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Article

EXOGENOUS GIBBERELLIC ACID AMELIORATES SALINITY-INDUCED MORPHOLOGICAL AND BIOCHEMICAL ALTERATIONS IN *Portulaca grandiflora*

Papel do Ácido Giberélico Exógeno na Melhora das Alterações Morfológicas e Bioquímicas Causadas Pela Salinidade em ***Portulaca grandiflora***

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ABSTRACT - An investigation was carried out to estimate the NaCl stress and ameliorative effects of Gibberellic Acid (GA_3) on *Portulaca grandiflora* Hook. A crop experiment was conducted (CRBD) where all the pots were irrigated to field capacity. The treatments were given as (T0) control without NaCl, (T1) 80 mM NaCl, (T2) 80 mM NaCl and 50 ppm GA_3, (T3) 80 mM NaCl and 75 ppm GA_3 and (T4) 80 mM NaCl and 100 ppm GA_3. The samples were collected at 90 DAS. It was found that plants subjected to salt stress generally showed a reduction of vegetative growth. GA_3 spraying on *Portulaca grandiflora* with 75 ppm showed a high amelioration effect on growth and on biochemical patterns, which enhanced salt tolerance. In *Portulaca grandiflora*, data showed that NaCl stress inhibited fresh and dry weight and further introduced significant deviation on some biochemical parameters. However, GA_3 partially ameliorated growth and some biochemical parameters of *Portulaca grandiflora* under NaCl stress.

Keywords: salt stress, biochemical and morphological changes, plant hormone.

RESUMO - Foi realizada uma pesquisa para estimar o estresse com NaCl e os efeitos benéficos do ácido giberélico (GA_3) na planta ***Portulaca grandiflora****. Realizou-se um experimento de cultura (CRBD) em que todos os vasos foram irrigados até atingirem a capacidade de campo. Os tratamentos foram administrados como controle (T0) sem NaCl, (T1) 80 mM de NaCl, (T2) 80 mM de NaCl e 50 ppm de GA_3, (T3) 80 mM de NaCl e 75 ppm de GA_3 e (T4) 80 mM de NaCl e 100 ppm GA_3. As amostras foram colhidas aos 90 DAS. Verificou-se que as plantas submetidas a estresse salino geralmente apresentavam redução do crescimento vegetativo. A pulverização com GA3 em* ***Portulaca grandiflora*** *com 75 ppm mostrou elevado efeito de melhora no crescimento e em padrões bioquímicos, o que aumentou a tolerância ao sal. Em* ***Portulaca grandiflora****, os dados mostraram que o estresse com NaCl inibiu o peso fresco e seco e, além disso, causou desvio significativo em alguns parâmetros bioquímicos. No entanto, o GA_3 parcialmente melhora o crescimento e alguns parâmetros bioquímicos de* ***Portulaca grandiflora*** *sob estresse com NaCl.*

Palavras-chave: estresse salino, mudanças bioquímicas e morfológicas hormônio vegetal.

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INTRODUCTION

Environmental stresses such as soil salinity or water stress can promote changes in a wide range of morphological, physiological, biochemical, and molecular processes in plants (Ashraf and Harris, 2013). In plant species, the increase in contents in sodium (Na^+) and chloride (Cl^-) ions is a main reason for deleterious effects of salt stress. Moreover, Cl^- ion is the most dangerous (Hasanuzzaman et al., 2013). Adaptation or tolerance of plants to salinity mainly depends on their ability to alter physiological traits, metabolic pathways, and molecular or gene linkages, in order to allow survival under stress (Gupta and Huang, 2014).

Salt-affected soil reclamation is necessary because, on a daily basis, salt intrusion in cultivable land is increasing throughout the world (Ghanaatian and Sadeghi, 2016). Over the past few years, many methods have been tested to overcome adverse effects of salinity stress on plants, namely mycorrhiza inoculation (Beltrano et al., 2013) hydrogen sulfide priming (Christou et al., 2013), ABA application (Osakabe et al., 2014) and salicylic acid treatment (Li et al., 2014). Previously, Ashraf et al. (2002) reported the combining effects of gibberellic acid and salt stress on the photosynthetic capacity of wheat plants.

