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Glycine betaine enhanced vegetative growth and photosynthetic pigments of salinity-stressed squash plants *Cucurbita pepo* L.

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ABSTRACT

Soil salinity has become a chronic problem and a major challenge facing agricultural producers. Exogenous application of osmo-protectants such as glycine betaine (GB) may be an effective solution to different abiotic stresses. Therefore, the current study investigated the effects of the exogenous application of GB on squash plants' vegetative growth and pigment content under salt stress. Salt treatments were applied using four doses of NaCl (0 control, 1000, 2000, and 3000 ppm). 15 days after sowing, foliar sprays of GB treatments (0 mM control, 25 mM, and 50 mM) were applied. The experimental design was completely randomized using 12 treatments (4 salt concentrations × 3 foliar treatments of GB) with 5 replicates. GB enhanced growth characteristics of squash plants (fresh and dry weights, shoot length, stem diameter, leaf area, and the number of leaves), which obviously decreased when the plants were exposed to different salt stress levels. Except for the leaf area of the studied plants, which was demonstrated to be enhanced at 50 mM in this study, 25 mM GB treatment was the most effective in enhancing vegetative growth. On the other hand, GB treatments enhanced the production of photosynthetic pigments. Either 25- or 50-mM GB application significantly improved chlorophyll and carotenoid contents of squash leaves grown under salinity stress conditions compared to stress-free plants, with the exogenous application of 50 mM GB being the most effective in this investigation. The results obtained from this research clearly show that foliar applications of GB may partially or totally mitigate the harmful impacts of NaCl salt stress on vegetative growth and photosynthetic pigments.

1. Introduction

Environmental stress is a topic of major scientific interest because of its negative effects on agricultural productivity. Due to anthropogenic activities, this has recently gotten significantly worse. Global problems have resulted in climatic change, which causes salt and drought stresses that ultimately decrease plant growth and crops. Also, the increase in temperature that is associated with climate change significantly raises the chances of drought risk, accelerating the causes of land degradation and deterioration. As a result, massive scientific efforts are underway to improve agricultural output under a variety of environmental stresses in order to meet rising global food demand. These abiotic stressors, such as salt, drought, cold, and heat, have a negative impact on the survival, growth dynamics, and yield of many important food crops. One of the major abiotic stressors that affects plant development and production, especially in arid and semi-arid environments, is salinity stress [1-4].

Salinity limits plants' capacity to utilize water, resulting in a decrease in growth rate as well as alterations in plant metabolic processes. Excessive salt ion concentrations inhibit plant growth and development by decreasing photosynthesis, respiration, total carbohydrate, fatty acid, and protein content while raising amino acid levels, particularly proline [5-6].

One of the most crucial processes that is impacted in plants by salinity is photosynthesis. This is a result of salinity's effects on the contents of chlorophyll (Chl) and carotenoids (Car), as well as PSII and PSI enzyme activity reduction. Salt stress affects photosynthetic enzymes, chlorophyll, and carotenoid levels. It damages the chloroplast membranes and destroys the structure of these organelles. Moreover, decreasing the rate of Rubisco carboxylation, and depressing photosystem II (PSII) activity, electron transport, and photophosphorylation activity [7-9].

The negative effects of salt stress on growth, yield and productivity in this popular vegetable have been thoroughly established and salinity led to a decrease in the number and area of the leaves, total yield, length and diameter of the fruits of squash plants. The species *Cucurbita pepo* L. is moderately tolerant to salinity, with limits for irrigation water and saturated extract, at 3.1 and 4.7 dS m^{-1} respectively. However, its response to this abiotic stress varies according to the plant genotype and developmental stage, environmental factors, intensity and duration of the stress, cultural practices, irrigation management, and edaphoclimatic conditions [10-11].

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Among the compatible solutes, GB is a particularly effective protectant (osmoregulatory substance) against abiotic stress. Studies of GB have focused on GB-mediated tolerance to various kinds of stress and at various stages of the life cycle of plants [12]. The exogenous application of GB may quickly penetrate through leaves and be transferred to other organs, where it can improve stress tolerance. Additionally, the mechanism of foliar application of GB when applied to leaves is translocated to meristematic tissues, in particular, flower buds and shoot apices, and then translocated to actively growing and expanding tissues. Thus, it improves the chlorophyll contents, stomatal conductance (gs), relative water content (RWC), water use efficiency (WUE), and membrane stability, which can cause an enhancement in crop functioning under salt stress conditions [12-15].

