the most vital Fabaceae vegetable crops. Beans are important legumes food due to their amplitude to produced significant amount of protein rich seed for human nutrition [20]. Snap bean plants classified as sensitive to salinity stress [21]. Approximately, 5-10% of production bean in Latin America and 20-30% in Middle East is affected by salinity stress [22].

Therefore, the aim of this study was to determine the effect of glycine betaine application on growth, physio-chemical analysis and yield of snap beans grown in salinity stress condition.

2. Materials and Methods

2.1. Plant material and stress application:

The snap bean (*Phaseolus vulgaris* L. cv. Bronco) seeds gotten from Vegetative Research Section, Horticulture Research Institute, Agriculture Research Centre, Giza, Egypt. This selection of Bronco cv. it's the most common bean cultivar in Egypt sensitive to salt stress. Seeds were surface disinfected with 0.1% HgCl_2 and were washed with deionized water. Seeds were sown on 25th September 2017 in 20 cm diameter pots filled with 8 kg acid washed sand soil and moistened with distilled water.

Two plants for each pot were exposed to control condition (0 mM NaCl) or treatments (GB, 50 mM NaCl, 100mM NaCl, 50 mM NaCl+ GB and 100 mM NaCl +GB). Saline treatment of each pot was supplied with 600 ml of 50, 100 mM NaCl with nutrient Hoaglant solution. NaCl solution was supplied once week while the irrigation applied twice a week.

Treatments were arranged in complete randomized blocks design with three replicates. Each replicate contain 6 pots in greenhouse of the Agricultural Botany Department, Faculty of Agriculture, Zagazig University, Sharkia Governorate, Egypt. Plants were sampled for chemical analysis at 45 day after sowing (DAS) and were growing until green yield.

2.2. Application of Glycine Betaine:

Snap bean plants subjected to one concentration of Glycine Betaine. The GB (98%) perchloric acid titration, (carboxymethyl) trimethyl ammonium inner salt, Oxyneurine, $\text{C}_5\text{H}_{11}\text{NO}_2$, MW: 117.15, were purchased from Sigma Aldrich Co. Glycine Betaine was applied in concentration 200 ppm. solution of GB was prepared by mixed 0.2 g/l of GB granular powder with distilled water and sprayed over the highest point of the plant covering everywhere throughout the plant's leaves, by utilizing Pressurized Spray Bottle with 0.1% Tween 20 as surface spreader and applied for three times on the plants at 15, 25 and 35 days after sowing.

Estimation of plant growth and green yield:

Three pots from each replicate were randomly taken at 45 days after sowing, plants were removed along with the sand and were put in a bucket filled with water. The sand particles were smoothly moved from the plants and measured root and shoot length. The plants were put in an oven at 70°C for 48h till constant dry weight was reached. Green pods of each treatment were harvested at proper maturing stage, counted and weighted.

Estimation physio-chemical constituents:

Chlorophyll pigments were extracted using 0.1 g fresh leaf by pure acetone according to Fadeel's method [23] and pigments contents were calculated using the formula adapted by [24]. Malondialdehyde (MDA) was determined out according to [25]. To determine accumulation of

proline we used method described by [26]. The method described by [27] used to estimate the water use efficiency (WUE) values. The membrane stability index (MSI) was determined by [5]. [28] method was used to estimate the total inorganic ions and electrolyte leakage (EL) in the leaves. The method described by [29] was used to estimate relative water content (RWC).

The concentrations of N, P, K and Na were estimated as follows: digested 0.2 g of dried leaf with sulphuric acid in the presence of H₂O₂ [30]. Total Na and K concentrations were measured directly using Flame photometer [31]. Total nitrogen determined using a microkjeldahl method according to [32]. Total phosphorus was determined colourmetrically using ascorbic acid method [33].

Determination of antioxidants activities:

The enzyme extraction was done by the protocol revealed by [34]. Catalase (CAT) was measured spectro-photo-chemically according to [35]. [36] Method was used to estimate the activity of peroxidase (POD) in bean leaves. Ascorbate peroxidase (APX) was measured spectro-photo-chemically according to [37]. Superoxide dimutase (SOD) activity was determine by recording the decrease in absorbance of superoxide-nitro blue tetrazolium complex by the enzyme [38].