In this present work, we report salinity stress amelioration by Gibberellic Acid (GA_3) in *Portulaca grandiflora* (Portulacaceae), a succulent plant, with colorful flowers, which is used as a garden plant in UAE. *Portulaca oleracea* is an annual succulent herb largely disseminated worldwide (Alam et al., 2014). Numerous works have been reported about the action of GA_3 to improve crop performance in normal conditions, and little enlightenment has been reported about its exogenous application during salt stress. Nevertheless, Iqbal and Ashraf (2013) have provided some details to demonstrate the ability of foliar pretreatment to overcome the deleterious effects of salt stress on wheat plants. Hamayun et al. (2010) reported effects of GA_3 application on soybean plants and explained its role in phytohormone levels under NaCl induced salt stress.

This study aims to assess the effect of GA_3 on the adverse effects of saline stress in *Portulaca grandiflora*, taking growth and some biochemical parameters into consideration.

MATERIALS AND METHODS

A greenhouse experiment was conducted to evaluate the morphological, physiological response of *Portulaca grandiflora*. under salt stress and the amelioration effect of GA_3 (Figure 1). The greenhouse experiment was carried out in Al-Foah Experimental Station (270N and 220S latitude and 510W and 570E longitude) of the College of Food and Agriculture, UAEU, in Alain city (160 km Eastern Abu Dhabi, the capital city of United Arab Emirates). Greenhouse temperature was 24±2 °C and relative humidity was 50-60% during the experimental period.

Three plants were planted per pot. Pots were watered to field capacity up to 90 days after planting (DAP), and leaching was avoided. The initial EC level of the soil was maintained by flushing each pot with the required volume of corresponding treatment solution at 45, 60 and 75 DAS. The position of each pot was randomized at four-day intervals to minimize spatial effects in the greenhouse. The seedlings were thinned to one per pot at 20 DAS. Plants were uprooted randomly at 90 DAS and used for determining growth, pigment composition and other biochemical constituents.

Growth parameters

After washing the plants in tap water, fresh weight was determined with an electronic balance (Citizen Scales PVT LTD., Model – XK3190-A7M) and the values were expressed in grams per plant. After fresh weight was measured, the plants were dried at 60 °C in hot air oven for 24 hours (to constant weight); three plants per treatment were measured, and the values are expressed in grams per plant.

Pigment constituents

Chlorophyll and carotenoid contents were extracted from the leaves and estimated according to the method of Arnon (1949). Carotenoid content was calculated using the formula of Kirk and

From the left: T0, T1, T2, T3 and T4.

Figure 1 - The five groups of different treatments of *Portulaca grandiflora* on the Al-Foah farm, UAEU.

Allen (1965) and expressed in milligrams per gram of fresh weight. Anthocyanin was extracted and estimated by the method of Beggs and Wellmann (1985).

Biochemical analysis

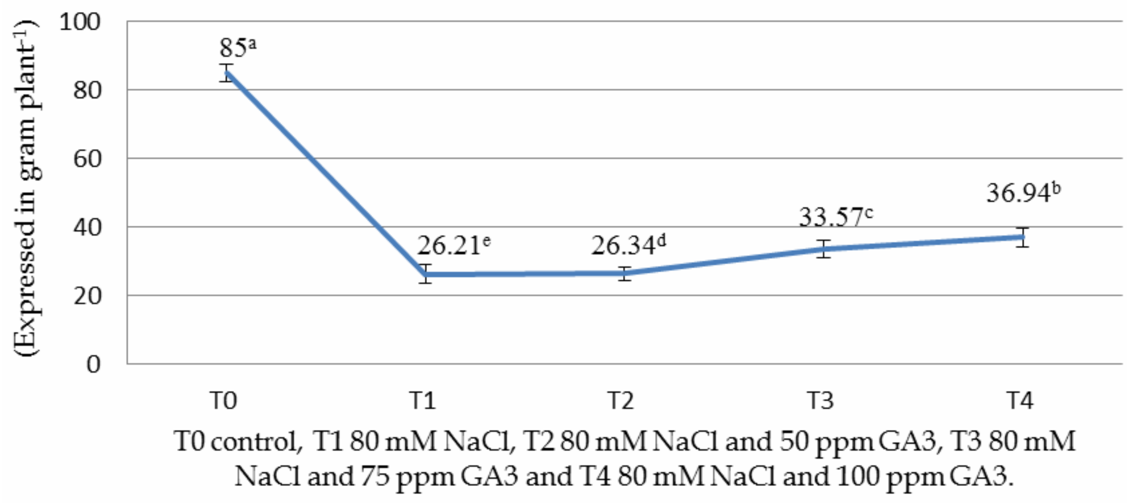
Proline was extracted and estimated following the method of Bates et al. (1973). Total phenol was estimated by the method of Malick and Singh (1980).

