

Research

The Role of Bacterial and Fungal Microbiomes in Enhancing Plant Tolerance to Environmental Stresses

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Abstract

In this study, a completely randomized design (CRD) in four treatments was applied: non-inoculated (control), PGPR-inoculated, fungal-inoculated and PGPR-fungal co-inoculated. The study showed a substantial increase in growth attributes of inoculated plants over the control. Plant height increased from 10.5 ± 0.6 cm to 20.3 ± 1.0 cm, root length from 8.2 ± 0.5 cm to 17.8 ± 0.9 cm, fresh weight from 2.0 ± 0.2 g to 5.4 ± 0.5 g, and dry weight from 0.40 ± 0.05 g to 1.20 ± 0.08 g. Inoculation with *B. licheniformis* also lowered the levels of oxidative stress markers, with MDA content dropping from 6.9 ± 0.3 to 2.5 ± 0.1 nmol/g FW and H_2O_2 content from 5.8 ± 0.2 to 2.3 ± 0.1 μ mol/g FW. Antioxidant enzyme activities were markedly enhanced, with SOD increasing from 24.5 ± 1.2 to 60.5 ± 2.0 U/mg protein, CAT from 18.0 ± 1.0 to 55.2 ± 1.8 U/mg protein, and POD from 19.5 ± 1.1 to 58.0 ± 1.7 U/mg protein. Physiological characteristics were also significantly enhanced, with proline content increasing from 2.0 ± 0.1 to 6.0 ± 0.3 μ mol/g FW and chlorophyll content from 1.3 ± 0.1 to 3.1 ± 0.2 mg/g FW. Root colonization studies validated the establishment of the inoculants, with CFU increasing from undetectable levels in the control to $5.6 \pm 0.3 \times 10^6$ g⁻¹ soil in the co-inoculated plants. These results indicate that combined PGPR and beneficial fungi inoculation is a feasible and sustainable approach to improve plant tolerance to stress under challenging environmental conditions.

Keywords: Plant Microbiome, Fungi, Drought Stress, Salinity Stress, Oxidative Stress, Antioxidant Enzymes

1. Introduction

Abiotic stresses like drought and salinity are significant limitations to agricultural production across the world. Such stresses negatively impact the growth and physiology of plants, resulting in lower yields and poor product quality [1]. While drought stress leads to limited availability of water, salinity stress causes ion imbalance and osmotic stress, both of which lead to oxidative stress in plants due to an excess of ROS [2].

In order for these plants to thrive under these stressful circumstances, they have been able to develop a range of physiological and biochemical defenses, including antioxidant protection, osmoprotective substances like proline, and stress-regulated gene activity. Unfortunately, such natural defense systems may not always be enough in order to provide sufficient protection [3].

During recent times, there has been an increase in research concerning the plant microbiome, which refers to those bacteria and fungi that occur within the rhizosphere and plants themselves. It has been seen that these microorganisms have a very important role in improving plant growth and stress tolerance [4]. The plant growth promoting rhizobacteria (PGPR), which include species like *Bacillus subtilis* and *Pseudomonas fluorescens*, help increase nutrient absorption, synthesize plant hormones, and affect the hormonal status of plants. On the other hand, the beneficial fungi, like *Trichoderma harzianum* and arbuscular mycorrhizal fungi, help develop the roots of plants [5].

In addition, microorganisms associated with plants play an important role in reducing oxidative stress through increased production of antioxidative enzymes, including SOD, CAT, and POD enzymes, which reduce the harm done by reactive oxygen species on plant cells. Also, they aid in the production of metabolic products like proline while ensuring chlorophyll synthesis [6].

It is now well understood that symbiosis between plants and the microorganisms associated with them plays a significant role in adapting to environmental stresses. There is evidence to show that inoculation with both bacteria and fungi together yields synergistic benefits for plant growth, outperforming separate inoculations [7].

Hence, the current study seeks to explore the potential of plant-associated bacteria and fungi, separately and collectively, in boosting plant resilience against drought and salinity stresses. The current study endeavors to explore the morphological, biochemical, and microbiological responses in an attempt to ascertain the performance of the plant microbiome in alleviating stress conditions.