Statistical analysis:

Statistically significant differences between means were compared at $P \leq 0.05$ using Duncan's Multiple Range Test. The statistical analysis was carried out using COSTAT computer software (CoHort Software version 6.303, Berkeley, CA, USA).

3. Results

3.1. Effect of GB on plant growth and productivity under NaCl salinity stress:

Data in Table 1 indicated that significant decreased in plant growth parameters (root length, shoot length and plant dry weight) and yield (No. of pod pot⁻¹ and green pod yield pot⁻¹) reflected the increased NaCl toxicity by increasing salt concentration. However, foliar spray with GB promoted growth parameters and yield. The interaction between GB application and NaCl concentrations (50 and 100 mM) ameliorative the bad effects of NaCl salinity and kept growth parameters and yield of bean plants less than the control.

Table 1. Effect of foliar application with glycine betaine on growth traits and yield of snap bean plants grown under NaCl salinity stress conditions during season 2017

NaCl (mM)	Glycine betaine (%)	Shoot length (cm)	Root length (cm)	Plant dry weight (g)	No of pod /pot	Green pod yield /pod
0.00	0.00	29.33 a	22.33 a	7.75 a	13.33 a	49.18 a
	0.02	29.67 a	23.07 a	8.08 a	14.67 a	52.50 a
50	0.00	20.08 c	14.99 c	4.74 c	6.33 c	30.03 c
	0.02	25.50 b	18.47 b	6.41 b	9.00 b	38.63 b
100	0.00	13.50 e	9.09 d	2.23 c	3.33 e	12.09 e
	0.02	18.27 d	14.11 c	3.92 b	5.00 d	24.48 d

Data in the interaction analyzed with Least Squares Means and means separated with Least significant difference.

The same letters in each column indicate not significant differences according to the LSD test ($P \leq 0.05$).

3.2. Effect of GB on leaf pigments, malondialhyde and free proline under NaCl salinity stress:

From data that illustrated in (Table 2), photosynthetic pigments Chl. a, b, and Carotenoids concentration in leaf tissues of bean plants were significantly decreased by increasing NaCl concentration, also the content of free proline and MDA were significantly increased. Maximum proline quantity was found in plants which were subjected to high concentration of NaCl (100mM)

in combined with GB application. Exogenous application of GB was ameliorative the undesirable effects of NaCl salinity. It significantly increased leaf pigments and proline however decreased MDA.

Table 2. Effect of foliar application with glycine betaine on photosynthetic pigments, malondialdehyde (MDA) and proline of snap bean plants grown under NaCl salinity stress conditions during season 2017

NaCl (mM)	Glycine betaine (%)	chlorophyll a (mg·g ⁻¹ FW)	chlorophyll b (mg·g ⁻¹ FW)	Carotenoids (mg·g ⁻¹ FW)	MDA (μmol·g ⁻¹ FW)	Proline (μg·g ⁻¹ DW)
0.00	0.00	1.22 b	0.62 a	1.10 a	0.69 c	13.43 e
	0.02	1.35 a	0.64 a	1.12 a	0.70 c	14.20 e
50	0.00	0.96 c	0.49 c	0.84 c	2.03 b	23.90 d
	0.02	1.01 c	0.54 b	0.89 b	1.69 b	27.80 c
100	0.00	0.84 d	0.40 e	0.75 e	2.63 a	34.33 b
	0.02	0.89 cd	0.45 d	0.78 d	1.87 b	38.50 a

Data in the interaction analyzed with Least Squares Means and means separated with Least significant difference.

The same letters in each column indicate not significant differences according to the LSD test ($P \leq 0.05$).