Statistical analysis

The data were analyzed (ANOVA and DMRT) by using SPSS. The values represent mean ±SD for three samples in each group. The values that do not share a common superscript are significantly different ($p<0.05$).

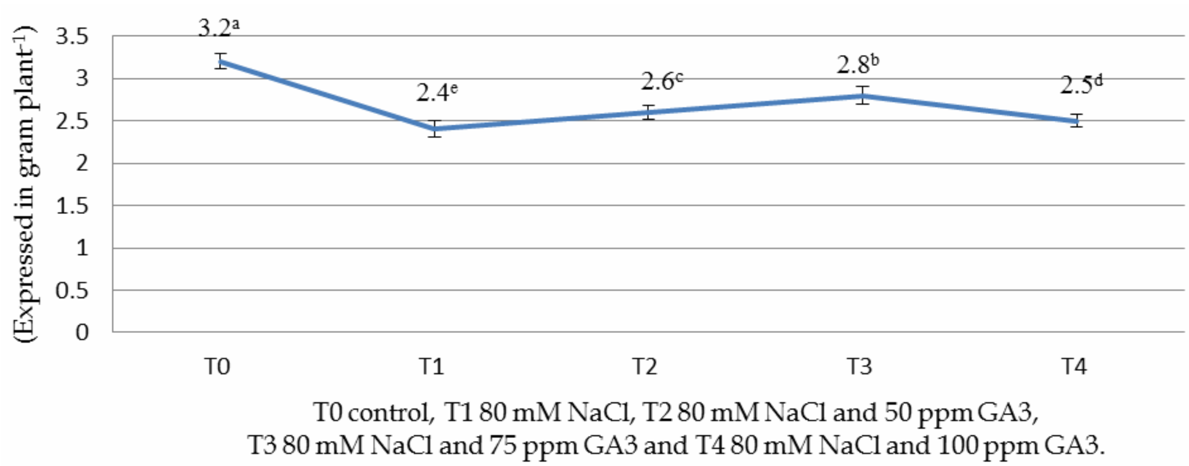
RESULTS AND DISCUSSION

Considering the morphological parameters, control plants (T0) showed the maximum value of fresh and dry weight, followed by T4 (80 mM NaCl + 100 ppm GA_3). All the three treatments sprayed with GA_3 displayed higher fresh and dry weight than T1, which under the salt condition, which had no application of GA_3 (Figures 2 and 3). Plants that were subjected to salt stress generally show a reduction of vegetative growth (Mukherjee et al., 2014). Our results are consistent with the reports of Iqbal and Ashraf (2013), which showed that gibberellic acid can enhance salt tolerance in wheat plants. Hamayun et al. (2010) reported that GA_3 application significantly promoted soybean plant length and plant fresh/dry biomass, which were markedly hindered by NaCl induced salt stress.



The values represent mean ±SD for three samples in each group. Values that are not sharing a common superscript are significantly different ($p<0.05$).

Figure 2 - Effect of individual and combined treatments of NaCl and GA_3 on plant fresh weight.

