ROLE OF CYTOKININS TO ALLEVIATE THE SALINITY STRESS THROUGH THE PRODUCTION OF SECONDARY METABOLITES

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ABSTRACT

Cytokinins are regarded as plant growth hormone which also alleviate abiotic stresses in plants. In this regard, we aerially sprayed the 8 days old seedlings of *Zea mays* prior stressed with NaCl, the growth of the seedlings was improved. The growth parameters included root length, shoot length, fresh weight and leaf area. We observed higher root length, shoot length, fresh weight and leaf area ratio after CK treatment compared to the seedlings stressed with NaCl only. We also assessed the biochemical profile of the seedlings in terms of the osmolytes also referred as secondary metabolites (total flavonoids, phenolic and proline). Interestingly, the CK treatment induced the higher production of Secondary metabolites in the seedlings compared to stress conditions. Thus the relative water content of the seedlings were also high after the CK treatment. Additionally, the prior stressed seedlings treated with CK also had higher level of IAA which worked synergistically with CK to produce higher enzymatic activity such as Catalase. Comparatively, the seedlings stressed with NaCl only had lower IAA level and thus the catalase activity was lowered. Thus, it was evaluated that CK treatment alleviates salinity stress through production of Secondary metabolites.

Keywords: Cytokinins, Secondary metabolites, Growth Parameters, Catalase activity

Introduction

Maize (*Zea mays*), commonly known as Corn, is a cereal grain pioneer domesticated by native peoples of southern Mexico about 10,000 years ago (Gul et al., 2020). The term maize is preferred in formal, scientific, and international usage as a common name because it refers specifically to this one grain, unlike *corn*, which has a complex variety of meanings that vary by context and geographic region (Khoury et al., 2022). Maize is widely cultivated throughout the world, and a greater weight of maize is produced each year than any other grain (Rizzo et al., 2022). In 2021, total world production of maize was 1.2 billion tones (Abbade, 2021). Maize is the most widely grown grain crop throughout the America, with 384 million metric tons grown in the United States alone in 2021 (Guzzon et al., 2021). In Pakistan maize is planted on an estimated area of 0.9 million hectare with an annual production of 1.3 million tones (Shaheen et al., 2022). Salinity is one of the important abiotic stresses that has been notably affecting the plant growth and yield (Ghosh et al., 2022). The continuous increase in salinity in arable land due to poor cultivation practices and weather trade have devastating worldwide consequences, and it was proposed that approximately 50% of arable land could be lost by mid of the 20^{th} Century (SENO et al., 2022) .To date, approximately a 125 million hectares of agricultural lands have already been severely suffering from salinity, thus it is considered a serious hazard to agriculture (Hossain et al., 2020). In Pakistan, a complete of 6.3 million hectares of land has been significantly stricken by salinity (Khan et al., 2022).

The plants have developed several tolerance mechanisms to overcome the salinity stress (Johnson and Puthur, 2021). Among those, the primary one is the homeostatic sustenance with the help of the osmotic the synthesis of osmolytes (Chiappero et al., 2021). Plants produce osmolytes, such as proline, soluble sugars, Flavonoids, Phenolics, Proline to defend the plant cells against the destructive effects of salt stress (Birhanie et al., 2022). Similarly, antioxidant molecules consisting of glutathione (Zhao et al.) reduce the reactive oxygen species produced under salinity stress (Dumanović et al., 2021).Proteins can also help in osmotic adjustment underneath salt strain (Krishnamoorthy et al., 2022).

Phytohormones are those hormones produced by plants to perform metabolic activities of it (Sivaramakrishnan and Incharoensakdi, 2020). Phytohormones play vital role in the plant reaction to salt stress by adaptation of plant growth and development (Zhao et al., 2021). There are nine plant hormones and are divided into two groups: growth promotion hormones and stress response hormones (Arnao and Hernández-Ruiz, 2021). The growth promotion hormones are composed of Auxin, Gibberellin (GA), Cytokinins (CKs), Brassinosteroids (BRs), and Strigolactones (SLs) (Ait-El-Mokhtar et al., 2022). Some of the growth promotion hormones also play an important role in stress condition, such as SLs and BRs (Zhao et al., 2021). The stress response hormones contain Abscisic acid (ABA), Ethylene, Salicylic acid (SA), and Jasmonic acid (JA) (Wang et al., 2020)

