

Brassinolide and Salicylic Acid Induced Antioxidant Enzymes, Hormonal Balance and Protein Profile of Maize Plants Grown under Salt Stress

¹Samia .M. El-Khallal, ¹Tahani A. Hathout, ²Abd El Raheim A. Ahsour and ²Abd-Almalik A. Kerrit

¹Botany Department, Faculty of Girls for Arts, Science and Education, Ain Shams University, Cairo, Egypt

²Biology Department, Faculty of Science, Al_Aqsa University, Gaza, Palestine

Abstract: Salt damage has a broad physiological spectrum affecting metabolic processes. Application of 0.25 ppm brassinolide (BR) and 0.15 ppm salicylic acid (SA) as seed soaking and foliar spraying greatly alleviate oxidative stress in maize plants grown under salt stress. Results showed that activity of antioxidant enzymes (superoxide dismutase, peroxidase, catalase and ascorbate peroxidase) significantly increased in maize shoots as compared with stressed control. The highest activity was recorded in BR-treated plants. Salt stress led to sharp decrease in the levels of IAA, GA₃ and Zeatin, while ABA level greatly increased in maize shoots. Application with both regulators increased these levels and BR-treated plants had higher hormonal content than SA treated plants under the same conditions. Also, BR and SA markedly increased content of total soluble proteins and nucleic acids (DNA and RNA) especially in shoots of 3 weeks old plants irrigated with well water + 50 mM NaCl. In addition, protein profile of maize shoots indicate that BR and SA regulate expression of salt-stress inducible proteins as well as induced de novo synthesis of specific polypeptides, which are anticipated to play an active role in salt resistance. Thus, both regulators induced the appearance of novel protein bands having molecular weights 215, 122, 108 KDa (induced by BR), 48, 23 (induced by SA) and 34 (induced by both regulators) and not found in proteins of stressed or un-stressed control. Other protein bands of molecular weights 100, 89, 43.5, 40.5, 24 and 22 KDa were detected in maize shoots in response to salt stress, BR and SA. Results in the present work revealed that treatment with BR and SA greatly alleviate the harmful effect of salt stress on maize plants by increasing levels of antioxidant enzymes, endogenous growth hormones and induced the appearance of salt defense proteins.

Key words: salt stress- brassinolide- salicylic acid and *Zea mays*.

INTRODUCTION

From a general point of view, high salinity, most commonly mediated by NaCl, in soil or irrigation water is one of the major abiotic stresses globally^[1]. Salinity leads to oxidative stress in plants due to production of reactive oxygen species (ROS) such as superoxide radical (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radical (OH[·]) and alkoxy radical. These ROS produced in the cell and interact with a number of cellular molecules and metabolites thereby leading to a number of destructive processes causing cellular damage^[2,3,4,5]. Production of ROS are detoxified by both non-enzymatic and enzymatic antioxidant systems which play an important role in salt tolerance^[6]. A number of plant hormones such as ethylene, abscisic acid, salicylic acid and steroids are involved in the regulation of plant antioxidant enzymatic system^[7]. On the other hand, physiologic or metabolic adaptations to salt stress at

the cellular level are the main response to molecular analysis and have led to the identification of a large number of genes induced by salt^[8]. Salt stress is a multigenic trait and a number of groups are responsible for encoding salt-stress proteins: (1) genes for photosynthetic enzymes, (2) genes for synthesis of compatible solutes, (3) genes for vascular-sequestering enzymes, and (4) genes for radical scavenging enzymes^[9]. Therefore, induction of specific protein is a common response of plants to various environmental stresses. High expression levels of *SALT* gene and protein were detected in the roots and shoots of rice plants treated with salt, heat, ABA and JA^[10]. In addition, the genetic stress hormone ABA is up-regulated by salinity and induces genes involved in salt and osmotic alleviation^[11]. ABA-inducible genes are predicted to play an important role in the mechanism of salt tolerance on rice^[12].

Corresponding Author: Samia .M. El-Khallal, Botany Department, Faculty of Girls for Arts, Science and Education, Ain Shams University, Cairo, Egypt
E-mail: Samia_moheb@yahoo.com

Brassinosteroids (BRs) are hydroxylated derivatives of cholestan, which play an essential role in plant growth and development by influencing various physiological responses^[13]. BRs are also recognized as regulators of transcription and translation^[14] thereby improving the level of total proteins and enzymes^[15,16]. Recently, Aroar *et al.*^[17] observed that treatments with brassinolide markedly reduced activities of antioxidant enzymes.

