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Research Article

Simultaneous Treatment of Different Gibberellic Acid Doses Induces Ion Accumulation and Response Mechanisms to Salt Damage in Maize Roots

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ABSTRACT

The present study was aimed to find out the changes in plant responses to salinity with GA3 treatments. With this aim, combinations effects of salinity (350 mM NaCl) and three different doses of gibberellic acid (100, 300 and 500 ppm) on physiological and biochemical analysis of maize (Zea mays L.) roots were studied in soil experiment. The obtained results showed that treatment of GA3 (300-500 ppm) caused a reduction in salt-induced damage, improvement in biomass yield, regulation in water status, increasing proline level, reactive oxygen species (ROS) scavenging capacity, induction of SOD (36.6% at 500 ppm), CAT (28.5% at 500 ppm), APX (3.18 and 3.26 fold at 100 ppm and 500 ppm), GST (2.83 and 2.59 at 100 ppm and 500 ppm) enzyme activities, while POX activity was decreased only at GA3 (31.3% at 500 ppm), and thus alleviation of the oxidative damage. The results indicated that the salt application had a negative effect on the macro and micronutrient concentrations in roots. Otherwise, N, Ca and P concentration was increased by gibberellic acid under salinity, while, K, Cu, Mn, Fe and Zn were reduced compared to salt treatment alone. To sum up, these results showed that GA3 could be used as a signal molecule in antioxidant enzyme regulation related with ions for increasing salt tolerance in maize under salinity.

Keywords: Gibberellic acid; Maize; Salinity; Antioxidant enzymes; Ions Gibberellic acid; Maize; Salinity; Antioxidant enzymes; Ions

INTRODUCTION

Maize (*Zea mays L.*), one of the most important cereal crops, is grown under a wide spectrum of soil and climatic conditions. This crop is an important C4 plant from the Poaceae family and moderately sensitive to salt stress; nonetheless, it has wide intraspecific genetic variations for salt resistance [1] Salinity imposes osmotic stress and ionic toxicity to maize, and it shows adverse effects on growth and development. Salinity inhibits growth and development of most plants. Inhibition of shoot and root development is the primary response to the stress. Growth, morphology, anatomy, and physiology of roots are affected by salinity. Due to changes in water and ion uptake by the roots [2], a decrease in plant root weight and length has been detected under salt stress [3].

Salinity is one of the most damaging environmental stress factors in the world that restrain plant life [4]. It limits both product efficiency in agriculture and it effects geographic distribution [5]. Soil salinity cause ionic stress and low osmotic potential leading to cell and even plant death. Beside this, salt stress can create physiological drought in plants. Furthermore, accumulation of ions like Na^+ and Cl^- in plant tissues leads to oxidative damage which depends on ROS (reactive oxygen species). ROS are free radicals that are formed endogenously in plants [6]. Excess generation of ROS such as superoxide, hydrogen peroxide and hydroxyl radical cause oxidative damages in the apoplastic compartment and damages of cellular membranes by lipid peroxidation [7].

Plants eleminate ROS accumulation during salinity stress by enzymatic superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), glutathione -s-tranferase (GST), peroxidase (POD), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR) and glutathione peroxidase (GPX) non enzymatic (ascorbate,

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glutathione (GSH), a-tocopherol, carotenoid and phenolic compounds) pathways [8]. However, very aspects of plant mechanisms remain unclear under salt stress despite much important research [9]. The essential nutrients effect growth, biomass and productions in plant roots [10]. The root system architecture is greatly influenced by soil conditions [11], including nutrient gradients and concentrations of nitrate and phosphorus [12]. Roots also affect the surrounding nutrient composition by the release of organic compounds that play a vital role in mineralizing nutrients [13]. Hence, understanding the mechanisms involved in plant nutrient uptake is of key importance for improving worldwide agricultural production and mitigating environmental risks [14]. The salinity had an effect on the mineral compositions in leaves, roots and soil [15].

Some researchers have used plant growth regulators (PGRs) for decreasing or alleveting the adverse effects of salinity. The plant growth regulators are of considerable importance in mitigating the negative effect of salt stress [16]. GA_3 are endogenous plant growth regulators, having tetracyclic, diterpenoid compounds [17] that plays role in plant growth and development. Plants treated with GA_3 have determined that there is an improvement in physiological; such as seed germination, leaf expansion, and start of flowering, nutrient uptake and fruit development. Otherwise, Achard et al. [18] have found that the DELLA protein family is the major GA_3 negative regulator in regulating environmental signals and other plant hormone signaling pathways. The main role of gibberellic acid in regulating plant growth under abiotic stress conditions associated with DELLA proteins retarding growth, due to that eliminating the harmful effects of salinity by stimulating the accumulation of antioxidant enzyme activities [19]. Nimir et al. [20] reported that GA_3 caused a reduction in Na concentration and partly decreased the concentration of other ions. These results agreed with exogenous application of GA_3 could reduce salinity stress byreducing Na accumulation and increasing uptake of other essential nutrients [21]. In literature, there are many studies related with gibberellic acid and oxidative damage in plants but the results are contradictory. While GA application under stress conditions in some plants increases some enzyme activities depending on the application differences, it causes a decrease in others and this situation varies depending on the method of simultaneous foliar, Hoagland and seed soaking applications [22-24]. For example, GA_3 application increased antioxidant enzyme activities in some plants (Brassica juncea, Capsium annum) under salt stress [25,26]. In contrast, it was reported that application of GA_3 GA_3 decreased the antioxidant enzyme activities in Brassica juncea [27]. In summary, this study was aimed to discuss and explain the work flow of antioxidant enzymes in detail, ion concentrations and other parameters under GA_3 application with salinity in maize roots.

