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Influence of foliar application of glycinebetaine on *Tagetes erecta* L yield cultivated under salinity conditions

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Abstract: Tagetes genus of *Composite* family consider one of the most favorite floriculture plant. Therefore, of particular interest examine the salt tolerance of this bedding and coloring agent plant. In this research, was report the role of glycinebetaine (GB) in attenuating the adverse impacts of salt stress in African marigold plant, along with their anti-oxidative capacities and biochemical attributes. The salt stressed African marigold (100 and 150 mM NaCl) was treated with GB at 200 mM, beside untreated control plants. According to the obtained results, the growth characters were negatively in salt stressed plants but a mitigate impact of GB were observed in this respect. Obviously, the morphological as well as some physiological characters were reduced with salinity treatments while GB treatment reverses these effects. Overall, the alleviate impact of GB on the negative impact of salt stress was enhanced through improving total phenolic and antioxidant enzyme activity. Further, it is concluded that GB concentration induces the activities of antioxidative enzymes which scavenged ROS increased under saline conditions.

Keywords: antioxidant activity; chlorophyll; glycinebetaine; membrane stability index; salt stress

1. Introduction

Tagetes erecta L. is an herbaceous plant contains small leaves and flowers in comparison to other marigolds. Moreover, African marigold are a valuable crop for controlling parasitic nematodes [1]. The eessential oils in addition to lutein (carotenoids), which used in the food supplements, manufacture of soap, perfumes, cosmetics, and pharmaceutical industries were extracted from the aerial parts [2].

Soil salinization and water irrigation in Saudi Arabia lands (arid and semi arid areas) causes a serious abiotic stress including degradation of agricultural lands, especially that controlling the growth and yield [3]. Further, salt stress changes an imbalance in the cytosolic ionic flow of cells and thus results in oxidative damage that affects the function of the lipid bilayer and the photosynthetic rate as well as the metabolism of cells [4]. Salinity decreased the productivity in numerous medicinal plants [5,6,7], and induces oxidative stress *via* increased ROS production which is related to tissue destruction [8]. The two defense mechanisms against salt were detoxify ROS by stimulating ROS scavenger enzymes like catalases, superoxides dismutase and peroxidases activities [9] and /or non-enzymatic antioxidants, i.e. osmoregulation GB, ascorbate, phenolics, tocopherol, reduced glutathione, and proline [10].

Chen and Murata [11] reported that GB is a compatible solute, water-soluble and non toxic at elevated levels has the roles of effective protection of plant cells against salinity stress through osmotic stress adjustment [12], protein stabilisation (Rubisco) [13], protection of the photosynthetic machinery [14], and decrease of oxygen radicals scavengers [15]. Seedling stage in rice gave a positive results but, a lack of reproductive effects by the adding of GB to the medium or by exogenous methods before subjection to NaCl [16,17]. Thus, the object of this work was to investigate the impact of GB application on plant development, productivity and quality of cultivated marigold flowers grown in saline conditions, highlighting carotenoids production (lutein) as an important product for flowering of this plant.

Materials and Methods

Experimental set-up

A potted greenhouse experiment was conducted at Taif University, Saudi Arabia through the seasons 2018 - 2019. GB Efficacy on salt stress mitigation of African marigold was evaluated. Plant seeds were soaked in a aerated solution of $CaSO_4$, 1 mM for 1 day and then germinated in darkness at 28 ºC for 2 days between two layers of filter paper. After 4 days, the seedlings were placed in the soil in 25 cm size pots. Physical characteristics of the soils were (sand, 77.21%, silt 7.99%, clay 14.80%) and the chemical soil properties were (pH 7.98, EC 2.65 dSm^{-1}, OM 0.14%, Na^+ 3.12, SO_4^{-2} 43.25, HCO_3 2.75 and Cl^- 0.42 $meqL^{-1}$, total N^+ 0.15% and PO_4^{3-} 0.037%). Pots were placed in a growth chamber charged with a NPK mineral fertilizer (17:17:17) at 5 g per pot and at a temperature of 26 °C /18 °C in light (200 W m^{-2}) and dark period, respectively with relative humidity at 70 %. NaCl levels were gradually increased to 100 and 150 mM with or without 200 mM GB as foliar application in 4 replicates.

