Role of Cytokinins in alleviating the Copper toxicity in maize plant

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Abstract

Plant growth hormones called cytokinins (CKs) also help plants recover from abiotic challenges such heavy metal stress. In this research, we aerially sprayed *Zea mays* seedlings with $CuSO_4$ stress. When the seedlings' growth was assessed, it had improved. Root length, shoot length, fresh weight, and leaf area growth kinetics were noted. Surprisingly, CKs treatment boosted the seedlings' greater synthesis of secondary metabolites compared to $CuSO_4$ treatment. Eventually, the seedlings relative water content was likewise high. Additionally, the seedlings treated with CKs and $CuSO_4$ stress had increased levels of IAA, which combined with CKs to create enhanced enzyme activity, including catalase and oxidase. Individually stressed seedlings from $CuSO_4$ treatments showed reduced IAA levels, which decreased the activity of the catalase and oxidase. So it was determined that the use of cytokinins reduced the effects of heavy metal stress by causing the creation of secondary metabolites.

Keywords: Cytokinins, Copper Sulphate, Secondary metabolites, Growth Kinetics, Enzymatic activity

Introduction

Heavy metals, which can be essential or non-essential, play a crucial part in plant development and growth, but their presence in excessive concentrations can be hazardous to plants, affecting their capacity to absorb and retain other non-essential elements (Asati et al., 2016). Heavy metals stress may produce a variety of distinct biochemical, physiological, and morphological changes in plants, as well as plant viability (Nguyen et al., 2021). Plants need these metals to carry out their metabolic functions such as photosynthesis, respiration and processes related to growth and development (Yruela, 2005). One of the heavy metals which is required in trace amount is copper (Guo et al., 2019).

It is a crucial component of plastocyanin and cytochrome, which aid in photosynthesis and respiration (Merchant et al., 2020). In plants, copper is necessary for enzymatic activities, seed formation, and chlorophyll content synthesis (Essa et al., 2021). However, the excess amount in younger tissues of plants typically exhibit chlorotic stripes at the tip of the leaf, bluish green streaks on the midrib on both sides, and other symptoms of copper deficiency (Rajendran et al., 2009). In excess amount of Cu which cause several disorders to plant can be altered by the phytohormones (Nazli et al., 2020). Phytohormones are small chemical signaling chemicals generated by plants to support healthy growth and development. Auxins, gibberellins, cytokinins, Strigolactones, abscisic acid, ethylene, brassinosteroids, jasmonates, and salicylates are examples of plant growth regulators (A Seif El-Yazal et al., 2015). Cytokinins are precursors of growth because they promote cell division in plant shoot and root systems (Davies, 2010). More than 100 genes associated with photosynthesis are controlled by CKs (Li et al., 2021). During their inhibition in plants, the seeds are prevented from germinating and put into dormancy; leaves, fruits, and flowers are fell off; and the stomata are closed (Xuan et al., 2020). The inhibition of hormone can affect the rate of photosynthesis under biotic and abiotic stress (Kosakivska et al., 2022).

Additionally, the phytohormones regulate the production of secondary metabolites such as flavonoids, phenolics and phyto-alexins (Jan et al., 2021). Flavonoids are a class of natural compounds with varying phenolic structures found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine (Sharma et al., 2020a). Due to their strong antioxidant qualities, plant polyphenols have gained growing attention (Gulcin, 2020). These substances are only formed or activated when the host plants come into contact with the parasite, and they inhibit the fungus from multiplying in hypersensitive tissues and soluble sugars (Soluble sugars are particularly sensitive to environmental changes that impact glucose transport from source organs to sink organs (Adedayo et al., 2022). They are stored by plants as hexose, which can be either glucose or fructose (Misra et al., 2022). However, little is known about CK's involvement in influencing the formation of antioxidant chemicals that promote plant development.

