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Research Article

Impact of Plant Growth Regulators and Fungicides on Non-Enzymatic Antioxidant Defenses System in *Arabidopsis thaliana*

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ABSTRACT

Abiotic and biotic stresses induce oxidative damage in plants, leading to excessive production of reactive oxygen species (ROS). The non-enzymatic antioxidant defense system plays a crucial role in mitigating oxidative stress. This study evaluates the effects of selected plant growth regulators (PGRs) and fungicides on non-enzymatic antioxidant components in *Arabidopsis thaliana*. Plants were treated with gibberellic acid (GA₃), salicylic acid (SA), and fungicides (carbendazim and mancozeb), individually and in combination. Parameters such as ascorbic acid, reduced glutathione (GSH), total phenolics, and flavonoids were quantified. Results showed that SA significantly enhanced antioxidant levels, while fungicides exhibited both stimulatory and inhibitory effects depending on concentration. Combined treatments showed synergistic effects in certain cases, improving antioxidant accumulation. Statistical analysis revealed significant variation ($p < 0.05$) among treatments. These findings suggest that PGRs and fungicides modulate the antioxidant defense system and may improve stress tolerance in plants.

INTRODUCTION

Plants are continuously exposed to a wide range of environmental stresses, including abiotic factors such as drought, salinity, temperature extremes, and chemical exposure, as well as biotic stresses caused by pathogens. These stress conditions lead to the excessive generation of reactive oxygen

species (ROS), including superoxide radicals, hydrogen peroxide, and hydroxyl radicals. While ROS function as important signaling molecules at controlled levels, their overaccumulation disrupts cellular homeostasis, resulting in oxidative damage to lipids, proteins, nucleic acids, and membranes. To counteract this oxidative stress,

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plants have evolved a sophisticated antioxidant defense system comprising both enzymatic and non-enzymatic components. Among these, non-enzymatic antioxidants such as ascorbic acid, glutathione, phenolic compounds, and flavonoids play a crucial role in directly scavenging ROS, maintaining redox balance, and protecting cellular integrity under stress conditions.

Plant growth regulators (PGRs) are key signaling molecules that regulate plant growth, development, and stress responses. Compounds such as gibberellic acid (GA₃) and salicylic acid (SA) are known to influence physiological and biochemical pathways, including antioxidant metabolism. Salicylic acid, in particular, is widely recognized for its role in inducing systemic acquired resistance (SAR) and enhancing plant tolerance against both biotic and abiotic stresses through modulation of antioxidant defense mechanisms. Gibberellic acid, although primarily associated with growth promotion, has also been reported to indirectly influence stress tolerance by regulating metabolic activities and cellular expansion.

Fungicides, extensively used in agricultural systems to control fungal pathogens, may also exert significant effects on plant physiology beyond their primary protective role. Chemicals such as carbendazim and mancozeb can influence oxidative metabolism by either inducing mild stress or altering antioxidant enzyme activities and metabolite levels. While fungicides help reduce pathogen-induced oxidative damage, their direct interaction with plant metabolic pathways may result in either enhancement or suppression of antioxidant systems depending on concentration, exposure duration, and plant species.

Arabidopsis thaliana, a well-established model organism in plant biology, provides an ideal system for studying biochemical and molecular

responses due to its small genome, short life cycle, and ease of genetic manipulation. Despite extensive research on antioxidant systems, there remains a significant gap in understanding the combined effects of plant growth regulators and fungicides on non-enzymatic antioxidant defense mechanisms. Therefore, the present study aims to investigate the individual and interactive effects of selected PGRs and fungicides on key non-enzymatic antioxidants in *Arabidopsis thaliana*, thereby providing insights into their potential role in enhancing plant stress tolerance and improving crop management strategies.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The experiment was conducted using *Arabidopsis thaliana* (Col-0 ecotype) grown under controlled environmental conditions with a temperature of 22 ± 2°C, relative humidity of 60–70%, and a photoperiod of 16 h light and 8 h dark. Seeds were surface sterilized and germinated on nutrient medium, after which healthy seedlings were transferred to pots containing a standardized soil mixture.



Fig No.01

Experimental Design



A completely randomized design (CRD) was used with the following treatments:

Treatment Code	Description
T0	Control
T1	GA ₃ (100 ppm)
T2	SA (1 mM)
T3	Carbendazim (0.1%)
T4	Mancozeb (0.2%)
T5	GA ₃ + Carbendazim
T6	SA + Mancozeb

Plants were monitored regularly for growth and physiological responses throughout the experimental period. Sampling was carried out at a fixed interval (21 days after treatment) to capture the biochemical changes induced by the treatments. Fully expanded leaves were collected, immediately processed, or stored under appropriate conditions to prevent degradation of metabolites. The collected samples were then subjected to biochemical analyses for quantification of non-enzymatic antioxidants, including ascorbic acid, glutathione, phenolics, and flavonoids, using standardized protocols.

