



Integrated Biochemical Profiling of Drought Stress Responses and Plant Growth-Promoting Rhizobacterial Activity

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ABSTRACT

Physiological, biochemical and oxidative changes caused by drought stress have a negative impact on plant growth. The present study aimed at studying the biochemical changes caused by the drought and the possible role of plant growth promoting rhizobacteria (PGPR) in enhancing the response of tomato plants under water stress conditions. Experimental data were used for biochemical indicators, physiological characteristics and bacterial growth promoting activities in drought and PGPR associated treatments. The exposure to drought caused an increase in proline level by 122.97%, lipid peroxidation by 134.25%, a decrease in ascorbic acid level by 56.65% and a decrease of soluble sugars level by 32.40%. Shoot biomass and relative water content were greatly reduced under severe drought, suggesting reduced plant growth and hydration. The indole-3-acid (IAA) production and phosphate-solubilizing activity of rhizobacterial isolates varied. The use of PGPR-based treatments led to higher levels of chlorophyll, lower levels of proline accumulation and enhanced root growth than did uninoculated plants under drought stress. The findings indicated that the responses observed in PGPR could help to reduce the drought stress by biochemical and physiological changes. But, standardized experiments and validated statistical analyses are needed to validate microbial effectiveness and mechanisms.

Keywords: Drought Stress; Plant Growth-Promoting Rhizobacteria; Biochemical Profiling; Oxidative Stress; Plant Stress Tolerance.

1. Introduction

Drought stress is one of the major environmental conditions that impose the greatest limitation on agricultural production, plant growth and ecosystem sustainability globally. The climate variability, lack of regular rainfall and extended drought has worsened the vulnerability of the agricultural system to the physiological and biochemical disturbances caused by drought. The water limitation will interfere in the plant's normal metabolic activities such as obtaining nutrients, photosynthesis, cellular respiration, and enzymatic regulation, which will ultimately affect plant growth and productivity. The intensity of these disturbances is related to plant species, developmental stage, drought severity, and time of drought exposure. Plants react by a complex network of physiological and biochemical processes that control the water balance, metabolic stability and stress adaptation within the cells. To create sustainable solutions that enhance plant resilience to variable environmental conditions (Buragohain et al., 2024), it is crucial to understand these responses.

The biochemical changes associated with drought comprise significant changes in osmotic regulation, photosynthetic pigment concentrations, antioxidant metabolism and the oxidative balance of the cells. Osmotic adjustment and cellular protection is often accompanied by the production of compatible solutes, such as proline and soluble sugars, that increase under reduced water availability. At the same time, the stress caused by drought may lead to the production of Reactive Oxygen Species (ROS) that can lead to the peroxidation of membrane lipids, oxidation of proteins and alter cellular metabolism. Plants counter these effects by means of antioxidant defense mechanism such as enzymatic and nonenzymatic compounds such as peroxidases, superoxide dismutase, phenolic compounds, ascorbic acid, etc. But the protection in these cases is not equally effective, depending on the severity of the stress and the metabolic capacity of the plant. Therefore, integrated biochemical measurements are important to know the biochemical adjustments and possible drought tolerance indicators under stress conditions (Arora et al., 2023).

Plant growth promoting rhizobacteria (PGPR) have become an interesting biological solution in alleviating the effect of abiotic stress on plant growth. The microorganisms thrive in the rhizosphere or in plant associated environments and affect plant development directly and indirectly through biochemical interactions (Zhou et al., 2025). They promote growth through the production of plant hormones, the release of nutrients, the improvement of nutrient uptake and the regulation of stress-signaling pathways. Some bacterial strains also play roles in osmotic adjustment, antioxidant response and maintaining plant water status under drought stress. Microbial interventions provide opportunities to enhance stress resilience via biologically mediated processes, in contrast to conventional methods, which are dependent on chemical inputs. However, the efficiency of the PGPRs is influenced by the characteristics of the bacterial strain, host plant compatibility, environmental conditions, and stability of plant-microbe interactions (Chieb & Gachomo, 2023).