A *Zea mays* is a major grain crop in the Poaceae family. Maize is a valuable commercial crop because it contains minerals, vitamins, fiber, oil, and embryonic proteins (Madhu, 2022). Maize oil is used by companies that manufacture soap and prepare meals (Narowska et al., 2019). Maize starch is commonly used as a diluent in cosmetics and the pharmaceutical industries (Garcia et al., 2020). In addition to nutrition, it includes phytochemical substances that can be used to treat chronic diseases like as liver ailments, stomach ulcers, and other digestive concerns (Awuchi et al., 2020). Pakistani farmers produce maize across 1.3 million hectares of various ecosystems (Hayat), ranging from 30 meters above sea level in Sindh's dry plains to around 3,000 meters above sea level in Gilgit-Karakoram Baltistan's Mountains (Malik and Azam, 2009), as part of a complicated irrigated crop cycle, and on a lesser scale in Punjab (Ali and Chaudhry, 1990). KPK (Khyber Pakhtunkhwa) cultivated 471,000 hectares of maize, yielding 915,000 tons each year (Ali et al., 2019).

The purpose of this study is to investigate the effects of CKs on maize development under Cu stress. In this context, we hoped to assess the involvement of CKs in the activation of secondary metabolites in plants, which then deal with oxidative damage caused by diverse abiotic or biotic stresses. This research would help to develop the integration of future research to deal with the many degrading effects of the environment.

Method and Materials

Plant materials and treatment conditions

The seeds of *Z. mays* were acquired from the Agriculture Department, Khyber Pakhtunkhwa. The seed sterilization and germination procedures were carried out in accordance with the previously reported approach. *(Yousaf et al., 2021)*. The treatment conditions adopted were as:

1. 2 mL of distilled water that has been sterilized was given to the control seedling.
2. In amount of 2 mL, Seedlings were sprayed with 150 mM concentration of $CuSO_4$.
3. In amount of 2 mL, Seedlings were sprayed with (10 µL) concentration of Cytokinins.
4. Seedlings were sprayed with 2 mL of 10 L Cytokinins and 150 mM CuSO4 in a combination.

Sample preparation

The sample extraction was prepared as previously described (Yousaf et al., 2022b). IAA content, Total Phenolics Content (TPC), Total Flavonoid Content (TFC), and Proline were all determined using the extract. The method for determining IAA through HPLC has already been reported(Yousaf et al., 2021). TPC, TFC, and proline were measured with a spectrophotometer as described earlier ().

Chlorophyll Content

SPAD-502 Plus (Konica Minolta, Japan was used to determine the total chlorophyll content of the sample.

Determination of Relative Water Content (RWC)

The relative water content was determined using the recently reported technique (Yousaf et al., 2022) (Yousaf et al., 2022a).

Results

Growth Kinetics

After 08 days of treatment, different growth parameter such as Root Length, Shoot Length, Fresh Weight and Leaf Diameter were noted in *Z mays* seedlings. The seedlings stressed with $CuSO_4$ show reduced shoot length which was induced under CK treatment. Interestingly, the stressed seedlings with $CuSO_4$ along with CK treatment also enhanced the shoot length. Similarly, under metal stress the root length was also decreased while the CK treatment enhanced it. Additionally, when CK and $CuSO_4$ both were applied to the seedlings, the root length was increased (Figure 1A-1B).

For the observation of the efficiency of photosynthesis, we observed the leaf area ratio which show the size of leaf lamina divided by total biomass of the seedlings. The CKs treatment increased the leaf area ratio and CK-$CuSO_4$ treatments also indicate higher growth of the leaf. In Contrast, the seedlings stressed with $CuSO_4$ alone produced lower leaf area ratio. Similarly, the fresh weight of CKs phenotype and CK-$CuSO_4$ phenotypes were high compared to seedlings stressed with $CuSO_4$, which remains low (Figure 1C-1D).

Secondary Metabolite production

Secondary metabolites such as IAA, TFC, TPC and Proline were measured in the seedlings stressed with $CuSO_4$ or CK or CK and $CuSO_4$. TPC was higher when CK was applied to seedlings while lower under $CuSO_4$ treatment. And CKs with the combination of $CuSO_4$ also induced the level of TPC compared with NaCl treated seedlings (Figure 2A).

