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Exploring the Impact of Water Stress and PGPR Inoculation on Morphological, Physiological, and Biochemical Parameters in Tomato Plants

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Abstract

This study explores the effects of varying water stress conditions on tomato plants, ranging from severe to mild stress levels, alongside the influence of soil inoculation with Plant Growth-Promoting Rhizobacteria (PGPR). The experiment utilized bacterial isolates including Bacillus amyloliquefaciens (SF14), Alcaligenes faecalis (ACBC1), and Bacillus aryabhattai (B11) to inves-tigate morphological, physiological, and biochemical parameters in the Campbell 33 tomato variety. The study aims to understand plant responses to water stress and evaluate the specific impact of PGPR bacterial strains on various aspects of plant performance. Parameters assessed encompassed root growth and weight, stem diameter, growth rate, leaf and flower count, water content, as well as above-ground and root fresh/dry weights, chlorophyll, anthocyanin, and flavonoid levels. Severe water stress led to decreases in morphological and physiological metrics, while bacterial inoculation showed a positive influence. Unexpectedly, bacterial inoculation reduced anthocyanin levels under severe stress, indicating intricate interactions between bacterial inoculation and plant responses. These findings provide valuable insights into sustainable agricultural practices by uncovering complex interactions among environmental factors, soil micro-biomes, and plant physiology.

Keywords : Inoculation, bacterial strains, anthocyanins, flavonoids, biochemical parameters.

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Introduction

In many parts of the world, including regions beyond the Mediterranean, the consequences of drought are profound. Crop failures, water scarcity, and diminished agricultural yields have become recurring issues, challenging the resilience of farming communities (Aghakouchak et al., 2021; Bond et al., 2019; Chivenge et al., 2015; Dolan et al., 2021). As nations grapple with the shared challenge of water scarcity, collaborative efforts are crucial to developing innovative strategies that address the complex interplay between climate change, water resources, and sustainable agriculture(Mani and Goniewicz, 2023; Pardoe et al., 2018).

In Morocco, groundwater constitutes a vital component of the hydraulic heritage (Bahir and Mennani, 2002). It's plays a crucial role in socio-economic development (Molle François, 2017). Groundwater resources serve as the key adjustment variable, meeting 90% of the drinking water requirements and supporting irrigation across almost 40% of the Kingdom's total irrigated area. This contribution exceeds 50% of the corresponding economic value generated (Bahir and Mennani, 2002). Reports from France-info (Franceinfo, 2023) and AFP (2022) (AFP, 2022) highlight that Morocco has been severely impacted by a drought lasting nearly forty years, raising concerns about a potential exacerbation of water shortages this year due to climate change and ineffective water management. According to the Ministry of Agriculture (2022), the drought is expected to escalate in Morocco until 2050 due to a decrease in rainfall (-11%) and rising temperatures (+1.3°C). This is anticipated to result in a reduction in the availability of irrigation water by more than 25%.

