

Effect of Gibberellic Acid, Paclobutrazol and Zinc on Growth, Physiological Attributes and the Antioxidant Defense System of Soybean (*Glycine max*) under Salinity Stress

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Abstract Soil salinity is one of the major abiotic stresses which caused significant reduction in the growth parameters, photosynthetic pigments and yield components of soybean plants. The present study aims to improvement of soybean production under saline conditions and also tries to elucidate the possible mechanisms of plant tolerance by using three different treatments (Gibberellic acid, Paclobutrazol and Zinc sulphate). The magnitude of reduction, increased by increasing salinity level. Application of the above mentioned treatments, in the absence and presence of NaCl, has greater changes in most of the assayed parameters where the adverse effects of salinity as regards the growth characters, photosynthetic pigments and yield components as well as soluble carbohydrates, soluble protein and oil contents in the yielded seeds were significantly mitigated by treatment with either GA₃, PBZ or Zn. Salinity caused significant decreases in the activities of endogenous gibberellic acid (GA₃) and indole acetic acid (IAA) while activities of both Jasmonic acid (JA) and Absciscic acid (ABA) were increased. Significant increases were observed in the activities of superoxide dismutase (SOD), peroxidase (POX), catalase (CAT) and glutathione reductase (GR) in shoots of salt stressed plants. Application of GA₃, PBZ or Zn caused great variations in the activities of endogenous phytohormones and antioxidant enzymes. Treatment with either GA₃, PBZ or Zn caused significant reduction in both lipid peroxidation and proline that observed in shoots and root of salinized soybean plants. The electrophoregram of protein pattern of the yielded seeds of the soybean in response to their pre-emergence treatment with different concentrations of NaCl (50 mM & 100 mM) with or without GA₃, PBZ or Zn appeared variations in the number of electrophoretic protein bands. A total of 23 bands was detected with different molecular weights ranging from 129 kDa to 9 KDa. Two newly formed protein bands have appeared, 1 of them (16 KDa) at 50 mM with Zn or PBZ and the other band (19 KDa) has appeared at 50 mM NaCl with PBZ. Generally, it could be concluded that application of either GA₃, PBZ or Zn have (to more extent) a beneficial regulatory role in plants grown under salt stress conditions.

Keywords Gibberellic acid, Paclobutrazol, Zinc, Antioxidant defense system, Soybean (*Glycine max*), Salinity stress

1. Introduction

Worldwide, 20% of total cultivated and 33% of irrigated agricultural lands are exacerbated by high salinity. Phenomena like low precipitation, high surface evaporation, irrigation with saline water, weathering of native rocks, and poor agricultural practices have increased the rate of soil salinization to 10% per annum. It has been predicted that more than 50% of the arable land would be salinized by the year 2050 [1].

High salt levels generate a two-component stress on plants:

an osmotic stress caused by reducing water availability in soil and an ionic stress due to imbalance of solutes in the cytosol [2]. Recent research has identified various adaptive responses to salinity stress at molecular, cellular, metabolic, and physiological levels, although mechanisms underlying salinity tolerance are far from being completely understood. This paper provides a comprehensive review of major research advances on biochemical, physiological, and molecular mechanisms regulating plant adaptation and tolerance to salinity stress [3].

Soybean (*Glycine max* L.) a legume species native to East Asia, is now widely grown as the primary oilseed crop in the world including in many regions in the world. It is noteworthy that China is currently the largest importing country for soybean despite being one of its origins. To meet the increasing demand for food, oil and protein resources,

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further increases in soybean production are essential [4].

When plants are exposed to salinity stress, reactive oxygen species (ROS) hydrogen peroxide (H_2O_2), (superoxide radicals (O_2^-), singlet oxygen (O^*) and hydroxyl radicals (OH^*) are generated in response to stress conditions. ROS can cause oxidative damage to many cellular components, including membrane proteins, lipids, chlorophyll and nucleic acids [5].

ROS are well- termed second messengers of cellular processes, including ability to tolerate environmental stresses [6, 7]. Whether ROS will act as signalling molecule or damaging depends on the delicate equilibrium between scavenging and ROS production. Due to the multifunctional roles of ROS, it is essential for the cells to switch the level of ROS tightly to prevent any oxidative injury. Detoxing of excess ROS or scavenging is achieved by an efficient antioxidative system comprising of the enzymic in addition to nonenzymic antioxidants [8]. The enzymic antioxidants include superoxide dismutase (SOD) is a major scavenger of O_2^- and its enzymatic action results in the production of H_2O_2 and O_2 . Then, H_2O_2 is scavenged by a variety of peroxidases (POX) or directly broken into water and oxygen by catalases (CAT), glutathione reductase (GR). Glutathione reductase activity regulates the redox potential of cells and is it play an important role in the physiological under oxidative stress. The role of GR is to protect the cell against oxidative stress effects by maintaining a high reduced glutathione membrane to-oxidized glutathione (GSH/GSSG) ratio [9, 10].

In situations of limited soil, water around the roots, some compounds such as proline accumulate within the cells to supply appropriate conditions for absorbing water. Proline, that's usually regarded as an osmoprotectant, is proven to be involved in tolerance mechanisms against oxidative stress, the main strategy that plants use to avoid harmful results of abiotic stresses [11].

Exogenous application of different plant growth regulators is a well-recognized strategy to alleviate stress-induced adverse effects on different crop plants by regulating a variety of physiobiochemical processes such as photosynthesis, chlorophyll biosynthesis, nutrient uptake, antioxidant metabolism, and protein synthesis, which are directly or indirectly involved in the mechanism of stress tolerance [12]. The incorporation of plant growth regulators (PGRs) during presoaking treatments in many crops has improved seed performance under saline conditions [13]. It is also possible that under highly saline conditions, naturally present hormones and seed soaking with plant growth regulators helps to ameliorate the adverse effects of salinity by supplying hormones for normal growth. Phytohormones are known to influence a number of physiological processes, including enzyme activation. One of the most effective ways to overcome the problems of salinity is the use of plant growth regulators. Chauhan *et al.* [14] suggested that plant growth regulators help in overcoming the harmful effects of salinity on growth by changing the endogenous growth

regulators which affect plant water balance.

This study was designed to investigate the effect of either GA_3 (as growth promoter), PBZ (as growth retardant) or Zn (as micronutrient), NaCl and their interactions on growth, yield and some metabolic activities in soybean plants hopping to elucidate the role of these substances in alleviating the adverse effects of salt stress.

2. Materials and Methods

2.1. Methods of Planting, Treatments and Collection of Samples

The seeds of soybean (*Glycine max* L. var. klark) were obtained from the Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. A pot experiment was carried out in Botanical farm, Fac. of Sci., Al-Azhar Univ., Cairo, Egypt. Seeds were sown in pots 45 cm diameter. Each pot was filled with 10 kg of clay loamy soil (40% clay, 35% silt & 25% sand) with 15 seeds/pot. Thinning was performed after 1 week later of germination leaving Ten plants per pot. The pots were divided into four sets representing the following; first set contains (control, 50 mM NaCl, & 100 mM NaCl), the second set contains (GA_3 , GA_3 + 50 mM NaCl & GA_3 + 100 mM NaCl), the third set (PBZ, PBZ + 50 mM NaCl, PBZ + 100 mM NaCl) and the fourth set (Zn, Zn + 50 mM NaCl, Zn + 100 mM NaCl). The seeds and the developed seedlings irrigated with fresh water (control), 50 mM NaCl and 100 mM NaCl. While, each of GA_3 (200 ppm), PBZ (200 ppm) and Zn (150 ppm of $ZnSO_4$) was applied as presoaking (two hours) and as foliar twice when plants at 48 and 72 days old. Samples were collected for analysis when the plants 58 (Stage I) and 82 (Stage II) days old. At the end of the growth season analysis of the yield as well as the yielded seeds from the different treatments and controls were done.

2.2. Phytochemical Contents

Photosynthetic pigments and carotenoids were estimated using the method of Vernon and Selly [15]. Contents of soluble protein of seeds were estimated according to the methods of Lowery *et al.* [16]. Contents of soluble carbohydrate of seeds were measured according to the method of Umbriet *et al.* [17]. The oil content of seed was determined according to the method of AOAC [18] using soxhelt apparatus and petroleum ether (40-60°C) as a solvent.

2.3. Assay of Enzymes Activities

Protein enzymes were extracted according to the method of Kherjee and Choudhuri [19]. Super oxide dismutase (SOD) activity was measured according to the method of Dhindsa *et al.* [20]. Peroxidase (POX) activity was assayed using the method of Bergmeyer [21]. Catalase (CAT) activity was assayed according to the method of Chen *et al.* [22]. Glutathione reductase (GR) activity was assayed according to the method of Karni *et al.* [23].

2.4. Endogenous Phytohormones

Levels of endogenous gibberellic acid (GA_3), Jasmonic acid (JA), indole acetic acid (IAA) and abscisic acid (ABA) were determined for all treatments and the controls using HPLC according to the method of Lee *et al.* [24].

2.5. Determination of Lipid Peroxidation

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content according to Hernaldez and Almansa [25], fresh weight samples (500 mg) were homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000g for 20 min at 4 °C. One ml aliquot of the supernatant was mixed with 3 ml of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA and incubated at 90 °C for 20 min. After stopping the reaction in an ice bath, samples were centrifuged at 10,000g for 5 min. The supernatant absorbance at 532 nm was then measured.

2.6. Determination of Proline Content

For determination of the proline content, shoot and roots were hand-homogenized in 3% of sulfosalicylic acid and centrifuged at 3000g at 4 °C for 10 min. The supernatants were used for proline estimation according to the method of Bates *et al.* [26].

2.7. Electrophoreses

Total soluble proteins of seeds were analyzed using sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Soybean seeds (0.5 g) were homogenized with 2 ml of a buffer containing 50 mM Tris (hydroxymethyl) aminomethane (Tris)-Glycine (pH 8.3), 0.5 ml sucrose, 50 mM EDTA, 0.1 ml KCl, 2 mM PMSF and 0.1% (v/v) 2-mercaptoethanol in a chilled pestle and mortar at 4 °C. The homogenate was centrifuged in a refrigerated centrifuge (Sigma, 2K15, Germany) at 14,000g for 10 min. Protein concentration in the supernatant samples was estimated according to the method of Lowery *et al.* [16]. The supernatants were stored in small aliquots at -40 °C for SDS-PAGE.

Supernatant samples (40 µg protein) were mixed with equal volumes of solubilizing buffer [62.5 mM Tris-HCl, pH 6.8, 20% (w/v) glycerol, 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol and 0.01% bromophenol blue] and heated for 4 min at 95 °C, and then it cooled on ice. Polypeptide pattern was analyzed on 12% SDS polyacrylamide gels according to the method of Laemmli [27], as modified by Studier [28]. The gels were stained with 0.25% Coomassie Brilliant Blue R-250 (Sigma) in 50% (v/v) methanol and 10% (v/v) acetic acid for 2 h and destained with 50% (v/v) methanol and 10% (v/v) acetic acid until the background was clear. The gels were photographed and scanned using a densitometer (GS- 710, Bio-Rad, USA) and analyzed with AlphaEaseFCTM ver. 4 software.

2.8. Statistical Analysis

We calculate sample size according to Raosoft, and all

statistical calculations were done using SPSS (statistical package for the social science version 20.00) statistical program at 0.05 level of probability [29]. Quantitative data with parametric distribution were done using Analysis of variance the One-way ANOVA and Post hoc-LSD tests (the least significant difference). The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered non-significant (NS) at the level of > 0.05, significant at the level of <0.05, 0.01 and highly significant at the level of < 0.001. Discriminant analysis, automatic and linear modelling were estimated to show the relationship between quantitative parameters [30].

3. Results and Discussion

3.1. Growth Characters

The obtained results (Figs. 1-7) showed a retarded growth in salt-stressed plants. Shoot length, root length, fresh and dry weights of both shoots and roots and the number of leaves/plant were significantly decreased under saline condition. Decreases in the aforementioned characters were increased with increasing the level of salinity. Many studies have shown that biomass partitioning between roots and shoots is strongly influenced by the most limiting resource under stress growth conditions, and resource deficiency is often ameliorated by increasing the biomass allocation to the part of the plant responsible for acquiring the most limiting resource [31, 32]. The reduction in root and shoot development may be due to the toxic effects of the NaCl used as well as unbalanced nutrient uptake by the plants competitors between Na^+ and Cl^- and further anions and cations may result in a reduced plant growth and yield [33, 34].

Results of the present study (Figs. 1-7) revealed that, with a few exceptions, the growth characters of salinized and non-salinized soybean plants were increased under the application of each of GA_3 , PBZ and Zn. The aforementioned increases, in most cases, were found to be statistically significant. These results are more obvious in plants grown at the first level of NaCl (50 mM). The adverse effects of salinity as regards the growth parameters were significantly alleviated by the application of growth regulators [35]. In this regard, the applications of gibberellins increase the seed germination percentage by attributing the fact that they increase the amino acid content in embryo and cause release of hydrolytic enzyme required for digestion of endospermic starch when seeds renew growth at germination. Gibberellic acid acts synergistically with auxins, cytokinins and probably with the other hormone, is what might be called a system approach, or synergism. The overall development of the plant is regulated by the growth hormones, nutrient and environmental factors [14]. Regarding the treatment with PBZ, Hajjhashemi and Kiarostami [36] suggest that PBZ treatment may be useful to improve the salt tolerance of wheat via reducing the negative

effect of salinity on vegetative growth. Also, Mahmoud [37] revealed that, under non saline conditions, treatments with paclobutrazol (75 ppm) generally enhanced most of the growth and yield characteristics represented by the length of shoot & root length, fresh and dry weights of shoots and roots/plant and seed index of soybean plants. Regarding the treatment with Zn, some investigations illustrate the potent effect of Zn in overcoming the adverse effects of salinity on plant growth and development. In this concern, Anita *et al.* [38] recorded significant values of growth and yield of *Vigna radiata* plants grown under saline conditions in response to treatment with Zn. Moreover, Aldinary [39] revealed that, under saline conditions 4000 & 8000 ppm NaCl, treatments with Zn (100 ppm), in cowpea plants, generally enhanced most of the growth and yield characteristics (shoot length, root length, fresh and dry weights of shoots and roots/ plant 100 seed weights).

3.2. Photosynthetic Pigments

As shown in figures (8 & 9), contents of chlorophyll a & b in leaves of soybean plants were significantly decreased in response to saline conditions. The decrease in chlorophyll contents was increased with increasing salinity level. At the same time, the obtained results (Fig. 10) showed that carotenoid content in leaves of soybean plants were significantly increased under salt stress conditions. Several investigators confirmed that salinity adversely affects the photosynthetic pigments of different plants (Almodares *et al.* [40] on sorghum, Abeer [41] on *Vigna sinensis* and Aldinary [39] on cowpea plants. Mane *et al.* [42] revealed that decrease in chlorophyll content under NaCl salinity to the disruption in cellular functions and membrane deterioration. Disrupted photosynthetic electron transport chain or stability of the pigment protein complex with increased activity of chlorophyllase may also be the reason for the decrease in chlorophyll content under saline conditions.

