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ORIGINAL ARTICLE

Mitigation of the Salinity Influences on Maize (*Zea mays* L.) Productivity by Exogenous Applications of Glycine Betaine

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A field experiment was carried out to estimate the advantageous efficiency of the foliar application of glycine betaine (GB) (10mM) on salt-stressed maize (*Zea mays* L.) plants. Salinity treatments (50 and 100 mM) were conducted using sodium chloride (NaCl).

The results showed that the increase in salinity levels led to a decrease in the maize shoot growth criteria (the leaves number per plant, leaf area index, shoot dry weight, plant height and ear height) and yield components (the ear diameter, ear length, kernels number per ear, seed weight per ear and the 100 seed weight). This effect was obvious with the 100 mM NaCl treatment for all measured parameters. On the other hand, the exogenous application of glycine betaine seemed to mitigate the negative effects of salt stress on the growth criteria and productivity of maize plants. So, it can be concluded that maize plants are not salt-tolerant, and the foliar application of GB might be useful as a possible growth regulator to ameliorate the growth and productivity of maize plants under salinity conditions.

Key words: Growth Criteria, Maize, Osmoregulation, Salt Stress, Yield

Salinity is considered to be an agricultural issue affecting the plants growth and production in the dry and semi-dry areas around the world decide it has become a predicament of serious concern (Alasvandyaria and Mahdavia, 2018). Due to limited rainfall and high evaporation rates, in addition to the poor quality of soil and bad water management, salinity stress has become an earnest impendent threat to plant production around the world. Despite this fact, it is generally assumed that salt stress occurs exclusively in the dry and semi-dry areas, while in reality, no climatic zone is exempt from this problem (Farooq *et al.*, 2016).

Maize (*Zea mays* L.) is considered the third crop in ranking after wheat and rice and it is grown in a broad range of soil types and environmental conditions. Maize is relatively susceptible to salinity stress, hence insight into the salt stress response of maize, its resistance techniques and overall handling choices may assist to innovate the planning process for an ameliorated maize attitude in the saline environments (Bose *et al.*, 2018).

Glycine betaine (GB) is an amphoteric quaternary ammonium compound, which has a significant role as a convenient solute for many plants under different kinds of environmental stresses such as salt, heat and drought stresses (Alasvandyaria and Mahdavia, 2018). Plant species have various abilities to synthesise GB: several plants like spinach and barley accumulate comparatively high levels of GB in their chloroplasts, while other plants such as Arabidopsis and tobacco are not able to synthesize GB (Sakamoto and Murata, 2002). It was found that the growth of plants that can naturally accumulate GB enhances under drought and salt stresses (Alasvandyaria and Mahdavia, 2018). In fact, GB was used as an exogenous application for limiting salt stress influences in plants, regardless of whether it synthesises GB or not, e.g. corn (Yang and Lu, 2005).

This study aims to investigate the effect of the foliar application of GB on the growth criteria and productivity of salt stressed maize plants (*Zea mays*).

MATERIALS AND METHODS

A field experiment was conducted in Egypt (30° 06' N and 31° 25' E) during the summer of 2018. The maize plant (*Zea mays*) seeds were supplied by the Ministry of Agriculture in Egypt. A homogenous lot of maize seeds were separately surface-sterilized by soaking them in 0.01% $HgCl_2$ for 3 minutes and further thoroughly rinsed in sterile water. The maize seeds was planted at 40 kg ha^{-1} rate (the recommended seeding rate) through the method of hand pulling drill. The plants were subjected to natural day/night conditions (minimum/maximum temperature and relative humidity were 29.2° C/33.2° C and 63%/68% respectively) at midday during the experimental period. All the plants were irrigated to field capacity by tap water.