The Phytohormones Cytokinins (CKs) were assigned for plant improvement (Ali et al., 2021). Over saline condition can be easily broke with the help of CKs (Trifunović-Momčilov and Motyka, 2021). Recently, functions of CKs in plant stress defense have been gradually characterized (Yu et al., 2021). Under salt stress, not only CKs homeostasis but also its signal transduction pathway is disturbed in a plant species dependent manner (Yu et al., 2020). In turn, through manipulating exogenous CKs application or endogenous CKs metabolism or signaling, plant function can also be diverse across species (Jogawat et al., 2021). In this review, we scientifically summarized this mutual regulation between CKs and salt stress (Yu et al., 2021). Considering the senescence-delaying effect of CKs, its roles in alleviating salt-induced senescence and maintaining crop yields are specifically highlighted (Trifunović-Momčilov et al., 2021). We also discussed here how CKs crosstalk with other phytohormones, including ABA and ethylene, to mediate salt response (Yu et al., 2021).

The prevailing examine is designed to access phenotypic responses of different water dropwort cultivars under salt strain and to select salt-tolerant and sensitive cultivars primarily based on phenotype among them (Niemann, 2019). Secondly, it aims to have a look at a few physiological parameters which includes enzymatic and the non-enzymatic antioxidant protection system, chlorophyll content material, and ionic homeostasis regarding the salt tolerance in selected tolerant and touchy cultivars of water dropwort (Ashraf and Munns, 2022). For these objectives, numerous parameters, which includes plant growth, fresh and biomass, relative water content material (RWC), chlorophyll content, production charge of ROS, osmolytes and antioxidant molecules concentration, and activities of antioxidant enzymes, had been studied (Ali et al., 2022).

Method and Materials

Plant materials and treatment conditions

Z. mays seeds were obtained from Agriculture Department, Khyber Pakhtunkhwa. The seed sterilization and the germination procedure was adopted accordingly the method described earlier(Yousaf et al., 2021). The treatment conditions adopted were as

1. Control seedling irrigated with sterilized distilled water in amount of 2 mL.
2. Seedlings sprayed with 150 mM concentration of NaCl in amount of 2 mL.
3. Seedlings sprayed with (10 µL) concentration of Cytokinins in amount of 2 mL.
4. Seedlings sprayed with 150 mM concentration of NaCl and 10 µL concentration of Cytokinins in amount of 2 mL each.

Sample preparation

The sample for extraction was made as described earlier (Yousaf et al., 2021). The extract was used to determine IAA content, Total Phenolics Content (TPC), Total Flavonoid Content (TPC) and Proline. For determination of IAA through HPLC, the method was explored already described (Yousaf et al., 2021). TPC, TFC and proline were determined using spectrophotometer Aluminum chloride method was used for the estimation of Total Flavonoid Content (TFC) in plant samples. Briefly, 500µL of the supernatant or plant extract was added with 100µL of 10% aluminum chloride, 100µL of 10% potassium acetate and 4.8 mL of 80% methanol. The mixture was shaken vigorously and incubated for 30 min. Optical density was noted at 415 nm after incubation. The Total Phenolic Contents (TPC) in the culture supernatant and plant samples were determined by Folin-Ciocalteu method. Briefly, to the 200µL of the sample extract, 800µL of Folin-Ciocalteu reagent, and 2 mL of 7.5% sodium carbonate was added. The total content was diluted to 7 volumes with distilled water and kept for incubation for 2 h.

Proline content and enzymatic activities such as Oxidase and Catalase activities were determined as described (Yousaf et al., 2021).

Chlorophyll Content

The full chlorophyll content material was measured with SPAD-502 Plus (Konica Minolta, Japan).

Determination of Relative Water Content (RWC)

Relative water content was analyzed using the method described as earlier (Yousaf et al., 2022).