The exogenous application of GB has been suggested as an alternative approach to genetic engineering to improve the productivity of crops under stress conditions. This may make using GB mitigate the negative effects of salt stress on plant development and yield an economically viable solution. Moreover, exogenous application of GB to leaves or roots has been indicated to increase the tolerance to various stresses of some plant species, including both non-accumulators and natural accumulators. Therefore, the main objective of this study was to assess the impact of glycine betaine (GB) on growth, and photosynthetic pigments of squash. Specifically, from this study hold potential significance in the development of sustainable management approaches for crop production, specifically under salt stress conditions [8,13,16,17].

2. Materials and Methods

2.1. Experimental setup

The study was conducted at King Abdulaziz University Nursery in Jeddah during the summer season of 2022 to evaluate the potential mitigating effect of glycine betaine foliar application on growth characteristics, and photosynthetic pigment of *Cucurbita pepo* L. under salt stress. Chemicals used in the present study such as glycine betaine (98% purity) were obtained from Sigma-Aldrich, USA, and NaCl (99.5% purity) from Honeywell (Fluka), Germany. Healthy squash seeds (Peru origin) were sown (one seed per pot). pots were filled with peat moss, sand, and vermiculite in a ratio of 1:2:1, respectively, and supplied with a NPK nutrient solution from the first day to day 15, then salinity treatments were established by adding different concentrations (0 control, 1000, 2000, 3000 ppm) of NaCl with a half-strength nutrient solution. Glycine betaine foliar sprayings (0 control, 25 mM, and 50 mM) were performed 15 days after sowing and every 10 days. Sprayings were applied in the early morning using a hand-held sprayer and plants were sprayed thoroughly to cover the leaves. Each solution was supplemented with 0.25% (v/v) Tween-20 as a surfactant. The experimental design was completely randomized with 12 treatments (4 NaCl concentrations × 3 glycine betaine rates) with 5 replicates. A random sample of three plants per treatment was tagged at the flowering stage to measure growth characteristics. Five fully expanded leaves (the sixth leaf from top) from each treatment were used to measure chlorophylls and carotene content.

2.2. Growth and physiological measurements

Plant length and stem diameter were measured using a meter scale and expressed in centimeters (cm). Shoot fresh weights (FW) and dry weights (DW) of were measured using an analytical balance and expressed in grams (g). Fresh samples were dried using a dry oven at 70 ○C until the weight became constant to record the dry weights. Leaf areas were measured using leaf area–leaf weight relationship as described by [18]. The leaves surface was carefully washed with distilled water. Ten leaf disks (6 mm) were dried in an oven at 70 °C for 72 h until constant weight to get disks dry weight (DDW). Total leaf area plant^{-1} was calculated using the following formula:

$$\text{Total leaf area plant}^{-1} = [\text{LDW}/\text{DDW}] \times \text{DA}$$

where LDW is the total leaf dry weight (g), DDW is the disk dry weight and DA is the disc area.

Chlorophyll a, b, and carotenoid contents were determined using the dimethyl formamide (DMF) method described by [19-20]. Ten leaf discs (6 mm diameter) were taken from leaf number six of each plant. Leaf discs were stored in separate eppendorf tubes with 1 ml of DMF for more than 48 h in the dark at 4 ^{0}C. Chlorophyll a and b contents were measured by the absorption at wavelengths of 647 nm and 664 nm, orderly using a Genesys 10S UV-vis spectrophotometer.

For calculation formulas, the concentration of chlorophyll a and b was calculated according to the following formulas [20]:

$$\text{Chlorophyll a (Chl a)} = 11.65\ A664 - 2.69\ A647 \quad (x)$$

$$\text{Chlorophyll b (Chl b)} = 20.81\ A647 - 4.53\ A664 \quad (xi)$$

From the sample disc area and the measured chlorophyll, a and b concentrations, chlorophyll a and b contents (μg mm^{-2}) were calculated using the following formulas:

$$\text{Chlorophyll a } (\mu g\ mm^{-2}) = (x) / \text{disc area}$$

$$\text{Chlorophyll b } (\mu g\ mm^{-2}) = (xi) / \text{disc area}$$

$$\text{Total Chlorophyll} = \text{Chl a} + \text{Chl b}$$

Total carotenoids content was determined by measuring the rate of absorption at wavelength of 480 nm via a Genesys 10S UV-vis spectrophotometer.