Two weeks from sowing, the plots were divided into two sets. The first set continued to be irrigated with normal water and was further divided into two subgroups serving as control (Cont.) and control + glycine betaine (Cont. + GB). The second set was irrigated with saltwater and divided into four subgroups: 50 mM NaCl (50 mM), 100 mM NaCl (100 mM), 50 mM NaCl + glycine betaine (50 mM+ GB) and 100 mM NaCl + glycine betaine (100 Mm+ GB). The plants were sprayed with glycine betaine (10 mM) every week during the experiment. Further, nitrogen and phosphorus fertilizers were applied at two stages (soon after planting and during stem elongation) as 150 kg ha^{-1} (urea) and 200 kg ha^{-1} (calcium phosphate) respectively.

Growth Measurements

The growth measurements (leaves number per plant, leaf area index, shoot dry weight, plant height and ear height) of all the treated and untreated plant, were taken at 10 weeks after planting (10 WAP), and shoot dry weight was measured at 6, 8, 10 and 12 weeks after planting (6, 8, 10 and 12 WAP). To measure the shoot dry weights of the plants, the shoot system was put into sacks and then left to dry in a drying oven at 80° C until the weight was stable (Carpýcý *et al*., 2009). To calculate the mean measurement of each treatment, three replicates were taken.

Yield Components

The yield components (the ear diameter, ear length, kernels number per ear, seed weight per ear and the 100 seed weight) of the all treated and untreated plants were taken at the end of the experiment (the harvest time). To calculate the mean measurement of each treatment, three replicates were taken.

Statistical Analysis

The main effect of factors (salt stress concentration, glycine betaine and growth stages) were evaluated using the Statistical Package for the Social Sciences (SPSS) version 11.0 computer software package (Daniel, 1995). A two-tailed P value < 0.05 was statistically significant. The paired sample t-test was used to compare the means with and without the treatment of glycine betaine. A one-way analysis of variance (ANOVA) was used to find the significant differences in the maize plant growth criteria and yield parameters responses among the different treatments with a level of significance of less than 5% ($p < 0.05$).

RESULTS

Changes in the Maize Growth Criteria

Plant growth is affected by various internal and external factors besides its genetic makeup and the evaluation of plant growth is an important tool for assessing the crop productivity in various crops. The data presented in Table 1 shows that the treatment with 50 and 100 mM NaCl caused considerable decrease ($p < 0.05$) in the growth vigour of the shoots (the leaves number per plant, leaf area index, shoot dry weight, plant height and ear height) below the control values. This reduction was remarkable with the 100 mM concentration. The GB application generally retracted the effect of either concentrations, but the values remained less than those of the untreated plants. The maximum and minimum values of growth vigour of shoots were obtained with the non-stressed plants plus GB and the 100 mM stress respectively.

Table 2 represents the shoot dry weight of the maize plants response to the two salt concentrations (50 and 100 mM NaCl), which increased in the same manner at all the growth stages (6, 8, 10 and 12 WAP). In relation to the control, the treatment of both with NaCl concentrations led to a notable reduction ($p < 0.05$) in the shoot dry weight, but the effect was more obvious with 100 mM than 50 mM. The application of GB on the two NaCl concentrations inhibits their negative influences on the shoot dry weight. The highest and lowest shoot dry weight values of maize plants were obtained with the non-stressed plants and the 100 mM stress respectively.

Changes in the Yield Components

The changes in the maize yield components (the ear diameter, ear length, kernels number per ear, seed weight per ear and the 100 seed weight) under the influence of salinity and their interaction with GB are shown in Table 3. There was a significant reduction ($p < 0.05$) in all the yield parameters as a result of the treatment of NaCl concentrations, but the effect was greater on 100 mM than 50 mM. On the other hand, the use of GB appeared to mitigate the effects of the salinity on the maize grain yield components. The maximum and minimum values of the maize grain yield components were obtained with the non-stressed plants plus GB and the 100 mM stress respectively.

