Advances in Biology & Earth Sciences
Vol.9, No.3, 2024, pp.368-373
https://doi.org/10.62476/abes93368

EFFECT OF VARIOUS SALTS ON ANTIOXIDANT PROTECTION AND CARBON METABOLISM OF COTTON

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Abstract. This study investigates the combined effects of sodium chloride (NaCl) and zinc sulfate ($ZnSO_4$) on antioxidant defense mechanisms and carbon exchange processes in cotton (*Gossypium hirsutum*) plants. Cotton plants were subjected to varying concentrations of NaCl and $ZnSO_4$ to evaluate their impact on superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APO) activities, as well as chlorophyll content, photosynthetic rate and nutrient uptake efficiency. Combined treatments showed synergistic effects, enhancing antioxidant defense and overall plant resilience. These findings suggest that $ZnSO_4$ can play a crucial role in alleviating the detrimental effects of salinity on cotton plants, offering valuable insights for improving crop productivity and stress tolerance under saline conditions.

Keywords: *Adaptation, stress factor, salts, ATP, chlorophyll.*

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Received: *1 October 2024;* ***Accepted:*** *27 November 2024;* ***Published:*** *5 December 2024.*

1. Introduction

Cotton (*Gossypium hirsutum*) is a vital crop in global agriculture, not only for its fiber but also for its seed oil and other by-products. The productivity and quality of cotton plants can be significantly affected by environmental stressors, including salinity and micronutrient availability. NaCl (sodium chloride) and $ZnSO_4$ (zinc sulfate) are two such factors that have profound effects on plant physiology (Gupta *et al*., 2012).

Salinity, primarily caused by NaCl, poses a significant challenge to plant growth and development. High salinity levels disrupt ion balance, leading to osmotic stress and oxidative damage. Excessive sodium ions can interfere with the uptake of essential nutrients like potassium and calcium, further exacerbating the stress. To combat these stresses, plants activate their antioxidant defense systems, which include enzymes like superoxide dismutase, catalase and peroxidase (Abro *et al*., 2024). These enzymes help neutralize reactive oxygen species (ROS) generated during salt stress, thereby protecting cellular components from oxidative damage. Understanding the impact of NaCl on these antioxidant defenses is crucial for developing strategies to improve plant resilience to salinity (Khan & Panda, 2018).

Furthermore, NaCl stress can affect various physiological processes, including photosynthesis and respiration. High salinity levels can lead to a decrease in chlorophyll

How to cite (APA):
Alakbarova, Sh.E. (2024). Effect of various salts on antioxidant protection and carbon metabolism of cotton. *Advances in Biology & Earth Sciences*, *9*(3), 368-373 https://doi.org/10.62476/abes93368

content, which in turn reduces the photosynthetic efficiency of plants. This reduction in photosynthetic activity can negatively impact carbon exchange processes, leading to decreased biomass production and overall plant growth. Additionally, NaCl stress can impair root development and function, affecting the plant's ability to absorb water and nutrients from the soil (Wu *et al.*, 2006; Seyidaliyev et al., 2024).

Zinc, an essential micronutrient, plays a critical role in various physiological processes in plants, including enzyme activation, protein synthesis and hormone regulation. $ZnSO_4$ supplementation can influence the carbon exchange processes, such as photosynthesis and respiration, by affecting the activity of zinc-dependent enzymes. Additionally, zinc is known to enhance the root absorption system, improving nutrient uptake efficiency and overall plant health. Zinc deficiency, on the other hand, can lead to stunted growth, chlorosis, and reduced crop yields (Alakbarova, 2024).

Therefore, investigating the effects of $ZnSO_4$ on cotton plants can provide insights into optimizing zinc nutrition for better growth and stress tolerance. Previous research has demonstrated that both NaCl-induced salinity and zinc deficiency pose significant threats to plant productivity. NaCl stress has been reported to reduce photosynthetic efficiency, disrupt membrane integrity and inhibit root growth (Babayev *et al*., 2023).

Moreover, the interaction between NaCl and $ZnSO_4$ and their combined effects on antioxidant defense mechanisms, carbon exchange processes and root absorption systems in cotton plants have not been thoroughly explored. This study aims to investigate these interactions and provide a comprehensive understanding of how NaCl and $ZnSO_4$ influence the growth and development of cotton plants. By elucidating these mechanisms, we hope to contribute valuable insights into enhancing cotton plant resilience and productivity under varying environmental conditions (Zafar *et al*., 2022).