Salicylic acid is an important signal molecule modulating plant response to stress. SA act as a potential non-enzymatic antioxidant as well as plant growth regulators, which play an important role in regulating a member of plant physiological processes including photosynthesis^[18]. Some earlier reports show that exogenous SA could ameliorate the damaging effects of heavy metals in rice^[19], salinity in wheat^[20] and drought in maize^[21]. In addition, the important protective action of SA probably reflects its ability to induce the expression of genes coding not only for PR-proteins, but also, for example, for genes encoding extension in *Arabidopsis* plants^[22]. Burkhanova *et al.*^[23] reported that SA induced synthesis of heat shock proteins in tobacco plants and fast activation of the 48KDa protein Kinase, which was identified as SIPK (salicylic acid induced protein kinase), demonstrating the involvement of SA in the induction of different antistress programs. SIPK is a new component in a Ca²⁺ and ABA-independent pathway that way lead to plant adaptation to hyperosmotic stress^[24]. Very recently, in spite of osmotic adaptation, Szepesi *et al.*^[25] reported that application by SA led to prolonged ABA accumulation and enhanced activity of aldehyde oxidase in *solanum lycopersicum*.

The present work was carried out to study the effect of presoaking and foliar spraying applications by brassinolide and salicylic acid on alleviating the oxidative stress induced by NaCl on maize plants. Therefore, changes in the activity of antioxidant enzymes, endogenous hormones, nucleic acids and protein profile in shoots of maize plants were investigated at two different stages of growth.

MATERIALS AND METHODS

Zea maize hybrid sweet corn (Merit v.) produced by Asgrow vegetable seeds company (USA). BR and SA were provided from Sigma company. 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, Ca²⁺, Mg²⁺, 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 3rd 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 automizer 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 was used for morphological and physiological studies.

Extraction and Assay of Antioxidant Enzymes:

Extraction was done as reported by Silavana *et al.*^[26]. One g of frozen plant tissue homogenized in mortar with 5ml special mixture buffer contained 50mM phosphate buffer pH 7.4, 1mM EDTA, 19 polyvinylpyrrolidone(PVP) and 5%(v/v) tritium x-100 under ice cold conditions. The homogenate was centrifuged at 10.000g for 20min and the supernatant were used for the assay of peroxidase (POX), superoxide dismutase (SOD), ascorbate peroxides (APOX) and catalase(CAT).

Activity of SOD was determined according to Silvana *et al.*^[26]. The activity was assayed by the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) as described by Becana *et al.*^[27] with some modification. In test tube 200 µL of enzyme supernatant + 300µL of K phosphate buffer pH 7.8 were added to 3.5 ml of O²⁻ generator mixture (14.3 mM methionine, 82.5 mM NBT and 2.2 mM riboflavin). Then shaken and placed the tubes under the direct lamp for 10min. Reading was carried out at wave length 560 nm, Blank and control were run in the same way but without illumination and enzymes, respectively. SOD activity unit defined as mg protein required to cause 50% inhibition of the reduction of NBT.

Peroxidase activity was assayed following the method of Kar and Mishra^[28]. Five ml of the assay mixture contained 300mM of phosphate buffer (pH 6.8), 50 mM catechol, 50 mM H₂O₂ and 1 ml of crude enzyme extract. The reaction was stopped by the addition of 1ml of 10% H₂SO₄. The colour was read at 430 nm, and the enzyme activity unit was expressed as the decrease in optical density g⁻¹F.wt h⁻¹.

Ascorbate peroxidase activity was assayed as described by Nakama and Asada^[29] using reaction mixture containing 50mM K- phosphate buffer (pH 7.0), 0.1 mM hydrogen peroxide, 0.5 mM ascorbate (reduced form) and 0.1mM EDTA. One ml of the reaction mixture was transferred directly into cuvette, and 100 µl from enzyme supernatant were added, and quickly measured the decrease in optical density within one minute at 290 nm. Ascorbate peroxidase activity unit defined as decrease in optical density g⁻¹ F.wt S⁻¹ under the assay conditions. Activity of catalase was estimated as mentioned by Chance *et al.*^[30] using reaction mixture consisting of 50 mM K-phosphate buffer pH (7.2) and 2 mM hydrogen peroxide. One ml of the reaction mixture was transferred directly into measuring cuvette, and 60µl from enzyme supernatant were added and quickly measured the decrease in optical density during one min at 240 nm. Catalase activity unit defined as decrease in absorbance at 240 nm g⁻¹ F. wt S⁻¹.