Table 1. Effect of salt stress and exgenous glycine betaine on maize growth criteria at 10 WAP

Treatment	**Plant height at maturity (cm)**	**Ear height above ground (cm)**	**Leaf area index**	**No. of leaves/plant (at maturity)**
	$\bar{x} \pm$ **SD**	$\bar{x} \pm$ **SD**	$\bar{x} \pm$ **SD**	$\bar{x} \pm$ **SD**
Cont.	165.5± 9.15	55.7± 4.75	1.347± 0.08	15.3± 2.52
Cont.+ GB	167.4± 10.31	57.4± 5.02	1.435± 0.06	16.7± 2.04
50mM	146.8± 7.54	46.6± 3.52	1.244± 0.05	13.7± 2.46
50 mM + GB	152.3± 7.18	50.8± 5.47	1.304± 0.11	14.2± 1.92
100 mM	141.5± 6.75	39.6± 6.23	0.941± 0.08	10.3± 1.71
100 mM + GB	149.3± 8.21	45.2± 6.44	1.176± 0.07	12.5± 2.12
ANOVA-One Way F (p-value)	12.901 (0.000)**	9.144 (0.000)**	30.506 (0.000)**	4.653(0.003)*

* *p*≤0.05; ** *p*≤ 0.01

Table 2. Effect of salt stress and exgenous glycine betaine on shoot dry weight of maize plants at the various growth stages

Treatment	Shoot dry weight (g)			
	6 WAP	8 WAP	10 WAP	12 WAP
	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD
Cont.	22.5 ± 3.52	42.6± 4.05	67.6 ± 5.52	95.3± 6.13
Cont.+ GB	21.4± 2.02	42.1± 3.62	66.8 ± 6.06	93.5± 5.02
50mM	16.7± 1.68	38.7± 3.08	58.6± 5.07	76.4± 7.08
50 mM + GB	20.6± 1.08	40.2± 2.12	63.7± 3.02	82.7 ± 4.13
100 mM	16.5± 1.46	32.4± 2.35	53.1± 4.07	69.4 ± 5.59
100 mM + GB	18.0± 2.07	36.4± 2.35	60.1± 4.07	78.4 ± 5.59
ANOVA-One Way F (p-value)	6.789 (0.001)*	12.905 (0.000)**	6.541 (0.001)*	20.530 (0.000)**

* $p \le 0.05$; ** $p \le 0.01$

Table 3. Effect of salt stress and exgenous glycine betaine on of yield components of maize plants

Treatment	Ear length (cm)	Ear diameter (cm)	Number of kernels/ ear	100 seed weight (g)	Seed weight/ear (g)
	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD
Cont.	12.6 ± 2.35	4.76± 0.74	421.6± 15.64	26.8± 3.57	77.5±6.37
Cont.+ GB	13.5 ± 2.34	5.45 ± 0.46	450.7± 12.56	28.5± 2.56	80.5± 7.45
50mM	10.7 ± 1.72	3.98± 0.72	317.9± 17.57	22.7± 1.24	60.4± 9.25
50 mM + GB	11.4 ± 0.98	4.41± 0.28	376.6± 22.45	25.8± 2.08	71.6±6.84
100 mM	7.42 ± 2.13	2.89 ± 0.32	289.8± 23.62	18.4± 2.45	55.8± 6.47
100 mM + GB	9.75 ± 2.02	4.11± 0.84	346.8± 18.21	23.6± 2.13	67.3±5.79
ANOVA-One Way F (p-value)	9.051 (0.000)**	19.231 (0.000)*	130.260 (0.000)*	23.182 (0.000)*	19.097 (0.000)*

* $p \le 0.05$; ** $p \le 0.01$

DISCUSSION

Plant growth is affected by various internal and external factors besides its genetic makeup and the evaluation of plant growth is an important tool for assessing the crop productivity in various crops. The results obtained in Table 1 and 2 showed that salt stress caused remarkable decrease ($p < 0.05$) in the growth vigour of the maize plant shoots (leaves number per plant, leaf area index, shoot dry weight, plant height and ear height).

