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Full Length Research Paper

Effects of gibberellic acid on growth and photosynthetic pigments of *Hibiscus sabdariffa* L. under salt stress

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Today, there is emerging focus on the importance of medicinal plants that have occupied a significant position in solving the health care problems in human life. Karkade is an important medicinal plant and is used for curing various illnesses. The aim of this study was to improve growth performance by enhancing the photosynthetic pigments and enzyme carbonic anhydrase (CA) activity of *Hibiscus sabdariffa* L. (cv. Sabahia 17) under NaCl stress. Under non-saline condition, application of GA_3 enhanced growth parameters (shoot length, shoot fresh weight (FW), shoot dry weight (DW), root FW, root DW, leaf area), relative water content (RWC), CA activity, anthocyanin and photosynthetic pigments (chlorophyll (Chl) *a*, Chl *b*, and total Chl). However, the application of GA_3 reduced the inhibitory effect of NaCl on growth attributes and photosynthetic pigments by inducing the enzymes CA activity and enhancing RWC. Therefore, it is concluded that GA_3 might help in the tolerance of plants to salt stress.

Key words: Carbonic anhydrase, Karkade, medicinal plant, photosynthetic pigments, relative water content, salinity.

INTRODUCTION

Medicinal plants are essential for human beings that utilize them for basic preventive and curative health care since time immemorial. *Hibiscus sabdariffa* L. (English: Red sorrel, Roselle; Arabic: Karkade, belongs to Malvaceae family) is widely cultivated in Egypt, China and Thailand for different purposes. The plant parts including seeds, leaves, fruits and roots are used in various foods such as wine, juice, jam, jelly, syrup, gelatin, pudding, cake, ice cream, tea, spice and other desserts. Roselle is a good source of natural antioxidants (protocatechuic acid and anthocyanins) that protect the body from damage by free radicals and lipid peroxidation (Tee et al., 2002; Liu et al., 2002; Ali et al., 2003, 2005). It is used for the treatment of several ailments, including high blood pressure, liver diseases, fever, urinary tract infection, pain of the muscles of the uterus and intestine (Herrera-Arellano et al., 2004; Akindahunsi and Olaleye, 2003; Auddy et al., 2003; Fatehi et al., 2003; Perry, 1980; Faraji and Tarkhani, 1999; Owolabi et al., 1995; Tanaka et al., 1993).

It is well established that plant hormones play an essential role in the regulation of signal transduction pathway involved in the induction of plant stress response. Under salt stress, seed germination, plant growth and net photosynthetic CO_2 uptake decreased with decreasing stomatal conductance and also, NaCl changes thylakoid membrane structure and decrease the contents of chlorophylls and carotenoids (Khavari-Nejad and Mostofi, 1998; Siddiqui et al., 2009; Hakim et al., 2010). Performance of plants could be improved by adopting the different strategies for exogenous application of plant hormones under environmental stress. To overcome this disorder, proper management practices and exogenous application of plant growth hormones could be very helpful in the field. The aim of this study was to study the effects of gibberellic acid on

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Table 1. Effects of GA_3 on shoot length, shoot FW, shoot DW, root FW, root DW and leaf area of *H. sabdariffa* L.

Treatment	Shoot length (cm)	Shoot FW (g)	Shoot DW (g)	Root FW (g)	Root DW (g)	Leaf area (cm^2)
0mM NaCl + 0M GA_3 (control)	17.00 ± 0.29^{b}	1.73 ± 0.15^{bc}	0.27 ± 0.01^{b}	1.53 ± 0.15^{b}	0.17 ± 0.01^{b}	43.15 ± 2.50^{bc}
0mM NaCl + 10^{-6} M GA_3	21.17 ± 0.44^{a}	3.41 ± 0.46^{a}	0.45 ± 0.02^{a}	1.87 ± 0.06^{a}	0.23 ± 0.02^{a}	73.56 ± 5.54^{a}
40mM NaCl + 10^{-6} M GA_3	17.17 ± 0.20^{b}	2.09 ± 0.22^{b}	0.27 ± 0.03^{b}	1.46 ± 0.02^{b}	0.16 ± 0.01^{b}	52.22 ± 2.35^{b}
80mM NaCl + 10^{-6} M GA_3	15.50 ± 0.50^{c}	1.51 ± 0.15^{bc}	0.19 ± 0.01^{c}	0.91 ± 0.13^{c}	0.10 ± 0.03^{c}	36.56 ± 2.88^{cd}
120mM NaCl + 10^{-6} M GA_3	14.23 ± 0.58^{c}	1.24 ± 0.04^{c}	0.15 ± 0.01^{c}	0.64 ± 0.03^{c}	0.06 ± 0.01^{d}	31.60 ± 1.83^{d}