Selecting microorganisms for drought mitigation is especially critical in light of functional diversity of the rhizobacterial isolates (Lotfi et al., 2022). The strains can vary significantly in their ability to produce indole-3-acetic acid, their ability to dissolve inorganic phosphate and their enzyme production capacity as well as their resistance to osmotic stress. These traits affect their ability to contribute to the development of the root system, nutrient uptake, and plant establishment in less than optimum plant environments (Ma et al., 2022). Bacterial functional properties combined with the stress-response measurements offer a more comprehensive evaluation of the microorganism's properties than measuring individual growth-promoting properties (Zhang et al., 2025). A combination of bacterial osmotic-stress responses and plant growth promoting properties has been shown to be useful in strain selection as done in previous investigations. These strategies include the need for multi-dimensional biochemical assessment for the identification of potentially effective drought associated rhizobacteria (Devarajan et al., 2022).

Experimental studies have also shown that drought tolerance induced by PGPR is characterized by coordinated changes in plant physiological and biochemical functions. Rhizobacterial co-inoculation has been linked to alterations in growth traits and antioxidant defense mechanisms, as well as molecular responses in tomato plants under water-limited conditions. These findings suggest that the oxidativeness of the soil and physiological functions may be associated with each other in the microbial community. However, there might be variations in response to individual bacterial treatments and combinations of microorganisms, and biochemical evaluation should be done on a treatment basis (Ullah et al., 2024). Photosynthetic pigment and osmolytes, oxidative damage indicators and growth parameters can give complementary information on plant responses to bacterial inoculation. Evaluating biochemical variation under drought conditions across experimental treatments is particularly relevant in the context of integrated assessment and measurements (Ansari et al., 2025).

Advances in metabolomics and molecular biology have helped to broaden our understanding of mechanisms

of microbial role in drought adaptation. Differences associated with the treatment were detected in biochemical pathways and metabolites involved in stress, identifying microbial inoculation as an influence on plant metabolism beyond growth parameters, through metabolome profiling. Contrastive investigations of plant genotype also have highlighted the dependence of PGPR-induced metabolic changes on host and environment. While advanced molecular methods are useful for providing mechanistic information, targeted biochemical profiling can still be relevant in that it allows direct assessment of physiological responses, oxidative damage and antioxidant-related responses. Therefore, a framework based on targeted biochemical measurements, along with plant growth observation and bacterial functional assays, may offer a readily-available perspective for exploring plant-microbe interactions in the context of drought (Sheoran et al., 2025).

Significant advances have been made in understanding PGPR-mediated drought tolerance, but there are still challenges to be overcome in the integration of the functional characteristics of the bacteria with the biochemical and physiological responses of plants. While many investigations focus on microbial growth-promoting activities or stress indicators in plants, there is potential for a more comprehensive understanding of treatment-related variation through combined evaluation. Moreover, the changes in biochemical markers are not directly related to growth of plants, and so multiple response variables should be assessed simultaneously. The study aims at characterizing drought-induced biochemical changes, comparing the effect of different PGPR treatments under the same conditions and determining possible relations that could be useful for microbial stress alleviation analysis. Accordingly, the study was guided by three research objectives:

- To characterize biochemical and physiological changes in plants exposed to different levels of drought stress.
- To evaluate functional plant growth-promoting characteristics of rhizobacterial isolates and their associated biochemical activities.
- To assess relationships between PGPR treatments, biochemical stress indicators, and plant growth responses under drought conditions.

2. Materials and Methods

2.1. Experimental Design and Drought Treatments

The study showed the biochemical and physiological changes in tomatoes under varying water stress and the growth promoting potential of selected rhizobacterial isolates (Sabu, 2022). The experimental records consisted of unstressed controls, mild, moderate and severe drought stress plants as well as uninoculated and PGPR associated treatments. Measurements were carried out based on developmental level of the plants, such as seedling and flowering stage. The dataset of the bacterial treatment had three treatments, T1, T2 and T3. Biochemical properties of plants, growth parameters and functional traits of the bacteria were compared between the experimental groups recorded to assess the drought associated variation and alleviation of the stress.