2. Materials and Methods

1. Study Design

The experimental work aimed at assessing the impact of the plant microbiome (comprising bacteria and fungi) on the stress resilience of plants to various environmental stresses such as drought and salinity. The plant groups used in the experiment included a control group without microbes, bacteria-inoculated, fungus-inoculated, and combination of both bacteria and fungi-inoculated plant groups. The groups of plants were then subjected to controlled environmental stresses that simulate drought and salinity conditions to determine the response and impact of the plant microbiome on stress resilience.

2. Plant Material

The seeds for the experiment were collected from an authentic agricultural source, guaranteeing that there was no contamination at the outset of the experiment that would alter the results. The seeds were subjected to surface sterilization according to a specific procedure, consisting of treating them with 70% ethanol for 1 minute and then with 1% sodium hypochlorite for 5 minutes. They were then washed extensively with distilled water [8].

3. Microbial Isolates

3.1 Bacterial Isolates

Plant Growth-Promoting Rhizobacteria (PGPR), including *Bacillus subtilis* and *Pseudomonas fluorescens*, were chosen because of their documented ability to promote plant growth and provide resistance to stress. These organisms were grown on nutrient agar plates maintained under sterile conditions at a temperature of 28°C for 24–48 hours to produce sufficient growth of bacteria [9].

3.2 Fungal Isolates

These beneficial fungi include *Trichoderma harzianum* and arbuscular mycorrhizal fungi. This is because of their vital contribution to plant development and their resistance to stresses. The fungi were grown on potato dextrose agar plates in a sterile manner, at suitable temperatures to ensure proper growth before being introduced to soil or seed [10].

4. Inoculation Procedure

The bacterial inoculum used in this study had an approximate concentration of 10^8 CFU/ml. The seeds were immersed in the bacterial inoculum for 2-4 hours before being planted. Fungi were thoroughly mixed with the soil prior to sowing of the seeds for easy establishment and mutual association with roots [11].

5. Growth Conditions

The plants were cultured in sterilized loam soil inside pots in order to prevent any possible contamination and ensure that there is accuracy during the experiment. These were the growth conditions used during the experiments: Temperature was set at $25\pm 2^{\circ}\text{C}$, photoperiod was 16/8 h of light/darkness, while watering involved using distilled water [12].

6. Stress Application

The drought stress treatment was achieved by applying water at levels between 30% and 40% of the field capacity, which reduced water availability and induced physiological stress reactions. The salinity stress was generated through the addition of NaCl solution to the irrigation water at concentrations of 100-150 mM [13].

7. Measured Parameters

7.1 Morphological Traits

Evaluation of plant morphological features was conducted in order to ascertain their efficiency in showing the growth performance of plants in stressful environment conditions. These features include plant height, root length, fresh weight, and dry weight.

7.2 Biochemical Analyses

Physiological indicators were used to test the physiological response to stress and microbial treatment. These include MDA and H_2O_2 for oxidative stress indicators. Other tests included the measurement of antioxidant enzymes like SOD, CAT, and POD because of their activity against reactive oxygen species. Metabolic indicators, on the other hand, were also used to measure the osmotic adjustment and photosynthesis efficiency through proline and chlorophyll content measurements.

8. Microbiological Analysis

Microbial colonization of roots was determined through serial dilutions and CFU enumeration to determine the presence of microbial communities associated with plant roots [14].

9. Statistical Analysis

Statistical analyses were performed using SPSS software. Data were presented as mean \pm SD. Differences among groups were assessed using one-way ANOVA, with post hoc testing where appropriate. The Pearson correlation coefficient was calculated to examine the correlations between variables. Significance level was set at $p < 0.05$.

3. Results

The data presented in Table 1 indicate a notable enhancement of morphological characters for the samples inoculated with microorganisms relative to the control samples. The joint application of bacteria and fungi demonstrated superior values across all the growth characteristics, which suggests an impressive synergistic interaction among them.