Extraction and Determination of Plant Hormones:

The method of extraction was adopted by Gazit and Bulmenfeld^[31]. The frozen plant material was blended in cold 85% methanol by an electric automixer with 85% methanol at about 0°C. Endogenous growth hormones was separated using ethylacetate after acidification to pH 2.5 using 1N HCl. The ethyl acetate fractions (IAA, GA, ABA) and aqueous fraction (cytokinins) were evaporated to dryness and dissolved in 5 ml HPLC methanol. 5µl of methanol extract was injected into HPLC apparatus with the following characters: column (C18- 3.5x 300 mm- silics- based packing materials, flow rate (1.0ml/min), absorbance detector(mode 1680) automade and filtration using 0.45µm filter.

Determination of Total Soluble Protein and Nucleic Acids:

Total soluble proteins was estimated using foline reagent according to the method described by Lowery *et al.*^[32]. Extraction of nucleic acids was carried out according to the method of Ogur and Rosen^[33]. This method is based on the elimination of the acid soluble fraction by cold perchloric acid (PCA) and the delipidation of tissue by treatment with alcohol-ether solvents. The insoluble fraction contains RNA and DNA which can be separated differentially by treatment with cold and hot PCA, respectively. DNA

was estimated by the DPA(diphenylamine) colour reaction as described by Burton^[34]. While RNA was determined colourimetrically by orcinol reaction as described by Dische^[35].

Protein Electrophoresis: Extraction of proteins was carried out in shoots of maize plants (5weeks old). SDS-PAGE was done according to the method of Lamli (1970)[36]. Gel was stained with commassi blue R250 and destained with 5% MeOH/acetic acid mixture. The lanes on the gel were scanned in Teinch soft laser scanning densitometer.

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^[37]. Means were compared by LSD at 5%.

RESULTS AND DISCUSSIONS

Results:

Activity of Antioxidant Enzymes: Results in Table (1) showed that activity of SOD in shoots of maize plants (3 and 5 weeks old) markedly increased with increasing salinity level. Treatment with BR and SA as presoaking and foliar spraying highly increased activity of SOD, as compared with stressed control. BR treatments showed the higher SOD activity than SA treatments. Also, activity of SOD in plants of 5 weeks old was higher than that of 3 weeks old.

Also, activity of peroxidase (POX) gradually increased in maize shoots of all treatments with increasing concentration and exposure time of salinity. The highest POX activity was recorded in shoots of SA-treatments of 3 weeks old, but at 5 weeks old, BR-treated plants have the highest activity (Table 1).

At 3 weeks old, CAT activity in shoots of maize plants treated or non- treated with BR or SA increased with increasing salt level, but the reverse was true at 5 weeks old. Among all treatments SA-treated plants recorded the highest activity as appeared in Table (1). However, activity of APOX greatly increased in all treatments in response to salt stress and bioregulators. The highest activity was noticed in plants presoaked and sprayed with 0.15ppm SA and irrigated with well water +100mM NaCl.

Endogenous Growth Hormones: Data in Figure (1) cleared that salt stress led to sharp change in the balance of endogenous hormone levels in maize shoots, as compared with non-stressed control. Levels of IAA,

Table 1: Change in the activity of antioxidant enzymes (SOD, POX, CAT and APOX) in shoots of maize plants (3 and 5 weeks old) presoaked and sprayed with 0.25ppm BR and 0.15ppm SA under salt stress.