3.3. Effect of GB on water use efficiency (WUE), membrane stability index (MSI), electrolyte leakage (EL) and relative water content (RWC) under NaCl salinity stress:

The presence of NaCl significantly reduced WUE, MSI and RWC by increasing NaCl concentration, while electrolyte leakage (EL percentage) was increased (Table 3). The maximum percentage of EL was found in plants subjected to 100 mM NaCl. While, treated of stressed plants with GB decreased the ionic leakage but kept it less than control plant. Sprayed plant with GB in the absence of NaCl stress enhanced WUE, MSI and RWC with no significant differences with control, while LE (%) was reduced with no significant differences with control. GB improved WUE, MSI and RWC under low concentration of NaCl and the values were in positive status than those plants grown under salinity alone.

Table 3. Effect of foliar application with glycine betaine on water use efficiency (WUE), membrane stability index (MSI), electrolyte leakage (EL) and relative water content (RWC) of snap bean plants grown under NaCl salinity stress conditions during season 2017

NaCl (mM)	Glycine betaine (%)	WUE	MSI (%)	EL (%)	RWC (%)
0.00	0.00	0.96 a	68.00 a	8.35 e	93.27 a
	0.02	0.97 a	69.80 a	8.13 e	94.27 a
50	0.00	0.62 c	48.43 c	10.48 c	71.17 c
	0.02	0.75 b	59.30 b	9.69 d	79.97 b
100	0.00	0.23 e	31.54 e	11.94 a	54.30 e
	0.02	0.35 d	41.03 d	10.91 b	63.93 d

Data in the interaction analyzed with Least Squares Means and means separated with Least significant difference.

The same letters in each column indicate not significant differences according to the LSD test ($P \leq 0.05$).

3.4. Effect of GB on leaf mineral concentration and K⁺/Na⁺ ratio under NaCl salinity stress:

Mineral nutrient concentration in snap bean leaves as function of NaCl and exogenous GB application are showed in (Table 4). NaCl levels and foliar spray with GB interacted with each other to bring significant changes in N, P, K and Na concentration. Na concentration was significantly increased in bean leaves in the presence of NaCl stress. However, plants which treated with GB had significant lower Na (%) compared to untreated plants under salinity stress conditions. Under saline levels there were reduction in N, P and K percentage. Application GB enhanced N, P and K concentrations under salinity stress conditions. Under salt stress condition K⁺/Na⁺ ratio was

significantly decreased, while application of GB individually or in combination with salt stress enhanced K^+/Na^+ ratio as compared to untreated plants.

Table 4. Effect of foliar application with glycine betaine on mineral concentration and K^+/Na^+ ratio of leaves of snap bean plants grown under NaCl salinity stress conditions during season 2017

NaCl (mM)	Glycine betaine (%)	N (%)	P (%)	K (%)	Na (%)	K^+/Na^+
0.00	0.00	1.78 a	0.48 a	1.87 b	0.54 e	3.48 b
	0.02	1.88 a	0.49 a	1.98 a	0.53 e	3.76 a
50	0.00	1.50 c	0.33 c	1.64 d	1.21 c	1.35 d
	0.02	1.64 b	0.41 b	1.75 c	1.07 d	1.63 c
100	0.00	1.25 d	0.22 e	1.45 e	1.58 a	0.92 d
	0.02	1.39 c	0.28 d	1.58 d	1.39 b	1.13 d

Data in the interaction analyzed with Least Squares Means and means separated with Least significant difference.

The same letters in each column indicate not significant differences according to the LSD test ($P \leq 0.05$).

3.5. Effect of GB on antioxidant enzyme under NaCl salinity stress:

Data in Table 5 showed that CAT, POX, APX and SOD activities were significant increased by increasing salinity stress compared to control plants. Application of GB individually increased insignificantly antioxidant enzymes as compared to untreated one. However, the maximum activities of antioxidant enzymes were recorded under the combination of 100 mM NaCl with GB followed by saline treatment (100 mM). The increase in antioxidant enzymes helps bean plants to overcome the undesirable effects of NaCl salinity stress.