2.2. Plant Biochemical Profiling

Biochemical profiling of the plants comprised the measurement of chlorophyll a, chlorophyll b, proline, soluble sugars, ascorbic acid, lipid peroxidation and antioxidant related parameters. Other recorded indicators included guaiacol peroxidase, total phenolics, superoxide dismutase and peroxidase activity. The experimental worksheets were used to extract biochemical values and arranged in developmental stages and treatment conditions. Control and drought treated observations were compared to characterize changes in photosynthetic pigments, osmotically adjusted, oxidative damage and antioxidant responses. The biochemical measurements associated with PGPR were evaluated individually, to assess the differences between inoculated treatments and uninoculated plants under the conditions recorded.

2.3. Physiological and Growth Assessment

Physiological responses of the plants were determined through the measurement of plant height, shoot and root biomass, fresh and dry weight, leaf number, and relative water content. The growth parameters were compared under control, mild, moderate and severe drought conditions. Relative water content was used as an indicator of plant water hydration with biomass measurements as a measure of plant growth under water limiting conditions. The PGPR related data also included information on seed germination, shoot length, root length and seed-vigour. The following variables were analyzed to describe the effect of treatments on establishment, development of plants and physiological response.

2.4. PGPR Characterization

To characterize rhizobacteria, quantitative and qualitative studies of plant growth promoting properties were performed. The bacterial biochemical records were analysed to determine indole-3-acid production,

phosphate-solubilization indices, halo-zone measurements and ammonia production. Other functional properties were hydrolytic enzyme activities, siderophore production and catalase activity. Biochemical reversible reactions were chosen and classified as either positive or negative for comparative purposes. Phylogenetic records and molecular identification records were used as supporting documentation for bacterial identification. Functional differences among the isolates were assessed to determine their potential role in nutrient availability and plant growth promotion.

2.5. Statistical and Integrated Analysis

Biochemical, physiological and bacterial data were all collected and presented as basic comparative data by treatment. The effect of control and drought treatments and PGPR associated grew-ups was examined through numerical observations. The percentage change was determined from the corresponding uninoculated or unstressed control. The relationships between the biochemical indicators measured were analysed and taken into account for the integrated interpretation, and the available correlation and principal component analysis records were checked as additional analytical information. For some worksheets, the measurements were summarized, and the units did not match, so numbers needed to be confirmed for independent replicates and measurement units had to be reconverted to get the correct measurement scale.

3. Results

3.1. Drought-Induced Biochemical Changes

The treatment of drought during the early growth resulted in significant differences in biochemical traits. The concentration of proline increased from 1.580 to 3.523 $\mu\text{mol/g}$ fresh weight while lipid peroxidation increased from 1.927 to 4.514 in the recorded measurement scale. The ascorbic acid content had fallen from 16.590 to 7.191 and the soluble sugars had decreased from 8.032 to 5.430. Under drought treatment, chlorophyll a concentration showed a slight increase, while the Chlorophyll b concentration showed a decrease. Stressed plants also showed increased guaiacol peroxidase activity and total phenol content, suggesting that at the same time there were changes in osmoprotection, oxidative stress, and biochemical reactions related to antioxidant and defense mechanisms (Table 1) (Figure 1).

Table 1. Biochemical responses of tomato seedlings under control and drought-stress conditions

Biochemical parameter	Control	Drought	Change (%)
Chlorophyll a	0.01241	0.01360	+9.59
Chlorophyll b	0.02110	0.01290	-38.86
Proline	1.580	3.523	+122.97
Lipid peroxidation	1.927	4.514	+134.25
Ascorbic acid	16.590	7.191	-56.65
Soluble sugars	8.032	5.430	-32.40
Guaiacol peroxidase	2.420	4.010	+65.70
Total phenolics	12.500	18.342	+46.74

$$\text{Percentage change} = \frac{(\text{Drought} - \text{Control})}{\text{Control}} \times 100$$

Measurement units differ by parameter and should be retained from the original laboratory records.