MATERIALS AND METHOD

Plant growth and experimental design

Seeds belonging to maize (*Zea mays L.*) M14G44 cultivar seeds, supplied from MAY-Agro seed company (Bursa), were used as the biological material of this present study. The seeds were sown in plastic pots, each of which (width 20 cm, length 12 cm, height 18 cm) contains 30-40 seeds and the mix ture of soil: clay: clay-loam in the ratio of 2:1:1, pH: 6.5. Seeds were placed in the dark for 5 days for germination. After germination, seedlings were grown in a growth room at 25°C (16 h day/8 h night photoperiod), light intensity of 350 µmol m–2 s–1 and watered with Hoagland solution for 8 days [28].

The pots were divided into eight treatments. Each one contained three pots (totally 40 plants). Treatments; Control (1), NaCl 350 mM (2), GA_3, 100 ppm (3), GA_3, 300 ppm (4), GA_3, 500 ppm (5), NaCl+GA_3, 100 ppm (6), NaCl+GA_3, 300 ppm (7), NaCl+GA_3, 500 ppm (8) and watered with Hoagland solution for 10 days (Figure 1). Then, the seedlings were harvested on the 10th day and the samples were preserved at -80°C. All GA_3 (100, 300, 500 ppm) concentrations were applied with Hoagland solution in soil medium.

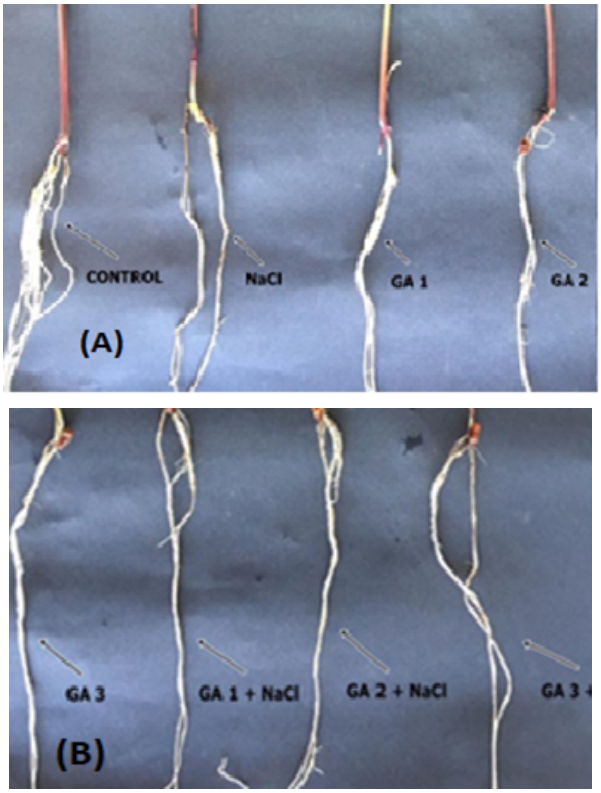

Figure 1: The effects of gibberelic acid treatment in maize (*Zea mays L.*) roots under salt stress. Control (1), NaCl 350 mM (2), GA_3 100 ppm (3), GA_3 300 mM (4), GA_3 500 ppm (5), NaCl +GA_3, 100 ppm (6), NaCl +GA_3 300 ppm (7), NaCl +GA_3, 500 ppm (8).

Physiological parameters and biomass yield

The relative water content (RWC) was calculated in accordance with Smart and Bingham [29]. Harvested roots were weighed to determine their fresh weights (FW). The seedlings were floated on de-ionized water for 5 h under low irradiance and then the turgid tissue was quickly blotted to remove excess water and their

turgid weights (TW) were determined. Dry weights (DW) were determined after the shoots were dried in an oven at 70°C for 72 h. In addition, root lengths were measured in all groups.

Measurement of malondialdehyde and hydrogen peroxide content

The level of lipid peroxidation in leaf samples was determined in terms of the malondialdehyde (MDA) content according to the method specified by Madhava and Sresty [30]. The MDA content, an end-product of lipid peroxidation, was determined by using the thiobarbituric acid reaction. The MDA concentration was calculated from the absorbance at 532 nm, and measurements were corrected for nonspecific turbidity by subtracting the absorbance at 600 nm. An extinction coefficient of 155 mM□1 cm□1 was used to determine the MDA concentration.

Hydrogen peroxide content the H2O2 content was determined according to Velikova et al. [31]. Fresh leaves (0.1 g) were homogenized in 5 ml of 0.1% trichloroacetic acid (TCA) and centrifuged at 12,000 rpm for 15 minutes. The supernatant (0.5 ml) was then mixed with 0.5 ml of buffer (10 mM potassium phosphate, pH 7) and 1 ml of 1 M potassium iodide. The absorbance reading was taken at 390 nm.

Measurement of proline content and hydroxyl radical scavenging activity

The proline contents of roots were determined according to Claussen [32]. For each treatment, 0.5 g leaf sample was ground in a mortar after addition of a small amount of glasspowder and 5 mL of a 3% (w/v) aqueous sulfosalicylic acid solution. The homogenate was filtered through two layers of glass-fibers. To the filtrate (1 ml), glacial acetic acid and ninhydrin reagent (1 ml each) were added. The closed test tubes containing the reaction mixture were kept in a boiling water bath for 1 h before the reaction was terminated at room temperature (22°C) for 5 min. The absorbance of the reaction mixture was determined at 546 nm. The proline concentration was determined from a standard curve and calculated on fresh weight basis (µg proline g-1 FW).