Results

Growth Kinetics

After 08 days of treatment, growth kinetics comprising of Root Length, Shoot Length, Fresh Weight and Leaf Diameter were observed *Z mays* seedlings. The seedlings stressed with 150 mM solution of NaCl revealed reduction in shoot length which became increased under CK treatment (10 uL). Interestingly, the CK treatment to stressed seedlings with NaCl also enhanced the shoot length. Similarly, the root length was also decreased under salinity stress while the CK treatment enhanced it. As expected, the root length was also increased when CK and NaCl both were applied to the seedlings (Figure 1A-1B).

In order to analyze the efficiency of photosynthesis, we observed the leaf area ratio which indicated the size of leaf lamina divided by total biomass of the seedlings. Interestingly, the CK treatment and CK-NaCl treatments induced higher growth of the leaf. However, the seedlings stressed with NaCl alone produced lower leaf area ratio. Resultantly, the fresh weight of CK phenotype and CK-NaCl phenotypes were high compared to seedlings stressed with NaCl (Figure 1C-1D).

Secondary Metabolite production

Secondary metabolites comprised on the production of IAA, TFC, TPC and Proline were measured in the seedlings stressed with NaCl or CK or CK and NaCl. TPC was higher when CK was applied to seedlings while the same was lower under NaCl treatment. CKs with the combination of NaCl also increased the level of TPC as compared to NaCl treated seedlings (Figure 2A).

The level of TFC and IAA was higher when CK was applied to seedlings while the NaCl reduced the same compared to control. CKs with the combination of NaCl also increased the level of TFC compared to control. Similarly, Proline level was remained high in CK seedlings while low in NaCl treated seedlings. Interestingly, the CKs with the combination of NaCl also enhanced the level of Proline content as compared to control. The IAA level was also high in CK and CK-NaCl phenotype while lowered in NaCl stressed seedlings (Figure 2B-2D).

Relative water Content

It was observed that CK treated seedlings had higher the Relative Water Content as compared to NaCl treated seedlings and Control. Additionally, the CK with NaCl also show increased the value of Relative Water Content compared to control (Figure 2E).

Chlorophyll content

Results revealed that seedlings treated with CK were increased the Chlorophyll Content as compared to NaCl treated seedlings and control. Furthermore the CK with NaCl in combination also increased the level of Chlorophyll Content as compared to NaCl treated seedlings and control (Figure 2F).

Enzymatic kinetics

Enzymatic activities included CAT activity and oxidase were determined. CK treated seedlings had higher CAT activity which was lowered in NaCl stressed seedlings. Furthermore, the CK-(Robinson et al., 1983)be lowered in CK and CK-NaCl treatments in the seedlings compared to control while it remained high in NaCl treated seedlings (Figure 3A-3B).

Discussion

Salinity is one of the important abiotic stresses that has been notably affecting the plant growth and yield (Majeed and Muhammad, 2019). Salt stress adversely impacts plants by limiting seed germination, growth, and development, flowering and fruiting (Kalleli et al., 2022). In this study, the 8 days old seedlings of maize were subjected to salinity stress by sprayed aerially with 150 mM of NaCl. Due to effect of salinity stress at concentration 150 mM, the shoot length of the maize seedlings was reduced. It was noted that the shoot length was lowered compared to control. Salinity stress significantly decreases the growth of the plant by inducing higher osmotic stress through ionic and nutrient imbalance (Shahid et al., 2020). These imbalances adversely manipulate the different biochemical and physiological activities responsible for optimal plant growth and development (Shahid et al., 2020). Similarly the Fresh weight of plants is also affected by the salt stress (Kumar et al., 2021). The fresh weight of plants become lower due to treatment of NaCl. Salinity stress inhibited the growth potential and decrease the chlorophyll content of plants, by increasing Na+ and Cl- ions which reduced the normal functioning to enhance the fresh weight of the plants, the fresh weight of the maize seedlings was also lowered under salinity stress (Ali et al., 2021). Growth parameter like root length is also becoming imbalance due to salt stress (Abbasi et al., 2016). Salinity adversely affects plant growth when salts accumulate in the root zone. High levels of salt in root affect seed germination and plant growth by osmotic stress, ion toxity and ion imbalance (ionic stress) (Safdar et al., 2019). Therefore, the root length was also reduced in the seedlings of maize under imposed salinity stress at concentration 150 mM

