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Brassinolide and Salicylic Acid Induced Growth, Biochemical Activities and Productivity of Maize Plants Grown under Salt Stress

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Abstract: Application by bioregulators brassinolide (BR) and salicylic acid (SA) as seed soaking and foliar spraying alleviate the harmful effect of NaCl stress on maize plants . In the present study, irrigation by well water plus 50mM or 100mM NaCl markedly decreased shoot growth parameters (length, number of leaves, leaf area, fresh and dry weights and RWC) of 3 and 5 weeks old maize plants as compared with plants irrigated with well water only. Treatments with 0.25ppm BR or 0.15ppm SA significantly increased these parameters under the same conditions of salt stress. Content of photosynthetic pigments (chl a, chl b, carotenoids and total pigments) significantly decreased in leaves of stressed plants and increased in BR and SA- treated plants. Levels of some compatible solutes (total soluble sugars, free amino acids and proline) significantly increased in shoots of salt stressed plants treated or untreated with both regulators. The highest level was recorded in shoots of BR –treated plants especially at 3 weeks old. In addition , presoaking and foliar spraying by BR and SA greatly increased content of N, P , K^+ , Ca^{+2}, and Mg^{+2} and decreased Na^+, Na^+/K^+ and Na^+/Ca^{+2} in maize shoots , as compared with stressed control. Levels of both nitrate and ammonia and activity of nitrate reductase greatly decreased in salt stressed plants, and significantly increased in BR and SA treated plants. Finally, maize yield including plant mass, number of grains, weight of grains and weight of 100 grains/plant gradually decreased with increasing salt concentrations. Application with both bioregulators greatly increased maize productivity and BR was higher than SA. Results revealed that application with BR and SA as seed soaking plus foliar spraying increased maize resistance against salt stress by enhancing growth, metabolic activities and productivity of grain yield.

Key words: Brassinolide - salicylic acid – salinity- maize - growth - metabolic activity and productivity.

INTRODUCTION

Corn (*Zea mays* L.) grow in more countries than any other cultivated crops. It is a major source of food for both Human and animals through the world. Improvement in corn relies on better understanding of the corn itself, including its genome, physiology and behavior in growth and development [1].

As a result of the human activities of last decades, soil salinity and drought are serious problems in both agricultural and natural ecosystems. About 20% of the cultivated area and nearly half of all irrigated lands in the world are affected by salinity and/or drought. In Gaza strip, fresh water for drinking is difficult, people use ground water (well water) in their day needs since 1985. In Gaza strip wells salinized water markedly increased because of increasing the demand in well water for drinking and irrigation in agriculture which led to sea introgen into ground water. Ministry of Agriculture in Gaza (2001) indicated that the better fresh well water contained Cl^- 112 ppm and TDS 549. Whereas high salinity well water used in agriculture contained Cl^- 2787 ppm and TDS 5060.

Salinity stress is the major environmental factors limiting plant growth and productivity. Salt stress causes a lot of physiological and biochemical changes including the accumulation of low weight solutes, such as proline and betaine commonly referred to as compatible solutes [2,3]. The ability of plants to tolerate salt is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast functions and maintains ions[4,5]. Essential pathways include those that lead to synthesis of osmotically active metabolites and certain free radicals scavenging enzymes that control ion and water flux and support scavenging oxygen radicals or chaperone[6,7].

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Out of various compounds exploited to alleviate the plant stress, the brassinolide (BR) and salicylic acid (SA) are recognized as a novel groups of phytohormones to regulate the plant growth and their productivity[8]. Brassinosteriods (BR) are a group of naturally occurring plant steroidal compounds with wide ranging biological activity that offer the unique possibility of increasing crop yield through both changing plant metabolism and protect plants from environmental stresses[9]. BRs are recognized as regulators of transcription and translation thereby improving the level of proteins[10]. Several BRs mainly brassinolide have been evaluated in the field and have produced significant yield increases in various crops[11,12]. Besides this, BRs have an ameliorative role on plant, under biotic and abiotic stresses[13,14,15,16,17].

