Research Article

Effect of Indole-3-Acetic acid and Gibberellic acid (GA3) on seeds germination, growth performance and biochemical constituents of *Zea mays* L. growing under the salt stress

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Abstract

Maize (*Zea mays* L.) crop is sensitive to salt stress, whereas salinity problem is a serious threat to its crop production in the Khyber Pakhtunkhwa. Here, we find out the effect of exogenously applied Auxin (IAA) and Gibberellin (GA3) on agronomic and biochemical constituents of maize (*Zea mays* L.) under the induced stress of Sodium Chloride (NaCl). Our main findings are as follows; (1) germination percentage of *Zea mays* L. is critical to salt stress than later developing stages. (2) IAA and GA3 relatively reduced the adverse influence of NaCl on *Zea mays* L. seed germination. (3). Growth factors were reduced at the seedling stage under NaCl+IAA, while GA3+NaCl relatively induced the growth of the seedling. (4) The response of biochemical constituents of *Zea mays* L. to salinity and plant growth regulators (IAA and GA3), showed that the NaCl+GA3 (20ppm NaCl+40ppm GA3) treatment induced the Chlorophyll a, b and Carotenoid a, b in treatment NaCl+20ppm GA3 and 30ppm NaCl+40ppm GA3, respectively. Treatment 30ppm NaCl+40ppm GA3 and 20ppm NaCl+20ppm induced the Protein and proline content comparatively to NaCl+IAA and Control treatment (without the application of IAA, GA3, and salinity). Little variations were observed in sugar content between the treatments and control, however, the relatively higher Sugar content was recorded in 10ppm NaCl+40ppm GA3 treatment. These findings revealed that GA3 plays an important role in certain of the negative influence of salt stress with may the protein accumulation which improves the permeability of the membrane and induced the micro and macro-nutrients levels.

Keywords: GA3; IAA; Salt Stress; *Zea mays* L.

Introduction

The occurrence of excess soluble salts in the soil refers to salt-affected soils or saline soli which suppresses plant growth [1]. Plants

regularly face abiotic stress environment during development and growth, such as freezing, drought, chilling, high temperatures, and salinity. Stress conditions

can delay growth and development, reduce production, and in extreme cases cause the death of plants [2]. Saline stress imposes effects on plant growth through the small osmotic prospective of topsoil and imbalance of nutrition [3]. Incensement of salt concentration in soil is a serious problem for crop production around the globe [4]. Salinity effect the plant functioning, morphology, and homeostasis, and reduced the plant biomass and yield [5]. Agricultural crops has to contract with variable abiotic and biotic stresses in around the world, which reduced the crops development and production [6]. Toxic intensities of Na in organs of plant effect the membranes and sub-cellular organelles, decreasing development, and inflicting irregular improvement earlier than plant mortality [7]. A few physiological procedures, for example, respiration and photosynthesis, nitrogen fixation, and starch metabolism are additionally influenced under saline conditions prompting to lose the productivity of crops.

Plant hormones take part in a very important role in a variable the protecting reaction contrary to various stresses of biotic and abiotic [8]. It also induced/suppressed the expression of various gene via antagonistic and/or synergistic events [9]. Plant growth regulators such as IAA is performing a crucial role in the plant growth for example lateral root formation, vascular tissues development, differentiation of cell, apical dominance and expression of genes [10]. Plant hormones also act as salts in crop plants and respond to them, and this is indicated regularly under various pressures [11]. The detrimental effect of salinity on seed germination may be due to a decrease in the internal levels of phytohormones [12]. Other researchers [13] have shown that the inhibitory effect of salt growth is reduced by the inclusion of tritium seeds with various plant growth regulators.