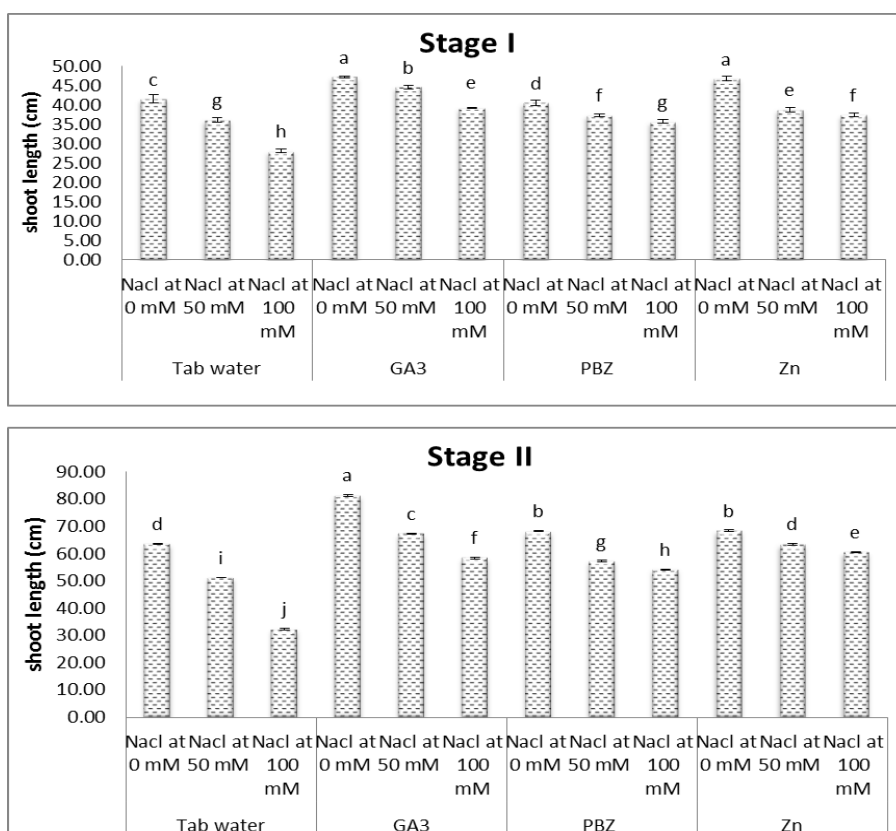


Figure (1). Effect of salinity, GA₃, PBZ, Zn and their interactions on shoot length of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

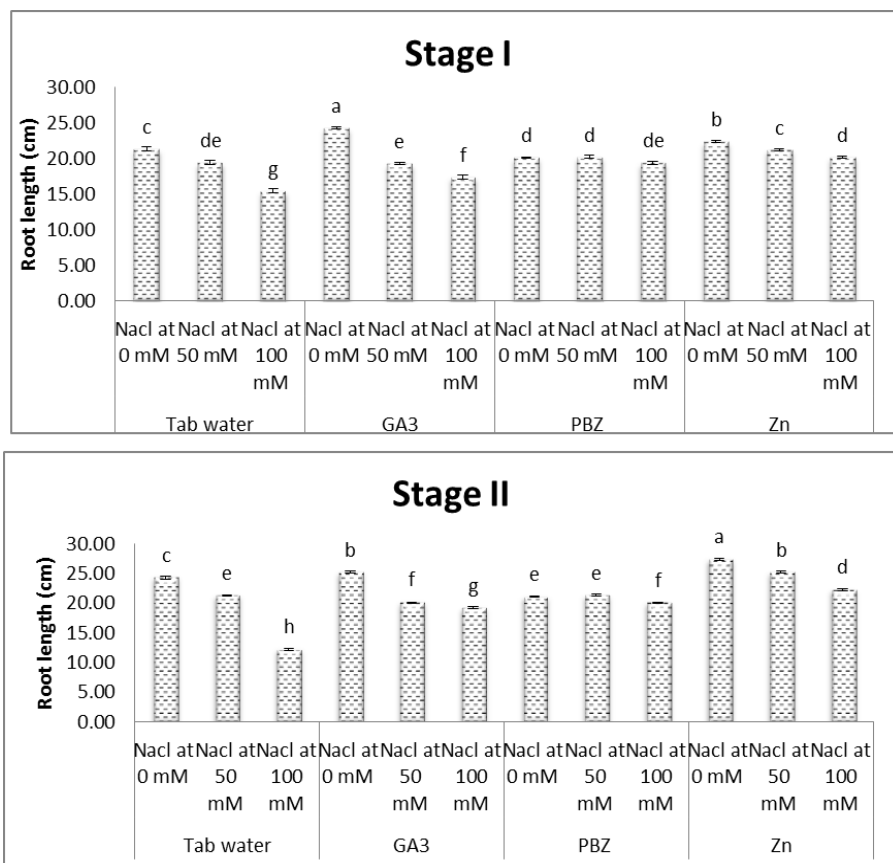


Figure (2). Effect of salinity, GA₃, PBZ, Zn and their interactions on root length of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

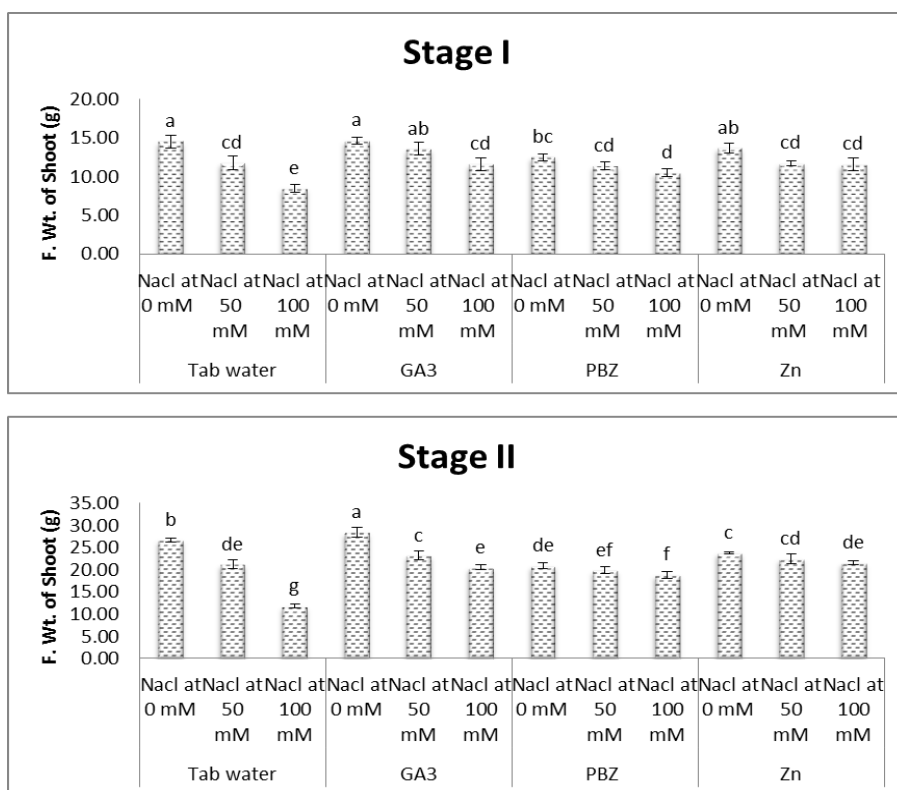


Figure (3). Effect of salinity, GA₃, PBZ, Zn and their interactions on fresh weight shoot of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

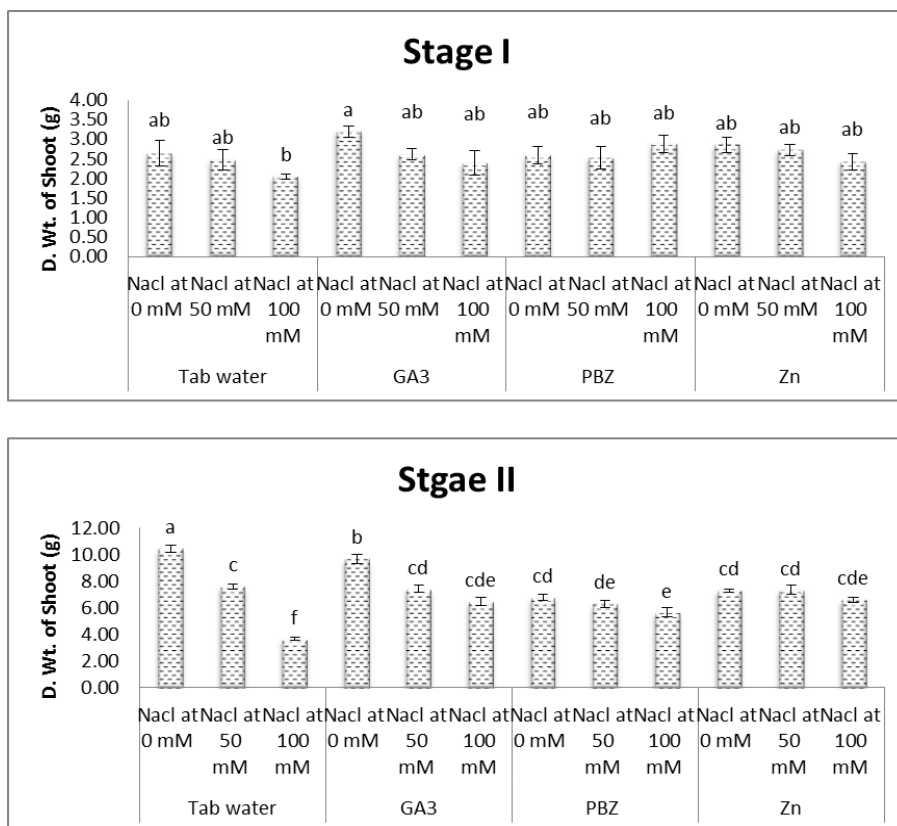


Figure (4). Effect of salinity, GA₃, PBZ, Zn and their interactions on dry weight shoot of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

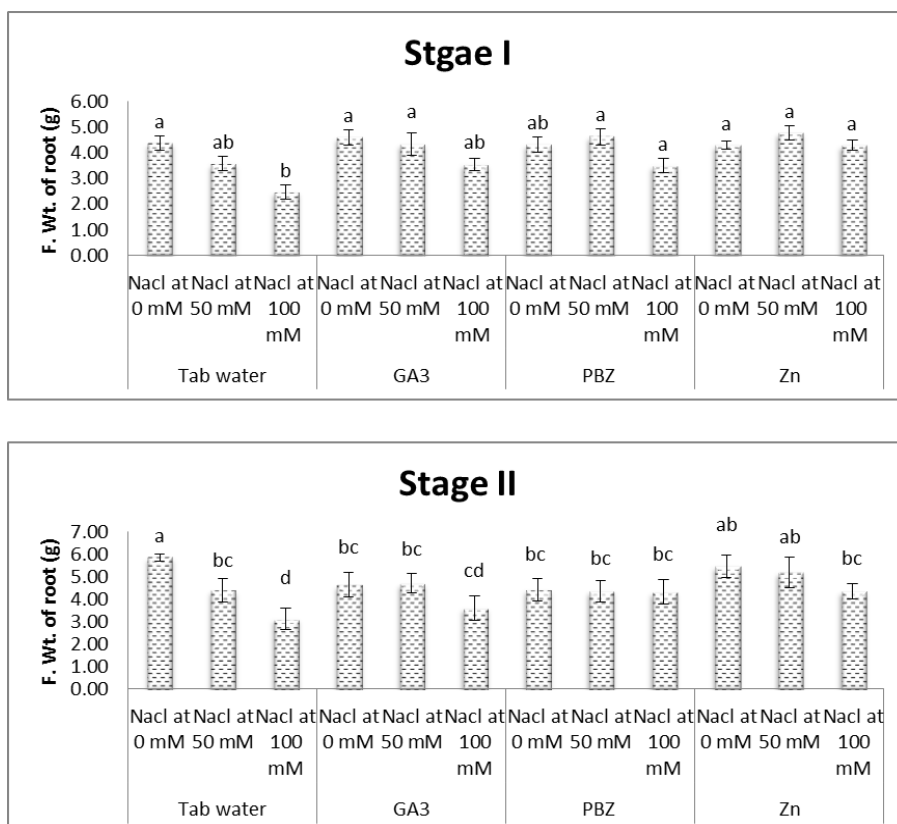


Figure (5). Effect of salinity, GA₃, PBZ, Zn and their interactions on fresh weight root of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

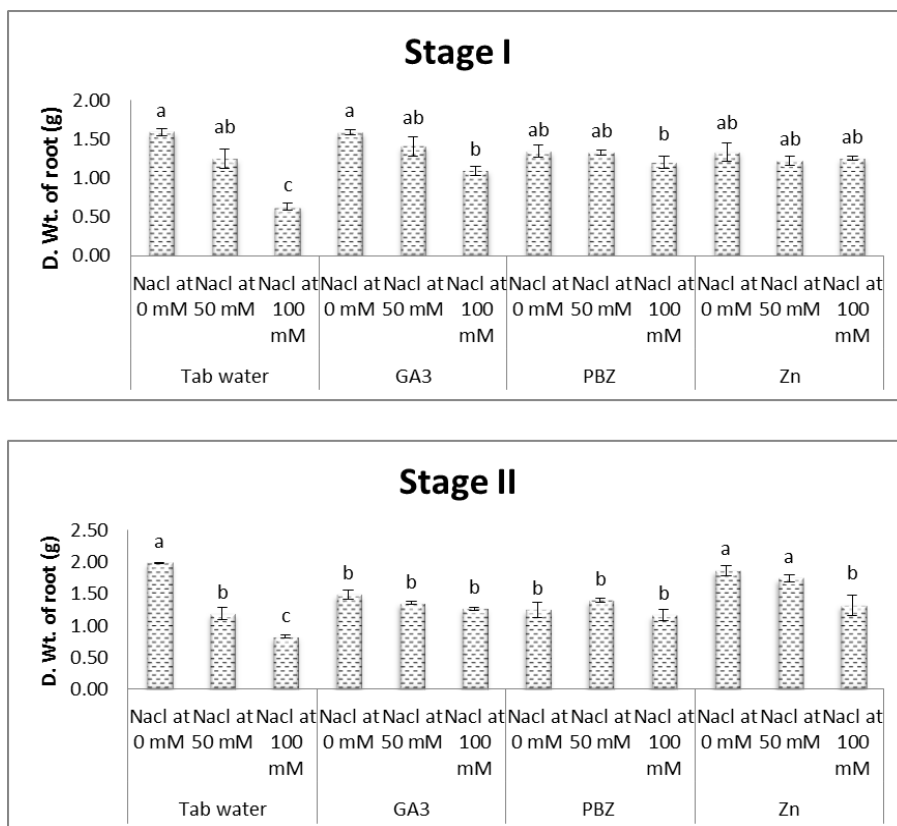


Figure (6). Effect of salinity, GA₃, PBZ, Zn and their interactions on dry weight root of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

