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Investigation of the Role of Gibberellic Acid in Salinity Tolerance of Upland Cotton Plant

Abstract

The salinity is the actual issue in the plant growth. It directly restricts the productivity of plants in the whole species. Using phytohormone(s) in seed priming is one of the most crucial treatment methods to protect plants from salinity.

A plant hormone called gibberellic acid (GA_3) is essential for controlling plant growth and development, especially in stressful situations. Determining the potential of seed priming with gibberellic acid under salt stress was the main goal of our study. We investigated the effects of varying GA_3 and NaCl concentrations on the levels of chlorophyll a, b, and carotenoids in cotton seedlings of the *Gossypium hirsutum* L. Agdash-3 genotype. This study also showed the effects of different GA_3 concentrations on the dynamics of polyphenol oxidase activity, which is crucial for the metabolism of phenolic substances and their involvement in plants' defense response. This investigation demonstrates that gibberellic acid regulates antioxidant enzyme activity and sustains chlorophyll a, b and carotenoid contents during salt stress circumstances which enhances resistance to salinity in cotton plants.

Keywords: *Salinity, Gibberellic acid (GA_3), Gossypium hirsutum, antioxidant enzymes*

Introduction

It is important to note that soil salinity is the biggest problem in agricultural development and this issue can affect negatively future generations. It restricts crop productivity and sustainability. Having a high salt content in the soil limits water uptake and leads to oxidative stresses and ion toxicity (Abdel-Hamid *et al*., 2014). Damaging membranes and reducing photosynthetic rate can occur due to salt-affected soil which makes stressful conditions for the plants. To detect and struggle with adverse effects, plants have been adapted to some morphological and physiological changes like getting a formation of relevant solutes to protect cell content, improved water utilization efficiency, and ROS detoxcification involving activating anti-oxidant systems (Augé et al., 2014)

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One of the most significant fiber crops in the world, cotton (Gossypium spp.) provides millions of farmers with a living and is an essential raw ingredient for the textile industry.
India, China, the United States, Brazil, and Pakistan are among the largest producers, with around 35 million hectares under cultivation in more than 80 nations. Over $50 billion is produced worldwide each year by the cotton business, which makes a substantial contribution to both industrial and rural economies.

Research

Cotton is important for sustainable agriculture in addition to its economic value. In contrast to many other primary crops, it resists drought and yields items like livestock feed and cottonseed oil, which increases its agricultural importance. Cotton is still extremely susceptible to salt stress though, which risks results and fiber quality in arid and semi-arid areas despite its ability to survive (Jenkins et al., 2018; Mammadova et al., 2024).

Soil salinity is one of the most significant abiotic elements influencing cotton production, especially in areas with extensive irrigation. The formation of too much salt, mostly sodium chloride (NaCl), in the soil, causes salinity stress, which impairs plants' ability to absorb water and maintain nutritional balance (Munns, Tester, 2008; Alizada et al., 2024). Normally, cotton is known as a tolerant plant in contrast to other crops. Despite having some biochemical alterations in the cotton against harmful effects, at the high salinity conditions, it can cause of reduction in production and fiber quality. Osmotic stress is one of the direct consequences of salinity on cotton plants, where a high concentration of salt in the soil impairs the plant's capacity to take in water Because excessive salt levels cause problems with seeds' ability to absorb water, they also negatively impact seed germination and the beginnings of seedlings (Sharif, *et al*., 2019).

Understanding how plants interpret stress signals and react to different environmental stressors is crucial. Endogenously generated chemical compounds known as phytohormones play an essential role in controlling the growth and production of plants. There are several phytohormones such as gibberellins, ethylene, abscisic acid (ABA), and jasmonic acid (JA) which are necessary in stimulating plants to become more resistant to different types of stress (Khan, N. et al., 2020).