Maize (*Zea mays* L.) belongs to the Poaceae family, after wheat and rice, and is the 3rd most important crop. It is developed in a wide range of climatic and soil conditions sensitive to salinization. In 2012, it is widely grown and cultivated on approximately one hundred and seventy-seven (177) million hectares worldwide, and total production is approximately 875 million tons. *Zea mays* L. is the essential crop that is the necessary required of oil and food for individual intake. It is also utilized as nourish for livestock all over the world but it mostly treated with salt stress. Saline stress is s key abiotic stress that prevents the growth and yield of crops. It is one of the world's oldest and most widely distributed environmental challenges. [14]. Never the less, extensive intr-specific genetic disparity for salt resistance present in *Zea mays* L. [15]. As indicated by [16] biphasic model of salt-stress induced reduction of growth, osmotic-stress in the earlier stage and toxicity of ions in the subsequent (second) stage are control the growth reduction in cereals, particularly wheat. [17] developed a similar model for saltiness-actuated maize development decrease, but ion toxicity and related development decrease that occur to a small degree earlier in *Zea mays* L. [18]. The *Zea mays* L. sensitivity is related with increased addition of sodium ion (Na⁺) to the leaves [17]. The present study is conducted to explore the effect of IAA and GA3 on seed germination, seedling growth, and biochemical constituents of *Zea mays* L. under salt stress. We expected that proper application of plant growth regulators (IAA and GA3) would reduce the effects of salt stress (NaCl).

Materials and Methods

Area of research

In order to determine the effects of different sodium levels and two plant regulators such as IAA and GA3 on germination, morphological and biochemical contents

experiments were conducted in Bacha Khan University, Charsadda.

Endogenous hormones and Salt

For this purposes plant regulators of IAA and GA3 and Sodium Chloride as salinity was used.

Plant materials and experimental design

Seeds of maize (*Zea mays* L.) cultivar Pop-1 were obtained from the agricultural research

center of Khyber Pakhtunkhwa, Pakistan. The experimental work was carried out in the field in October using a randomized complete block design (RCBD) in natural light conditions with an average temperature of 22° C. The experiment consisted of 16 clay pots divided into 6 sets. The treatment set was as follows in (Table 1).

Table 1. Details list of various treatment

1. Control (T0-only distilled water)	9. 20ppm NaCl+40ppm IAA
2. 10ppm S	10. 30ppm NaCl+40ppm IAA
3. 20ppm S	11. 10ppm NaCl+20ppm GA3
4. 30ppm S	12. 20ppm NaCl+20ppm GA3
5. 10ppm NaCl+ 20ppm IAA	13. 30ppm NaCl+20ppm GA3
6. 20ppm NaCl+20ppm IAA	14. 10ppm NaCl+40ppm GA3
7. 30ppm NaCl+20ppm IAA	15. 20ppm NaCl+40ppm GA3
8. 10ppm NaCl+40ppm IAA	16. 30ppm NaCl+40ppm GA3

S= Salt (NaCl), ppm= parts per million, AH= Auxin Hormone (IAA), GH= Gibberellin Hormone (GA3)

Germination percentage

The emergence of plumule was taken as index of germination. Initiation and completion of germination was recorded

daily. The germination percentage was recorded daily for 10 days and germination percentage was calculated with the following formula:

$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated seeds}}{\text{Number of total seeds}} \times 100$$

Measurement of agronomic parameters

Certain agronomic parameters were observed in field as shoot length by (Measurement scale), root length by (Measurement scale), fresh and dry weight through (Weight Machine), leaf area, leaf no, root no (Measurement scale) of each samples were determined.

Biochemical analysis

Soluble proteins determination

Soluble protein extraction and assessment was achieved followed the method of [19].

Protein extraction

Combine with 0.2 g of ice-covered cold pastel and mortar in 5 ml of potassium phosphate buffer (0.1 m, pH = 7). Homogenize or filter extract with a cheese

cloth or a glass. The extract is placed in the freezer for cooling. After cooling, the extract is concentrated at 4000 rpm for 10 minutes (centrifugal at 12000 rpm for 20 min if there is a cooling concentration with excessive speed). The supernatant is moved to a test tube, the test tube is inspected, and then the extract volume is adjusted with a buffer with 5 ml. For dilution, take 0.2 ml of this extract and load 4.8 ml of buffer (this extract will provide the occasion).