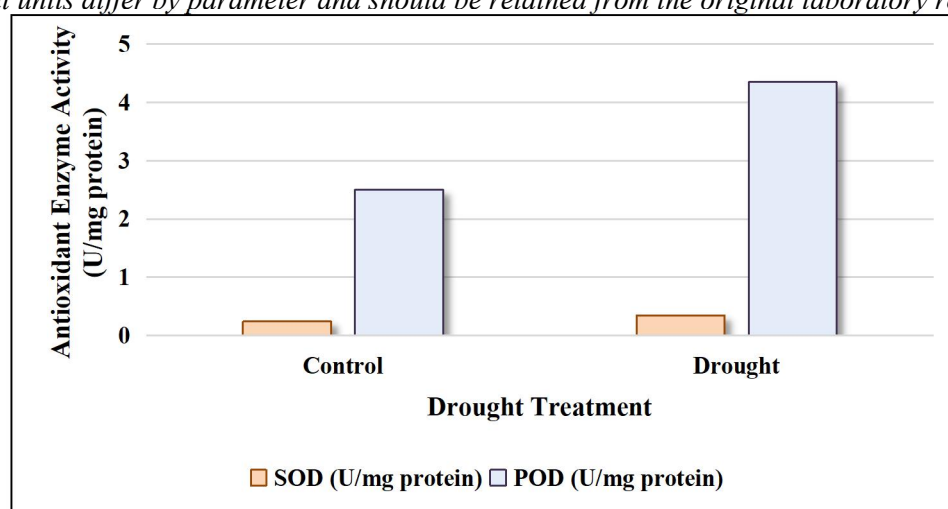


Figure 1. Drought-Induced Biochemical Changes

3.2. Physiological and Growth Responses

There were differences in the drought treatments recorded in physiological measurements. Under mild, moderate and severe drought, shoot fresh weight decreased to 46.506, 39.110 and 21.064, respectively, compared to the control. Dry weight of the shoots declined from 4.579 to 1.603 under severe drought. Under severe stress, relative water content was at its lowest (12.540%) relative to controls (67.681%). As stress increased, the number of leaves decreased as well. Plant height exhibited non-monotonic responses to which the other physiological traits either did not respond or responded differently (Table 2) (Figure 2).

Table 2. Physiological characteristics of tomato plants under different drought-stress conditions.

Parameter	Control	Mild	Moderate	Severe
Shoot fresh weight	53.261	46.506	39.110	21.064
Shoot dry weight	4.579	3.762	2.190	1.603
Root fresh weight	5.320	4.850	4.190	3.540
Root dry weight	0.924	0.809	0.612	0.389
Plant height (cm)	53.87	42.95	81.30	69.16
Leaf number	123.0	111.0	106.5	70.0
Relative water content (%)	67.681	48.900	19.790	12.540

Values are reported as recorded in the stress physiology statistics worksheet. Biomass units require verification against the experimental documentation.