Hydroxyl radical scavenging activity. The ability of fusiformis extracts to scavenge the hydroxyl radical generated by Fenton reaction was measured according to the modified method given by Kim et al. [33], where A0 is the absorbance of the control reaction and A1 is the absorbance in the presence of resveratrol sample. The scavenging activity on hydroxyl radicals:

[(A0 – A1)/A0 × 100]

Measurement of antioxidant enzyme activities

SOD enzyme activity: The superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed by its ability to inhibit the photochemical reduction of nitrotetrazolium blue chloride (NBT) at 560 nm [34].

CAT enzyme activity: The catalase (CAT; EC 1.11.1.6) activity was assayed in a reaction mixture (2 ml) containing 50 mM K-phosphate buffer (pH 7.0). Then, 12.2 mM H2O2 was added, and the reaction wasstarted by adding 100 µl supernatant. The activity was determined by monitoring the degradation of H2O2 at 240 nm over 2 min against a supernatant-free blank. Enzyme-specific activities were expressed as µmol of H2O2 oxidized min-1 mg-1 protein [35].

POX enzyme activity: The peroxidase POX (EC 1.11.1.7) activity was based on the method described by Herzog and Fahimi [36]. The increase in the absorbance at 465 nm due to oxidation of diaminobenzidine (DAB) was followed for 1 min. One unit of POX activity was defined as 1 µmol H2O2 decomposed in 1 min.

APX enzyme activity: The ascorbate peroxidase (APX; EC 1.11.1.11) activity was measured according to Nakano and Asada [37]. The assay depended on the decrease in absorbance at 290 nm as ascorbate was oxidized.

GST enzyme activity: The glutathione-s-transferases (GST; EC 2.5.1.18) activity was determined by the method of Habig et al. [38] by following the increase in absorbance at 340 nm due to the formation of the conjugate 1-chloro-2,4-dinitrobenzene (CDNB) using as substrate at the presence of reduced glutathione (GSH).

Measurement of ion concentration

Ion concentration of roots was determined by [39]. The roots were washed for 1 min in deionised water, dried at 65°C for 48 h. The dried shoot samples were then ground and approximately 0.2 g of the ground samples were ashed at 550°C for 12 h for determination of total N, P, K, Mg, Ca, Fe, Cu, Mn, Zn concentrations. Total nitrogen (N) in digests was determined by the Kjeldahl method [40]. P concentrations were determined using the photocolormetric method described by Murphy and Riley [41]. K, Ca, Mg, Fe, Zn, Cu and Mn also determined by "Varian, 220FS" atomic absorption spectrophotometer [42].

Statistical analysis

The experiment was conducted in a completely randomized design and the measurements were performed with 6 replicates (n=6). Statistical variance analysis of the data was performed using ANOVA and the differences among treatments were compared using Tukey's post-hoc analysis with the least significant differences at the 5% level. In all the figures, the spread of the values is shown as error bars representing standard errors of the means.

RESULTS AND DISCUSSION

Effects of gibberellic acid on physiological parameters

Analysis of variance of data for fresh and dry weights of and roots, root length and relative water content of maize root (Table 1) showed that Salinity in the root zone had a significant adverse effect on these growth attributes (P<0,05). The results indicated that applied NaCl inhibited the plant growth, and cause a decrease in fresh and dry weights in the salinity condition compared to control. On the bases of our results, the root lenght of maize was reduced by salinity as compared to control group (1.97 fold). GA_3 treatments (100, 300 and 500 ppm) did not cause any change in these values compared to control. However, combined of GA_3 and salt treatment increased lenght value but the most increase

(38.19%) at 300 ppm GA_3 under salinity compared to the salt treatment alone (Table 1). In the present study, Table 1 shows that salt treatment increased the fresh weight and decreased dry weight of roots in maize plants as compared to the control groups. However, 100 and 300 ppm of GA_3 doses significantly increased the dry weight of root compared to control, but it progressively declined with further increases in GA_3 and salinity. On the other hand, 100 ppm GA_3 treatment alone did not cause any change in these values, whereas 300 and 500 ppm increased this values under the salinity condition (Figure 1). Increases in fresh weight of salt stressed plants in response to GA_3 may be related to the induction of protective role of membranes that increase the tolerance of plant to damage. From these results, it was clearly showed that in parallel with the statements of other researchers, salt stress had a negative effect on root length, fresh weight and dry weight of maize root (Table 1). However, GA_3 treated maize roots had an increase root lenght and weight value because GA_3 encourages cell division and cell elongation under salinity. Similarly, Kaya et al. [43] reported that exogenous GA_3 application promoted lenght and weight of wheat and tomato plants.

Table 1: The effects of gibberellic acid treatment on dry and fresh weight, lenght, relative water content of roots of maize (Zea mays L.) under salt stress. Control (1), NaCl 350 mM (2), GA_3 100 ppm (3), GA_3 300 mM (4), GA_3 500 ppm (5), NaCl+GA_3, 100 ppm (6), NaCl+GA_3 300 ppm (7), NaCl+GA_3, 500 ppm (8).