The findings of this research could have broader implications for other crops grown in saline soils and areas with micronutrient deficiencies. Understanding these mechanisms can aid in developing agronomic practices and biotechnological interventions to mitigate the adverse effects of salinity and zinc deficiency, ultimately leading to improved crop performance and sustainability (Qamer *et al*., 2021). The aim of our article is to study the effect of high concentrations of NaCl from chlorine salts and $ZnSO_4$ from sulfate salts on the antioxidant defense system and carboxylating enzymes in the active phases of development of the ontogenesis of the Ganja-182 variety of cotton *Gossypium hirsutum L.*

2. Material and methods

The seeds of the Ganja-182 variety of Cotton (*Gossypium hirsutum* L) were included in the State Register of Breeding Achievements of the Republic of Azerbaijan. Patent № 00238 issued a certificate of authorship. Mutant - 5/8 x Mutant - 6/34= was obtained by directional selection on the hybrid obtained from hybridization. Seedlings were grown in pots in a growth chamber under controlled conditions (temperature regime of 25ºC, 16/8-h photoperiod and relative humidity of 75%).

- The experiment was conducted using a completely randomized design with 2 treatments and 3 replicates per treatment.
- Treatments included:
- Control (no NaCl or $ZnSO_4$)
- NaCl at concentrations of 50, 100 and 200
- $ZnSO_4$ at concentrations of 50, 100 and 200

Superoxide Dismutase (SOD) Activity: Enzyme activity was measured using the nitro blue tetrazolium (NBT) reduction method. Leaf tissues were homogenized in a buffer containing 50 mM phosphate buffer (pH 7.8), 1 mM EDTA, and 1% polyvinylpyrrolidone (PVP). The homogenate was centrifuged and the supernatant was used for the assay. SOD activity was expressed as units per milligram of protein. *Catalase (CAT) Activity:* CAT activity was determined using the method described by Aebi (1984). Leaf tissues were homogenized in a buffer containing 50 mM phosphate buffer (pH 7.0). The decomposition of H_2O_2 was measured spectrophotometrically at 240 nm and CAT activity was expressed as units per milligram of protein.

Chlorophyll Content: Chlorophyll a and b were extracted from leaf tissues using 80% acetone. The absorbance was measured at 663 nm and 645 nm, respectively. The concentrations were calculated using Arnon's (1949) equations and expressed as milligrams per gram of fresh weight.

Carotenoid Content: Carotenoid content was determined by measuring the absorbance at 470 nm in the chlorophyll extract and calculated using Lichtenthaler's (1987) formula. *Total Protein Content*: Protein was extracted from leaf tissues using a buffer containing 50 mM phosphate buffer (pH 7.0) and 1 mM EDTA. The total protein content was determined using the Bradford assay (1976) with bovine serum albumin (BSA) as the standard and expressed as milligrams per gram of fresh weight.

Extraction and Quantification: ATP and ADP were extracted from fresh leaf tissues using a perchloric acid extraction method. The extract was neutralized with potassium hydroxide. The concentrations of ATP and ADP were determined using a luciferase-based assay (ATP determination kit) and a coupled enzymatic assay, respectively (Qamer *et al*., 2021).

Results were expressed as nanomoles per gram of fresh weight. Data were analyzed using Microsoft Excel 2016 software.

3. Results and Discussion

The amount of photosynthetic pigments chl a, chl b and carotenoids (car) decreases depending on time and salt concentration. Their amount increases to true leaves phase (TLP) at a salt concentration of 100 mM and decreases slightly from blossom phase (BLP). Such similarity is also observed at a concentration of 200 mM, but with values approximately 10-15% lower than the values at a concentration of 100 mM.

In explaining the results given in Diagram 1, we encountered an interesting fact. We determined that at a NaCl concentration of 100 mM, the Σ xl/kar ratio also increases in parallel with the increase in the xl a/b ratio.

As can be seen from this, it is important to compare the Pn values and the xl a/b and Σ xl/kar ratios. Thus, the change in these three parameters under BLP occurs as follows: xl a/b in the presence of NaCl at concentrations of 100 and 200 mM, respectively, 1.0 and 1.0, the ratio Σ xl/kar 1.44 and 1.42, In the presence of ZnSO4, for NaCl it was equal to 1.14 and 1.03 and for the parameters Σ xl/kar – 3.95 and 2.1.

It can be concluded that the greater part of plant resistance to stress is determined by the ratios of the above parameters.