Treatments	SOD		POX		CAT		APOX	
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
Well water								
H ₂ O	1.88b	2.90c	217c	390c	0.50c	171.6	0.26c	0.58c
BR	2.01a	3.25a	323b	814a	1.55b	203.6	0.93a	1.00a
SA	1.59c	3.18b	427a	464b	1.74a	208.5	0.73b	0.89b
LSD at 5%	0.07	0.06	37.0	31.6	0.06	0.06	0.009	0.005
Well water +50mM NaCl								
H ₂ O	2.36c	3.11c	285c	558c	2.12b	100.5c	0.47c	0.74c
BR	3.52a	4.44a	425b	916a	5.53a	155.2b	1.69a	1.18a
SA	3.03b	4.35b	564a	607b	5.44a	182.1a	1.50b	1.72b
LSD at 5%	0.09	0.09	32.0	20.4	0.12	0.12	0.04	0.05
Well water +100mM NaCl								
H ₂ O	2.23c	3.37c	452c	445c	4.47c	49.7c	0.76c	1.26c
BR	3.78a	4.67a	595b	1053a	7.60a	137.0b	1.88b	2.15b
SA	3.35b	4.49b	693a	793b	6.24b	166.4a	1.93a	2.48a
LSD at 5%	0.10	0.01	19.4	43.7	0.10	0.10	0.02	0.009

* SOD activity unit mg protein⁻¹, POX activity unit change in optical density.g⁻¹Fwt.h⁻¹, APOX activity unit decrease in absorbance at 290nm g⁻¹ Fwt.S⁻¹ and CAT activity unit decrease in absorbance at 240nm g⁻¹ Fwt.S⁻¹.

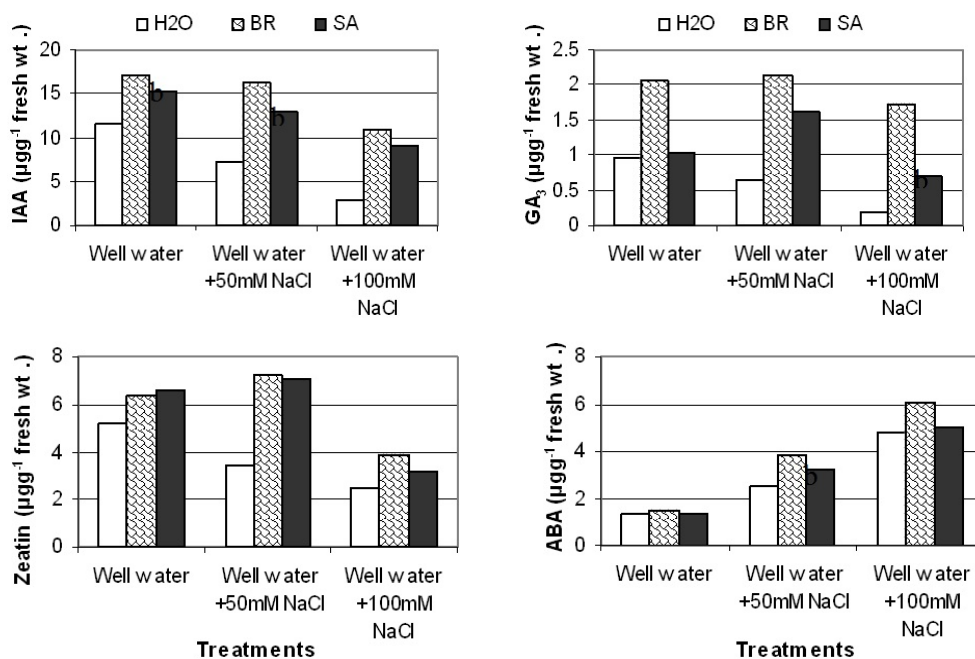


Fig. 1: Change in endogenous growth hormones (μg g⁻¹ fresh wt.) in shoots of maize plants (3 and 5 weeks old) presoaked and sprayed with 0.25ppm BR and 0.15ppm SA under salt stress.

GA₃ and Zeatin significantly decreased, while ABA level greatly increased in maize shoots by increasing salt levels. Application with BR and SA alleviate the decline in the level of growth promoting hormones as compared with non-treated plants, BR- treated plants had higher hormonal content than SA – treated plants under the same conditions. Among all treatments, BR-treated plants irrigated with well water + 50mM NaCl was the highest hormonal content.

Application with BR and SA alleviate the decline in the level of growth promoting hormones as compared with non-treated plants, BR- treated plants had higher hormonal content than SA – treated plants under the same conditions. Among all treatments, BR-treated plants irrigated with well water + 50mM NaCl was the highest hormonal content.