Salicylic acid (SA) naturally occurs in plants in very low amounts and participates in the regulation of physiological processes such as stomatal closure, nutrient uptake, protein synthesis, inhibition of ethylene biosynthesis and transpiration[18,19,20]. It has been identified as an important signaling element involved in establishing the local and systemic disease resistance response of plants after pathogen attack[21]. Also ,SA play an important role in abiotic stress tolerance and considerable interests have been focused on SA due to its ability to induce a protective effect on plants under stress[22]. Many studies support that SA induced increases in the resistance of wheat[23,24,25,26] and maize[27,28]to salinity and osmotic stress, respectively.

Finally, it is clear that BR and SA mediated physiological changes in plant, which play a vital role during environmental stresses. Therefore, this work was designed to study the possibility of both regulators to alleviate the retarding effect of salt stress and increased maize salt resistance. Application of BR and SA as seed soaking and foliar spraying on growth, nutrient uptake, some compatible solutes, nitrogen metabolism and productivity of maize plants irrigated with either well water or well water plus different levels of NaCl were investigated.

MATERIALS AND METHODS

Zea maize hybrid sweet corn (Merit v.) produced by Asgrow vegetable seeds company (USA). BR and SA were provided from Sigma. The water used in the present experiment was obtained from a known well, which is used for drinking and agricultural irrigation in Gaza strip. The well water analyzed for Na^+, K^+, P, Ca^{2+}, Mg^{2+}, Cl^- and total dissolved solid (TDS) had the following values (80.5, 3.99, 0.001, 25.7, 30.5, 361.0 and 1217.0 ppm) respectively.

A lot of homogenous maize seeds were washed thoroughly with tap water, then surface sterilized with 1% Na. hypochlorite solution for 2 minutes and finally rinsed with distilled water several times. Maize seeds were divided into 3 groups and soaked for 12 hours in distilled water, 0.25 ppm BR and 0.15 ppm SA, respectively. After air drying for 24 hours, five seeds were sown in 40 cm diameter pots containing 35kg loamy sandy soil (4:1) mixed with 5g calcium super – phosphate. Thirty five pots were divided into 3 groups (15 pots / group), each group was as follows:

1. Plants presoaked and sprayed with distilled water.
2. Plants presoaked and sprayed with 0.25ppm BR.
3. Plants presoaked and sprayed with 0.15ppm SA.

Each group was sub-divided into other three subgroups, the first one was irrigated with well water (control), the second was irrigated with well water +50mM NaCl and the third one was irrigated with well water + 100mM NaCl (2 liters once every 3 days). On the other hand, plants of 1st, 2nd , and 3ed groups were sprayed twice (at 2 and 4 weeks old) with distilled water , 0.25ppm BR and 0.15ppm SA mixed with 1ml of 0.1% tween 20. Spraying was carried out by an outomizer starting from the top down to the base of the plant continuously until the solution falls down of the leaves. Five replicates of the different treatments at 3 and 5 weeks old (1st and 2nd stages, respectively) were used for morphological and physiological studies.

Determination of Certain Metabolites: The photosynthetic pigments were determined by the spectrophotometric method as recommended by Metzner *et al*[29]. Total soluble sugars after extraction by 80% aqueous ethanol were determined using the phenol–sulphoric method according to Dubois *et al*[30]. Free amino acids were determined using ninhydrin according to Muting and Kaiser[31]. However, proline was extracted and measured from maize tissue according to the method of Bates *et al*[32].

Determination of Some Macro Elements: Extraction of minerals was carried out using nitric, sulfuric and perchloric acid according to the method described by Chapman and Pratt[33]. Total nitrogen was determined using the colorimetric method described by Yeun and Follord[34]. Calcium was determined using Ca^{2+} labkit according to Stern and Lewis[35] after some modification. To 150 µl of the sample, add 1ml of reagent A (Ethanolamine buffer 0.5 mM/L) and 1ml of reagent B (Cresolphthaleine 0.62 mM/L and hydroxyginoleine 69 mM/L) were added and mixed. The optical density at 575nm was recorded after 5 minutes of incubation time at room temperature. Also, magnesium was determined using Mg^{2+} labkit according to Grindler[36] with some modifications. To 100 µL of the sample, 2 ml of freshly prepared mixture of 1

volume of reagent B (Amino methyl propanol 1 mM/L and EDTA 0.21 mM/L) with 1 volume of reagent B (Calmagite 0.3 mM/L) were added and well mixed. Optical density at 520nm was recorded after 5min. Phosphorus was measured according to the method of Jackson[37]. However, potassium and sodium were determined photometrically using a flame photometer (JENWAY CO UK FPF 7) as mentioned by Jackson[37].