Gibberellic acid's (GA_3) capacity as a basic plant hormone has been shown in several studies. Gibberellins (GAs), are essential for the germination of seeds and seedling development, stem and the length of the root, growing leaves and flowering. Because they control a variety of metabolic processes, the activity of multiple enzymes, and gene regulation (Miceli, A. et.al., 2019). Exogenous GA_3 treatment improves ion adoption, photosynthetic activity, effectiveness of water use, stomatal activity, and other phytohormone balance (Alonso-Ramírez et al., 2009). GA_3 also reduces lipid peroxidation and increases the effectiveness of antioxidants and osmoprotectants to mitigate the severe impacts of stress factors (Rady, et al., 2021.)

Several studies have demonstrated a relationship between GAs and JAs in both favorable and unfavorable circumstances. According to Achard *et al*. (2008) DELLA proteins build up whereas GA signaling decreases in response to cold stress, reducing plant development. According to Wingler et al. DELLA proteins can attach to JAZ proteins, which stimulates jasmonate-responsive genes. JA–GA interaction under extreme conditions is revealed by this result. Their stimulating and inhibitory properties in plants are similar to those of other plant hormones, and their antagonistic or synergistic effects with regard to other crop hormones are well documented (Soto, A. et al., 2012).

The goal of this study was to find out how gibberellic acid helps cotton plants become more tolerant of salinity. Our study tried to shed light on the possible use of GA_3 as a tactic for reducing salinity stress in cotton farming by assessing its effects on growth, physiological indicators, and stress-related reactions (Wingler, Tijero, Müller, Yuan, Munné-Bosch, 2020).

Material and Methods

The upland cotton- *Gossypium hirsutum* Agdash-3 genotype, which was obtained from the Genetic Resources Institute of the Ministry of Science and Education, Republic of Azerbaijan, was used for the tests. Cotton genotype seeds were placed in 7 cm plastic cups filled with perlite after being pre-treated for eight minutes with 0.2% potassium permanganate. In a growth chamber, the

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seedlings were cultivated at 22–24°C, 65–75% humidity, and 4800 lux of light. The duration of the lighting was 12/12 hours, day and night. Throughout the whole growth period, beginning with the initial days of seedling formation, Steiner's solution was utilized as a nutritional medium with the addition of 150 ppm and 300 ppm of GA_3. Analysis was done on embryonic cotyledons that were three weeks mature. We also used a mixture of GA_3 and NaCl (8.7 g of salt per 1 liter in each stage) in the first, second, and third stages to measure how these components affect plant growth (Ahmad, Kamal, Singh, Ashfaque, Alamri, Siddiqui, Khan, 2021).

Catalase activity-The Mosheva gasometric approach was used to measure catalase activity (Mosheva, 1982). After measuring half a gram of plant product, 20 ml of purified water and 0.5 g of $CaCO_3$ were gradually added to homogenize the mixture. Catalase activity was then tested when the extract was moved to an Erlenmeyer flask, a Landolt vessel with a side neck. Five milliliters of 3% were added to the extract to initiate the reaction, and a magnetic stirrer was used to mix it continuously. After two minutes, the measurement was completed. Following this period, the burette scale san be used to read the amount of oxygen emitted. The pure sample's enzyme activity was measured in milliliters per second*g (Amrahov, Mammadova, Allahverdiyeva, Aliyev, Alizada, Aghazada, Ojagverdiyeva, Mammadov, 2023).

Peroxidase activity-In reference to Chance and Maehly (Chance & Maehly, 1955), peroxidase activity was measured. In an ending amount of 4 ml, the reaction mixture contained 500µl of enzyme extract, 0.1 M Na-phosphate buffer, pH 7,2, 1 mM EDTA, 30 mM H2O2, and 50 mM guaiacol. At 440 nm, tetraguaiacol production was observed. The extinction value of tetraguaiacol (26.6mM −1 * cm−1) was used to determine the concentration of the compound. The expression for POD activity was $\Delta A590 * g^{-1} * min^{-1}$.

