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Research Article

Synergistic Application of Gibberellic Acid and *Pseudomonas Aeruginosa* for Sustainable Plant Growth under Drought Conditions

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ABSTRACT

The present study investigates the influence of water stress and inoculation with plant growth-promoting rhizobacteria (PGPR), specifically *Pseudomonas aeruginosa*, as well as the interaction of gibberellic acid (GA3) with PGPR on mung bean (*Vigna radiata*). The experiment was carried out at the University of Karachi to evaluate approaches for improving crop production under water stress conditions. The results indicate that PGPR inoculation and GA3 application help mitigate the negative effects of drought stress on tested plants. PGPR colonization of the rhizosphere enhanced plant growth and antioxidant responses, while GA3 treatment proved beneficial for cell elongation and chlorophyll contents. The combination of PGPR, drought and GA3 treatments showed the best performance, suggesting that these methods can be effectively used to increase crop productivity in water-stress environments. These findings contribute to understanding plant adaptation strategies and provide potential solutions for sustainable agriculture in dry and semi-arid areas.

Keywords: Detoxification strategies; Mycotoxin contamination; Moisture content analysis; Thin Layer Chromatography; Sustainable food safety

INTRODUCTION

Water stress is a major constraint for the growth and yield of crops, particularly in arid and semi-arid areas, causing severe economic losses in agriculture. This kind of abiotic stress modifies plant water relations at the cellular and whole-plant level and therefore leads to specific and nonspecific reactions and harm. It has been demonstrated that inoculation of plants with native beneficial microorganisms can increase drought-tolerance of crops in such regions (Seleiman et al., 2021).

As per the research of Zhong et al. (2025), Gibberellic acid (GA3) has been applied by root absorption and its treatment has been proven to increase the elongation zone length by increasing both the number of cells and the final cell length. However, De Souza and MacAdam (2001) pointed out that in dwarf leaves, the shorter elongation zone is mainly the result of a decrease in final cell length. Although elongation zone lengths are different among dwarf, control, and GA3-treated leaves blades, an inverse correlation between gibberellic acid content and peroxidase activity has been recognized for a long time. For example, application of gibberellic acid to dwarf maize leaves or to internodes of the pea (*Pisum sativum*) resulted in elongation and the alteration of the activity of certain peroxidase isozymes, but not in an overall alteration of the complement of peroxidase isozymes in the tissue (Sardoei et al., 2024). Furthermore, Hoagland and Boyette (2024) mentioned that gibberellic acid may promote growth by increasing cell elongation in certain plant species and by increasing both cell elongation and cell division. The rhizosphere is a thin layer of soil that surrounds plant roots, plays an important role in root function and metabolism (Garcia et al., 2001). A multitude of micro-organisms are present in this area, including bacteria, fungi, protozoa and algae, but the bacteria are the predominant ones.

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Plant species selectively recruit bacterial species that promote their vigor by secreting organic compounds in the form of root exudates (Bhattacharyya and Furtak, 2022). As bacteria are abundant and competitive for root colonization, they must be exerting a major influence on plant physiology (Barriuso et al., 2008). Moreover, rhizospheric associations between plants and microbes are a major determinant for plant health and soil fertility (Mulugeta, Gelaw and Rabuma, 2025).

A number of environmental stresses, including drought and salt stress, impair the electron transport system and lead to the accumulation of reactive oxygen species (Kanojia and Dijkwel, 2018). However, H_2O_2, O_2 and OH generated as activated oxygen radicals are susceptible to accumulation under water deficit stress, which can cause damage to the photosynthetic apparatus. Oxidative damage in plant is suppressed by antioxidants including superoxide dismutase (SOD), ascorbate peroxidase, ascorbic acid and glutathione (Mishra et al., 2023). These reactive species can cause damage within chloroplasts, leading to a cascade of damaging effects such as destruction of chlorophyll, lipid peroxidation and loss of proteins (Kesawat et al., 2023). Leguminous crop such as mung bean (*Vigna radiata*) also called moong bean/green gram is a native to the Indian subcontinent and are grown mainly in India, China and Southeast Asia as well as in hot, dry areas of Southern Europe and the Southern United States. They are consumed in both savory and sweet dishes and are a rich source of protein, fiber, antioxidants and phytonutrients (Diatta et al., 2024).