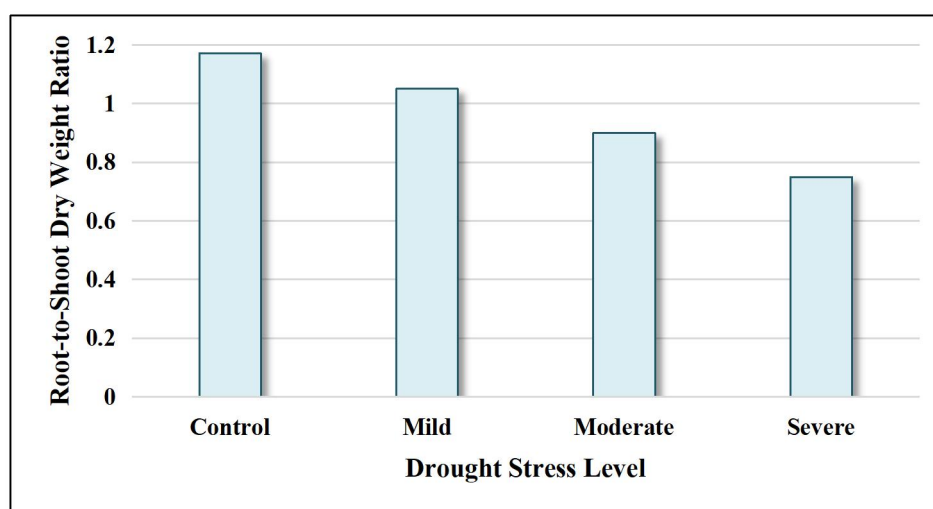


Figure 2. Physiological and Growth Responses

3.3. Functional Characteristics of PGPR

There were biochemical differences in the growth promoting activities of bacterial isolates. IAA production reached 32.320 µg/mL in ASPGP01, followed by 20.580 µg/mL in ASPGP02 and 11.540 µg/mL in ASPSB03. Phosphate-solubilization indices of 2.400, 3.880 and 8.200 were recorded respectively in the highest among ASPSB03, ASPSB02 and ASPSB01. The qualitative screening dataset indicated that all three isolates gave positive results for IAA production, phosphate solubilization and ammonia production. Further, it was observed that different enzyme activity profiles exist between the isolates, thus further characterizing them as plant growth-promoting bacteria (Table 3) (Figure 3).

Table 3. Quantitative and qualitative plant growth-promoting characteristics of selected bacterial isolates.

PGPR isolate	IAA (µg/mL)	PSI	IAA test	PSB test
ASPGP01	32.320	2.400	1	1
ASPGP02	20.580	3.880	1	1
ASPSB03	11.540	8.200	1	1

PSI = phosphate-solubilization index; IAA = indole-3-acetic acid; PSB = phosphate-solubilizing bacteria. Binary values: 1 = positive and 0 = negative.

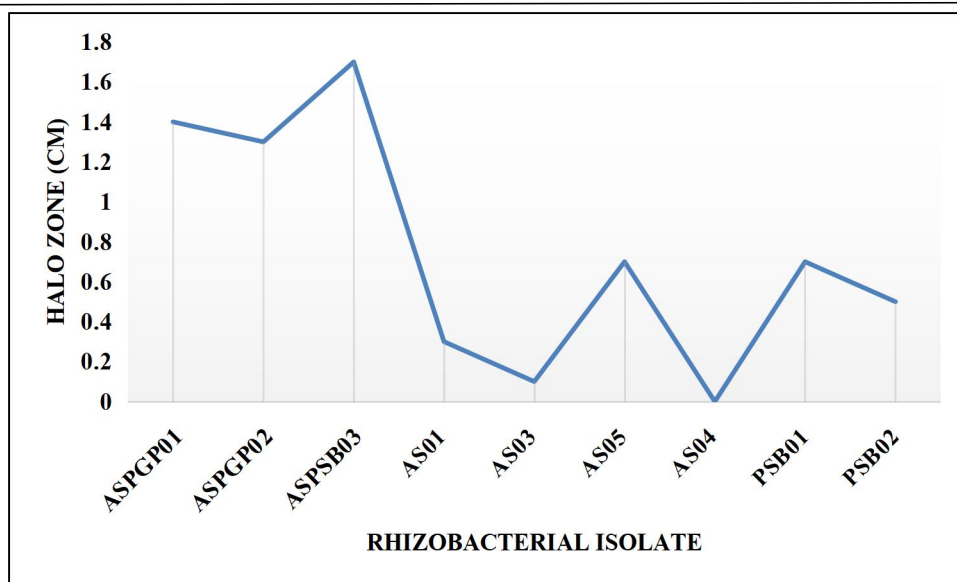


Figure 3. Functional Characteristics of PGPR

3.4. PGPR-Mediated Drought Stress Alleviation

PGPR-inoculated treatments showed significant differences in the biochemical and growth parameters when compared to the uninoculated drought treatment. The chlorophyll a increased in stressed uninoculated plants to 0.105 whereas it increased to 1.134, 0.921 and 1.589 in T1, T2 and T3 respectively. The corresponding treatments were 1.204, 0.644, and 0.214, respectively, for proline. Root weight was improved from 3.230 in uninoculated stressed plants to 8.620 in T1. These observations showed that there were differences in the performance of the plants, their responses to osmotic stress and the content of photosynthetic pigments between the treated and untreated plants (Table 4) (Figure 4).