Estimation of Nitrate, Ammonia and Nitrate Reductase Activity: Nitrate (NO_3^-) was estimated by phenol disulphonic acid method as described by Snell and Snell[38]. Ammonia (NH_4^+) was estimated colourmetrically by the method adopted by Naguib[39]. Nitrate reductase (NR) was extracted using 0.2M phosphate buffer pH (7.4) containing 10^{-3} M EDTA and 5mM cystein according to Aslam *et al*[40]. Activity of NR was assayed following the method of Malik and Sight[41]. NR activity unit defined as µg NO_2 produced by g^{-1} Fwt h^{-1}.

Statistical Analysis: Five replicates of the different treatments were used in the present investigation and the data presented in this work was carried out for two successive years 2002-2003 and 2003-2004. The results were analyzed statistically using the one way analysis of variance (ANONVA) as described by Snedecor and Cochran[42]. Means were compared by LSD at 5%.

RESULTS AND DISCUSSIONS

Results:

Change in Morphological Criteria: Data in Table (1) appeared that growth parameters (shoot length, number of leaves, leaf area and fresh and dry weights of shoot) and RWC of 3 and 5 weeks old maize plants significantly decreased with increasing the level of NaCl as compared with control. Application with BR and SA (0.25 and 0.15 ppm, respectively) as seed soaking and foliar spraying greatly alleviate the retarding effect of NaCl on maize plants. It is clear that BR and SA increased shoot growth of maize plants irrigated with well water or well water + NaCl. But, treatment with BR was more effective than SA under the same conditions. RWC content in maize leaves significantly decreased in leaves of 3 and 5 weeks old maize plants irrigated with well water + 50 and 100 mM NaCl. Treatments with BR and SA highly increased RWC in maize leaves as compared with stressed or non-stressed controls. SA- treated plants have RWC than BR- treated plants of both stages.

Change in Metabolic Activities: The photosynthetic pigments (chl a, chl b, carotenoids and total pigments) significantly decreased in leaves of maize plants under salt stress, and reached to minimum values with well water + 100mM NaCl (Table 2). Treatment with BR highly increased chl a, chl b and total pigments in maize leaves as compared with stressed or non-stressed controls, while SA highly increased carotenoids especially in leaves of 5 weeks old maize plants.

Salt stress significantly increased TSS in shoots of maize plants treated or un-treated with BR and SA (Table 3). Plants irrigated with well water + 50mM NaCl have higher TSS than that of plants irrigated with well water or well water +100mM NaCl. The highest value of TSS was recorded in SA –treated plants. Total free amino acids gradually decreased in shoots of 3 weeks old plants with increasing the concentration of NaCl. But, the reverse was true in shoots of 5 weeks old maize plants (Table 3). At 3 weeks old, BR-treated plants have the highest value of free amino acids, while SA-treated plants of 5weeks old recorded highest free amino acids. On the other hand, proline markedly increased in shoots of maize plants treated or un-treated with both regulators. The highest increment of proline was noticed in 3 weeks old maize plants treated with BR.

Change in Some Macro Elements: In response to salt stress, content of some elements (N, P, K^-, Ca^{+2} and Mg^{+2}) significantly decreased in maize shoots as compared with non-stressed control (Table 4). Application with BR and SA markedly increased content of these elements in shoots under the same conditions of stress. BR- treated plants had higher content of N, P and Mg^{+2} than that of SA –treated plants. While high values of Ca^{2+} and K^+ were found in shoots of SA-treated plants.

Sodium content and ratios of Na^+/K^+ and Na^+/Ca^{2+} progressively increased with increasing concentrations and exposure time of NaCl. These increments greatly reduced in maize shoots as the result of presoaking and spraying with either BR or SA. The highest reduction was obtained in plants treated with SA.