Table 1. Effect of Microbiome on Morphological Traits under Stress

Group	Plant Height (cm)	Root Length (cm)	Fresh Weight (g)	Dry Weight (g)
Control	10.5 \pm 0.6	8.2 \pm 0.5	2.0 \pm 0.2	0.40 \pm 0.05
Bacteria	14.7 \pm 0.8	12.5 \pm 0.7	3.5 \pm 0.3	0.75 \pm 0.06
Fungi	16.2 \pm 0.9	14.1 \pm 0.8	4.1 \pm 0.4	0.90 \pm 0.07
Bacteria + Fungi	20.3 \pm 1.0	17.8 \pm 0.9	5.4 \pm 0.5	1.20 \pm 0.08

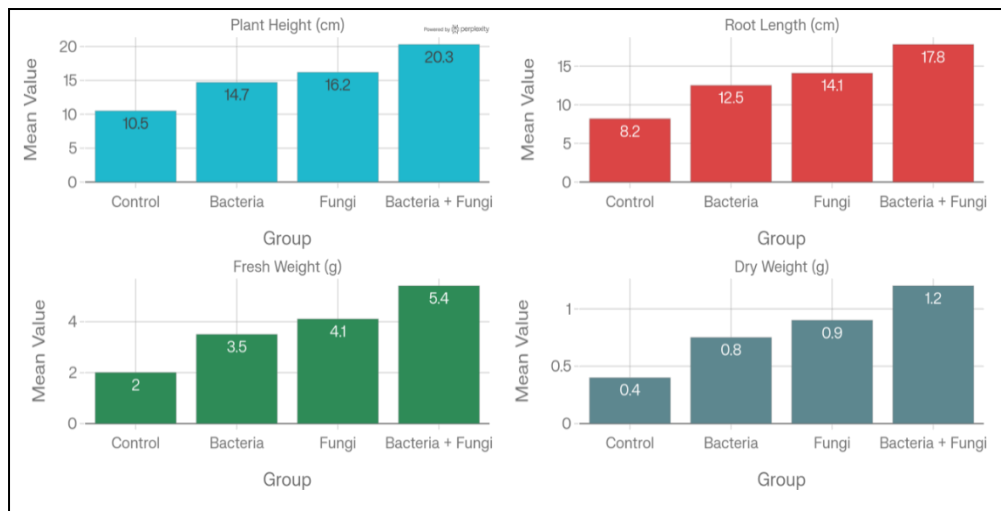


Figure 1. Illustrates a graphical comparison of plant height, root length, fresh weight, and dry weight among all experimental groups.

From Table 2, it is evident that there was a considerable increase in oxidative stress in the control group as a result of environmental stress. This, however, was effectively reduced by the microbial interventions with lower amounts being noted in the combined treatment.

Table 2. Oxidative Stress Markers (MDA and H₂O₂)

Group	MDA (nmol/g FW)	H ₂ O ₂ (μmol/g FW)
Control	6.9 ± 0.3	5.8 ± 0.2
Bacteria	4.3 ± 0.2	3.7 ± 0.2
Fungi	4.0 ± 0.2	3.4 ± 0.1
Bacteria + Fungi	2.5 ± 0.1	2.3 ± 0.1

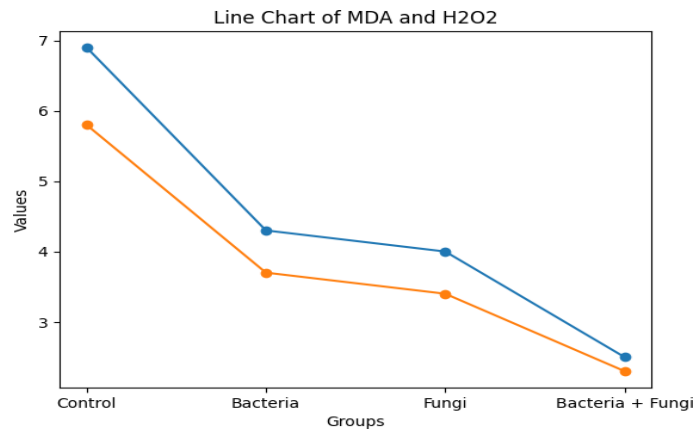


Figure 2. Presents the comparison of oxidative stress marker levels across all groups.

