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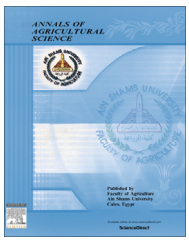

Influence of exogenous application of some phytoprotectants on growth, yield and pod quality of snap bean under NaCl salinity

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Abstract Snap bean is a salt sensitive plant and suffers from losses in yield and pod quality with any little increase of salt concentration in irrigation water. In order to study the effect of salicylic acid (SA), spermidine (Spd), and glycine betaine (GB) as phytoprotectants on enhancing growth, yield and pod quality of snap bean under different levels of NaCl salinity, an outdoor pot experiment was conducted in 2012 and 2013 seasons. Salinity was applied as NaCl form at 0 and 2000 ppm. The concentrations of foliar treatments were two levels for each treatment; the first level was 0 mM which served as control, and the second level was 1 mM SA, 0.5 mM Spd and 5 mM GB, in addition to their combinations. NaCl salinity at 2000 ppm reduced most of vegetative growth parameters such as plant f.w., leaf area ratio and leaf area index, which in turn reflected on the reduction of pods no./plant and yield f.w./plant and an increase in the fruit abscission percentage. Pod moisture % decreased under 2000 ppm NaCl which reduced pod f.w., and increased pod curvature %. Under 2000 ppm NaCl, GB at 5 mM and all its combinations increased membrane stability index, total soluble sugars and total soluble proteins concentration, while reducing free amino acids concentration, which were concomitant with decreasing pod curvature %. Meanwhile, application of SA at 1 mM, GB at 5 mM, and GB5 + SA1 + Spd0.5 increased leaves and pods no./plant, pod moisture %, and pod f.w., which reflected on increasing green pod yield.

Introduction

Snap bean (*Phaseolus vulgaris* L.) is one of the most important vegetable crops, and is classified as a salt sensitive plant and suffers from growth and yield loss between 10% and 50% at salinity level from 1 to 3 dSm^{-1} (Maas and Hoffman, 1977). There was a 85% growth reduction on a dry weight basis of bean plants subjected to 96 mM NaCl (Wignarajah, 1990). About 20–30% of the bean-production areas in the Middle East including Egypt are affected by soil salinity, which could be caused by (1) poor irrigation water which contains considerable amounts of salts that accumulate in the soil surface layer, (2) poor drainage, (3) poor water management, and (4)

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low rainfall and high evaporation rate, which led to capillarity rise of salts from underground water into the root zone (Bayuelo-Jiménez et al., 2002).

Salinity affects almost every aspect of the physiology and biochemistry of plants and leads to water deficit, and causes ion imbalance of the cellular ions resulting in osmotic stress and ion toxicity which, significantly reduces membrane permeability, plant growth and yield (Tuteja et al., 2012). The osmotic stress is associated with lack of cell wall extension and expansion leading to cessation of the growth. The ionic effect includes interference with nutrient imbalance and lowering the net photosynthetic rates in the affected plants (Khadri et al., 2007). NaCl salinity causes reduction in carbohydrates supplied by photosynthesis that are important for cell growth, which reflected on restriction in water availability and imbalance in nutrients uptake by plants (Tuteja et al., 2012). Salinity reduces the ability of plants to utilize water and causes not only a reduction in growth rate but also changes in plant metabolic processes (Munns, 2002). Common bean is known to exclude Na^+ from the shoot by re-absorption of Na^+ from the xylem, and translocate Cl^- to leaves. High leaf Cl^- concentrations reduce growth by altering the nutritional balance of the plant, affecting CO_2 assimilation, and altering water relations (Bayuelo-Jiménez et al., 2003). The Cl^- concentration of leaf tissue increased linearly with increasing external NaCl concentration. Activity of Rubisco enzyme was decreased by up to 40% at high leaf Cl^- concentration (Seemann and Critchley, 1985).

Salt tolerance mechanisms in plants are classified into cellular homeostasis, stress damage control, and growth regulation (Zhu, 2001). As a consequence of salinity stress, tolerant plants often activate cell signaling pathways including those that lead to synthesis of ABA, osmoprotectants active metabolites (amino acids, sugars, GB and polyamines), specific proteins, and certain free radical scavenging enzymes (Manchanda and Garg, 2008).

Glycine betaine is thought to protect the plant by stabilizing macromolecules and by balancing water potential between the plant cell and the environment. GB is mainly localized in chloroplasts and plays a vital role in chloroplast adjustment and protection of thylakoid membranes, thereby maintaining photosynthetic efficiency and plasma membrane integrity (Tuteja et al., 2012). Exogenous application of GB mitigates the adverse effects of environmental stresses in some plant. For example, in common beans, GB-treated plants exhibited a slower decrease in leaf water potential during drought stress and fully alleviated the adverse effects of water deficit on CO_2 absorption and chlorophyll fluorescence, while it had little or no effect on shoot biomass or pods yield (Ashraf and Foolad, 2007). Foliar application of GB on pea plants under drought stress increased number of leaves per plant, pods number per plant and green pods yield. While it has a little effect on total soluble sugars concentration in leaves, it led to an increase in total free amino acids concentration in pea leaves (Osman, 2015).

Salicylic acid is an endogenous growth regulator, actively involved in germination, plant growth, photosynthesis, stomatal conductance, flower induction, fruit ripening, ions uptake and transport (Shakirova, 2007), and protection of plants against multiple environmental stresses such as salinity, freezing, heavy metals, and osmotic stress (Pál et al., 2013). SA affects ethylene biosynthesis and stomatal movement, enhances the level of photosynthetic pigments and photosynthetic rate and modifies the activity of some of the important enzymes as well (Yusuf et al., 2013). Salicylic acid induces activation of protein kinase in tobacco exposed to osmotic stress suggesting its role in anti-stress mechanisms (Ahanger et al., 2014). Exogenous application with SA has been reported to enhance the efficiency of several developmental, physiological, and biochemical processes. It has been reported that exogenous application of SA enhances protection of photosynthetic pigments in barley and the maintenance of membrane integrity (El-Tayeb, 2005), significantly decreased the lipid peroxidation induced by NaCl salinity (Pál et al., 2013).

Polyamines (putrescine, spermidine, and spermine) are low molecular weight nitrogenous compounds found in all plants, and implicated in various developmental processes, as well as responses to various environmental stresses (Yamaguchi et al., 2006; Ahanger et al., 2014). Polyamines are positively charged at physiological pH. This property allows polyamines to interact with negatively charged macromolecules such as DNA, RNA, proteins and phospholipids, which make them involved in the regulation of physiochemical properties of cell membranes, structure and functions of nucleic acids and modulation of enzyme activities. Polyamines are implicated in a wide range of regulatory processes such as promotion of growth, cell division, DNA replication and cell differentiation (Groppa and Benavides, 2007). Polyamines serve as messengers of stress signals. As a result of acid neutralizing and antioxidant capability, polyamines show anti-senescence, anti-stress effects, and membrane and cell wall stabilizing abilities. Exogenous application of polyamines has been suggested as an effective approach for enhancing stress tolerance of crops and crop productivity as well (Ahanger et al., 2014).

Throughout the last few decades, exogenously applied phytoprotectants such as osmoprotectants, polyamines, plant hormones, antioxidants, signaling molecules, and trace elements were used to provide a significant protection in plants subjected to environmental stresses. Yet, the signal transduction pathways and the precise mechanisms of protection are still unclear. The proper dose and interval of treatment of the exogenous protectants and the appropriate methods of application need to be studied more precisely (Hasanuzzaman et al., 2015).

Since snap bean plants are sensitive to salinity stress, so the present study was designed to investigate the potential effects of the exogenously applied salicylic acid, spermidine and glycine betaine and their combinations on enhancing growth, yield quality and maximizing productivity of snap bean plants, exposed to slightly NaCl salinity stress (2000 ppm). This concentration of NaCl has a slightly effect on most plants, but it has a moderate effect on snap bean plants.

Materials and methods

A pot experiment was conducted during the two growing seasons of 2012 and 2013 under outdoor conditions in acid washed sandy soil, at the experimental farm, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, in order to investigate the effect of foliar application with salicylic acid, spermidine and glycine betaine and their combinations as ameliorating compounds on yield quality and productivity of snap bean plants under two levels of NaCl salinity.

Experimental design and treatments

Seeds of snap bean (*P. vulgaris* L.) cv. Bronco were obtained from Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt. Ten seeds were sown on 1st of March during 2012 and 2013 seasons in 15-liter plastic pot filled with 14 kg acid washed sandy soil. The pot dimensions were 30 cm diameter top, 25 cm diameter base and 26 cm depth. Seedlings were thinned to three homogeneous seedlings after 10 days from germination. The seedlings were watered with Hoagland solution (Hoagland and Arnon, 1950).

