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## Salicylic acid ameliorates salinity tolerance in maize by regulation of phytohormones and osmolytes

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**Abstract:** Salinity is one of the most widespread stresses responsible for water and soil pollution across the globe. Salicylic acid (SA) has a major role in defence responses against various abiotic stresses. In the current study, SA (0.05 mmol) influences were evaluated in mitigation of the negative impact of salinity (40 and 80 mmol NaCl) in the maize plant. NaCl stress-induced significant accumulation of organic osmolytes (total soluble sugars (TSS), total soluble protein (TSP), and proline) by 35.6, 66.2, and 89.2%, respectively, with 80 mmol NaCl. In addition, salinity is also responsible for the elevated accumulation of inorganic osmolytes ( $\text{Na}^+$  and  $\text{Na}^+/\text{K}^+$  ratio) by 202.4% and 398.8%, respectively, and for the reduction in the  $\text{K}^+$  and  $\text{Ca}^{2+}$  levels by 48.6% and 58.9%, respectively, with 80 mmol NaCl. Moreover, salinity stress reduced phytohormones (indoleacetic acid (IAA) and gibberellic acid (GA3)) by 48.8% and 59.8%, respectively, with 80 mmol NaCl; however, abscisic acid (ABA) was increased by 340.5% with 80 mmol NaCl. Otherwise, SA application caused an additional enhancement in TSS, TSP, proline,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , IAA, and GA3 contents but decreased the  $\text{Na}^+$ ,  $\text{Na}^+/\text{K}^+$  ratio, and ABA to an appreciable level. In conclusion, SA pre-soaking mitigates the negative impact of NaCl toxicity in maize through the regulation of phytochromes and various organic and inorganic osmolytes, which may ameliorate salinity tolerance in maize.

**Keywords:** soil salinisation; salt stress; osmoprotectant; adaptation; salt-sensitive C4 species; organic and inorganic solutes; *Zea mays* L.

Plants are affected by several abiotic stresses, among them salinity stress being the major cause of reduced crop growth and production (Jalil and Ansari 2019). High salinisation restricts agricultural productivity through destructive influences on seed germination, seedling growth, root development, flowering, and fruit setting (Chakdar et al. 2019). In addition, the damage impact of salt stress on plant growth may be contributed with either specific ion effect, osmotic effect, induction of oxidative stress, or nutritional imbalance (Sharma et al. 2019). Such impacts may result in plant performance deterioration and impaired membrane stability. Moreover, the first threat that plants can face under salinity is the reduction of medium water potential, causing the dehydration of tissues (El-Katony et al. 2019). Furthermore, salinity may encourage closure of stomata, subsequently injuring the photosynthetic apparatus and enhancing the reactive oxygen species generation (ROS), resulting in the oxidative injury of various cellular constituents,

expressed in lipid peroxidation, protein degradation, enzyme activity disruption, and DNA damage (Riffat and Ahmad 2018).

Osmolytes are organic (such as glycine betaine, proline, sugars, and proteins) and inorganic (such as  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{PO}_4^{3-}$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ) osmoprotectant solutes that promote the potential of cells to preserve water without obstructing the regular metabolism (Sharma et al. 2019). The main purposes of these osmoprotectants are to regulate the enzyme activity, scavenge the ROS, prevent the membrane disintegration, and balance the ionic transport across the cell membrane (Riffat and Ahmad 2018). Moreover, these osmolytes maintain plant cellular functions, and this is considered the major strategy of plants for stress adaptation (Elhakem 2019). Moreover, previous studies have investigated the osmolyte role in abiotic stresses such as salinity (Riffat and Ahmad 2018) and drought (Elhakem 2019).

Phytohormones play a significant role in different biochemical and physiological processes in plants. Their role in alleviating environmental stresses is critical in endowing plants with tolerance under unfavourable conditions (Sharma et al. 2019). Amongst all phytohormones, abscisic acid (ABA) is a prominent regulator complicit in the stimulation of cellular adaptation to salt stress. ABA may interrupt the stomatal activity and later initiate the stress signaling response (Koo et al. 2020). Other phytohormones such as auxin (IAA), cytokinins (CK), gibberellins (GA), ethylene, and brassinosteroids have additionally been investigated to be implicated in the adaptation mechanism and the relief of the damaging impact of salt stress (Shaki et al. 2019).

Salicylic acid (SA) is a plant phenolic phytohormone, existing ubiquitously in plants, and it has been recognised as a molecule essential to contribute to the abiotic stress response (Sharma et al. 2019). The fundamental mechanisms of SA-induced tolerance of abiotic stress involve SA-mediated (1) osmolyte accumulation of proline, glycine betaine, amines, and soluble sugars, which could help in osmotic homeostasis maintenance; (2) enhanced ROS scavenging activity; (3) regulation of mineral ion uptake; (4) regulation of other hormone pathways, and (5) improved production of secondary metabolites, such as phenolics, terpenes, nitrogen, and sulfur compounds (Koo et al. 2020). Several investigations had suggested that SA elevated the maize plant's resistance to osmosis (Darko et al. 2019), drought (Elhakem 2019), salinity (El-Katony et al. 2019), and heavy metals (Sharma et al. 2020).