Polyphenol oxidase activity-Polyphenol oxidase activity was measured by tracking the oxidation of a 0.05 M catechol solution at 590 nm. The reaction took place in a 0.1 M potassium phosphate buffer at pH 7.2, following the method described by Yermakov et al. (1987). The enzyme activity was expressed in units per minute per gram of fresh weight (U/min*g FW).

Chlorophyll a, b, and carotenoids-The Wellburn method was used to determine the levels of carotenoids, chlorophyll a, and b (Wellburn and Lichtenthaler, 1984). In a mortar, 20 milliliters of 80% acetone were mixed with one gram of freshly cut leaf tissue that had been sliced into tiny pieces. A filter paper of F grade was next applied to filter the homogenate. The resultant filtrate's optical density was measured at 440, 644, and 662 nm. The results were measured in mg/g of fresh material (Wellburn, Lichtenthaler, 1984).

Results

Under various conditions, such as salt stress (150 mM NaCl), and gibberellic acid (GA) administrations (150 ppm and 300 ppm), the catalase activity (ml/sec*g) is illustrated in this bar chart. Furthermore, it shows the relationship between GA and salt stress to comprehend the possible moderating effects of GA on the catalase activity of the Agdash-3 genotype. (Fig 1)

Treatment with 150 mM NaCl significantly reduces the catalase activity, which is 40% lower than the control. In contrast, treatment with 150 ppm GA_3 increases catalase activity to around 85% of the control phase, which is greater than salt-stressed plants but a few less than the control. Though not as noticeable as with 150 ppm GA_3, 300 ppm GA_3 shows a small increase in activity and approaching 75% of the control level when comparing with plants during NaCl stress. Consequently, 150 ppm GA_3 restores enzyme activity at its peak and successfully opposes the negative effects of stress caused by NaCl. In terms of lowering salt stress, this last condition suggests that 150 ppm GA_3 is more advantageous than 300 ppm GA_3 since it results in a greater recovery in catalase function ninety percent of control in comparison to the 300 ppm GA_3 phase (Fig 1) (Al-Harthi, Bafeel, El-Zohri, 2021).

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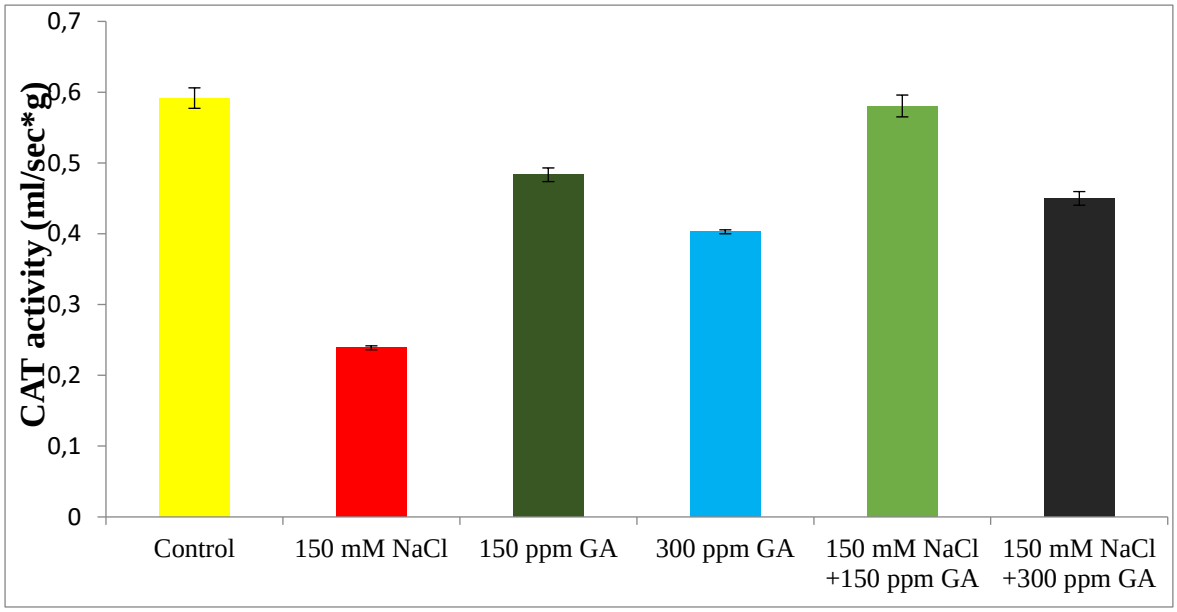