The concentrations of foliar application treatments were two levels for each treatment; the first level was 0 mM which served as control, and the second level was 1 mM salicylic acid (SA), 0.5 mM spermidine (Spd) and 5 mM glycine betaine (GB), and a mixture of their combinations which revealed below. Plants were sprayed four times with 8-day intervals starting at the growth stage 14 (unfolding of second trifoliate leaf) of BBCH scale which was used to identify the phenological development stages of a plant (Lancashire et al., 1991):

1. Control (sprayed with tap water)
2. SA at 1 mM (SA1)
3. Spd at 0.5 mM (Spd0.5)
4. Spd at 0.5 mM + SA at 1 mM (Spd0.5 + SA1)
5. GB at 5 mM (GB5)
6. GB at 5 mM + SA at 1 mM (GB5 + SA1)
7. GB at 5 mM + Spd at 0.5 mM (GB5 + Spd0.5)
8. GB at 5 mM + SA at 1 mM + Spd at 0.5 mM (GB5 + SA1 + Spd0.5)

For salinity levels, pots were divided into two groups, the first was irrigated with full strength Hoagland nutrient solution to serve as control plants (0 ppm NaCl). The second group was the same plus 2000 ppm NaCl in the nutrient solution. The salinity treatment started at the growth stage 15 of BBCH scale (unfolding of third trifoliate leaf). Treatments were arranged in a complete randomized block design with three replicates.

Vegetative growth characteristics

Plant height, plant fresh weight, number of leaves per plant, leaf area ratio (LAR), and leaf area index (LAI) were recorded at full bloom stage (50 days after sowing). Leaf area ratio and leaf area index were calculated according to the equations of Hunt (1990) as follows:

$$\text{Leaf area ratio} = \frac{\text{Total leaf area per plant}}{\text{Total dry weight per plant}}$$

$$\text{Leaf area index} = \frac{\text{Total leaf area per plant}}{\text{Total ground area per plant}}$$

Total leaf area per plant was determined by Image-pro plus software (version 6.2, Media Cybernetics Inc., USA) using digital images of the plant leaves.

Plant water status measurement

The leaf relative water content (RWC) was determined in the full expanded first and fourth leaf from the top of the plant to study and track plant water status at bloom stage (50 days after sowing) in young and old leaves. Ten leaf blades (disks with 10 mm in diameter) were punched with a borer from a set of leaves into a reweighed sealed vial. After the fresh weight had been obtained, the disks were floated for 6 h in distilled water in covered Petri dishes kept at low light intensities and in a constant temperature room (20 °C), until they became fully turgid. The disks were surface dried, returned to the same vial and reweighed to obtain the turgid weight. Finally, the leaf disks were oven dried at 80 °C to a constant weight (almost 12 hours) and weighed again to obtain the dry weight. The RWC on a percentage basis was calculated using the equation of Schonfeld et al. (1988).

$$\text{RWC } (\%) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Membrane stability index (MSI)

Membrane stability index was estimated according to Sairam et al. (1997). 0.1 g of unblemished full expanded first and fourth leaf from the top of the snap bean plant were cut into disks of uniform size and taken in test tubes containing 10 ml of double distilled water in two sets. One set was subjected to 40 °C for 30 min and its conductivity was recorded (C1) using a conductivity meter (LYS – DRLANGE). Second set was kept in a boiling water bath (100 °C) for 15 min and its conductivity was also recorded (C2).

$$\text{Membrane stability index} = (1 - (C1/C2)) \times 100$$

Flowering and yield components

Number of flowers per plant, total number of pods per plant (marketable and unmarketable pods), fruit abscission percentage, treatment-control fruit set ratio, harvest index, and the green pods yield as fresh weight per plant were calculated as average per plant for each pot. Harvest index (HI) was calculated as the ratio between the dry weight of the marketable pods and plant total dry weight (Hunt, 1990). Fruit abscission percentage was calculated as follows:

$$\text{Fruit abscission } \% = \left(1 - \frac{\text{Total number of pods}}{\text{Total number of flowers}}\right) \times 100$$

Treatment-Control fruit set ratio (Fruit set sharing %): Sharing percentage of the treatment in fruit set over control was calculated by the following equation:

$$\text{Fruit set sharing } \% = \left(\frac{\text{Fruit set } \% \text{ of treatment}}{\text{Fruit set } \% \text{ of control}} - 1\right) \times 100$$

The green pods yield was harvested at the optimum marketable stage of pod growth (50% of pods have reached typical length of the BBCH scale (Lancashire et al., 1991)). The yield was weighed at every harvesting date to obtain yield per plant and average of pod weight per plant. Images of marketable

pods were analyzed by Image-pro plus software (version 6.2, Media Cybernetics Inc., USA) to calculate the average of pod length, pod width and the percentage of pod curvature angle. The pod curvature angle was measured using a protractor tool provided by Image-pro plus software, which was used to calculate the percentage of pod curvature angle as follows:

$$\text{Pod curvature } \% = \left(180 - \frac{\text{Pod curvature angle}}{180}\right) \times 100$$

The descriptive scale of immature pod curvature of *P. vulgaris* by IBPGR (1982) was straight, slightly curved, medium curved, strong curved, and very strong curved, used to indicate to pod quality. This descriptive scale depended on human sense, which was less accurate, for more accuracy, the digital image provided for previous degree of pod curvature was analyzed and calculated using the same method described above. The calculated data were used as a reference scale for pod curvature %, which was 0% for straight, slightly curved = 11%, medium curved = 20%, strong curved = 29%, and very strong curved = 42%.

The percentage of marketable pod moisture content using oven method (A.O.A.C., 2005) was calculated as follows:

$$\text{Pod moisture } \% = \left(\frac{\text{Pod f.w.} - \text{Pod d.w.}}{\text{Pod f.w.}}\right) \times 100$$

Biochemical analyses

Leaf and pod samples were collected at 60 days after sowing to determine total free amino acids, total soluble protein, and total soluble sugars. Total soluble sugars and total free amino acids were extracted from 1 g leaf and pod tissues separately by 80% hot ethanol by the modified method of Irigoyen et al. (1992) and Katoch (2011) respectively. The homogenate was centrifuged at 10,000 rpm for 10 min, and the supernatant was collected. The pellet was re-extracted twice with 3 ml of 80% ethanol, then vortexed and centrifuged. The supernatants were combined and stored at −20 °C until free amino acids and total soluble sugar concentration determination. Total free amino acids were determined according to the method described by Swamy (2008). The pink color developed was measured using a spectrophotometer (Mapada UV 1200) at 570 nm. The total soluble sugars in the ethanol-soluble fractions were determined by the method of Sadasivam and Manickam (2010). After sample vacuum dried, dissolved in deionized water, deproteinized, and centrifugation at 10,000 rpm for 5 min, 1 ml of supernatant was reacted with 4 ml freshly prepared anthrone reagent (100 mg anthrone + 50 ml 95% H_2SO_4) at 100 °C for 10 min. After cooling on ice, the total soluble sugar concentration was determined at 620 nm by a spectrophotometer using glucose as standard. Total soluble protein was determined in leaf and seed extracts using the method of Bradford (1976).

Statistical analysis

Data of the two seasons were arranged and statistically analyzed using CoStat software (version 6.4, CoHort Software, USA) according to the method described by Gomez and Gomez (1984). Two-way analysis of variance (ANOVA) was used to test for significant differences among foliar application substances, salinity and their interactions at $P < 0.05$, followed by Tukey's HSD test. One-way ANOVA was used to reveal significant differences across foliar application substance treatments within individual salinity level while a post hoc Tukey's HSD test was used to test for significant differences between individual treatments means. Spearman correlation coefficients between biochemical constituents of snap bean leaves and pods, RWC, MSI, and different yield attributes under different NaCl salinity levels were calculated using XLSTAT Addinsoft version 2016 (Addinsoft, NY).

Results

Vegetative growth characteristics

Plants subjected to NaCl salinity at 2000 ppm comparing with 0 ppm NaCl had a significant reduction in plant fresh weight, LAR and LAI as overall salinity mean in both seasons and plant height in the first season, whereas leaf number per plant was insignificant (Table 1). Although the significance test between the individual foliar treatments (SA, Spd, and GB) and their combinations for leaf number per plant were insignificant under the two levels of salinity, the individual treatment of GB at 5 mM and GB5 + SA1 + Spd0.5 recorded the highest values comparing with other treatments under the two levels of NaCl salinity, except for SA at 1 mM which recorded the highest value under 2000 ppm salinity and the lowest value under zero salinity (Table 1).