Additionally, when CK was administered to seedlings, the level of TFC and IAA increased while the level of $CuSO_4$ decreased similarly to control. TFC levels were higher in the presence of CKs and $CuSO_4$ compared to the control. Proline levels were reduced in $CuSO_4$-treated seedlings but remained high in CK seedlings. It's interesting to note that the CKs combined with $CuSO_4$ increased the level of proline content in comparison to the control. IAA levels were low in $CuSO_4$ stressed seedlings but high in the CK and CK-$CuSO_4$ phenotypes (Figure 2B-2D)

Relative water Content

In comparison to $CuSO_4$-treated seedlings and Control, it was calculated that CK-treated seedlings had greater Relative Water Content. Additionally, compared to the control, the CK with $CuSO_4$ also showed an elevated value for Relative Water Content. (Figure 2E)

Chlorophyll content

Results showed that seedlings treated with CK had higher chlorophyll contents than seedlings treated with $CuSO_4$ or the control. Additionally, compared to $CuSO_4$ treated seedlings and the control, the combination of CK and $CuSO_4$ boosted the level of Chlorophyll Content (Figure 2F)

Enzymatic kinetics

Oxidase and CAT activity were among the enzymes whose activities were measured. $CuSO_4$ stress caused a decrease in CAT activity, which was greater in CK treated seedlings. Furthermore, seedlings treated with CK-$CuSO_4$ displayed increased CAT activity compared to control. In contrast, when seedlings are treated with $CuSO_4$ or CK, the oxidase activity remains high in both cases (Figure 3A-3B).

Discussion

One of the most significant abiotic stressors that has had a negative impact on plant development and production is heavy metal stress(Tuteja et al., 2011) . By restricting seed germination, growth, and development, as well as blooming and fruiting, copper stress has a negative effect on plants (O'Brien et al., 2021). In this study, 150 mM of $CuSO_4$ was sprayed aerially onto 8-day-old maize seedlings to cause heavy metal metal stress. The 150 mM $CuSO_4$ stress had the effect of shortening the shoot length of the maize seedlings. It was noticeable that the shot length was less than it was for the control. Because of the nutritional imbalance caused by Cu toxicity, plant deve(Chrysargyris et al., 2019)lopment is greatly reduced(Chen et al., 2022) . The many biochemical and physiological metabolisms that are responsible for the best possible plant growth and development are adversely affected by these imbalances(Khan et al., 2022) . The Cu stress also has an impact on the Fresh Weight of plants(Saleem et al., 2020) . Due to the treatment of Cu, plants' fresh weight decreases(Chrysargyris et al., 2019) . Stress from copper lowered plant chlorophyll concentration, hindered growth potential, and impaired normal function, which increased the fresh weight of the plants(Parveen et al., 2020) . Under significant metal stress, the fresh weight of the maize seedlings decreased as well(AbdElgawad et al., 2020) . Due to Cu stress, growth parameters including root length are also becoming unbalanced(Godoy et al., 2021) . Plant development is negatively impacted by heavy metal stress when Cu toxics build up in the root zone(Sandeep et al., 2019) . Metal-induced effects of high Cu levels on plant growth and seed germination in roots zone(Ahmad et al., 2020) . Due to metal toxicity, high Cu levels in roots have an impact on seed germination and plant development(Fahad et al., 2019) . Therefore, with 150 mM of applied Cu stress on maize seedlings, the root length was likewise decreased(Godoy et al., 2021) .

Additionally, by decreasing the leaf width and the number of chlorophyll cells, the copper stress disrupts the normal operation of the leaf(Mir et al., 2021) . The photosynthetic process was severely hampered by heavy metal stress, which also resulted in decreased leaf development, decreased root growth, and decreased stomatal conductance(Rai et al., 2021) . Chlorophyll is a photosynthetic pigment that gives plants their green colour and aids in photosynthesis, which is how plants make their own food(Babarinsa, 2021) . Due to an increase in copper concentration in plants, the Cu stress decreased the amount of chlorophyll present (Qin et al., 2022). Stomata close as a result of Cu stress, which restricts the process of photosynthesis (Sharma et al., 2020b). Osmotic stress is increased by copper, which negatively affects the actions of numerous stomatal enzymes involved in reducing carbon dioxide (CO_2) (Mareri et al., 2022). In these research, copper stress was used to lower the chlorophyll content in maize seedlings. The negative effects of the Cu stress on relative water content (RWC). Commonly, relative water content decreased as Cu toxicity increased. The relative water content of the leaves decreased as a result of Cu stress, and this loss of turgor limits the amount of water that is available.