Soluble protein estimation

Add 0.1 ml of the sensitive extract to the check tube. Add 5 ml Bradford essay and shake. To empty the reagent, take 0.1 ml buffer and add 5 ml Bradford Ace reagent.

The spectrophotometer measures a 5 particle density (OD) of 595 nm.

Extraction and estimation of total carbohydrates

Sugar estimation of fresh leaves was done following the method of [20].

Extraction

0.2 g of a fresh sample of leaves had been crushed in a 10ml distilled water mortar. Afterwards the crushed material was centrifuged for 10 minutes at 1000rpm. The supernatant was then gathered and the residue discarded in another test tube.

Estimation

In a test tube 0.5 ml of plant extract was selected and 5 ml of anthrone reagent was applied. The tubes were then heated for about 15 minutes in a water bath, and cooled instantly in ice-cold water. The green color that is read against reagent blank is obtained

at 620 nm. The reagent anthrone was taken blank as the reagent. The carbohydrate content was measured in micrograms per ml. The quantity was then recorded in microgram per milligram fresh weight.

Extraction and estimation of chlorophyll content

Extraction and Estimation of chlorophylls were done by the method of [21].

Extraction and estimation

The fresh mass of leaf was removed, then the leaves the leaves were dissolved in 3 ml of 80 percent acetone in a small amount of acid-washed sand and centrifuged for 5 min at 1000 rpm. The litter is then washed 3 times with 1 ml of 80 percent acetone. The supernatants are then mixed with acetone and set to 7 ml. These results are then reported at a visual density of 663 nm, 645 nm, 480 nm and 510.

Equations

Chlorophyll a. (mg/g) = $12.7 A_{663} - 2.69 A_{645}$

Chlorophyll b. (mg/g) = $22.9 A_{645} - 4.68 A_{663}$

Total Chlorophyll = $20.2 \times A_{645} + 8.02 \times A_{663}$

Carotenoids $\left(\frac{\text{mg}}{\text{g}}\right) = 7.6 D_{480} - 1.49 D_{510} \times \frac{V}{D} \times 1000 \times W^3$

Results and Discussion

Germination of seed under different treatments of salinity and plant hormones

Soil salinity, Salinity+IAA reduced the germination percentage of *Zea mays* L. cultivars Pop-1 as compared to control and Salinity+GA3 treatments (Table 2). Highest germination percentage showed under the treatment of 10ppm NaCl+40ppm GA3 (7.7 ± 0.701), 10ppm NaCl+ 20ppm GA3 (7.5 ± 0.631), while minimum germination percentage was recorded in 30ppm NaCl+20ppm IAA (4.5 ± 0.601) and 20ppm NaCl+20ppm IAA (4.5 ± 0.571). This indicates that GA3 reduced the adverse effect of salt stress on seed sprouting of *Zea mays* L. comparatively to IAA. Similar results documented by [22-25] stated that GA3 reduced the influence of salt stress in

crops. [26] documented that Auxin induced fresh and dry biomass of seedling, hypocotyl length and hypocotyl dry weight, but did not control on seed germination percentage and root length under salt stress. [27] documented that the GA3 has a better salt resistance by increasing the germination percentage, whereas high salt concentration could be alleviated by the presence of GA3 [28].

The effects of salinity-plant hormones interactions on root and shoot length

Results of salt stress revealed a reduction of the root and shoot length of *Zea mays* L. (maize) compared to control, saline stress+IAA, whereas salt stress+GA3 revealed improvement in shoot and root length. The maximum value of shoot length was observed on 20ppm NaCl+40ppm GA3

(15.43±5.2190) (9.2±2.4033) and minimum in treatment 30ppm NaCl (5.63±1.683). Higher root length was recorded in treatment 10ppm NaCl+40ppm GA3 (10.7666±5.232), while shorter root length observed in treatment 30ppm salt+40ppm IAA (4.18±1.2703) (Table 2). This indicated that salt stress decreased the root and shoot length, but the application of GA3 decline the negative effects of saline stress. Saline anxiety reduced the water absorption ability of plants, which leads to a decline in stem and radical length [29]. [30] documented that the shoot development significantly reduced at 10 dS/m in a greater concentration of salt stress. As the IAA applications in the rising tips and stele of roots [31] is most likely maintained by acropetal transport from the seed or shoot tissues, endogenous levels of auxin may reduce rapidly plumule-radical length [32]. [33] initiate that GA3 can help to elongation of root under sodium chloride. The effects of salinity on alfalfa sprouting was increased in salt concentration caused a decline in different characters (i.e. root and shoot length). The inhibitory effect of salinity on seedling growth and development rate, as previously observed by [34].