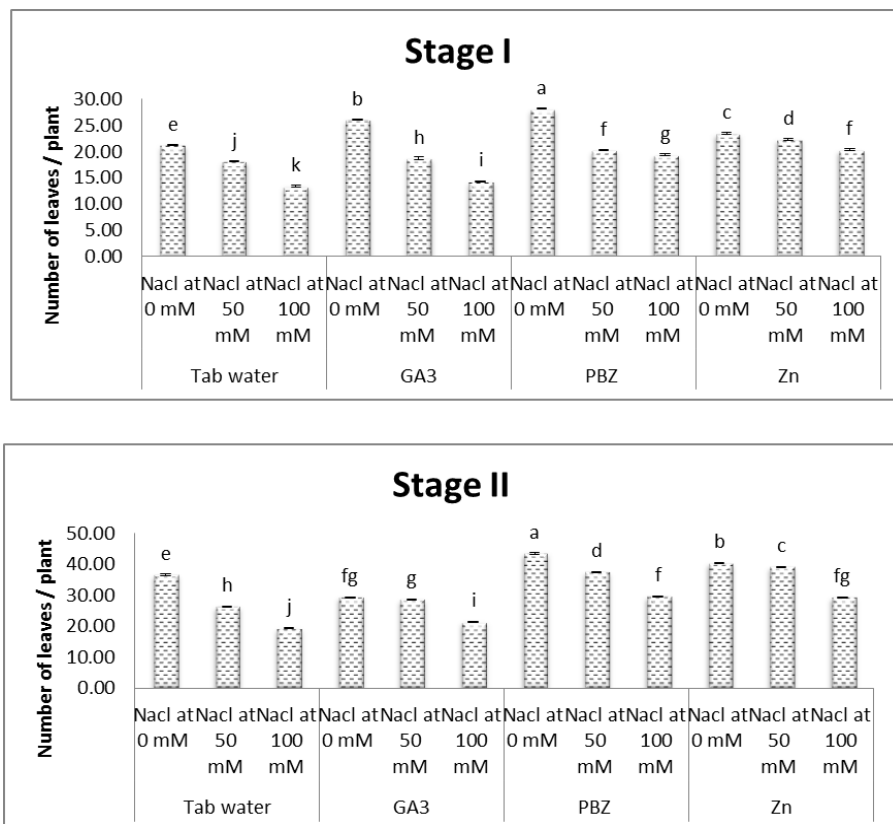


Figure (7). Effect of salinity, GA₃, PBZ, Zn and their interactions on number of leaves/soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

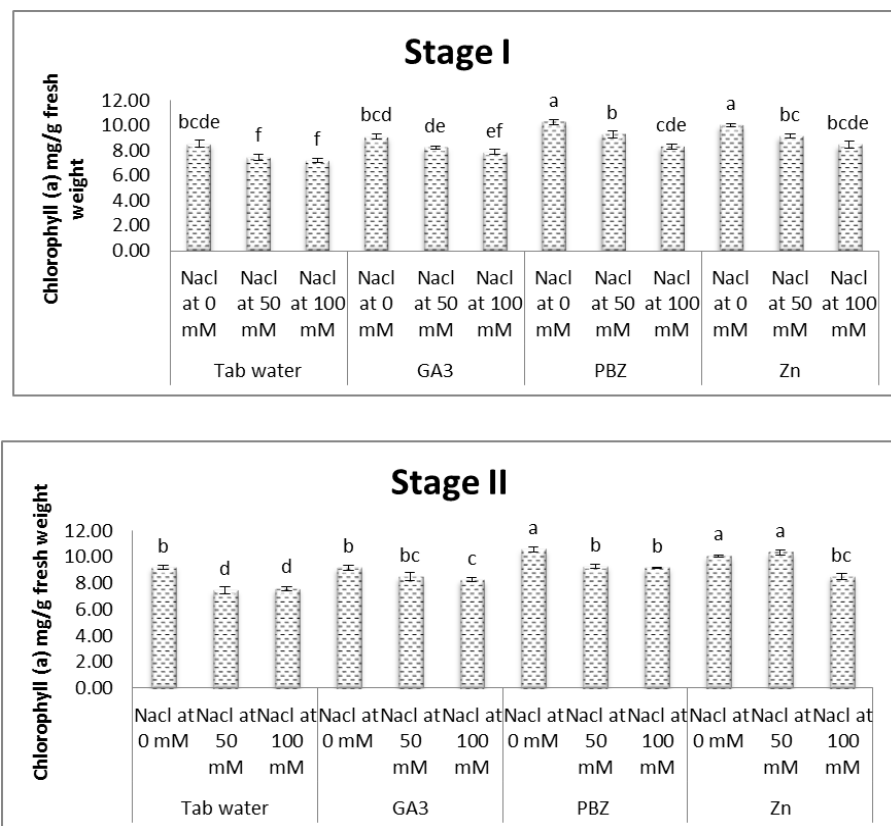


Figure (8). Effect of salinity, GA₃, PBZ, Zn and their interactions on the chlorophyll (a) content (mg/g fresh weight) of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

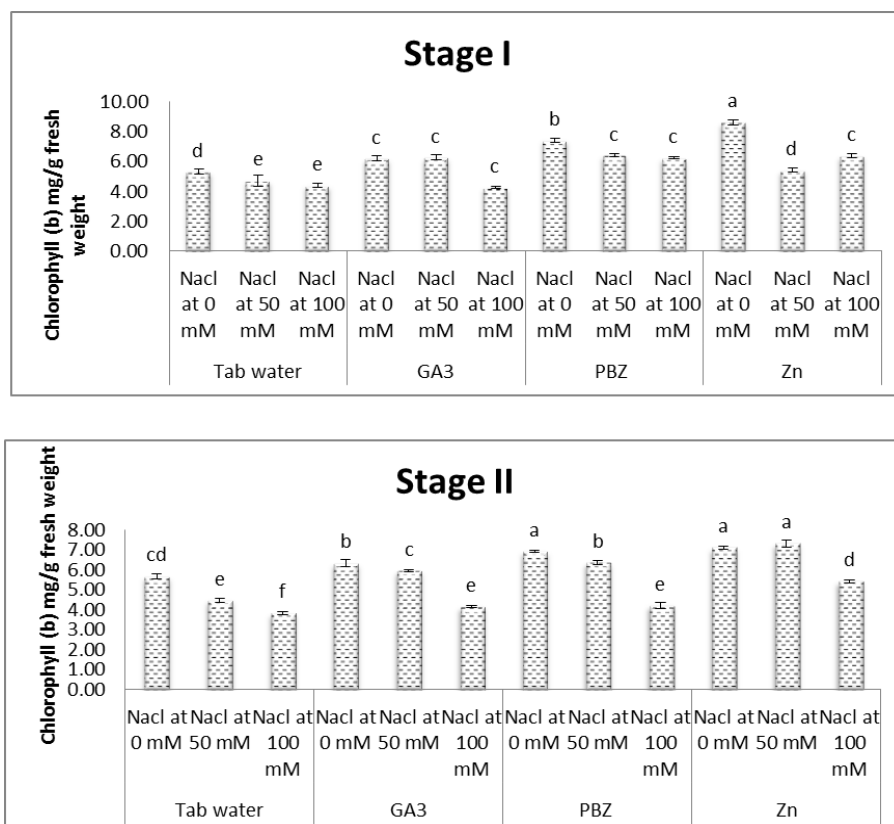


Figure (9). Effect of salinity, GA₃, PBZ, Zn and their interactions on the chlorophyll (b) content (mg/g fresh weight) of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

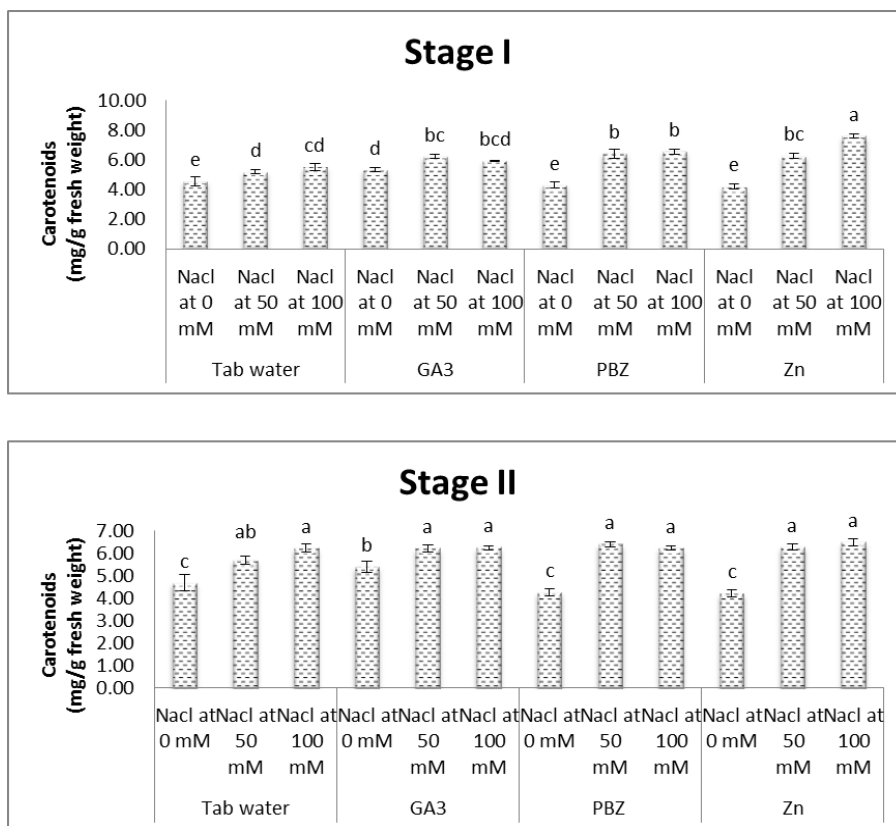


Figure (10). Effect of salinity, GA₃, PBZ, Zn and their interactions on the carotenoid content (mg/g fresh weight) of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

On the other hand, results presented in figures (8-10) revealed that the reduction in photosynthetic pigments caused by NaCl was alleviated to a high extent by the application of PBZ, Zn and GA₃. It was found from the obtained results that application of both PBZ or Zn was more effective than GA₃ in mitigating the adverse effect of salinity on photosynthetic pigments contents. This was the case throughout the two stages of growth. Results revealed also that treatment with either GA₃, PBZ or Zn markedly increased the carotenoid content than that observed in salt stressed plants. This was the fact throughout the duration of the experiment and under the different salinity levels. In this concern, in *Trigonella foenum-graecum* leaves, a decrease in chlorophyll a as well as chlorophyll b was observed with a significant reduction of 30 percent in total chlorophyll content at 60 mM NaCl concentration as compared to control [42]. Anjali and Aruna [43] proved that presoaking *Trigonella foenum-graecum* seeds in GA₃, has improved chlorophyll a, chlorophyll b and total chlorophyll contents in leaves as compared to unprimed seeds grown at 60 mM NaCl concentration. Zeid [44] also reported an alleviation of adverse effects of salinity on chlorophyll content in barley with GA₃ treatment.

It is evident from the above mentioned data that, in the present investigation, treatment with either PBZ or Zn was observed to be more effective as compared to GA₃ with respect to chlorophyll contents. Similar observations of mitigative effects of both PBZ and Zn on chlorophyll

contents in plants grown under saline or drought conditions have been reported by other workers Chauhan *et al.* [14] and Mahmoud [37] suggested that plant growth regulators help in overcoming the harmful effects of salinity on growth by changing the endogenous growth regulators which affect plant water balance.

3.3. Antioxidant Enzymes

In the present study (Figs. 11-14), significant increases were observed in the activities of SOD, POX, CAT and GR in the shoots of soybean plants under salt stress conditions. Moreover, it was found that the activities of antioxidant enzymes were increased with increasing salinity level. Even under optimal conditions, many metabolic processes produce ROS. The production of toxic oxygen derivatives is increased as a result of all types of abiotic or biotic stresses. Plants possess efficient systems for scavenging active oxygen species that protect them from destructive oxidative reactions [45].

As part of this system, antioxidant enzymes are key elements in the defense mechanisms. Changes in the activities of the antioxidant enzymes under saline conditions has been reported by several investigators, increased in the case of salt-tolerant cotton [46], shoot cultures of rice [47], cucumber [48] and wheat [32]. In this respect, the effects of salt stress on the antioxidant enzymes are very complex and depend on the treatment time, plant species and genotypes [49]. Shen *et al.* [50] found that the activities of catalase

(CAT), peroxidase (POX), superoxide dismutase (SOD) and hydrogen peroxide were increased under stress conditions on soybean plants.

The enzyme is involved in dismutation of superoxide radicals to hydrogen peroxide and oxygen [51]. In the present study, SOD activity has increased with increasing NaCl level concentration and a treatment period (Fig. 11). Soybean plants exhibited much more elevated SOD activity and for that reason could possess better oxygen free radicals scavenging certain capacity minimizing the oxidative damage. Published reports also showed that demonstrated that over expression of SOD led to efficient, stress protection against NaCl stress in plants such as *B. maritima* and *B. vulgaris* [52], cotton [46], maize [53].

Superoxide dismutase action leads to the production of H_2O_2 a highly toxic molecule to living cells, which needs to be eliminated from plant cells in subsequent reactions. Under such stress conditions enzymes, for example CAT and POX are usually activated and active in the removal of H_2O_2 scavenging enzyme in plant leaves [53]. Our results showed that enzyme activities of CAT, POX elevated considerably under salt stress conditions over their controls. High catalase activity boosts, the cell membrane stability by decreasing H_2O_2 content under NaCl stress conditions [54]. We demonstrated that, rise in CAT, POX activities follows elevated SOD activity under salt stress. Our results offer the hypothesis that antioxidative enzymes play a main protective role within the detoxing of O_2 and H_2O_2 by scavenging process with coordination of SOD [55, 56].

Glutathione reductase (GR) is a vital enzyme involved with converting of facets of GSSG to GSH under ecological stress conditions [13]. Within our study, the GR activity increased with NaCl concentration, with an increased prominent raise being observed with control plants. Raise in activity under Salinity stress seemed to be proven in the past reports on cucumber [48] and barely [55].