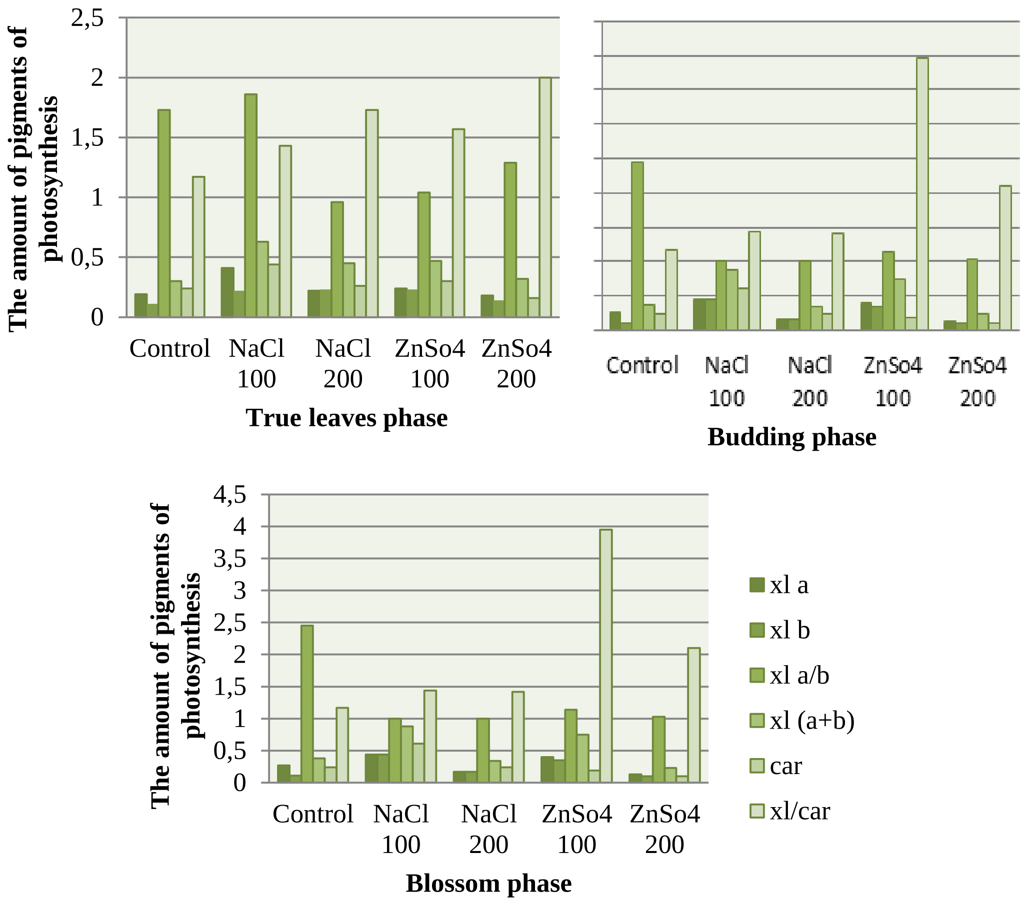

Diagram 1. The influence of chlorine and sulfate salts on the dynamics of changes in gas exchange parameters in leaves in the initial phases of cotton plant development (mM)

Although a similar trend was observed in BP, an increase in the ratio Σ xl/car was observed, which is possible only under conditions when the number of cars is less than the number of Σ xl.

It was concluded that the plant under BlP is more resistant to stress than under BP, that is, with increasing age of the plant and the level of stress, the plant's reaction to them also increases.

During ontogenesis changes in ATP and ADP, as well as NAD and NADP molecules in cotton leaves under salt stress, of particular interest is the study of the kinetics of this change depending on the duration of stress.

In order to determine the dynamics of changes in the amount of ATP and ADP in cotton leaves grown under salinity conditions, studies were conducted in the TLP, BP and BLP of plant development. The results obtained are presented in the diagrams below.