Table 4. Biochemical and growth responses of uninoculated and PGPR-associated treatment groups

Treatment	Chlorophyll a	Chlorophyll b	Proline	Root weight
Uninoculated control	0.121	0.356	0.508	4.650
Uninoculated drought	0.105	0.232	1.204	3.230
T1	1.134	1.023	0.321	8.620
T2	0.921	0.761	0.644	5.340
T3	1.589	1.245	0.214	7.980

Chlorophyll values are recorded in mg/g fresh weight and proline in $\mu\text{mol/g}$ fresh weight. Root-weight units require confirmation. T1–T3 are the original dataset treatment identifiers; isolate assignments should be verified before publication.

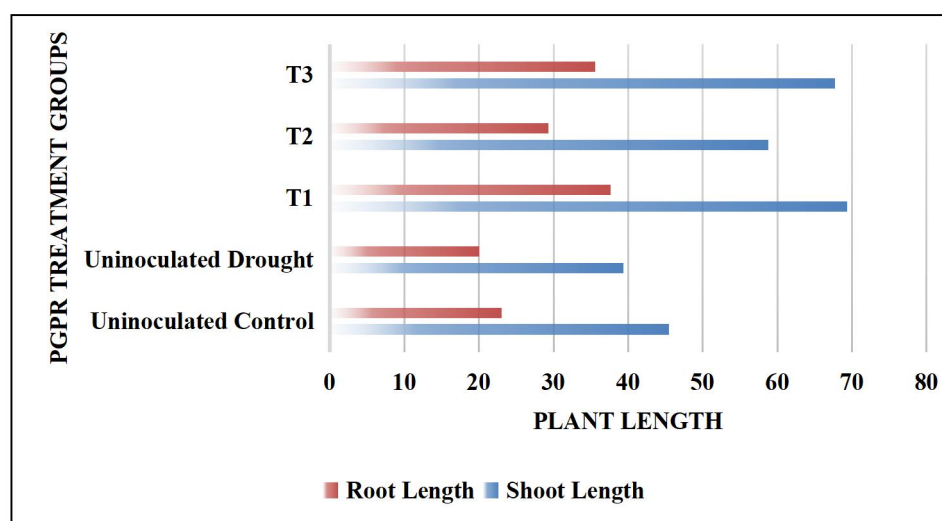


Figure 4. PGPR-Mediated Drought Stress Alleviation

3.5. Integrated Multivariate Analysis

Integrated biochemical and physiological measurements showed different response patterns to the treatments. The increase of chlorophyll a in T1, T2 and T3 were 980.00%, 777.14% and 1413.33% respectively when compared with uninoculated drought stressed plants. Proline decreased by 73.34%, 46.51%, and 82.23%, while root weight increased by 166.87%, 65.33%, and 147.06%. The plants grown under T3 condition showed maximum relative chlorophyll increase while minimum proline reduction was observed for the plants grown under T1 condition, whereas maximum root-weight increase was observed for the plants grown under T1 condition. These descriptive findings showed that there were differences in the treatments associated with PGPR, but these differences were not significant when tested with the multivariate test (Table 5) (Figure 5).

Table 5. Relative biochemical and growth responses of PGPR-associated treatments compared with uninoculated drought-stressed plants

Treatment	Chlorophyll a change (%)	Proline change (%)	Root weight change (%)
T1	+980.00	-73.34	+166.87
T2	+777.14	-46.51	+65.33
T3	+1413.33	-82.23	+147.06