In general, the overall mean of foliar treatments under NaCl salinity levels showed that the vegetative growth of snap bean responded positively to foliar treatments (SA, Spd, GB and their combinations) comparing with control (untreated plants). Maximum significant values in plant height were for the applications of Spd0.5 + SA1, GB and its combinations. The highest significant values in plant fresh weight were recorded by the application of GB5 + SA1 + Spd0.5 and SA1 respectively. Leaf area ratio and leaf area index recorded the highest mean values (99 and 104 for LAR; 2.2 and 1.7 for LAI in the 2nd season) by foliar application of GB5 + SA1 + Spd0.5 and Spd0.5 respectively under 2000 ppm NaCl salinity. This increase in LAR under the treatment with GB5 + SA1 + Spd0.5 (99 cm^2/g in the 2nd season) is referred mainly to the increase in its value under 2000 ppm NaCl (115 cm^2/g in the 2nd season), comparing with its value under 0 ppm NaCl (84 cm^2/g), where plants responded differentially under the different levels of salinity. On the other hand, the highest mean value of Spd at 0.5 mM under NaCl salinity levels (104 cm^2/g in the 2nd season) is raised mainly to the increase in its value under 0 ppm NaCl which recorded 108 cm^2/g in the 2nd season (Table 1). This observation indicated that Spd at 0.5 mM has a positive effect on vegetative growth only under non-stressed condition.

Plant water status and membrane stability index

Salinity affected membrane stability index more than relative water content as presented by overall salinity mean of MSI and RWC for first and fourth leaf (Table 2). The MSI in fourth leaf from the top of the plant in 2000 ppm NaCl showed the highest values (72.8% and 70.4% in both seasons) comparing with plants grown in zero NaCl salinity (67.1% and 65% in

Table 1 Influence of salicylic acid (SA), spermidine (Spd), glycine betaine (GB) and their combinations as foliar application under different levels of NaCl salinity (0 and 2000 ppm) on vegetative growth parameters of snap bean plant in both seasons (2012 and 2013).

Foliar treatments (mM)	Plant height (cm)	Plant f.w. (g)	Leaf no./plant	Leaf area ratio (cm^2/g)	Leaf area index	Plant height (cm)	Plant f.w. (g)	Leaf no./plant	Leaf area ratio (cm^2/g)	Leaf area index
	1st season					2nd season				
NaCl at 0 ppm										
Control	14.5 d	14.5 b	7.0 a	98 b	1.4 a	13.1 c	13.6 b	6.6 a	96 b	1.3 c
SA1	15.2 cd	21.5 ab	6.6 a	90 bd	1.6 a	14.0 c	21.1 ab	6.2 a	89 cd	1.6 bc
Spd0.5	18.6 bc	20.6 ab	7.4 a	114 a	2.4 a	17.2 b	19.7 ab	6.8 a	108 a	2.2 ab
Spd0.5 + SA1	22.4 a	18.4 ab	8.0 a	95 bc	1.6 a	20.7 a	17.6 b	7.4 a	92 bc	1.4 c
GB5	20.0 ab	16.3 b	9.8 a	113 a	2.6 a	19.0 ab	15.8 b	9.0 a	104 a	2.3 a
GB5 + SA1	19.6 ab	16.0 b	7.6 a	65 e	1.5 a	18.6 ab	15.3 b	7.0 a	62 e	1.3 c
GB5 + Spd0.5	20.5 ab	24.4 ab	8.8 a	88 cd	1.9 a	19.6 ab	23.1 ab	8.4 a	87 cd	1.8 ac
GB5 + SA1 + Spd0.5	18.7 ac	28.6 a	9.4 a	86 d	2.4 a	17.5 b	27.7 a	9.0 a	84 d	2.3 ab
Mean	18.6 A	20.0 A	8.1 A	93.5 A	1.9 A	17.5 A	19.2 A	7.6 A	90.3 A	1.7 A
NaCl at 2000 ppm										
Control	14.0 d	11.0 a	7.8 a	36 f	0.4 d	13.3 e	9.7 c	7.2 a	34 f	0.4 e
SA1	17.4 c	17.9 a	8.6 a	82 c	1.5 b	16.5 cd	16.4 ab	8.0 a	78 c	1.4 b
Spd0.5	16.5 c	12.7 a	7.4 a	103 b	1.3 b	15.8 d	12.2 bc	7.0 a	99 b	1.2 bc
Spd0.5 + SA1	18.7 b	16.8 a	6.8 a	77 cd	0.8 c	17.7 bc	16.0 ac	6.4 a	73 cd	0.8 d
GB5	18.8 ab	13.0 a	8.2 a	68 de	0.8 c	18.4 ab	12.3 ac	7.8 a	66 d	0.8 d
GB5 + SA1	19.4 ab	14.9 a	7.2 a	57 e	1.2 b	18.8 ab	14.2 ac	7.0 a	57 e	1.1 c
GB5 + Spd0.5	17.0 c	14.2 a	6.6 a	100 b	1.2 b	16.3 cd	13.6 ac	6.2 a	98 b	1.1 c
GB5 + SA1 + Spd0.5	19.8 a	20.0 a	8.0 a	127 a	2.3 a	19.3 a	18.8 a	7.4 a	115 a	2.2 a
Mean	17.7 B	15.0 B	7.6 A	81.2 B	1.0 B	17.0 A	14.2 B	7.1 A	77.5 B	1.0 B
Mean of foliar treatments under NaCl salinity levels										
Control	14.2 d	12.8 c	7.4 a	67 d	0.9 c	13.2 d	11.7 c	6.9 a	65 d	0.9 d
SA1	16.3 c	19.7 ab	7.6 a	86 c	1.5 bc	15.2 c	18.8 ab	7.1 a	84 c	1.5 bc
Spd0.5	17.5 bc	16.7 bc	7.4 a	108 a	1.8 ab	16.5 bc	16.0 bc	6.9 a	104 a	1.7 ab
Spd0.5 + SA1	20.5 a	17.6 ac	7.4 a	86 c	1.2 bc	19.2 a	16.8 bc	6.9 a	82 c	1.1 cd
GB5	19.4 ab	14.7 bc	9.0 a	90 bc	1.7 ab	18.7 a	14.1 bc	8.4 a	85 c	1.5 bc
GB5 + SA1	19.5 a	15.4 bc	7.4 a	61 d	1.4 bc	18.7 a	14.8 bc	7.0 a	60 e	1.2 bd
GB5 + Spd0.5	18.7 ab	19.3 ac	7.7 a	94 b	1.6 bc	18.0 ab	18.3 ab	7.3 a	93 b	1.5 bc
GB5 + SA1 + Spd0.5	19.2 ab	24.3 a	8.7 a	106 a	2.3 a	18.4 a	23.2 a	8.2 a	99 a	2.2 a

Means followed by different letters are significantly different at $P < 0.05$ level; Tukey's HSD test. Where f.w. = fresh weight. Capital letters for mean of NaCl salinity level, whereas lowercase letters for interaction between NaCl level and foliar treatment.

both seasons), whereas for the first leaf, MSI significantly decreased by 46.7% in the 2nd season under 2000 ppm NaCl comparing with 51.2% for plants grown under zero NaCl salinity. The values of relative water content under all salinity levels for the first leaf were higher than its values at the fourth leaf, whereas the opposite observation was recorded with MSI, where the values of MSI for the first leaf were lower than its values at the fourth leaf (Table 2).

All foliar treatments led to an increase in the values of RWC and MSI in both first and fourth leaf comparing with control (Table 2). The highest significant values in MSI for both first and fourth leaf were for GB5 + SA1 application under 0 ppm NaCl salinity, while under 2000 ppm NaCl salinity the highest significant values in MSI were for GB5 + Spd0.5 application. The overall mean of foliar treatments under NaCl salinity levels shows that GB at 5 mM and its combinations with other foliar treatments recorded the highest significant values in MSI in the first and fourth leaf in addition to Spd0.5 + SA1 application in the fourth leaf (Table 2).