The content of nitrate and ammonium significantly decreased in shoots of 3 and 5 weeks old maize plants irrigated with well water + 50 or 100 mM NaCl (Table 5). Presoaking and foliar spraying with BR and SA markedly increased content of nitrate and ammonia in maize shoots under the same conditions of stress. On the other hand, activity of NR decreased in shoots of stressed- maize plants as compared with non-stressed control. BR-.treated plants recorded the highest activity of NR.

Change in Productivity: Data in Table (6) appeared that salt stress significantly decreased yield of maize plants compared with control. It is clear that shoot mass, number of grains, weight of grains and harvest

Table 1: Change in shoot growth criteria of maize plants presoaked and sprayed with 0.25 ppm BR and 0.1 ppm SA under salt stress.

Treatments	length (cm)		No. of leaves /plant		Leaf area /plant (cm^2)		Fresh wt. (g/plant)		Dry wt. (g/plant)		Relative water) content (RWC)	
Stages	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Well water												
H_2O	24.0c	45.3c	7.30b	9.2b	50.6c	149.0c	22.0c	200.0c	2.2b	32.2a	80.8c	83.5c
BR	32.5a	60.8a	8.60a	11.0a	66.3a	175.1a	30.6a	292.0a	3.3a	29.0b	87.1a	88.8b
SA	30.8b	56.1b	7.80b	10.8a	60.6b	171.0b	28.5b	270.6b	3.1a	26.9c	84.6b	90.2a
LSD at 5%	0.82	1.74	0.61	0.32	2.65	3.32	0.4	20.91	0.30	0.55	2.30	1.55
Well water +50mM NaCl												
H_2O	20.5c	34.2b	7.0c	8.0b	39.8b	105.5c	14.4c	140.0c	1.24c	16.21b	62.8c	66.3c
BR	27.0a	46.3a	8.2a	10.2a	48.6a	138.0a	20.6a	210.6a	1.86a	21.0a	68.7b	75.7b
SA	25.6b	44.7a	7.6b	10.1a	49.5a	134.5b	18.5b	195.0b	1.73b	20.5a	72.8a	80.4a
LSD at 5%	0.87	1.92	0.54	0.26	5.18	3.30	0.62	12.72	0.25	0.66	2.42	2.14
Well water +100mM NaCl												
H_2O	16.0c	23.3c	6.6v	7.2c	30.1c	86.5c	7.6c	95.1b	0.81c	11.1c	54.5	58.6c
BR	21.2a	28.3b	7.3a	9.3a	39.2a	105.7a	12.9a	140.0a	1.43a	15.2a	63.8bc	71.6b
SA	19.0b	30.6a	7.3a	8.9b	35.5b	99.5b	12.1b	134.5a	1.36b	14.3b	68.7a	76.6a
LSD at 5%	0.43	1.48	0.77	0.25	2.4	3.5	0.53	22.6	0.05	0.43	2.83	2.78

Table 2: Change in photosynthetic pigments (mg g^{-1} fresh wt.) in leaves of maize plants presoaked and sprayed with 0.25ppm BR and 0.15ppm SA under salt stress.

Treatments	Chl a		Chl b		Carotenoids		Total pigments	
Stages	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Well water								
H_2O	0.42c	0.73b	0.23c	0.47b	0.08c	0.12c	0.73c	1.32c
BR	0.67a	0.87ab	0.35a	0.63a	0.16b	0.63b	1.18a	1.65b
SA	0.56b	0.97a	0.30b	0.69a	0.17a	0.69a	1.03b	1.69a
LSD at 5%	0.010	0.015	0.010	0.014	0.005	0.008	0.009	0.025
Well water +50mM NaCl								
H_2O	0.16b	0.65b	0.11c	0.41c	0.05c	0.08c	0.32c	1.14b
BR	0.36a	0.94a	0.21a	0.60a	0.13b	0.15b	0.70b	1.69a
SA	0.37a	0.88a	0.20b	0.53b	0.16a	0.17a	0.73a	1.58a
LSD at 5%	0.011	0.012	0.009	0.012	0.004	0.008	0.013	0.018
Well water +100mM NaCl								
H_2O	0.14c	0.46b	0.02c	0.21b	0.02c	0.05c	0.25b	0.72c
BR	0.33a	0.56ab	0.09b	0.31b	0.09b	0.11b	0.61a	0.98b
SA	0.31b	0.72a	0.11a	0.48a	0.11a	0.13a	0.62a	1.33a
LSD at 5%	0.005	0.018	0.004	0.011	0.004	0.004	0.011	0.021