Results in figures (11-14) revealed that, under saline and normal conditions, treatment with either GA_3 , PBZ or Zn, in most cases markedly reduced the over increases in the activities of POX, CAT and GR, however the activities of SOD was increased than that of the corresponding controls. Similar results were obtained in the work of Aldinary [39] who found that treating cowpea plants with either PBZ or Zn increased the activities of SOD in shoots of plants grown in either saline or non-saline conditions. Tuna *et al.* [57] reported that POX, CAT activity in salt-stressed maize plants was decreased by exogenous application of GA_3 .

3.4. Endogenous Phytohormones

Results of the present investigation (Fig. 15) showed that salinity greatly affected the activities of the endogenous phytohormones in shoots of soybean plants. Under the first level of salinity (50 mM NaCl), the content of both JA and ABA was markedly increased than that of the control ones. At the same time, the content of both GA_3 and IAA was significantly decreased in response to the first level of NaCl. At the second level of NaCl (100 mM), significant decreases were observed in the content of JA, GA_3 and IAA, while the content of the ABA was found to be highly significantly increased. In this regard, El-Khallal *et al.* [58] found that salt stress led to sharp decrease in the levels of IAA, GA_3 and Zeatin, while ABA level greatly increased in maize shoots. 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 and cytokinins. Thus, reduction in shoot growth of tomato plants is probably related to hormonal signals generated in response to salt stress as suggested by Ghanem *et al.* [59].

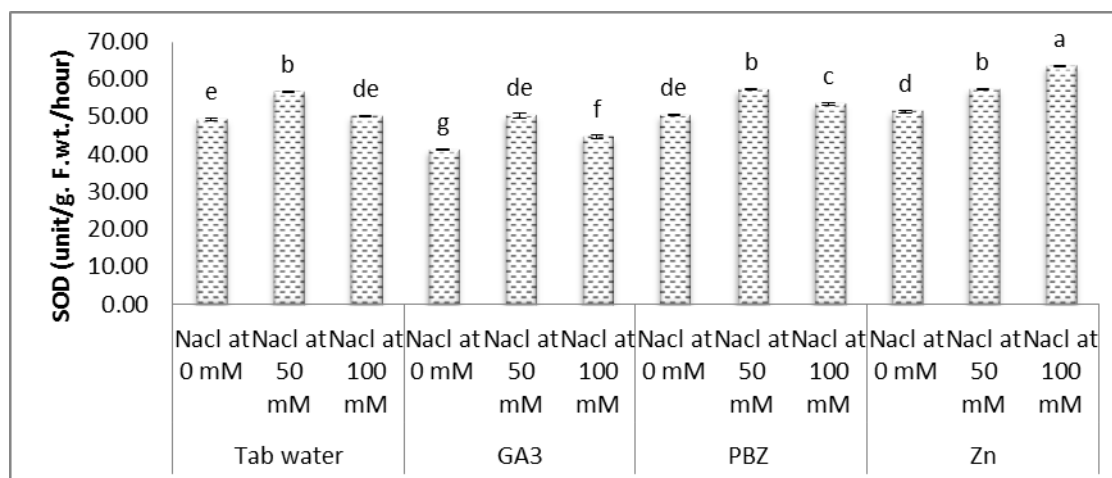


Figure (11). Effect of salinity, GA_3 , PBZ, Zn and their interactions on the activity of superoxide dismutase (SOD) (unit/g. F.wt./hour) of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

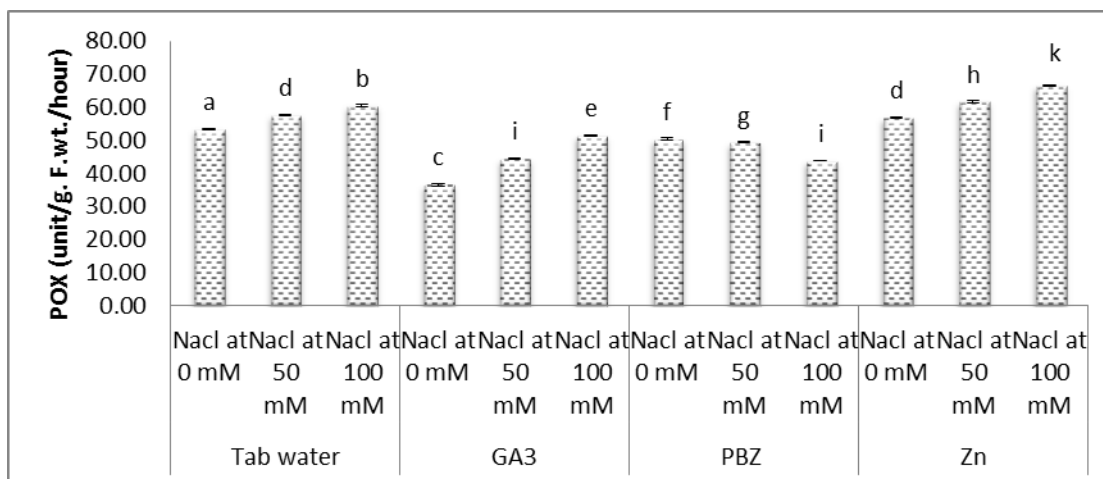


Figure (12). Effect of salinity, GA₃, PBZ, Zn and their interactions on the activity of peroxidases (POX) (unit/g. F.wt./hour) of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

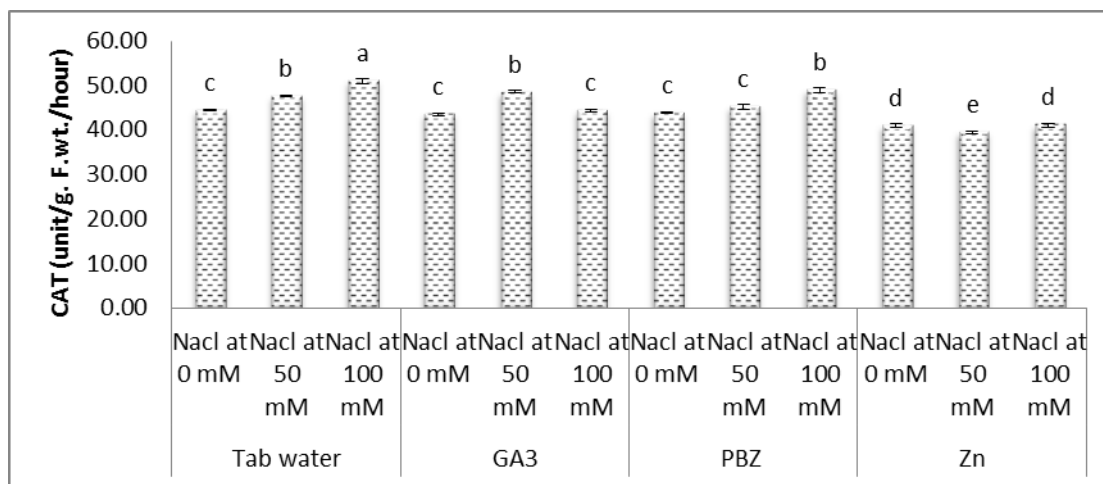


Figure (13). Effect of salinity, GA₃, PBZ, Zn and their interactions on the activity of catalase (CAT) (unit/g. F.wt./hour) of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

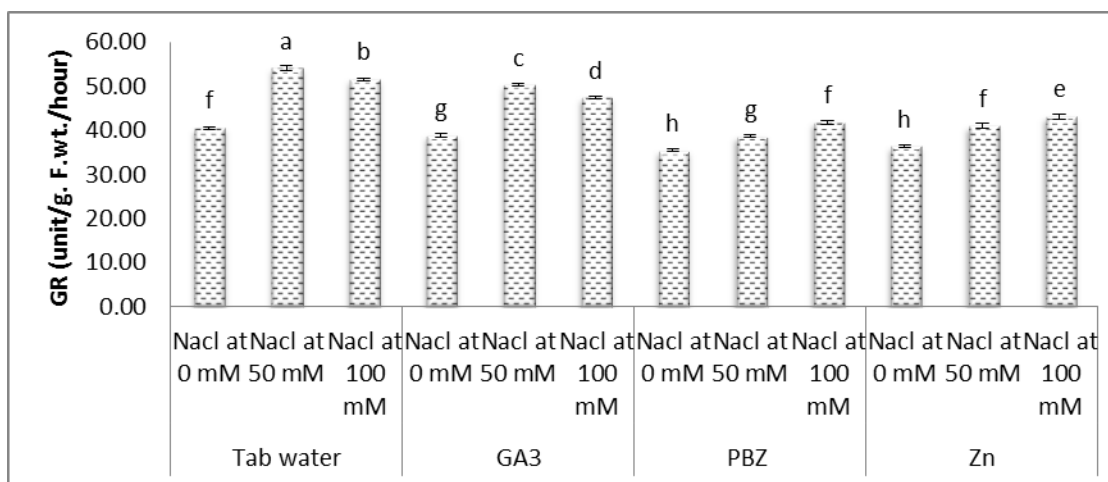


Figure (14). Effect of salinity, GA₃, PBZ, Zn and their interactions on the activity of glutathione reductase (GR) (unit/g. F.wt./hour) of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

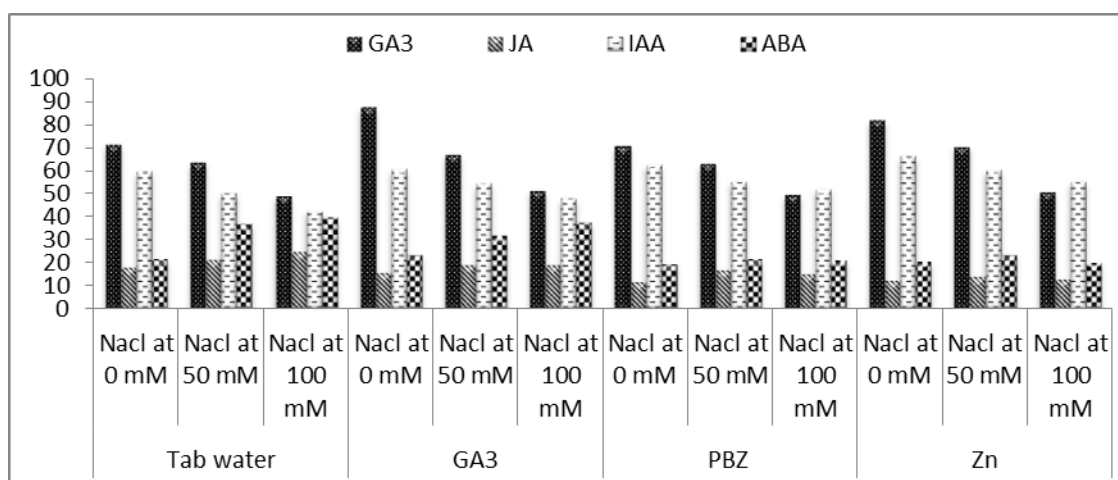


Figure (15). Effect of salinity, GA₃, PBZ, Zn and their interactions on contents of endogenous hormones of soybean plants

Results of the present study (Fig. 15) revealed that the adverse effects of salinity as regards the endogenous GA₃ and IAA were markedly alleviated by treatment with either GA₃, PBZ or Zn. This was the case with plants grown in either saline or non-saline conditions. On the other hand, significant decreases in the content of JA and ABA were observed in plants grown in salinized conditions due to application of GA₃. Treatment with PBZ or Zn was found to be more effective than that of GA₃ in decreasing the contents of endogenous JA and GA₃ in salinized soybean plants. The potent effect exogenous application of GA₃, PBZ or Zn as regards the activities of endogenous phytohormones was investigated by others. In this concern, Aldinary [39] found that treating cowpea plants with either PBZ or Zn markedly increased the endogenous contents of GA₃ and IAA, while the content of endogenous ABA was decreased. This was the case with plants grown under saline conditions.

3.5. Lipid Peroxidation

Lipid peroxidation represented by the determined content of malondialdehyde (MDA) as shown in figure (16). Significant increases were observed in the contents of MDA in the shoots. This was the case throughout the duration of the experiment and under the two applied levels of salinity. In this regard, Hulusi *et al.* [60] under the effect of NaCl treatment MDA content increased significantly during the experimental period in sesame cultivars as compared to control groups Naureen and Naqvi [61] measured H₂O₂ and MDA concentrations in salt stressed wheat plants that are oxidative stress indicators. H₂O₂ caused membrane damage fasten the Haber-Weiss reaction by production of hydroxyl radical led to increase lipid peroxidation. Our results are also in agreement with those of Shalata and Neumann [62] reported that salt-stress increased the accumulation of lipid peroxidation products produced by interactions with damaging active oxygen species in stems of tomato plants.

Verma and Mishra [63] reported that salinity caused a reduction in seedling growth and biomass accumulation, which was parallel to that caused by increased superoxide

(2O⁻), hydrogen peroxide (H₂O₂) levels, lipid peroxidation and electrolyte leakage in leaf tissues.

On the other hand, the results of the present study (Fig. 16) revealed that treatment with either GA₃, PBZ or Zn significantly decreased the content of MDA (lipid peroxidation) in shoots of soybean plants. It is worth to mention that treatment with PBZ or Zn was found to be more effective than GA₃ in decreasing the MDA content in shoots of soybean plants. This was the case with plants grown in either saline or non-saline conditions and under the two applied levels of salinity. The inhibitory effect of sufficient concentrations of Zn application on the production of these injurious components in saline conditions has been reported by other authors [64-66]. Zinc plays a key role in controlling the generation and detoxification of free oxygen radicals and subsequent lipid membrane oxidation [67]. It has been demonstrated that Zn ions have a strong inhibitory effect on membrane bound, NADPH oxidase [68].

3.6. Free Proline

The obtained results (Figs. 17 & 18) showed that, free proline content in both shoots and roots of soybean plants was significantly increased under salt stress conditions. Increases in the proline contents were more obvious with increasing the level of salinity. This was the case throughout the duration of the experiment. Ashraf and Foolad [69] reported that in organisms ranging from bacteria to higher plants, there is a strong correlation between increased cellular proline levels and the capacity to survive under salt stress. In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub cellular structure (membrane and proteins) scavenging free radicals and buffering cellular redox potential under stress conditions. The obtained results are in agreement with Amini and Ehsanpour [70] in tomato, Radyukina *et al.* [71] in *Geum urbanum* L. and Lobato *et al.* [72] in *Vigna unguiculata* L. 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 Sharma *et al.* [73].