Flowering and yield components

Flowers number per plant, fruit abscission percentage, and harvest index as overall mean recorded the highest significant values under NaCl salinity at 2000 ppm comparing with 0 ppm NaCl as presented in Table 3. On the other hand, the total number of pods per plant and the pods yield as fresh weight per plant recorded the highest values as overall mean under 0 ppm NaCl salinity application comparing with 2000 ppm NaCl salinity. Individual treatment of combined GB5 + SA1 + Spd0.5 recorded the highest values in flowers number per plant, total number of pods per plant, sharing percentage in fruit set over control, harvest index, and the pods yield as fresh weight per plant, whereas it recorded the lowest value in fruit abscission percentage in both seasons under 0 ppm NaCl salinity. While under 2000 NaCl salinity the best foliar applications recorded the highest values in flowers number per plant, total number of pods per plant, sharing percentage in fruit set over control and the pods yield as fresh weight per plant were SA at 1 mM, Spd0.5 + SA1 and

Table 2 Influence of salicylic acid (SA), spermidine (Spd), glycine betaine (GB) and their combinations as foliar application under different levels of NaCl salinity (0 and 2000 ppm) on relative water content (RWC) and membrane stability index (MSI) of snap bean 1st and 4th leaf in both seasons (2012 and 2013).

Foliar treatments (mM)	RWC 1st leaf	RWC 4th leaf	MSI 1st leaf	MSI 4th leaf	RWC 1st leaf	RWC 4th leaf	MSI 1st leaf	MSI 4th leaf
	1st season				2nd season			
	NaCl at 0 ppm							
Control	83.3 a	83.6 a	34.6 d	59.4 b	81.9 b	80.5 a	33.1 c	55.8 c
SA1	90.4 a	89.1 a	39.0 cd	61.7 ab	87.9 ab	87.4 a	38.3 c	59.3 bc
Spd0.5	93.5 a	90.3 a	38.6 cd	62.3 ab	91.6 a	89.7 a	36.3 c	60.1 bc
Spd0.5 + SA1	88.5 a	87.0 a	47.2 bd	72.3 ab	87.5 ab	84.8 a	44.1 bc	70.6 ab
GB5	89.8 a	87.2 a	70.4 ab	68.0 ab	87.5 ab	84.8 a	67.0 a	64.6 ac
GB5 + SA1	90.7 a	84.8 a	75.1 a	75.2 a	87.0 ab	83.7 a	71.4 a	73.5 a
GB5 + Spd0.5	91.5 a	85.0 a	63.5 ab	65.0 ab	88.5 ab	83.4 a	62.4 a	65.0 ac
GB5 + SA1 + Spd0.5	93.2 a	87.5 a	60.4 abc	73.1 ab	91.8 a	85.6 a	57.4 ab	70.5 ab
Mean	90.1 A	86.8 A	53.6 A	67.1 B	88.0 A	85.0 A	51.2 A	65.0 B
	NaCl at 2000 ppm							
Control	82.7 a	83.6 a	33.7 c	57.8 c	81.3 b	80.3 a	31.1 d	54.5 c
SA1	83.5 a	87.5 a	38.0 c	67.6 bc	81.7 b	85.1 a	35.3 d	66.2 bc
Spd0.5	90.4 a	88.4 a	44.3 bc	76.6 ab	88.4 ab	87.0 a	41.0 cd	73.2 ab
Spd0.5 + SA1	88.6 a	85.7 a	42.1 bc	74.5 ab	87.5 ab	84.0 a	40.3 cd	73.0 ab
GB5	83.7 a	86.9 a	46.4 bc	77.7 ab	81.4 b	84.7 a	44.7 bd	74.3 ab
GB5 + SA1	90.0 a	84.6 a	63.4 ab	73.0 ab	86.7 ab	83.2 a	58.8 ab	71.4 ab
GB5 + Spd0.5	88.9 a	84.5 a	71.3 a	83.1 a	87.9 ab	83.8 a	67.5 a	80.4 a
GB5 + SA1 + Spd0.5	92.4 a	87.3 a	55.2 ac	72.3 ab	91.6 a	85.3 a	54.5 ac	70.4 ab
Mean	87.5 A	86.0 A	49.3 A	72.8 A	85.8 B	84.2 A	46.7 B	70.4 A
	Mean of foliar treatments under NaCl salinity levels							
Control	83.0 b	83.6 a	34.2 c	58.6 b	81.6 c	80.4 a	32.1 c	55.2 c
SA1	87.0 ab	88.3 a	38.5 c	64.7 ab	84.8 bc	86.3 a	36.8 bc	62.7 bc
Spd0.5	92.0 ab	89.3 a	41.5 c	69.4 a	90.0 ab	88.4 a	38.7 bc	66.6 ab
Spd0.5 + SA1	88.5 ab	86.3 a	44.6 bc	73.4 a	87.5 ac	84.4 a	42.2 b	71.8 a
GB5	86.8 ab	87.0 a	58.4 ab	72.8 a	84.4 bc	84.7 a	55.8 a	69.4 ab
GB5 + SA1	90.4 ab	84.7 a	69.2 a	74.1 a	86.8 ac	83.5 a	65.1 a	72.5 a
GB5 + Spd0.5	90.2 ab	84.8 a	67.4 a	74.1 a	88.2 ab	83.6 a	64.9 a	72.7 a
GB5 + SA1 + Spd0.5	92.8 a	87.4 a	57.8 ab	72.7 a	91.7 a	85.4 a	56.0 a	70.4 ab

Means followed by different letters are significantly different at $P < 0.05$ level; Tukey's HSD test.
Capital letters for mean of NaCl salinity level, whereas lowercase letters for interaction between NaCl level and foliar treatment.

GB5 + SA1 + Spd0.5. Control treatment under 2000 NaCl salinity recorded the highest values in flowers number per plant, fruit abscission percentage and harvest index.

The overall mean of foliar treatments under NaCl salinity levels shows that all studied flowering and yield parameters in Table 3 increased to the highest significant levels under GB5 + SA1 + Spd0.5 treatment comparing with other foliar treatments, except for fruit abscission percentage parameter, where the same treatment recorded the lowest significant value.

Neither NaCl salinity nor foliar treatments affected significantly pod length and width parameters (Table 4). Although there are no significant differences between overall mean of pod curvature % under NaCl salinity levels, the pod curvature % increases with concomitant increase in NaCl salinity. The highest value in pod curvature % was recorded with control plants under 2000 ppm NaCl salinity. All foliar treatments decreased the percentage of pod curvature under all levels of NaCl salinity comparing with its control. Individual treatment of GB at 5 mM or combined with SA at 1 mM or Spd at 0.5 mM were the most effective treatments in decreasing the pod curvature % especially, under 2000 ppm NaCl salinity (Table 4). Pod fresh weight is the only significant parameter between all pod parameters as overall mean, whereas the highest significant value was under 0 NaCl salinity. The percentage of pod moisture content decreased simultaneously with increase in NaCl salinity. All foliar treatments led to decrease in the percentage of pod moisture content comparing with its control under 0 ppm NaCl salinity, whereas under 2000 ppm NaCl salinity the opposite results for foliar treatments were recorded (Table 4). These contrasted results of pod moisture % under the two levels of NaCl salinity led to insignificant differences between overall means of pod moisture %.

Biochemical content changes

NaCl salinity at 2000 ppm decreased the concentration of total free amino acids in leaves and pods of snap bean as overall mean comparing with its value under 0 ppm NaCl salinity (Table 5). All foliar treatments, especially for GB at 5 mM and all its combinations, led to an increase in the concentration of total free amino acids in leaves and pods of snap bean plants under 0 ppm NaCl salinity comparing with its control. In contrast, under NaCl salinity at 2000 ppm the values of most individual foliar treatments insignificantly decreased comparing to

Table 3 Influence of salicylic acid (SA), spermidine (Spd), glycine betaine (GB) and their combinations as foliar application under different levels of NaCl salinity (0 and 2000 ppm) on reproductive parameters and yield components of snap bean plant in both seasons (2012 and 2013).