Table 5. Effect of foliar application with glycine betaine on activities of catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX) and superoxide dismutase (SOD) of snap bean plants grown under NaCl salinity stress conditions during season 2017

NaCl (mM)	Glycine betaine (%)	CAT (A564 $\text{min}^{-1} \cdot \text{g}^{-1}$ protein)	POX (A564 $\text{min}^{-1} \cdot \text{g}^{-1}$ protein)	APX (A564 $\text{min}^{-1} \cdot \text{g}^{-1}$ protein)	SOD (A564 $\text{min}^{-1} \cdot \text{g}^{-1}$ protein)
0.00	0.00	60.20 e	0.64 d	50.33 e	3.25 e
	0.02	61.33 e	0.66 d	51.07 e	3.55 e
50	0.00	73.60 d	1.29 c	69.57 d	6.39 d
	0.02	79.67 c	1.39 b	73.20 c	7.23 c
100	0.00	82.33 b	1.37 b	82.53 b	7.82 b
	0.02	88.20 a	1.50 a	89.83 a	8.74 a

Data in the interaction analyzed with Least Squares Means and means separated with Least significant difference.

The same letters in each column indicate not significant differences according to the LSD test ($P \leq 0.05$).

4. Discussion

Salinity stress is one of the major environmental problems affecting agriculture crop production all over the world. Different estimates indicated that 7% of the total land surface, proximally, 1000 million hectare of the earth is saline [39]. The salinity problem is critical especially in arid and semiarid regions. In the arid and semiarid regions, poor irrigation water which contain many salts caused salinity by accumulation of those salts on the soil surface. Also, high evaporation rate, low rainfall and poor water management cause saline problem in this area [20]. In this study, NaCl salinity stress at (50 and 100 mM) significantly decrease growth parameters of bean plants i.e., root and shoot length and plant dry weight as well as pods yield (Table 1).

A large amount of investigation suggested that increase accumulation level of GB in plants enhanced stress tolerance [40]. However, function of GB relatively only high levels of GB in some

plants under stress condition. It has been showed that the accumulation and/or synthesis of GB in many plants is comparatively lower under salt stress [41]. In the present study, exogenous application of GB overcome the unfavorable effects of salt stress and may be practical access to enhance salt tolerance at bean plants.

Plants subjected to GB were shown healthy and dark green compared to untreated plants. When exposed to salt stress, plant growth parameters has been decreased, this may be attributed to the inhibition of available water absorption, exaggerated accumulation of Na^+ or Cl^- , decreased in essential nutrient uptake and toxic intermediate components such as ROS [42], roots and shoots length and plant dry weight were higher in GB treatment under salt stress conditions than untreated plants may be due to that GB alleviated the undesirable effect of salt stress on growth parameters of bean plants. Exogenous application of GB to plants, which accumulated little or none of these compounds may help to decrease the adverse effect of salinity stress [17, 43].

In our study salinity stress significantly decreased bean growth as well as green yield was noted in (Table1). The results are in agreement with those found by [44, 45, 46] with a lot of plant species. The toxic effect of NaCl salinity stress negatively affected plant productivity by increasing accumulation of Na^+ and Cl^- in bean plants [20, 47]. However, exogenous application of GB increased significantly green yield under salinity stress condition. Those results in agreement with those obtained by [48, 49] on tobacco and sunflower, respectively.

Chlorophyll content was higher in plants which treated with GB under salt stress than untreated plants. This result proposed that GB may assume an explicit job in managing the salt stress prompted senescence in leaves [50]. Also, [51] reported that foliar application with GB increase chlorophyll content under salt stress. Although other reports indicated that application of GB enhanced ameliorative of chlorophyll content under salt stress by decreased activity of chlorophyllase which degrading chlorophyll [52]. decreased toxic ion, such as Na^+ content, increased magnesium content which needed for chlorophyll synthesis, and increased K^+ content [53] increased cytokinin concentration, which inhibits degradation of chlorophyll [54].

In present study, lipid peroxidation was estimated as malondialdehyde (MDA) content, this being a biochemical pointer of stress, where it hinders the generation of biomass and decline conceivable outcomes of plant adjustment to push [55]. MDA Content in 50 and 100 mM NaCl treatments were higher than its content of other treatments (Table 2), a reality clarified by the high receptive oxygen species (ROS) concentrations created in this treatment. This could decidedly impact at the biomass of the plants which subjected to 50 and 100 mM NaCl combined with GB.