Change in Total Soluble Protein and Nucleic Acids:

As shown in Table 2 salt stress significantly increased TSP in shoots of maize plants treated or un-treated with BR and SA. Plants irrigated with well water + 50 mM NaCl had higher TSP than that of plants irrigated with well water or well water +100mM NaCl. The highest values of TSP were recorded in BR-treated plants. Concerning nucleic acids, results appeared that content of DNA significantly decreased in maize plants under salt stress. Both regulators (BR and SA) markedly increased DNA in shoots of 3 weeks old plants, as compared with stressed control. BR-treated plants had the highest content. However level of RNA increased in maize shoots in response to salt stress or /and growth regulators, but at 100mM NaCl, this level decreased in 5 weeks old plants as compared with 3 weeks old plants. The highest RNA content was recorded in BR- treated plants (5 weeks old) irrigated with well water+ 50 mM NaCl (Table 2).

Change in Protein Profile: The results of SDS – PAGE electrophoretic patterns of proteins extracted from shoots of maize plants treated or non-treated with BR and SA are shown in photo (1). The relative molecular weights and % of the polypeptide bands are recorded in Table 3. It is clear that 14 bands of molecular weights 75, 66, 54, 50, 45, 42, 39, 32, 29, 27, 26, 19, 13 and 69 KDa were found in soluble proteins extracted from different treated plants and could be considered as main bands (these bands were not recorded in table 3). However, protein bands of 148 and 110 KDa appeared only in soluble proteins of un- treated plants (well water).

In response to 50M NaCl, maize plants induced the appearance of 4 new proteins having molecular weights 205, 144, 112 and 10 KDa. While one band of 210 KDa were recorded in shoots of maize plants in

response to 100mM NaCl. Application with BR and SA as seed soaking and foliar spraying induced the appearance of novel protein bands having molecular weights 215, 122, 108 KDa (induced by BR), 48, 23 (induced by SA) and 34 (induced by both regulators) and not found in proteins of stressed or un-stressed control. Finally, protein bands of molecular weights 100, 89, 43.5, 40.5, 24 and 22 KDa were detected in maize shoots in response to salt stress, BR and SA.

Discussion: Salt damage has a broad physiological spectrum affecting metabolic processes. The over production of ROS and the presence of oxidative damage due to salt stress have been reported^[2,4,5]. Thus, the extent of oxidative stress is determined by the amount of ROS (O₂⁻, H₂O₂ and OH⁻) in plant cells, and the balance in the activity of antioxidant enzymes (SOD, APOX, POX and CAT) is crucial for suppressing toxic ROS levels^[38,39]. In the present study, high activity of antioxidant enzymes especially SOD, POX and APOX in shoots of maize plants treated with BR and SA (Table 1) indicate that both regulators greatly activate defense system in order to alleviate oxidative damage induced by salt stress. Therefore the enhancement of SOD activity in salt stressed plants may be attributed to the high induction of specific SOD isoenzymes, which contribute to the tolerance against salt stress^[40,41]. High activity of POX is a common response to various oxidative stress factors^[42]. Induction in POX activity in stressed cells reflect the change in mechanical properties of the cell wall , which in turn , could be related to the salt adaptation process since the properties are known to be modified by salt stress.

Activity of H₂O₂ scavenging enzymes significantly changed in response to NaCl stress. According to our results, APOX and POX probably had more important role in H₂O₂ detoxifying than CAT, although CAT together with APOX and POX play detoxifying role in plant. On the other hand, reduction in CAT activity in shoots of 5 weeks old plants may be due to the prevention of new enzyme synthesis^[43] or catalase photo-activation^[44]. At the molecular level, NaCl-stimulated CAT activity through activation of *Cat*₂ and *Cat*₃ genes in *Nicotiana plumbaginifolia*^[45]. However, Induction of APOX in maize shoots under salt stress (Table 1) may have even dramatic effect on the protection of plants against stress as compared with CAT. This induction led to generation of H₂O₂ at the intercellular space of the plant which appears to be diffused first into the cytosol and then into peroxysome in which CAT is typically found^[46]. According that APOX might be a key enzyme for the decomposition of H₂O₂ especially under CAT inactivation.

Table 2: Change in total soluble protein and nucleic acids (DNA & RNA) in shoots of maize plants (3and 5 weeks old) presoaked and sprayed with 0.25ppm BR and 0.15ppm SA under salt stress.