The agriculture in Morocco is regularly confronted with frequent periods of drought as well as constraints in terms of water resources, which should have negative repercussions on plant growth and crop productivity. Mediterranean regions experience intense drought periods, leading to the expansion of arid zones (Medyouni et al., 2021). Given the gradual depletion of irrigation water resources globally, it is crucial to study the effects of water deficit on water-intensive plants, such as tomatoes (Solanum lycopersicum)(Chaudhary et al., 2019; Hoshikawa et al., 2021). Water scarcity and increasing competition for water resources between agriculture and other sectors require the exploration of new irrigation strategies adapted to semi-arid Mediterranean regions. These strategies should be capable of reducing irrigation water consumption while maintaining agricultural production. The effects of water stress have been the subject of numerous studies on various crops. The development, growth, and productivity of plants can be impacted by water deficit conditions, depending on the intensity, timing, and duration of stress, as well as genotype, as observed in tomatoes (Solanum lycopersicum L.) (Ben Ayed et al., 2022; Hoshikawa et al., 2021; Medyouni et al., 2021). Currently, many studies have explored alternative methods to improve agricultural production while reducing environmental contamination risks. Among these approaches, the use of microorganisms has been studied (Kalozoumis et al., 2021; Ullah et al., 2016). These microorganisms have the ability to colonize plant roots or interact directly or indirectly with them in a significant manner. Among beneficial microorganisms, we find bacteria known as "Plant Growth Promoting Rhizobacteria" (PGPR), which generally promote plant growth(Essalimi et al., 2022; Haque et al., 2020). Some of these PGPR bacteria are used as inoculants to enhance root development by producing certain phytohormones such as auxins, including indole acetic acid (IAA), cytokinins, and gibberellins(Andryei et al., 2021; Tahiri et al., 2021). Numerous studies have demonstrated that these rhizobacteria also play a role in plant protection by reducing the harmful effects of pathogens through the synthesis of specific antibiotics (Beneduzi et al., 2012; Santoyo et al., 2021). Furthermore, it has been shown that these bacteria play a significant role in drought stress tolerance, in addition to their ability to increase biomass and root growth (Kamal, 2018; Kumar et al., 2021; Wei et al., 2022). The principal aim of this study is to analyze the impact of water stress and PGPR bacterial strains on specific morphological, physiological, and biochemical parameters in tomato crops. Subsequently, the goal is to elucidate the interconnections among these diverse parameters and the applied treatments.

Materials and Methods

Methodology for Cultivating Campbell 33 Tomatoes under Controlled Experimental Conditions

In this specific trial, plant materials comprising seeds and plants of the tomato variety Campbell 33 were utilized. For sowing operations, to ensure sterility, the peat used in the experiment underwent autoclaving for one hour. The cells were then meticulously filled with sterilized peat under pressure to achieve the optimal planting substrate structure. Subsequent to this, a pre-watering phase was executed. The seeding process transpired in trays filled with peat within a controlled environment under glass. Immediate irrigation followed the sowing, and subsequent watering occurred bi-daily. Upon reaching the 4-leaf stage, the plants underwent transplantation into pots containing a blend of 1/3 peat, 1/3 sand, and 1/3 topsoil. Consistently, from sowing to transplanting, the plants received a uniform amount of water. Subsequently, water stress was induced through four distinct irrigation treatments:

T0: Fully irrigated control (500 ml per plant every 2 to 3 days).
T1: Dry control (irrigated at transplanting), constituting severe stress.
T2: 40% of water requirements (200 ml per plant every 2 to 3 days), indicating moderate stress.
T3: 70% of water requirements (350 ml), representing mild stress.

Post-application of these treatments, watering resumed every two days, adhering to the previously established irrigation parameters.

Bacterial Inoculation and Water Stress Interactions in Campbell 33 Tomato Plants

The bacterial strains employed in this experiment consist of Bacillus amyloliquefaciens (SF14), Alcaligenes faecalis (ACBC1), and Bacillus aryabhattai (B11), sourced from the plant protection laboratory collection at the National School of Agriculture in Meknes. To facilitate bacterial multiplication, a solid LB medium served as the culture medium. Further, the prepared medium underwent autoclaving at 121°C for 20 minutes to ensure sterility.

The bacterial strains were then inoculated into six Petri dishes, each containing the previously prepared liquid LB medium. These plates were incubated in a 28°C environment for 48 hours to foster bacterial growth. Upon opening the Petri dishes, 3 to 4 ml of sterile distilled water were aseptically added to each dish. The resulting mixture was carefully blended to recover the bacteria, and the solutions were subsequently filtered through filter paper before being collected in test tubes.

The bacteria, now in solution form, were directly introduced into the soil by incorporating them into the irrigation water. The experimental design followed a completely randomized setup, with water stress and bacterial strains serving as the studied factors. Each experimental combination underwent replication three times for robust analysis and reliable results. On each of the separate irrigation treatments mentioned above, we inoculated these three types of bacteria:

ACBC1 : Alcaligenes faecalis bacterial strain
B 11 : Bacillus aryabhattai bacterial strain
SF 14 : Bacillus amyloliquefaciens bacterial strain
W.I : Without bacterial inoculation.