Table 3: Change in some compatible solutes (mg/g fresh wt.) in shoots of maize plants presoaked and sprayed with 0.25ppm BR and 0.15ppm SA under salt stress.

Treatments	Total soluble sugars		Free amino acids		Proline	
Stages	1st	2nd	1st	2nd	1st	2nd
Well water						
H_2O	32.6c	38.1c	3.50c	4.43c	0.18c	0.08c
BR	104.5a	111.0b	5.07a	6.07b	0.35a	0.18a
SA	93.3b	118.6a	4.77b	7.08a	0.21b	0.10b
LSD at 5%	3.12	4.60	0.12	0.069	0.009	0.007
Well water +50mM NaCl						
H_2O	74.0c	76.7c	1.78c	5.16c	0.23c	0.12c
BR	114.8b	134.6b	2.93a	6.34b	0.48a	0.28a
SA	126.8b	145.6a	2.05b	7.81a	0.32b	0.22b
LSD at 5%	3.81	4.37	0.09	0.067	0.010	0.015
Well water +100mM NaCl						
H_2O	64.6c	68.5c	1.06c	5.96b	0.36a	o.35a
BR	83.91b	95.8a	2.03a	6.58ab	0.67a	0.51a
SA	96.20a	98.9a	1.75b	7.14a	0.44b	0.41b
LSD at 5%	3.80	3.87	0.08	0.106	0.01	0.016

Table 4: Change in mineral contents (mg g^{-1} dry wt.) in shoots of maize plants presoaked and sprayed with 0.25ppm BR and 0.15ppm SA under salt stress.

Treatments	N		Ca^{2+}		Mg^{2+}		P		K^+		Na^+		Na^+/K^+		Na^+/Ca^{2+}	
Stages	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Well water																
H_2O	3.40c	4.23c	2.7c	4.91c	2.31c	3.80c	0.50c	0.56c	6.71c	17.5a	14.4a	10.9a	2.15	0.62	5.33	2.27
BR	6.02a	7.88a	4.1b	6.50a	5.70a	5.51a	0.65a	0.74a	10.0b	21.6a	12.5c	8.3c	1.25	0.39	3.06	1.41
SA	4.48b	6.76b	5.0a	5.82b	5.56b	4.50b	0.59b	0.62b	11.5a	19.5b	13.3b	8.5b	1.27	0.44	2.66	1.60
LSD at 5%	0.09	0.06	0.12	0.12	0.01	0.014	0.02	0.011	0.05	0.30	0.12	0.08				
Well water +50mM NaCl																
H_2O	2.47c	4.84c	1.60c	4.06b	1.02c	3.30	0.48c	0.63c	3.1c	19.8c	21.2	17.6a	0.84	0.89	13.3	4.40
BR	4.85a	6.65a	3.61b	4.74a	3.12a	4.81	0.78a	0.87a	4.0a	23.8a	21.7	15.3b	5.43	0.64	6.03	3.26
SA	3.77b	5.61b	4.00a	3.92c	2.44b	3.5	0.52b	0.84b	3.7b	21.3b	19.7	14.0c	5.31	0.66	4.93	3.59
LSD at 5%	0.06	0.14	0.03	0.01	0.01	0.011	0.03	0.02	0.04	0.23	0.25	0.13				
Well water +100mM NaCl																
H_2O	1.34c	3.16c	1.03c	3.80a	0.90c	2.06c	0.38c	0.64c	2.2c	21.1c	24.7a	25.8a	11.3	1.23	24.7	6.82
BR	3.92a	5.80a	2.90b	3.96a	2.71a	3.71b	0.71s	0.77a	2.8a	23.3a	20.5b	17.3b	7.32	0.74	19.8	4.55
SA	1.51b	4.24b	3.06a	3.42b	1.83b	4.06a	0.44b	0.74b	2.5b	22.6b	21.2b	16.1c	8.46	0.71	7.07	4.74
LSD at 5%	0.04	0.09	0.07	0.08	0.01	0.012	0.02	0.02	0.09	0.18	0.88	0.15				

Table 5: Change in nitrate (NO_3^-), ammonia ($NH4^-$) and nitrate reductase(NR) activity in shoots of maize plants presoaked and sprayed with 0.25ppm BR and 0.15ppm SA under salt stress.