Means of a given data followed by the same letter(s) are not statistically different (P<0.05).

growth parameters, photosynthetic pigments and enzyme carbonic anhydrase activity in *H. sabdariffa* L. cv. Sabahia 17 under NaCl stress.

MATERIALS AND METHODS

The green house experiment was performed at the Department of Botany and Microbiology, King Saud University, Riyadh, KSA. The seeds of Karkade (*H. sabdariffa* L. cv. Sabahia 17) were obtained from the Sabahia Horticulture Research Station, Alexandria, Egypt. Before sowing, healthy seeds were surface sterilized with 1% sodium hypochlorite for 10 min, then vigorously rinsed with sterilized double distilled water (DDW). Five seeds were sown (1.5 to 2 cm deep) in plastic pots (6 cm in diameter), filled with perlite, supplied with Raukura's nutrient solution (Smith et al., 1983). The pots were arranged in a simple randomized design with a single factor and 4 replicates. The treatments included: (i) 0 mM NaCl + 0 M GA_3 (control), (ii) 0 mM NaCl + 10^{-6} M GA_3, (iii) 40 mM NaCl + 10^{-6} M GA_3, (iv) 80 mM NaCl + 10^{-6} M GA_3, (v) 120 mM NaCl + 10^{-6} M GA_3. Nutrient solution was applied at 100 mL pot^{-1} every 2 days. When the plants were at the stage of 2 to 3 true leaves, the treatment was added to the pots with experimental Karkade plants. The plots received irrigations (200 mL DDW in each pot) every 3 days to keep the perlite moist. The plants were sampled randomly from each experiment pot at 45 days after sowing to assess their growth characteristics (shoot length $plant^{-1}$, shoot fresh weight (FW) $plant^{-1}$, shoot dry weight (DW) $plant^{-1}$, root FW $plant^{1}$, root DW $plant^{-1}$ and leaf area $plant^{-1}$), relative water content (RWC), carbonic anhydrase (CA) activity, anthocyanin and photosynthetic pigments (chlorophyll (Chl) *a*, Chl *b* and total Chl).

The shoot length was measured by using a meter scale after removal from the pots. The plants were then placed in an oven run at 60°C for 48 h. These dried plants were weighed to record the plant dry weight. The leaf area was measured with the help of portable area meter, LI-COR, Model LI-3000, USA.

The RWC was expressed as percentage of the water content at a given time and tissue as related to the water content at full turgor (Slatyer, 1967). The relative water content was calculated using the following formula given by González and González-Vilar (2001):

$$RWC (\%) = [(FW - DW) / (TFW - DW)] \times 100$$

The activity of (CA: EC 4.2.1.1) was determined by the method of Dwivedi and Randhawa (1974). The leaf samples were cut into small pieces and suspended in cystein hydrochloride solution. The samples were incubated at 40°C for 20 min. The pieces were blotted and transferred to the test tubes containing phosphate buffer (pH 6.8), followed by the addition of alkaline bicarbonate solution and bromothymol blue indicator. The test tubes were incubated at 50°C for 20 min. After the addition of 0.2 ml of methyl red indicator, the reaction mixture was titrated against 0.05 N HCl. The results were expressed as [μmol (CO_2) kg^{-1} (FW) s^{-1}].

