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Influence of Exogenous Application of Glycine Betaine on Growth and Ion Accumulation in Strawberry Plants under Saline Condition

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ABSTRACT

Glycine betaine (GB) plays a crucial role in plants and in their response to abiotic stress. This experiment was conducted to evaluate the application of glycine betaine (GB) and its ability to alleviate the effects of salinity stress (SS) on fruit yield and ion accumulation in strawberry (*Fragaria × ananassa* Duch cv. Paros). Three levels of SS (0, 20, and 40 mM NaCl) and GB (0, 5, 10 mM) were used on the plants in a greenhouse experiment. The results indicated that increasing the salinity level reduced the yield and altered the dynamism of ion accumulation. Leaf area, relative water content (RWC), leaf fresh weight, and yield decreased under salinity stress (36.7%, 9.2%, 28%, and 41%, respectively), especially at 40 mM NaCl. Under SS, there was an increase in Na content of the roots, fruits, and leaves (78%, 54%, and 78%, respectively) as well as in K content of the fruits (50%), but with a decrease in the K content of the leaves (29%) and the roots (25%), and P content of the leaves (55%). Overall, salinity increased the Na content, but reduced the K/Na ratio. Salinity and glycine betaine interactions had a significant effect on the Na content of the roots and leaves, the K content in the leaves, and K/Na ratio in the leaves and roots. At 40 mM NaCl, using 10 mM GB reduced the leaf and root Na content by 22% and 30%, respectively. Although the application of exogenous GB on strawberry changed the pattern of ion accumulation, it was not effective in diminishing the adverse effects of salinity stress on strawberry plants cv. 'Paros'.

Introduction

Strawberry plants (*Fragaria×ananassa* Duch.) represent an important commercial fruit crop, with increasing cultivation areas worldwide that should meet rising demands. The species is considered susceptible to NaCl salinity stress (Keutgen, 2009). Nonetheless, it can be grown successfully in different parts of Iran, from the north to the south, with a range of cold to tropical climates (Morgan, 2006). There are hundreds of strawberry cultivars being commercially produced around the world. The greenhouse production of strawberry in Iran has expanded extensively over the past few decades, and hydroponic systems are progressively being applied. The major cultivated cultivar in Iran is 'Paros', a short-day cultivar, which is widely cultivated in the west of Iran and is resistant to powdery mildew and leaf spot diseases (Ebrahimi, 2012).

While salinity is becoming an increasing environmental problem for crop production

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(Tang et al., 2015), negative effects of NaCl salinity on strawberry plant growth and fruit productivity have been reported (D'Anna et al., 2003). Salt stress results in the development of leaf necrosis and accelerated leaf senescence, thereby reducing photosynthetic capacity in plants. This adversely affects assimilation of carbohydrates for fruit production. There are several reports on different responses of strawberry cultivars to salinity stress. Salt stress reduces fresh and dry matter of the whole plant body and affects photosynthetic-active leaf areas. The NaCl salinity stress, at high levels, diminishes total leaf area per plant due to a decrease in leaf number. The presence of NaCl in the root medium induced an increase in Na and Cl concentrations in all organs of several plants in research (Giuffrida, 2001, Keutgen and Keutgen, 2003, Saied, 2005 and Keutgen, 2009). So far, finding a way to alleviate the influence of salinity stress on strawberry has drawn much attention. Glycine betaine (GB) has been reported to accumulate in the cytoplasm as an osmolyte in a wide range of plants under environmental stress conditions. In previous research, it showed stabilizing, protective actions on enzymes and membranes under unfavorable, stressful conditions (Ashraf and Foolad, 2007; Wyn Jones, 1981). Compatible solutes, such as glycine betaine, are known to play a role in osmotic adjustment in many crops, as they accumulate under environmental stresses. Exogenous applications of glycine betaine have reportedly ameliorated the effects of drought, salt and temperature-related stresses. The most prominent effects of exogenous glycine betaine applications were reportedly on tomato plants, whereby the accumulation of Na^+ and Cl^- decreased as a result of the applications. Plant growth tends to be seriously inhibited by the addition of glycine betaine to the growing media. There have been reports on negative responses by plants to exogenous applications of glycine betaine (Heuer, 2003). Endogenous GB levels in strawberry leaves were reportedly evaluated during acclimation to cold temperatures (Rajashekar, 1999). While previous studies have sought to evaluate the effects of GB application on plants, there is little information on the strawberry response to salinity. The aim of this study was to evaluate the ability of GB application to alleviate the side effects of salinity on strawberry plants.