Fig 1. Catalase (CAT) activity in Agdash-3 genotype exposed to various concentrations of GA and NaCl

This bar chart exhibits peroxidase activity for several conditions. By converting hydrogen peroxide (H_2O_2) into oxygen and water, peroxidase, called POX, an essential defense enzyme, aids plants in coping with damage caused by oxidative stress. (Fig 2)

150 mM NaCl significantly lowers the lowest level of activity of peroxidase in every approach (50–60% drop compared to control). At 150 mM NaCl, peroxidase activity significantly decreases (by roughly 50%). However, the 150 ppm GA_3 dosage significantly increases peroxidase activity (by around 20-30%) in comparison to the salt-stressed category. Peroxidase activity dramatically increased in the 150 mM NaCl + 300 ppm GA_3 method comparison with all other procedures, surpassing the control by 10 to 20 percent. This indicates that the maximum stimulation of peroxidase activity occurs at a higher GA_3 level (300 ppm GA_3) under saline circumstances (Achard, Gong, Cheminant, Alioua, Hedden, Genschik, 2008). (Fig 2)

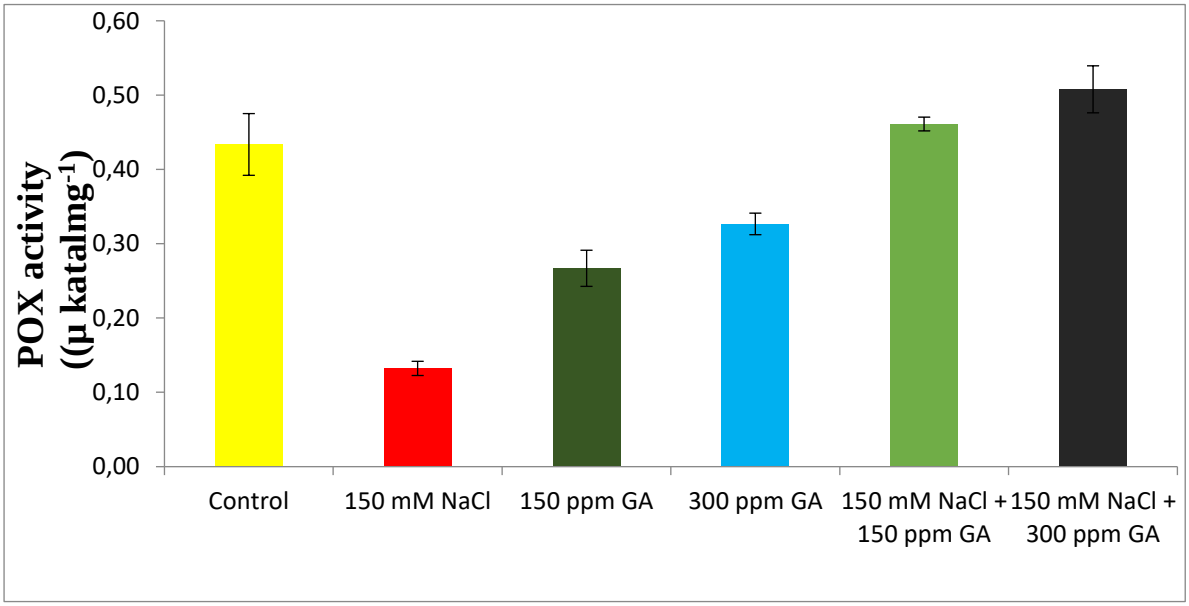

Fig 2. Peroxidase (POX) activity in the Gossypium hirsutum Agdash-3 genotype faced with varying levels of GA and NaCl

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This bar graph displays the activity of polyphenol oxidase (PPO). PPO is a specific enzyme that is essential to plant defense systems, especially when plants are under stress. It does this by accelerating the conversion of polyphenols to quinones, that can protect plants from infections and oxidative stress. (Fig 3) (Misratia, Ismail, Hakim, Musa, Puteh, 2013).