Maize (*Zea mays* L.) is one of the essential cereal crops. Moreover, it is a fundamentally valuable global food crop (Riffat and Ahmad 2018). The plant is considered as a moderately salt-sensitive C4 species, with broad genotypic variability that permits its cultivation in an extensive range of soils, as well as below variable conditions of climate (El-Katony et al. 2019). Thus, the current study was undertaken to investigate the effectiveness of SA exogenous application in relieving the injurious effects of NaCl stress on some phytohormones and osmolytes in maize.

## MATERIAL AND METHODS

**Plant growth conditions.** This investigation was implemented in Egypt (30°06'N, 31°25'E) in the summer of 2020. The maize (*Zea mays* L.) kernels were provided by the Ministry of Agriculture (Egypt).

A symmetrical group of maize kernels was sterilised by soaking in 0.01% HgCl<sub>2</sub> and then rinsed in sterile water. The kernels were divided into two sets: the kernels of the 1<sup>st</sup> were soaked in distilled H<sub>2</sub>O, and the 2<sup>nd</sup> set was soaked in salicylic acid (0.05 mmol). The kernels were thoroughly planted at the recommended seeding rate (40 kg/ha rate) through the hand-pulling drill method. The maize plants were exposed to a normal day/night conditions (maximum/minimum relative humidity and temperature were 69/62% and 34/27 °C, respectively, at midday) over the experimental period. All the maize plants were irrigated with tap water to field capacity.

**Salt treatment.** At four weeks from planting, the 1<sup>st</sup> set was subdivided into three groups: control (0 mmol), 40 mmol NaCl (40 mmol), and 80 mmol NaCl (80 mmol). The 2<sup>nd</sup> set was subdivided to three groups: control + salicylic acid (0 mmol + SA), 40 mmol NaCl + salicylic acid (40 mmol + SA), and 80 mmol NaCl + salicylic acid (80 mmol + SA). After planting and during stem elongation, phosphorus and nitrogen fertilisers were applied at two stages as calcium phosphate (200 kg/ha) and urea (150 kg/ha), respectively. During the experiment time, the unstressed plants (control) were irrigated by tap water to the field capacity, and the stressed plants were irrigated with saltwater. For the measurement of the phytohormones and osmolytes, three samples were chosen.

## Organic osmolyte determination

**Total soluble sugar accumulation.** TSS was extracted and measured in the treated and untreated maize leaves at six weeks after planting (6WAP) depending on the method described by Yoshida et al. (1976). TSS was extracted by overnight submersion of dry tissue in 10 mL of 80% (v/v) ethanol at 25 °C with periodic shaking. TSS was measured by reacting 0.1 mL alcoholic extract with freshly prepared anthrone reagent (3.0 mL) for 10 min in a boiling water bath, and the cooled samples were detected in a Spectronic 21D spectrophotometer (Kyoto, Japan) at 625 nm. Glucose series (0, 20, 40, 60, 80, and 100 µmol) were applied for the standard curve.

**Total soluble protein level.** TSP was extracted and estimated in the stressed and unstressed maize leaves at 6WAP by the method described by Bradford (1976). Saline phosphate buffer was prepared by mixing 10 mmol Na<sub>2</sub>HPO<sub>4</sub>, 2.7 mmol KCl, 2 mmol KH<sub>2</sub>PO<sub>4</sub>, and 1.37 mmol NaCl. The pH 7.2 was maintained using 62.5 mmol Tris HCl. TSP determination was carried

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out by extracting the 0.5 g fresh weight (FW) of leaves in saline phosphate buffer. After that, the solution was centrifuged, and the supernatant was obtained. The dye stock was dissolved to an equal volume of supernatant, swirled, and then kept for 30 min in an incubator. The absorbance was noted using a spectrophotometer (UV-1100, Kyoto, Japan) at 595 nm. A standards series (10 to 50 µg mL<sup>-1</sup> of bovine serum albumin) was prepared for the standard curve.

**Proline content.** Proline was measured in the treated, and untreated maize leaves at 6WAP by the ninhydrin-based colorimetric method as described by Lee et al. (2018). The fresh leaves (0.5 g) were ground, and 20 µL of 1% (w/v) sulfosalicylic acid was added per mg FW tissue. After centrifuging at 15 000 rpm for 5 min at 4 °C, the supernatant was separated and blended with acidic ninhydrin (1.25% [w/v] ninhydrin in 80% [v/v] acetic acid) in 1:2 ratio and incubated at 95 °C for 30 min. The absorbance was measured using a spectrophotometer at 510 nm, and the proline concentration was calculated using the proline standard curve.

**Inorganic osmolyte determination.** Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup> ions were evaluated in the maize plants leaves at 6WAP, according to Wolf (1982). A total of 0.5 g of the dry leaf was incubated in 5 mL H<sub>2</sub>SO<sub>4</sub> all-night and heated in the digestion block at 350 °C for 30 min. After that, the mixture was cooled; H<sub>2</sub>O<sub>2</sub>, 1 mL was added and heated again for 20 min. These procedures were reduplicated until the pure solution was achieved and filtered, and the volume was made up to 50 mL by distilled H<sub>2</sub>O. Then, the extract was used for Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup> determination using a flame photometer (Jenway PFP-7, Newbury, UK). A standard series (10, 20 to 100 ppm of Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup>) was prepared for the standard curve.