Additionally, the salt stress disturb the normal functioning of leaf by reducing the leaf diameter and reduction of chlorophyll cells (Delfine et al., 1998). Salt stress was badly affect photosynthesis, therefore leading to plant growth degradation, decreases leaf growth, reduced root growth, and reductions of stomatal conductance (Mimouni et al., 2016). Chlorophyll is a photosynthetic pigment that gives plants their green color, and it helps plants create their own food through photosynthesis. The salt stress reduced the chlorophyll content by increasing the concentration of salts ions in plants. Due to salt stress the closure of the stomata takes place, which lead to limiting photosynthesis mechanism (Chaves et al., 2009). NaCl increase osmotic stress which effects adversely the activities several stomatal enzymes involved in carbon dioxide (CO_2) reduction (Hnilickova et al., 2021) .In the current studies, the chlorophyll content was reduced in the seedling of maize under imposed salinity stress. Relative water content (RWC) adversely affect by the salt stress. Relative water content frequently ($P<0.05$) reduced with the increasing salt stress (Meguekam et al., 2021) . Salt stress induced a decrease in the relative water content of the leaves, which show a loss of turgor that causes limiting of water availability (Cocozza et al., 2013).

Under stress conditions such as salt stress, the plants produce secondary metabolites which increase the osmotic potential of the cell (Sunita et al., 2020). The secondary metabolites includes proline, flavonoids, and Phenolics. Proline, is an amino acid, which plays a vital role in plants growth. It protects the plants from several stresses and also helps plants to recover from stress applied to it (Hayat et al., 2012). Similarly, flavonoid and Phenolics improve tolerance of NaCl stress through the scavenging of free radicals, a process that decreases oxidative stress (Gill and Tuteja, 2010). In this regard, the seedlings under imposed salinity stress had lower production of proline, flavonoids and Phenolics. IAA induced the osmotic protection in salt stressed plants which was also supported by decreased proline content and enhanced soluble sugar, soluble protein, and total free amino acid contents in the roots, stem, and seeds (Abdel Latef et al., 2021). The Phytohormones Cytokinins (CKs) were assigned for plant improvement. Over saline condition can be easily broke with the help of CKs. Interestingly, the CK treatment alleviated the salt stress in the seedlings by producing higher growth. To produce optimal growth in the seedlings of maize, the CK enhanced the production of secondary metabolites such as IAA, TFC, TPC and Proline. Which in turn scavenge the ROS production at the result of salinity stress (Hatami et al., 2021). Furthermore enzymatic activity like CAT activity enhanced by the treatment of salt stress (Ding et al., 2019). When NaCl applied to the Z mays seedlings the CAT activity induced. Additionally, the oxidase activity decrease by applying the salt stress (Che et al., 2022). Therefore, in this study, it has been estimated that CK treatment is essential for the plants to alleviate abiotic stresses such as salt stress.

Conclusion

Cytokinins (Buckler and Stevens) are the plant growth inducing hormone which have a role in the alleviating abiotic stresses. In this regard, we aerially sprayed the 8 days old maize seedlings with CK or 150 mM salt stress or both. Resultantly, the growth of the seedlings were found to be lowered as the shoot length, root length, fresh weight and leaf area were reduced. However, in combination of CK and NaCl, the growth parameters of the seedlings were high. This increase in growth parameters in the seedlings was due to the higher production of secondary metabolites such as proline, flavonoids, and Phenolics content which scavenged the ROS. Therefore, it was concluded that CK was involved to alleviate the stress condition of saline in the seedlings by the production of secondary metabolites.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

HF performed the experiments, IB wrote draft manuscript. MJY and IB supervised the research work and proposed the idea. MJY improved the draft manuscript. NA and MB carried out some experimental procedures. DF performed statistical analyses.