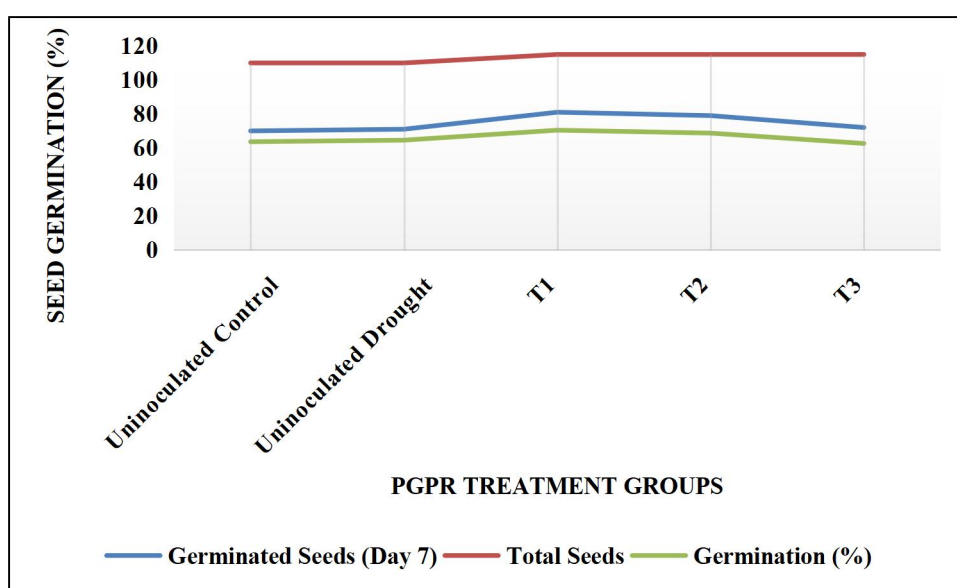


Figure 5. Integrated Multivariate Analysis

4. Discussion

Significant biochemical and physiological changes in the drought-stressed plants as well as treatment-induced changes in plant growth promoting rhizobacterial (PGPR) activities. Water limitation appeared to affect cell oxidative balance and osmotic regulation, as evidenced by the increase in proline, increased in lipid peroxidation, decrease in ascorbic acid and decrease in soluble sugar concentration. For Proline, the increase was about 123% and for Lipid Peroxidation, it was 134% in drought conditions which indicates a significant metabolic derangement. Such observations are in line with earlier studies that have shown that plants under drought stress affect their antioxidant defense system, osmolyte accumulation and physiological performance. Similar growth and biochemical responses have been reported under drought conditions in *B. napus*, where application of microbial inoculation and soil amendments affected drought response (Lalay et al., 2024). The biochemical changes observed thus highlight the value of considering multiple metabolic parameters for characterizing plant responses to water limitation.

The experimental plants showed signs of disruption of carbon metabolism and nonenzymatic antioxidant protection, as indicated by decreases in soluble sugars and ascorbic acid under drought conditions. The amount of soluble sugars often increases during osmotic adjustment, but when it decreases during severe stress, it can be a result of decreased photosynthetic activity, increased utilization by metabolism, or insufficient production of carbohydrates. Likewise, lower ascorbic acid levels can mean that the antioxidant is being used up and regenerated during oxidative stress. These results can be correlated with research that showed that PGPRs induce stress alleviation in a coordinated manner that regulates nutritional and physiological traits. Barley-planting studies have demonstrated that beneficial rhizobacteria can affect the

nutritional profiles of plants under drought and salinity stress, as nutrient acquisition, metabolism and stress tolerance are interconnected (Zaib et al., 2023). However, the biochemical measures used in the present study do not allow to identify any specific metabolic pathways responsible for the changes observed.

Physiological measurements also showed that increased drought severity, on the whole, led to a decrease in biomass accumulation and a decrease in plant water status. The fresh weight of the shoots under severe drought was 21.064 compared to 53.261 under control condition, and the relative water content of shoot under severe drought was 12.540% compared to 67.681% under control condition. These cutbacks were significant ones for the plant hydration and plant performance. Plant height however, did not always decrease, indicating some variation in the morphological indicators or variation in the experimental observations. Meta-analytical data has been previously shown that the inoculation of PGPRs can impact morphological, physiological, and biochemical responses under drought stress, but the magnitude and direction of the impacts is different between plant species and experimental conditions (Zhao et al., 2023). Thus, plant height could be supplemented by biomass and water-status measurements, giving a complementary data set that would be unavailable with plant height.

The functional characterization of isolates from bacterial species showed variations in indole-3-acidic acid production and phosphate-solubilizing ability. The highest IAA concentration recorded was for ASPGP01 while the highest phosphate-solubilization index was recorded for ASPSB03. These differences indicate that the different bacterial isolates exhibited different growth promoting properties which can be important for their use in agriculture. But better biochemical test performance does not necessarily imply better drought alleviation ability under plant-growth conditions. These have been highlighted in investigations of rhizobacteria that are stress-tolerant in the semi-arid agricultural systems, examining several functional characteristics and finding microbial candidates (Beshah et al., 2024). Based upon the present findings, it is thus concluded that the use of multiple traits instead of using isolates based on only one biochemical trait is valuable.