Membrane lipid peroxidation and electrolyte leakage in higher plants are commonly indicated by free radical which induced membrane damage or retrogradation when plants are subjected to environmental stresses [56]. Our study showed that exogenous GB decreased the EL and MDA in salt-stressed leaves of bean. These results suggested that GB mitigated lipid peroxidation and facilitated the maintenance of membrane functions under salt stress conditions. This facilitation could be referred to the GB stimulate the responses of antioxidant which protect plant from oxidative damage [57, 58].

Proline additionally modified in plants exposed to salinity it adds to osmotic adjustment. Plants for the most part accumulate suitable osmolytes, for example, proline and GB because of salt stress [44]. Improving accumulation of proline in plants grown under saltiness stress might be an adjustment to make up for the vitality for growth and survival and in this way enable the plant to endure stress [59]. A more elevated amount of proline accumulation coming about because of GB application under saltiness has been found in tomato [60] and rice [50] under ordinary conditions accumulation of cell proline has been found from around 5 percent of the amino acid pool, while under stress conditions accumulation of Cellular proline has been found up to 20– 80 percent this may increased synthesis and decreased degradation in many plant species [61]. The mechanisms which free proline decreases ROS harm and expanding plant resistance are that proline decreases NaCl-saltiness stress by detoxification of ROS created because of NaCl harming. Free proline may physically extinguish singlet oxygen or respond specifically with hydroxyl radicals [62].

Salinity stress decreased water relations such as relative water content and water use efficiency while, exogenous application of GB improve these items. Water status is highly sensitive to salinity and therefore prevalent at responses of the plant to stress [63]. It is well known that salinity stress decreased hydraulic conductivity of root, resulting in reduced water flow from roots to shoots, even in osmotically adjusted plants [64]. Photosynthesis is coupled with transpiration rate of plants, and the transpiration inhibition is a reliable and quick measure of toxic effects of salt [65, 66]. The reduced in RWC in plants grown under salt stress proposed that NaCl diminished translocation of water from roots to shoots and caused a toxic impact on the development of bean. The decline in water translocation coming about because of saltiness stress may cause lessen in leaf water content, which would result in conclusion of stomatal to safeguard their water status [64]. Our outcomes demonstrated that GB application expanded RWC in salt-stressed plants. This proposed GB could expand the water translocation from roots to shoots by expanding hydraulic conductivity lastly increment the RWC and transpirations rate under salt stress [64].

Increased salt focus in the region of the root system can meddle with mineral nourishment of plants and limit vegetable yield because of saltiness or osmotic value of the soil solution. Availability of nutrient to plant affected by salt stress from numerous points. It modifies binding, maintenance and change of soil nutrients and influences the nutrients absorption or uptake by roots because of ions antagonism and decreased growth of root. Plant nutrients metabolism disrupted by salinity, fundamentally through water stress, thus decreasing the utilization efficiency of nutrients. Many symptoms existing in some species-specific such as necrosis and burns of leaf edges in the presence of high concentrations of salts that may due to the accumulation of some ions such as Na^+ and Cl^- [67]. Plants mineral nutrition based on the activity of membrane transporters, which take an interest in the ions transport from the soil into the plant and direct their conveyance inside and between cells [4, 68, 69]. At least Changes in membranes may lead to turmoil in cells chemical composition and can therefore be appear as symptoms of some essential elements deficiency, similarly as it happens in the absence of salts [70]. High concentrations of salts negatively affect the absorption of other elements such as N, P, K^+ , Ca^+ [70, 71].