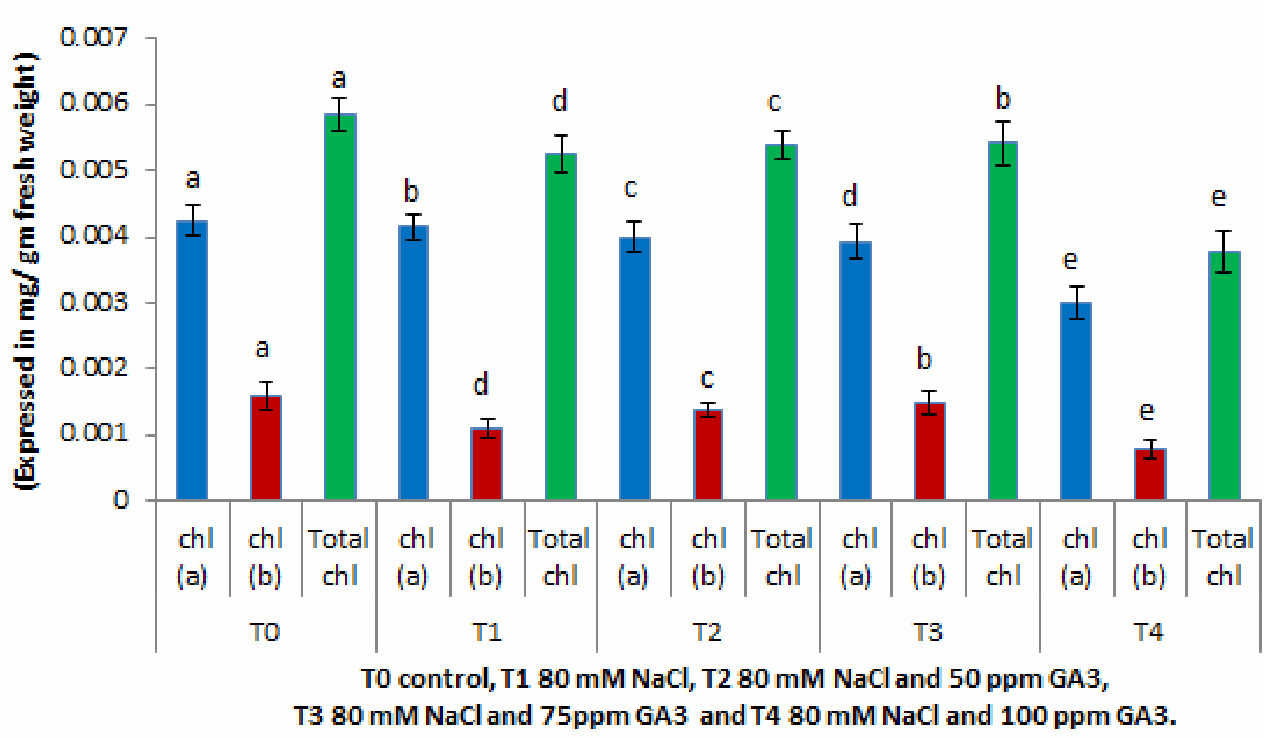


The values represent mean ±SD for three samples in each group. Values that are not sharing a common superscript are significantly different ($p<0.05$).

Figure 3 - Effect of individual and combined treatments of NaCl and GA_3 on plant dry weight.