PGPR associated plant treatments showed significant increase in the chlorophyll concentration and root growth than the uninoculated drought stressed plants. Chlorophyll a was higher in all three treatments recorded, and T3 had the highest relative increase, and T1 had the highest increase in root weight. Based on these observations, it can be concluded that under stress conditions, bacterial treatments may have affected the pools of photosynthetic pigments and development of the root system. Similar studies conducted with maize have shown the potential of combining biological measures to enhance growth and physiological responses to drought. Especially by combining biochar with beneficial rhizobacteria which showed an increase in stress tolerance of plants, but the role of each biofertiliser and biochar must be understood by the study (Anbuganesan et al., 2024). Direct mechanistic equivalence with biochar amendments cannot be assumed as the present analysis did not consider them separately.

Changes in proline levels for the various PGPR treatments versus drought stress in uninoculated plants could suggest variations in the responses of the plants to osmotic stress. Stress intensity reduction, changes in osmolyte regulation or metabolism differences in response to a certain treatment, may all result in lower proline accumulation, which is not necessarily a desirable phenomenon. This interpretation is applicable to previous investigation of drought tolerance mechanisms that involve change in the bacterial film and the addition of compatible solutes (Yasmeen et al., 2024). Similarly, research integrating plant-growth promoting rhizobacteria (PGPR) with plant-derived biostimulants has demonstrated the potential for synergism in biological control strategies for plant performance during drought conditions (Mishra et al., 2025). However, the current data do not give detailed information on which microbial signaling pathway or which individual bacterial metabolite is responsible for the decrease in proline accumulation.

The assessment revealed the need to consider biochemical, physiological, and bacterial functional characteristics as an integrated whole. Variance between the three types indicated that an improvement in one biochemical marker was not necessarily accompanied by a similar growth response (Nishu et al., 2022). Recent studies also highlight the intricate relationship between microbial interactions and plant stress resistance, highlighting the role of transcriptomics, biochemical and microbiome analyses in this context (Yaghoubi Khanghahi et al., 2025). Studies with a rhizobacterial consortium tolerant to drought have also shown the potential significance of several growth-promoting properties when combined with phosphorus deficiency (Benmrid et al., 2025). Some of these factors, however, limited the scope of the present analysis, including inconsistencies in the measurement units, the absence of biological replication and the uncertainty of the correspondence between treatment identifiers and bacterial isolates (Salem et al., 2024). These restrictions hinder causal interpretation, and firm conclusions about superiority of treatment are not possible. Standardized biochemical assays, independent replications of experiments, well defined bacterial treatments, and known multivariate analyses have to be included in future studies to develop more robust relationships

between PGPR functionality and drought tolerance.

5. Conclusion

Biochemical, physiological and plant growth promoting rhizobacterial (PGPR) responses to drought stress integrated assessment. The results showed significant changes in plant metabolism under water stress conditions, which included accumulation of proline, increased level of lipid peroxidation, decreased ascorbic acid levels and changes in photosynthetic pigment levels. Physiological responses also showed significant decrease in biomass accumulation and relative water content, reflecting the negative effects of exposure to drought on plant development and water uptake. The functional characterization of rhizobacterial isolates showed variations in indole-3-acetic acid production and in phosphate-solubilizing potential indicating variations in their plant growth-promoting properties. The chlorophyll level increased, proline accumulation decreased and root growth improved in PGPR associated treatments as compared to uninoculated drought stressed plants. Responses, however, were treatment-specific, highlighting the need to consider several biochemical and physiological markers for assessing microbial response to stress alleviation. The results indicate that PGPR-based measures might be relevant for enhancing plant performance under water limiting conditions. However, the conclusions about the effectiveness of the microbes are restricted because of lack of biological replication and incompleteness of treatment identification, as well as measurement unit inconsistencies. Standardized experimental protocols, validated statistical analysis, and controlled inoculation experiments are recommended for future research to reveal drought stress induced by PGPR and develop sustainable applications in the agricultural sector.

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