**Phytohormone determination.** Phytohormones were evaluated for the stressed and unstressed maize plants at 6WAP, according to Müller and Munné-Bosch (2011). The fresh leaves (0.2 g) were extracted in a solution containing acetic acid (1%), isopropanol (79%), and methanol (20%). The samples were kept on ice for 30 min, sonicated for 10 min, and centrifuged at 4 °C for 10 min at 13 000 rpm. Then, the supernatant was exposed to a new cycle of extraction and, after that, injected into an LC-MS/MS system (Infinity Series, Agilent 1200, California, USA) attached to a mass spectrometer of triple quadrupole (Agilent Technologies, Model 6430). The Agilent Eclipse Plus chromatographic column (RRHD, 2.1 × 50 mm, 1.8 µm) was used at 0.3 mL/min flow rate and con-

nected to a triple quadrupole mass spectrometer. The mass spectrometer was worked through the alternating negative and positive modes, according to the retention time for every hormone, and the samples scanned in a multiple reaction mode to evaluate the ABA, IAA, and GA3 hormones. The produced mass spectra were processed using Mass Hunter programming (Agilent Technologies, California, USA) to acquire the extracted chromatograms of each transition and to get the zone values that showed each hormone abundance. Standard curves were applied to turn the zone values to µg hormone/g FW.

**Statistical analysis.** The data analysis was performed using the Statistical Package for Social Sciences (SPSS, version 16.0) software (SPSS Inc., Illinois, USA) (Daniel et al. 1995). A two-way analysis of variance was performed to obtain the significant variations in the responses of measured parameters among the various treatments with a significance level of less than 5% ( $P < 0.05$ ).

## RESULTS

**Salicylic acid pre-soaking enhanced the accumulation of organic solutes under NaCl stress.** The effects of NaCl and SA exogenous application on organic solutes in the maize leaves were shown in Figure 1. Salt stress enhanced the accumulation of TSS in the flag leaves of treated maize plants (Figure 1A). This enhancement was more remarkable ( $P < 0.05$ ) with the 80 mmol (35.6%) than 40 mmol treatment (24.3%). SA treatment caused additional accumulation in TSS levels by 39.4% and 59.3% under 40 and 80 mmol + SA treatments, respectively, as compared to their respective controls.

In relation to the untreated plants, salinity stress-induced TSP production (Figure 1B) in maize leaves. The TSP level was remarkably increased ( $P < 0.05$ ) by 40.6% and 66.2% under 40 and 80 mmol NaCl stress, respectively. SA exogenous application caused additional accumulation in TSP content with the by 70.3% and 77.5% with 40 and 80 mmol + SA treatments, respectively, over the untreated plants. The maximum and minimum values of TSP ( $7.4 \pm 1.29$  and  $13.1 \pm 2.06$ ) were observed with 0 and 80 mmol + SA treatments, respectively.

Similarly, proline content in the maize leaves was significantly improved with the increasing of NaCl concentration as compared to unstressed plants (Figure 1C). The improvement was more noticeable ( $P < 0.05$ ) with the 80 mmol (89.2%) than 40 mmol