While they are not as widespread as some other types of beans, like chickpeas and black beans, mung beans can be extremely healthy. The mung bean was domesticated in Persia (modern-day Iran), where its wild progenitor (*Vigna radiata* subsp. *sublobata*) is found (Sangiri et al., 2007). This study demonstrated effect of drought stress on mung bean plants could be alleviated by inoculation with PGPR and treatment with GA3 to increase the growth and antioxidant activities of mung bean plants.

MATERIALS AND METHODS

A pot experiment was conducted at the greenhouse of the Department of Botany, University of Karachi, Pakistan, to compare the growth of mung beans in different treatments under drought stress conditions. Twenty clay pots were used in these experiments in which for each treatment (Control, Drought, PGPR, GA3 and Drought+PGPR+GA3) four replicates were used. PGPR treatments were provided at the rate of10 ml after sowing seeds, and then waited for the plants to become about 1.5cm tall or the two-leaf stage. Plants were allowed to grow under normal growth conditions and regularly water was given for whole month.

Total chlorophyll and Carotenoid analysis

Total chlorophyll and carotenoids were extracted by boiling 0.05 g of tested leaves material chopped into small pieces in 5 mL of 80% acetone. The supernatant was collected and the absorbance of the supernatant was measured at 663 nm, 645 nm and 470 nm respectively. The concentrations of total chlorophyll and carotenoids (mg/g fresh weight of leaves tissue) were then calculated from the following formula (Longjam et al., 2018).

$$Chl\ a: 11.75 \times A_{663} - 2.35 \times A_{645}$$

$$Chl\ b: 18.61 \times A_{645} - 3.96 \times A_{663}$$

$$\text{Carotenoid } (_{X+C}) = \frac{1000 \times A_{470} - 2.27(Chl\ a) - 81.4(Chl\ b)}{}$$

Carbohydrate

For carbohydrate content, 0.05 g of leaves tissue was crushed in Tris buffer, centrifuged and the supernatant was collected. In the case of analysis, 1 mL of the extract was added to 2 mL of water and 5 mL of anthrone reagent and boiled for 10 min at 100°C and cooled. The absorbance was taken at 620 nm and the carbohydrate concentration was estimated by reference to a standard curve. This method can be used to quantify total carbohydrate in the leave samples (Hamid et al., 2011).

Protein

About 0.01 g of leaves tissue was mixed in 3 mL of chilled phosphate buffer and centrifuged at 5000 rpm for 10 minutes. The supernatant was taken and 1 mL of extract was mixed with 2ml Bradford reagent. The mixture was left and incubated at room temperature for a period of 5 minutes before the absorbance was read at 595 nm (Rahman et al., 2025a).

Phenolic content

The total phenolic content was calculated using the Folin Ciocalteu reagent (Rahman et al. 2016) with slight modification. 0.01g leaves tissue in small pieces was crushed in 5ml (80%) ethanol, then centrifuged it at 3000 rpm for 10 minutes. Collect the

supernatant, then take 1ml of diluted extract, add 5ml of Folin reagent and 4ml of sodium carbonate. Incubate at room temperature at 30°C in dark conditions and then note the O.D. at 795nm. Standard curve was prepared with gallic acid for the calculation of phenolic content in µg mL-1.

Peroxidase activity

Peroxidase activity (POD) was calculated using the method by Rahman et al. (2025b). The enzymes were obtained by grinding 100 mgmL−1 fresh leaf tissues in a cooled 0.1 M phosphate buffer (pH 6.5) and centrifugation at 5000 rpm for 15 minutes. The sample extract (0.1 mL) was combined with a solution of pyrogallol (3 mL, 0.05 M), and the spectrophotometer was calibrated to zero at 430 nm. Subsequently, 0.5 mL of a 1% H2O2 solution was included, and the the variation in optical density was measured for as long as 3 min.

H_2O_2 scavanging activity (%)

The leaves sample's ability to scavenge hydrogen peroxide was assessed using the method outlined by Raman et al., (2021). Prepared a 0.1 M phosphate buffer solution with a pH of 7.4. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer. Leaf aliquots of 10 mg mL-1, were combined with 0.6 mL of the H_2O_2 solution (40mM) then adjust the final volume to 3 mL. The absorbance of the reaction mixture was measured at 230 nm on spectrophotometer. Phosphate buffer lacking $H_2O_2$was used as a blank.

RESULTS AND DISCUSSION

The results demonstrated that the application of PGPR significantly enhanced the dry root weight, shoot weight and leaves number of mung bean plants as compared with the control treatment. Similarly, GA3 treatment also improved plant growth parameters; however the combined application of PGPR and GA3 exhibited the highest increase in overall plant growth, particularly in fresh root and shoot biomass. In contrast, drought-stressed plants showed a marked reduction in all growth parameters, indicating the detrimental effects of water deficit on mung bean growth and development (Figure 1).