Comprehensive Measurement of Morphological, Physiological, and Biochemical Parameters in Campbell 33 Tomato Plants under Experimental Conditions

Parameters measured during our experiment include morphological, physiological, and biochemical aspects:

Morphological Parameters:

a. Root Length: Root length is evaluated using a centimeter-graduated ruler.
b. Stem Diameter: The basal part of the stem's diameter is measured with a stainless steel hardened digital caliper, boasting a sensitivity of 0.001 millimeters.
c. Growth Rate: The growth rate is determined by the length of the stem, measured with a graduated ruler. Stem length measurements are expressed in centimeters.

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d. Number of Leaves: Visual counting with the naked eye is employed to ascertain the number of leaves.
e. Number of Flowers: The number of flowers is determined through direct visual counting.

Physiological Parameters:

a. Chlorophyll Content: Chlorophyll content is measured twice. The first measurement is taken using the "CCM 200 plus" chlorophyll meter, and the second is conducted 10 days later using the dualex. DUALEX Scientific is a handheld leaf-clip sensor that uses fluorescence and light transmission to assess leaf status. It measures the optical absorbance of the leaf's epidermis in the UV range by detecting chlorophyll fluorescence. Additionally, it determines the leaf's chlorophyll content by using light at different wavelengths, specifically in the red and near-infrared (NIR) spectrum.
b. Above-ground and Root Fresh Weight: This parameter is measured using an industrial scale.
c. Above-ground and Root Dry Weight: Plants are placed in an oven at 70°C for 48 hours to measure dry weight. The dry weight is then measured using an industrial scale.
d. Water Content: Water content (expressed as a percentage) is calculated using the formula Te = (Pf - Ps / Ps) * 100, where Te is water content, Pf is fresh weight, and Ps is dry weight.

Biochemical Parameters:

a. Anthocyanin Content: Anthocyanin levels are measured directly on the leaves using the dualex.
b. Flavonoid Content: Flavonoid content is directly measured on the leaves using the dualex.

Statistics

The comprehensive dataset is presented using means accompanied by standard deviation values. Statistical analysis of the results was conducted with Minitab software to investigate the variance (ANOVA) in morphophysiological and biochemical characteristics related to both water stress and the applied bacteria. In cases of significant variability, a Tukey post hoc test at a significance level of $p < 0.05$ was applied. To ensure the robustness of subsequent statistical analyses, the Kolmogorov-Smirnov test was meticulously employed to assess the normality and homogeneity of variance for all variables. For a deeper understanding of the relationships among key variables, Principal Component Analysis (PCA) was employed. This analysis focused on the variables studied across various treatments, not only assessing their variability and correlations but also revealing patterns of similarities and differences within samples based on the treatment type. To better visualize distinct groups, Hierarchical Cluster Analysis (HCA) was applied. The PCA was performed using JMP Pro 14 software from SAS, Cary, NC, USA, allowing a nuanced exploration of the multidimensional dataset and providing insightful perspectives into the complex dynamics inherent in the study parameters.

Results

Effect of Water Stress and Bacterial Strains on Morphological Parameters

Figure 1 shows that severe water stress without soil bacterial inoculation (T1, WI) resulted in a remarkably significant reduction in root length, averaging 11.33 ± 1.57bc. In contrast, moderate water stress (T2) and light stress (T3) had no significant impact on root length, with averages nearly identical to those observed under normal irrigation (T0) 18.50 ± 2.2abc, 17.67 ± 2.08abc for T2, and 22.17 ± 2.77abc for T3.

Soil Bacterial inoculation with Alcaligenes faecalis (ACBC1) exhibited a positive effect on root length, particularly in conjunction with light water stress, reaching an average of (30.67±2.02a), surpassing even the inoculation with this bacterium under normal irrigation, severe, and moderate water stress conditions. Likewise, the Bacillus aryabhattai bacterial strain significantly increased root length compared to the treatment without WI inoculation, particularly under light and severe water stress, with mean values of (29.50±3.01ab) and (22.00±2.76abc), respectively.

The soil inoculation by Bacillus amyloliquefaciens significantly impacted the increase in root length under severe water stress, whereas its effect was not significant under normal irrigation conditions, moderate and light water stress.