Foliar treatments (mM)	F. no.	Pods no./plant	Fruit Abs.%	FSS %	HI	Yield f.w./plant	F. no.	Pods no./plant	Fruit Abs.%	FSS %	HI	Yield f.w./plant
	1st season						2nd season					
NaCl at 0 ppm												
Control	19 ab	6.6 b	65 a	–	19 a	15 b	18 a	6.2 ab	66 a	–	22 a	14 d
SA1	19 ab	10 ab	46 bc	53	23 a	18 b	18 a	9.4 ab	49 c	52	28 a	16 cd
Spd0.5	16 ab	8.2 ab	49 bc	46	26 a	24 ab	16 ab	7.6 ab	51 bc	41	29 a	23 bc
Spd0.5 + SA1	16 ab	6.8 b	58 ac	18	27 a	16 b	16 a	6.6 ab	58 ac	21	29 a	15 d
GB5	16 ab	6.4 b	59 ab	15	18 a	16 b	15 ab	6.0 b	61 ab	13	25 a	16 cd
GB5 + SA1	15 b	7.6 b	49 bc	44	27 a	17 b	14 ab	7.0 ab	51 bc	43	31 a	16 cd
GB5 + Spd0.5	18 ab	9.4 ab	48 bc	47	24 a	29 a	17 b	9.0 ab	48 bc	50	32 a	27 ab
GB5 + SA1 + Spd0.5	21 a	11.6 a	45 c	57	28 a	34 a	20 a	10.6 a	46 c	55	33 a	32 a
Mean	17 B	8.3 A	52 B	–	24 B	21 A	17 B	7.8 A	54 B	–	29 B	20 A
NaCl at 2000 ppm												
Control	23 a	6.8 a	70 a	–	43 a	10 b	22 a	6.6 a	70 a	–	40 a	10 b
SA1	23 a	8.8 a	61 a	30	42 a	20 a	21 a	8.2 a	62 b	29	40 a	19 a
Spd0.5	19 ab	6.2 a	68 a	6	26 a	11 b	19 ab	6.0 a	68 ab	7	33 a	10 b
Spd0.5 + SA1	21 a	8.4 a	59 a	35	34 a	15 ab	20 a	7.8 a	62 b	29	38 a	14 ab
GB5	19 ab	6.4 a	67 a	10	35 a	16 ab	19 ab	6.2 a	67 ab	11	37 a	15 ab
GB5 + SA1	18 ab	5.6 a	69 a	4	36 a	13 ab	18 ab	5.5 a	69 ab	5	38 a	12 ab
GB5 + Spd0.5	16 b	5.4 a	66 a	12	35 a	11 b	15 b	5.2 a	66 ab	15	38 a	11 b
GB5 + SA1 + Spd0.5	21 a	8.4 a	60 a	33	33 a	20 a	20 a	7.8 a	61 b	30	37 a	20 a
Mean	20 A	7.0 B	65 A	–	35 A	15 B	19 A	6.7 B	66 A	–	38 A	14 B
Mean of foliar treatments under NaCl salinity levels												
Control	21 a	6.7 c	67 a	–	31 a	12 c	20 a	6.4 ab	68 a	–	31 a	12 c
SA1	21 ab	9.4 ab	54 b	41	32 a	19 b	20 a	8.8 ab	55 c	40	34 a	18 b
Spd0.5	18 ab	7.2 bc	58 ab	26	26 a	17 bc	17 ab	6.8 ab	59 bc	24	31 a	16 bc
Spd0.5 + SA1	19 ab	7.6 bc	59 ab	27	31 a	15 bc	18 ab	7.2 ab	60 ac	25	34 a	14 bc
GB5	18 ab	6.4 c	63 ab	13	26 a	16 bc	17 ab	6.1 b	64 ab	12	31 a	15 bc
GB5 + SA1	16 b	6.6 c	59 ab	24	31 a	15 bc	16 b	6.3 b	59 bc	24	35 a	14 bc
GB5 + Spd0.5	17 ab	7.4 bc	57 ab	30	29 a	20 b	16 b	7.1 ab	57 bc	33	35 a	19 b
GB5 + SA1 + Spd0.5	21 a	10 a	52 b	45	31 a	27 a	20 a	9.2 a	54 c	43	35 a	26 a

Means followed by different letters are significantly different at $P < 0.05$ level; Tukey's HSD test. Where F.no. = flowers number per plant, Pods no./plant = total number of pods per plant, Fruit Abs.% = fruit abscission percentage, FSS % = sharing percentage in fruit set over control, HI = harvest index, and yield f.w./plant = the pods yield as fresh weight per plant.
Capital letters for mean of NaCl salinity level, whereas lowercase letters for interaction between NaCl level and foliar treatment.

its control in leaves, whereas in pods the maximum significant value for the concentration of total free amino acids was for control plants. In addition, the results of total free amino acid concentration in pods of control plants may vary significantly under the two tested levels of NaCl salinity, which recorded the highest significant value under NaCl salinity at 2000 ppm (6.5 mg g^{-1} f.w. in the first season), whereas under 0 ppm NaCl salinity recoded the lowest one (0.8 mg g^{-1} f.w. in the first season). GB at 5 mM and all its combinations (especially for GB5 + Spd0.5 treatment) as mean of foliar treatments under NaCl salinity levels led to an increase in the concentration of total free amino acids in leaves and pods of snap bean (Table 5).

Although there are no significant differences between overall means of the concentration of total soluble proteins under NaCl salinity tested levels in pods of snap bean plants, there was a significant difference between their values in leaves (Table 5). Not only the concentration of total soluble proteins as overall mean in pods were not affected by NaCl Salinity levels, but also there were no significant differences between foliar treatments on the concentration of total soluble proteins. In contrast, the concentration of total soluble proteins in leaves exhibits a significant difference as affected by foliar treatments under 0 ppm NaCl salinity. Furthermore, under 2000 ppm NaCl salinity, the foliar treatments led to an increase in the concentration of total soluble proteins in leaves but did not reach the significance level. Exogenously applied spermidine at 0.5 mM alone or combined with salicylic acid at 1 mM insignificantly reduced the values of total soluble proteins in pods of snap bean than its control under 2000 ppm NaCl salinity. Results of mean of foliar treatments under NaCl salinity levels showed that salicylic acid at 1 mM recorded the highest significant value in leaves over all other treatments (Table 5).

The concentrations of total soluble sugars as overall mean for leaves and pods were highly significant under 0 ppm NaCl salinity which were 11.7 and 20.5 mg g^{-1} f.w. in first season respectively, compared with their values under 2000 ppm NaCl salinity which were 2.0 and 4.1 mg g^{-1} f.w. in first season respectively (Table 5). The concentrations of total soluble

Table 4 Influence of salicylic acid (SA), spermidine (Spd), glycine betaine (GB) and their combinations as foliar application under different levels of NaCl salinity (0 and 2000 ppm) on pod parameters of snap bean plant in both seasons (2012 and 2013).

Foliar treatments (mM)	Pod length (cm)	Pod width (mm)	Pod curvature %	Pod f.w. (g)	Pod moisture %	Pod length (cm)	Pod width (mm)	Pod curvature %	Pod f.w. (g)	Pod moisture %
	1st season					2nd season				
NaCl at 0 ppm										
Control	10.0 a	9.1 a	13.2 a	3.8 ab	92.1 a	9.7 a	9.1 a	12.4 a	3.3 a	93.4 a
SA1	9.8 a	8.0 a	12.5 a	3.3 b	89.2 ab	9.7 a	7.8 a	10.6 a	3.1 a	87.5 a
Spd0.5	9.9 a	7.8 a	10.3 a	4.5 a	90.3 ab	9.7 a	7.6 a	10.8 a	4.1 a	90.0 a
Spd0.5 + SA1	10.2 a	8.2 a	9.2 a	3.7 ab	86.6 b	10.0 a	8.2 a	9.5 a	4.0 a	89.8 a
GB5	10.0 a	9.0 a	9.9 a	3.0 b	91.3 ab	9.7 a	9.1 a	10.6 a	3.1 a	91.5 a
GB5 + SA1	10.6 a	8.7 a	9.3 a	3.2 b	89.9 ab	10.3 a	8.6 a	9.3 a	3.3 a	89.9 a
GB5 + Spd0.5	10.6 a	9.4 a	13.1 a	4.6 a	90.4 ab	10.8 a	9.1 a	12.1 a	4.9 a	89.7 a
GB5 + SA1 + Spd0.5	11.1 a	9.3 a	9.9 a	4.6 a	91.7 a	10.8 a	9.1 a	9.7 a	4.9 a	91.4 a
Mean	10.3 A	8.7 A	10.9 A	3.8 A	90.2 A	10.1 A	8.6 A	10.6 A	3.9 A	90.4 A
NaCl at 2000 ppm										
Control	9.8 a	8.7 a	16.3 a	2.6 b	85.1 b	9.5 a	8.6 a	16.4 a	2.7 b	83.8 b
SA1	10.6 a	9.0 a	11.8 ab	3.8 a	91.4 ab	10.4 a	8.8 a	12.0 ab	3.9 a	91.4 a
Spd0.5	10.6 a	8.3 a	12.4 ab	2.8 ab	90.4 ab	10.7 a	8.1 a	12.8 ab	3.0 ab	91.1 a
Spd0.5 + SA1	10.6 a	8.6 a	9.8 b	3.0 ab	91.7 ab	10.4 a	8.2 a	10.3 ab	3.0 ab	91.3 a
GB5	11.2 a	7.9 a	9.8 b	3.9 a	92.8 a	11.0 a	7.8 a	9.7 b	3.6 ab	92.5 a
GB5 + SA1	9.5 a	9.0 a	7.9 b	2.8 ab	85.7 ab	9.4 a	8.9 a	7.8 b	2.8 b	90.9 ab
GB5 + Spd0.5	9.5 a	7.9 a	8.1 b	3.0 ab	91.5 ab	9.5 a	7.6 a	8.3 b	3.0 ab	91.2 a
GB5 + SA1 + Spd0.5	10.4 a	8.3 a	10.5 b	3.5 ab	90.7 ab	10.3 a	7.9 a	9.8 b	3.6 ab	92.6 a
Mean	10.3 A	8.5 A	10.8 A	3.2 B	89.9 A	10.2 A	8.2 A	10.9 A	3.2 B	90.6 A
Mean of foliar treatments under NaCl salinity levels										
Control	9.9 a	8.9 a	14.7 a	3.2 b	88.6 a	9.6 a	8.9 a	14.4 a	3.0 b	88.6 a
SA1	10.2 a	8.5 a	12.1 ab	3.5 ab	90.3 a	10.1 a	8.3 a	11.3 ab	3.5 ab	89.4 a
Spd0.5	10.2 a	8.1 a	11.4 ab	3.6 ab	90.3 a	10.2 a	7.9 a	11.8 ab	3.5 ab	90.5 a
Spd0.5 + SA1	10.4 a	8.4 a	9.5 ab	3.4 ab	89.2 a	10.2 a	8.2 a	9.9 b	3.5 ab	90.5 a
GB5	10.6 a	8.4 a	9.8 ab	3.5 ab	92.0 a	10.4 a	8.4 a	10.2 b	3.4 ab	92.0 a
GB5 + SA1	10.0 a	8.9 a	8.6 b	3.0 b	87.8 a	9.8 a	8.7 a	8.6 b	3.1 b	90.4 a
GB5 + Spd0.5	10.1 a	8.6 a	10.6 ab	3.8 ab	90.9 a	10.1 a	8.4 a	10.2 b	3.9 ab	90.5 a
GB5 + SA1 + Spd0.5	10.7 a	8.8 a	10.2 ab	4.0 a	91.2 a	10.6 a	8.5 a	9.7 b	4.3 a	92.0 a