The Chl was extracted from fresh leaves of experimental plants using the DMSO method based on Barnes et al. (1992). Chl absorption in the extract was measured using UV-VIS spectrophotometer. Contents of the Chls were calculated using the following formulas:

$$Chl\ a = 14.85\ A_{664.9} - 5.14\ A_{648.2}$$

$$Chl\ b = 25.48\ A_{648.2} - 7.36\ A_{664.9}$$

$$Total\ chlorophyll\ (a + b) = 7.49\ A_{664.9} + 20.34\ A_{648.2}$$

The anthocyanin content was determined by incubating leaf sample overnight in 150 µL of methanol acidified with 1% HCl (v/v). After the addition of 100 µL of distilled water, anthocyanins were separated from chlorophylls with 250 µL of chloroform. Total anthocyanins were determined by measuring the A530 and A657 of the aqueous phase using a spectrophotometer. By subtracting the A657 from the A530, the relative amount of anthocyanin was calculated as OD/g.FW (Neff and Chory, 1998).

Statistical analysis

The data were analysed statistically with SPSS-11 statistical software (SPSS Inc., Chicago, IL, USA). Mean was statistically compared by Duncan's multiple range test (DMRT) at P>0.05%.

RESULTS

Application of GA_3 (10^{-6}M) significantly enhanced all growth attributes as compared to control under non-stress (Table 1). The data presented in Table 1 revealed that plants fed with GA_3 and 40 mM NaCl showed the lowest inhibition of all growth characteristics. Application of GA_3 and 40 mM of NaCl decreased shoot length by 18.89, shoot FW by 38.71, shoot DW by 40, root FW by 21.92, root DW by 30.43 and leaf area by 29.01% when compared to GA_3 application alone. However, application of GA_3 with 40 mM of NaCl showed parity with the control for shoot length, shoot FW, shoot DW, root FW, root DW and leaf area.

Application of GA3 alone GA_3 alone enhanced RWC, Chl *a, b,* total Chl, anthocyanin and enzyme CA activity as compared to control (Figures 1 and 2). The