Results of the present study (Figs. 17 & 18) revealed that treatment with either GA₃, PBZ or Zn significantly decreased the content of proline in both shoots and roots of soybean plants. It is worth to mention that treatment with PBZ or Zn was found to be more effective than GA₃ in decreasing the contents proline in both shoots and roots of soybean plants. This was the case with plants grown in either saline or non-saline conditions. In this regard, contrary to our results, Aldinary [39] found that treating salinized-cowpea plants with either PBZ or Zn increased the content of proline in both shoots and roots. Anjali and Aruna [43] presented that when *Trigonella foenum-graecum* plants were grown under saline environment, leaf proline was found to increase by about 90 percent as compared to control. However, plant growth regulators (PGR) treated plants of *Trigonella foenum-graecum* exhibited significant reduction in leaf proline content as compared to untreated seeds grown under 60 mM NaCl concentration, maximum reduction being observed with GA₃, followed by Kinetin and BA, although, the contents were found to be slightly higher than that of control plants.

3.7. Yield and Yield Components

Figures (19 & 20) showed the difference in including a number of pods/plant, number of seeds/plant and weight of 100 seed for each treatment as well as controls. Also, figures (19 & 20) demonstrate the content of soluble proteins, soluble carbohydrates and oil content in the yielded seeds for each treatment and controls. All the aforementioned parameter was significantly reduced under saline conditions. The magnitude of reduction, increased by increasing salinity level. These results are in accordance with other investigations. In this regard, Weria *et al.* [74] revealed that, in soybean plants, salinity (0, 33, 66 and 99 mM NaCl) caused a significant reduction in the number of pods/plant, number of seeds/plant and weight of seeds/plant. Sofy [75] revealed also that, treating lentil plants with either 20% or 30% sea water resulted in, significant decreases in the contents of total soluble carbohydrates, soluble protein in yielded seeds. Ayman *et al.* [76] abstracted that salinity caused significant reduction in the number of pods / plant, number of seeds/plant, weight of 100 seed, contents of soluble proteins and total oil content in the yielded seeds of soybean plants.

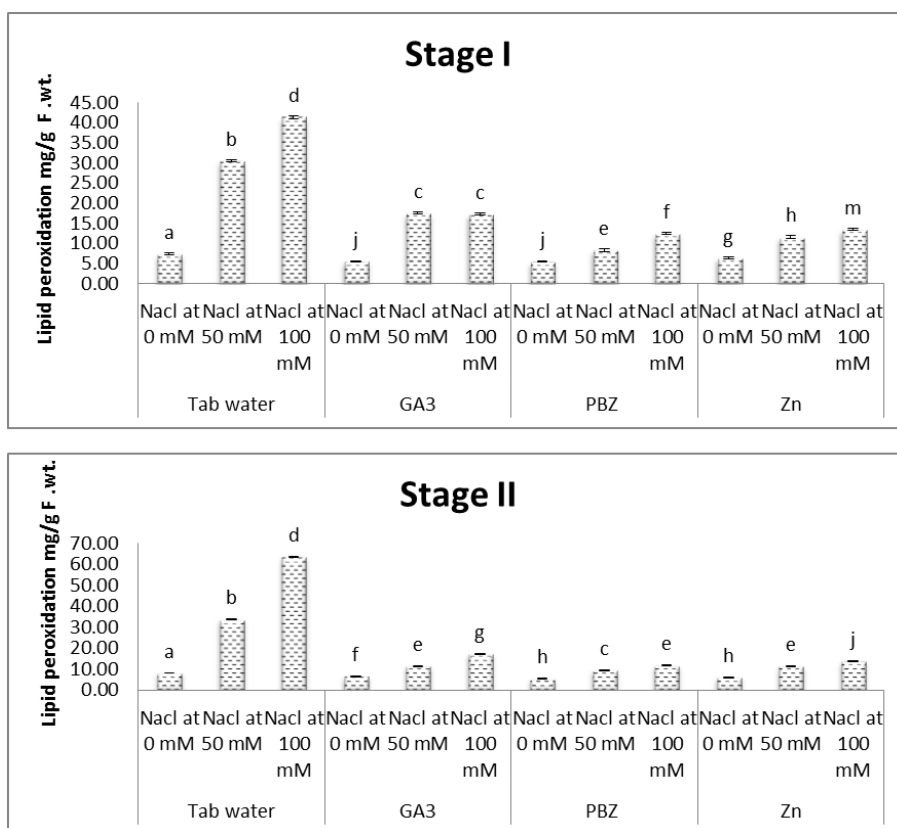


Figure (16). Effect of salinity, GA₃, PBZ, Zn and their interactions on the lipid peroxidation in shoots (mg/g F.Wt.) of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

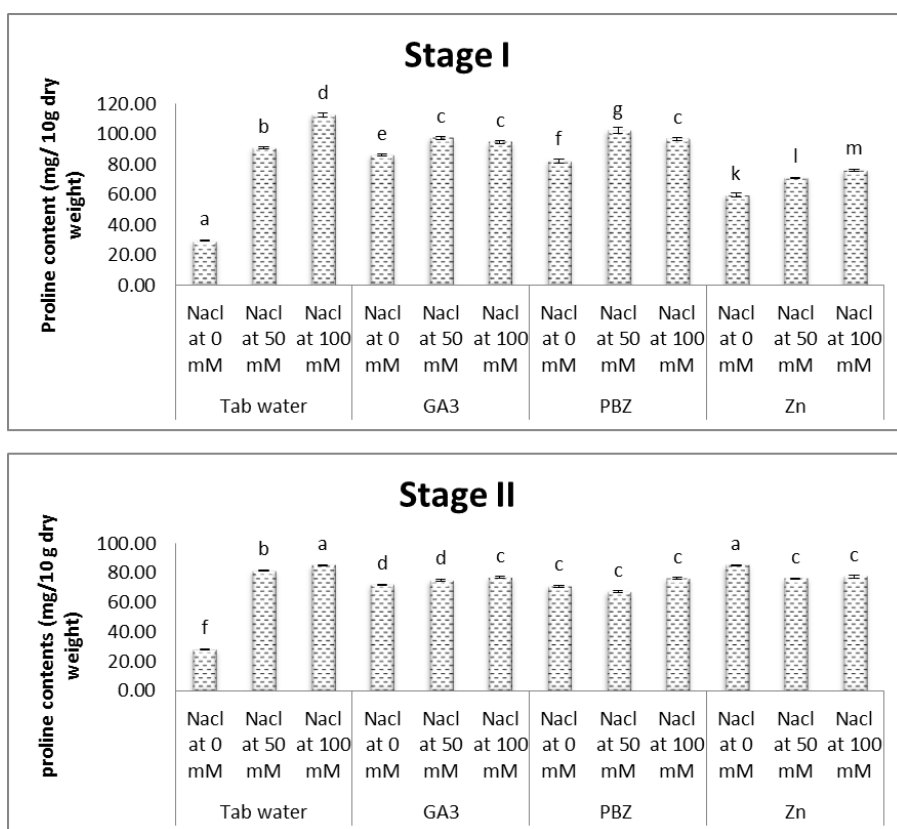


Figure (17). Effect of salinity, GA₃, PBZ, Zn and their interactions on the total proline content in shoot (mg/g dry weight) of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

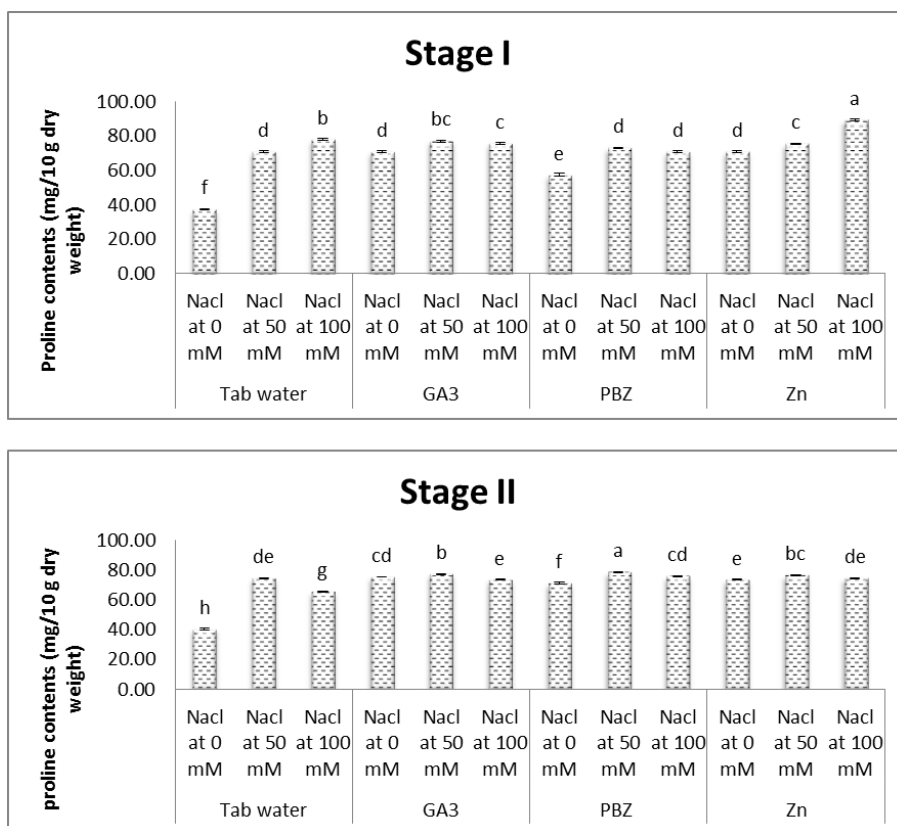


Figure (18). Effect of salinity, GA₃, PBZ, Zn and their interactions on the total proline content in root (mg/g dry weight) of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

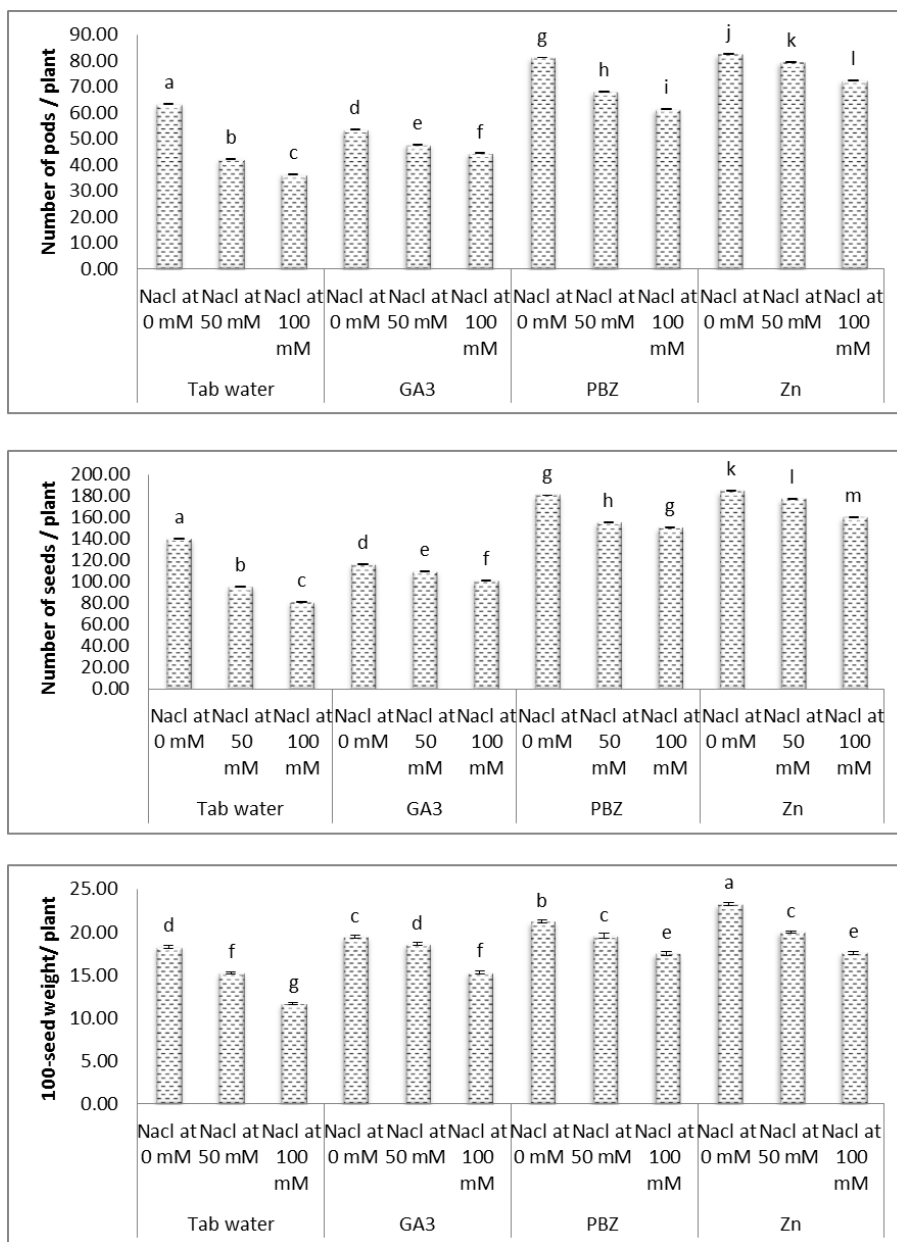


Figure (19). Effect of salinity, GA₃, PBZ, Zn and their interactions on number of pods, number of seeds and 100-seed weight of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