Total carotenoids concentration was calculated according to the following formula:

$$\text{Total carotenoids (car)} = [1000A480 - 0.89\ (\text{Chl a}) - 52.02\ (\text{Chl b})] / 245 \quad (xii)$$

From the sample disc area and the measured total carotenoid concentration, total carotenoids content (μg mm^{-2}) was calculated using the following formula:

$$\text{Total carotenoids } (\mu g\ mm^{-2}) = (xii) / \text{disc area}$$

2.3. Statistical analysis

All data were subjected to an analysis of variance (ANOVA) procedures in Genstat statistical package (version 11) (VSN International Ltd, Oxford, UK). Difference between means was compared using Duncan's multiple range test.

3. Results

3.1. Growth characteristics

The statistical analysis revealed significant differences in the FW of leaves that decreased with increasing salinity when compared to the control (Table 1). The salinity concentration of 3000 ppm showed the highest effect on the FW of leaves at an average of (FW = 16.37 g), while the FW of leaves in the control treatment was (FW = 31.78 g). Furthermore, the results also showed that there was a significant effect on the FW of leaves when using GB. The FW of leaves at the treatment of GB50 mM concentration (FW = 25.66 g) was lower than that at the treatment of GB25 mM concentration (FW = 28.07 g) and higher when compared with the control (FW = 22.75 g).

Aside from the statistical findings, it was clear that the salinity with GB treatments significantly affects the FW of leaves. The greatest impact was observed on the FW of the leaves under GB25 mM treatment with salt concentration of 1000 ppm (FW = 32.97 g), while GB50 treatment had the least impact under salinity of 3000 ppm (FW = 16.79 g). Under a salinity of 2000 ppm, there was a noticeable difference between the treatments of GB25 mM concentration (FW = 27.01 g) and the treatment of GB50 mM concentration (FW = 20.53 g) on the FW of the leaves.

As shown in Table (1), it was observed that there were significant differences in the DW of leaves and the weight decreased with increasing salinity compared to the control. The salinity concentration of 3000 ppm showed the highest effect on the DW of leaves (DW = 2.690 g) as compared with the control (DW = 4.151 g). In addition, the results clarified that there was a significant effect on the DW of leaves when using GB. The GB25 mM concentration had a higher effect on the DW of leaves (DW = 3.864 g) than the GB50 mM concentration (DW = 3.533 g) when compared to the control (DW = 3.312 g). Meanwhile, the results revealed that the salinity treatments with GB had a significant effect on the DW of leaves. The highest DW of leaves (4.207 g) was observed under treatment at GB25 mM concentration with a salinity concentration of 1000 ppm, while the lowest DW (2.617 g) was noticed under treatment at GB50 mM concentration with a salinity concentration of 3000 ppm. Also, there was no significant difference between treatments at GB50 mM and GB0 mM concentrations under a salinity concentration of 3000 ppm on the DW of leaves.

The results of the statistical analysis showed that there were significant differences in the FW of plant stems, which decreased with increasing salinity concentrations compared to the control (Table 1). The salt concentration of 3000 ppm showed the highest effect on the FW of stems (FW = 17.32 g) compared to the control (FW = 34.53 g). Moreover, the results clarified that there was a significant effect on the FW of stems when using GB. The FW of stems at GB25 mM concentration was (FW = 27.57g), which was higher than that at GB50 mM concentration (FW = 26.51 g) and the control (FW = 25.44 g). The highest FW of stems (FW = 33.36 g) was detected with the treatment of GB25 mM under 1000 ppm salt concentration. On the other hand, the recorded FW of stems (FW =17.21 g) under treatment at a GB 50 mM concentration with a salinity of 3000 ppm was remarkably low. The treatments at GB25 mM (FW = 24.32 g) and GB50 (FW = 24.11 g) under a salinity concentration of 2000 ppm showed no significant difference in the FW of stems.