	Dry weight (g)	Fresh weight (g)	Length (cm)	Rwc
C	0.047 ± 0.014^{a}	0.48 ± 0.10^{a}	48.75 ± 3.77^{a}	29.22 ± 3.25^{a}
NaCl	0.032 ± 0.011^{b}	0.71 ± 0.12^{b}	24.66 ± 3.50^{b}	23.74 ± 2.66^{b}
GA1	0.049 ± 0.007^{a}	0.46 ± 0.41^{a}	44 ± 4.14^{c}	29.84 ± 2.15^{a}
GA2	0.055 ± 0.019^{c}	0.51 ± 0.13^{c}	47.66 ± 4.76^{a}	32.94 ± 7.58^{c}
GA3	0.036 ± 0.003^{d}	0.59 ± 0.14^{d}	44.16 ± 4.44^{c}	26.65 ± 6.23^{d}
GA1+NaCl	0.042 ± 0.009^{e}	0.46 ± 0.16^{a}	34 ± 6.32^{d}	27.39 ± 1.67^{d}
GA2+NaCl	0.042 ± 0.009^{e}	0.60 ± 0.11^{e}	39.83 ± 8.30^{e}	27.04 ± 1.21^{d}
GA3+NaCl	0.041 ± 0.0131^{e}	0.70 ± 0.21^{f}	35.5 ± 5.68^{d}	36.2 ± 2.85^{e}

Table 2. The effects of gibberellic acid treatment on ion concentration roots of maize (Zea mays L.) under salt stress. Control (1), NaCl 350 mM (2), GA3 100 ppm (3), GA3 300 mM (4), GA3 500 ppm (5), NaCl+GA3, 100 ppm (6), NaCl+GA3 300 ppm (7), NaCl+GA3, 500 ppm (8).

MACRO(%)						MICRO (ppm)				
	%N	%P	%K	%Mg	%Ca	%Na	Cu	Mn	Fe	Zn
C	1.327^{b}	0.273^{ab}	1.170b	1.219^{a}	0.344^{e}	0.850^{g}	12^{a}	17.6^{ef}	550.8^{d}	81.7^{a}
NaCl	1.056^{h}	0.240^{cc}	0.723^{e}	1.027^{b}	0.471^{b}	2.711^{b}	11^{b}	26.8^{a}	732.6^{a}	53.9^{b}
GA1	1.159^{d}	0.237^{cc}	1.167^{c}	0.927^{c}	0.319^{f}	0.695^{h}	10.8^{b}	25.2^{b}	571.3^{c}	53c
GA2	1.123^{e}	0.290^{a}	1.235^{a}	0.794^{e}	0.268^{g}	0.898^{f}	10^{c}	15.5^{g}	540.2^{e}	50.1^{e}
GA3	1.464^{a}	0.252^{bd}	1.114^{d}	0.931^{c}	0.416^{d}	1.181^{c}	8.5^{d}	19.6^{c}	586^{b}	51.4^{b}
GA1+NaCl	1.075^{g}	0.255^{bc}	0.503^{h}	0.866^{d}	0.618^{a}	2.744^{a}	8.9^{d}	17.3^{f}	444.5^{g}	33.5^{g}
GA2+NaCl	1.243^{c}	0.210^{e}	0.689^{f}	0.867^{d}	0.318^{f}	2.373^{d}	9.1^{d}	18.7^{d}	458.6^{f}	23.1^{h}
GA3+NaCl	1.114^{f}	0.221^{dc}	0.560^{g}	0.745^{f}	0.456^{c}	2.570^{c}	7.9^{e}	18.1d^{e}	441^{h}	48.2^{f}

Relative water content is an important indicator of the plant water status under stress to establish the adequate amount of water needed for metabolism. In the present study, salt stress inhibited relative water content (18.75%) in maize roots but all GA_3 treatment alone did not change it significiantly (Table 1). Nevertheless, GA_3 treatment alleviated reduction in RWC values caused by salinity, while the highest increase was at 500 ppm (52. 48%) (Table 1). As a result of our study, GA_3 treatment mitigated the adverse effects of salt stress. Korkmaz et al. [16] determined that with the application of GA_3 in rapeseed plant, it provides the damage of salt stress by protecting the plant water balance.

Effects of gibberellic acid on enzymes

Analysis of variance of data for antioxidant enyzmes of roots showed that salinity had a significant adverse effect on these paramaters ($P<0,05$). In maize roots, 350 mM NaCl application increased MDA value by 2.5 fold compared to the control groups, although GA_3 application alone did not affect it at all concentrations (Figure 2). In the present study, GA_3 treatment under salt stress led to a 42.26, 50.1 and 49.05% decrease in MDA values respectively, as compared to the salt treatment

alone (Figure 2). Salinity increases lipid peroxidation and leads to accumulate MDA content in plants. In maize roots, GA_3 application minimized salt induced oxidative damage by supressing MDA content. This result is also parallel with the report of Khan et al. [44].

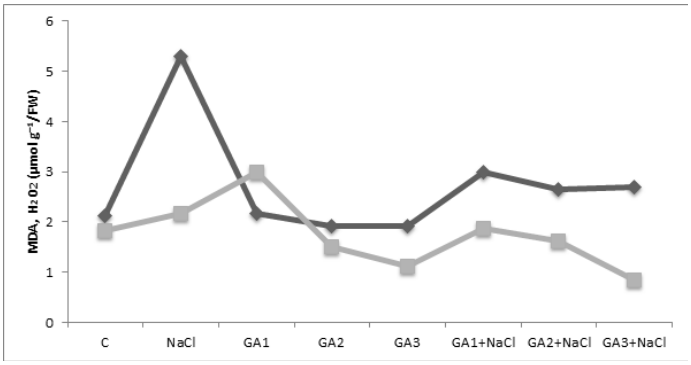

Figure 2: The effects of gibberelic acid treatment on MDA, H2O2 content of maize (*Zea mays L.*) roots under salt stress. Control (1), NaCl 350 mM (2), GA_3 100 ppm (3), GA_3 300 mM (4), GA_3 500 ppm (5), NaCl+GA_3, 100 ppm (6), NaCl+GA_3 300 ppm (7), NaCl+GA_3, 500 ppm (8)Columns with different letters represent significantly different (P<0.05) values.