Seedling fresh and dry biomass

Salinity significantly decreases the fresh and dry mass of the maize plant, whereas IAA sprayed has relatively little influence as compared to GA3 treatment (Table 2). Maximum fresh and dry weight was observed for treatment 20ppm NaCl+40ppm GA3 (1.64±1.364) and 20ppm NaCl+40ppm GA3 (0.57±0.2895), respectively. Minimum fresh and dry biomass were recorded for treatment 10ppm NaCl+40ppm IAA (0.36±0.1472) and 10ppm NaCl+20ppm IAA (0.16±0.0785). Such results are consistent with the findings of [35], [36], & [37], according to which

salinity substantially decreases plant fresh and dry weight. Similarly, [38] also documented that the GA3 and NAA pre-harvest spray increased Banarasi Karaka's fruit size, weight and yield.

Effect of IAA and GA3 on leaf area, leaf number and root number under salt stress

The influence of plant regulator on root number, leaf area and leaf number of *Zea mays* L. cultivars Pop-1 showed in (Table 3). The maximum leaf area and leaf observed for 10ppm NaCl+40ppm GA3 (21.26±3.4523), and 30ppm NaCl+40ppm IAA (5.5±2.5099) respectively. The lower value of leaf area and leaf numbers were recorded for 10ppm NaCl+20ppm IAA (12.31±6.6739) and 30ppm NaCl+40ppm IAA (2.03±0.5163), respectively. The higher number of roots was recorded for 20ppm NaCl+ 40ppm IAA (8.16±2.8577), while minimum numbers of roots were noted in 30ppm NaCl+40ppm IAA (3.66±2.3380). These results showed variability, whereas relatively the GA3 effect higher on leaf area and leaf number, while IAA has a strong influence on root numbers of *Zea mays* L. (Table 3). Such results are reported by other researchers, in sensitive genotypes, significantly affect the leaves numbers [39], and decrease in leaf area [40]. This may be due to a decrease in chlorophyll content, relatively low photosynthesis, net assimilation of carbon dioxide, and low conductivity [41]. The reduction of leaf area and leaf numbers may due to uptake of lower water and nutrient-limited under the saline conditions [42]. GA3 generally reduced the adverse influence of saline stress, because plant growth regulators are normally effect the development and growth of plants. They control seed sprouting, leaf expansion stem elongation, and flowering in plants [43].

Table 2. Effect of plant growth regulators (IAA and GA3) on the NaCl induced changes in the percent (%) seed germination, shoot and root length, fresh and dry biomass of *Zea mays* L.