Treatments	Total soluble protein (mg ⁻¹ g dry wt)		DNA (μgg ⁻¹ dry wt)		RNA (μgg ⁻¹ dry wt)	
	1 st	2 nd	1 st	2 nd	1 st	2 nd
Well water						
H ₂ O	0.17c	0.36c	7.1b	11.2c	25.2C	28.6
BR	0.33a	0.64a	9.3a	18.8a	38.8b	43.1
SA	0.24b	0.55b	8.2a	16.5b	45.4a	37.6
LSD at 5%	0.03	0.005	1.23	0.36	1.12	2.05
Well water +50mM NaCl						
H ₂ O	0.11b	0.31c	5.5c	9.7	29.7	31.6
BR	0.18a	0.46a	7.8a	17.5a	41.2	48.7
SA	0.19a	0.42b	6.5b	15.4	38.6	44.3
LSD at 5%	0.009	0.005	0.84	0.31	1.72	2.17
Well water +100mM NaCl						
H ₂ O	0.08b	0.24b	3.7c	8.5	33.8	33.2
BR	0.12a	0.40a	6.3a	15.3	47.6	39.6
SA	0.09b	0.41a	6.1a	13.4	41.8	36.1
LSD at 5%	0.007	0.005	0.69	0.27	2.20	2.47

Table 3: Relative molecular weights (MW) and bands % of soluble proteins extracted from shoots of maize plants (5 weeks old) soaked and sprayed with BR and SA under salt stress.

MW KDa	Control			BR (0.25ppm)			SA (0.25ppm)		
	Well water	Well water + 50mMNaCl	Well water + 100mMNaCl	Well water	Well water + 50mMNaCl	Well water + 100mMNaCl	Well water	Well water + 50mM NaCl	Well water+ 100mMNaCl
215				3.6					
210			1.1						
205		1.0							
148	1.1								
144		1.6							
122				1.2					
112		1.5							
110	1.0								
108				1.3	1.2				
100	1.4	1.2	0.9	1.3	1.1				
89	1.3	1.2	1.0	0.9	1.1		1.9	1.1	
48							1.22		
43.5	2.7	2.2	1.5						3.2
37	3.2			2.4			1.8		2.5
34				2.2	2.6	3.5	2.7	3.5	4.0

Table 3: Continue

30.5	3.3	2.5	3.5	4.0	3.0	2.7
24	2.4	2.4	2.0	1.5	2.2	
23					3.1	2.7
22	2.4	1.6			1.7	1.8
10	1.5					

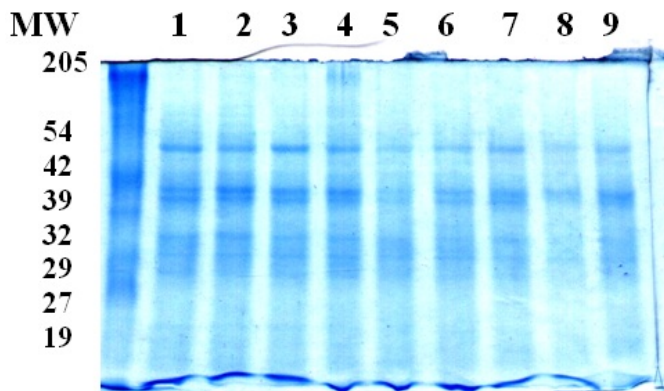


Photo 1: SDS-PAGE of proteins extracted from shoots of maize plants (5 weeks old) presoaked and sprayed with sprayed with BR and SA under salt stress.

Lane 1 = control(well water).

Lane 6= BR+ 100mM NaCl.

Lane2= well water+50mM NaCl.

Lane 7= SA+ well water.

Lane3= well water+100mM NaCl

Lane 8= SA+ 50mM NaCl.

Lane 4= BR+ well water.

Lane 9= SA+ 100mM NaCl.

Lane 5= BR+ 50mM NaCl.