Materials and Methods

The short-day strawberry (*Fragaria × ananassa* Duch. cv. 'Paros') was obtained from the Agricultural Center of Kermanshah, Iran. Well-rooted strawberry daughter plants, similar in their sizes, were selected and cultivated in containers (13 cm diameter) filled with 1.5 kg of culture medium that contained perlite and coco peat (1:1 V: V). After planting, sufficient irrigation was administered until the plants were well-established, with the salinity treatment being performed four weeks after cultivation. A hydroponic system was used with an open nutrition system. The plants were fertilized every day with mono potassium phosphate, N-P-K (18-18-18) and calcium nitrate alternately. Microelements were added using Brexil Combi fertilizer (Valagro, Mexico) as foliar sprays, weekly, and pH value of the solution was adjusted to 5.8-6.2 using $HNO^{-}{}_{3}$. A factorial experiment was arranged based on a completely randomized design with four replications. Four weeks after planting until the end of the experiment, which corresponded to three months in the growing season, NaCl treatments were applied by irrigating each plant with 0, 20 and 40 mM NaCl solutions, three times a week (Seyed Lar Fatemy, 2009; Keutgen, 2008). Soil leaching was performed on each plant once a week to prevent the accumulation of salt. Three GB treatments (Dora Agri-Tech, China) (0, 5 and 10 mM) were applied as foliar sprays by a hand sprayer, 10 days after the first stress was administered on plants (Hamani, 2021, Heure, 2003, Rajashekar, 1999 and Xing 2001). Fruits were harvested at an optimal stage of physiological maturity when 90% of the fruit surface had reached a full red color.

At the end of the experiment, the plants were collected from each treatment to measure fresh and dry weights of the fruits, leaves and roots, total leaf area, and leaf count per plant. The samples were placed in an oven at 70 °C for 48 hours. Relative water content and electrolyte leakage percentage were determined according to Yamasaki et al. (1999) and Lutts et al. (1996), respectively. The sodium, phosphorus and potassium contents were measured in the dried leaves, roots and fruits according to Wahing et al. (1989) and Chapman and Pratt (1961). Concentrations of sodium and potassium were analyzed using a flame photometer. The phosphorus concentration was determined by a spectrophotometer.

Data were analyzed with the MSTATC statistical package (MSTATC, Michigan State University, East Lansing, MI) according to a factorial experiment on a completely randomized design. Mean values were compared using Duncan's new multiple range test (DNMRT).

Results

The analysis of variance showed that the salinity stress caused a decrease in fruit yield, leaf fresh weight, relative water content and leaf area in strawberry cv. 'Paros' (Table 1). The effect of salinity on fresh and dry weight of roots and shoots, leaf dry weight, electrolyte leakage percentage and leaf count were not significant ($p<0.05$). However, salinity stress significantly influenced fruit yield, fresh leaf weight, relative water content and leaf area. The decrease in leaf area, leaf fresh weight, relative water content, and yield were statistically significant ($p<0.01$) at a salinity level of 40 mM (36.78%, 28%, 9.2%, and 41%, respectively) (Table 2). The highest and lowest leaf areas were observed in response to 0 and 40 mM NaCl treatments, respectively. There were also significant differences in leaf relative water content (RWC) between the control and the other treatments, as the salinity reduced RWC (Table 2).

Table 1. Analysis of variance of the effect of salinity treatments and glycine betaine on morphological and physiological indices of *Fragaria ananassa*

Source of variance	df	Mean of squares					
		Root dry weight (g)	Root wet weight (g)	Shoot dry weight (g)	Shoot wet weight (g)	Leaf dry weight (g)	Leaf wet weight (g)
Salinity (S)	2	0.34^{ns}	12.21^{ns}	5.82^{ns}	1.24^{ns}	4.39^{ns}	2.20**
Glycine betaine(GB)	2	0.02^{ns}	0.89^{ns}	2.13^{ns}	0.04^{ns}	0.43^{ns}	0.27^{ns}
S × GB	4	0.21^{ns}	7.37^{ns}	14.53^{ns}	0.16^{ns}	1.71^{ns}	0.14^{ns}
Error	27	0.39	7.39	6.33	0.49	2.13	0.19
CV (%)		30.44	18.81	28.95	11.19	24	9.17

Source of variance	df	Mean of squares				
		Yield (g/plant)	Relative water content (%)	Leaf area (mm^2)	Leaf number	Electrolyte leakage percentage (%)
Salinity (S)	2	34619.16**	124.53**	6291213109.02**	0.10^{ns}	3.098^{ns}
Glycine betaine(GB)	2	2620.14*	7.35^{ns}	7729624.96^{ns}	0.07^{ns}	13.49^{ns}
S × GB	4	812.13^{ns}	1.80^{ns}	446929018.28^{ns}	0.002^{ns}	8.37^{ns}
Error	27	599.922	8.42	742876022.68	0.06	8.27
CV (%)		11.86	4.45	30.96	9.49	16.10