Plants create secondary metabolites that boost a cell's tolerance to a certain stress while under stressful conditions, such as Cupper stress (Akhi et al., 2021). Flavonoids, phenolics, and proline are among the secondary metabolites (Badiaa et al., 2020). An amino acid called proline is essential for the development of plants (Ghosh et al., 2022). It shields plants from various pressures and also aids in their recovery after being subjected to stress (Yaashikaa et al., 2022). Similarly, by scavenging free radicals, flavonoid and phenolic compounds increase resistance to $CuSO_4$ stress (Chowdhary et al., 2021). In this regard, proline, flavonoids, and phenolics synthesis were decreased in seedlings under Cu stress (Badiaa et al., 2020). Increased proline concentration, soluble sugar, soluble protein, and total free amino acid levels in the roots, stem, and seeds all contributed to IAA's ability to induce osmotic protection in heavy metal-stressed plants (Feng et al., 2021). Cytokinins (CKs), a class of phytohormones, were designated for plant enhancement (Aremu et al., 2020). With the aid of CKs, heavy metal stress conditions are readily broken (Das et al., 2022). It's interesting to note that the CK therapy increased growth, which reduced Cu stress in the seedlings (Li et al., 2022). The CK increased the formation of secondary metabolites such IAA, TFC, TPC, and Proline in the seedlings of maize to generate the best growth possible (Aguirre-Becerra et al., 2021). It then scavenges the ROS generation brought on by Cu stress (Zhang et al., 2022). Furthermore, the treatment of Cu stress increased enzymatic activity such as CAT activity (Zeng et al., 2019). The CAT activity was induced when $CuSO_4$ was administered to the seedlings of Z. mays (Mazaheri-Tirani et al., 2021). Applying $CuSO_4$ also causes a reduction in oxidase activity (Umar et al., 2022). As a result, it has been determined in this study that CK therapy is necessary for plants to recover from abiotic challenges such heavy metal stress.

Conclusion

Cytokinins, which are phytohormones that stimulate plant development, are crucial in reducing abiotic stresses such as heavy metal toxicity. Therefore, 150 mM of $CuSO_4$, CK, or both were aerially applied to 8-day-old maize seedlings. Because of the reduction in shoot length, root length, fresh weight, and leaf area, it was noticed that the growth of the seedlings was slowed. However, the seedlings' growth characteristics were high whether CKs were used alone or in conjunction with $CuSO_4$. This improvement in growth parameters in the seedlings was brought on by the enhanced synthesis of secondary metabolites such proline, flavonoids, and phenolic content, which scavenged the ROS. Thus, it was determined that CKs produced secondary metabolites such IAA, Proline, Flavonoids, and Phenolics that reduced the Heavy Metal stress in the seedlings.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