Treatment	Percent (%) Germination	Shoot length (cm)	Root length (cm)	Fresh biomass (gm)	Dry biomass (gm)
Control (T0)	7.0±1.414	7.1±1.2263	9.12±11.95	0.89±0.361 3	0.45±0.213 0
10ppm S	6.3±0.701	6.45±1.4652	8.2±3.6959	0.62±0.16	0.30±0.169 3
20ppm S	6.5±0.771	5.60±1.0667	6.2±3.4979	0.54±0.142 6	0.28±0.149 4
30ppm S	6.5±0.601	5.63±1.683	6.85±3.5274	0.73±0.426 3	0.37±0.376 6
10ppm S & 20ppm AH	6.5±0.701	7.61±1.230	7.45±1.986	0.75±0.639 4	0.16±0.078 5
20ppm S & 20ppm AH	4.5±0.571	8.10±1.592	6.314±3.706	0.58±0.534 7	0.34±0.327 2
30ppm S & 20ppm AH	4.5±0.601	8.56±0.985	6.85±1.886	0.56±0.378 7	0.29±0.123 8
10ppm S & 40ppm AH	7.0±0.701	6.61±1.874	5.58±2.5763	0.36±0.147 2	0.20±0.143 8
20ppm S & 40ppm AH	5.5±0.702	8.01±1.0666	6.6±3.2655	0.51±0.313 9	0.19±0.057 9
30ppm S & 40ppm AH	5.5±0.721	6.88±1.9823	4.18±1.2703	0.53±0.092 0	0.16±0.073 6
10ppm S & 20ppm GH	7.5±0.631	10.48±2.863 8	9.28±3.8478	0.57±0.274 6	0.23±0.141 4
20ppm S & 20ppm GH	5.5±0.612	12.95±9.897 2	4.65±2.3355	0.88±0.170 1	0.47±0.925 2
30ppm S & 20ppm GH	6.5±0.721	14.75±6.011 2	9.566±5.530	0.99±0.683 9	0.46±0.437 1
10ppm S & 40ppm GH	7.7±0.701	12.8±5.3508	10.7666±5.23 2	0.86±0.407 1	0.38±0.166 0
20ppm S & 40ppm GH	5.5±0.571	15.43±5.219 0	9.2±2.4033	1.64±1.364	0.57±0.289 5
30ppm S & 40ppm GH	6.5±0.700	13.81±4.965 2	10±5.0382	0.73±0.252 5	0.40±0.204 8

S= Salt (NaCl), ppm= parts per million, AH= Auxin Hormone (IAA), GH= Gibberellin Hormone (GA3), Cm= centimeter

Table 3. Effect of plant growth regulators (IAA and GA3) on the NaCl induced changes in the leaf area, leaf number, and number of roots of *Zea mays* L

Parameters	Leaf area	Number of leaf	Number of roots
Control (T0)	15.05±9.3818	2.5±0.5477	5.33±1.9663
10ppm S	18.9±4.7547	4.33±2.0655	6.33±0.8164
20ppm S	14.80±5.968	4.16±1.4719	4.83±1.7224
30ppm S	16.21±5.4985	4.33±2.1602	6.16±1.4719
10ppm S & 20ppm AH	12.31±6.6739	4.66±1.8618	5.00±0.8944
20ppm S & 20ppm AH	15.4±4.5624	4.66±2.1602	4.16±1.1690
30ppm S & 20ppm AH	15.2±5.1586	4.00±0.8944	6.5±1.3784
10ppm S & 40ppm AH	18.33±3.7712	2.33±0.1516	5.5±2.1679
20ppm S & 40ppm AH	17.26±1.2706	2.5±0.5477	4.83±1.7224
30ppm S & 40ppm AH	15.41±4.9321	2.03±0.5163	3.66±2.3380
10ppm S & 20ppm AG	16.93±4.9192	4.16±2.3166	5.5±1.3784
20ppm S & 20ppm AG	18.13±6.8593	5.33±1.8618	4.5±1.3784
30ppm S & 20ppm AG	15.18±7.3643	4.66±2.1602	5.00±1.7885
10ppm S & 40ppm AG	21.26±3.4523	4.16±1.4719	7.16±2.7868
20ppm S & 40ppm AG	19.68±4.0999	4.83±1.9407	8.16±2.8577
30ppm S & 40ppm AG	20.75±7.3306	5.5±2.5099	6.33±2.1602

S= Salt (NaCl), ppm= parts per million, AH= Auxin Hormone (IAA), GH= Gibberellin Hormone (GA3)

Effect of IAA and GA3 on chlorophyll a/b and Carotenoid contents under salinity stress