Among of each of the treatments, 150 mM NaCl (red bar) exhibits the largest increase in PPO activity, rising by almost 25% in comparison to the control. This means a salt unbalance stimulates an effective PPO reaction by increasing the stress response and engaging the protective mechanism. With 150 ppm GA_3, polyphenol oxidase (PPO) activity clearly drops about fifteen percent, but with 300 ppm GA_3, it increases by roughly 10%, reaching levels that are nearly identical to the control. In comparison to 150 mM NaCl alone, PPO activity falls by eight to twelve percent in the final two treatments, 150 mM NaCl + 150 ppm GA_3 and 150 mM NaCl + 300 ppm GA_3. This suggests that GA_3 supports in controlling PPO activity during salt stress, avoiding over-activation while preserving the plant's defensive reaction. (Fig 3) (Chance, Maehly, 1955).

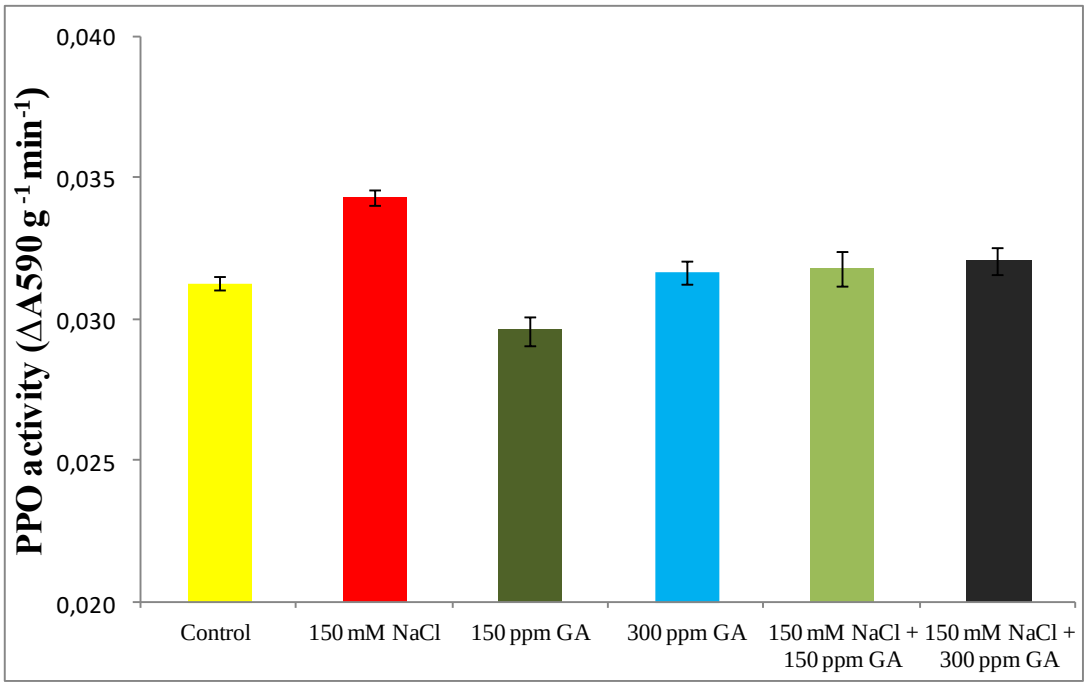

Fig 3. The activity of polyphenol oxidase (PPO) of Gossypium hirsutum Agdash-3 genotype exposed to different concentrations of GA and NaCl