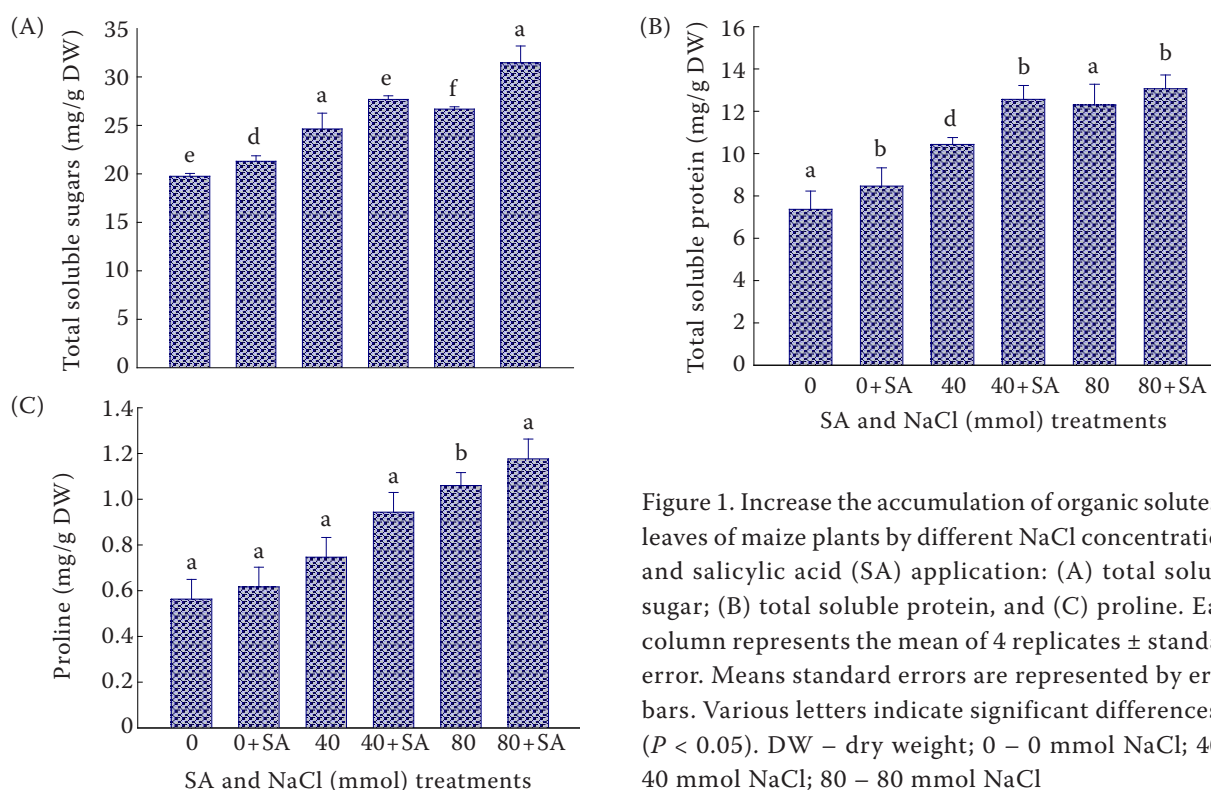


Figure 1. Increase the accumulation of organic solutes in leaves of maize plants by different NaCl concentrations and salicylic acid (SA) application: (A) total soluble sugar; (B) total soluble protein, and (C) proline. Each column represents the mean of 4 replicates  $\pm$  standard error. Means standard errors are represented by error bars. Various letters indicate significant differences at ( $P < 0.05$ ). DW – dry weight; 0 – 0 mmol NaCl; 40 – 40 mmol NaCl; 80 – 80 mmol NaCl

(33.9%). Additionally, supplementation of SA induced a further accumulation in the proline content by 67.8% and 106.7% with 40 and 80 mmol + SA treatments, respectively, relative to control plants.

**Salicylic acid application regulated the uptake of inorganic solutes under salinity.** The influences of salinity stress and SA treatment on the inorganic solute's accumulation were represented in Figure 2. In relation to the control values, NaCl application resulted in a noticeable increase ( $P < 0.05$ ) in  $\text{Na}^+$  level and  $\text{Na}^+/\text{K}^+$  ratio. These accumulations were more noticeable with the 80 mmol (202.4% and 398.8%) than 40 mmol level (98.3% and 193.5%) in  $\text{Na}^+$  level and  $\text{Na}^+/\text{K}^+$  ratio, respectively (Figure 2A, C). Moreover, the plants treated with NaCl plus SA showed less increase in  $\text{Na}^+$  level and  $\text{Na}^+/\text{K}^+$  ratio under 80 mmol + SA (44.4% and 87.2%) than 40 mmol + SA (24.4% and 44.6%), respectively. Otherwise, the highest ( $1.16 \pm 0.75$  and  $2.35 \pm 1.07$ ) and lowest ( $0.36 \pm 0.12$  and  $0.32 \pm 0.11$ )  $\text{Na}^+$  and  $\text{Na}^+/\text{K}^+$  values were observed with 80 mmol and 0 mmol + SA applications, respectively.

Furthermore, the addition of NaCl decreased the  $\text{K}^+$ , and  $\text{Ca}^{2+}$  levels in stressed maize plants leaves. Compared to their respective controls, the reduction was more significant ( $P < 0.05$ ) with the 80 mmol (48.6% and 58.9%) than 40 mmol (34.5% and 39.6%) in  $\text{K}^+$  and

$\text{Ca}^{2+}$  levels, respectively (Figure 2B, D). On the other hand, SA supplementation to stressed plants repaired the  $\text{K}^+$  and  $\text{Ca}^{2+}$  levels but remained below the control values. Additionally, the  $\text{K}^+$  and  $\text{Ca}^{2+}$  maximum ( $1.11 \pm 0.36$  and  $0.63 \pm 0.08$ ) and minimum values ( $0.49 \pm 0.21$  and  $0.23 \pm 0.05$ ) were detected with 0 mmol + SA and 80 mmol treatments, respectively.

**Salicylic acid application enhanced phytohormones under salt stress.** The impact of salt stress and SA application on the phytohormone level in maize leaves was shown in Figure 3. Salinity stress reduced the IAA content by 34.2% and 48.8% with 40 and 80 mmol treatments, respectively (Figure 3A). Plants treated with NaCl in combination with SA revealed less decrease of 17.8% and 27.7% in IAA level under 40 and 80 mmol + SA treatments, respectively, in relation to control plants.

Similarly, there were significantly lower levels of GA3 in NaCl-treated maize plants as relative to the control (Figure 3B). GA3 level was reduced by 38.5% and 59.8% under 40 and 80 mmol NaCl stress, respectively. However, the SA application maintains the GA3 level at 16.2% and 27.9% with 40 and 80 mmol + SA treatments, respectively, in relation to control plants.