Source of variance	df	Mean of squares					
		Root Na	Leaf Na	Fruit Na	Root K	Leaf K	Fruit K
Salinity (S)	2	26133934.33**	204.20**	216.75*	7659377.77**	9914877.77**	52754460.02*
Glycine betaine(GB)	2	1633779*	21.11^{ns}	8.39^{ns}	1735277.77^{ns}	2335277.77^{ns}	33192249.36^{ns}
S × GB	4	1472540.33**	76.15**	20.13^{ns}	848761.11^{ns}	17033377.77**	8467547.69^{ns}
Error	27	361883.22	13.51	61.10	528681.48	4107048.14	11779791.21
CV (%)		10.37	6.18	14.49	14.28	11.99	28.81

Source of variance	df	Mean of squares					
		Root P	Leaf P	Fruit P	Root K/Na ratio	Leaf K/Na ratio	Fruit K/Na ratio K
Salinity (S)	2	423.10*	560.77**	7.77^{ns}	2.31**	7.30**	1.84^{ns}
Glycine betaine(GB)	2	3575.77^{ns}	379.77*	287.16^{ns}	0.026^{ns}	0.06^{ns}	3.42^{ns}
S × GB	4	29.95^{ns}	155.37^{ns}	183.56^{ns}	0.058*	3.31*	0.53^{ns}
Error	27	84.17	91.54	153.10	0.014	0.89	1.61
CV (%)		29.11	25.42	30.87	12.48	19.20	30.68

**: Significant at the 0.01 *: Significant at the 0.05 ns: Non significant

Table 2. Effects of salinity treatments on morphological and physiological indices in *Fragaria ananassa*

Salinity treatment (mM)	Vegetative growth index			
	Yield (g/plant)	Relative water content (%)	Leaf area (mm^2)	Leaf wet weight (g)
S1 (0 mM)	246.7[a]	68.76 [a]	110435.42[a]	28.00[a]
S2 (20 mM)	228.30 [a]	64.69[b]	89031.25[b]	21.12[b]
S3 (40 mM)	145.44 [b]	62.40[b]	69808.01[b]	20.15[b]

†Based on Duncan's new multiple range test ($p<0.05$), mean values in each row with the same letter are not significantly different. Abbreviations: df, degree of freedom; P, phosphorus; K, potassium; Na, Sodium; CV, coefficient of variation

The results of variance analysis showed the significant effects of salinity stress on different parameters of strawberry cv. 'Paros' (Table 1). The presence of NaCl in the root increased the Na, K and P concentrations in all plant organs. The results indicated that salinity significantly ($p<0.05$) increased Na in the roots (78%), leaves (78%), and fruits (54%) at 40 mM NaCl. The highest Na content was observed in response to severe stress levels. The highest level of Na was observed in the roots and the lowest level of Na was measured in the leaves and fruits. Na content in the fruits was lower than in other organs (Fig. 1, 2 and 3).

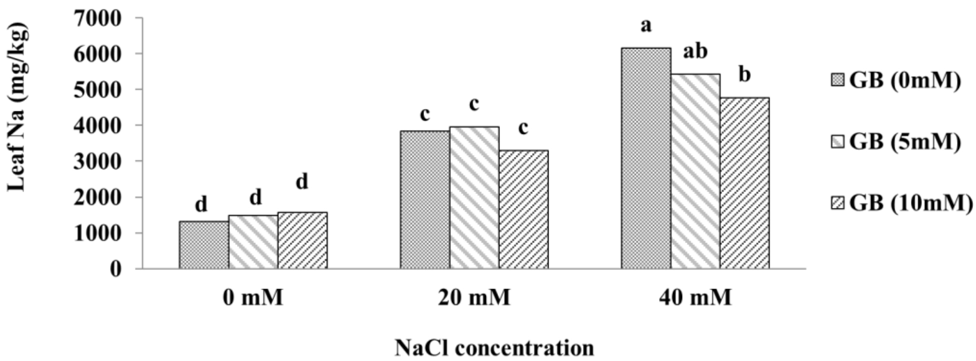

Fig. 1. Effects of salinity (NaCl) and glycine betaine treatments on leaf Na content. Abbreviations: Na, Sodium; Cl, chloride; GB, glycine betaine. Different letters denote a significant difference based on Duncan's multiple range test

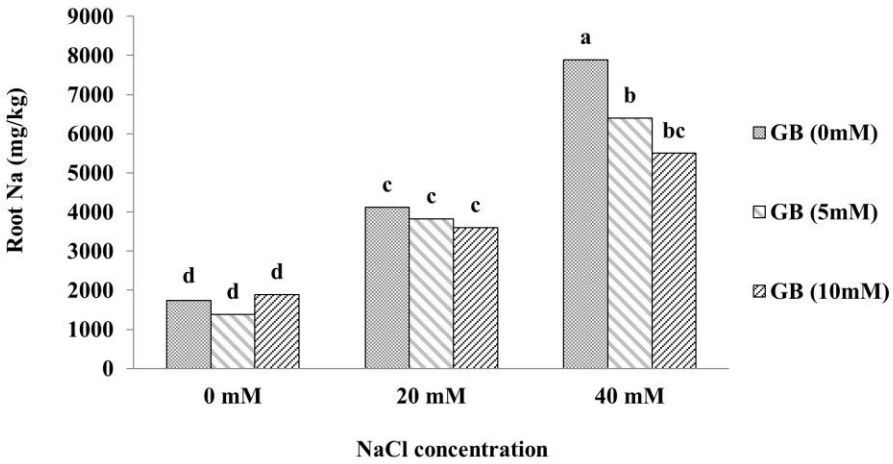

Fig. 2. Effects of salinity (NaCl) and glycine betaine treatments on root Na content. Abbreviations: Na, Sodium; Cl, chloride; GB, glycine betaine. Different letters denote a significant difference based on Duncan's multiple range test