The table 3 indicates a significant increase in the activity of the antioxidant enzymes when there was inoculation of the microbes in comparison to the control treatment. This is seen from the high activity level in the combined treatment sample.

Table 3. Antioxidant Enzyme Activities

Group	SOD (U/mg protein)	CAT (U/mg protein)	POD (U/mg protein)
Control	24.5 ± 1.2	18.0 ± 1.0	19.5 ± 1.1
Bacteria	39.8 ± 1.5	34.6 ± 1.3	37.9 ± 1.4
Fungi	45.2 ± 1.6	40.1 ± 1.5	42.3 ± 1.3
Bacteria + Fungi	60.5 ± 2.0	55.2 ± 1.8	58.0 ± 1.7

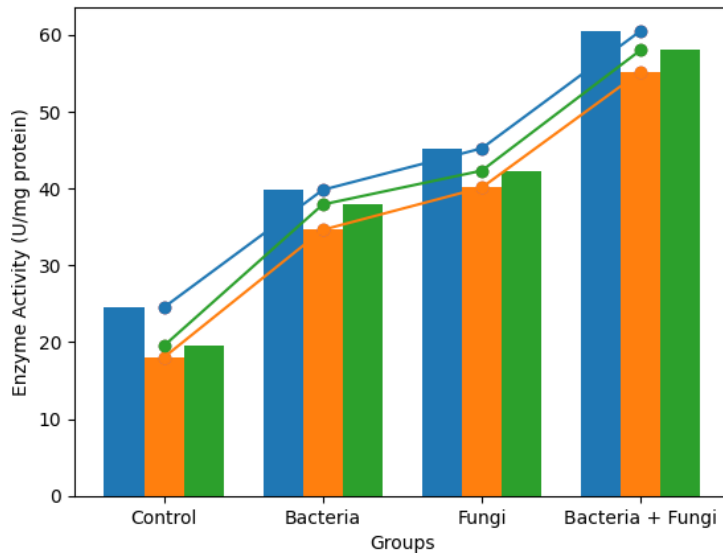


Figure 3. Illustrates the antioxidant enzyme activity patterns (SOD, CAT, POD) among treatments.

Table 4 demonstrates metabolic improvement in treated plants. Higher proline levels suggest improved osmotic adjustment, whereas increased chlorophyll suggests improved photosynthetic efficiency. Combined treatment resulted in the highest level of metabolic enhancement.

Table 4. Metabolic Parameters

Group	Proline ($\mu\text{mol/g FW}$)	Chlorophyll (mg/g FW)
Control	2.0 ± 0.1	1.3 ± 0.1
Bacteria	3.7 ± 0.2	2.0 ± 0.1
Fungi	4.1 ± 0.2	2.4 ± 0.1
Bacteria + Fungi	6.0 ± 0.3	3.1 ± 0.2