Figure 2 demonstrates that hydrogen peroxide level was increased by 18.47% significiantly under salt treatments. When GA_3 was applied to salt treated plants, there was a noticeable decrease by 45.87% with 500 ppm GA_3 as compared to salt stress alone (Figure 2). It's well known that CAT is an important antoxidant enzyme that converts H2O2 to water in peroxysomes. In our results, GA_3 treatment decreased this activity by 62.85, 74.28 and 57.14% as compared to control groups in maize roots. Similarly, GA_3 exogenous application showed that hydrogen peroxide (H2O2) content was decreased in bean root (Phaseolus vulgaris L. cv. Naz), but it caused an increase with GA_3 application under salt stress [45].

In our research, ~1.71 fold decrease, in comparison to the control group, was observed in salt treated maize roots (Figure 3). On the contrary, GA_3 treatment alone did not affect proline levels at 100 and 300 ppm but 500 ppm increased this level by 2.2 fold as compared to salt stress alone. However, the proline level was increased by GA_3 plus salt treatment by (47.92, 66.86, 79.05%) as compared to salinity.

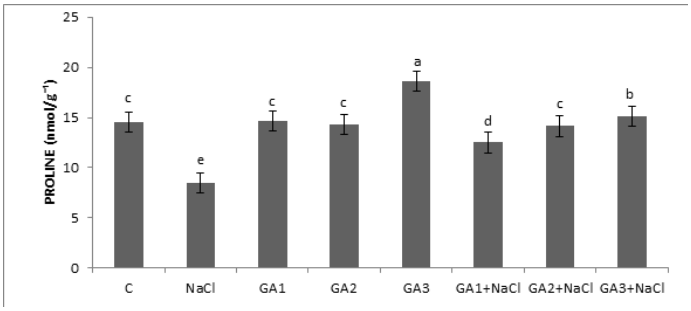

Figure 3: The effects of gibberelic acid treatment on prolin content of maize (*Zea mays L.*) roots under salt stress. Control (1), NaCl 350 mM (2), GA_3 100 ppm (3), GA_3 300 mM (4), GA_3 500 ppm (5), NaCl+GA_3, 100 ppm (6), NaCl+GA_3 300 ppm (7), NaCl+GA_3, 500 ppm (8). Columns with different letters represent significantly different (P<0.05) values.

Proline protects membrane stability and mitigates the effect of NaCl on cell membrane. In our results, salinity reduced proline levels and increased damage in maize roots (Figure 3). Neverthless, GA_3 application induced proline values significiantly and maintained water balance in roots. These results are also in agreement with the RWC and physiological parameters. Similarly, Wang et al. [46] reported that oak leaves had an increased proline level under GA_3 and salt stress. It could be suggested that in this experiment, GA_3 induced lower osmotic potential and maintain water balance to cope with salinity.

Figure 4 illustrates that plants treated with salt showed low hydroxyl radical scavenger capacity. However, GA_3 treatment alone decreased this level by (45.84, 46.95, 44.9%) as compared to salt treatment alone. From this result, it could be suggested that gibberellic acid may play role as an antioxidant and under stress conditions. Moreover, this capacity induced significiantly (16.8, 23.0, 24.3%) with increased concentration of GA_3 under salt treatment, as compared to the salt treatment alone. This finding showed that GA_3 could behave as a signal molecule to increase stress tolerance in maize roots.

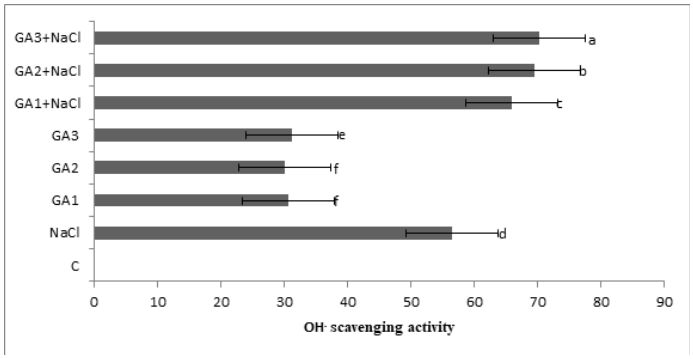

Figure 4: Time course effects of gibberelic acid treatment on hydroxyl radical (OH) scavenging activity of maize (*Zea mays L.*) roots under salt stress. Control (1), NaCl 350 mM (2), GA_3 100 ppm (3), GA_3 300 mM (4), GA_3 500 ppm (5), NaCl+GA_3, 100 ppm (6), NaCl+GA_3 300 ppm (7), NaCl+GA_3, 500 ppm (8). Columns with different letters represent significantly different (P<0.05) values.

In the present study, salt treatment alone decreased SOD activity (29%) in maize roots. SOD activity modulates superoxide radical content and removes to hydrogen peroxide and decreases the risk of OH. Radical formation. This result is parallel with the findings of Zhu et al. [4] who reported that okra seedlings had a decreased SOD activity under salinity. Otherwise, all GA_3 treatment alone decreased the SOD enzyme activity, compared to the control groups (Figure 5). However, in our results, exogenous application of GA_3 increased the activity of SOD activity under salt stress (18.8, 22.7, 36.6%). In contrast with this result, Chakrabarti and

Mukharji [47] also reported that SOD activity decreased in Vigna radiata by GA_3 spraying application.