Growth and flower characters

Shoot length in cm, branch number per plant, shoot and root fresh and dry weights (g/plant), flower number $plant^{-1}$ and flower weight per plant (fresh and dry) taken in this experiment. Leaf number/plant and its area (cm^2) were followed; leaf blade areas were established by a digital picture analysis as reported by Matthew et al. [18].

Chlorophyll and carotenoid assessment

Chlorophyll was determined as described by Shabala et al. [19], while total carotenoid concentrations (Cx+c) were estimated by methods of Lichtenthaler [20].

Relative Water Content (RWC).

Methods of Weatherley [21] were applied to measure Relative Water Content on the basis of the equations: (FW-DW) / (TW-DW) x 100, in which FW : fresh weight, TW: turgid weight when saturated with distilled water for 24 h at 4 °C, and DW: dry weight.

Membrane Stability Index (MSI)

Samples of leaves were collected from mid-plant for determining the ion leakage as reported by Sairam et al. [22]. The ion leakage was determined as Membrane Stability Index according to the formula: MSI = $(1 - (C_1/C_2)) \times 100$.

Total Phenolics

Leaf powdered samples (1 g) were extracted in 80 % methanol and assaying the total phenolics using Folin-Ciocalteu reagent as described by McDonald et al. [23], expressed as g GAE kg^{-1} DW.

Anti-oxidation enzyme assays

The determination of the anti-oxidant enzymes SOD, CAT and POX assay in leaf extract and the soluble protein levels were analyzed by Bradford's method [24].

The activity of SOD (EC 1.15.1.1) was estimated by the determination of its capacity to inhibit photo-chemical degradation of tetrazolium nitroblue (NBT) as reported by Giannopolitis and Ries [25].

The activity of CAT (EC 1.11.1.6) was measured spectrophotometrically according to Clairbone [26].

The activity of POX (EC 1.11.1.7) was assayed as described by Shanon et al. [27].

The expression of enzymatic activities was given in μmol min^{-1} mg^{-1} protein.

Potassium and Sodium Contents

Dried marigold leaf was wet digested to estimate potassium and sodium content as mentioned by Jackson [28].

Statistical analysis

Experiments were carried out twice in four replicates and the ANOVA will be performed with the program MSTAT. Means were separated using Duncan's multiple range tests at a significance level of 0.05.

Results and Discussion

Analysis of variance (ANOVA) for the data on vegetative characters: height of plant, number of branch, number of secondary branch, FW and DW (g /plant) of African marigold shoot and root showed that salinity stress treatments (100 and 150 mM NaCl) significantly reduced these parameters (Table 1). The reduce in leaf number and leaf area was the response to the effect of salinity, described similar outcomes (Table 2). While, when GB at 200 mM was applied exogenously, an improvement and enhancing of the previous parameters.

Similarly, flower attributes; i.e. flower number per plant, fresh and dry flower weight (g/flower) sharply reduced by salinity, while the applications of 200 mM GB improved it especially at 100 mM NaCl (Table 2). Parvin et al. [29] mentioned that, GB application was enhanced leaf numbers and reduced salinity. The injure impacts of salinity causes disturbance in some metabolic, reduction of net photosynthesis, decline in water availability, imbalance of ionic, enlargement inhibition of cell or impairment of meristematic activity [30,31]. Same results have already been mentioned by Ali et al. [32], Hassan and Ali [3] and Alotaibi et al. [33] on *S. chinensis* (Link) Schneider, Ali and Hassan [5] on Chamomile, Hassan et al. [34,35] and Ali et al. [36] on *Rosa damascena*, Mansour and Ali [37] and Ali and Hassan, [5] on *Calendula officinalis* L. Recently, Attia et al. [7] recorded a decrease in damask rose by salt stress treatments may be due to nutrition imbalance statues and K, Ca and Mg reduction in the photosynthetic organs.