Treatments	NO_3^-(mgg^{-1} dry wt.)		NH_4^-(mgg^{-1} dry wt.)		NR(unit/µg NO_2 produced g^{-1} fresh wt.)	
Stages	1st	2nd	1st	2nd	1st	2nd
Well water						
H_2O	0.17c	0.36c	0.89c	1.90c	67.1b	33.92c
BR	0.33a	0.64a	1.93a	2.34a	83.5a	45.19b
SA	0.24b	0.55b	1.76b	2.09b	72.3b	50.65a
LSD at 5%	0.03	0.005	0.028	0.17	7.17	3.18
Well water +50mM NaCl						
H_2O	0.11c	0.31c	0.69c	1.88b	52.0b	18.63b
BR	0.18b	0.46a	0.98a	2.05a	65.2a	38.14a
SA	0.19a	0.42b	0.83b	1.90b	57.1b	32.02a
LSD at 5%	0.009	0.005	0.021	0.066	6.72	7.77
Well water +100mM NaCl						
H_2O	0.08c	0.24c	0.51b	1.46c	40.4b	15.88b
BR	0.12a	0.40b	0.67a	1.92a	50.5a	26.94a
SA	0.09b	0.41a	0.62a	1.81b	48.6a	26.56a
LSD at 5%	0.007	0.005	0.053	0.040	3.63	2.68

Table 6: Productivity of maize plants (13 weeks old) presoaked and sprayed with 0.25ppm BR and 0.15ppm SA under salt stress.

Treatments	Mean shoot mass (g/plant)	%	Mean No. of ears/plant	%	Mean No. of grains/plant	%	Wt. of grains (g/plant)	%	Wt. of 100 grains (g)	%	Harvest index (HI)	%
Well water												
H_2O	247b		1.6b		896c		151c		16.9c		0.61c	
BR	296a	120	2.0a	125	1121a	125	225a	149	20.1a	119	0.76a	125
SA	293a	119	2.0a	125	1032b	115	190b	126	18.4b	109	0.65b	106
LSD at 5%	27.3		0.38		123.1		16.2		0.59		0.03	
Well water +50mM NaCl												
H_2O	120b		1.0b		690c		90c		13.0c		0.75c	
BR	154a	128	1.4a	140	833a	121	147a	163	17.6a	135	0.95a	127
SA	146a	122	1.2b	120	786b	114	121b	134	15.4b	118	0.83b	111
LSD at 5%	13.8		0.11		55.9		9.6		1.03		0.03	
Well water +100mM NaCl												
H_2O	81b		0.8b		421c		25b		5.9b		0.31b	
BR	106a	131	1.3a	163	539a	128	40a	160	7.4a	125	0.38a	122
SA	95a	117	1.0a	125	478b	114	37a	148	7.7a	130	0.39a	126
LSD at 5%	11.3		0.37		43.3		5.0		0.88		0.04	

index decreased by 28%, 21%, 63% and 27% in plants irrigated with well water and 100mM NaCl. Presoaking and spraying with BR and SA enhanced yield parameters of maize plants, as compared with stressed control. Treatments with BR and SA increased number of grains by 21% and 14 % and weight of grains by 63% and 34 %, respectively in plants irrigated with well water + 50mM NaCl. Finally results appeared that BR was more effective than SA for increasing productivity of maize plants irrigated with well water +NaCl.