The results of the statistical analysis of the DW of plant stems showed a significant difference, which decreased with increasing salinity compared to the control (Table 1). The saline concentration of 3000 ppm had the greatest impact on the DW of stems (DW = 1.638 g), whereas the control had the least favourable results (DW =3.792 g). Meanwhile, the results revealed that there was a significant effect on the DW of stems when using GB. On average, the effect of GB50 mM concentration on the dry weight of stems was lower (DW = 2.636 g) than the effect of GB25 mM concentration (DW = 2.861 g).

Furthermore, the results demonstrated that the salinity with GB treatments had a significant effect on the DW of stems. It was discovered that the DW of stems has the greatest impact under the treatment effect of GB25 mM at salinity of 1000 ppm with an average DW of 3.823 g, whereas the treatment effect of GB50 mM at 3000 ppm concentration has the lowest result on the DW of stems (DW =1.650 g).

Table 1. Effect of exogenous spray applications of glycine betaine (GB) on leaf fresh weight (LFW) and dry weight (LDW), stem fresh weight (SFW) and dry weight (SDW) of squash plants grown under different NaCl concentrations.

Treatments		LFW (g)	LDW (g)	SFW (g)	SDW (g)
Salinity (ppm NaCl)	GB				
0	0 mM	29.33b	3.93bc	33.90ab	3.56b
	25 mM	32.93a	4.17abc	34.80a	3.85ab
	50 mM	33.08a	4.35a	34.88a	3.96a
1000	0 mM	29.46b	3.89c	26.97d	2.85c
	25 mM	32.97a	4.21ab	33.36b	3.82ab
	50 mM	32.25a	4.15abc	29.85c	3.07c
2000	0 mM	19.24cd	2.95d	23.97e	1.74de
	25 mM	27.01b	4.09abc	24.32e	2.02d
	50 mM	20.53c	3.02d	24.11e	1.86de
3000	0 mM	12.97e	2.47e	16.93f	1.51e
	25 mM	19.36cd	2.98d	17.82f	1.75de
	50 mM	16.79d	2.62e	17.21f	1.65de

Values are the means of three replicates. Mean values in each column followed by a different lower-case-letter are significantly different by Duncan's multiple range test at $P \leq 0.05$.

The findings revealed that there were significant differences in the stem diameter when compared to the control and the stem diameter decreased with increasing salt concentrations (Table 2). The major impact on stem diameter (4.856 mm) was seen at a salt level of 3000 ppm. No significant difference was observed in stem diameter at a salinity concentration of 1000 ppm (SD = 4.812 mm) and the control (SD = 5.684 mm). However, the statistical results showed that there was no significant effect on the stem diameter when using GB. The effect of GB25 mM treatment on stem diameter was relatively higher (SD = 5.478 mm) than the effect of GB50 mM (SD = 5.439 mm) when compared to the control at GB0 mM concentration (SD = 5.241 mm). Furthermore, the results also demonstrated that the treatments of salinity with GB have significant differences on the stem diameter. It showed

that the treatment of GB25 mM at 1000 ppm salt concentration had the highest impact on the stem diameter (5.900 mm), meanwhile, the GB50 mM at 3000 ppm concentration was the lowest impact (SD = 4.852 mm). Also, the effects at the treatments of GB25 mM (SD = 5.17 mm) and GB50 mM (SD = 5.15 mm) under a 2000 ppm salt concentration both showed no significant difference on the stem diameter.

Statistical analysis revealed that there were significant differences in the plant length when compared to the control and these differences decreased with increasing salt concentrations (Table 2). The salinity concentrations at 1000 ppm (PL = 13.08 cm) and 2000 ppm (PL = 10.49 cm) had significantly different effects on the plant length, even though the salinity concentration at 3000 ppm had the greatest effect on the plant length (PL = 9.60 cm). However, the results also explained that there was a significant effect on the plant length when using GB. The GB25 mM had the highest effect on the plant length (PL = 14.58 cm), while there was no significant difference between concentrations of GB0 mM (PL = 11.38 cm) and GB50 mM (PL = 11.74 cm) on the plant length. The treatments of salinity with GB had significant differences on the plant length. It was clear that the effect of the treatment at GB25 mM under a salinity concentration of 1000 ppm had the highest impact on the plant length (PL = 13.99 cm), whereas, at the G50 mM treatment under a salinity of 3000 ppm the effect was relatively low (PL = 9.58 cm). On the other hand, the effect of the treatments at GB50 mM (PL = 10.72 cm) and GB25 mM (PL = 10.75 cm) under a salinity concentration of 2000 ppm had no significant differences on the plant length.