In the current study, the ABA level was enhanced under salt stress to 156.7% with 40 mmol and 340.5% with



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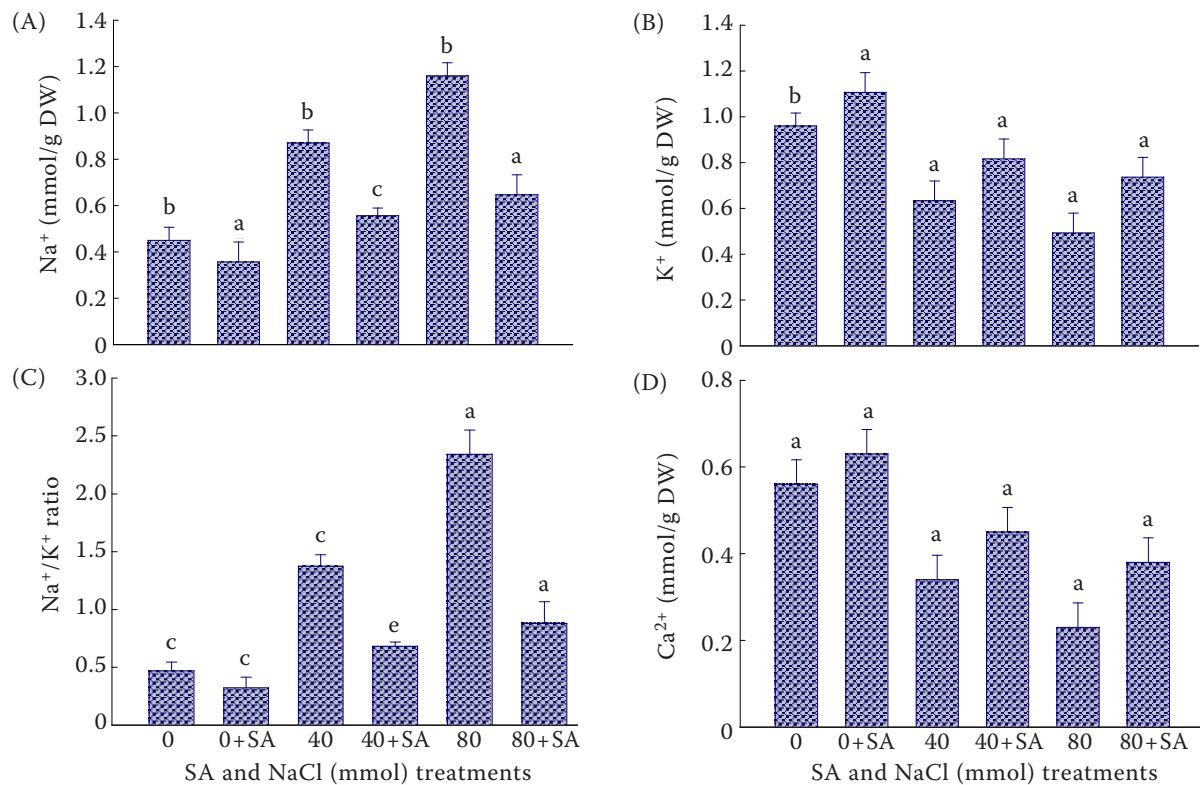


Figure 2. Salicylic acid (SA) application regulates the ion uptake of maize plants treated with different NaCl concentrations: (A)  $\text{Na}^+$ ; (B)  $\text{Na}^+/\text{K}^+$  ratio, (C)  $\text{K}^+$  and (D)  $\text{Ca}^{2+}$ . Each column represents the mean of 4 replicates  $\pm$  standard error. Mean standard errors are represented by error bars. Various letters indicate significant differences ( $P < 0.05$ ). DW – dry weight; 0 – 0 mmol NaCl; 40 – 40 mmol NaCl; 80 – 80 mmol NaCl

80 mmol stress over that in control plants (Figure 3C). On the other hand, SA treatment maintained the ABA level at 51.3% and 86.4% with 40 and 80 mmol + SA applications, respectively.

## DISCUSSION

During the salt stress in plants, several physiological and biochemical changes occur inside the cells and tissues. When the concentration of the solutes in the root region increases, the soil water potential reduces, leading to prevent plant in maintaining turgor by improving the accumulation of osmolytes in the cytosol, which have a major role in both osmotic adjustment and osmoprotection under various abiotic stresses (Elhakem 2019, Sharma et al. 2020).