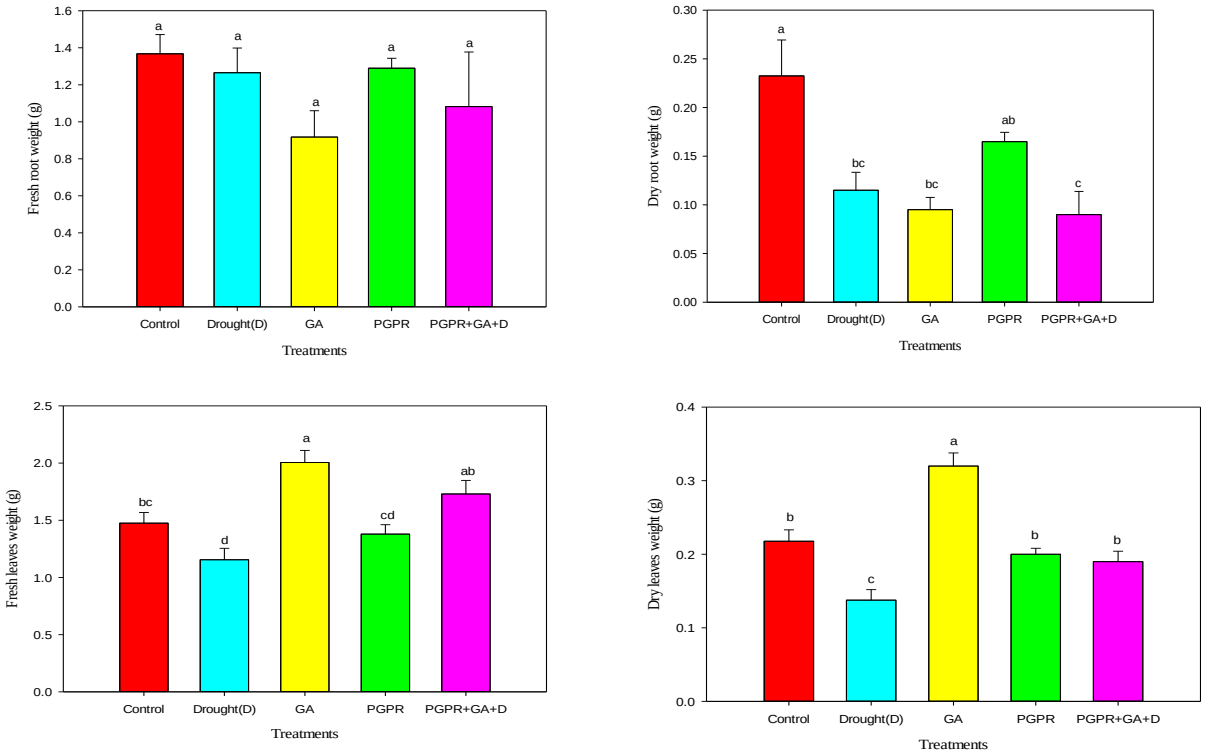


Figure 1. Effect of PGPR and GA3 on growth parameters of mung bean (*Vigna radiata*) under drought stress.

Photosynthetic pigment analysis revealed that chlorophyll a and b contents were highest in plants

treated with GA3 alone, whereas carotenoid content remained comparatively lower. The improvement in chlorophyll content may be associated with the stimulatory effect of GA3 on plastid ultrastructural development, chlorophyll preservation and delayed leaves senescence. These findings were supported by the increased net photosynthetic rate observed in GA3 treated tested plants, followed by PGPR as compared to the control. Moreover, the combined treatment of PGPR and GA3 under drought stress resulted in the maximum accumulation of photosynthetic pigments relative to drought-stressed plants. Conversely, drought treatment alone significantly reduced chlorophyll content in mung bean plants during vegetative growth (Figure 2).