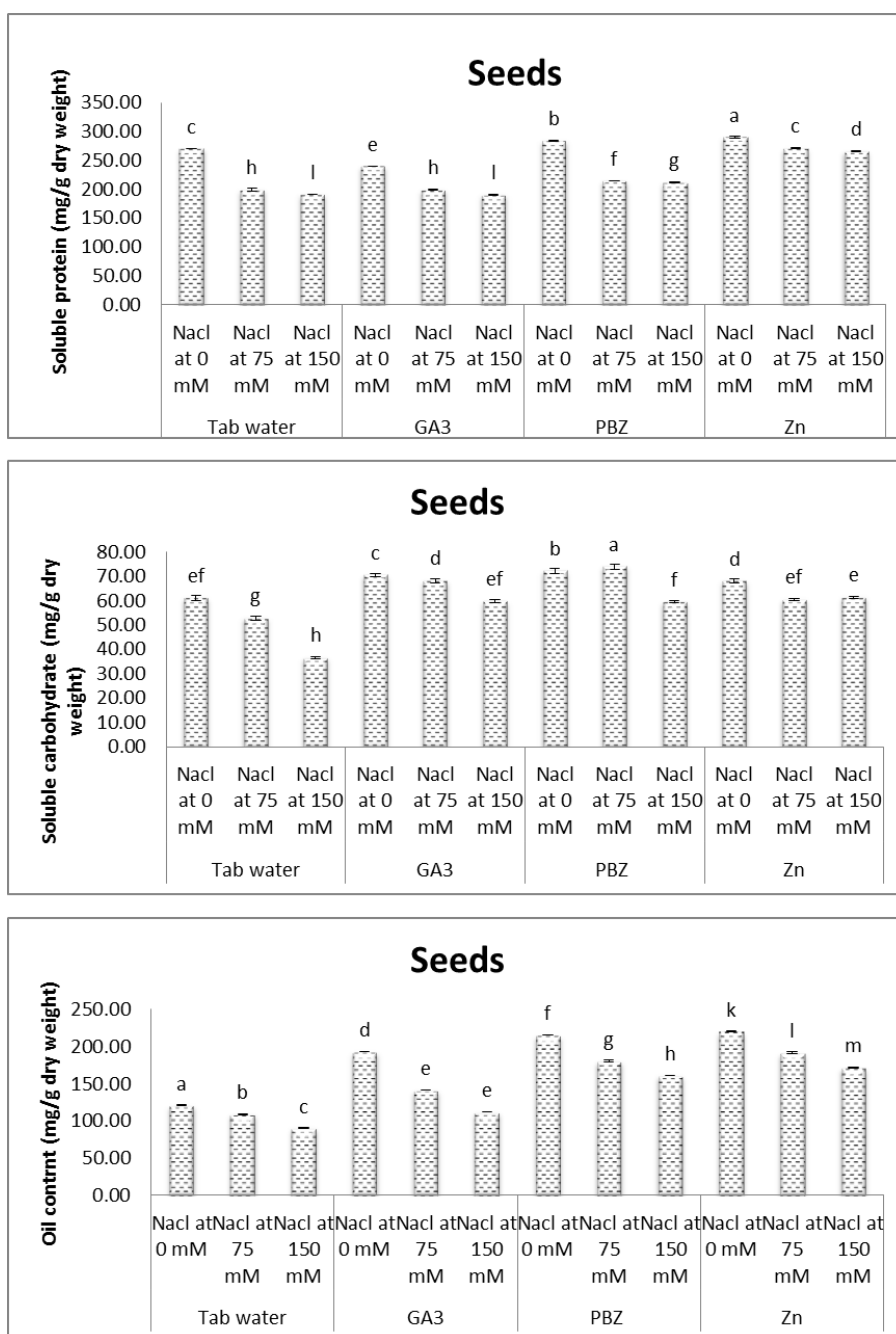


Figure (20). Effect of salinity, GA₃, PBZ, Zn and their interactions on soluble protein, soluble carbohydrate and oil content (mg/g dry weight) of soybean plants. The bars with the same letters are not significantly different at $P \geq 0.05$

The results of the present study (Figs. 19 & 20) revealed that treatment with either GA₃, PBZ or Zn significantly increased the number of pods/plant, number of seeds/plant, weight of 100 seed, contents of soluble proteins, soluble carbohydrates and total oil content in the yielded seeds of soybean plants. It was observed that treatment with PBZ and Zn was found to be more effective than GA₃ (Figs. 19 & 20). In this regard, Aldinary [39] application of either PBZ or Zn significantly increased the number of pods/plant, number of seeds/plant, weight of 100 seed, contents of soluble carbohydrates and soluble proteins in the yielded seeds of cowpea plants grown under stress conditions.

3.8. Protein Profile in the Yielded Seeds

The total soluble proteins in the yielded seeds of the soybean plant under the different treatments were separated electrophoretically using an SDS-PAGE technique in order to find out the treatments variation in specific accumulation salt induced protein upon the salt stress condition (Fig. 21 & Table 1). The major variations are expressed as changes in appearance or disappearance of some bands in the yielded seeds of the soybean in response to their pre-emergence treatment with different concentrations of NaCl (50 mM & 100 mM) with or without GA₃, PBZ or Zn (Tables 1-3). A

total of 23 bands were detected with different molecular weights ranging from 129 kDa to 9 kDa. These protein bands have distributed into 7 monomorphic bands (30.4%) and 16 polymorphic bands (69.6%) (Table 1).

The electrophoretic protein pattern (Fig. 22) of the yielded seeds presoaked in GA₃, Zn or PBZ and then treated with salinity stress 50 mM or 100 mM showed the disappearance of 7 protein bands (negative markers related to salinity stress compared with corresponding control) with molecular weight ranging between 129 and 24 kDa (Table 2), where the highest number of disappeared protein bands (6 bands) showed at salinity stress 100 mM without GA₃, PBZ or Zn, and the lowest number (1) showed at salinity stress 50 mM with PBZ. While, there are no negative protein bands at salinity stress 50 mM with Zn. On the other hand, there are 2 protein bands considered as newly formed protein bands (specific positive markers related to salinity tolerance compared with corresponding control) have appeared (Table 2), 1 of them (16 KDa) appeared in the yielded seeds at 50 mM with Zn or PBZ. The other band (19 KDa) has appeared at salinity stress 50 mM with PBZ.

Also, there are 4 protein bands considered as positive markers related to the treatments with GA₃, Zn or PBZ and not disappeared at different levels of salinity stress (50 mM & 100 mM), with molecular weight ranging between 112 and 39 kDa (Table 3). One protein band (45 KDa) related to GA₃, Zn and PBZ, 3 protein bands (112 & 73 KDa) related to Zn and PBZ, and 1 protein band (39 KDa) related to Zn only. The protein bands disappeared at different levels of salinity stress (50 mM & 100 mM) with GA₃ or PBZ. While, the 4 protein bands related to Zn, 1 of them (112 KDa) still appeared at salinity stress 50 mM and 100 mM, two of them (73 & 39 KDa) still appeared at salinity stress 50 mM only and disappeared at salinity stress 100 mM, and the last one (45 KDa) still appeared at salinity stress 1000 mM only.

The variations in protein profile could show information about tolerance mechanism of plants growing under salt stress. The tolerance reaction may be resulted from rapid synthesis or fewer degradation of responsive proteins to salinity stress specifically for the proteins that have a very greater molecular weight [77]. One possible reason behind appearance of some proteins under salt stress would be that the gene (s) responsible for certain proteins have been completely enhanced because of stress [78]. It's also achievable that the genes were not completely suppressed, but inhibited because of stress and finish recovering from the inhibition wasn't achieved [78]. The specifically synthesized protein under salt stress seems to possess a role in supplying adaptation to plants.

El-Farash *et al.* [79] studied the expression of 12 different protein bands, which were induced in salt stressed tomato plants. They reported that the expression of these proteins was genetically regulated, depending on the salt concentration. Hassanein [80] reported that NaCl treatment

of peanut plants (*Arachis hypogaea*) promoted induction (127 and 52 kDa) or repression (260 and 38 kDa) in the synthesis of few polypeptides.

Variation in the protein pattern via the look of new bands and disappearance from the others, of various applications of salt stress would indicate either enhancement or repression of gene expression during these treatments. This may affect the created proteins as a result of salt stress, either around the transcription or post-transcription levels of gene expression [81, 82]. Soybean plants growing under salinity stress might be adapted to such stress through promoted a suitable variation in the protein banding patterns. Supporting this view the result that obtained by Sobhanian *et al.* [82], who reported that 19, 22 and 14 from 340, 330 and 235 bands obtained from soybean leave, hypocotyl and roots, correspondingly were up and lower controlled by treatment with 40 mM NaCl for just one week. Their results say metabolic process related proteins were mainly lower-controlled because of NaCl treatment recommended these proteins lead to each organ in adaptation to saline conditions. Phytohormones are essential for the ability of plants to adapt to abiotic stresses by mediating a wide range of adaptive responses [83, 84]. They often rapidly alter gene expression by inducing or preventing the degradation of transcriptional regulators via the ubiquitin proteasome system [85].

3.9. Automatic Linear Modeling and Discriminant Analysis

Finally, when used the yielded weight as a target; we observed that the accuracy of all data about 99.4%. The effect of target weight of 100 seed, the wide line is very effective as oil content in seeds, the number of leaves at stage II, the number of leaves at stage I, activity of catalase, shoot length at stage I, fresh weight of shoot at stage I, root length at stage II, and root length at stage I. Also, the coefficient of target yield weight the positive blue color wide line is effective as oil content in seeds, and the number of leaves at stage II, activity of catalase, shoot length at stage I, fresh weight of shoot at stage I, and root length at stage II. While, negative orange color wide line is effective as the number of leaves at stage I, and root length at stage I (Fig. 23 a-d).

So, use of discriminant analysis that led to the conclusion when use different treatments of GA₃ 200 ppm, PBZ 200 ppm, Zn 150 ppm in pre-soaking and foliar application on soybean plants, we resulted that the Zn 150 ppm is better application then PBZ 200 ppm, and the lowset effect is GA₃ 200 ppm when treated with fresh water.

While, when treated with different irrigated salinity on soybean plants, we resulted that the Zn 150 ppm + 50 mM NaCl, is a good treatment to alleviate the stress condition while the low effect treatment to alleviate the stress condition is GA₃ 200 ppm + 100 mM NaCl as shown in figure (24).

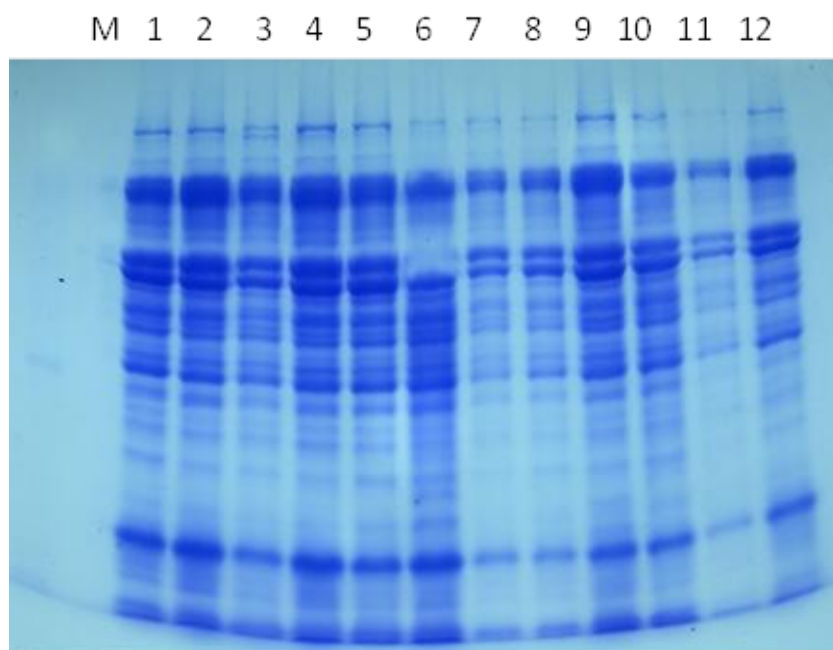


Figure (21). SDS-PAGE protein banding pattern of the yielded seeds of the soybean in response to their pre-emergence treatment with different concentrations of NaCl (50 mM & 100 mM) with or without GA₃, PBZ or Zn. (1) control, (2) GA₃, (3) PBZ, (4) Zn, (5) Zn + 50 mM NaCl, (6) PBZ + 50 mM NaCl, (7) 50 mM NaCl, (8) GA₃ + 100 mM NaCl, (9) Zn + 100 mM NaCl, (10) PBZ + 100 mM, (11) 100 mM NaCl and (12) GA₃ + 50 mM NaCl

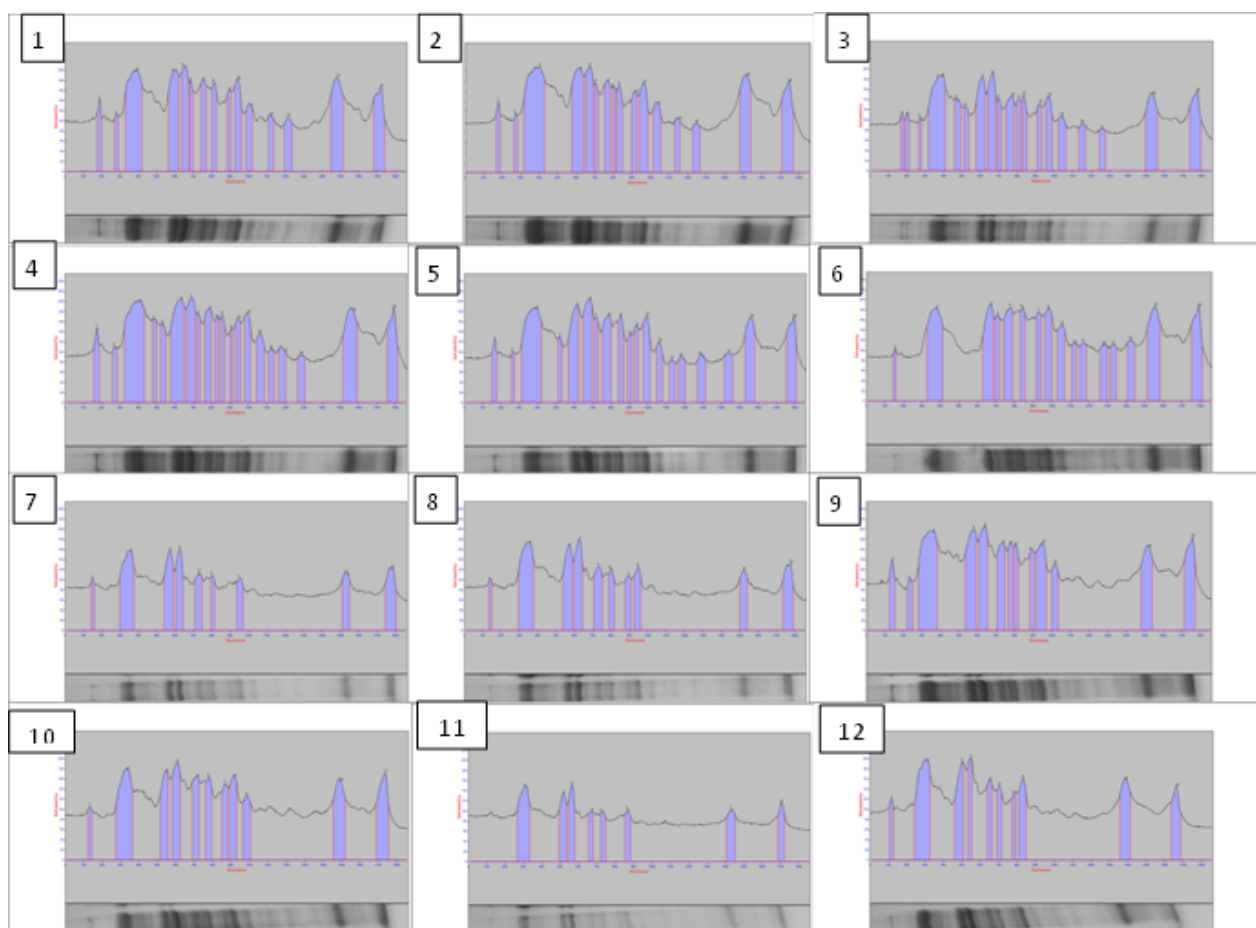


Figure (22). Electrophoregram of protein pattern of the yielded seeds of the soybean in response to their pre-emergence treatment with different concentrations of NaCl (50 mM & 100 mM) with or without GA₃, PBZ or Zn. (1) control, (2) GA₃, (3) PBZ, (4) Zn, (5) Zn + 50 mM NaCl, (6) PBZ + 50 mM NaCl, (7) 50 mM NaCl, (8) GA₃ + 100 mM NaCl, (9) Zn + 100 mM NaCl, (10) PBZ + 100 mM, (11) 100 mM NaCl and (12) GA₃ + 50 mM NaCl