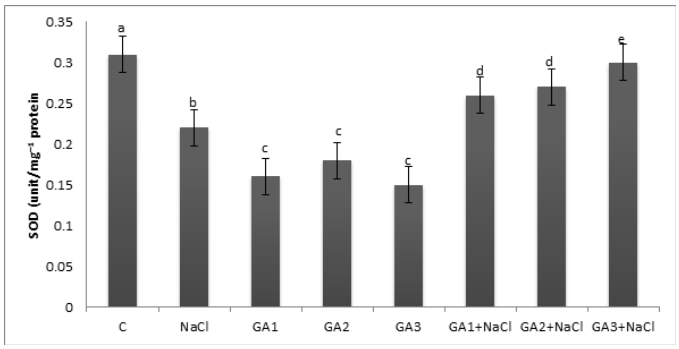

Figure 5: The effects of gibberelic acid treatment on SOD enzyme activity of maize (*Zea mays L.*) roots under salt stress. Control (1), NaCl 350 mM (2), GA_3 100 ppm (3), GA_3 300 mM (4), GA_3 500 ppm (5), NaCl+GA_3, 100 ppm (6), NaCl+GA_3 300 ppm (7), NaCl+GA_3, 500 ppm (8). Columns with different letters represent significantly different (P<0.05) values.

Similar to SOD activity, CAT level was decreased under salinity (20%), whereas it was increased only by combination of 500 ppm GA_3 and salt treatment by 28.57% (Figure 6). In parallel with this result, it was found that priming application of GA_3 increases the CAT activity in peper seeds under salinity [26].

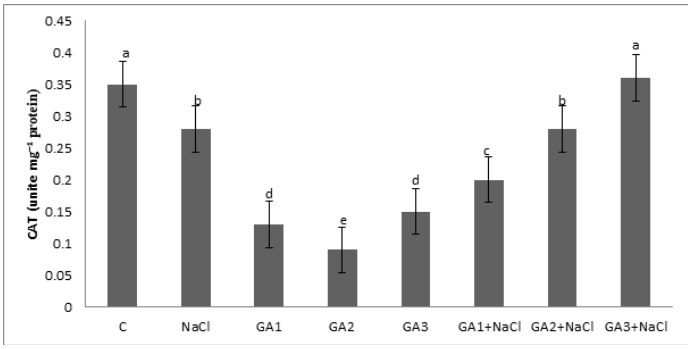

Figure 6: The effects of gibberelic acid treatment on CAT enzyme activity of maize (*Zea mays L.*) roots under salt stress. Control (1), NaCl 350 mM (2), GA_3 100 ppm (3), GA_3 300 mM (4), GA_3 500 ppm (5), NaCl+GA_3, 100 ppm (6), NaCl+GA_3 300 ppm (7), NaCl+GA_3, 500 ppm (8). Columns with different letters represent significantly different (P<0.05) values.

According to our results, POX enzyme activity was not changed by salinity by in maize roots. However, GA_3 treatment alone increased this activity 2.04, 2.64 and 1.52 fold as compared to control groups (Figure 7). This reaction could be related with the lignification role of POX enzyme besides catalyzing hydrogen peroxide in plant structure. Otherwise, 100 ppm and 300 ppm GA_3 and salt application increased this level, compared to salt stress, but this was reduced by 31.37% at 500 ppm. In contrast with this result, Tuna et al. [19]. Reported that POX enzyme activity was decreased with 100 ppm GA_3 (spray) under salinity in maize leaves. It's clear that the effects of GA_3 depend on application method and plant organ as mentioned before.

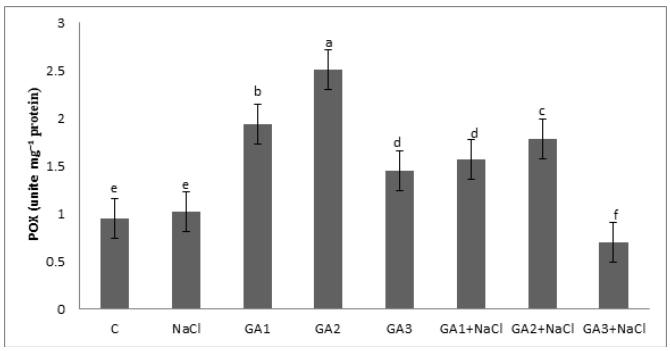

Figure 7: The effects of gibberelic acid treatment on POX enzyme activity of maize (*Zea mays L.*) roots under salt stress. Control (1), NaCl 350 mM (2), GA_3 100 ppm (3), GA_3 300 mM (4), GA_3 500 ppm (5), NaCl+GA_3, 100 ppm (6), NaCl+GA_3 300 ppm (7), NaCl+GA_3, 500 ppm (8). Columns with different letters represent significantly different (P<0.05) values.

APX enzyme activity was increased by 50% under salinity as compared to control groups. This result is also agreement with the report of Abdel Gawad et al. [48] in maize roots. Although this activity was positive for roots to scavenge ROS under salinity but only the increased APX activity was not efficient to cope with the severe NaCl stress. Also, all GA_3 treatment alone did not affect the APX enzyme activity. However, salt and GA_3 treatment increased this activity by 3.18, 2.38 and 3.26 fold as compared to salt stress alone (Figure 8). In contrast with this finding, Ahmad et al. [21] showed that GA_3 sprayed (75 ppm) Brassica juncea plants showed decreased APX enzyme activity under salinity. It could be suggested that the higher concentration of GA_3 was more effective to increase APX activity for detoxfying the hydrogen peroxide. Similar to POX activity, there is an important point to be underlined that spraying GA_3 gives different result from dissolving in nutrient solution in plants.