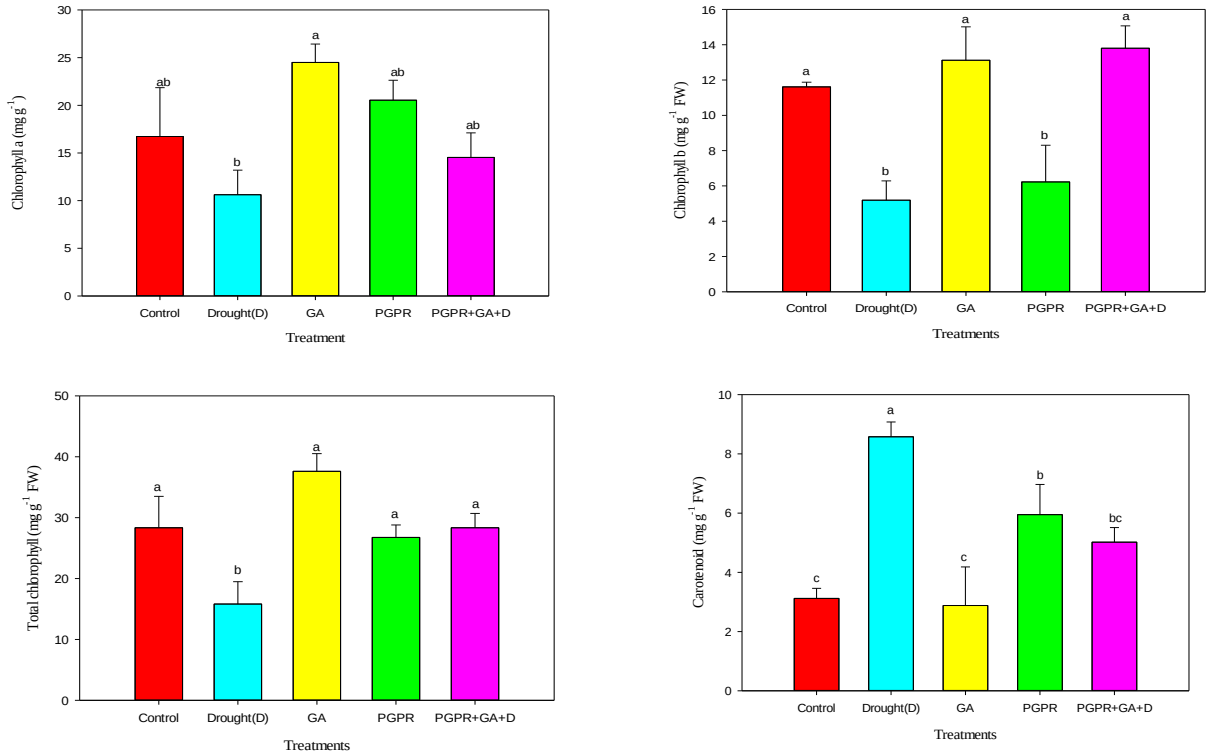


Figure 2. Effect of PGPR and GA3 on photosynthetic pigments of mung bean (*Vigna radiata*) under drought stress.

It also reduced chlorophyll concentration under drought stress may impair light-harvesting efficiency and photosynthetic activity ultimately affecting plant productivity. Biochemical analysis further indicated that GA3 treatment alone significantly increased carbohydrate, phenolic and protein contents while reducing H_2O_2 accumulation, followed by PGPR treatment. However, the combined application of PGPR and GA3 under drought stress conditions showed the best improvement in plant survival and stress tolerance as compared with individual treatments (Figure 3). These findings suggest that the synergistic interaction between PGPR and GA3 effectively mitigates the adverse effects of drought stress by enhancing photosynthetic efficiency, osmolyte accumulation and antioxidant defense mechanisms in mung bean plants. Therefore, the combined use of PGPR and GA3 may serve as a promising strategy for improving drought tolerance and sustaining crop productivity under water-limited conditions.

The present results demonstrate that drought stress severely impairs mung bean growth and physiology, but PGPR and GA3, particularly in combination, alleviate these negative effects. The reduction in growth parameters under drought is attributed to decreased cell turgor and photosynthetic inhibition (Song et al., 2025). In this study increased root weight under drought is a classic drought avoidance strategy to access deeper water while, single GA3

application had little effect on roots, which aligns with reports that gibberellins primarily target shoot growth and leaf expansion (Mander & Liu 2010). Kumar et al., (2022) demonstrated that PGPR can enhance crop yields by promoting drought stress tolerance through hormone production or by adjusting plant performance. PGPR likely improved plant growth through IAA production, phosphate solubilization, and ACC deaminase activity, which lowers stress ethylene (El-Saadony et al., 2024). Similarly, GA3 promotes cell elongation and division, thus partially reversing drought-induced growth inhibition (Litvin et al., 2016).

