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THE EFFECT OF HEAT AND SALINITY STRESS ON THE CONTENT PROLINE AND HYDROGEN PEROXIDE IN LEAVES OF COTTON PLANT

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Abstract

This study investigated the effect of high temperature and salt stress on proline and hydrogen peroxide (H₂O₂) accumulation in the leaves of cotton (*Gossypium barbadense* L.) variety Surxon-103. Exposure to elevated temperature led to a significant increase in proline content and H₂O₂ levels, indicating the activation of stress-related biochemical responses. Although chloride and sulfate salinity increased H₂O₂ content, proline levels remained relatively stable. In contrast, carbonate salinity acted as the most severe stress factor, characterized by a maximal increase in H₂O₂ and a pronounced decrease in proline content. The results suggest that these parameters can be used as important indicators of heat and salt stress tolerance in cotton plants.

Keywords: Cotton (*Gossypium barbadense* L.), heat stress, salt stress, Proline and hydrogen peroxide

Introduction

Abiotic stresses such as salinity and high temperature severely affect the growth and productivity of cotton (*Gossypium barbadense* L.) by disrupting physiological and metabolic processes (Zhang et al., 2020). One of the major adaptive responses to these stresses is proline accumulation, which functions in osmotic adjustment and stabilization of cellular structures (Ashraf & Foolad, 2007). At the same time, stress conditions stimulate the overproduction of reactive oxygen species (ROS), particularly hydrogen perox-

ide (H₂O₂), which serves as both a signaling molecule and an indicator of oxidative stress. Excessive H₂O₂ accumulation can cause oxidative damage to membranes and proteins, leading to impaired cellular functions (Hasanuzzaman et al., 2013). The ability of cotton genotypes to regulate proline metabolism and H₂O₂ detoxification varies significantly and is closely associated with stress tolerance (Iqbal et al., 2019). Therefore, this study investigates changes in proline and H₂O₂ content in the leaves of the cotton variety Surxon-103 under heat and salinity stress conditions.

Method

Plant Material and Heat Stress Treatment

The seeds were initially delinted by short-term treatment with concentrated 96% H₂SO₄. Subsequently, the seeds were soaked in distilled water for several hours. The prepared seeds were then placed on moistened filter paper. Half of the filter papers were transferred into beakers corresponding to three different salinity models, while the remaining half was reserved for cultivation under heat stress conditions. All experimental variants were incubated in a sterilized growth chamber at 28 °C for a period of seven days.

Cotton (*Gossypium barbadense* L.) variety Surxon-103 was grown under controlled conditions at 30 °C (control). Heat stress was applied by exposing plants to 45 °C. Fully expanded leaves were collected from control and heat-stressed plants for biochemical analysis.

Seeds of the cotton (*Gossypium barbadense* L.) variety Surxon-103 were grown under thermostat-controlled conditions using three different salinity models (NaCl, Na₂SO₄, and Na₂CO₃), each applied at two concentrations (100 and 200 mM NaCl; 100 and 200 mM Na₂SO₄; and 100 and 200 mM Na₂CO₃).

Proline determination. Proline content was determined using the ninhydrin method described by Bates et al. (1973). Fresh leaf tissue (0.5 g) was homogenized in

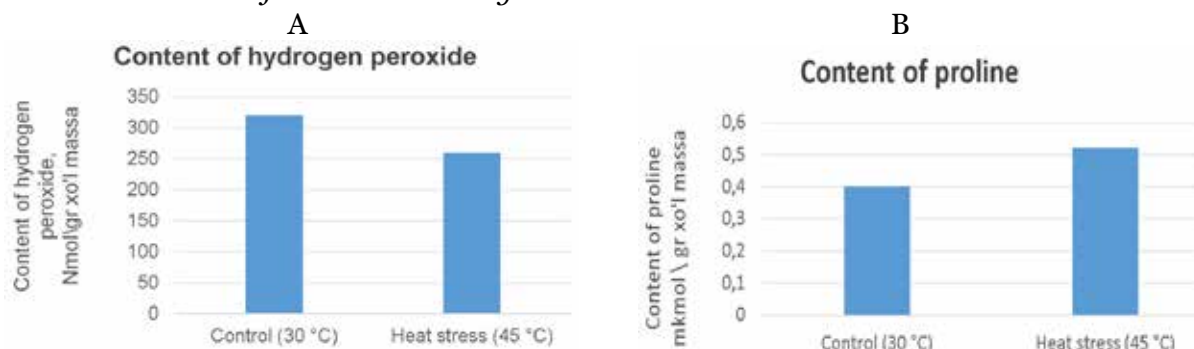
3% sulfosalicylic acid, reacted with acid ninhydrin, incubated at 95 °C for 1 h, and absorbance was measured at 520 nm. Results were expressed as μmol g⁻¹ fresh weight.