In a nutshell, the soil inoculation by the three bacteria employed in this trial led to a substantial increase in the root system under different stress conditions (severe and light), while the inoculation had no impact under normal irrigation conditions and moderate water stress.

Figure 2 showls that severe water stress without bacterial inoculation (T1, WI) led to a highly significant reduction in stem diameter, averaging 2.77d ± 0.27. Moderate water stress (T2, WI) also resulted in a noteworthy decrease in stem diameter compared to normal irrigation (5.77c ± 0.32), albeit less severe than the impact observed with severe water stress. Conversely, light water stress (T3) exhibited no significant impact on root length, demonstrating an average of 7.18abc ± 1.13, nearly identical to the measurement observed under normal irrigation (7.28abc ± 0.53).

Soil Bacterial inoculation with Bacillus aryabhattai B 11 did not exhibit a substantial impact on stem diameter increase across various stress conditions. In contrast, the Alcaligenes faecalis bacterial strain (ACBC1) demonstrated a positive influence on stem diameter increase, particularly in conjunction with mild T3 water stress. It surpassed the average stem diameter obtained under normal irrigation conditions without inoculation (T0 WI) (7.28abc±0.53), reaching an average of (8.03a±0.76). This effect was consistently observed, even when compared to severe T1 WI (2.77d±0.27), normal T2 WI (5.77c±0.32), and mild T3 WI (7.18abc±1.14) conditions.

Similarly, the soil inoculation of the Bacillus amyloliquefaciens SF 14 bacterial strain in plants subjected to mild water stress exhibited a noteworthy increase in stem diameter when compared to all other treatments without soil inoculation. The average stem diameter recorded was 7.62ab±0.31, highlighting the significant positive impact of Bacillus amyloliquefaciens SF 14 under light water stress conditions.

In summary, the soil inoculation with the bacterial strains Bacillus amyloliquefaciens and Alcaligenes faecalis resulted in a considerable enhancement of stem diameter under stress conditions, specifically in moderate and mild water stress scenarios. Conversely, the soil inoculation had no discernible impact under conditions of normal irrigation and severe water stress.

In general, the various levels of water stress induced a significant reduction in plant growth rate, registering an average of (52.30abc±0.85) under light stress, (49.67abc±1.15) for moderate stress, and (47.07c±2.57) for severe water stress, as opposed to plants under normal irrigation conditions, which exhibited an average growth rate of (56.07a±1.85) (Figure 3).

Under light water stress conditions, all three inoculated bacteria—SF14, AcBc1, and B11—demonstrated a notably positive impact on growth rate compared to non-inoculated plants facing the same stress. For added precision, it is worth noting that the soil inoculation of SF14 (55.47a±2.34) and B11 (56.00a±1.00) under light water stress resulted in a growth rate similar to that of normally irrigated and non-inoculated plants (T0, WI) (56.07a±1.85).

In general, the diverse levels of water stress led to a notable decrease in the average number of leaves, with values of (125.00b±11.50) observed under light stress, (130.00b±12.50) for moderate stress, and (39.00c±8.50) for severe water stress. This is in contrast to plants subjected to normal irrigation conditions, which displayed an average leaf count of (174.00a±10.58).

Notably, bacterial soil inoculation exhibited no significant impact on leaf number increase under either normal irrigation or severe water stress, yielding similar averages. However, a substantial effect was observed under moderate and light water stress conditions. Specifically, under light water stress, the two bacteria SF14 and ACBC1 displayed significant impact, while under severe water stress, the three bacteria B11, SF14, and ACBC1 demonstrated a noteworthy influence surpassed that observed with non-inoculated irrigation (Figure 4).