Table 2. Effect of exogenous spray applications of glycine betaine (GB) on stem diameter (SD) and plant length (PL) of squash plants grown under different NaCl concentrations.

Treatments		SD (mm)	PL (cm)
Salinity (ppm NaCl)	GB		
0	0 mM	5.63abcd	13.50ab
	25 mM	5.86ab	13.85a
	50 mM	5.95a	13.88a
1000	0 mM	5.35bcde	12.50c
	25 mM	5.90ab	13.99a
	50 mM	5.81abc	12.77bc
2000	0 mM	5.26cdef	10.00de
	25 mM	5.17def	10.75d
	50 mM	5.15def	10.72d
3000	0 mM	4.73f	9.50e
	25 mM	4.99ef	9.73e
	50 mM	4.85ef	9.58e

Values are means of three replicates. Mean values in each column followed by a different lower-case-letter are significantly different by Duncan's multiple range test at $P \leq 0.05$.

It was observed that the number of leaves decreased as the salinity concentration increased. Statistical analysis revealed a significant difference in the number of leaves under different treatments (Figure 1). A salinity concentration of 3000 ppm had the highest effect on the number of leaves (NOL = 11.56 leaves), whereas a salinity of 1000 ppm (NOL =15.67 leaves) had no significant difference compared to the control (NOL =16 leaves). There was a significant effect on the number of leaves when using GB. The GB25 mM concentration (NOL =14.58 leaves) had the most effect on the number of leaves, but there were no significant differences between the GB25 (NOL =14.58 leaves) and G50 mM (NOL = 14.08 leaves) treatments when compared to the control GB0 (NOL = 13.25 leaves).

Furthermore, the results revealed that the salinity treatments with GB had significant differences on the number of leaves. The treatment at GB25 mM under 1000 ppm salt concentration had the highest impact on the number of leaves, with an average of (NOL = 16.67 leaves), whereas at GB50 mM under 3000 ppm salinity, the number of leaves was (NOL = 12 leaves). In addition, there was no significant difference in the number of leaves at the treatment concentration of GB25 mM (NOL = 13.33 leaves) under a salinity concentration of 2000 ppm and under 3000 ppm (NOL = 12.33 leaves).

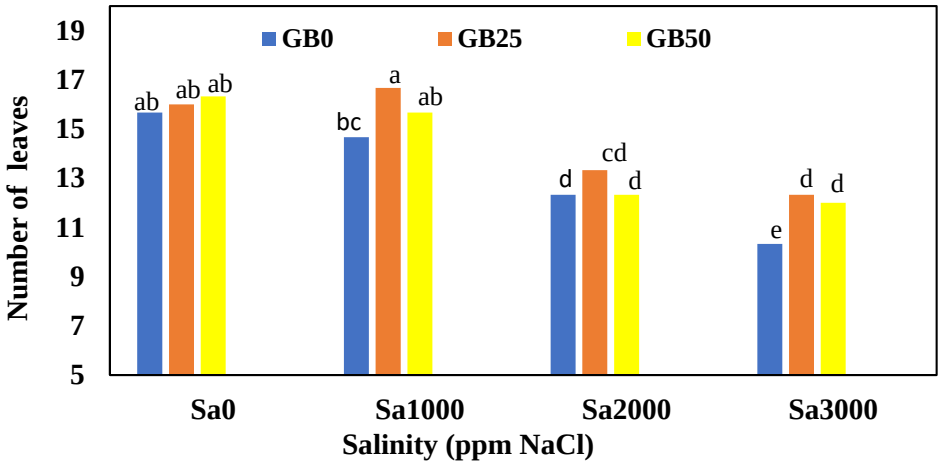

Figure 1. Effect of treatments with salinity concentrations (0, 1000, 2000, 3000 ppm) and glycine betaine (0, 25, 50 mM) on number of leaves in squash plants at (38) DAS.

The statistical findings clear that there were significant differences in the LA of leaves that decreased with increasing salinity compared to the control (Figure 2). The salinity concentration of 3000 ppm showed the highest effect on the LA at an average of (LA = 347.2 mm), as compared with the control (LA = 468.5 mm). In addition, the results also showed that there was a significant effect on the LA of leaves when using GB. The LA at the treatment of GB50 mM (LA = 442.7 mm) was higher than that at the treatment of GB25 mM concentration (LA = 419.3 mm) when compared with the control (LA = 366.2 mm).