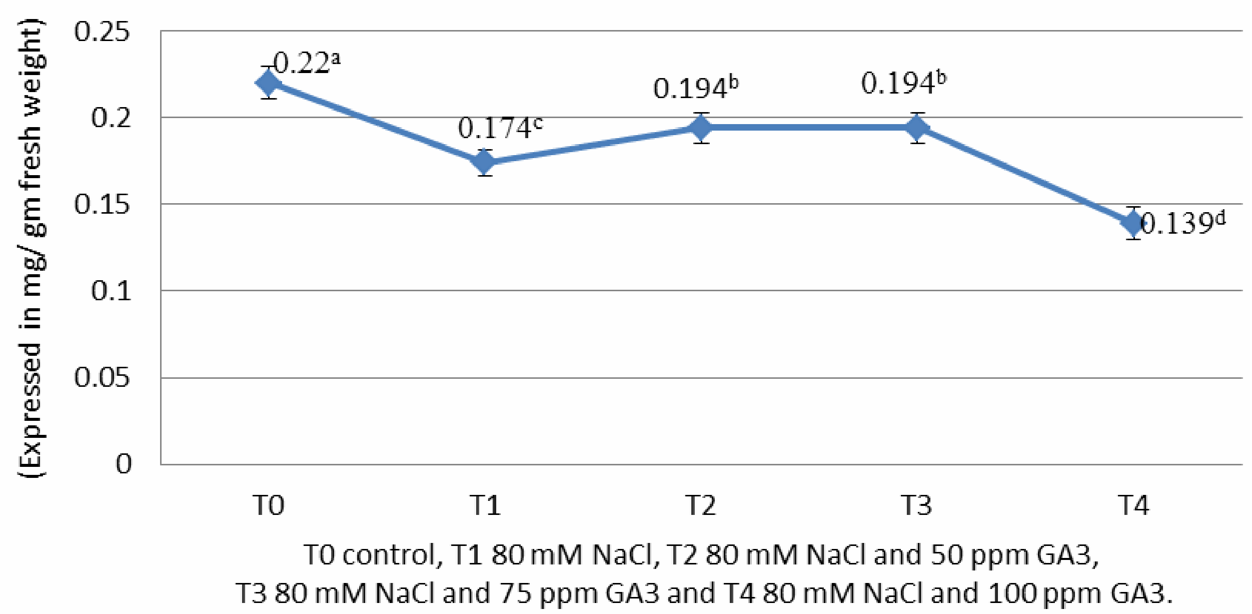
The effects of GA_3 on the photosynthetic pigments of *Portulaca grandiflora* (Figures 4, 5 and 6) showed that, as regards control (T0), salinity reduced chlorophyll contents, yet this pigment content slightly increased after GA_3 application. Treatment 3 (80 mM NaCl+75 ppm GA_3), showed the highest effect of GA_3 application on chlorophyll content. Moreover, treatment 4 (80 mM NaCl+100 ppm GA_3) revealed a negative effect of GA_3 application on chlorophyll content (which decreased under salinity). It should be noted that Athar et al. (2015) showed that glycinebetaine can improve photosynthesis in canola, which was initially reduced under salt stress. The reduction in pigment contents during salt stress can be attributed to the inhibition of chlorophyll biosynthesis as a result of the enhancement in ethylene production as explained by Khan (2003). The increase in chlorophyll levels in salt stressed plants under GA_3 is in accordance with the previous reports of Shah (2007) in mustard plants.

In treatment 2 (80 mM NaCl+50 ppm GA_3) and treatment 3 (80 mM NaCl+75 ppm GA_3), carotenoid content also increased significantly after spraying with GA_3, yet and antagonist pattern was found in treatment 4 (80 mM NaCl+100 ppm GA_3). Spraying GA_3 at 50 ppm had no effect on anthocyanin content, but there was a significant increase with 75 ppm (treatment 3) (higher than the control – treatment 0). However, anthocyanin content decreased in treatment 4 (Figures 4, 5 and 6). This pattern was found to be similar to the one in the report of Pinheiro et al. (2008) about the reduction in pigment accumulation in salt-stressed castor bean plants. The application of gibberellic acid under salinity enabled the *Hibiscus sabdariffa* plants to restore the altered pigments concentrations induced by NaCl (Ali et al., 2012).



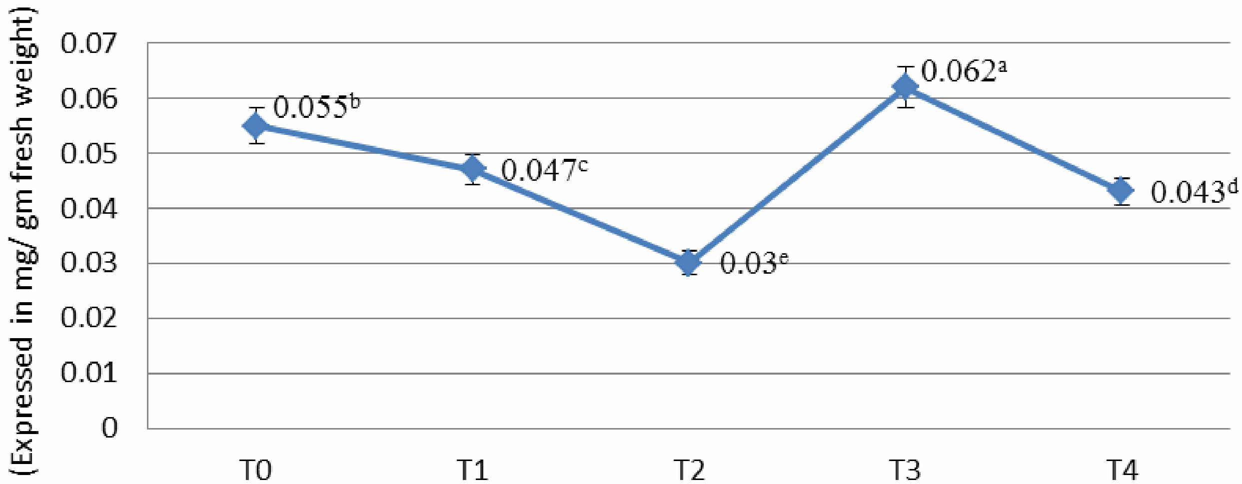
The values represent mean ±SD for three samples in each group. Values that are not sharing a common superscript are significantly different (p<0.05).