Increase in the activity of antioxidant enzymes in shoots of maize plants treated with BR and SA led to an up regulation of the genes controlling the synthesis of these enzymes or an increased activation of constitutive enzymes pools in plants under stress conditions. Thus, growth regulator – treated plants have high resistance to the oxidative damage as compared with un-treated plants. Induction in antioxidant enzyme activities in BR- treated plants was reported by Li and Van- Staden^[47], Nunez *et al.*^[48] and Ozdemir *et al.*^[49] using different plants . BR play an important role in detoxifying the toxicity of ROS by enhancing activity of antioxidant enzyme in water- stressed wheat plants, making the plants more resistant to stress and improved the recovery growth of plants after stress^[50]. However, activation of these enzymes induced by pretreatment with SA may contribute to its antistress effects in plants. Sakhabutdinova *et al.*^[51]found that SOD and POX are involved in a range of defense reactions induced by SA in salinized wheat plants. Antioxidant system contribute substantially to SA- induced adaptation to subsequent stress conditions. It is clear from these results that salt tolerance capacity of maize plants treated or untreated with both regulators is

closely related with the maintains of specific activity of antioxidant enzymes.

Phytohormones play critical roles in regulating plant responses to stress. Under the effect of salinity levels, the endogenous growth hormones IAA, GA₃ and zeatin content decreased, while ABA content markedly increased. These results appeared that salt stress led to sharp changes in the balance of endogenous hormones which associated with the accumulation of ABA and decrease in the level of IAA, GA₃ and cytokinins. Thus, reduction in shoot growth of maize plants^[52] is probably related to hormonal signals generated in response to salt stress as suggested by Ghanem *et al.*^[53]. It is very likely that the large drop in CK contents in salinized plants (Fig 1) could contribute to the progression of senescence under stress. This decrease may be the result of reduced transport of root-synthesized CK, and/or increased breakdown of CKs^[54,55]. On the other hand, the strong decrease observed in leaf zeatin content can also may be explained by an induction of cytokinin oxidase activity. The role of this enzyme in plant could be the maintenance of an optimal level of cytokinin for plant adaptation under stress conditions (e.g. shift in shoot to root assimilate

partitioning) and/or resetting a cytokinin signaling system to a basal level^[56].

ABA is a genetic stress hormone that has multiple functions, including induction of genes involved in osmotic stress protection^[11]. Salt-induced ABA mediated the inhibition of leaf expansion and limited the accumulation of Na⁺ and Cl⁻ in leaves^[57]. ABA could also provoke carbohydrate accumulation by putatively blocking sucrose export from mature leaves, contributing to osmotic adjustment during the early phase of salinity^[58]. On the other hand, a high ABA level was less maintained in BR and SA treated plants providing the development of antistress reactions. Maintaining high level of ABA in growth regulators-treated plants under salt stress promotes protective reactions, which decreased injurious effects on growth and accelerate growth resumption. Finally, ABA induce late-embryogenesis abundant proteins (LEA), osmoprotectants and osmolyte biosynthesis genes^[59,60].

Salinity significantly reduced IAA levels in shoots of maize plants, which could cause delayed acquisition of resource necessary for generative growth and seed development^[61]. Also, it was reported that reduction in GA₃ and Zeatin levels can promote membrane deterioration by allowing a more rapid lipid peroxidation^[62]. Application with BR and SA prevented the reduction effect of salinity on the content of growth promoters in maize shoots (Figure 1). These results revealed that both regulators diminished changes in phytohormone levels in stressed plants. Thus promotion in growth of hormone-treated plants could be attributed to its effect on hormonal balance between the values of growth promoters and inhibitors. Similar results were obtained by Hathout^[63], Zaky^[64] and Uprati and Murti^[65] on wheat, *Vicia faba* and bean plants, respectively. On the other hand, results may indicate that BR act as mediator between specific genes responsible for initiation and stimulation of growth and processes of metabolism as suggested by Metzger^[66]. Also, studies of the BR signaling pathway and BR gene –regulating properties indicate that there is cross-talk between BR and other hormones (ABA, JA and ethylene), including those with established roles in plant defense responses^[67]. In addition, SA reduced the damaging action of salinity on plant growth and accelerate reparation of the growth processes mediated by maintaining high level of IAA, CKs and ABA, which in turn induce a wide spectra of antistress reactions in plants^[68].

Recently, Ghanem *et al.*^[53] reported that exogenous or endogenous hormones could improve crop salt tolerance by delaying both senescence during the initial phase of salinity and the subsequent toxic effects.