TSS are significant components of the signaling passage, which interconnect the stress pathways that form a complex network and manage the plants' metabolic responses (Sharma et al. 2019). The current investigation showed that salinity stress enhanced the TSS content in maize plants. This result has been reported in many investigations with maize plants

(Riffat and Ahmad 2018, El-Katony et al. 2019). During the conditions of high drought or salinity, various sugar contents usually elevate even if there is a decrease in the assimilation of  $\text{CO}_2$  (Sharma et al. 2019). TSS has a considerable role in ROS scavenging, osmotic homeostasis maintenance, membrane stabilisation, and various other important functions in plants under abiotic stresses (Elhakem 2019, Sharma et al. 2020). Furthermore, Sharma et al. (2019) stated that the noticeable improvement in carbohydrate levels could as well be due to the photosynthetic mechanism of activation in stressed plants. SA triggers sugar accumulation in plants under abiotic stress conditions (Sharma et al. 2020). The SA application influence on leaf TSS of maize plants was significant in both treated and untreated plants. This finding was reported in other studies, i.e., in tomato (Ahmadi et al. 2018) and maize (El-Katony et al. 2019). In addition, SA treatment could result in metabolic pathway activation, which exhausts soluble sugars that cause the growth replenishment of stressed plants (El-Katony et al. 2019). In this regard, Sharma et al. (2019) reported that the sig-

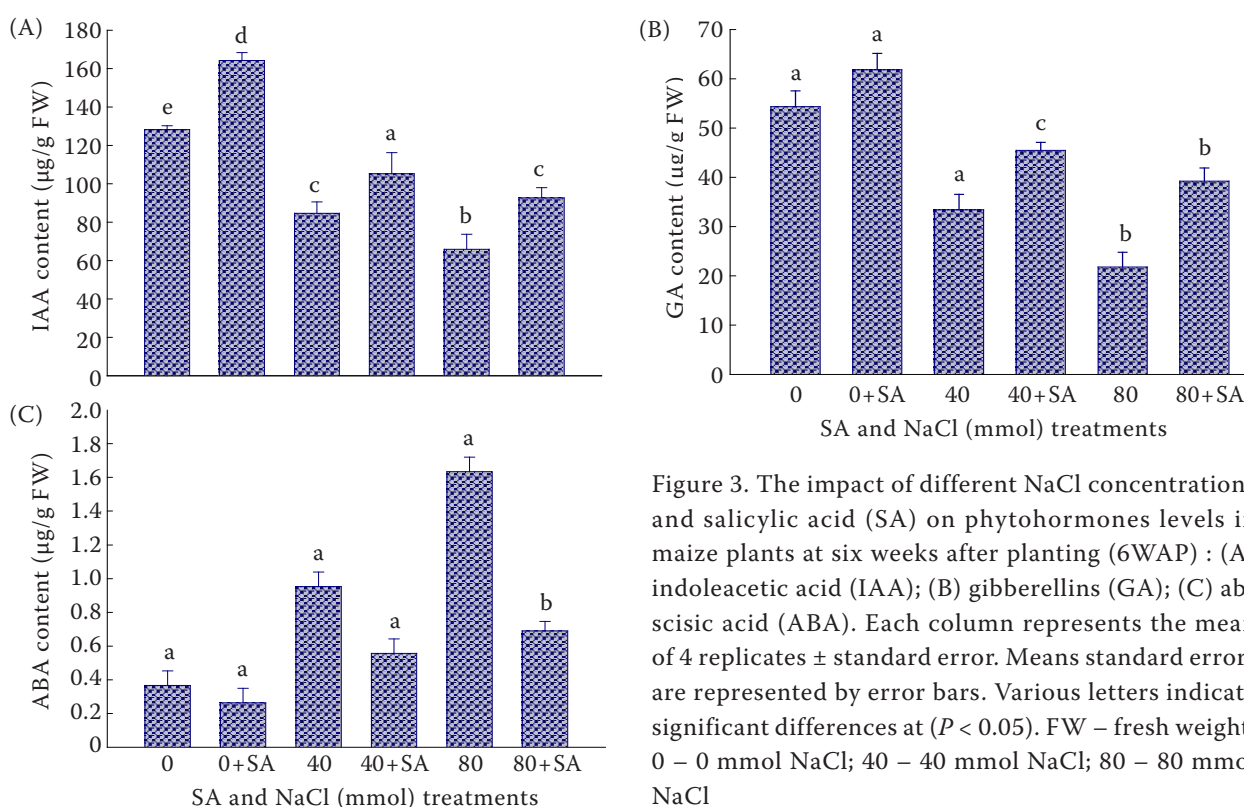


Figure 3. The impact of different NaCl concentrations and salicylic acid (SA) on phytohormones levels in maize plants at six weeks after planting (6WAP) : (A) indoleacetic acid (IAA); (B) gibberellins (GA); (C) abscisic acid (ABA). Each column represents the mean of 4 replicates  $\pm$  standard error. Means standard errors are represented by error bars. Various letters indicate significant differences at ( $P < 0.05$ ). FW – fresh weight; 0 – 0 mmol NaCl; 40 – 40 mmol NaCl; 80 – 80 mmol NaCl

nificant improvement in carbohydrate levels could as well be due to the recovery of the photosynthetic apparatus in stressed plants.

Various abiotic stresses enhance the soluble protein accumulation in plants that may supply nitrogen storage for utilisation and support in osmotic adjustment (Ahmed et al. 2019). The soluble proteins help in raising the level of nitrogen that enhances growth and development in plants under stress conditions (Riffat and Ahmad 2018). The current study showed an increase in TSP content with increasing NaCl level. Increased protein content was also reported in many plants, i.e., lettuce and maize (Ahmed et al. 2019, Jain and Vaishnav 2019). In addition, Cai and Gao (2020) concluded that exceptional expression of stress proteins is a major strategy in maintaining integrity, original configuration, and topology of cellular membrane components to preserve their normal functioning under salt stress. In this respect, Jain and Vaishnav (2019) reported a considerable negative relationship between total protein level, cell, and membrane stability.