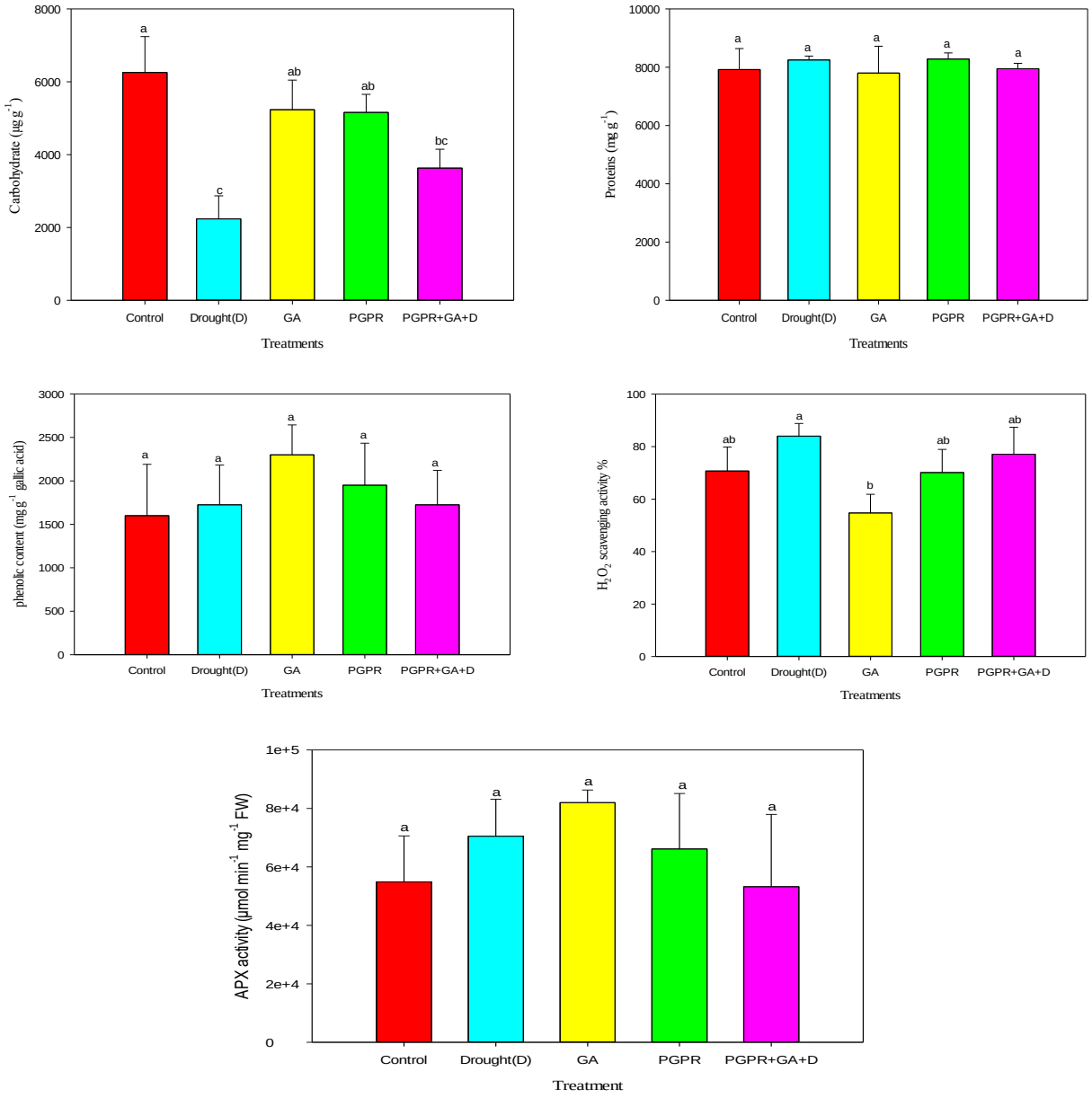


Figure 3: Effect of PGPR and GA3 on physio-biochemical parameters of mung bean (*Vigna radiata*) under drought stress.