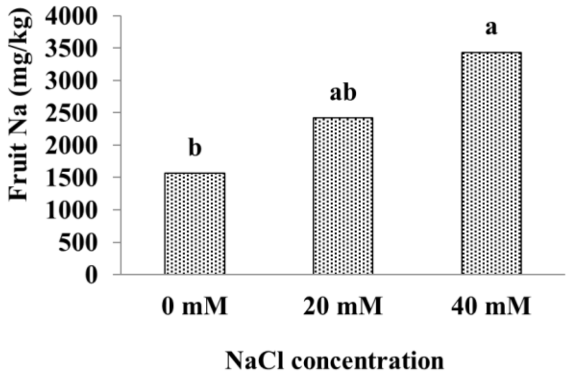

Fig. 3. Effects of different levels of salinity (NaCl) on fruit Na content. Abbreviations: Na, Sodium; Cl, chloride. Different letters denote a significant difference based on Duncan's multiple range test

Salinity stress influenced K and P contents in the roots, leaves and fruits at the salinity levels of 20 and 40 mM NaCl ($p<0.05$). Potassium content in the control plants was higher than that of Na. The results indicated that increasing the level of salinity stress caused a decrease in the K content of the roots (19% and 25%) in response to 20 and 40 mM NaCl, respectively, and the application of 40 mM NaCl reduced the accumulation of ions in the leaves (29%) significantly ($p<0.05$). The same treatment increased the K content in the fruits by 50%. Among the different organs, the highest concentration of K was found in the leaves and fruits, and the lowest content was observed in the roots. The strawberry plants showed an increase of K in the fruits under salinity stress (Fig. 4, 5 and 6).

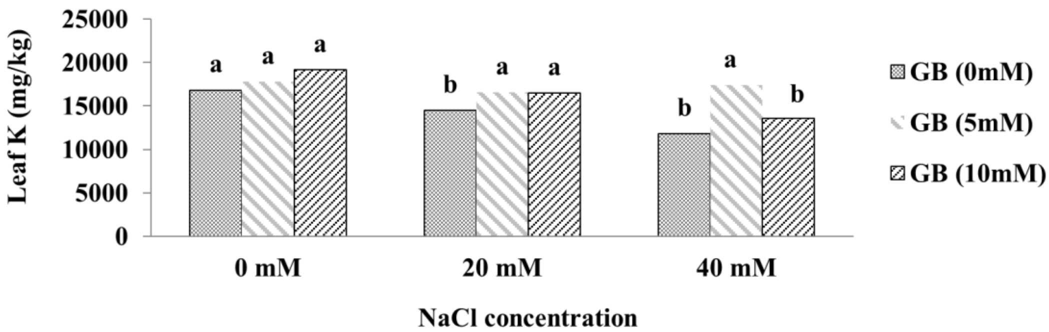

Fig. 4. Effects of salinity (NaCl) and glycine betaine treatments on leaf potassium content. Abbreviations: Na, Sodium; K, potassium; Cl, chloride; GB, glycine betaine. Different letters denote a significant difference based on Duncan's multiple range test

Increasing the salinity level led to a significant decrease in K/Na ratio. The K/Na ratio decreased in response to 40 mM salinity levels in the roots (77%) and leaves (86%). The highest K/Na ratio was found in the control treatment in the roots and leaves. The K/Na ratio in the leaves was higher than that of the roots (Fig. 7 and 8). There was a significant decrease in the P concentration of the leaves (55%) at 40 mM NaCl. The P concentration in the leaves decreased significantly after exposure to salinity ($P<0.05$) (Fig. 9 and 10).

The results indicated that GB application decreased fruit yield (13%) significantly at 5 mM (Fig. 11). The effect of GB application on ion accumulation in both leaves and roots was significant. GB application reduced the Na content in the leaves and roots (22% and 30%, respectively) at 40 mM NaCl (Fig. 1 and 2). Moreover, the application of 5 and 10 mM GB increased the K content of the leaves in response to 20 and 40 mM NaCl significantly. The 10 mM concentration of GB reduced the K content in the roots (13.7%) under salinity (Fig. 4 and 12). The low concentration of GB was effective only in response to an increase in the leaf K content in response to 20 and 40 mM NaCl (Fig. 4). However, 5 mM GB increased the P content in the leaves (Fig. 13) and increased the K/Na ratio in the roots of the control plants (Fig. 7).