Means followed by different letters are significantly different at $P < 0.05$ level; Tukey's HSD test. Where f.w. = fresh weight.
Capital letters for mean of NaCl salinity level, whereas lowercase letters for interaction between NaCl level and foliar treatment.

sugars in pods were higher than its values in leaves. Exogenous application of GB at 5 mM and its combinations with other treatments reduced the values of total soluble sugars in leaves, whereas the same treatments led to an increase in total soluble sugars in pods of snap bean plants. Moreover, under 2000 ppm NaCl salinity plants treated with GB at 5 mM and its combinations with other treatments had higher total soluble sugar concentrations in the leaves and pods, compared with untreated plants (control). Exogenously applied Spd0.5 + SA1 recorded the highest significant values of the concentration of total soluble sugars in leaves under 0 ppm NaCl salinity, which in turn reflected on its significant value as mean of foliar treatments under NaCl salinity levels (Table 5).

Correlation analysis

The correlation coefficient matrix between biochemical constituents of snap bean leaves and pods, RWC, MSI, and different yield attributes under 0 ppm and 2000 ppm NaCl salinity is presented in Tables 6 and 7 respectively. When eliminating the addition of NaCl (control plants, Table 6), the correlation of green pods yield was strong positive with plant f.w. (0.91), pods no./plant (0.8), and RWC at 1st leaf, while it has a moderate positive correlation with total free amino acids and total soluble proteins concentrations in leaves (0.47 and 0.45 respectively). Furthermore, the green pods yield had a strong negative correlation with fruit abscission percentage (−0.7). Fruit abscission percentage had a significant negative correlation with other parameters, where it was strongly correlated with pods no./plant (−0.85), and plant f.w. (−0.79), in addition to it was highly correlated with RWC at 4th leaf (−0.5), and total soluble proteins in leaves (−0.55), while it had a moderate correlation with total amino acids concentration in pods (−0.47). Leaves no./plant had a strong positive correlation with MSI at 1st leaf (0.67) and total free amino acids concentration in leaves (0.66). Pods no./plant had a positive correlation with plant f.w. (0.93), RWC at 1st leaf, and the concentration of total soluble proteins in leaves (0.5). The concentrations of free amino acids in leaves and pods were highly correlated with MSI in both first leaf and fourth leaf, which in turn correlated with total soluble sugars concentration in pods and leaves no./plant. Leaves no./plant had a positive significant correlation with yield of the green pods (Table 6).

Addition of NaCl salinity at 2000 ppm decreased the values of correlation coefficient (Table 7) comparing with its values

Table 5 Influence of salicylic acid (SA), spermidine (Spd), glycine betaine (GB) and their combinations as foliar application under different levels of NaCl salinity (0 and 2000 ppm) on total free amino acids, total soluble proteins, and total soluble sugars (TSS) concentrations of snap bean leaves and pods in both seasons (2012 and 2013).

Foliar treatments (mM)	Amino acids (mg g^{-1} f.w.)		Proteins (mg g^{-1} f.w.)		TSS (mg g^{-1} f.w.)		Amino acids (mg g^{-1} f.w.)		Proteins (mg g^{-1} f.w.)		TSS (mg g^{-1} f.w.)	
	Leaf	Pod	Leaf	Pod	Leaf	Pod	Leaf	Pod	Leaf	Pod	Leaf	Pod
	1st season						2nd season					
NaCl at 0 ppm												
Control	3.1 b	0.8 c	2.0 b	0.7 a	12.7 bc	18.2 bc	2.9 bc	0.8 c	2.0 bc	0.7 a	11.4 bd	16.6 cd
SA1	2.6 b	3.4 ac	3.4 ab	1.0 a	14.1 ab	14.9 c	2.7 c	3.2 ac	3.2 ab	0.9 a	13.3 ab	14.2 d
Spd0.5	4.7 ab	2.7 bc	3.3 ab	0.8 a	13.1 b	23.1 ab	4.5 ac	2.6 bc	3.0 ac	0.8 a	12.1 ac	20.2 ac
Spd0.5 + SA1	4.5 ab	4.4 ac	2.1 b	1.2 a	17.0 a	18.0 bc	4.4 ac	4.0 ac	2.0 bc	1.1 a	15.2 a	17.1 bd
GB5	4.9 ab	4.5 ac	2.4 ab	1.2 a	8.4 d	22.0 ab	4.6 ac	4.2 ab	2.1 bc	1.0 a	8.0 d	20.3 ac
GB5 + SA1	5.2 ab	5.5 ab	1.9 b	0.9 a	9.6 cd	25.6 a	5.0 ab	5.2 ab	1.9 c	0.9 a	9.1 cd	23.4 a
GB5 + Spd0.5	5.8 a	7.0 a	4.0 a	0.7 a	9.6 cd	23.2 ab	5.4 a	6.5 a	3.8 a	0.7 a	9.6 cd	22.1 ab
GB5 + SA1 + Spd0.5	4.8 ab	3.5 ac	2.3 ab	1.1 a	8.7 d	19.1 bc	4.5 ac	3.3 ac	2.3 bc	1.0 a	8.1 d	18.7 ad
Mean	4.4 A	4.0 A	2.7 A	1.0 A	11.7 A	20.5 A	4.2 A	3.7 A	2.5 A	0.9 A	10.8 A	19.1 A
NaCl at 2000 ppm												
Control	3.9 ab	6.5 a	1.7 a	1.1 a	1.6 b	3.4 a	3.6 ab	6.1 a	1.6 b	1.0 a	1.4 b	3.1 bc
SA1	3.8 ab	1.8 bc	3.1 a	1.3 a	1.7 ab	4.5 a	3.5 ab	1.7 c	2.9 a	1.2 a	1.6 ab	3.9 ac
Spd0.5	3.6 ab	1.4 c	2.1 a	0.9 a	2.0 ab	3.3 a	3.5 ab	1.4 c	2.0 ab	0.8 a	1.9 ab	3.1 bc
Spd0.5 + SA1	2.8 b	1.8 bc	1.7 a	0.6 a	1.6 b	3.1 a	2.6 b	1.7 c	1.6 b	0.6 a	1.5 b	3.0 c
GB5	3.4 ab	2.9 bc	2.3 a	1.0 a	1.9 ab	4.6 a	3.4 ab	2.6 bc	2.2 ab	0.9 a	1.8 ab	4.2 ac
GB5 + SA1	3.7 ab	2.9 bc	2.3 a	1.2 a	2.0 ab	4.7 a	3.6 ab	2.9 bc	2.3 ab	1.0 a	1.8 ab	4.5 a
GB5 + Spd0.5	4.2 a	4.2 ab	2.1 a	1.2 a	2.5 ab	4.3 a	4.0 a	4.2 ab	1.9 ab	1.1 a	2.3 ab	4.0 ac
GB5 + SA1 + Spd0.5	2.8 b	4.2 ab	2.0 a	1.7 a	2.6 a	4.5 a	2.7 b	4.0 b	1.9 ab	1.4 a	2.4 a	4.3 ab
Mean	3.5 B	3.2 B	2.2 B	1.1 A	2.0 B	4.1 B	3.4 B	3.1 B	2.1 B	1.0 A	1.8 B	3.8 B
Mean of foliar treatments under NaCl salinity levels												
Control	3.5 b	3.6 ab	1.9 b	0.9 a	7.2 bd	10.8 cd	3.3 bc	3.4 bc	1.8 c	0.8 a	6.4 bd	9.8 bc
SA1	3.2 b	2.6 b	3.3 a	1.2 a	7.9 ab	9.7 d	3.1 c	2.5 bc	3.1 a	1.0 a	7.4 ab	9.0 c
Spd0.5	4.2 ab	2.0 b	2.7 ab	0.9 a	7.6 bc	13.2 ac	4.0 ac	2.0 c	2.5 ac	0.8 a	7.0 ac	11.7 ab
Spd0.5 + SA1	3.6 ab	3.1 b	1.9 b	0.9 a	9.3 a	10.6 cd	3.5 bc	2.9 bc	1.8 c	0.8 a	8.4 a	10.0 bc
GB5	4.1 ab	3.7 ab	2.3 ab	1.1 a	5.2 e	13.3 ac	4.0 ac	3.4 bc	2.2 bc	1.0 a	4.9 d	12.2 ab
GB5 + SA1	4.5 ab	4.2 ab	2.1 ab	1.1 a	5.8 de	15.2 a	4.3 ab	4.0 ab	2.1 bc	0.9 a	5.5 cd	14.0 a
GB5 + Spd0.5	5.0 a	5.6 a	3.0 ab	1.0 a	6.0 ce	13.8 ab	4.7 a	5.3 a	2.8 ab	0.9 a	5.9 bd	13.1 a
GB5 + SA1 + Spd0.5	3.8 ab	3.8 ab	2.2 ab	1.4 a	5.6 de	11.8 bd	3.6 ac	3.7 ac	2.1 bc	1.2 a	5.3 d	11.5 ac