Table (1). Effect of two concentrations (50 and 100 mM) of NaCl salt stress on the soybean yielded seeds protein banding patterns with or without GA₃, PBZ or Zn. (+) presence of the band, (-) absence of the band and (M.W) Molecular weight

Band No.	M.W (KD)	Control	GA ₃	PBZ	Zn	Zn + 50 mM NaCl	PBZ + 50 mM NaCl	50 mM NaCl	GA ₃ +100 mM NaCl	Zn +100 mM NaCl	PBZ +100 mM	100 mM NaCl	GA ₃ + 50 mM
1	129	+	+	+	+	+	+	+	+	+	+		+
2	125	-	-	+	-	-	-	-	-	-	-	-	-
3	112	+	+				-	-	-		-	-	-
4	88	+	+	+	+	+	+	+	+	+	+	+	+
5	79	-	-	+	+	-	-	-	-	-	-	-	-
6	73	-	-				-	-	-	-	-	-	-
7	61	+	+	+	+	+		+	+	+	+	+	+
8	56	+	+	+	+	+	+	+	+	+	+	+	+
9	53	+	+	+	+	+	+						
10	47	+	+	+	+	+	+	+	+	+	+	+	+
11	45	-				-	-	-	-		-	-	-
12	43	+	+	+	+	+	+	+	+	+	+	+	+
13	39	-	-	-			-	-	-	-	-	-	-
14	36	+	+	+	+	+	+		+	+	+		+
15	33	+	+	+	+	+	+	+	+	+	+	+	+
16	30	+	+	+	+	+	+			+	+		
17	27	+	+	+	+	+	+						
18	24	+	+	+	+	+	+						
19	21	-	-	-	+	+	+	-	-	-	-	-	-
20	19	-	-	-	-	-		-	-	-	-	-	-
21	16	-	-	-	-			-	-	-	-	-	-
22	13	+	+	+	+	+	+	+	+	+	+	+	+
23	9	+	+	+	+	+	+	+	+	+	+	+	+
Total		15	16	19	20	19	16	9	10	13	11	8	10

Table (2). Specific negative and positive protein bands related to salinity tolerance in the yielded seeds of the soybean in response to their pre-emergence treatment with different concentrations of NaCl (50 mM & 100 mM) with or without GA₃, PBZ or Zn compared with corresponding control. (+) presence of the band and (-) absence of the band. Color sign refers to the negative and positive bands

Specific positive markers related to salinity tolerance									
Band No.	M.W (KD)	Zn + 50 mM NaCl	Zn +100 mM NaCl	PBZ + 50 mM NaCl	PBZ +100 mM	GA ₃ + 50 mM	GA ₃ +100 mM NaCl	50 mM NaCl	100 mM NaCl
1	19	-	-		-	-	-	-	-
2	16		-		-	-	-	-	-
Total positive markers		1	0	2	0	0	0	0	0
Specific negative markers related to salinity tolerance									
1	129	+	+	+	+	+	+	+	
2	61	+	+		+	+	+	+	+
3	53	+		+					
4	36	+	+	+	+	+	+		
5	30	+	+	+	+				
6	27	+		+					
7	24	+		+					
Total negative markers		0	3	1	3	4	4	5	6

Table (3). Specific positive protein bands in the yielded seeds of the soybean in response to their pre-emergence treatment with GA₃, PBZ or Zn and not disappeared at different levels of salinity stress (50 mM & 100 mM). (+) presence of the band and (-) absence of the band. Color sign refers to the positive bands

Band No.	M.W (KD)	Zn	Zn + 50 mM NaCl	Zn +100 mM NaCl	PBZ	PBZ + 50 mM NaCl	PBZ +100 mM	GA ₃	GA ₃ + 50 mM	GA ₃ +100 mM NaCl
1	112	+	+	+	+	-	-	-	-	-
2	73	+	+	-	+	-	-	-	-	-
3	45	+	-	+	+	-	-	+	-	-
4	39	+	+	-	-	-	-	-	-	-
Total positive markers		3	3	2	3	0	0	1	0	0

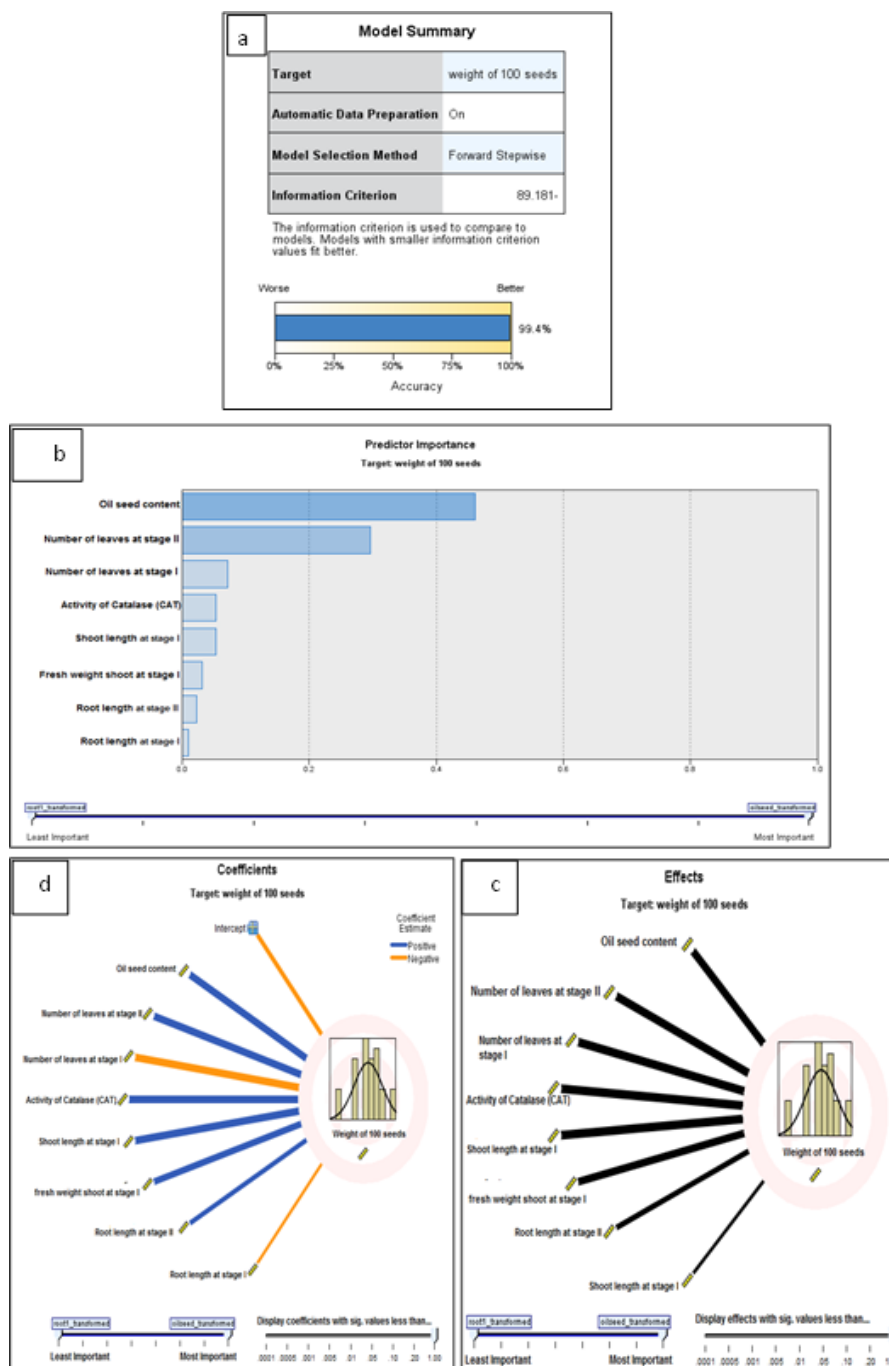


Figure (23). (a-d): Automatic linear modelling of the yielded seeds of the soybean in response to their pre-treatment with different concentrations of NaCl (50 mM & 100 mM) and or without GA₃, PBZ or Zn

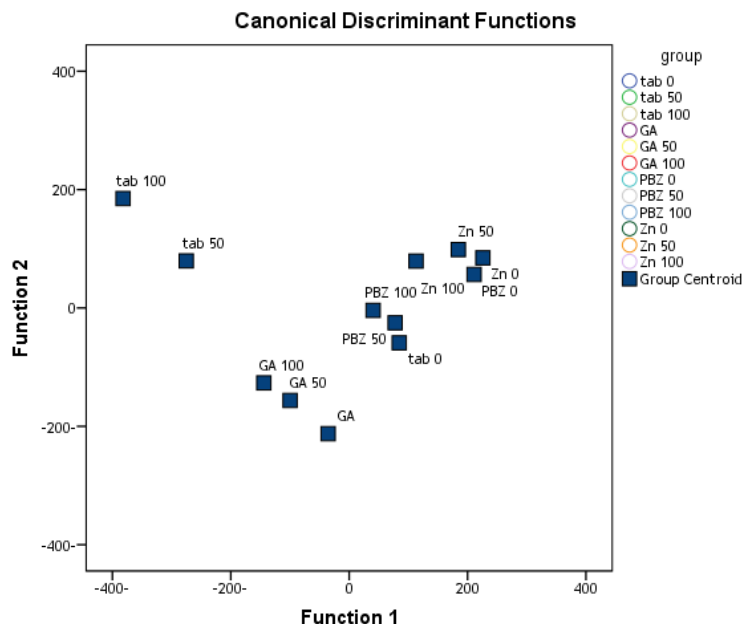


Figure (24). Discriminant of the yielded seeds of the soybean in response to their pre-treatment with different concentrations of NaCl (50 mM & 100 mM) and or without GA₃, PBZ or Zn

4. Conclusions

Based on present findings, it was concluded that the use of GA₃, PBZ and Zn greatly improving and often maximizing the majority of the growth characteristics and yield of soybean plants grown either in saline or non-saline conditions via enhancement the majority of the physiological processes from the treated plants. It had been found in the obtained results that application of either PBZ or Zn was more efficient than GA₃ in mitigating the adverse after effect of salinity.

REFERENCES

- [1] Pooja, S. and Rajesh, K. (2015): Soil Salinity: A Serious Environmental Issue and Plant Growth Promoting Bacteria as One of the Tools for Its Alleviation. Saudi Journal of Biological Sciences, 22, 123-131.
- [2] Conde, A.; Chaves, M.M.; and Gero's, H. (2011): Membrane transport, sensing and signaling in plant adaptation to environmental stress. Plant Cell Physiol. 52, 1583-1602.
- [3] Bhaskar, G. and Bingru, H. (2014): Mechanism of Salinity Tolerance in Plants: Physiological, Biochemical, and Molecular Characterization. International Journal of Genomics Volume 2014, 1- 18 pages.
- [4] Lee, G.A.; Crawford, G.W.; Liu, L.; Sasaki, Y. and Chen, X. (2011): Archaeological soybean (*Glycine max*) in East Asia: does size matter? PLoS One. 6, e26720.
- [5] Ahmad, P. and Umar, S. (2011): Oxidative Stress: Role of Antioxidants in Plants, Studium Press, New Delhi, India.
- [6] Neill, S.; Desikan, R. and Hancock, J. (2002): "Hydrogen peroxide signaling," Current Opinion in Plant Biology, vol. 5, no. 5, pp. 388-395.
- [7] Yan, J.; Tsuchihara, N.; Etoh, T. and Iwai, S. (2007): "Reactive oxygen: species and nitric oxide are involved in ABA inhibition of stomatal opening," Plant, Cell and Environment, vol. 30, no. 10, pp. 1320-1325.
- [8] Noctor, G. and Foyer, C.H. (1998): "Ascorbate and glutathione: keeping active oxygen under control," Annual Review of Plant Biology, vol. 49, pp. 249-279.
- [9] Zaefyzadeh, M.; Quliyev, R.A.; Babayeva, S.M. and Abbasov, M.A. (2009): "The effect of the interaction between genotypes and drought stress on the superoxide dismutase and chlorophyll content in durum wheat landraces," Turkish Journal of Biology, vol. 33, no. 1, pp. 1-7.
- [10] Chen, Q.; Zhang, M. and Shen, S. (2010): "Effect of salt on malondialdehyde and antioxidant enzymes in seedling roots of Jerusalem artichoke (*Helianthus tuberosus* L.)," Acta Physiologiae Plantarum, vol. 33, no. 2, pp. 273-278.
- [11] Tatar, O. and Gevrek, M.N. (2008): Influence of water stress on proline accumulation, lipid peroxidation and water content of wheat. Asian Journal of Plant Sciences, 7: 409-412.
- [12] Akram, N.A. and Ashraf, M. (2013): Regulation in Plant Stress Tolerance by a Potential Plant Growth Regulator, 5-Aminolevulinic Acid. J Plant Growth Regul, Volume 32, Issue 3, pp 663-679.
- [13] Gurmani, A.R.; Bano, A.; Din, J.; Khan, S.U. and Hussain, I. (2009): Effect of phytohormones on growth and ion accumulation of wheat under salinity stress. African Journal of Biotechnology, 8(9): 1887-1894.
- [14] Chauhan, J.S.; Tomar, Y.K.; Singh, N.I.; Ali, S. and Debarati (2009): Effect of growth hormones on seed germination and