In addition, pre-soaking in SA further enhanced the accumulation of TSP in maize leaves. This result was in agreement with preceding studies in various plants, i.e., *Zea mays* L. and *Limonium bicolor* Mill.

(El-Katony et al. 2019, Liu et al. 2019). In this regard, Nahrjoo and Sedaghatpour (2018) found that SA foliar application caused remarkably enhanced protein, antioxidant enzymes, catalase activity, and proline content in rosemary plants under salinity stress. Another report by Liu et al. (2016) found that SA treatment improved the contents of soluble protein, proline, soluble sugars, and antioxidant enzyme activities in *Nitraria tangutorum* L. exposed to saline conditions.

Proline, an amino acid, is one of the most distinguished organic osmolytes which accumulates in plant tissues exposed to various abiotic stresses (Sharma et al. 2020, Sohag et al. 2020). The obtained result indicated that salt stress-induced a noticeable enhancement in the proline level in maize plants. The elevation of proline observed in salt-treated plants indicates an adaptive strategy for the regulation of the transcript levels of salinity, stress proteins, subcellular structure stability, osmotic adjustment, and cellular adaptation to salinity stress, which are investigated by various studies (Riffat and Ahmed 2018, Cai and Gao 2020). These results indicated that the improvement in proline content might be from the metabolic responses involved in the translocation pathway, which help in recognition of the different abiotic stresses to induce the physiological responses (Elhakem 2019,

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Sohag et al. 2020). Furthermore, Sharma et al. (2019) showed that the function of proline as a signaling compound for mitochondrial function regulation influences cell proliferation through the activation of specific genes, which are necessary for recovery from stress, and the enhancement in proline level helps in maintaining the integrity of membranes through decreasing oxidation of lipids by ROS and protecting the cellular redox potential.

SA was found to improve the proline synthesis under NaCl stress (Ahmadi et al. 2018). The obtained results demonstrated that pre-soaking treatment of SA induced more accumulation in the proline levels in maize leaves. This finding was in agreement with other studies (Ahmad et al. 2018, Sharma et al. 2019). The enhancement in proline metabolism is correlated with the stress tolerance mechanism in plants under salinity (Ahmadi et al. 2018). Furthermore, Khan et al. (2013) investigated that 0.5 mmol SA application elevated the *Triticum aestivum* L. plant's tolerance to heat stress by improving the proline level in response to SA application increased activity of glutamyl kinase and reduced activity of proline oxidase. In this regard, Ahmad et al. (2018) elucidated that the SA treatment enhanced the antioxidant enzyme activity as well as the proline levels in salt-stressed *Vicia faba* L. Furthermore, it was argued that enhanced proline accumulation by SA treatment promoted the assimilation of nitrogen and improved photosynthesis (Sharma et al. 2019).

Plants accumulate higher amounts of  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{Ca}^{2+}$  ions under salt stress causing severe ion toxicity (Chakdar et al. 2019). Besides, toxicity to a particular ion differs among various plant species (Cai and Gao 2020). Ion toxicity could alter the cytoplasmic metabolic processes and might cause injurious impacts on photosynthesis (Chakdar et al. 2019). NaCl stress led to an increase in  $\text{Na}^+$  and  $\text{Na}^+/\text{K}^+$  ratio but a decrease in  $\text{Ca}^{2+}$  and  $\text{K}^+$  uptake in the current study. Comparable results were confirmed with El-Katony et al. (2019) in *Zea mays* L., Cai and Gao (2020) in *Chenopodium quinoa* (Willd.). According to Ahanger and Agarwal (2017),  $\text{Na}^+$  and  $\text{K}^+$  share identical physicochemical composition due to which competition occurs between both ions for their uptake. In addition, Ahmad et al. (2018) showed that  $\text{Na}^+$  uptake increased with increasing NaCl level, which ultimately induced a reduction in  $\text{Ca}^{2+}$  and  $\text{K}^+$  uptake. Furthermore, mineral uptake is one of the main adaptations that confers tolerance of stress (Ahanger and Agarwal 2017).

SA can significantly regulate the uptake and metabolism of essential mineral nutrients to enhance growth and development in environmentally stressed plants (El-Katony et al. 2019). Besides, the SA protective role in membrane safety, as well as ion regulation and nutrient uptake, has also been investigated (Jini and Joseph 2017). SA application decreased the  $\text{Na}^+$  level and  $\text{Na}^+/\text{K}^+$  ratio but enhanced  $\text{Ca}^{2+}$  and  $\text{K}^+$  uptake in the NaCl-stressed maize plant. Comparable results have been reported in *Carthamus tinctorius* L. (Shaki et al. 2019) and *Zea mays* L. (El-Katony et al. 2019). In this respect, Ahmad et al. (2018) proved that the improvement in the mineral nutrients uptake could be due to the SA-stimulated activity of  $\text{H}^+$ -ATPase enzyme and the beneficial correlation between SA and the uptake of mineral nutrients advocated the mitigating role of SA against salt stress. In another investigation, SA treatment to low and high saline soils enhanced the growth, antioxidant enzyme activity, nutrient value, yield, and salinity tolerance of rice plants (Jini and Joseph 2017). Based on these results, it is suggested that SA protective effects on NaCl stress-induced oxidative injury in maize leaves could be associated with its regulatory roles in the organic and inorganic solutes levels.