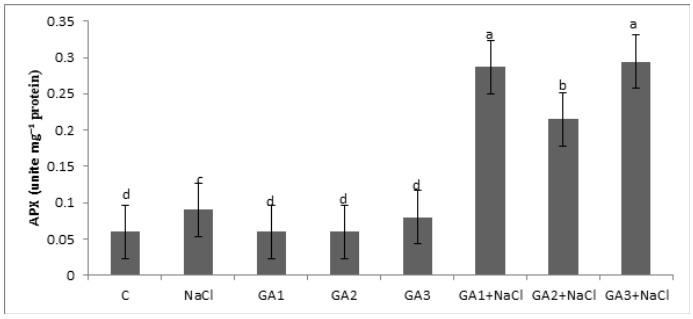

Figure 8: The effects of gibberelic acid treatment on APX enzyme activity of maize (*Zea mays L.*) roots under salt stress. Control (1), NaCl 350 mM (2), GA_3 100 ppm (3), GA_3 300 mM (4), GA_3 500 ppm (5), NaCl+GA_3, 100 ppm (6), NaCl+GA_3 300 ppm (7), NaCl+GA_3, 500 ppm (8). Columns with different letters represent significantly different (P<0.05) values.

On the basis of our results, Figure 9 shows that total GST enzyme activity was reduced by salt treatment in maize roots.

Otherwise, all GA_3 treatment with salinity increased this activity by 2.83, 2.50 and 2.59 fold as compared to salinity respectivelly. GA_3 applications alone decreased this level as compared to control groups in literature, there is no report with the effect of gibberellic acid on mode of action of this enzyme (GST) in vivo. Although there are few studies about the effects of hormones on GST enzyme activity (ABA, SA, NO) [49,50].

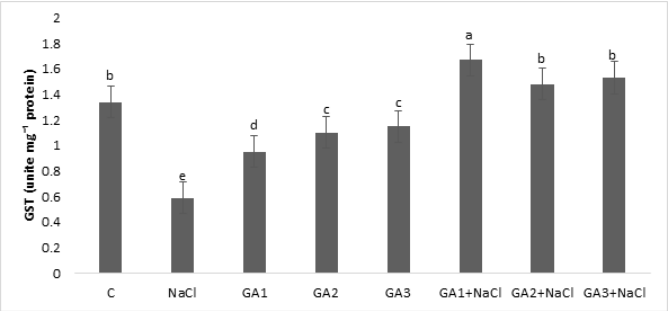

Figure 9: The effects of gibberelic acid treatment on GST enzyme activity of maize (*Zea mays L.*) roots under salt stress. Control (1), NaCl 350 mM (2), GA_3 100 ppm (3), GA_3 300 mM (4), GA_3 500 ppm (5), NaCl+GA_3, 100 ppm (6), NaCl+GA_3 300 ppm (7), NaCl+GA_3, 500 ppm (8). Columns with different letters represent significantly different (P<0.05) values.

Effects of gibberellic acid on ion concentration

Analysis of variance of data for ion concentrations of maize root (Table 2) showed that salinity in the root zone had a significant adverse effect on these ion concentration attributes (P<0,05). In our research, the nitrogen (N) concentration was reduced salt treatment by (20.42%) as compared to control (Table 2). However, the N level was increased by GA_3 plus salt treatment by (1.79, 17.70, 5.49%) as compared to salinity. In this study, N concentration was increased in plants grown in 500 ppm GA_3 application, due to the synthesis of proteins required to avoid salt stress. Salt concentrations without GA_3 caused reduction in free proline accumulation and treating plants with GA_3 increased free proline content due to its alleviating role over salinity. Similar findings, GA_3 treatment counteracted adverse effects of salinity with accumulation of more free proline which maintained membrane permeability and protein increased by macro and micronutrient absorption [1]. It has been further proved to play an important role in the GA signaling pathway [51].

Phosphorus (P) concentration was decreased by NaCl application 14.3% compared to the control groups. In other studies, increasing salt concentrations also reduced the amount of phosphorus compared to the control [52]. GA_3 applied to salt treated plants, there was a noticeable increase by 61.5% with 100 ppm GA_3, while was decreased 16.2% with 500 ppm GA_3 as compared to salt stress alone (Table 2). In the interaction between salinity and GA_3 treatments had been increased P concentration in plants compared to the salt stress alone [20]. This increase can be explained by growth parameters and increase in biomass.

In maize roots, 350 mM NaCl application decreased potassium (K+) concentration by 38.2% compared to the control groups. Yasar et al. [53] carried out a study examining the salt stress-tolerance of water melon genotypes and indicated that the plants selectively took up Na and K ion under salt stress conditions, and the sensitive genotypes promoted the uptake of Na ions rather than K ions. Moreover, under salinity stress conditions, with increasing salt concentration, the K ion concentration decreases in salt sensitive plants [54]. High concentrations of sodium can inhibit the uptake of K by plants through the antagonism between these two ions as well as potassium ion leakage when Ca substitutes for Na in the cell membranes [52]. A favorable increase in K concentration was recorded in the root of salt stressed plants as a result of GA_3 application. In the present study, GA_3 application alone by (61.41, 70.81, 54.08%) affected all concentrations as compared to the salt treatment (Table 2). Although GA_3 treatment under salt stress led to a 30.42, 4.7 and 22.54% decrease in K concentration respectively, (Table 2). The influence of GA_3 on the salinity mechanism of ions uptake may be related to its effect on membrane permeability and rate of ion entry through the membrane, or enhances their translocation to the shoot [47]. In the present study, GA_3 causes to a decrease in K concentration; from this result, it can be thought that GA_3 application provides tolerance to salt stress by replacing Na with K.