Effect of Water Stress and Bacterial Strains on Physiological Parameters

The diverse water stress levels exhibited a detrimental effect on chlorophyll content, particularly evident in the case of severe water stress. The averages

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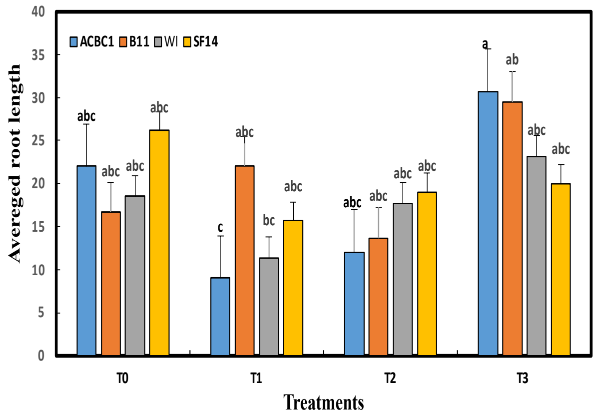

Figure 1. Impact of water stress and soil bacterial inoculation on root length (T0: normal irrigation; T1: severe water stress; T2: moderate water stress; T3: mild water stress. ACBC1: Alcaligenes faecalis bacterial strain, B 11: Bacillus aryabhattai bacterial strain, SF 14: Bacillus amyloliquefaciens bacterial strain, WI: no soil bacterial inoculation).

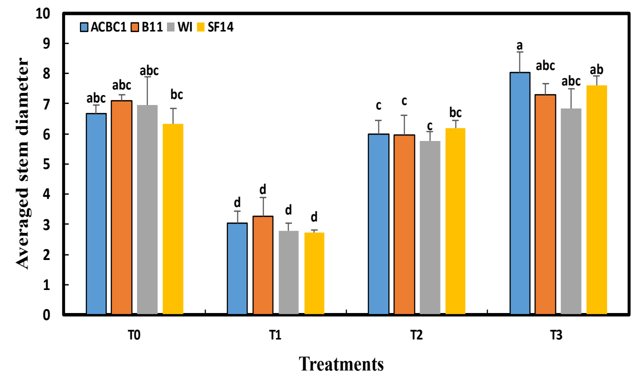

Figure 2. Impact of water stress and soil bacterial inoculation on stem diameter (T0: normal irrigation; T1: severe water stress; T2: moderate water stress; T3: mild water stress. ACBC1: Alcaligenes faecalis bacterial strain, B 11: Bacillus aryabhattai bacterial strain, SF 14: Bacillus amyloliquefaciens bacterial strain, WI: no soil bacterial inoculation).

were 22.36abc ± 3.75 under light water stress, 21.11abc ± 4.11 under moderate water stress, and 12.15c ± 2.66 under severe water stress, contrasting with the average of 26.87ab ± 3.62 observed under normal irrigation conditions. For increased precision and to emphasize average differences, the chlorophyll content of plants subjected to mild water stress and inoculated with Alcaligenes faecalis (ACBC1) was 26.89ab ± 2.85, while that of those inoculated with Bacillus aryabhattai (B11) was 29.39a ± 2.89, and with Bacillus amyloliquefaciens (SF14) was 28.70ab ± 2.22. All these values exceeded the chlorophyll content of non-inoculated plants experiencing the same type of stress, measuring 22.36abc ± 3.75, and even surpassed those under different stress conditions. In summary, it can be concluded that soil inoculation induced a positive response in chlorophyll content (Table 1).

Regarding fresh root weight, a notable reduction in average root weight (3.66d ± 1.26) was observed solely under severe water stress. In contrast, plants exposed to moderate stress (9.64abcd ± 1.15) and light stress (10.39abcd ± 1.83) maintained root weights comparable to those of plants receiving normal irrigation. In the case of all three stress types, the introduction of the three bacteria Alcaligenes faecalis (ACBC1), Bacillus amyloliquefaciens (SF14), and B11 (Bacillus aryabhattai)—elicited a positive impact on fresh root weight when compared to the non-inoculated treatments (WI). The same results were observed on aerial fresh weight. Although water stress and soil bacterial inoculation were applied, neither exhibited a significant effect on root dry weight and air dry weight, as reflected in their similar mean values. This suggests that the imposed water stress levels and soil bacterial inoculation did not elicit discernible changes in the measured weights.

A reduction in water content was noted in plants exposed to both moderate and severe water stress, recording averages of 81.22abcd±3.74 and 68.95cd±3.08, respectively. In contrast, plants under light water stress did not exhibit a significant decline in water content, registering 85.89a±4.39—an average nearly identical to that of plants receiving regular irrigation, at 89.57a±3.22.