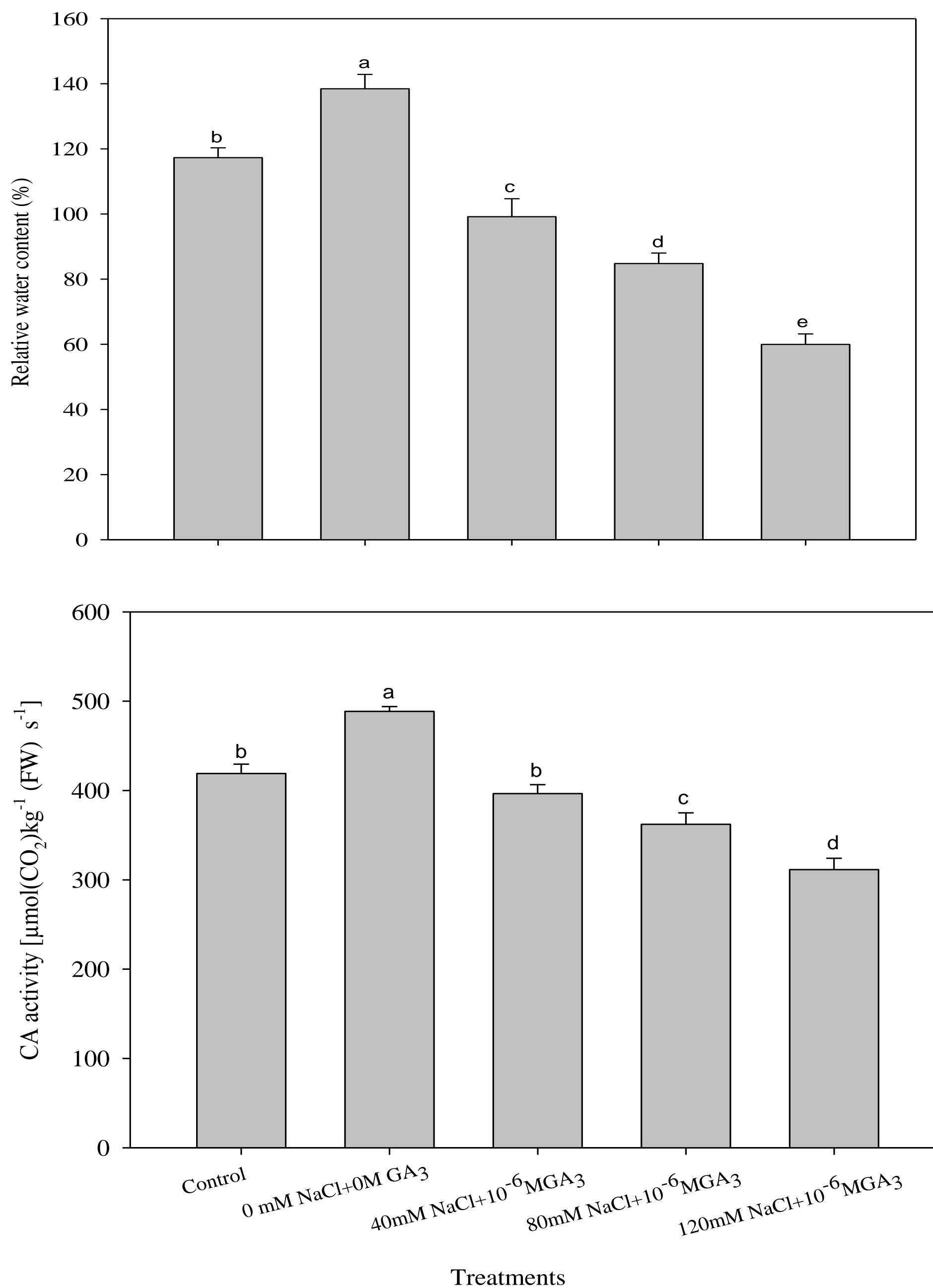

Figure 1. Effects of GA_3 on relative water content and carbonic anhydrase activity of *H. sabdariffa* L. Means of a given data followed by the same letter(s) are not statistically different ($P<0.05$).

alleviating effect of GA_3 was found to be maximum at 40 mM of NaCl. Application of GA_3 with 40 mM NaCl decreased RWC by 18.82, CA activity by 28.36, Chl *a* by 15.73, Chl *b* by 13.59, total Chl by 14.58 and anthocyanin by 41.25% as compared to GA_3 alone. However, application of GA_3 with 40 mM of NaCl exhibited statistically similar CA activity, Chl *a,* Chl *b* and total Chl, except RWC and anthocyanin content with the control.

DISCUSSION

Salt stress inhibits the plant growth by adversely damaging the various plant metabolisms (Siddiqui et al., 2009, 2010; Gunes et al., 2007, Khan et al., 2010). In this study, the application of GA_3 10^{-6} M was found to be more effective in alleviating the adverse effect of stress induced by 40 mM NaCl. The alleviating effects of GA_3