Phytohormones are very major constituents imparting tolerance to the plants toward abiotic stresses (Sharma et al. 2019). In this experiment, salinity stress significantly reduced IAA content (Figure 3A). Supporting our results, Shaki et al. (2019) stated that the IAA was decreased in safflower under 100–200 mmol NaCl treatment. Another report by Ahmad et al. (2018) demonstrated a reduction in IAA level by 36.60% in salt-stressed *Vicia faba* L. NaCl stress can affect the homeostasis of IAA due to the modifications in the metabolism and distribution of IAA (Kumar et al. 2015). In addition, the external application of SA improved the IAA content in maize plants. The same results were investigated by Ahmad et al. (2018). IAA is known to enhance the extensibility of cell wall, and so increased IAA levels under SA application may increase the extensibility of leaf cells, which is implicated in growth maintenance under conditions of transiently decreased hydration from salinity stress (Shaki et al. 2019).

GAs have a significant role in response to abiotic stresses, i.e., salt, cold, drought, and osmotic, which has become growingly evident, and reduction of GA levels and signaling appears to contribute to decreased plant growth under abiotic stresses (Kumar et al. 2015). Our results distinctly confirmed that

GA3 levels in maize plants were influenced by NaCl stress (Figure 3B). These results agreed with those obtained by Shaki et al. (2019) with safflower plants. In another study, Iqbal and Ashraf (2013) reported that GA3 application caused an increase in grain yield of two spring wheat cultivars, and this increase in yield was referred to GA3, resulted in modification of ion uptake and partitioning (in roots and shoots) and hormone stability under salt condition. Furthermore, the SA application increased the GA3 level in maize plants. In this respect, Shaki et al. (2019) demonstrated that SA may have a major role in some of the physiological processes contributing to GA biosynthesis and action.

ABA is known as a major phytohormone conferring tolerance to abiotic stress (Koo et al. 2020). In this study, salt stress-induced a noticeable increase in ABA content in maize plants (Figure 3C). In this matter, Chen et al. (2020) reported that alternations in ABA levels in individual roots and guard cells were detected after exposure to different amounts of salinity and humidity. Besides, ABA may activate the gene expression regulating the wax synthesis and stomatal closure, assisting limitation of water loss from saline condition (Zhao et al. 2017). On the other hand, SA treatment reduced ABA content to appreciable levels. A similar finding was obtained by Ahmad et al. (2018) with *Vicia faba* L. In this context, Koo et al. (2020) declared that ABA antagonistically manages SA-mediated defense signaling, and under certain abiotic stress conditions, i.e., salt and freezing stresses, SA and ABA together appeared to be able to positively regulate the response of stress tolerance. In another study, Liu et al. (2019) found that during germination of salt-stressed *Limonium bicolor* Mill., the seeds treated with SA had high levels of  $\alpha$ -amylase activity and GA, but low ABA, and they concluded that the treatment of SA upregulated the expression of the key genes implicated in GA biosynthesis while downregulating those implicated in ABA biosynthesis, so triggering an appropriate hormonal balance between ABA and GA that promote seed germination under NaCl stress. Based on these findings, it is suggested that SA being an important signaling phytohormone cross-talks with other hormones, including IAA, GA3, and ABA, and so can influence their synthesis.

Our results suggest that salinity stress causes alterations in the phytohormones and osmolytes in maize plants. Various natural osmoprotectants get accumulated in the maize plant to overcome the adverse

influences of saline conditions. Among these organic osmolytes, TSS, TSP, and proline have the upmost importance. SA application enhanced more accumulation in the organic osmotic adjustment solutes and thus induced salt tolerance in maize plants. Furthermore, salinity increased the  $\text{Na}^+$  level and  $\text{Na}^+/\text{K}^+$  ratio but lowered the beneficial inorganic osmolytes ( $\text{K}^+$  and  $\text{Ca}^{2+}$ ) in the maize plants. However, SA exogenous application decreased the  $\text{Na}^+$  and  $\text{Na}^+/\text{K}^+$  ratio to appreciable levels and improved the  $\text{K}^+$  and  $\text{Ca}^{2+}$  uptake, which were injured by salt stress conditions. Moreover, salinity reduced some promoter phytohormones (IAA and GA3) but increased the ABA level in maize plants. SA treatment mitigated the impact of salinity on the phytohormones by enhanced IAA and GA3 as well as reduced ABA levels. Based on the above results, it is recommended that SA at 0.05 mmol is highly influential in regulating phytohormones and osmolytes (organic and inorganic) for enhancing salt tolerance potential in maize plants.

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