The variations in the response of crops to salt stress tolerance was reported in many studies (Alasvandyari *et al*., 2017; Gebreegziabher and Qufa 2017; Umar *et al*., 2017). In this respect Gul *et al*. (2016) demonstrated that the growth vigour (the shoot length and the fresh and dry weights of the shoot) of the two maize cultivars were reduced under saline conditions (100 mM NaCl). Furthermore, Salama *et al.* (2015) found that salt stress (150 mM NaCl) decreased all the growth parameters (the fresh mass, dry mass and the relative growth rates of the shoots and roots) of wheat plants. Also, Alasvandyaria and Mahdavia (2018) reported that there was a great reduction in the fresh weight of shoots and roots in safflowers with the treatment of 10, 30 and 60 mM NaCl.

However, the salt-incited osmotic pressure is the real reason for decrease in development at the beginning stages of salinity condition, however subsequently the accumulation of sodium ions occurs in the leaves and diminishes plant development (Rahnama *et al*., 2010). More salt assemblage in the root system reduces the water potential of the soil solution and entirely decreases the plant root water conductivity (Abbasi *et al.,* 2015). Therefore, the cell membrane permeability decreases as well as the water influx to the plant is considerably diminished (Munns, 2002). Moreover, Abbasi *et al*. (2015) found that the transpiration rate, leaf water potential, relative water content, water maintenance, water take-up and water use proficiency decreased in jute plants treated with lower salinity condition. Moreover, Shannon *et al.* (1998) stated that the division and stretching of the cell were scientifically influenced by any reduction in the turgor pressure. The various investigations detected that the cell growth is firstly conformed to their turgor potential and the decrease in turgor pressure is one of the real reasons for suppression of plant growth under salt conditions, as in the case of *Shepherdia argentea* (Qin *et al*., 2010), rice (Moons *et al*., 1995) and maize (Cramer *et al*., 1996).

Furthermore, the obtained results indicated that the foliar application of GB rerated the salt stress influences on the growth vigour of the shoots. In this regard, Alasvandyaria and Mahdavia (2018) found an increase in the fresh weight of shoots and roots of safflower plants with the application of GB (30 and 60 mM) under salt stress conditions. Moreover Salama *et al*. (2015) stated that the treatment with GB (25, 50, 100 mM) mitigated the pernicious impacts on the growth vigour of the salt stressed wheat plants.

Indeed, GB is an essential regulator, which accumulates in the cell as a critical osmolyte and mitigates the cell from injury against salt, dryness, heat and oxidative conditions. This has been checked through many studies at the physiological, biochemical and molecular levels (Alasvandyari *et al*., 2017; Murmu *et al*., 2017). Furthermore, it had been found that GB had accumulated in the chloroplasts of many plant species and various kinds of microorganisms in response to the various environmental stresses, especially salinity and drought (Ranganayakulu *et al*., 2013). Concurrently, GB promotes the flow of water into the cells to preserve the osmotic equilibrium of the intracellular and manage the signal transduction cascade under different stress conditions (Ranganayakulu *et al*., 2013). Also, GB could provide adaptation strategies in the osmotic adjustment mediating through Na^+/K^+ differentiation, that includes membrane consistency and defence enzymes, which substantially contributes toward plant salinity tolerance (Singh *et al*., 2015). According to Ranganayakulu *et al.* (2013), GB might have a role in the protection of the thylakoid membrane, consequently preserving the photosynthesis efficiency to enhance the plant growth under the influence of salt stress.

Changes in the Yield Components

Salinity is known to be one of the most dangerous ecological factors determining the productivity of crop plants. Salinity causes a reduction in the functional leaf area and the plant growth, which greatly affects the yield in many crop plants (Salama *et al*., 2015). Our results in Table 3 showed that there was a significant reduction ($p < 0.05$) in all the yield parameters of maize plants (the ear diameter, ear length, kernels number per ear, seed weight per ear and the 100 seed weight) as a result of the NaCl concentration treatments (50 and100 mM). These results were consistent with many other studies on maize plants, such as Abdullah *et al*. (2001), Schubert (2009) and Kaya *et al*. (2013). In this regard, Kaya et al. (2013) reported that the grain number of maize plants had reduced under salt stress during the reproductive phase, which significantly decreased the grain yield.