The ACBC1 bacterium (Alcaligenes faecalis) was the soil inoculation that demonstrated an additive impact, and this increase was particularly pronounced in plants subjected to severe (84.97ab±1.78) and moderate (81.22abcd±3.74) water stress. This suggests that ACBC1's positive influence on the observed variables becomes more evident and substantial under higher levels of water stress, highlighting its potential as a beneficial factor in mitigating the adverse effects of water scarcity on plants.

Effect of Water Stress and Bacterial Strains on Biochemical Parameters

Water stress conditions, evidenced by averages of 0.79a±0.01 under severe stress (T1) and 0.43e±0.01 under moderate stress. In contrast, light water stress exhibited no discernible impact on anthocyanin content (0.45de±0.01), registering an average akin to that observed under normal irrigation 0.45de±0.04. This implies that the intensity of water stress plays a pivotal role in influencing anthocyanin production, with more pronounced effects observed under moderate and severe stress levels compared to the negligible impact under light stress. Likewise, the soil inoculation with the three bacteria employed in our experiment led to a decrease in the average anthocyanin levels under severe water stress. This unexpected outcome prompts further exploration into the interactions between soil bacterial inoculation and the plant's response to severe water stress, as it contradicts the common trend observed in stress-induced anthocyanin accumulation (Table 2).

Similar outcomes were observed for flavonoid content, mirroring the trends seen with anthocyanins. This parallel response indicates a degree of consistency in the plant's biochemical reactions to varying water stress conditions. Flavonoids, recognized for their antioxidant properties and role in plant defense, appear to be influenced in a manner akin to anthocyanins under the tested stress levels.

To enhance precision and elucidate correlations among the studied variables—specifically, the biochemical, physiological, and morphological parameters—alongside the various stressors and inoculation methods applied, Table 3 and Figure 6 elucidate the outcomes of the principal component analysis (PCA). The load diagram, in particular, visually illustrates how each variable is captured by the principal components. In this comprehensive analysis, the first principal component emerges as a robust factor, effectively encapsulating 54.40% of the inherent variability in the dataset. This substantial proportion implies that the first principal component serves as an almost exhaustive summary of the interrelationships between the variables, with the treatments accentuating its pivotal role in explaining the observed patterns. While the second principal component contributes a slightly smaller fraction, it still plays a significant role, accounting for an additional 13.70% of variability. The combined contribution of the first and second components reaches approximately 68%, signifying that these two components alone offer a comprehensive understanding of all the information embedded in the dataset, as illustrated in Figure 6 and figure 7. Upon examining the bacterial strains utilized, we observed divergent correlations with the parameters under investigation. Some strains exhibited positive correlations, while others displayed negative correlations. The nature of these correlations was found to be contingent upon both the specific stress applied and the type of bacteria introduced into the soil (ACBC1, B11, and SF14). Additional details about the correlations can be found in Table3, providing a more in-depth look at the relationships between the variables studied.

To enhance the visualization of population classifications based on the conducted treatments (water stress and soil inoculation), a Hierarchical cluster analysis (HCA) was conducted (Figure 8). This involved projecting individuals onto PC1 and PC2, as depicted in previous findings. Cluster 1 signifies the treatment group encompassing T0 (normal irrigation) and T3 (light water stress), while Cluster 2 represents the treatment group associated with T2 (moderate water stress). On the other hand, Cluster 3 encapsulates the population sub-