Results showed that higher photosynthetic pigments (chlorophyll a, b) were recorded in GA3 application followed by PGPR inoculation as compared to control plants. Our results supported by researchers who revealed that PGPR maintains chlorophyll by producing antioxidants and siderophores that improve Fe nutrition (Vaghela et al., (2025), while GA3 delays senescence by upregulating chlorophyll biosynthesis

genes (Wang et al.,2025). These measures counteract the chlorophyll breakdown and stomatal closure triggered by drought, promoting improved photosynthetic efficiency. Maximum carotenoids content was noted in drought stress as compared to all treatments. Rady et al., (2021) explained that carotenoids are vital pigments that act as antioxidants, safeguarding the photosynthetic system, especially during water scarcity when chlorophyll concentrations frequently decline. Cai, et al., (2024) reported that the external application of gibberellins enhances carotenoid concentrations in plants facing drought stress, aiding in better stress resistance. The drought-induced plant had the lowest carbohydrate (CHO) content, while all other treatments exhibited the highest amounts in our research. This increment of carbohydrate content indicated as these treatments serve as a protective measure to sustain metabolic processes, improve water uptake, and boost resilience, thereby averting the swift loss of carbohydrates that happens in untreated plants under drought stress (Admassie et al., 2022).

Generally protein content was reduced during drought stress due to closure of stomata and declined of photosynthetic machinery (Haghpanah et al., 2024), but mistakenly it was found higher in drought induced plant as well as in all treatments. GA3 application leads to increased protein content because it serves as an anti-stress agent, enhancing the production of osmoprotectants, antioxidants, stress-adaptive enzymes and sustaining metabolic activity in drought stress (Lamlom et al., 2025). Furthermore, Lim and Kim (2013) found same result that protein levels are elevated in PGPR-inoculated plants under drought stress mainly because these bacteria promote tolerance strategies by stimulating stress-adaptation protein synthesis and minimizing metabolic disturbances. These bacteria enhance plant protein production by increasing nutrient and water absorption and releasing protective compounds that inhibit protein degradation.

Plants possess a comprehensive defense mechanism of antioxidant enzymes that eliminate excessive ROS to safeguard the photosynthetic system and improve resilience to stress (Rahman et al., 2016). GA functions as a remarkable antioxidant that improves plants' capacity to endure different biological and abiotic stresses (Bao et al., 2020), while PGPR assist plants in strengthening their antioxidant defense mechanisms to fight against oxidative stress (Jakubowska et al., 2025). In this study, the amount of phenolic content and ascorbate peroxidase (APX) was greater in alone and Co-application of GA3 and PGPR as contrasted to control. This increased level of total phenols and the enhanced function of ascorbate peroxidase (APX) in plants exposed to both gibberellic acid and plant growth-promoting rhizobacteria (PGPR) under drought stress represent a synergistic defensive strategy. These therapies function collaboratively to reduce oxidative harm and improve the plant's physiological adaptability (Fu et al., 2023; Jabborova et al., 2021).

Maximum hydrogen peroxide (H_2O_2) scavenging activity was measured in drought stressed plant to mitigate excessive oxidative damage resulting from the overproduction of reactive oxygen species (ROS) (Bhardwaj et al., 2021). The reduced H2O2 scavenging activity was assessed in GA3 and PGPR inoculated plant under drought stress suggests a diminished requirement for excessive antioxidant production due to lower oxidative damage in the plants. This reduced scavenging activity signifies that the mitigation strategy of plant was so effective that they produce less ROS, in comparison to stressed plants that were not treated with any treatment (Yadav et al., 2024; Vurukonda et al., 2016).

We concluded that PGPR and GA3, alone and in combination, improved mung bean growth, photosynthesis, and antioxidant defense under drought stress. The combined application showed the strongest effect, indicating synergism between microbial and hormonal treatments. This integrated approach offers a practical, sustainable option to enhance crop productivity and resilience in water-limited environments threatened by climate change.

CONCLUSION

The researcher found that the application of PGPR and GA3 in alleviating the adverse impact of drought stress on mung bean plants. The findings indicate that the plant growth and antioxidant reaction, as well as the photosynthetic ability can be improved with the help of the colonization of the plants by the application of PGPR and the use of GA3 under the conditions of water scarcity. However, the performance of the combination of the treatment

factors was greatest with the combination of both the treatment of the drought stress and the GA3, suggesting that the combination of the two integrated treatments can actually perform well in the case of enhancing the crop productivity in the water-stressed condition. The results are important to the knowledge of plant adaptation mechanisms and can provide possible solutions for sustainable agriculture in the dry and semi-arid areas. Furthermore, the study identifies possibilities to enhance crop resilience and productivity by using positive microorganisms and plant growth regulators to meet an increasing common and severe frequency of droughts as a result of climate change. The holistic management approaches discussed in this research has the potential to improve food security and advance agricultural systems that are eco-friendly.

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