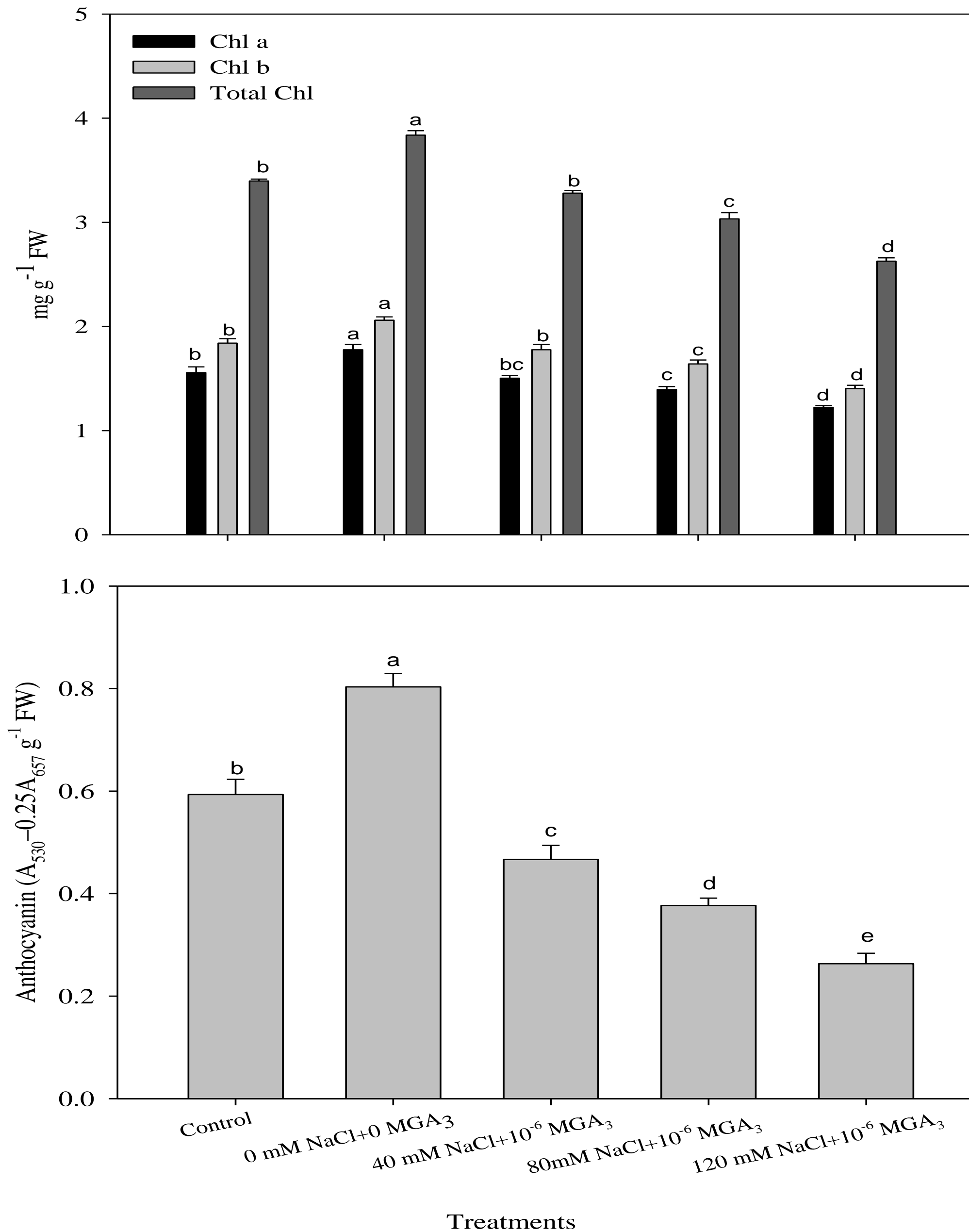

Figure 2. Effects of GA_3 on Chl *a*, *b*, total Chl and anthocyanin content of *H. sabdariffa* L. Means of a given data followed by the same letter(s) are not statistically different ($P<0.05$).

might be due to its role in the enhancement of CA activity, the enzyme which catalyzes the reversible hydration of CO_2 to HCO_3^-. This in turn, could be responsible for the reversal of altered plant growth inhibited by salinity. Salinity inhibits the RWC in plants. However, RWC is closely related to leaf water potential and finally to yield (Lafitte, 2002). Moreover, the application of GA_3 induced the RWC under non-saline condition (Figure 1). Under salt stress, minimum reduction of RWC was found at GA_3 with 40 mM of NaCl. This indicated that GA_3 maintained water balance in leaf. So, the result suggested that GA_3 helped the plant to avoid the water stress induced by salinity.

Reddy and Vora (1986) reported that salinity causes the inhibition of pigments due to instability of protein complex and destruction of Chl by inducing the activity of chlorophyllase, Chl degrading enzyme. In this study, the application of GA_3 significantly enhanced the Chl *a*, *b*, total Chl and antocyanin under non-saline condition (Figure 2). Under salinity, the application of GA_3 helped in the restoration of altered pigments concentrations induced by NaCl (Figure 2). It might be due to its

ameliorating role in primary growth potential, CA acivity and RWC. Salt stress suppressed the activity of CA enzyme due to inactivation of rubisco which sequentially reduces the net photosynthetic carbon metabolism, leaf Chl concentration and photosynthetic efficiency (Seeman and Critchley, 1985; Soussi et al., 1998). Interestingly, in this study, the application of GA_3 significantly alleviates the adverse effects of salt stress by increasing the activity of CA enzyme (Figure 1).

Conclusion

The results of this study shows that less reduction of plant growth, RWC, enzyme CA activity and pigments through the application of GA_3 might enhance salt tolerance of *H. sabdarifai* cv. Sabahia 17. This observation may be important for the improvement of the medicinal properties of this plant under salt stress conditions, especially in arid and semi-arid regions.

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