Discussion: Plant growth regulators, both natural or synthetic are wildly applied in agriculture and are used increasingly in manipulate plant growth and yield. Phytohormones can alleviate the retarding effect of salinity on plant growth as reported by Hathout[43], EL-Khallal and Nafie[44]and Anuradha and Rao[45]. Reduction in growth of 3 and 5 weeks old maize plants grown under salt stress may result from its effect on dry matter allocation, ion relations, water status, biochemical reactions and/ or a combination of many physiological factors[46]. The reduction in leaf area of maize plants under salt stress can be considered as a voidance mechanisms, which minimize water loss when stomata closed .It is known that reduction in leaf area in salt – stressed plants can be explained by a decrease in leaf turgor, changes in cell wall properties and a decreased in photosynthetic rate[47]. The susceptibility of maize plants to high concentration of NaCl is demonstrated by leaf necrosis, growth reduction and loss of fresh mass as shown in our results. These effects are probably due to an excessive increase and translocation of Na^+ and Cl^- ions to the leaf tissue, which cause alterations in the osmotic potential[5].

Application by 0.25 ppm BR and 0.15 ppm SA markedly increased growth of maize plants in comparison with stressed or non-stressed controls (Table 1). These results are in agreement with those obtained by Zaky[48] on *Vicia faba*; Vardhini and Rao[49]on sorghum, Ozdemir *et al*.[50]on rice and Khodary[51]on maize. Seed application with BR is sufficient to reduce the impact of salt stress on growth by restored pigment levels, increased nitrate reductase, nucleic acids and proteins as reported by Anuradha and Rao[52]on rice. Also, SA-treatment reduced damaging action of salinity on plant growth and accelerates growth processes after removal stress factors[20,22]. Induction in growth parameters of maize plants treated with bioregulators might be related to the induction of assimilating area, photosynthetic pigments and protein biosynthesis which consequently delayed leaf senescence which is induced by salt stress.

Relative water content (RWC) reflects the water status in plants; it is one of the important factors affecting plant growth and stress resistance. The decrease in RWC in salt –stressed maize plants may indicate a loss of turgor pressure that resulted in limited water available for cell expansion process[53]. Reduction in leaf turgidity of salt – stressed maize plants appeared to be alleviated by BR and SA which keep water within leaves and increase solute accumulation in the cytosol.

The ability of plants to tolerate salt is determined by multiple biochemical pathways that facilitate retention and/or acquision of water, protect chloroplast function and maintain ion homeostasis[6]. Salt stress affect photosynthetic components such as enzymes, chlorophyll and carotenoids, and this depend on the severity and duration stress[54,55]. In addition, reduction in the content of chl a and b in leaves of salt stressed maize plants could be attributed to increased activity of ion accumulation [56]. However, decrease in carotenoids lead to degradation of B-carotene and formation of Zeaxanthins, which are apparently involved in protection against photoenhibition [57]. Application by BR and SA as presowing and foliar spraying was effective in increasing level of pigments in leaves of maize plants grown under salt stress. High levels of these pigments might be explained by the fact that BR and SA had a protective effect on leave ultrastructure and preventing nucleus and chloroplast degradation[58], which delay leaf senescence /abscission. It has been reported that BR and SA caused increases in photosynthetic rate and carboxylating enzymes[52,59,51].

Accumulation of some compatible solutes (TSS , proline and free amino acids) in stressed plants produced lower solute potential , which allows plant cell to maintain a higher water content than the corresponding control. These solutes play an important role in plants under stress conditions, where major functions of sugars are osmoprotection and/or osmotic adjustment as reported by Parida *et al*.[60]. Accumulation of sugars in leaves of stressed plants especially treated with BR may be related to its reduced growth as result of NaCl inhibit sugar translocation or may be related to the greater energetically cost for osmotic adjustment[61]. In salt stressed maize plants, SA might be assumed to inhibit polysaccharide hydrolyzing enzyme system and/or accelerate the incorporation of soluble sugars into polysaccharides[51]. In addition, results cleared that presoaking and spraying with BR and SA significantly induced ability of maize to counteract salt stress by increasing levels of free amino acids and proline, which have been associated with a salt adaptive mechanism. Thus, induction in maize growth treated with either BR or SA may be related to high level of proline, which could serve as a reserve

supply of C and N for later recovery of the plants following stress.