Aside from the statistical analysis, it was clear that the salinity with GB treatments significantly affects the LA. The greatest impact was observed on the LA under GB50 mM treatment with salinity concentration of 1000 ppm (LA = 486.9 mm), while GB25 treatment had the least effect under salinity of 3000 ppm (LA = 349.2 mm). Also, there was a noticeable difference between the treatments of GB25 mM concentration (LA = 483.4 mm) under salinity of 1000 ppm and the treatment of GB50 mM concentration (LA = 389.1 mm) under 2000 ppm on the LA of the leaves.

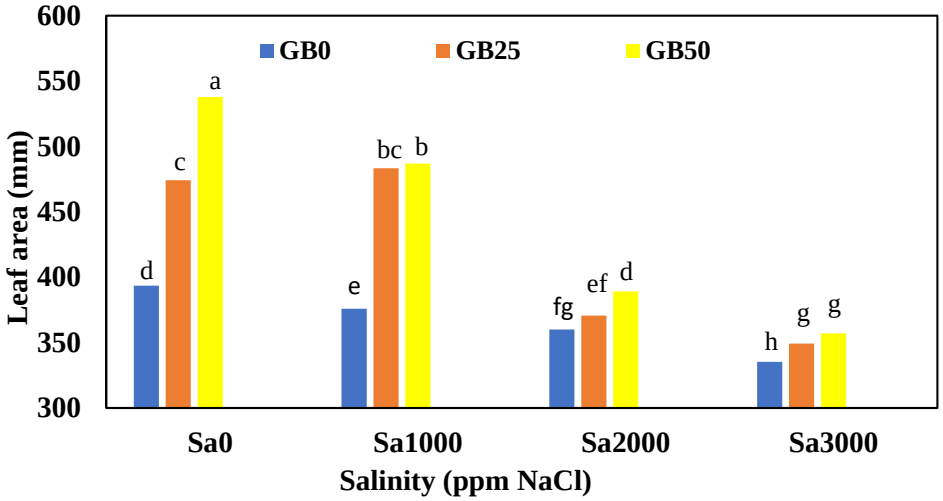

Figure 2. Effect of treatments with salinity concentrations (0, 1000, 2000, 3000 ppm) and glycine betaine (0, 25, 50 mM) on leaf area in squash plants at (38) DAS.

3.2. Photosynthetic pigments

The results of the statistical analysis showed that there were significant differences in carotenoids by which decreased with increasing salinity effect compared to the control (Table 3). The salinity concentration of 3000 ppm showed the highest effect on the carotene (carotene = 0.65 µg mm^{-2}), as compared with the control (carotene = 0.81 µg mm^{-2}). Furthermore, the results also showed that there was a significant effect on the carotenoids when using (GB). The effect of GB50 mM treatment on carotenoids was relatively higher (carotene = 0.84 µg mm^{-2}) than the effect of GB25 mM (carotene = 0.79 µg mm^{-2}) when compared to the control at GB0 mM concentration (carotene = 0.57 µg mm^{-2}). As well as the results also demonstrated that the treatments of salinity with GB have significant differences on the carotene. It showed that the treatment of GB50 mM at 1000 ppm salt concentration had the highest impact on the carotenoids (carotene = 0.86 µg mm^{-2}), meanwhile, the GB25 at 3000 ppm concentration was the lowest impact (carotene = 0.76 µg mm^{-2}). Also, the effect at the treatments of GB50 mM (carotene = 0.81 µg mm-2) under 2000 ppm and GB25 mM (carotene = 0.79 µg mm^{-2}) under 1000 ppm salinity concentration both showed no significant difference on the carotene.