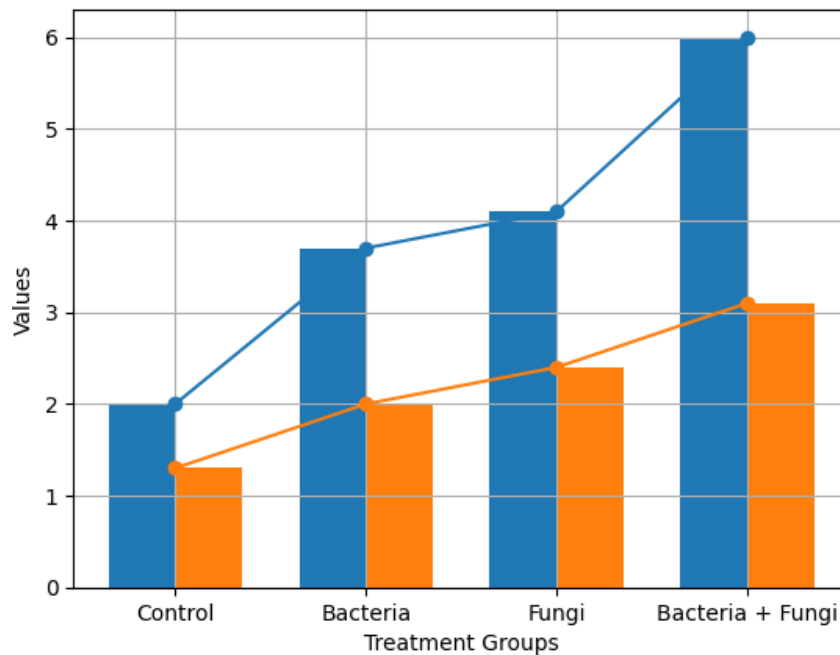


Figure 4. Shows variations in proline and chlorophyll content across treatments.

Table 5 validates the colonization of roots with microbes in inoculated plants. The results with the highest CFU count belong to the combined treatment method, demonstrating the efficacy of microbial colonization.

Table 5. Root Microbial Colonization

Group	CFU ($\times 10^6$ g ⁻¹ root)
Control	0.2 \pm 0.05
Bacteria	2.7 \pm 0.2
Fungi	2.5 \pm 0.2
Bacteria + Fungi	5.6 \pm 0.3

4. Discussion

The findings from the research reveal that plant-associated microbiome, characterized by PGPR and fungi, is instrumental in increasing plant resistance against adverse conditions such as drought and salt stress [15]. All the different microbial treatments increased plant growth and physiological efficiency than those from the control sample, with bacteria and fungi combined providing the greatest benefits, demonstrating synergism between microbes [16].

With regard to morphological features table 1, it is evident that increased plant height, root length, and biomass have been noted due to increased efficiency in nutrient uptake by the plants as well as water use [17]. It can be noted that these results are caused by the use of PGPR like *Bacillus subtilis* and *Pseudomonas fluorescens* that secrete plant growth hormones such as auxins [18]. Moreover, the arbuscular mycorrhizal fungi are involved in the enlargement of the root absorptive surface, thus ensuring greater access to water and nutrients [19].

As far as the oxidative stress markers table 2, are concerned, the marked decrease in MDA and H₂O₂ content in the plants treated with microbes suggests their ability to protect the plant from the damages caused by oxidative stress [20]. This is probably owing to the stimulation of the plant's antioxidant defense mechanism that prevents the production of ROS leading to the destruction of cell membranes, proteins, and DNA [21].

Antioxidant enzyme activity results table 3 reinforce the hypothesis, as a significant increase in SOD, CAT, and POD activity in plants undergoing treatment was noted when compared to the control [22]. The role of SOD is to break down the superoxide radical into hydrogen peroxide that is further broken down into water and oxygen by CAT and POD enzymes [23]. Therefore, induction of the enzymatic process represents an increased physiological capability in defending against stress [24].

With respect to the metabolic factors table 4, the increase in proline levels in the experimental plants is considered an important factor of adaptation because proline functions as an osmoprotectant and prevents the degradation of cellular proteins and membranes [25]. In addition, the high amount of chlorophyll in the leaves indicates the ability to maintain photosynthesis [26].

The results of root colonization table 5 demonstrated a successful growth of microbes after inoculation of the treatments, with the greatest CFU values in the combined treatment [27]. This suggests an intense symbiotic relationship between plants and microbiome that will enhance the nutrient exchanges and biochemical communication, increasing plant stress tolerance [28].

In summary, it can be concluded that the plant microbiome not only serves as a growth enhancer but also as a biological defense system, improving the tolerance of plants to environmental stresses [29]. The dominance of the joint treatment method underscores the complementary relationship between bacteria and fungi, lending credence to the principle of microbial synergy [30].

In addition to this, the findings are supported by past research conducted on the efficacy of plant-associated microbes to enhance agricultural productivity under adverse conditions and serve as a potential replacement for chemical fertilizers [31].

5. Conclusion

Plants are more tolerant to environmental stress due to better growth, lower oxidative stress, better antioxidant activity, and efficient metabolism because of microbiome in plants. The combined

application of bacteria and fungi resulted in the best synergy, demonstrating the high sustainability of this method for increasing plant resistance to abiotic stresses.

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