Under salt stress , content of some macroelements (N , P , K^- , Ca^{+2}, Mg^{+2}) significantly decreased in maize shoots especially at 5 weeks old and irrigated with well water + 100mM NaCl (Table 4). Results revealed that salt stress affects many metabolic and growth aspects mainly due to sodic toxicity, where Na^+ competes with K^+ on active metabolic sites leading to depressed growth expressed as dry matter production[62,56,63]. On the other hand, results cleared that presoaking and spraying with BR and SA were effective in counteracting the toxic level of Na^+ by increasing mineral uptake and utilization. Thus increase in N content in shoots of hormone- treated plants could be attributed to high uptake of inorganic -N such as nitrate from soil and its assimilation. However, increase in K^+ uptake in shoots of 5 weeks old plants could be important for osmotic adjustment and enzyme activity. It has been reported that K^+ takes part in maintaining a higher cytosolic K^+/Na^+ ratio, which is a key requirement for plant growth and high salt conditions[64]. Shabala *et al*.[65] reported that salt tolerance in crops was determined by their ability to exclude Na^+ and Cl^- from shoots and maintain high shoot K^+ level.

In response to salt stress, nitrate and ammonia significantly decreased in shoots of maize plants. These results indicated that reduction in growth of stressed maize plants is mainly related to the decrease in nitrate uptake and its assimilation. Low level of nitrate in maize shoots caused proportional decrease in nitrate reductase activity. Abd-ELbaki[66]reported that NR activity and NR- mRNA were reduced by salt stress in maize seedling. On the other hand, reduction in nitrate acquisition may control growth of maize through restriction of amino acids supply for protein synthesis or vice versa. While increase in nitrate uptake in BR and SA - treated plants might be related to the increased level of Ca^{2+}, which has been shown to increase activity of nitrate transporter under saline conditions. Moreover, increase in nitrate level in hormone - treated plants seems to protect the NR enzymes against the action of proteases and/or inhibitors besides triggering the do novo synthesis of NR protein by induction of NR gene expression[67]. Also, reduction in ammonia level in shoots of salt stressed plants might be attributed to the high activity of ammonia assimilation enzymes especially glutamate dehydrogenase, which play an important role in ammonia assimilation under stress conditions[8]. Finally treatments of BR and SA which induced nitrate and ammonia levels in maize shoots may induced concomitant increases in the activity NR. From these results, it appeared that NaCl primarily alters growth of maize plants concomitant with reduction of N accquision which lead to negative feedback mechanisms. BR and SA applications are able to overcome the adverse effects of salt stress mediated by restoring the metabolic alterations imposed by NaCl.

Salinity is a major factor limiting the crop productivity in the semi-arid areas of the world[68] . Reduction in grain yield of stressed maize plants might be attributed to the rapid reduction in leaf photosynthetic pigments and assimilates. Therefore, translocation of assimilates from stem to grains is the main source as well as limiting factor for growth and development of grain. According to Munns[69]salinity reduces plant productivity first by reducing plant growth during the phase of osmotic stress and subsequently by inducing leaf senescence during the phase of toxicity when excessive salt is accumulated in transpiring leaves. Salinity decreased grain yield and dry matter production of wheat[70] and rice[71].

On the other hand , results revealed that reduction in maize yield under salt stress may be related to the high level of ABA[72], which play two roles in the developing of grains: (1)decrease grain mass by decreasing endosperm cell number and (2) induce grain abortion thereby reducing the number of grains competing for assimilates [73]. While, increase in maize productivity as the result of presoaking and spraying by BR and SA might be related to the increase in photosynthetic products which constitute an improved supply source for sinks, leading to increase grain yield. BR increased crop yields through changing plant metabolism and protect plants from environmental stresses[9]. Also, BR have potential to improve pool yield of stressed bean plants mediated through an influence on cytokinin content and nitrogenase activity in the nodulated roots[75]. On the other hand, the ability of SA to increase dry mass may have significant and over coming the yield barrier arising from conditions of limited water availability[59]. Finally, our result indicated that presoaking and foliar spraying with BR and SA significantly increased salt resistance of maize plants irrigated with well water plus NaCl by enhancing metabolic activities and induction of grain yield.

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