The analysis of variance showed that GB significantly influenced the yield, root Na and leaf

P, but it did not show a significant effect on any of the growth parameters when salt stress was adversely effective on the strawberry plants (Table 1). The interaction of GB and salinity stress had a significant effect on Na content in the roots and leaves, the K content in the leaves and the K/Na ratio in the leaves and roots (Table 1).

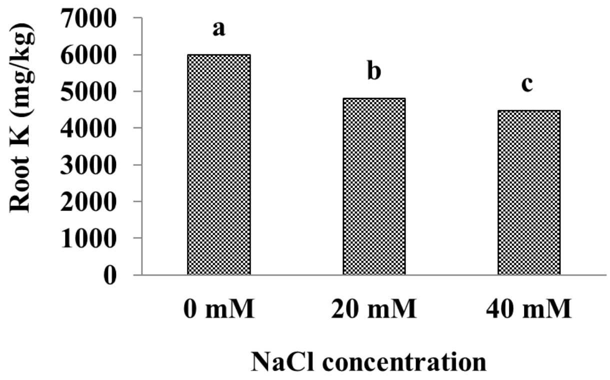

Fig. 5. Effects of different levels of salinity (NaCl) on root potassium content. Abbreviations: Na, Sodium; K, potassium; Cl, chloride. Different letters denote a significant difference based on Duncan's multiple range test

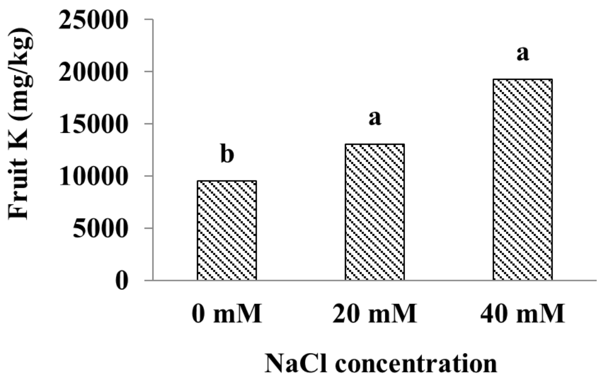

Fig. 6. Effects of different levels of salinity (NaCl) on fruit potassium content. Abbreviations: Na, Sodium; K, potassium; Cl, chloride. Different letters denote a significant difference based on Duncan's multiple range test

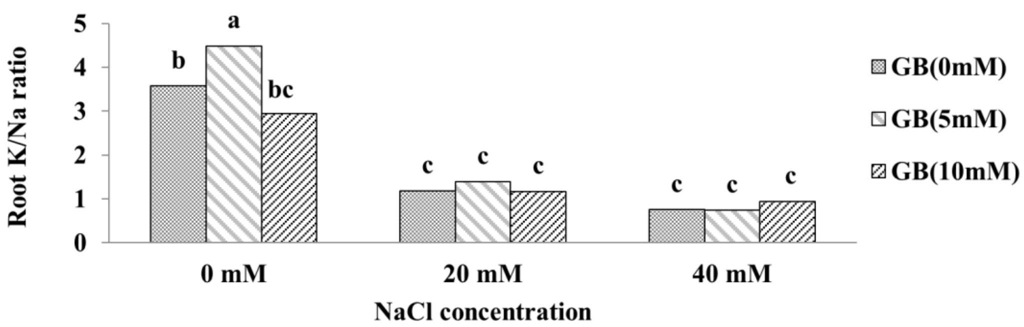

Fig. 7. Effects of salinity (NaCl) and glycine betaine treatments on root K/Na ratio. Abbreviations: Na, Sodium; K, potassium; Cl, chloride; GB, glycine betaine. Different letters denote a significant difference based on Duncan's multiple range test

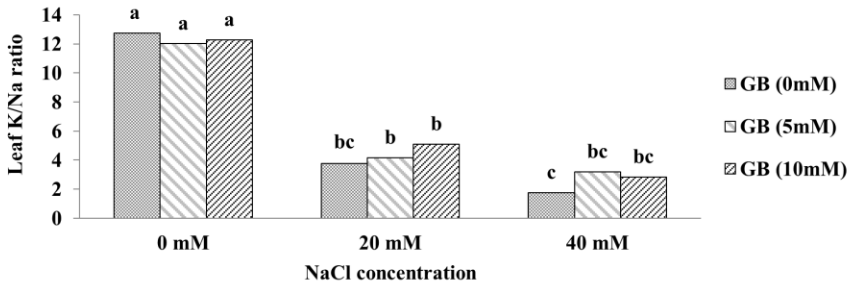

Fig. 8. Effects of salinity (NaCl) and glycine betaine treatments on leaf K/Na ratio. Abbreviations: Na, Sodium; K, potassium; P, phosphorus; Cl, chloride; GB, glycine betaine. Different letters denote a significant difference based on Duncan's multiple range test