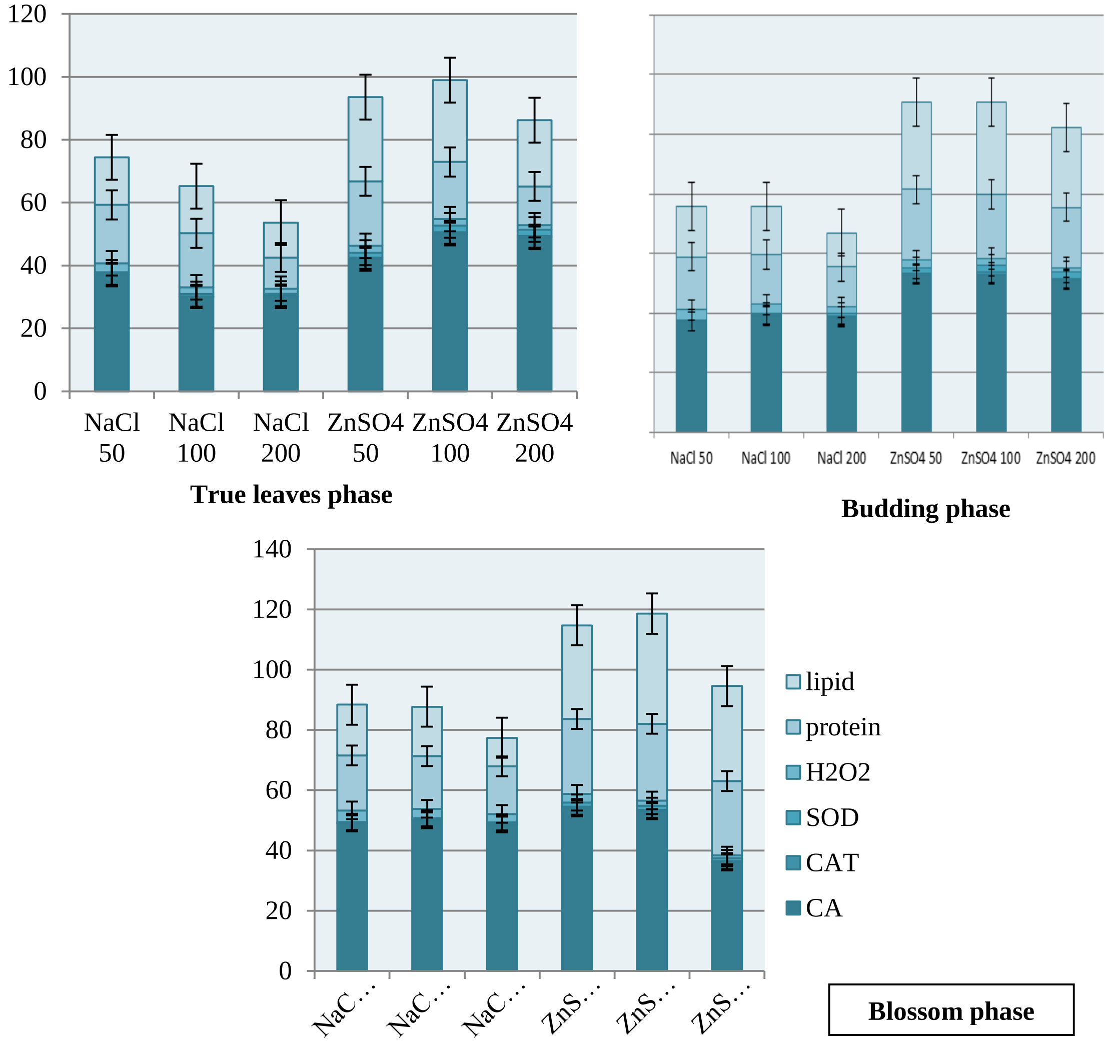

Diagram 2. The effect of chlorine and sulfate salts on the dynamics of changes in the amount of biopolymers in the leaves during the active growth phases of the cotton plant (mM)

During salt stress, respiration and photosynthesis are seriously affected. At high salt concentration, the entry of Na+ ions into the cytosol, the absorption of photons and the disruption of the END have a negative effect on the generation of ATF and NADFH molecules in FS II and the enzymatic activity of the Calvin cycle.

4. Conclusion

The reduction in total protein content and ATP levels under NaCl stress indicates a decline in metabolic activity and energy status. Salinity stress can lead to protein degradation and reduced ATP synthesis due to impaired mitochondrial function. $ZnSO_4$ treatment improved protein content and ATP levels, likely due to zinc's role in stabilizing protein structures and maintaining enzyme activities involved in energy metabolism. The combined treatment showed better biochemical status than NaCl alone, suggesting that zinc supplementation supports better metabolic function under stress.

The findings of this study have broader implications for improving cotton plant resilience and productivity under saline conditions. By elucidating the interactions between NaCl and $ZnSO_4$, this research provides valuable insights into optimizing zinc nutrition to enhance antioxidant defense, photosynthesis and nutrient uptake. Future research should explore the molecular mechanisms underlying the observed physiological changes, including the regulation of antioxidant enzyme genes and the role of signaling pathways in stress responses. Additionally, field studies are needed to validate the findings under natural growing conditions and assess the practical applications of zinc supplementation in saline soils.

Investigating the long-term effects of combined NaCl and $ZnSO_4$ treatments on cotton yield and fiber quality would provide valuable information for agricultural practices. Understanding the potential trade-offs between stress tolerance and crop productivity is essential for developing sustainable farming strategies in saline environments.

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