The amounts of carotenoids, chlorophyll a, and chlorophyll b (in µg/ml) under various conditions have been shown in this graph. (Fig 4)

In contrast to chlorophyll b and carotenoids, the chlorophyll a level in this figure demonstrated a strong favorable influence of GA_3 and NaCl. Chlorophyll a levels declined by roughly 22 percent during 150 mM NaCl stress in comparison to the control. However, as in comparison with NaCl stress alone, the amount of chlorophyll raised by 18% when 150 ppm GA_3 was added, and by 28% when 300 ppm GA_3 was added. Under all circumstances, levels of carotenoid and chlorophyll b stayed largely constant, with very slight variations of between five and ten. Approximately 30% of the pigment was lost overall due to salt stress, however up to 40% of the missing pigment was recovered with the use of 300 ppm GA_3. Nevertheless, the combination of high GA_3 and NaCl stress further decreased pigment content, most likely as a result of overstress. (Fig 4)

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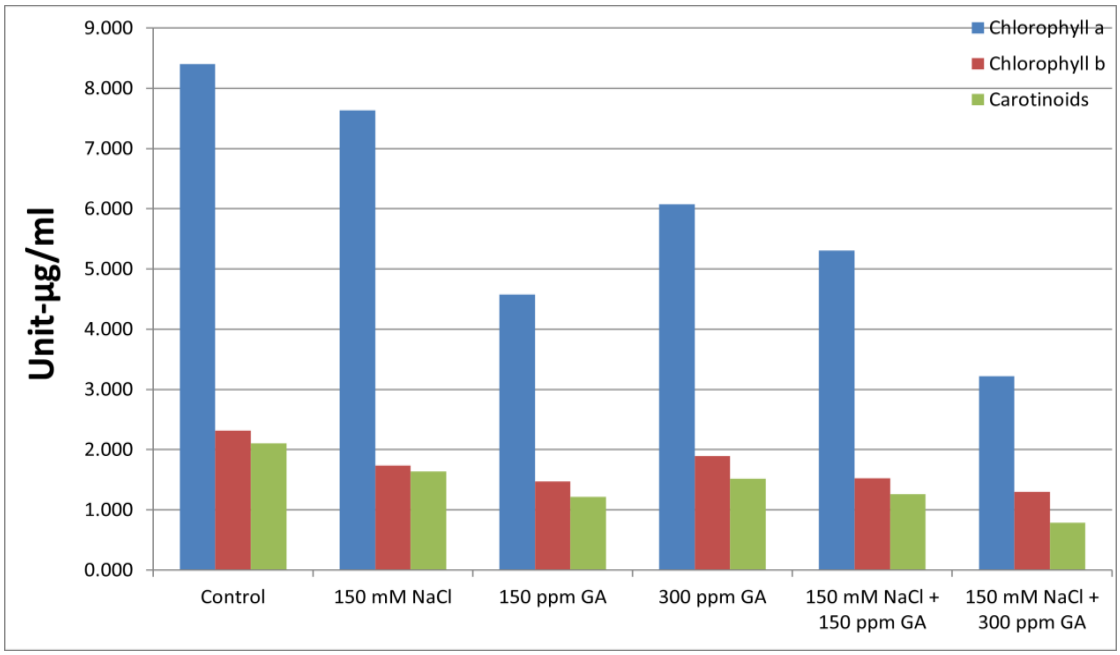

Fig 4. The impact of varying GA and NaCl concentrations on the levels of carotenoids, chlorophyll a, and b in the Gossypium hirsutum Agdash-3 genotype.

Investigating the impact of 150 mM (NaCl) and 150,300 ppm (GA_3) concentrations of GA_3+NaCl on the morphological characteristics and antioxidant enzymes of cotton plants was the goal of this research. We aimed to find out how these substances affect plant development and antioxidant enzyme activity.

Following phytohormone therapy, analyses were conducted to measure the length of the roots and shoots as well as the activity of the enzymes.