H₂O₂ determination. H₂O₂ content was measured according to Velikova et al. (2000). Fresh leaves (0.5 g) were extracted with 0.1% TCA, and absorbance was recorded at 390 nm after reaction with potassium iodide. H₂O₂ content was expressed as μmol g⁻¹ fresh weight.

Results

In this study, the effect of high temperature stress on oxidative processes in the cotton variety Surxon-103 was evaluated through content of proline and H₂O₂ (Fig. 1). Proline levels in the leaf tissues of Surxon-103 were compared under 30 °C (control) and 45 °C (heat stress) conditions. Exposure of the cotton variety Surxon-103 to high temperature stress (45 °C) resulted in notable changes in oxidative stress-related parameters in leaf tissues. Proline content increased from 0.40 under control conditions (30 °C) to 0.52 under heat stress, indicating a 30% increase relative to the osmoprotective compound. In contrast, H₂O₂ levels decreased under stress conditions, declining from 319.7 in the control to 260 at 45 °C, corresponding to an 18.7% reduction compared to the control.

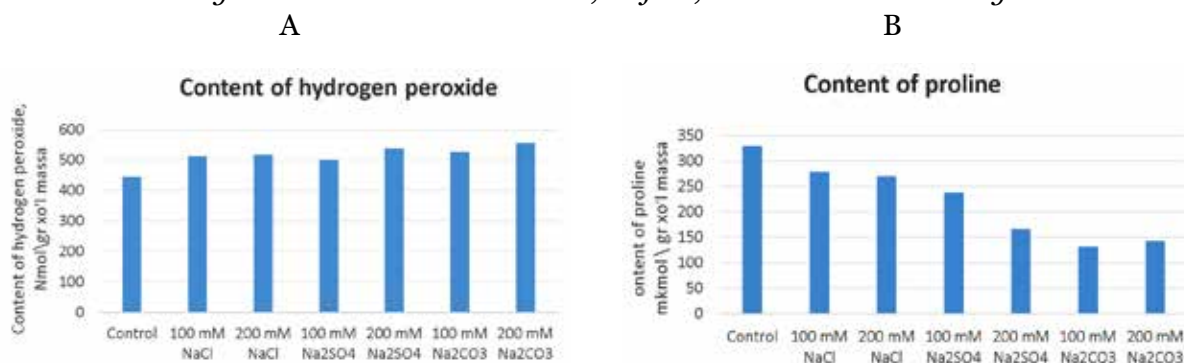
Figure 1. A) Hydrogen peroxide (H₂O₂) and B) proline contents in the leaves of the cotton variety Surxon-103 under heat stress conditions



Under chloride salinity conditions, an increase in H₂O₂ content in the Surxon-103 cotton cultivar compared to the control indicated the occurrence of oxidative stress; however, the minimal change in proline content under these conditions suggests that osmotic adjustment and antioxidant defense mechanisms remained relatively stable (Fig. 2).

Under sulfate salinity at 200 mM, the increase in H₂O₂ content to 538.2 nmol g⁻¹ indicated severe oxidative stress affecting cellular membranes. Concurrently, the decrease in proline content to 166 μmol g⁻¹ at this concentration confirmed that the plant's osmotic and antioxidant defense mechanisms were insufficiently activated.

Figure 2. A) Hydrogen peroxide (H_2O_2) and B) proline contents in the leaves of the cotton variety Surxon-103 under chloride, sulfate, and carbonate salinity conditions



The obtained results revealed that carbonate salinity was the most detrimental. Experimental data showed that exposure of the Surxon-103 cotton cultivar to 100 mM and 200 mM Na_2CO_3 led to an increase in H_2O_2 content to 526.3 and 555.1 $nmol\ g^{-1}$, respectively, while proline content decreased to 131 and 142 $\mu mol\ g^{-1}$. This represents up to a 50% reduction compared to the control. Moreover, the pronounced increase in H_2O_2 levels accompanied by a significant decrease in proline content further confirms that the plant's osmotic adjustment and antioxidant defense mechanisms were not adequately activated under carbonate salinity stress.