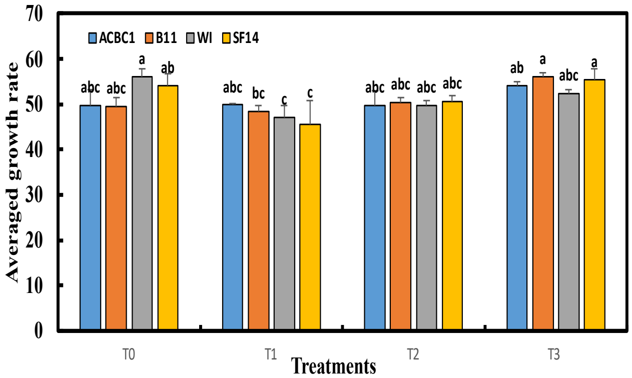

Figure 3. Impact of water stress and soil bacterial inoculation on growth rate (T0: normal irrigation; T1: severe water stress; T2: moderate water stress; T3: mild water stress. ACBC1: Alcaligenes faecalis bacterial strain, B 11: Bacillus aryabhattai bacterial strain, SF 14: Bacillus amyloliquefaciens bacterial strain, WI: no bacterial soil inoculation).

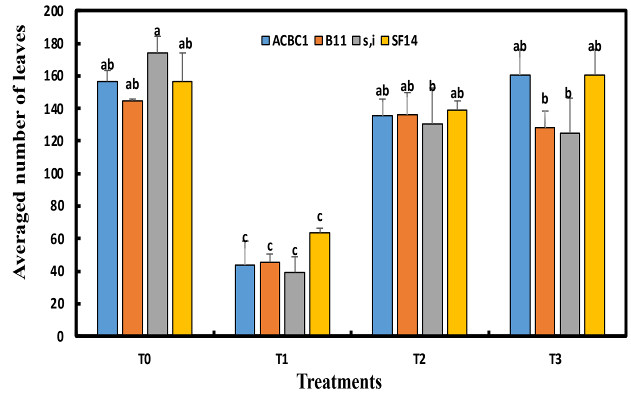

Figure 4. Impact of water stress and soil bacterial inoculation on number of leaves (T0: normal irrigation; T1: severe water stress; T2: moderate water stress; T3: mild water stress. ACBC1: Alcaligenes faecalis bacterial strain, B 11: Bacillus aryabhattai bacterial strain, SF 14: Bacillus amyloliquefaciens bacterial strain, WI: no soil bacterial inoculation).

jected to T1 (severe water stress). Notably, the first cluster is characterized by plants displaying elevated morphological and physiological growth alongside lower bioclimatic parameters. The second cluster exhibits average morphological and physiological parameters. In contrast, the third cluster is distinguished by elevated levels of biochemical parameters coupled with diminished morphological and physiological growth (Figure 8). This analysis provides a comprehensive view of the distinct treatment effects on population clusters, elucidating how different treatments contribute to variations in morphological, physiological, and biochemical aspects among the studied populations.

Discussion

In this study, we investigated the impact of water stress and soil inoculation with bacterial strains exhibiting plant growth-promoting properties (PGPR) on various morphological, physiological, and biochemical parameters in tomato plants. This research aims to shed light on plant responses to water stress while assessing the specific influence of PGPR bacterial strains on different aspects of plant performance. Examined parameters include morphological features such as root growth and weight, as well as physiological and biochemical measurements such as chlorophyll levels, anthocyanins, and flavonoids. The multidimensional approach adopted in this study seeks to provide a comprehensive understanding of plant adaptation mechanisms to water stress, while evaluating the potential benefits of PGPR bacterial strains. The obtained results will contribute to enhancing sustainable agricultural practices by uncovering the complex interactions between environmental factors, soil microbiome, and plant physiology.

In morphological terms, our findings indicate that water stress initiates a decline in root length, particularly evident after surpassing a stress threshold of 40% (severe water stress). This reduction in root elongation aligns with the outcomes of (Franco et al., 2011; Koch, 2019), which similarly emphasized a decrease in root growth associated with diminishing osmotic potential. The observed reduction in root length under water stress conditions underscores the plant's adaptive response to conserve water in times of scarcity (Franco et al., 2011). The agreement with previous research adds weight to the validity of our results and suggests a commonality in the impact of water stress on root morphology across different studies (Gambetta et al., 2020; Zia et al., 2021).