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

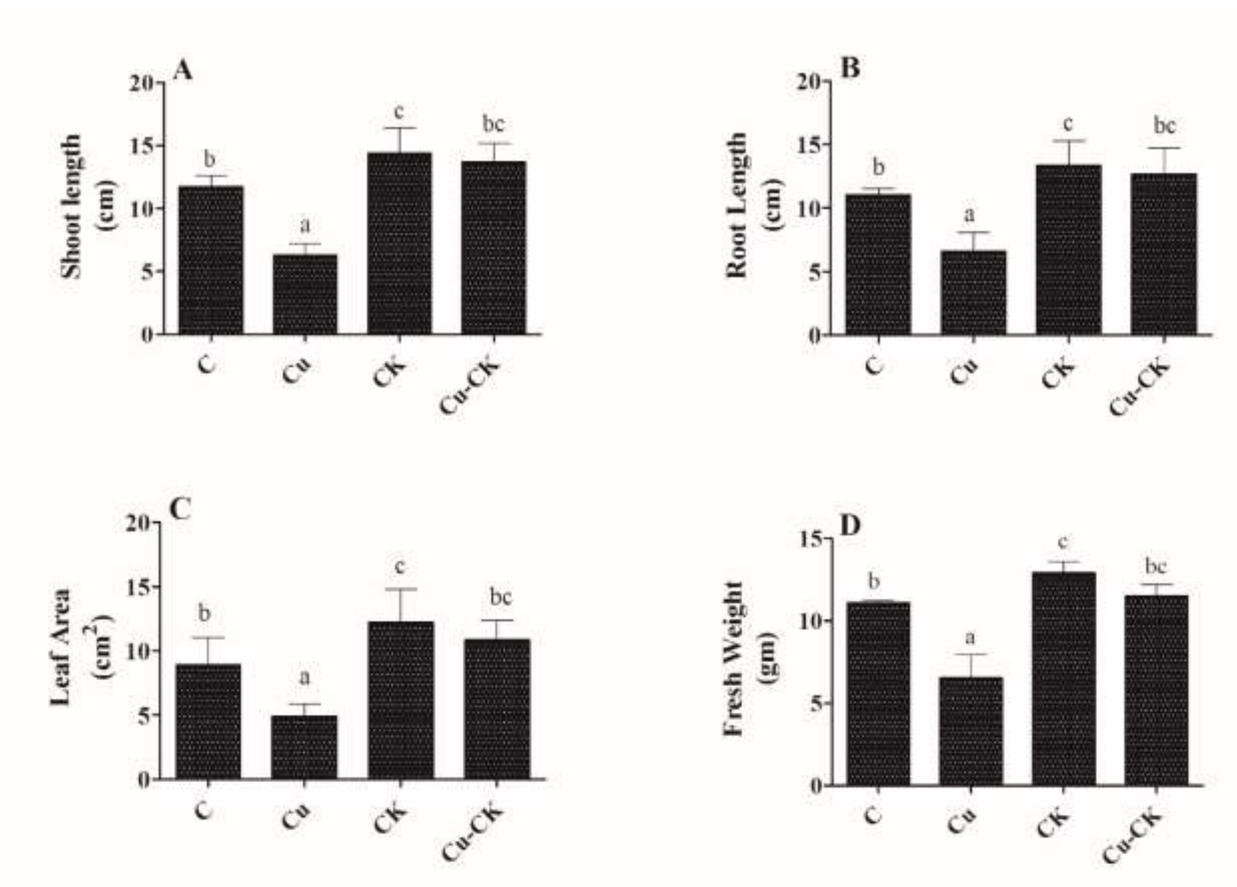
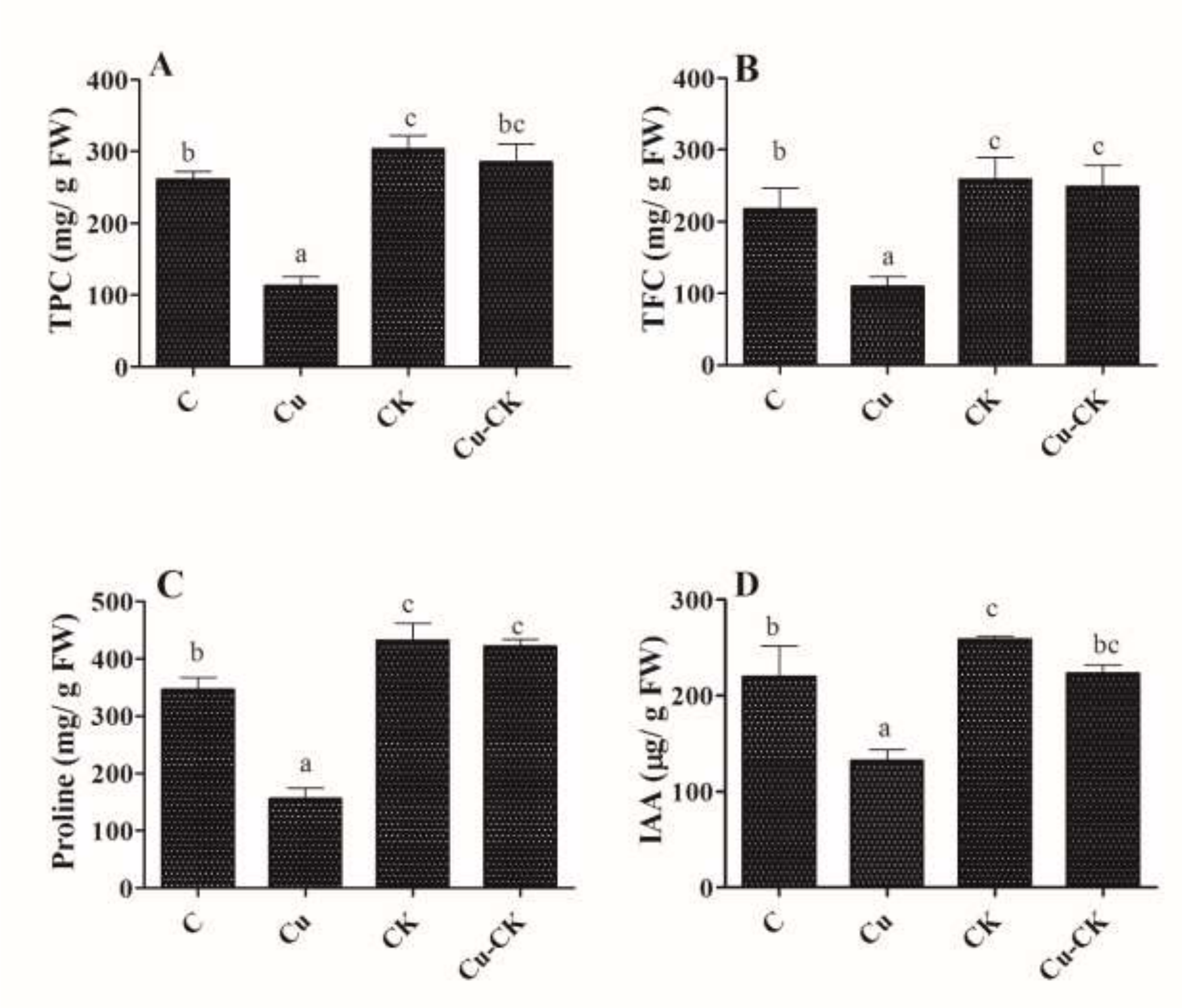