A considerable reduction of RWC, chlorophyll, carotenoids content and MSI of marigold plants were detected in salt stressed plants (Fig. 1 and 2). Although lower value of phenolics compounds was registered in unstressed plants, NaCl treatment enhanced phenolics (Fig. 3). However, GB treatment noticeably enhanced phenolics more than unstressed or stressed plants. Moreover, the carotenoids detected in marigold plants were enhanced [38]. Glycinebetaine foliar application can enhance abiotic constraint tolerance of several plants and subsequently improve growth and productivity, so if applied to photosynthetic organs, it is well absorbed by foliar tissues localized in cytosol and translocate to chloroplasts [39] and also taken up *via* roots [40].

Salinity alters water and osmotic potential and disturbed in Na^+/K^+ (Table 3), GB rehabilitates it and reduces the oxidative stress and assists in reducing electrolyte leakage [41,42,43]. Based on these findings, it was found that foliar treatment with GB improved the tolerance to salinity and to strongly maintained in the plant growth parameter (shoot and root fresh and dry weights).

Uunder various abiotic stresses, it is scientifically proven that both enzymatic and non-enzymatic antioxidants have a major effect as scavengers of ROS, which are necessary for plant resistance mechanisms [44].

Results of the analysis of variance showed that salinity and plant interaction for CAT, POD and SOD were statistically significant. Activity of all studied enzymes in marigold leaves increased with increasing the salt stress (150 mM) treatments (Table 3). The antioxidants increased significantly when applying GB treatment, which resulted in increased scavenging activity, which had an important protective role on the growth of African marigolds and improved the ability of its leaves to photosynthesize against salt stress. Improving the systems of antioxidant defense in response GB treatment was scavenged ROS and enhanced the stability of membrane [45]. GB are able to proteins stabilizer, lipids of membrane, structures of cell, cell turgor maintainace, adjustment of osmotic pressure, nitrogen storage, and redox metabolism to ROS scavenging under salinity [12]. Finally, the amino acid derivative GB is protective of higher plants from salt and osmotic stress in various ways: through osmotic adjustment [12], oxygen releasing stabilizer PS-II [46], membranes and protein quaternary structures [14], and the enzymes RUBISCO [13].

Conclusion

Based on the obtained findings, a conclusion can be drawn the growth and flowering characters were significantly decreased by salt stress treatments, while GB application promoted the growth as well as flowering attributes in stressed plants. In the same context, RWC, MSI, pigments content and phenolics content were also improved due to GB treatment. Accordingly, the oxidative stress decreased and salt stress inhibitory impacts on African marigolds were reduced as a result of GB treatment. This indicates that GB not only nullified the impact of salt stress, but also significantly improved growth, physio-biochemical parameters in addition to changes non-enzymatic and enzymatic antioxidants activities in plants of African marigold.

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Table 1. Effect of Glycinebetaine on morphological growth of *Tagetes erecta*, L cultivated under two-salinity stress.

Treatments	Plant height (cm)	Main branch number/plant	Secondary branch number/plant	Shoot FW (g plant)	Shoot DW (g/plant)	Root FW (g/plant)	Root DW (g/plant)
Control	62.56a ± 2.59	8.24a ± 0.29	25.02a ±1.23	154.56a ± 5.06	39.25a ±1.41	9.36a ± 0.14	3.21a± 0.08
Saln. 100 mM	52.87c±2.3	6.25b±.0.34	16.25c±1.36	122.47d±6.25	26.05d±2.36	5.89d±0.12	2.12c±0.07
Saln. 150 mM	48.68±3.14	4.98c±.0.28	15.12d±2.04	119.14e±4.69	28.69c±1.89	5.12d±0.26	1.89d±0.09
Saln. 100 mM + 200 mM GB	54.69bc±2.15	7.99a±.0.39	20.54b±1.58	136.58b±8.69	29.58±b1.27	7.36b±0.31	2.86b±0.04
Saln. 150 mM + 200 mM GB	50.67bc±3.65	6.85b±0.43	19.92b±2.06	133.56c±7.36	28.69c±2.36	6.99b±0.37	2.54c±0.06

Salin; salinity, GB; glycinebetaine. All data are means ± S.D. (*n=14*). The means in a column with the different letters are statistically different from the others based on Duncan's multiple range test at *P = 0.05*.