GA3 induced chlorophyll a and b content of *Zea mays* L. under the saline stress compared to control and IAA+salt stress. The maximum chlorophyll a and b contents were found for treatment 20ppm NaCl+40ppm GA3 (Chl-a 2.35 and Chl-b 0.72mg/g), while minimum chlorophyll a content recorded in treatment 20ppm NaCl (0.03mg/g) and chlorophyll b in control (T0-0.03mg/g) (Fig. 1). Higher content of Carotenoid a and b was found in treatment 10ppm NaCl+20ppm GA3 and 30ppm NaCl+40ppm GA3, whereas lower carotenoid content a and b was found in Control (T0) and 30ppm NaCl (Fig. 2). These results indicate that plants subjected to salinity with a combination of plant growth regulators (GA3) show promotion in photosynthetic pigment, whereas little influence of IAA was observed of treatment

NaCl+IAA. Similarly, [16] reported that sodium chloride stress has fewer effects on chlorophyll a and chlorophyll b contents. Such as in soybean plant treatment with GA3 improved the carotenoid contents and induced the photosynthesis ratio [44].

Effect of IAA and GA3 on protein, proline content, and sugar under salinity condition

Protein and Proline content values of *Zea mays* L. cultivars Pop-1 showed fluctuations with treatments of Salt, Salt+IAA, and Salt+GA3 (Fig. 3a), whereas little variations in sugar content were observed (Fig. 3b). Salt stress+GA3 (30ppm NaCl+40ppm GA3) induced the protein content as compared to Control and Salt+IAA treatment. Maximum proline content was observed for treatment 20ppm NaCl+20ppm GA3, while sugar records showed little variations between the treatments whereas higher value recorded for 10ppm NaCl+40ppm GA3. The lower value of

sugar content was recorded in 10ppm NaCl+40ppm GA3. This indicated that the higher salt stress reduced the sugars contents *Zea mays* L. cultivars Pop-1. The lower soluble sugar content of leaves was produced in plants of saline field and may the sugar formation in plants reduced under salinity stress [45]. [46] also reported that total sugar reduced on high concentration 12dSm-1 and increase in low concentration 8dSm-1. Non reducing-sugar declined with inducing the salt stress in all cultivars in *Oryza sativa*. Similarly, [47] documented that the influence of salinity on various varieties of *Triticum aestivum* L. (Wheat) under in vivo condition. He stated that the protein contents of leaves was a significantly decrease with increase salt stress as

compared to non-saline condition. GA3 when treated together with Pb for a long time affected the quantity of soluble protein in a helpful way [48]. The accumulation of IAA reduced the respiration rate and the soluble sugar, proline, soluble protein, and malondialdehyde in *C. camphora* leaves when compared with the cadmium stress with no addition of IAA [49]. The application of IAA and GA3 increased the accumulation of protein content and carbohydrates content. This accumulation of carbohydrates due to GA3 and IAA treatment might be connected with the efficiency of the photosynthetic apparatus, which leads to enlargement in plant productivity and dry matter production [50].

Figure 1. Effect of plant growth regulators (IAA and GA3) on the NaCl (saline stress) induced variations in the Chlorophyll a and b content of *Zea maize* L.

Figure 2. Effect of plant growth regulators (IAA and GA3) on the NaCl induced variability in the Carotenoid a and b content of *Zea mays* L.

Figure 3. Effect of IAA and GA3 on the NaCl induced changes in the Protein and proline content of *Zea mays* L.

Conclusion

From the current study, it can be concluded that Sodium Chloride (NaCl) stress effect the seeds germination, seedling growth, and biochemical constituents of *Zea mays* L. However, the applications of plant growth regulators foliar spray on *Zea mays* L. reduced the adverse effect of NaCl (salinity). Out of two plant growth regulators (IAA and GA3) examined the GA3 induced seed germination, seedling growth, and biochemical constituents such as Chlorophyll a, b, Carotenoid a, b, Protein, Proline and Sugar under the saline stress. These results revealed that the toxic influence of salt stress was reduced by the combination of the plant growth regulators-GA3.

Authors' contributions

Conceived and designed the experiments: F Asad & N Rahman, Performed the experiments: N Rahman & F Asad, Analyzed the data: N Rahman & F Asad, Contributed materials/ analysis/ tools: Naveen Dilawar, Wisal, Muhammad Shakeel & M Ghazal, Wrote the paper: F Asad & N Rahman.

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