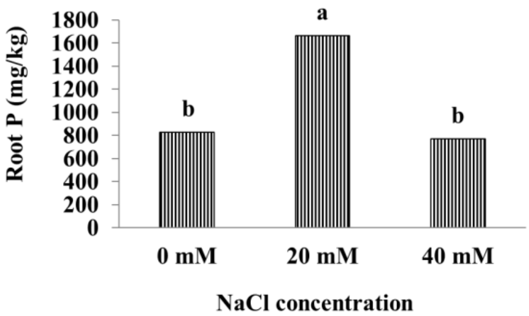

Fig. 9. Effects of different levels of salinity (NaCl) on root phosphorus content. Abbreviations: Na, Sodium; P, phosphorus; Cl, chloride. Different letters denote a significant difference based on Duncan's multiple range test

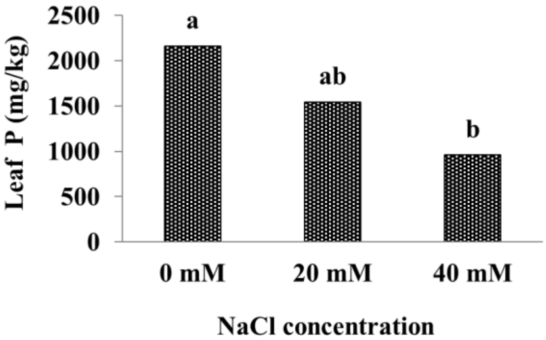

Fig. 10. Effects of different levels of salinity (NaCl) on leaf phosphorus content. Abbreviations: Na, Sodium; K, potassium; P, phosphorus; Cl, chloride. Different letters denote a significant difference based on Duncan's multiple range test

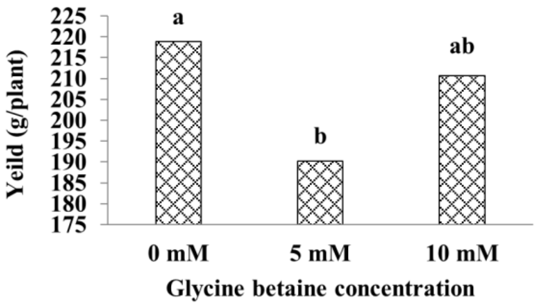

Fig. 11. Effects of glycine betaine on yield, under salinity stress. Different letters denote a significant difference based on Duncan's multiple range test

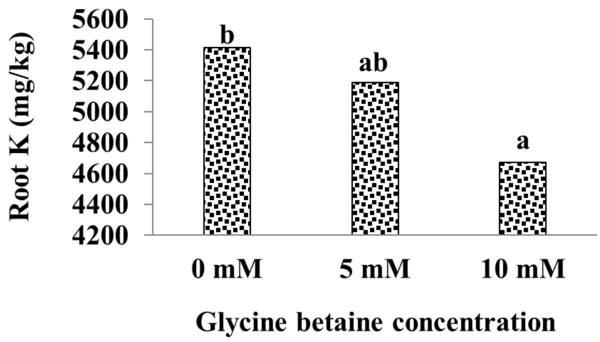

Fig. 12. Effects of glycine betaine different levels on root potassium content under salinity stress. Abbreviations: K, potassium. Different letters denote a significant difference based on Duncan's multiple range test

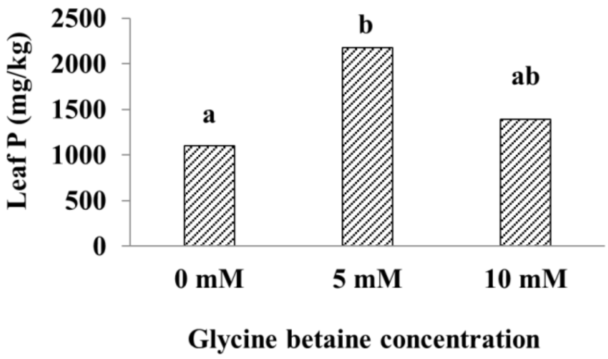

Fig. 13. Effects of glycine betaine different levels on leaf phosphorus content under salinity stress. Abbreviations: Na, Sodium; K, potassium; P, phosphorus; Cl, chloride. Different letters denote a significant difference based on Duncan's multiple range test