Discussion

In the present study, heat stress (45 °C) caused a 30% increase in proline content accompanied by an 18.7% decrease in H_2O_2 levels in the leaf tissues of the cotton variety Surxon-103. A similar response pattern has been reported in several plant species, particularly in heat-tolerant genotypes.

Ashraf and Foolad (2007) investigated the role of proline in stress tolerance in cotton and wheat and reported that exposure to high temperature resulted in a significant increase in leaf proline content, while oxidative stress markers such as H_2O_2 and malondialdehyde (MDA) were reduced in tolerant cultivars. The authors concluded that elevated proline levels contributed to enhanced reactive oxygen species (ROS) scavenging capacity, thereby limiting H_2O_2 accumulation. This finding closely aligns with the inverse relationship between proline and H_2O_2 observed in Surxon-103.

Similarly, Hasanuzzaman et al. (2014) studied heat stress responses in rice (*Oryza sativa*) and demonstrated that heat-toler-

ant varieties accumulated higher amounts of proline under elevated temperature conditions, whereas H_2O_2 levels were significantly lower compared to sensitive genotypes. Their results indicated that proline not only functions as an osmoprotectant but also plays a crucial role in maintaining redox homeostasis by supporting antioxidant enzyme activity, particularly catalase and peroxidase.

In another study, Wang et al. (2016) examined heat stress effects in maize (*Zea mays*) leaves and observed that increased proline accumulation under high temperature was associated with a decrease in H_2O_2 content. The authors attributed this reduction to the activation of enzymatic antioxidants and the direct ROS-scavenging ability of proline. They further reported a negative correlation between proline concentration and H_2O_2 levels, suggesting coordinated regulation of osmolyte synthesis and oxidative stress control.

Taken together, these studies support the results obtained in the present work, where increased proline accumulation in Surxon-103 under heat stress was accompanied by reduced H_2O_2 levels. This response indicates the effective activation of protective mechanisms, including osmotic adjustment and antioxidant defense, which collectively contribute to the mitigation of heat-induced oxidative damage. Therefore, the Surxon-103 variety can be considered to possess a relatively high capacity for maintaining oxidative balance under elevated temperature conditions.

Wang and colleagues investigated the biochemical response of rice (*Oryza sativa* L.) seedlings under NaCl, Na_2SO_4 , and Na_2CO_3 salinity conditions. Based on their findings, the authors emphasized that Na_2CO_3 salinity

represents the most detrimental stress factor compared with chloride and sulfate salinity. Under Na₂CO₃ treatment, a sharp increase in H₂O₂ content accompanied by a decrease in proline levels was observed in the seedlings, indicating the induction of severe oxidative stress.

Although chloride and sulfate salinity increased H₂O₂ content, proline levels remained relatively stable. In contrast, carbon-

ate salinity acted as the most severe stress factor, characterized by a maximal increase in H₂O₂ and a pronounced decrease in proline content. This pattern confirms that the plant's osmotic adjustment and antioxidant defense mechanisms were not adequately activated under carbonate salinity stress. These findings provide an important scientific basis for evaluating salinity tolerance and for use in breeding and selection studies.

References

- Ashraf, M. & Foolad, M. R. (2007). Roles of *glycine betaine* and *proline* in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, – 59(2). – P. 206–216. DOI: <https://doi.org/10.1016/j.envexpbot.2005.12.006>
- Hasanuzzaman, M., et al. (2014). *Proline and antioxidant defense mechanisms in plant abiotic stress tolerance*. Fundamental and Applied Agriculture. URL: <https://www.f2ffoundation.org/faa/index.php/home/article/download/259/245>
- Ashraf, M. A., Riaz, M., Arif, M. S., Rasheed, R., Iqbal, M., Hussain, I., ... (2019). *The role of nonenzymatic antioxidants in improving abiotic stress tolerance in plants*. In *Plant Tolerance to Environmental Stress* (P. 129–144). CRC Press.
- Zhang, H., et al. (2020). *OsProDH Negatively Regulates Thermotolerance in Rice by Modulating Proline Metabolism and ROS Scavenging*. *Rice*. URL: <https://thericejournal.springeropen.com/articles/10.1186/s12284-020-00422-3>
- Wang D., Xu M., Xu T., Lin X., Musazade E., Lu J., Yue W., Guo L., Zhang Y. Specific physiological responses to alkaline carbonate stress in rice seedlings // *Functional Plant Biology*. 2024. – Vol. 51. – No. 10. DOI: 10.1071/FP23161.

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