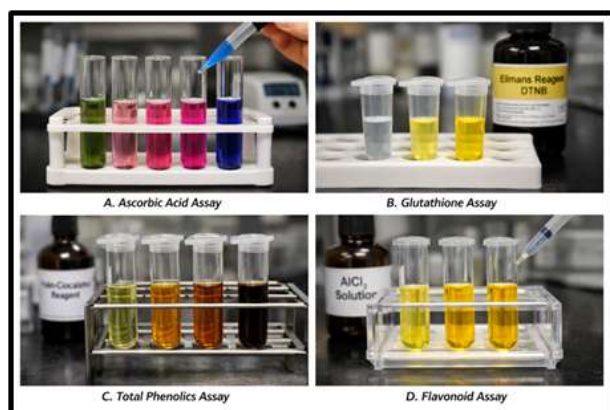


Fig no.02

Overall, the experimental setup was carefully structured to ensure uniform growth conditions, precise treatment application, and reliable data collection, thereby enabling accurate assessment of the individual and combined effects of plant growth regulators and fungicides on antioxidant defense mechanisms in *Arabidopsis thaliana*.

RESULTS

Effect on Ascorbic Acid Content

Treatment	Ascorbic Acid (mg/g FW)
T0	2.15 ± 0.12
T1	2.85 ± 0.15
T2	3.45 ± 0.18
T3	2.40 ± 0.11
T4	2.30 ± 0.13
T5	3.10 ± 0.16
T6	3.60 ± 0.20

Reduced Glutathione (GSH)

Treatment	GSH (μmol/g FW)
T0	1.80 ± 0.10
T1	2.20 ± 0.12
T2	2.90 ± 0.14
T3	2.00 ± 0.11
T4	1.95 ± 0.09
T5	2.50 ± 0.13
T6	3.05 ± 0.17

Total Phenolics:

Treatment	Phenolics (mg GAE/g FW)
T0	5.50 ± 0.30
T1	6.80 ± 0.35
T2	8.20 ± 0.40
T3	6.00 ± 0.28
T4	5.90 ± 0.32
T5	7.20 ± 0.36
T6	8.50 ± 0.45

Flavonoids content:

Treatment	Flavonoids (mg QE/g FW)
T0	3.20 ± 0.18
T1	4.10 ± 0.20
T2	5.30 ± 0.25
T3	3.60 ± 0.19
T4	3.50 ± 0.17
T5	4.50 ± 0.22
T6	5.60 ± 0.28

DISCUSSIONS

The present study clearly showed that plant growth regulators and fungicides significantly influence the non-enzymatic antioxidant defense system in *Arabidopsis thaliana*. Among the treatments, salicylic acid (SA) exhibited the most pronounced effect, leading to a substantial increase in ascorbic acid, glutathione, total phenolics, and flavonoids. This enhancement can be attributed to the well-established role of SA in activating defense signaling pathways, particularly systemic acquired resistance, which stimulates the biosynthesis of antioxidant metabolites through pathways such as the phenylpropanoid pathway. Gibberellic acid (GA₃), although primarily associated with growth promotion, also contributed to moderate increases in antioxidant levels, suggesting its indirect involvement in stress modulation and metabolic regulation.

Fungicide treatments showed differential responses, indicating their dual role in plant systems. Carbendazim exhibited a slight stimulatory effect on antioxidant accumulation, possibly due to mild stress induction that activates defense mechanisms. In contrast, mancozeb displayed relatively lower antioxidant enhancement, suggesting potential oxidative stress at the applied concentration. Notably, the combined treatments revealed synergistic interactions, particularly in the SA + mancozeb treatment, which resulted in the highest levels of antioxidant compounds. This indicates that salicylic acid may counteract the oxidative effects of fungicides while simultaneously enhancing the plant's defense capacity.

The increased accumulation of phenolics and flavonoids across treatments highlights the activation of secondary metabolic pathways, which are crucial for ROS scavenging and stress adaptation. Similarly, elevated levels of ascorbic acid and glutathione suggest improved redox

homeostasis and enhanced detoxification capacity. Overall, the findings emphasize that modulation of non-enzymatic antioxidants is a key mechanism through which plants respond to chemical treatments and environmental stress. These results are consistent with previous studies reporting the role of signaling molecules and agrochemicals in regulating oxidative metabolism.

CONCLUSION

In conclusion, the present investigation confirms that plant growth regulators and fungicides play a significant role in modulating the non-enzymatic antioxidant defense system in *Arabidopsis thaliana*. Salicylic acid emerged as the most effective treatment, markedly enhancing the levels of key antioxidants such as ascorbic acid, glutathione, phenolics, and flavonoids, thereby improving the plant's capacity to mitigate oxidative stress. Fungicides demonstrated variable effects, with carbendazim showing mild stimulatory action and mancozeb exhibiting comparatively lower enhancement, indicating that their impact is concentration-dependent and may induce oxidative stress under certain conditions.

Importantly, the combined application of plant growth regulators and fungicides resulted in synergistic effects, suggesting that integrated chemical treatments can be strategically used to enhance plant stress tolerance. The study highlights the critical role of non-enzymatic antioxidants in maintaining cellular redox balance and protecting plants from oxidative damage. These findings provide valuable insights for improving agricultural practices and developing stress-resilient crops. Future research should focus on molecular-level investigations and field-based validation to further elucidate the mechanisms underlying these interactions and their practical applications in crop management.



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