The statistical analysis showed that there were significant differences in the Chlorophyll A that decreased with increasing salinity effect when compared to the control (Table 3). The salinity concentrations at 1000 ppm (Chll A = 2.26 µg mm^{-2}) and 2000 ppm (Chll A = 2.16 µg mm^{-2}) had significantly different effects on the Chlorophyll A, even though the salinity concentration at 3000 ppm had the greatest effect on the Chlorophyll A (Chll A = 2.01 µg mm^{-2}). Furthermore, the results also showed that there was a significant effect on the Chlorophyll A when using (GB). The GB50 mM had the highest effect on Chlorophyll A (Chll A = 2.47 µg mm^{-2}) than the effect of GB25 mM (Chll A = 2.34 µg mm^{-2}) when compared to the control at GB0 mM concentration (Chll A = 1.81 µg mm^{-2}). The treatments of salinity with GB had significant differences on the Chlorophyll A. It was clear that the effect of the treatment at GB50 mM under a salinity concentration of 1000 ppm had the highest impact on the Chlorophyll A (Chll A = 2.52 µg mm^{-2}), whereas at the treatment of G25 mM under a 3000 ppm the effect was relatively low (Chll A = 2.29 µg mm^{-2}). On the other hand, the effect of the treatments at GB50 mM (Chll A = 2.31 µg mm^{-2}) under salinity concentration of 3000 ppm and GB25 mM (Chll A = 2.30 µg mm^{-2}) under a 2000 ppm had no significant differences on the Chlorophyll A.

It was observed that Chlorophyll B decreased as the salinity concentration increased. The statistical analysis revealed a significant difference in the Chlorophyll B under different treatments (Table 3). A salinity concentration of 3000 ppm had the highest effect on the Chlorophyll B (Chll B = 0.80 µg mm^{-2}), whereas a salinity of 2000 ppm (Chll B = 0.86 µg mm^{-2}) and at 1000 ppm (Chll B = 0.90 µg mm^{-2}) had significant difference compared to the control (Chll B = 0.96 µg mm^{-2}). In addition, the results clarified that there was a significant effect on the Chlorophyll B when using (GB). The GB50 mM had the highest effect on Chlorophyll B (Chll B = 0.99 µg mm^{-2}) than the effect of GB25 mM (Chll B = 0.94 µg mm^{-2}) when compared to the control at GB0 mM concentration (Chll B = 0.72 µg mm^{-2}). Meanwhile, the results revealed that the salinity treatments with GB had a significant effect on the Chlorophyll B of leaves. The highest Chlorophyll B (Chll B = 1.01 µg mm^{-2}) was observed under treatment at GB50 mM concentration with a salinity concentration of 1000 ppm, while the lowest effect (Chll B = 0.91 µg mm^{-2}) was noticed under treatment at GB25 mM concentration with a salinity concentration of 3000 ppm. Also, there was no significant difference between treatments at GB50 mM (Chll B = 0.92 µg mm^{-2}) under a salinity concentration of 3000 ppm and GB25 mM (Chll B = 0.92 µg mm^{-2}) under 2000 ppm on the Chlorophyll B.

Table 3. Effect of exogenous spray applications of glycine betaine (GB) on carotene, Chlorophyll A (Chl. A) and Chlorophyll B (Chl. B) of squash plants grown under different NaCl concentrations.

Treatments		Carotenoids	Chl. A	Chl. B
Salinity (ppm NaCl)	GB	µg mm^{-2}	µg mm^{-2}	µg mm^{-2}
0	0 mM	0.69f	2.11f	0.84f
	25 mM	0.82c	2.42c	0.97c
	50 mM	0.91a	2.65a	1.06a
1000	0 mM	0.61g	1.89g	0.76g
	25 mM	0.78cde	2.29e	0.94cde
	50 mM	0.86b	2.65f	1.01b
2000	0 mM	0.56h	1.79h	0.71h
	25 mM	0.77de	2.30de	0.92de
	50 mM	0.81cd	2.39cd	0.96cd
3000	0 mM	0.42i	1.43i	0.57i
	25 mM	0.76e	2.29e	0.94e
	50 mM	0.77de	2.31de	0.92de

Values are means of three replicates. Mean values in each column followed by a different lower-case-letter are significantly different by Duncan's multiple range test at $P \leq 0.05$.

4. Discussion

The current study indicated that foliar exogenous application of GB treatment significantly increased growth characteristics (e.g., shoot fresh and dry weights, shoot length and stem diameter, leaf area, and the number of leaves) that were obviously decreased when exposed to different salinity levels (1000, 2000, and 3000 ppm NaCl) in squash plants. These findings supported previous research that found salt stress reduced leaf area, limited water absorption, and disrupted metabolic processes [21]. However, the current acceleration of biomass and growth accumulation may result from the benefits of the foliar application of GB on the photosynthetic process under salinity stress. The treatments of 25 or 50 mM GB significantly increased the growth characteristics, including shoot fresh and dry weights (Table 1), shoot length and stem diameter (Table 2), number of leaves (Figure 1), as a result of increased photosynthetic activity and the metabolic product when compared to those of control conditions.