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Table 2. Effect of Glycinebetaine on leaf and flower growth of *Tagetes erecta* L cultivated under two-salinity stress.

Treatments	Leaf number (plant)	Leaf area (cm^2)	Number of Flowers $Plant^{-1}$	Flower fresh weight (g $flower^{-1}$)	Flower dry weight (g $flower^{-1}$)
Control	280.25a±6.25	2.71a±0.06	7.92a±0.21	7.72a±0.28	2.08b±0.06
Saln. 100 mM	221.36c±5.32	1.87c±0.07	5.02c±0.15	5.25c±0.43	1.56d±0.05
Saln. 150 mM	198.57d±3.98	1.63d±0.06	3.24d±0.23	4.47d±0.42	1.24e±0.04
Saln. 100 mM + 200mM GB	279.98a±4.85	2.64 a ±0.08	7.98a±0.31	7.04a±0.36	2.98a±0.03
Saln. 150 mM + 200mM GB	242.15b±5.85	2.23b±0.05	6.87b±0.24	6.04b±0.39	1.86c±0.07

Salin; salinity, GB; glycinebetaine. Values are means ± S.D. (*n=14*). The means in a column with the different letters are statistically different from the others based on Duncan's multiple range test at $P = 0.05$.

Table 3. Effect of Glycinebetaine on antioxidant enzyme activities, Na and K content of *Tagetes erecta* L cultivated under two-salinity stress.

Treatments	SOD (units min^{-1} mg^{-1} protein)	CAT (µmol min^{-1} mg^{-1} protein)	POX (µ mol min^{-1} mg^{-1} protein)	Na (mg g^{-1} FW)	K (%)
Control	2.37a±0.14	2.43a±0.06	24.12a±1.09	2.31e±0.08	2.34a± 0.07
Saln. 100 mM	1.74c±0.16	1.61d±0.08	16.08d±1.21	3.24b±0.07	2.26b±0.08
Saln. 150 mM	1.91b±0.12	1.82c±0.06	18.57c±1.57	4.52a±0.06	2.21c±0.06
Saln. 100 mM + 200mM GB	2.34a±0.09	2.37b±0.04	22.18b±1.26	2.63d±0.05	2.33a±0.07
Saln. 150 mM + 200mM GB	2.35a±0.10	2.42a±0.05	23.86a±1.21	2.76c±0.08	2.38a± a0.09

Salin; salinity, GB; glycinebetaine. The means in a column with the different letters are statistically different from the others based on Duncan's multiple range test at $P = 0.05$.

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Figure legends

Fig.1. Response of salt stress and glycinebetaine on A; relative water content (RWC) and B; membrane stability index (MSI) of African marigold plants. Vertical bars are the standard error. The same letter is not statistically different (*P* = 0.05).

Fig.2. Response of salt stress and glycinebetaine on A; chlorophyll content and B; carotenoids content of African marigold plants. Vertical bars are the standard error. The same letter is not statistically different (*P* = 0.05).

Fig.3. Response of salt stress and glycinebetaine on total phenolics content of African marigold plants. Vertical bars are the standard error. The same letter is not statistically different (*P* = 0.05).