All authors contributed to the article and approved the submitted version.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

Figures

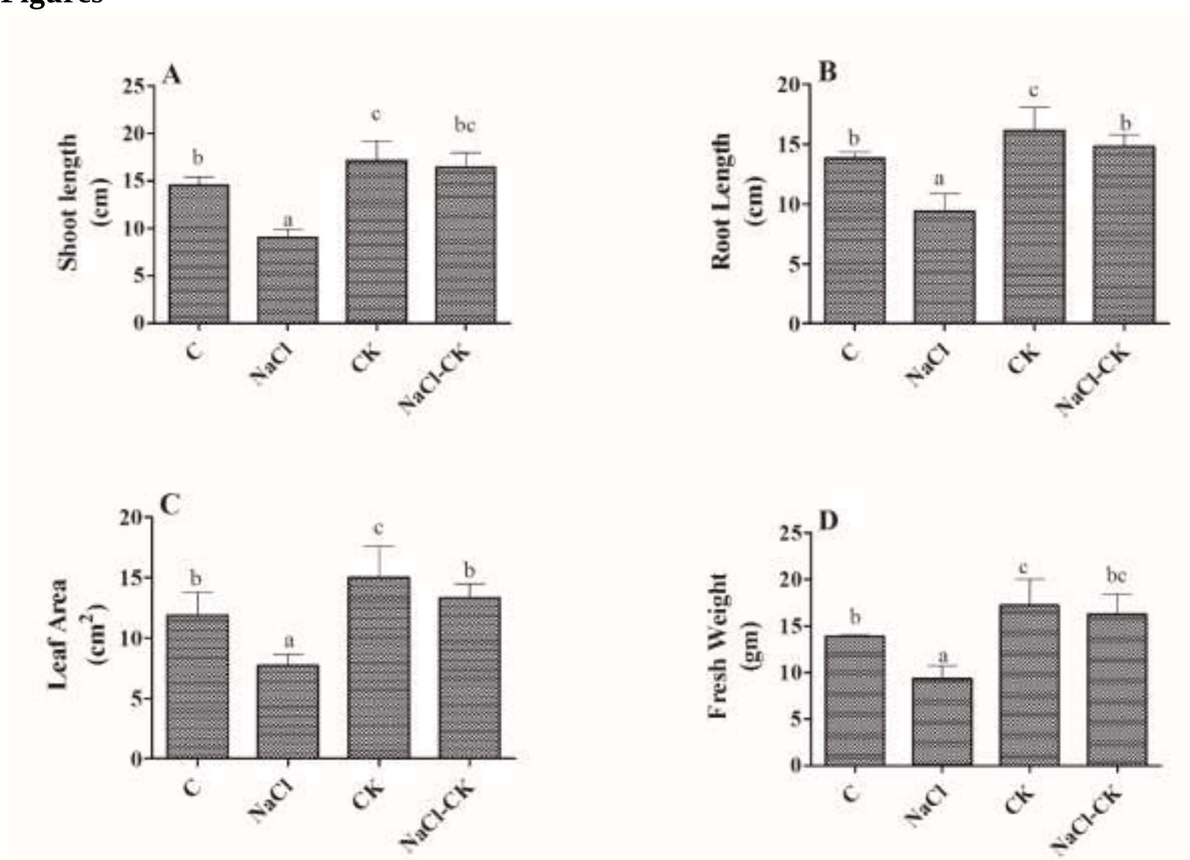
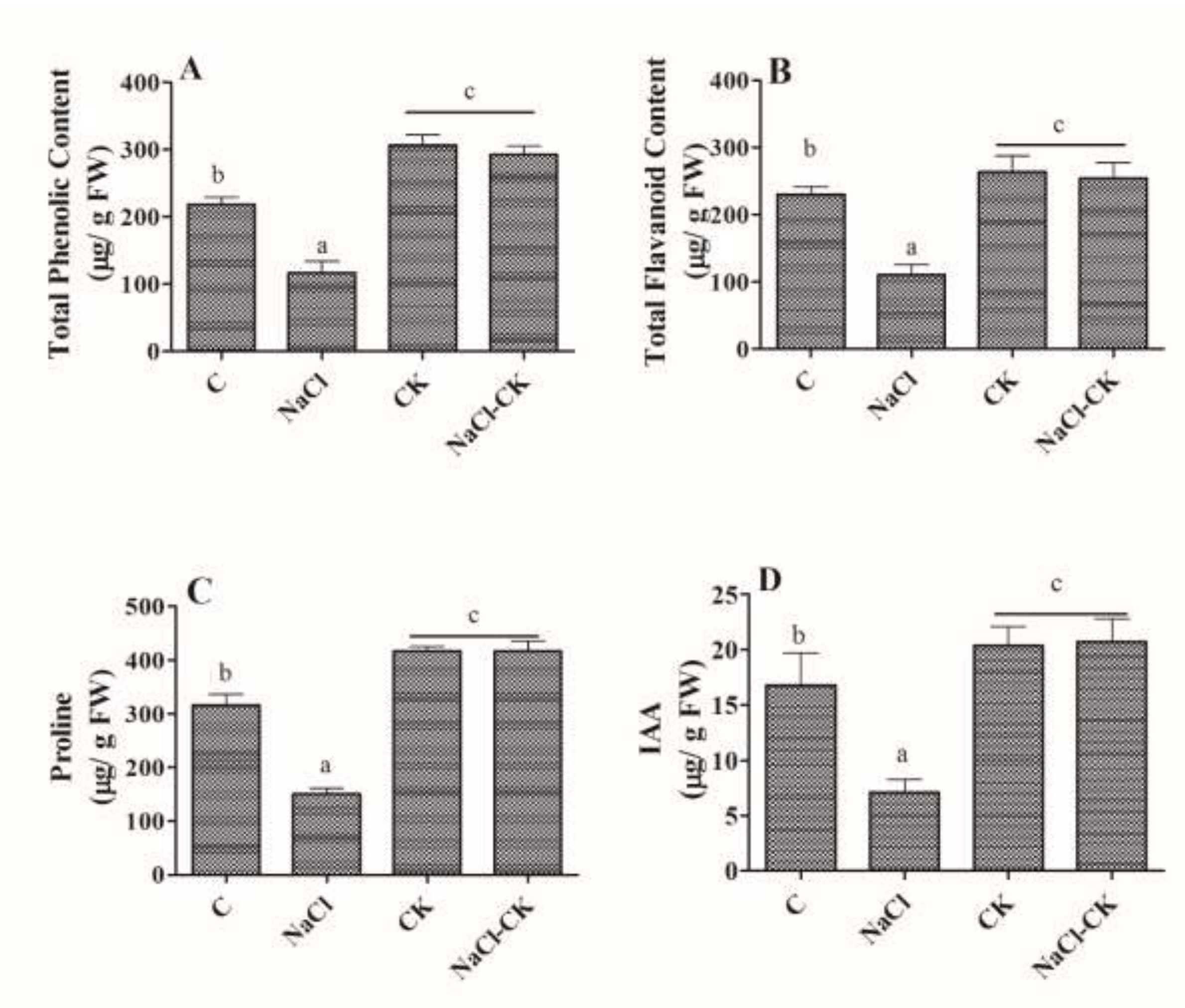