Changes in total soluble protein and nucleic acids in maize shoots may potentially alter the transitional process or the transcriptional one via modulating the activity of some enzyme responsible for enhancement of protein synthesizing machinery. The increase in soluble proteins in stressed shoots might be related to the requirement for an increased supply of glutamate, probably via protein degradation to induce accumulation of proline^[69]. In addition, increase in soluble proteins in BR and SA treatments has to be preceded by activation to transcribing nuclear DNA to RNA- dependent RNA polymerase^[70]. On the other hand, analysis of the plants proteome is an important amendment to the analysis of the genome, because gene expression is altered under adaptation to environmental stresses^[71]. Results showed that the appearance of 34 differentially regulated proteins in soluble proteins extracted from shoots of maize plants treated with both regulators and irrigated with well water or well water plus 50 or 100 mM NaCl. The results revealed that salinity and/or growth regulators altered the protein synthesis patterns, and this might predict the presence of several osmoresponsive genes which may be involved in adaptation to salt stress. Change in protein synthesis due to changes in the efficiency of mRNA translation, transcription, transport and stability. Liu and Zhu^[72] reported that accumulation of the specific proteins under salt stress may reflect the physiological reactions to a combination of ions in salt tolerance, osmotic adjustment, Na⁺/K⁺ homeostasis and Ca²⁺ mediated signal transduction.

Accumulation of proteins of molecular weights 23 and 22KDa which induced by SA had a possible role in salt adaptation and osmotic adjustment as reported by Parida *et al.*^[73]. Lopes *et al.*^[74] recorded that protein of 22KDa and its mRNA in the leaves of *Raphanus sativus* in response to salt stress or water deficit. Concerning protein band of molecular weight 26KDa, results showed that intensity of this protein increased in response to salt stress with or without growth regulators.

Induction of 26 KDa protein in maize plants has been speculated to represent osmotin that is involved in the rapid accumulation of proline and glucine betain during stress^[75]. Therefore, the gene coding for controlling the synthesis of the major osmoprotectants in plants is regulated by salt treatment^[76]. In maize plants, Zorb *et al.*^[77] detected three groups of differentially regulated proteins in roots and shoots under salt stress: A) proteins which are involved in protein biosynthesis and protein modifications by Kinase, (B) enzymes of the carbon metabolism and (C) enzymes of the nitrogen metabolism. In addition, several protein kinase and phosphatase are involved in response to salt stress^[78].

Protein profile of maize shoots indicate that BR and SA may regulate the expression of salt-stress inducible proteins as well as induced de novo synthesis of specific polypeptides, which are anticipated to play an active role in salt resistance. Also, involvement of growth regulators in the induction of alteration in protein patterns was attributed to their role in controlling cell division in the apical meristems by regulating certain genes namely *proliferin* or *cyclins*^[79,80]. BR altered gene expression involved in the stimulation of protoplasmic drought tolerance in leaf cells of *Sporobolus staphianus*^[81] and induced changes in protein profile of wheat^[82] and *Vicia faba*^[64] in response to water and salt stress, respectively. In addition, exogenous application by SA play a key role in coordinating and regulating several plant functions through their varied induction for growth responses –related genes within the target plant cells^[83]. Thus, results cleared that proteins of molecular weights of 48.0, 34.5 and 30.0 KDa which induced only by SA treatments may have functional roles in stimulating or blocking various metabolic events related to plant defense. Also, SA induce synthesis of heat shock proteins in tobacco plants and fast activation of 48KDa protein Kinase in suspension cell culture of tobacco under osmotic stress^[84]. In addition 48KDa protein was identified as salicylic acid induced protein Kinase (SIPK) that is activated by various stress stimuli. SIPK is a new component in a Ca²⁺ and ABA – independent pathway that may led to plant adaptation to hyperosmotic stress as reported by Hoyous *et al.*^[24] Moreover, results revealed that salt protein of 10KDa which induced in maize shoots by salt stress and BR completely disappeared in SA treatments. Hashim *et al.*^[85] found that protein of 10KDa disappeared in salinized rice roots treated with SA. The formation of specific protein bands in maize in response to NaCl, BR and SA appeared to be a reflection in alteration of gene expression machinery along the genomic make up DNA. Finally, results indicated that treatment with BR and SA greatly alleviate the retarding effect of salt stress on maize plants by increasing levels of antioxidant enzymes and endogenous growth hormones and induced the appearance of salt defense proteins. Thus, this work recommended that application by BR and SA especially as presoaking and foliar spraying was effective in overcoming salt stress on maize plants.

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