Figure 4 - Effect of individual and combined treatments of NaCl and GA_3 on chlorophyll content.



The values represent mean ±SD for three samples in each group. Values that are not sharing a common superscript are significantly different (p<0.05).

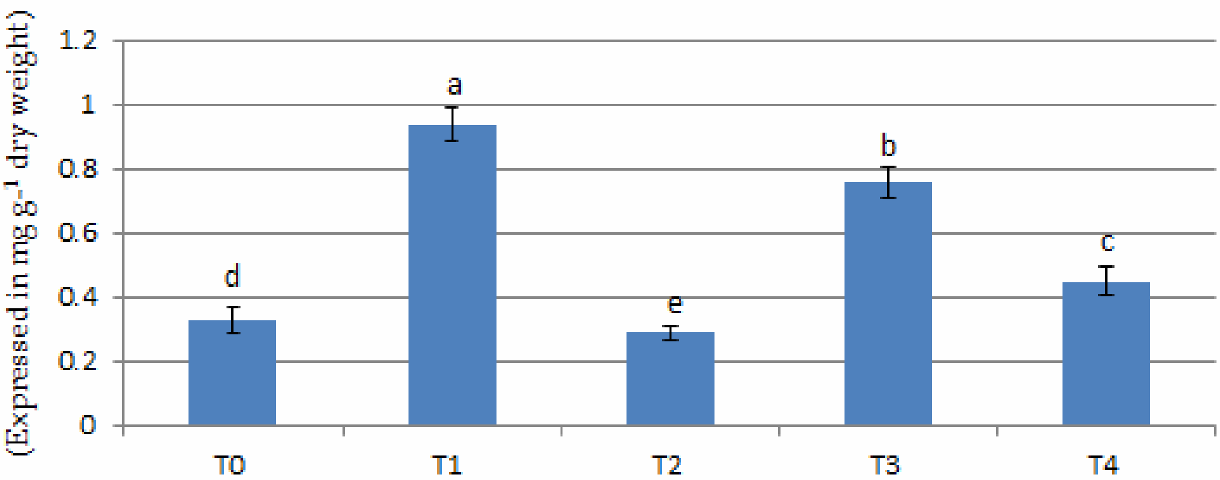
Figure 5 - Effect of individual and combined treatments of NaCl and GA_3 on carotenoid content.



The values represent mean ±SD for three samples in each group. Values that are not sharing a common superscript are significantly different ($p<0.05$).