Figure 1

Determination of shoot length, root length, leaf area and fresh weight in the seedlings of maize after stressed with NaCl or Cytokinin or NaCl and Cytokinin. Experiment was repeated three times for validation.



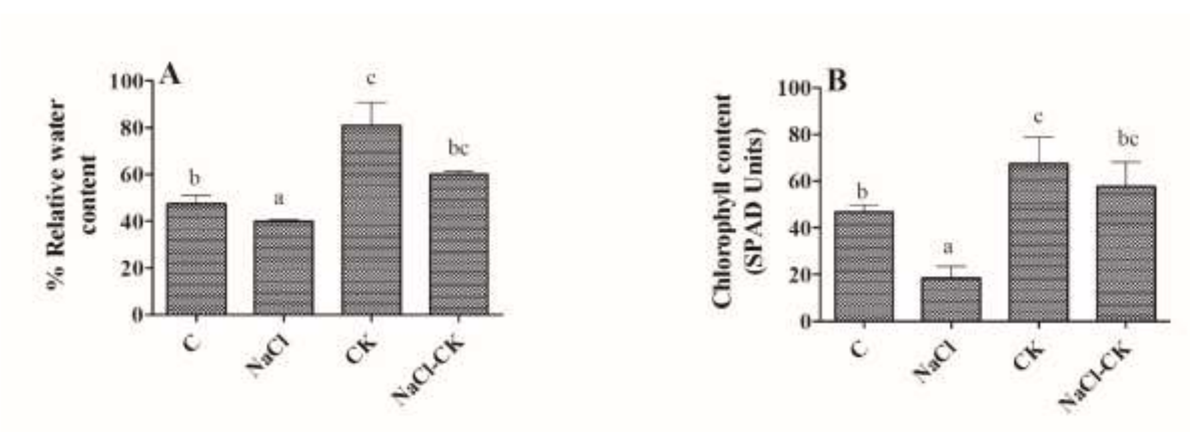


Figure 2

Determination of Total Phenolics Content, Total Flavonoid Content, Proline, IAA, Relative Water Content and Chlorophyll content in the seedlings of maize after stressed with NaCl or Cytokinin or NaCl and Cytokinin. Experiment was repeated three times for validation.

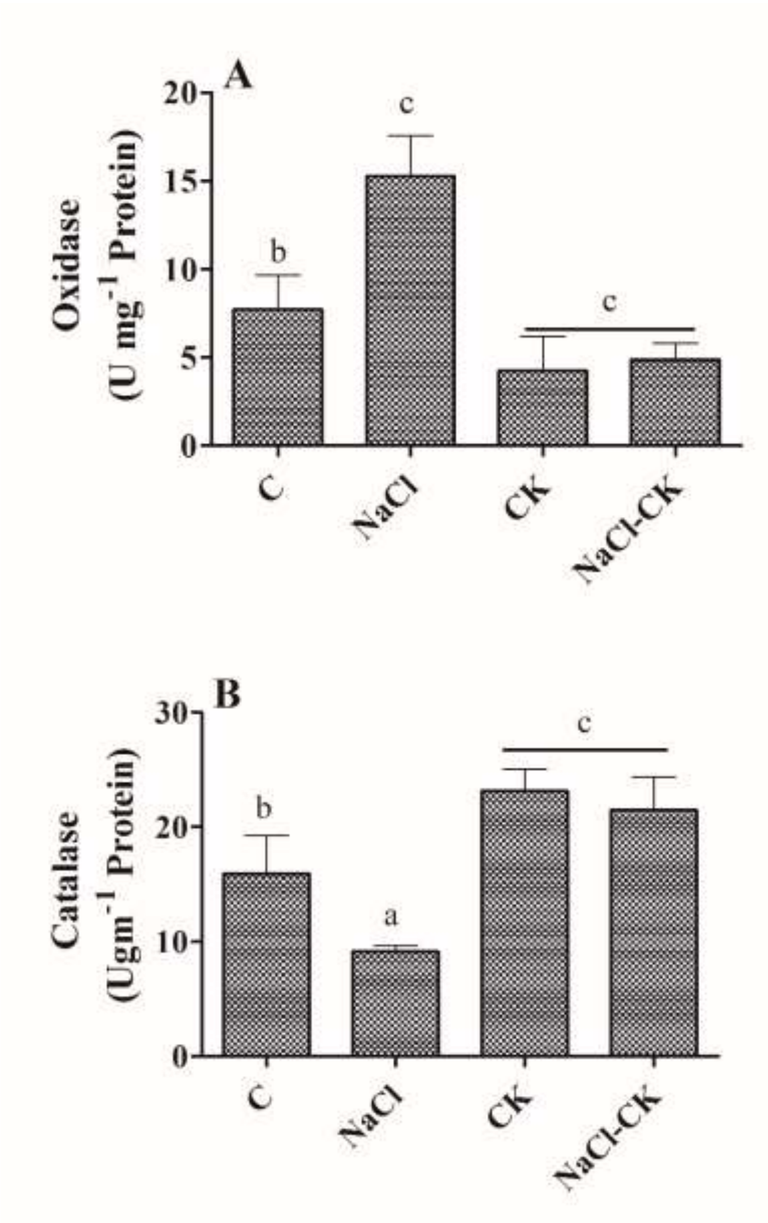


Figure 3

Determination of Oxidase and Catalase Activity in the seedlings of maize after stressed with NaCl or Cytokinin or NaCl and Cytokinin. Experiment was repeated three times for validation.

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