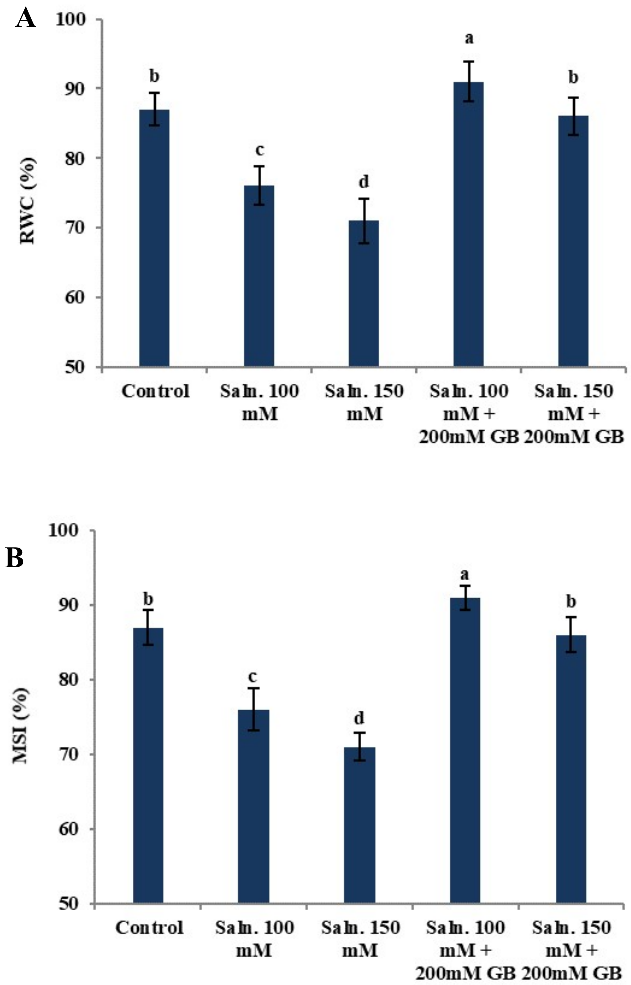
A
100
90
80
70
60
50
RWC (%)
b
c
d
a
b
Control
Saln. 100 mM
Saln. 150 mM
Saln. 100 mM + 200mM GB
Saln. 150 mM + 200mM GB
B
100
90
80
70
60
50
MSI (%)
b
c
d
a
b
Control
Saln. 100 mM
Saln. 150 mM
Saln. 100 mM + 200mM GB
Saln. 150 mM + 200mM GB

Fig. 1

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Fig. 2

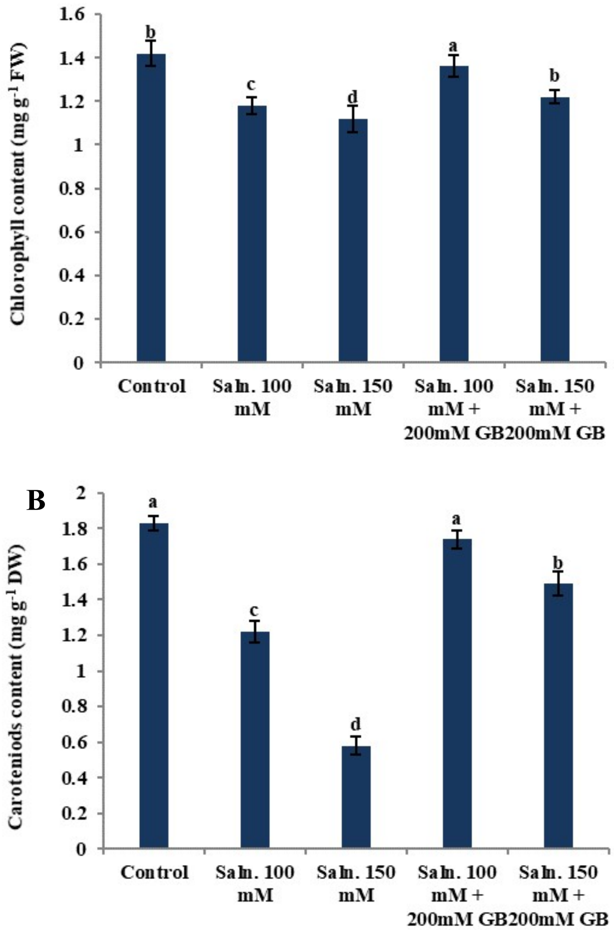

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Fig. 3