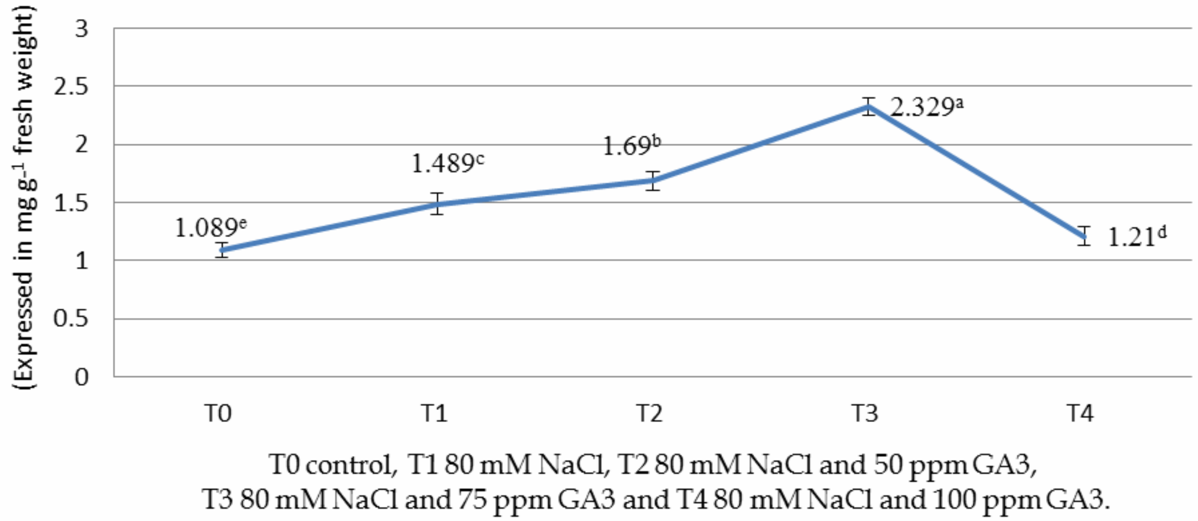
Figure 6 - Effect of individual and combined treatments of NaCl and GA_3 on anthocyanin content.

Proline was found to increase in treatment 1. It declined under the application of GA_3 sprayed in treatment 2 and raised in treatment 3. In treatment 4, proline content decreased about 0.45. Between treatments 2-4, treatment 2 showed the lowest value (0.29). By contrast, proline content decreased in treatment 2 because of the application of GA_3, and there was a reduction in the effect of salt stress as the plants acquired tolerance. The content of total phenol increased in treatments 1 and 2. It also increased in treatment 3 more than the control T0, whereas it decreased in treatment 4. These results confirmed that total phenol content increased with the application of GA_3 (Figures 7 and 8).



The values represent mean ±SD for three samples in each group. Values that are not sharing a common superscript are significantly different ($p<0.05$).

Figure 7 - Effect of individual and combined treatments of NaCl and GA_3 on proline content.



The values represent mean ±SD for three samples in each group. The values that do not share a common superscript are significantly different ($p<0.05$).

Figure 8 - Effect of individual and combined treatments of NaCl and GA_3 on total phenol content.

In stress conditions, proline acts as an osmoprotectant; it plays an important role in osmotic balancing, and its overproduction can play a significant role against salt stress (Surekha et al., 2014). Free Proline and soluble sugar accumulation in the leaves under stress conditions is of utmost importance for plant adaptation during stress (Tan et al., 2006). Exogenous treatment with GA3 reduced the deleterious effects of NaCl salinity in maize plants with proline accumulation, which, in turn, helped the plants to maintain membrane permeability (Tuna et al., 2008). There was an increase in phenol contents in salt treatment, which is the opposite finding in the reports of Yuan et al. (2010), who found a reduction in total phenolic contents of 5 and 7 day-old radish sprouts treated with 10 and 50 mM of NaCl. The reason might be the difference in the age of plants: the present study reported findings for 90 day plants, which will increase phenolics. There are previous reports of differential response of plants in phenolic accumulation during different growth stages (Choi et al., 2006; Barros et al., 2007; Ashraf et al., 2010). However, as it an antioxidant, the increase in total phenol contents can be correlated with the gibberellic acid contribution towards stress protection.

The application of gibberellic acid in salt-stressed *Portulaca grandiflora* ameliorates salt tolerance, accelerating the photosynthetic performance and some parameters of the biochemical pathway. Spraying of GA_3 on *Portulaca grandiflora* with 75 ppm has a high amelioration effect on growth possibly by altering some biochemical pathways, which enhanced salt tolerance. Thus, it can be concluded that exogenous application of GA_3 reduce the deleterious effects of salt stress and enhances tolerance to salinity in *Portulaca grandiflora*. Studies with more samples and also field evaluations are needed to ascertain this conclusion.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

C. A. J. planned the study, participated in lab works and interpreted the data. S. A. S. A. S. and S. S. S. A. S are students and they worked in this study as part of their senior project research; they participated in sample collection, lab works and writing of the manuscript. G. A. R. assisted in lab works and analysis of data and K. S. S. participated in interpretation of data and helped in manuscript preparation.

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