Discussion

Salinity treatment reportedly decreased the total leaf area in both "Korona" and "Elsanta" cultivars, which might be due to a decrease in leaf count. In strawberry plants, cv. 'Elsanta', single leaf area was significantly reduced to a size comparable to that of cv. 'Korona'. Leaf area was also significantly reduced in cv. 'Elsanta' due to the excessive appearance of necrosis, which was typical of Cl stress (Chaves et al., 2009; Keutgen, 2009). Based on previous research, RWC decreased significantly in the leaves of both cultivars under NaCl stress which contradicts the findings of this research. It seems that any reduction in leaf area due to stress can be related to a decrease in leaf count, slower cell division and less cellular elongation. Such a behavior would be typically the earliest response of glycophytes to salt stress. Reducing the leaf area may reduce total plant transpiration and, therefore, can be considered as a strategy of salinity tolerance. The decrease in photosynthetic area was not only due to a decrease in leaf area, but was also connected to growth-inhibiting effects of NaCl (Chaves et al., 2009; Keutgen, 2009). The decrease in leaf area was in agreement with previous studies by Saied et al. (2003), Khayyat (2009), Keutgen (2009), Turhan and Eris (2007) on other cultivars of strawberry.

In a similar research, salinity stress reduced the fruit yield and the leaf fresh weight in 'Camarosa' cultivars (Lolaee, 2013). In another research, salinity stress reduced the dry weights of roots, shoots, and the fresh weight of fruits in 'Selva' cultivar (Mazloomi, 2011). Salt stress reduced the fresh weight, dry weight, and the photosynthetic-active leaf area, especially in cv. 'Elsanta' (Keutgen, 2009). The negative influences of NaCl salinity on strawberry growth and fruit productivity have been reported by D'Anna et al. (2003), Keutgen (2008), Seyed Lar Fatemy (2009) and Yusefi (2010). Similarly, a report showed that salinity reduced fruit yield in 'Korona' and 'Elsanta' strawberry cultivars, compared to the control (Keutgen, 2009). Increasing the level of salinity reduced flower and fruit counts, thereby resulting in a lower yield. Also, salt stress increased leaf necrosis and accelerated its senescence, thereby reducing not only the photosynthetic capability of the plants but also the assimilation of available carbohydrates for fruit production (Giuffrida et al., 2001; Keutgen, 2003; Saied et al., 2005). A reduced level of water availability induced numerous physiological and biochemical changes in all plant organs. Gas exchange in the leaves is usually limited which, in turn, reduces carbon assimilation. Changes in the distribution of photo-assimilates can reduce vegetative growth and severely delay the development of plant reproductive organs (Gehrmann, 1985; Singer et al., 2003).

We also observed a decrease in RWC due to salinity stress. The water content of the plant organs was significantly influenced by the NaCl levels in the root medium. The water content increased in the roots, crown and petioles under NaCl stress. However, the water content decreased significantly in the leaves of both 'Korona' and 'Elsanta' cultivars (Keutgen, 2009). RWC is considered as an important criterion of plant water status. In addition, leaf RWC reflects the general metabolic activity in tissues (Flower and Ludlow, 1986).

The effects of salinity stress on different parameters of strawberry (cv. 'Paros') were measured in this study. Similarly, salinity (NaCl) increased the concentration of Na in the roots, shoots and fruits of strawberry (cv. 'Selva') (Mazloomi, 2011) which is similar to the results of this research. Based on other cases of research, the presence of NaCl in the root medium caused an increase in Na concentrations in all organs of the plant. An accumulation of Na increased in both 'Korona' and 'Elsanta' cultivars as a result of NaCl treatments in all plant organs, but no significant difference was found in the total Na content of plants in both cultivars. In general, cv. 'Korona' maintained a high level of Na in its roots and petioles (Keutgen, 2009). The data presented herein showed that the presence of NaCl in the growth medium can alter the nutrient content in the shoots and roots, which supports previous findings by Saied et al. (2003) who reported that salt stress can increase Na and Cl level in the roots and shoots of strawberry.

Salinity stress influenced K and P contents in the roots, leaves and fruits at different salinity levels. The K content decreased in the roots by NaCl treatment, which was not in agreement with the results of another similar case of research, wherein K uptake by the roots reportedly increased when the plants were exposed to NaCl (Kaya et al., 2001). The K concentration decreased in the roots under salt stress, while it increased in the fruits and was not affected in the shoots (Mazloomi, 2011). Also, Heuer (2003) indicated that salinity significantly reduced the K concentration in the leaves and roots of tomato plants. Similar to our findings, the 'Elsanta' cultivar under NaCl stress showed a significant increase in K content in the fruits and petioles (Keutgen, 2009).

Increasing the salinity level led to a significant decrease in the K/Na ratio. This can be attributed to the ability of the roots to purge Na and translocate them to the shoots, which would imply a better acclimation capacity. Salinity reduced the K/Na ratio in the roots and shoots of strawberry (Khayyat, 2009). Chauhan et al. (1980) observed that in saline stress-sensitive cereals, the K/Na ratio decreased in salt treatment, whereas potassium application increased this parameter. The Na/K ratio of plant organs was very small in the control groups under NaCl stress. The highest Na/K ratio was found in the roots, followed by lower values in the crowns and leaves (Keutgen, 2009). Exposure of plants to saline solutions increased the Na content significantly in the leaves and roots of tomato and strawberry (Heuer, 2003; Keutgen, 2009). In strawberry, cv. 'Selva', under saline conditions, the K concentration and K/Na ratio decreased, but the Na concentration increased and the P concentration was not affected (Seyedlar-Fatemy, 2009 and Khayyat, 2009). The NaCl-induced accumulation of Na and Cl, as well as the decrease in K content, are commonly observed in most species after exposure to salt stress (Aghaleh et al., 2011).