Fig.5. Impact of NaCl and NaCl+GA_3 on the development of AP-317 cotton genotype.
Table 1. Root and shoot length of the AP-317 genotype.

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Treatment	Root Length (Mean ± SD)	Shoot Length (Mean ± SD)
Control	12.617 ± 1.66	29.711 ± 2.62
150 mM NaCl	8.815 ± 0.90	14.353 ± 0.22
150 ppm GA	8.708 ± 1.30	35.764 ± 0.83
300 ppm GA	4.946 ± 0.60	29.255 ± 3.22
150 mM NaCl + 150 ppm GA	6.731 ± 1.15	18.049 ± 0.34
150 mM NaCl + 300 ppm GA	5.612 ± 0.24	13.799 ± 1.28

This table shows that plant growth had been affected by salt stress, which reduced root length and substantially inhibited shoot elongation. This demonstrates how salt inhibits the growth of plants. Shoot length dramatically rose at 150 ppm GA_3, greater than especially the control group. But like the NaCl treatment, the root length decreased. According to the concentration of 300 ppm, too much GA_3 promotes shoot growth but inhibits root elongation. In the treatment of GA_3 (150ppm) with NaCl (150 mM) showed that GA_3 did not mitigate root growth inhibition and obviously salt-stressed plants were not improved by high GA_3 (300 ppm) concentrations. (table 1) (Mosheva, 1982).

Discussion

Our consequences showed that gibberellic acid (GA_3) modulates enzymatic antioxidant activity and preserves chlorophyll and carotenoid levels under salt stress scenarios, which enhances the salinity tolerant of cotton Agdash-3 genotype. The results are consistent with other research that highlights the role of plant hormones in reducing the negative impacts from stress and maintaining development across salty conditions (Rady et al., 2021).

The differential effect of GA_3 on the antioxidant enzyme function was one of the main conclusions. When compared with plants exposed to NaCl stress solely, the introduction of GA_3, especially at 150 ppm, resulted in an important restoration in catalase and when 300 ppm GA_3 (with NaCl) was added, peroxidase activity increases while catalase activity decreases, suggesting that peroxidase carries the majority of the 300 ppm GA_3 concentration's load. (Fig 1 and 2). Furthermore, 150 ppm GA_3 restored catalase activity better than 300 ppm, demonstrating that there is an ideal concentration at which GA_3 management would not provide any more advantages. Miceli et al. (2019), who documented a comparable pattern in other crop varieties, support these findings (Yermakov, Arasimov, Yarosh, 1987).

PPO activity responded differently, with the maximum enzyme activity being induced by NaCl treatment independently (Fig 3). If PPO activity decreases, this indicates that GA_3 does not significantly affect PPO. An increase in PPO activity under salinity conditions indicates an increase in quenols. However, treatment with GA_3 stabilizes this amount (Fig 3). According to Yermakov et al. (1987), PPO's function in stress reduction and protection against oxidative damage is consistent with its increased activity under salt stress. The decrease in PPO activity following GA_3 therapy, however, raises the possibility that gibberellic acid controls PPO levels to avoid increased oxidation and preserve cellular homeostasis. Other research that focused on hormone-mediated stress adaptation in plants are consistent with GA_3's capacity to control PPO activity without over-activating it (Sharif et al., 2019).

Conclusion

The study also discovered that using GA_3 considerably reduced the decrease in chlorophyll a level based on salt stress (Fig 4). Although levels of carotenoid and chlorophyll b were mostly constant during treatments, GA_3 treatments particularly at 300 ppm helped to partially restore the amount of chlorophyll a (Fig 4). According to this finding, GA_3 could help to preserve photosynthetic efficiency under challenging circumstances by maintaining chlorophyll-protein

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complexes and lowering oxidative stress (Wellburn & Lichtenthaler, 1984). Nevertheless, overstimulation and elevated demands on the metabolism caused another drop in pigment level as a result of excessive GA_3 treatment, especially when linked with NaCl (Fig 4).

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