Moreover, Hutsch *et al*. (2014) stated that salt stress reduced photosynthesis and thus limited the assimilate export from source to sink organs, which is considered to be the main reason for unsatisfactory kernel setting and decrease in the grain number and weight. Another reason for the poor kernel setting under salinity conditions is the decrease in the acid invertase enzyme activity in the growing maize seeds, which is mainly associated to the capacity of sink. Whatever decrease in the enzyme activity would restrict the maize seeds number, owing to increased abortion of the kernel (Farooq *et al*., 2016).

On the other hand, the use of GB seemed to mitigate the effects of the salting stress on the maize grain yield components. The maximum and minimum values of the maize grain yield components were obtained with the non-stressed plants plus GB and the 100 mM stress respectively. These results can be compared to earlier studies on a range of crop species due to salt stress, such as soybean (Rezaei *et al.*, 2012) and wheat (Kotb and Elhamahmy, 2014). In contrast, some investigations reported that the application of GB foliar did not affect the cotton productivity (Meek *et al*., 2003). In this regard, (Kotb and Elhamahmy, 2014) stated that the foliar application of GB at 50 mM after 30, 45, 60 and 75 days from sowing represented a useful management strategy for osmotic adjustment and antioxidative capacity, which improved the resistance of wheat plants and enhanced their yield under salinity conditions.

Moreover, the improving impacts of the external application of GB on the 100-seed weight of maize plants under salinity stress conditions were in agreement with the observations in other studies on many crops under various stresses, such as sunflowers (Iqbal *et al*., 2008) and wheat (Aldesuquy *et al.*, 2013; Kotb and Elhamahmy, 2014). On the other hand, the seed yield increase after the GB application might be related to the larger seeds and greater number of filled seeds. However, Rezaei *et al.* (2012) stated that the use of GB as an external application to salt-stressed soybean plants increased the seed yield that was associated with the larger number of seeds per plant.

Indeed, the beneficial effect of GB on the growth and yield of many crops could be because it plays a role in cell protection, as a nutritive and an osmolyte or an antioxidant compound, in response to various stress conditions (Iqbal *et al.,* 2008; Aldesuquy *et al.,* 2013; Kotb and Elhamahmy, 2014). It plays a role in cell protection against oxidative stress by modulating the activities of the antioxidant enzymes and bringing changes in water relations, and it alleviates cell membrane injury by decreasing the membrane lipids oxidation and enhancing the mineral ion homeostasis under salinity conditions (Longxing *et al.,* 2012). In addition, the application of GB has been observed to have slightly increased the photosynthesis, reduced the photorespiration, increased stomatal conductance and resulted in more efficient gas exchange (Makela *et al*. 1998). This results in more availability of CO_2 for photosynthesis and the ability to avoid possible water use efficiency (Bergmann and Eckert, 1984), photo-inhibition (Makela *et al*., 1999), chloroplast volume (Rajasekaran *et al*., 1997), chloroplast ultra-structure (Makela *et al*., 2000) and enhanced chlorophyll content (Blunden *et al*., 1997), which was reflected in the plant growth and productivity under high salinity conditions.

CONCLUSIONS

The results of this investigation indicate that the maize plants are not salt tolerant. Consequently, salt stress caused various effects on their growth and productivity, which led to significant reduction in the measured growth parameters and the yield components. Furthermore, the external application of GB caused a remarkable improvement in the growth and productivity of maize plants and might have a significant role in modifying the salt response of plants. It is thus proposed that GB could be used as a possible growth regulator to ameliorate the growth and productivity of maize plants under salinity conditions.

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