Means followed by different letters are significantly different at $P < 0.05$ level; Tukey's HSD test. Where f.w. = fresh weight.
Capital letters for mean of NaCl salinity level, whereas lowercase letters for interaction between NaCl level and foliar treatment.

Table 6 Coefficients of correlation between biochemical constituents of snap bean leaves and pods, RWC, MSI, and different yield attributes for the combined two seasons under 0 ppm NaCl salinity.

Variables	(01)	(03)	(04)	(05)	(06)	(08)	(09)	(10)	(11)	(14)
01-Plant f.w.		0.93***	−0.79***	0.91***	0.71***	0.09	0.20	0.25	0.26	−0.20
02-Leaves no./plant	0.40	0.19	−0.11	0.51**	0.41	0.67***	0.49*	0.66***	0.46*	−0.60**
03-Pods no./plant	0.93***		−0.85***	0.80***	0.63***	0.04	0.13	0.04	0.18	−0.19
04-Fruit Abs.%	−0.79***	−0.85***		−0.70***	−0.80***	−0.26	−0.32	−0.31	−0.47*	0.20
05-Yield f.w./plant	0.91***	0.80***	−0.70***		0.69***	0.21	0.21	0.47*	0.27	−0.42
06-RWC 1st leaf	0.71***	0.63***	−0.80***	0.69***		0.29	0.39	0.51**	0.40	−0.18
07-RWC 4th leaf	0.39	0.37	−0.50**	0.25	0.75***	−0.21	0.00	−0.01	−0.04	0.28
08-MSI 1st leaf	0.09	0.04	−0.26	0.21	0.29		0.72***	0.75***	0.76***	−0.72***
09-MSI 4th leaf	0.20	0.13	−0.32	0.21	0.39	0.72***		0.63***	0.57**	−0.22
10-Leaf amino acids	0.25	0.04	−0.31	0.47*	0.51**	0.75***	0.63***		0.76***	−0.48*
11-Pod amino acids	0.26	0.18	−0.47*	0.27	0.40	0.76***	0.57**	0.76***		−0.31
12-Leaf proteins	0.52**	0.50**	−0.55**	0.45*	0.43*	−0.14	−0.40	0.12	0.36	0.04
13-Pod proteins	0.09	0.00	−0.02	−0.10	0.27	0.27	0.60**	0.06	0.12	0.12
14-Leaf TSS	−0.20	−0.19	0.20	−0.42	−0.18	−0.72***	−0.22	−0.48*	−0.31	
15-Pod TSS	−0.07	−0.14	−0.20	0.23	0.36	0.70***	0.45*	0.84***	0.57**	−0.53**

* Refer to significant correlation at $P < 0.1$ level.
** Refer to significant correlation at $P < 0.05$ level.
*** Refer to significant correlation at $P < 0.01$ level.

Table 7 Coefficients of correlation between biochemical constituents of snap bean leaves and pods, RWC, MSI, and different yield attributes for the combined two seasons under 2000 ppm NaCl salinity.

Variables	(02)	(03)	(04)	(05)	(06)	(08)	(11)	(12)	(13)	(14)
01-Plant f.w.	0.14	0.54**	−0.84***	0.83***	0.50*	0.26	−0.13	0.22	0.49*	0.28
02-Leaves no./plant		0.54**	−0.08	0.54**	−0.24	−0.34	−0.02	0.54**	0.44*	−0.02
03-Pods no./plant	0.54**		−0.59**	0.63**	−0.07	−0.59**	−0.17	−0.08	0.17	−0.37
04-Fruit Abs.%	−0.08	−0.59**		−0.76***	−0.37	−0.13	0.23	−0.02	−0.23	−0.21
05-Yield f.w./plant	0.54**	0.63**	−0.76***		0.18	0.11	−0.18	0.41	0.45*	0.18
06-RWC 1st leaf	−0.24	−0.07	−0.37	0.18		0.61**	−0.06	−0.09	0.25	0.76***
07-RWC 4th leaf	0.49*	0.42	−0.50**	0.50**	0.44*	−0.01	−0.56**	0.37	0.10	0.35
08-MSI 1st leaf	−0.34	−0.59**	−0.13	0.11	0.61**		0.23	0.24	0.28	0.82***
09-MSI 4th leaf	−0.41	−0.52**	−0.17	−0.10	0.40	0.65***	−0.19	0.06	−0.31	0.51**
10-Leaf amino acids	−0.11	−0.53**	0.48*	−0.53**	−0.17	0.17	0.36	0.25	0.27	0.14
11-Pod amino acids	−0.02	−0.17	0.23	−0.18	−0.06	0.23		−0.32	0.49*	0.20
12-Leaf proteins	0.54**	−0.08	−0.02	0.41	−0.09	0.24	−0.32		0.36	0.23
13-Pod proteins	0.44*	0.17	−0.23	0.45*	0.25	0.28	0.49*	0.36		0.51**
14-Leaf TSS	−0.02	−0.37	−0.21	0.18	0.76***	0.82***	0.20	0.23	0.51**	
15-Pod TSS	0.45*	−0.14	−0.03	0.47*	0.20	0.59**	0.29	0.70***	0.66***	0.55**

* Refer to significant correlation at $P < 0.1$ level.
** Refer to significant correlation at $P < 0.05$ level.
*** Refer to significant correlation at $P < 0.01$ level.

for control plants (Table 6). Green pods yield had a strong positive correlation with plant f.w. (0.83), while it has a high positive correlation with pods no./plant (0.63), leaves no./plant (0.54), and RWC at 4th leaf. Finally, green pods yield has a moderate correlation with total soluble proteins (0.45), and total soluble sugars (0.47) concentrations in pods. Total soluble sugars concentration in leaves was strongly correlated with RWC at 1st leaf (0.76), and MSI at 1st leaf (0.82), while the concentration of total soluble sugars in pods had a strong correlation with total soluble protein concentrations in both leaves and pods (0.7 and 0.66 respectively).

Discussion

Pods number and weight as the major yield parameters reflect the plant performance during previous growth stages, which depend mainly on the vigorous of vegetative growth and flowering status (Osman, 2015). Exposure to salinity stress at any stage of plant development led to reduce vegetative and reproductive growth constituents in most legume crops. Most legumes are sensitive or moderately sensitive to salinity (Khan and Basha, 2015).