Table 2 illustrates that plants treated with salt showed hight magnesium (Mg+) concentration, in comparison to the control groups. Also GA_3 treatment alone decreased this level by (9.73, 22.68, 9.34%) as compared to salt treatment alone. Moreover, this capacity decreases significiantly (15.6, 15.6, 27.4%) with increased concentration of GA_3 plus salt treatment, as compared to the salt treatment alone. Exogenous application of GA_3 could reduce salinity stress by reducing Na+ accumulation and increasing uptake of Mg+ [21]. This increase can be explained by the increase in chlorophyll amount in the leaves by transporting (Mg+) to the above ground parts of the plant with the application of GA_3, and also the fact that it encourages root development and causes an increase length and dry biomass significantly recovered when GA_3 was added to salt-stressed plants [55].

Compared to the control treatments, Ca concentration significantly decreased (50%) under salinity stress that this decrease lowers role of calcium signals required for salt tolerance. Higher salt concentrations caused to reduce intake of Ca ion and ion imbalance in plant. These results are in agreement with those of Tuna et al. [19], Balkaya et al. [56] and Ekbic et al. [51] who reported that increasing salt application decreased Ca concentration in plant tissue. However, under salinity stress conditions, with increasing salt concentration, the Ca concentration increases in salt tolerant plants [51]. Calcium is known to improve the negative effects of salinity on plants [57]. Tuna et al. [19] reported that sodium ions may compete with calcium ions for membrane-binding sites and high calcium levels

can protect the cell membrane from the adverse effects of salinity. Ca plays an important role in the salt-tolerant signal pathway, as a second messenger. In the present study, GA_3 treatments also reduced the Ca concentration compared to salt stress alone (32.2, 43, 11.7%). Salt and 100 ppm GA_3 treatment increased this concentration by 31.2% compared to salt stress alone. These results indicated that application of low levels of GA_3 can regulate plant growth and have positive effects [4]. These results showed that the decrease in Ca ion under salt stress of GA_3 application can be explained by the fact that GA_3 changes cation with Ca.

Sodium (Na) concentration significantly decreased with GA_3 treatment (74.36, 66.87, 56.43%) as compared to the salt groups alone. Application of GA_3 to salinity stressed plants caused a partial decrease in the adverse effect of salinity. Specifically, 300 ppm GA_3 treatment under salinity decreased Na (12,46%) as compared to salt alone (Table 2). In parallel with our findings; exogenous application of GA_3 could reduce salinity stress by reducing Na accumulation in rice [21].

Copper, zinc, iron, and manganese are the ingredients of some of the important antioxidant enzymes. These of the metals are essential elements for plant growth. The most common isoforms of SOD known in the literature are copper–zinc containing superoxide dismutase (Cu/Zn-SOD), manganese containing (Mn-SOD) and iron containing (Fe-SOD) [58] Cu ion concentration was decreased with GA_3 treatment 1.8, 9.09 and 22.7% as compared to salt groups in maize roots. In parallel with our findings, Abdel-Hamid and Mohamed [59] determined that salinity reduced copper concentration in Hordeum vulgare plants. However, GA_3 plus salt treatment was decreased by 19, 17.3, 28.2% compared to salt stress.

On the basis of our results, table shows that Mn ion concentration activity was increased by salt treatment (Table 2). In contrast with this result, Tuna et al. [19] reported that salinity had reduced Mn concentration in maize. In our study; 100 ppm GA_3 applications increased this level as compared to control groups. Otherwise, all GA_3 treatment with salinity decreased this concentration by 35.4, 30.2 and 32.4% as compared to salinity respectivelly.

On the basis of our results, Table 2 shows that Fe concentration was increased by salt treatment. Otherwise; GA_3 applications decreased this level as compared to salt stress. Compared with the control, Fe the concentration had been increased by irrigation using saline water while by GA_3 unaffected [60] in maize. Similarly, all GA_3 treatment with salinity decreased this concentration by 1.6, 1.6 and 1.7 fold as compared to salinity respectivelly. Under salt stress, Fe-SOD activity increased only slightly in cytosolic and mitochondrial fractions and significantly in chloroplastic fraction [61]. For instance, the induction of total SOD and Fe-SOD activities has been recorded in the leaves of bean and tolerant pea [62].

In our results, Zn concentration was not changed salinity aplication compared to GA_3 aplications in maize roots. It was also show that Zn concentration decreased with elevated soil salinity on wheat, rice, and pepper plants [63]. However, GA_3 treatment decreased this concentration 35.1, 38.7 and 37% as compared to control groups. In according to Zn concentration; GA_3 and salt application was reduced compared to salinity stress by 37.8, 57.1, 10.6% (Table 2). Particularly, Ramezani and Shekafandeh, [64] reported that the application of GA_3 and Zn on plant growth and development.

CONCLUSION

In conclusion, there was a slight reduction in the activities of SOD, CAT and GST, whereas POX was increased and APX was non changed under alone GA_3 treatment. This result shows that plants with treated with GA_3 alone did not produce hydrogen peroxide and had no need to induce antioxidant defence response. Neverthless, all combination of GA_3 and salinity induced SOD, APX, CAT and GST activities (except POX at 500 ppm), relative water content and biomass yield by playing as a signal role. This result clearly showed that simultanous treatment of GA_3 and salinity promotes antioxidant enzyme acitivites (especially GST enzyme, which needs more analysis) in maize roots. In addition, ion concentrations contents (Cu^+, Zn^+, Mn and Fe^+) were reduced by GA_3 under salinity, whereas (N^+, P) were induced. Otherwise, Ca and Mg were reduced with GA_3 alone. It could be suggested that GA_3 protected maize roots from salinity by inducing cofactors (Cu^+, Mn, Fe^+, Zn^+) of SOD enzymes spesifically. According to the results of the present study, it can be concluded that GA_3 plays role in regulating the salt stress in maize, and suggested that GA_3 could be used as a potential stress inhibitor with the appropriate concentration and application method.

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