Figure 1

Determination of the lengths of the shoots, roots, leaf area, and fresh weight in maize seedlings that have been stressed with either $CuSO_4$ or Cytokinins, or both $CuSO_4$ and Cytokinins. For verification, the experiment was conducted three times.



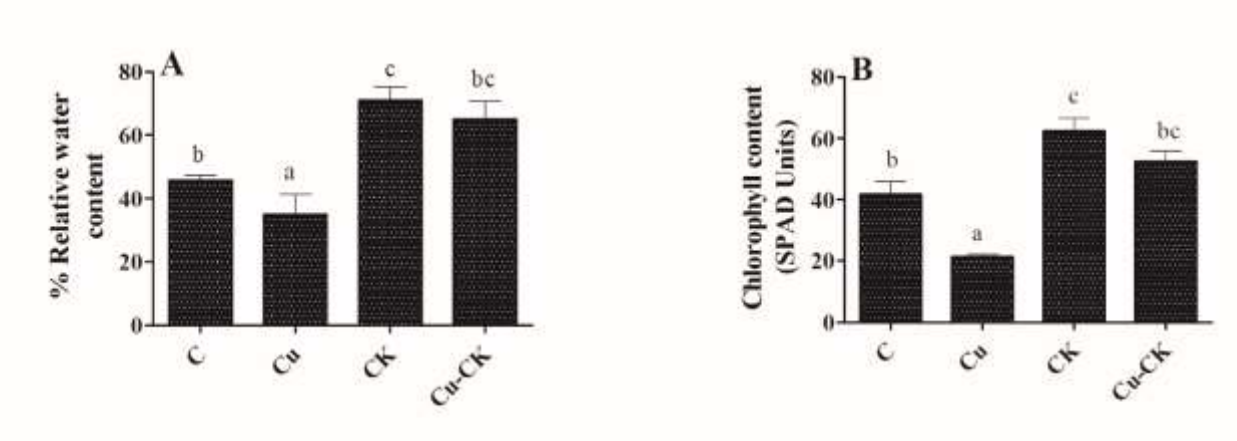
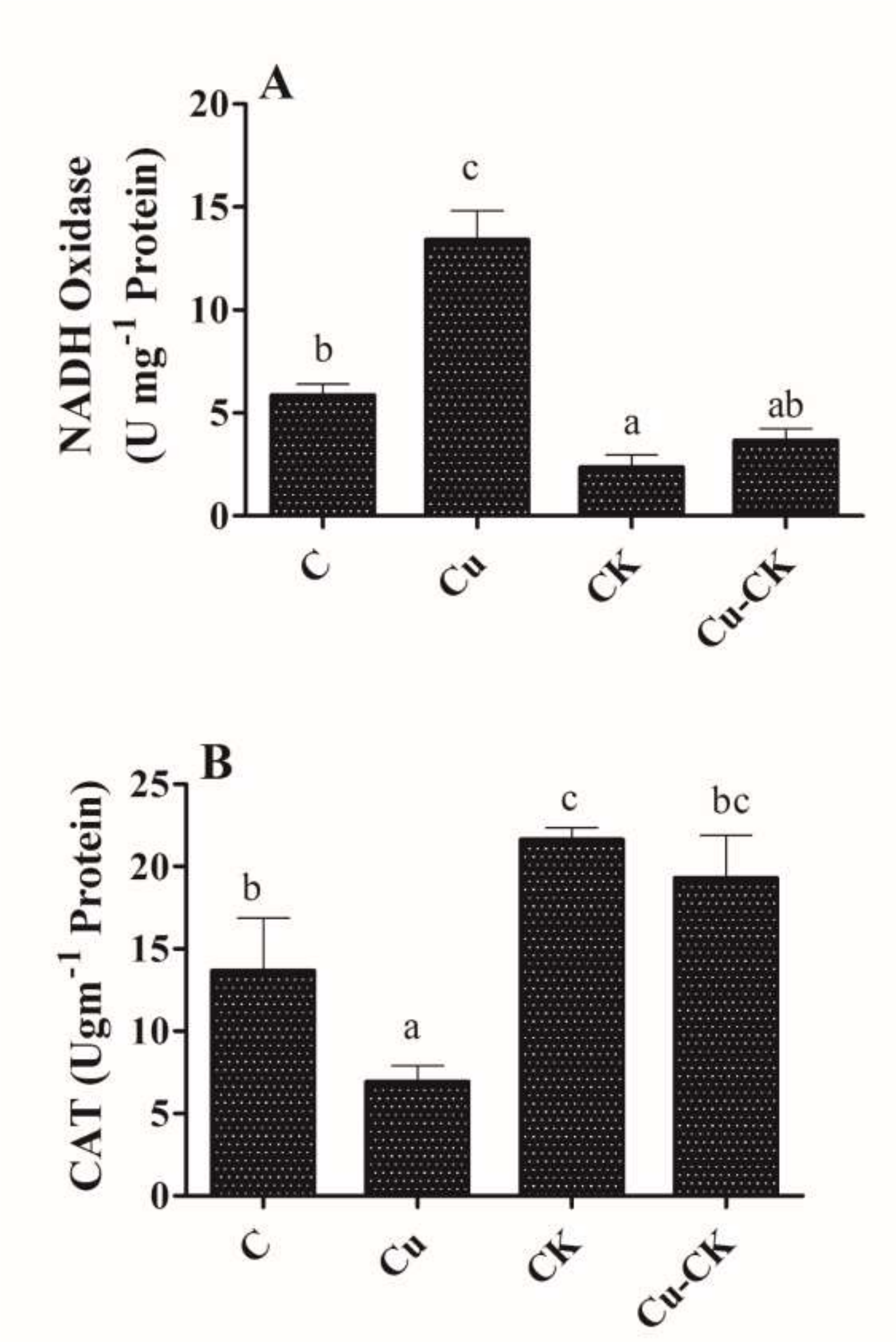


Figure 2

Proline, IAA, Relative Water Content, Total Phenolics Content, and Chlorophyll Content were determined in maize seedlings that had been stressed with either $CuSO_4$ or Cytokinins, or both $CuSO_4$ and Cytokinins. For verification, the experiment was run three more times.


A
NADH Oxidase (U mg^{-1} Protein)
0
5
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b
c
a
ab
C
Cu
CK
Cu-CK
B
CAT (Ugm^{-1} Protein)
0
5
10
15
20
25
b
a
c
bc
C
Cu
CK
Cu-CK

Figure 3

Analyzing the activity of the enzymes oxidase and catalase in maize seedlings that have been under stress from either $CuSO_4$ or cytokinins, or both $CuSO_4$ and cytokinins. For verification, the experiment was run three more times.

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