In the present study, when snap bean plants exposed to NaCl salinity as individual application led to a reduction in LAI, LAR and insignificant increase in leaves no./plant (Table 1), which reflects the high reduction percentage in the average of leaf area under salinity stress. Although NaCl salinity increased the leaves no./plant, which led to an increase in flowers number per plant and consequently, increased pods number per plant (Table 3), application of NaCl increased the fruit abscission ratio, which reflects on reducing the marketable pods per plant, and also increased harvest index (Table 3), which indicates that NaCl treatment redirected the assimilates to produce more pods than vegetative growth. Reduction in LAR and LAI reduced plant fresh weight (Table 1). The correlation coefficient in Table 6 revealed that, under NaCl application, any increase in plant fresh weight led to an increase in pods no./plant, which in turn highly positive correlated with green pods yield as fresh weight per plant. RWC had a positive moderate correlation with green pods yield, which directed from any increase in RWC led to an increase in leaves no./plant and a decrease in fruit abscission percentage (Table 7). MSI does not have any good correlation with green pods yield, but it has a highly positive correlation with RWC, which in turn has a direct effect on yield. The higher percentages of MSI consider as indication to membranes integrity, which reflect on maintaining its functions under diverse conditions. Since osmotic stress reduces cell water content, so increasing values of MSI led to an increase in RWC values. These results are in good agreement with those reported in bean plants by Bayuelo-Jiménez et al. (2012), who found that, application of NaCl reduced LAR, leaf photosynthetic rate, stomatal conductance and reduced transpiration rate which in turn reduced relative growth rate, and by Kaymakanova and Stoeva (2008) who recorded adverse effects of salinity on leaf area, plant height, number of leaves, and root length. Also Howladar (2014) recorded decline in bean leaves area per plant, plant dry mass, pods number and pods yield, total leaf chlorophyll content, water use efficiency, RWC, and MSI under salinity stress. In addition to the adverse effects of salinity on reducing biomass of *Phaseolus* plants, Khan and Basha (2015) reported that leaf water content and turgor potentials were also decreased in all tested species. A decrease in membrane stability reflects the extent of membrane lipid oxidative damage caused by reactive oxygen species formed under stress.

Increasing the levels of total soluble sugars and free amino acids is the major factor for maximizing vegetative growth, which reflects on number of pods per plant and pods yield of pea plants (Osman and Abd El-Gawad, 2013). In this regard, data in Table 6 revealed that in non-stressed plants, both total free amino acids and total soluble sugars concentrations in leaves have a high positive and negative correlation respectively with MSI. On contrarily, under stress conditions (Table 7), only total soluble sugars concentration had a strong positive correlation with MSI. The amount of total soluble sugars in leaves of non-stressed plants was 12.7 mg g^{-1} f.w., while under NaCl salinity reduced to 1.6 mg g^{-1} f.w. in the

1st season (Table 5). These observations suggest that total soluble sugars with small amount can protect cell membranes from damage, leading to an increase in MSI under stressed and non-stressed conditions. The reduction in total soluble sugars in leaves under saline conditions could be referred to the reduction occurred in stomatal conductance which reduces the photosynthetic rate and reduces photo-assimilates as previously mentioned by many researchers (Kaymakanova and Stoeva, 2008; Bayuelo-Jiménez et al., 2012), or this reduction in TSS could be referred to that most assimilates transform from soluble carbohydrates to structural carbohydrates when directed to produce more leaves with small area under NaCl treatment. In this connection, Carvalho et al. (2016) mentioned that full mature leaves become net sources of photo-assimilates to the newer developing leaves.

NaCl application slightly increased total free amino acids in leaves, while in pods, it markedly increased the amount of total free amino acids from 0.8 mg g^{-1} f.w. for non-stressed plants to 6.5 mg g^{-1} f.w. for stressed plants (Table 5). This observation suggests that the maintaining of pods growth under salinity conditions in sensitive plants depends mainly on free amino acids concentration in pods.

The positive effects of SA on plant growth and productivity are more pronounced under salinity conditions. Since, it led to an increase in all vegetative parameters comparing with its control especially for plant height, LAR and LAI (Table 1), consequently an increment in all pod parameters especially for pod weight and pod moisture %, which reflected on yield increase to twofold than its control (Table 3). On the contrary, under non-stressed conditions, SA had some negative effects on LAR, and leaf no./plant (Table 1), and all pod parameters (Table 4). These negative effects could be referring to decrease the amount of free amino acids in leaves and total soluble sugars in pods comparing with its control (Table 5). Since, bean plants are sensitive to salinity, so the amount of photo-assimilates will be reduced according to inhibition in photosynthesis. Application of SA under saline conditions did not alleviate the amount of TSS to its level in non-stress conditions, which suggests that, the increase in vegetative growth under NaCl treatment comparing with its control could be referred in part to that most TSS serving as raw material to synthesize structural carbohydrates. This suggestion was supported by Khan et al. (2010) who reported that exogenous salicylic acid enhanced the photosynthetic pigments and the maintenance of membrane integrity (El-Tayeb, 2005), and by Nahar et al. (2015) who mentioned that SA helped in maintaining a higher Rubisco activation state and maintained better PSII function and photosynthesis, which reflect on increase in the photosynthesis and fixation of carbon dioxide. SA not only has a positive effect on photosynthetic parameters, but also alters the activities of antioxidants enzymes and reduces the generation of reactive oxygen species, which induces protective effects on plants under salinity (Najafabadi et al., 2013). SA is involved in the synthesis of kinase protein which plays an important role in division and differentiation of cells (Ahanger et al., 2014).

Spermidine has a positive effect on vegetative growth parameters under stressed and non-stress conditions (Table 1), which reflects its importance in growth and development of plants under most environmental conditions. Increasing both MSI and RWC by Spd application under all NaCl levels to reach the highest values over all other treatments (Table 2), suggests that, spermidine can act as a potent protectant against abiotic stress (Nahar et al., 2015). Spermidine led to an increase in yield under non-stress conditions, which refers mainly to its effect on increasing pods number per plant (Table 3) and pod f.w. (Table 4). Unfortunately, the concentration of spermidine tested in this study did not increase yield significantly under NaCl stress, but when mixed with SA increased the yield 1.5 fold, while the highest yield was obtained when mixed with SA and GB (Table 3). Increasing pod f.w. under non-stress conditions suggests that, spermidine plays a vital role in managing pod total solids, since pod moisture percentage decreases comparing with its control (Table 4). Spermidine modulates ion balance of the cell and interacts with anionic molecules, such as DNA, RNA, proteins, and membrane lipids as it has polycationic nature at physiological pH (Marco et al., 2012).

The maximum vegetative growth was obtained by GB application under non-stress conditions, and also enhanced the snap bean vegetative growth under saline conditions, but not to the maximum status (Table 1), which may be referred to that GB increased MSI (Table 2), which reflected on increasing stability of thylakoid membranes and increasing photosynthetic rate and in turn increasing the photo-assimilates concentration in leaves and pods (Table 5). In this regard, Tuteja et al. (2012) reported that GB protects the photosystem II complex by stabilizing the association of the extrinsic PSII complex proteins under salt stress. Not only GB application led to an increase in MSI, but also led to a slightly increase in RWC. Levels of RWC increased when SA or Spd combined with GB application, especially for application of combined GB5 + SA1 + Spd0.5 under 2000 ppm NaCl salinity (Table 2). This increase in RWC under saline conditions when plants treated with GB, in turn led to an increase in pod moisture % and pod f.w. (Table 4) which reflected on green pods yield (Table 3). These observations suggest that GB at 5 mM alone or with its combinations with SA at 1 mM and Spd at 0.5 mM enhanced the water flow from roots to shoots by increasing the hydraulic conductivity, which reflect on increasing RWC (Hu et al., 2012).

Conclusion

Results of the current study concluded that, snap bean plant cv. Bronco is sensitive to saline stress, since it decreased LAR, LAI, plant fresh weight, MSI and green pods yield. Application of GB5 + SA1 + Spd0.5, GB5 + Spd0.5, and Spd0.5 respectively under non-stress conditions increased the green pods yield to about twofold of control. While for snap bean plants growing under salinity conditions, it could be suggested using the application of combined GB at 5 mM + SA at 1 mM + Spd at 0.5 mM or SA at 1 mM for multiplying the green pod yield or using the mixture of GB at 5 mM + SA at 1 mM + Spd at 0.5 mM for increasing both yield and quality (decrease pod curvature %) of the green pods.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aoas.2016.05.001.

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