Strawberry cultivars usually differ in their responses to salt stress (Badawwi et al., 1990). The Na concentration in the roots was more than that in the leaves. The current research showed that strawberry plants maintained a high level of Na in the roots, thereby resulting in a low level of Na in the leaves and fruits. Contrary to the current results, a small increase in leaf Na content was observed in strawberry plants cv. 'Rapella' (Awang and Antherton, 1994) and 'Korona' (Keutgen, 2009) under NaCl stress.

The leaf count in the 'Paros' cultivar was not reduced under NaCl stress. Na absorption increased in the plants after exposure to NaCl, which subsequently caused a decrease in the K content (Hasegawa et al., 2000). Khayyat (2009) explained that under NaCl stress, Na content increased in various plant parts. Jacoby (1999) explained that K accumulation reflected plant adaptation to salinity. Not only K and Na amounts, but also the K/Na ratio can be used as a physiological parameter for evaluating sensitivity to NaCl stress (De Lacerda et al., 2005). A low K/Na ratio indicates metabolic disorders such as a decrease in protein synthesis and enzyme activities (Wu et al., 2013). K ions have an osmotic function and prevent Na influx into the roots and shoots (Barragan et al., 2012).

The yield, root Na and leaf P in strawberry (cv. 'Paros') were influenced by GB, but not under salt stress. In a similar case of research, the exogenous GB application caused a decline in the accumulation of Na content, accompanied by a heightened accumulation of K content, which led to an increase in the K/Na ratio in soybean plants under salt stress (Malekzadeh, 2015). However, the mechanism by which GB caused this decline in Na and increased K accumulation needs further research. A few possible explanations could be the biological membranes of plants, and transporters for absorption of K from the growth medium (Chaum and Kirdmanee, 2010). In fact, these play an important role in protecting cellular K/Na ratios (Farooq et al., 2008). GB retains the integrity of cellular membranes for appropriate actions by enzymes and proteins under adverse levels of environmental stress (Raza et al., 2007). Furthermore, GB protects diverse transporters for a natural function under drought stress (Mahmood, 2009). It can be inferred that GB maintains a protective function in discriminating Na versus K under saline conditions. GB can increase the vacuolar output in the roots of salt-stressed plants for the accumulation of more Na, which was studied earlier on rice (Rahman et al., 2002).

There was a decrease in fruit yield by the application of GB and its inability to improve the morphological and physiological characteristics under salinity stress. Osmotic adjustment at the physiological level is an adaptive mechanism involved in salinity tolerance, which permits the maintenance of turgor pressure under salt stress (Yang et al., 2014). Under saline conditions, osmotic adjustment is achieved either by ion uptake or by the accumulation of compatible solutes. A compatible solute, such as GB, is known to play a key role in the process of osmotic adjustment in many crops, as it accumulates under environmental stresses (Evelin et al., 2012). In a study on the influence of GB application on salt-stressed tomato plants, it was observed that 1 mM GB did not affect the control plants, whereas adding 5 mM GB severely reduced the leaf fresh weight of the control plants. Applying 5 mM GB to salt-stressed plants severely reduced root fresh weight (74%) after 21 days, compared to the control plants (Heuer, 2003).

Regarding the plant response to GB application, the results of this research did not comply with previous findings by Rajashekar (1999) which explained that GB levels increased in response to cold-acclimation in strawberry plants. Also, the current results did not support previous observations by Weibing (2001) who applied GB exogenously to Arabidopsis plants as a foliar spray, so that the freezing tolerance increased from -3.1 to -4.5 °C. However, the exogenous application of GB on tomato plants caused a slower accumulation of Na and Cl, while plant growth was seriously inhibited by adding GB to the growth media. The deleterious effects of GB were probably the result of reduced osmotic adjustment, because of the inhibitory properties of proline and ion accumulation, which can be attributed to the toxic effects of GB (Heuer, 2003).

Conclusion

GB reduced the Na concentration in the roots and leaves, but it was not effective in improving vegetative growth and fruit yield. Given that the 'Paros' cultivar was able to protect leaves more efficiently from Na accumulation, it seems that this cultivar is relatively tolerant to salinity. Therefore, the exogenous application of GB could change the pattern of ion accumulation and nutrient balance, although these could not alleviate the adverse effects of salinity stress and failed to induce any significant levels of salinity tolerance in the strawberry plants.

Conflict of interest

The authors declare no conflict of interest for this work.

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