- seedling growth of black gram and horse gram. Journal of American Science, 5(5): 79-84.
- [15] Vernon, L.P. and Selly, G.R. (1966): The chlorophylls. Academic press. New York and London.
- [16] Lowery, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951): Protein measurement with the folin reagent. J. Biol. Chem., 193: 265-275.
- [17] Umbriet, W.W.; Burris, R.H.; Stauffer, J.F.; Cohen, P.P.; Johsen, W.J.; Lee page, G.A.; Patter, V.R. and Schneicter, W.C. (1969): Manometric techniques, manual describing methods applicable to the studs of tissue metabolism. Burgess publishing co., U.S.A; P.P.239.
- [18] AOAC (1980): Association of official agriculture chemists "official methods of analys" 13th Edition, Washington, DC, USA.
- [19] .Kherjee, S.P. and Choudhuri, M.A. (1983). Implication of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedling. Physiol. Plant, 58: 166-170.
- [20] Dhindsa, R.; Plumb–Dhindsa, P. and Thorpe, T. (1981): Leaf senescence correlated permeability, lipid peroxidation and decreased levels of superoxide dismutase and catalase. J. Exp. Bot., 32: 93-101.
- [21] Bergmeyer, H.U. (1974): in Methods of Enzymatic Analysis 2nd ed., vol. 2, pp, 458-459, Academic Press, New York.
- [22] Chen, Y.; Cao, X.D.; Lu, Y.X. and Wang, R. (2000): Effects of rare earth metal ions and their EDTA complexes on antioxidant enzymes of fish liver. Bull. Environ. Contam. Toxicol., 65: 357- 365.
- [23] Karni, L.; Moss, S. and Tel-OR, E. (1984): Glutathione reductase activity in heterocysts and vegetative cells of cyanobacterium *Nostoc muscram*. Arch. Microbiol., 140: 215-217.
- [24] Lee, B.; Martin, P. and Bangerth, F. (1989): The effect of sucrose on the levels of abscisic acid, indole acetic acid and zeatin/zeatin riboside in wheat ears growing in liquid culture.. Physiol. Plant., 77: 73-80.
- [25] Hernandez, J.A. and Almansa, M.S. (2002): Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. Physiol. Plant, 115: 251–257.
- [26] Bates, L.S.; Waldren, R.P. and Teare, I.D. (1973): Rapid determination of free proline for water stress studies. Plant Soil, 39: 205-207.
- [27] Laemmli, U.K. (1970): Cleavage of structural head of bacteriophage. Nature; 227: 680-685.
- [28] Studier, F.W. (1973): Analysis of bacteriophage T, early RNAs and proteins of slab gel. Journal of molecular Biology; 79: 237-248.
- [29] Snedecor, G.M. and Cochran, W.G. (1982): Statistical methods-7th edition, Iowa state Univ., Press, Ames., Iowa, USA., pp. 325-330.
- [30] Härdle, W. and Simar, L. (2007): Applied Multivariate Statistical Analysis. 2nded, Springer, 420pp.
- [31] Hutchings, M.J. and John, E.A. (2004). The effects of environmental heterogeneity on root growth and root/shoot partitioning. Ann. Bot.-London, 94: 1-8.
- [32] Sharaf, A.M. (2010): Improvement Growth, and Yield of Wheat Plants Grown Under Salinity Stress by Using Silicon. Journal of American Science; 6(11); 559-556.
- [33] De Pascale, S.; Maggio, A. and Barbieri, G. (2005): Salinization affects growth, yield and mineral composition of cauliflower broccoli. European Journal of Agronomy, 23: 254-264.
- [34] Keutgen, A. and Pawelzik, E. (2008): Quality and nutritional value of strawberry fruit under long term salt stress. Food Chemistry, 107: 1413-1420.
- [35] Hoque, M. and Haque, S. (2002): Effects of GA₃ and its mode of application on morphology and yield parameters of mungbean (*Vigna radiata* L.). Pak. J. Biol. Sci., 5: 281-283.
- [36] Hajihashemi, S. and Kiarostami, K. (2007): Effects of paclobutrazol and salt stress on growth and ionic contents in two cultivars of wheat. PAK.J.Biol.Sci., 1; 10(1):41-48.
- [37] Mahmoud M.M. (2014): Response of soybean plants to exogenously applied with Ascorbic acid, Zinc Sulphate and Paclobutrazol. Report and Opinion 2014;6(11).
- [38] Anita, S.A.; Shahid, U. and Mishra, S.N. (2012): Boron and zinc response on growth in *Vigna radiata* L. wilczek var. pusavishal under salinity. Journal of Plant, Animal and Environmental Sciences ISSN 2231-4490.
- [39] Aldinary, M.M. (2015): The protective effect of presoaking seed and foliar treatments on growth and metabolism of *Vigna sinensis* L plants under salt stress condition. M.Sc. Faculty of Science. Al-Azhar University, Cairo, Egypt.
- [40] Almodares, A.; Hadi, M.R. and Dosti, B. (2008): Effect of salt stress on growth parameters and carbohydrates contents in sweet sorghum. Research J. Envir. Sci., 2(4): 298-304.
- [41] Abeer, A.A. (2009): Increasing tolerance of *Vigna sinensis* L. to salt stress using an organic acid and polyamine. Ph. D. Thesis, Ain Shams Univ. Egypt.
- [42] Mane, A.V.; Karadge, B.A. and Samant, J.S. (2010): Salinity induced changes in photosynthetic pigments and polyphenols of *Cymbopogon nardus* (L.) Rendle. Journal of Chemical and Pharmaceutical Research, 2(3): 338-347.
- [43] Anjali, R. and Aruna, R. (2014): Improvement of Salt Tolerance in *Trigonella foenum-graecum* L. var. PEB by Plant Growth Regulators. Journal of stress physiology & biochemistry Vol. 10 No. 2, 136-143.
- [44] Zeid, I.M. (2011): Alleviation of seawater stress during germination and early growth of barley. International Journal of Agriculture: Research and Review, 1(2): 59-67.
- [45] Foyer, C.H.; Lelandais, M. and Kunert, K.J. (1994): Photooxidative stress in plants, Physiol. Plant., 92: 696-717.
- [46] Meloni, D.A.; Oliva, M.A.; Martinez, C.A. and Cambraia, J. (2003): Photosynthesis and ability of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress, Environ. Exp. Bot., 49: 69–76.
- [47] Fadzilla, N.M.; Finch, R.P. and Burdon, R.H. (1997): Salinity oxidative stress and antioxidant responses in shoot cultures of rice, J. Exp. Bot. 48, 325-331.
- [48] Lechno, S.; Zamski, E. and Tel-Or, E. (1997): Salt

- stress-induced responses in cucumber plants, *J. Plant Physiol.*, 150: 206-211.
- [49] Zhu, Z.; Wei, G.; Li, J.; Qian, Q. and Yu, J. (2004): Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). *Plant Sci.*, 167: 527-533.
- [50] Shen, X.; Zhou, Y.; Duan, L.; Li, Z.; Eneji, E. and Li, J. (2010): Silicon effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and ultraviolet-B radiation. *Journal of Plant Physiology* 167;1248–1252.
- [51] Sajjad, Z. and Hassan P. (2012): "Changes of antioxidant enzymes in oilseed rape in response to salinity stress". *International Journal of Agricultural and Crop Sciences*, 7: 398-403.
- [52] Bor, M., Ozdmir, F. and Turkan, I. (2003): The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritima* L. *Plant Soil*, 164: 77.
- [53] Neto, A.D.A.; Prisco, J.T. and Emeas-Filho, J. (2006): Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environ. Exp. Bot.*, 56: 87-94.
- [54] Esfandiari, E. and Shekari, F. (2007): The effect of salt stress on antioxidant enzymes activity and lipid peroxidation on the wheat seedling. *Not Bot Hort Agrobot Cluj*, 35: 48-56.
- [55] Liang, Y.; Chen, Q.; Liu, Q.; Zhang, W. and Ding, R. (2003): Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). *J. Plant Physiol.*, 160: 1157-1164.
- [56] Badawi, G.H.; Yamauchi, Y.; Shimada, E.; Sasaki, R.; Kawano, N. and Tanaka, K. (2004) : Enhanced tolerance to salt stress and water deficit by overexpressing superoxide dismutase in tobacco (*Nicotiana tabacum*) chloroplasts. *Plant Sci.*, 166: 919-928.
- [57] Tuna, A.L.; Kayab, C.; Dikilitas, M. and David, H. (2008): The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. *Environmental and Experimental Botany*, 62: 1-9.
- [58] El-Khallal, S.M.; Hathout, T.A.; Ashour, A.A. and Kerit, A.A. (2009): Brassinolide and salicylic acid induced growth, biochemical activities and productivity of maize plants grown under salt stress. *Research Journal of Agriculture and Biological Sciences*, 5(4): 380-390.
- [59] Ghanem, M.E.; Albacete, A.; Martinez- Andujar, C.; Acosta, M.; Romero-Arandaz, R.; Dodd, I.C.; Iltis, S. and Pérez-Alfocea, P. (2008): Hormonal changes during salinity-induced leaf senescence in tomato (*Solanum lycopersicum* L.). *J. Exp. Bot.*, 59(11): 3039-3050.
- [60] Hulusi, K.; Melike, B.; Filiz, Z. and Ismail, T. (2007): The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environmental and Experimental Botany* (60) 344–351.
- [61] Naureen, G. and Naqvi, F.N. (2010): Salt tolerance classification in wheat genotypes using reducing sugar accumulation and growth characteristics. *Emir. J. Food Agric.* 22(4): 308-317.
- [62] Shalata, A. and Neumann, P.M. (2007): Exogenous Ascorbic Acid (Vitamin C) Increases Resistance to Salt Stress and Reduces Lipid Peroxidation. *Journal of Experimental Botany*, 52, 2207-2211.
- [63] Verma, S. and Misra, N. (2005): Putrescine Alleviation of Growth in Salt Stressed *Brassica juncea* by Inducing Antioxidative Defense System. *Journal of Plant Physiology*, 162, 669-677.
- [64] Zago, M.P. and Oteiza, P.I. (2001): The antioxidant properties of zinc: interactions with iron and antioxidants. *Free Rad Biol Med* 31:266-274
- [65] Tavallali, V.; Rahemi, M.; Eshgi, S.; Kholdebarin, B. and Ramezani, A. (2010): Zinc alleviates salt stress and increases antioxidant enzyme activity in the leaves of pistachio (*Pistacia vera* L. Badami) seedlings. *Turk J Agri For* 34:349-359.
- [66] Weisany, W.; Sohrabi, Y.; Heidari, G.; Siosemardeh, A. and Ghassemi- Golezani, K. (2012): Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). *Plant Omic J* 5:60-67.
- [67] Alloway, B.J. (2008): Zinc in soils and crop nutrition. IZA and IFA Publisher, Belgium and Paris, pp 30-50.
- [68] Kawano, T.; Kawano, N.; Muto, S. and Lapeyrie, F. (2002): Retardation and inhibition of the cation-induced superoxide generation in BY-2 tobacco cell suspension culture by Zn^{2+} and Mn^{2+} . *Plant Physiol* 114:395-404.
- [69] Ashraf, M. and Foolad, M.R. (2007): Roles of glycine betaine and proline in improving plant abiotic resistance. *Environmental and Experimental Botany*, 59(2):206-216.
- [70] Amini, F. and Ehsanpour, A.A. (2005): Soluble proteins, proline, carbohydrates and Na/K changes in two tomato (*Lycopersicon esulentum* Mill.) cultivars under in vitro salt stress. *American J. of Biochemistry and Biotechnology*, 1(4):212-216.
- [71] Radyukina, N.L.; Ivanov, Y.V.; Kartashov, A.V.; Shevyakova, N.I.; Rakitin, V.Y.; Khryanin, V.N. and Kuznetsov, V.I.V. (2007): Inducible and constitutive mechanisms of salt stress resistance in *Geum urbanum* L. *Russian J. of Plant Physiology*, 54:612-618.
- [72] Lobato, A.K.S.; Oliveria, C.F.; Costa, R.C.L.; Santos Filho, B.G.; Cruz, F.J.R. and Laughinghouse, I.V. (2008): Biochemical and physiological behaviour of *Vigna unguiculata* L. Walp. Under water stress during the vegetative phase. *Asian Journal of Plant Sci.*, 7(1):44-49.
- [73] Sharma, D.K.; Dubey, A.K.; Manish, S.; Singh, A. K.; Pandey, R.N. and Anil, D. (2013): Effect of paclobutrazol and putrescine on antioxidant enzymes activity and nutrients content in salt tolerant citrus root stock sour orange under sodium chloride stress. *Journal of Plant Nutrition*; (36):1765-1779.
- [74] Weria, W.; Yousef, S.; Gholamreza, H.; Adel, S. and Kazem, G. (2012): Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). *POJ*. 5(2):60-67.
- [75] Sofy, M.R. (2011): Physiological and Biochemical Responses of Plant to Growth Regulators and Stress Condition. Ph.D. Thesis, Faculty of Science. Al-Azhar University, Cairo,

Egypt.

- [76] Ayman, S.; Sobhy, S.; Akihiro, U.; Hirofumi, S. and Celaleddin, B. (2015): Evaluation of salt stress effect on seed yield and quality of three of soybean cultivars Azarin J. of Agric. Vol. 2. Issu 5, 138-141.
- [77] Arefian, M.; Vessal, S. and Bagheri, A. (2014): Biochemical Changes and SDS-PAGE Analyses of Chickpea (*Cicer arietinum* L.) Genotypes in Response to Salinity During the Early Stages of Seedling Growth. J. Biol. Environ. Sci., 8(23), 99-109.
- [78] Amal, A.M. (2005): Two-dimensional Electrophoresis of Soluble Proteins and Profile of Some Isozymes Isolated from Maize Plant in Response to NaCl. Res. J. Agric. and Biol. Sci. 1(1): 38-44.
- [79] El-Farash, E.M.; El-Enamy, A.E. and Mazen, A. (1993): Influence of genotype and NaCl on the levels of growth, proteins, proline, free amino acids, viability and protein regulation in tomato callus cultures. Physiol Plant 4:345-352.
- [80] Hassanein, A.M. (1999): Alterations in protein and esterase patterns of peanut in response to salinity stress. Biol Plant 42:241-248.
- [81] Rashed, M.A.; Abo-Doma, A.; El-Rashidy, H. and Khaled, K.M.A. (2006): Molecular genetic characterization for some loci controlling salt tolerance in *Sorghum bicolor* (L). Egyptian Journal of Genetics and Cytology 35: 145-155.
- [82] Sobhanian, H.; Razavizadeh, R.; Nanjo, Y.; Ehsanpour, A.A.; RastgarJazii, F.; Motamed, N. and Komatsu, S. (2010): Proteome analysis of soybean leaves, hypocotyls and roots under salt stress. Proteome Sci., 8(1):19.
- [83] Amal, M.E. and Heba, I.M. (2014): The Effect Of The Exogenous Gibberellic Acid On Two Salt Stressed Barley Cultivars. Euro. Sci. J.10(6), 228-245.
- [84] Peleg, Z. and Blumwald, E. (2011): Hormone balance and abiotic stress tolerance in crop plants. Current Opinion in Plant Biology, 14 (3), 290-295.
- [85] Santner, A. and Estelle, M. (2010): The ubiquitin–proteasome system regulates plant hormone signaling. The Plant Journal, 61, 1029-1040.