High concentrations of salts caused Na^+ accumulation in plant tissues and a large potassium efflux from plant tissues [72], which influences cytosolic K^+ homeostasis and plant survival [73]. Therefore, maintenance high concentrations of cytosolic K and appropriate Na^+/K^+ balance may be considered the most fundamental salt tolerance mechanisms in plants Therefore, maintenance of high [58]. In the present study bean stressed plants accumulated more Na^+ and less K^+ and have higher Na^+/K^+ ratio than control plants this might be the aftereffect of conceivable hostility among K^+ and Na^+ [74]. On the other hand exogenous application of GB improved K^+ concentration and decreased Na^+ concentration compared to plants grown under salinity stress without GB application. These outcomes showed that GB may assume a job in keeping up cytosolic K^+ homeostasis by suppressing Na^+ upgraded apoplastic stream to lessen Na^+ uptake [75] what's more, moderating NaCl prompted K^+ efflux from the cell [58]. In this respect application of GB reducing accumulation of Na^+ and alleviation of NaCl-induced K^+ loss on plants grown under salinity stress as reported in tomato leaves [60], rice seedlings [76], and barley [77].

Mitigating the effect of application of glycine betaine on salinity stress is viewed as associated with scavenging free radicals and in protecting antioxidant enzymes [78]. Our results indicated that, salt stress caused increased the activity of SOD also, foliar application of GB improved its activity. These outcomes suggested that GB-improved in SOD activity may have protected membrane stability from saltiness prompted oxidative harm. This is affirmed by a past study by [79] who indicated that application GB increased the activity of SOD in wheat stressed plants.

In addition to SOD, a more elevated amount of security against oxidative harm ought to require quick expulsion of H_2O_2 by other scavenging systems, thus decreased H_2O_2 toxicity and the formation of the highly toxic OH^- [2]. CAT, POX and APX are the major H_2O_2 scavenging enzymes in plants, which are involved in the degradation of H_2O_2 into water and oxygen [80]. Ou results indicated that antioxidant enzymes activity (i.e. CAT, POX and APX) increased in plants under NaCl salinity stress while application of GB led to more increased in antioxidant enzymes activity under

NaCl salinity stress (Table 5). These results agreed well with those of [50] who found that GB increased antioxidant enzymes under salt stress. These outcomes recommended that exogenous GB could contribute to detoxifying H₂O₂ by enhancing antioxidant enzymes activity under salt stress.

As the best treatment, GB was beneficial in mitigating the deleterious effects of salinity stress on snap bean plants (Tables 1–5). Agreeing with our results, many studies have reported a significant improvement of plant growth by GB application under salinity stress conditions in many crop plants [50, 51, 56, 63, 75]. In the present study, the growth and yield improvements by GB was coincided with the maintenance of higher leaf photosynthetic pigments. The increase in these parameters of salt stressed snap bean plants with GB application, could be attributed to its ability to modify cell wall metabolism by improving tissue extensibility and enhancing cell enlargement. Another probable mechanism of GB is the increase of leaf rigidity by making it rougher in texture [11]. GB enhanced the osmotic pressure in plants under elevated salt and control conditions [13]. The increased yield by GB application under salt stress might be attributed to the available water by GB in plant tissue, leading to an increase in nutrients solubility in tissue cell to maximize metabolic mechanisms as possible and synthesize more metabolites/photosynthates for snap bean [11]. However, GB supplementation to salt stressed plants exhibited improved RWC due to water retention in plant tissues. The improved RWC in plants as a result of exogenous GB application was also observed by other researchers [81]. Results of this study showed that exogenous GB application increased the activities of CAT, POX, SOD and APX in snap bean leaves under salt stress. Exogenous GB application alleviated the adverse effects of salinity, most likely by scavenging ROS and protecting enzymatic antioxidants [78, 82] have suggested that GB overcome the adverse influences of oxidative stress by activating or stabilizing ROS scavenging enzymatic antioxidants and/or suppressing the ROS production by a mechanism that is still unknown. [83] have shown that exogenous GB application improves CAT activity in a salt sensitive rice cultivar under salinity stress. APX activity was also increased by GB application in plants subjected to salt stress, indicating H₂O₂ scavenging role of GB [81].

5. Conclusion

This study suggests that exogenous application of GB enhanced the response of bean plants to 50 and 100 mM NaCl salinity stress. The valuable impact of exogenous use of GB to plants under saltiness stress might be ascribed to its protective peroxidation-linked membrane deterioration and scavenging free radicals and the support of K⁺ homeostasis by preventing NaCl-induced K⁺ misfortune. In addition, GB activates the antioxidant system under normal and saline condition.

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