The 25 mM exogenous application of GB was the most effective in all growth characteristics except the leaf area of the studied plant, which was shown to be enhanced at 50 mM in this study. Our results on squash growth characteristics were in harmony with those of, who concluded that exogenous GB application increased dry and fresh weights of roots and shoots in maize and safflower plants grown under salt stress. Also, the foliar application of GB improved production, water absorption, nutrient uptake, and their translocation into growing plant parts. This might encourage the development and growth of biomass and more leaves. [8,22-24]. These results were supported by, who showed a significant increase in stomatal conductance values of tomato, maize, and eggplant, respectively, when GB was administered as a foliar spray to salt-stressed plants [25-27]. According to higher turgor pressure in stomatal guard cells may result in increased stomatal conductance in salt-stressed plants induced by GB foliar treatment. demonstrated that exogenous application of GB to salt-stressed maize plants enhanced growth, leaf water content, net photosynthesis, and the apparent quantum yield of photosynthesis. The exogenously applied GB increased the proportions of water bound in the cell owing to its hydrophilic characteristic, which in turn improved the turgor pressure in guard cells and led to an increase in stomatal conductance. However, the increased yield by GB application during salt stress might be attributed to availability of water by GB in plant tissue, leading to enhanced solubility of nutrients in tissue cells, which allowed the squash plant to maximize its metabolic processes and photosynthetic compounds [15,26,28,29].

As reported in the current work, salinity stress can have negative effects on both the efficiency of photosynthetic activity and the production of photosynthetic pigments [30]. However, foliar feeding of GB significantly improved the chlorophyll and carotenoid contents of squash leaves grown under salinity stress conditions. Exogenous application of 25- or 50-mM GB significantly enhanced photosynthetic pigments compared to stress-free controls (Table 3), with exogenous application of 50 mM GB being the most effective in this investigation. The preservation of endogenous water availability may be the cause of the increase in photosynthetic pigments caused by GB treatment. Additionally, by stabilizing the activity of proteins under salinity stress, GB can defend the photosynthetic apparatus. These increases in chlorophyll and carotenoid contents are consistent with authors who reported that GB treatment on plants grown under salt stress led to an increase in photosynthetic pigments [31-32]. This is also consistent with the previous reports [33–38].

5. Conclusions

This study revealed that using glycine betaine at different concentrations could improve plant growth and development and increase photosynthetic rates in plants grown under salt stress. Treatments with glycine betaine increased the development of shoot fresh and dry weights, shoot length and stem diameter, leaf area, and leaf number of squash plants, which were considerably decreased when subjected to a range of salt stresses. Exogenous treatments of 50 mM GB significantly increased squash leaves' chlorophyll and carotenoid contents under salt-stress conditions.

Author Contributions

Conceptualization, E.H.A. (Esraa H. Alharbi), R.S.J. (Rewaa S. Jalal), and S.M.H. (Saad M. Howladar); investigation, E.H.A. (Esraa H. Alharbi), R.S.J. (Rewaa S. Jalal), and S.M.H. (Saad M. Howladar) ; data curation, E.H.A. (Esraa H. Alharbi), and S.M.H. (Saad M. Howladar); formal analysis, E.H.A. (Esraa H. Alharbi), R.S.J. (Rewaa S. Jalal), and S.M.H. (Saad M. Howladar); methodology, E.H.A. (Esraa H. Alharbi), and S.M.H. (Saad M. Howladar) ; resources, E.H.A. (Esraa H. Alharbi), R.S.J. (Rewaa S. Jalal), and S.M.H. (Saad M. Howladar) ; software, E.H.A. (Esraa H. Alharbi), and S.M.H. (Saad M. Howladar) ; writing—original draft, E.H.A. (Esraa H. Alharbi), and S.M.H. (Saad M. Howladar) writing—review and editing, E.H.A. (Esraa H. Alharbi), and S.M.H. (Saad M. Howladar). All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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