Concerning stem diameter, moderate and severe water stress induce a significant reduction in stem diameter. These observations align with the findings of Daaboul's study (2015) (Daaboul, 2015) on poplar, where severe stress resulted in a diameter decrease. Additionally, our results harmonize with the findings of other studies, including those by (Alordzinu et al., 2022; Andryei et al., 2021; Nahar and Ullah, 2012; Nemeskéri and Helyes, 2019) Alordzinu et al. (2021), Nahar and Ullah (2012), and Nemeskéri et al. (2019). Fenglan et al. (2019) further corroborated these outcomes through a report on changes in cell size and tissue hydration. The report suggests that as soil water diminishes below field capacity, reaching the point of near-permanent wilting, plant growth is generally constrained (Chandrasekaran et al., 2021). The consistency among these studies reinforces the reliability of our observations and underscores the shared impact of water stress levels on stem diameter across diverse plant species. Understanding these responses contributes to our broader comprehension of plant physiological adjustments to varying water availability, with implications for optimizing crop management practices under different water stress conditions.

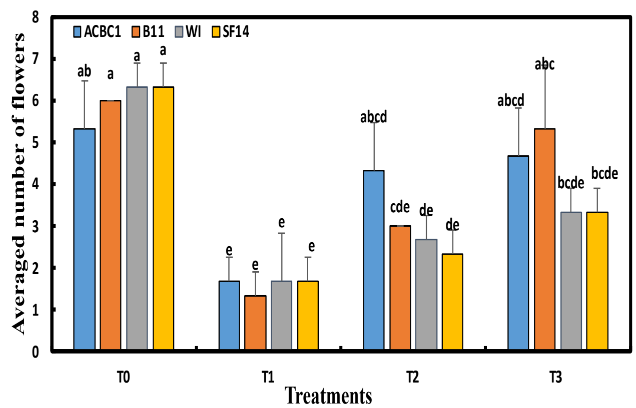

Figure 5. Impact of water stress and bacterial soil inoculation on number of flowers (T0: normal irrigation; T1: severe water stress; T2: moderate water stress; T3: mild water stress. ACBC1: Alcaligenes faecalis bacterial strain, B 11: Bacillus aryabhattai bacterial strain, SF 14: Bacillus amyloliquefaciens bacterial strain, WI: no bacterial soil inoculation).

The obtained results highlight a reduction in the height of water-stressed plants when compared to their non-stressed counterparts, aligning with the conclusions drawn by (Alordzinu et al., 2022) This diminished plant height can be ascribed to a decline in the relative water content of the leaves caused by the imposition of water stress, leading to a decrease in cell turgor pressure (Mani and Goniewicz, 2023). Consequently, the curtailed turgor pressure impedes cell elongation, culminating in a noticeable reduction in overall plant growth, as supported by studies such as (Seleiman et al., 2019; Toumi et al., 2022). The observed decrease in plant height under water stress conditions underscores the direct impact of water availability on the fundamental processes governing cell expansion and turgor-driven growth. This aligns with established physiological principles, where water stress disrupts the delicate balance needed for optimal cell elongation, ultimately affecting the plant's stature (Chandrasekaran et al., 2021).

Our findings underscore that water stress induces a reduction in the number of leaves, aligning with similar results obtained by (Daaboul, 2015). This reduction in leaf count can be interpreted as a morphological adaptation of plants to cope with water stress, as highlighted by (Koch, 2019; Toumi et al., 2022). The effect of water stress is to reduce the number of flowers, and as stress increases, the number of flowers decreases. This result is in line with the findings

Table 1. Influence of water stress and soil bacterial inoculation (T0: normal irrigation; T1: severe water stress; T2: moderate water stress; T3: mild water stress. ACBC1: Alcaligenes faecalis bacterial strain, B 11: Bacillus aryabhattai bacterial strain, SF 14: Bacillus amyloliquefaciens bacterial strain, WI: no soil bacterial inoculation) on physiological parameters (chlorophyll content, fresh root weight, aerial fresh weight, dry weight of roots, air-dry weight, and water content).

		TO	*T1*	*T2*	*T3*
Chlorophyll content	**WI**	26.87ab ± 3.62	12.15c ±2.66	21.11abc ± 4.11	22.36abc ±3.75
	ACBC1	25.46abc ± 4.75	14.78bc ±1.21	25.84abc ± 4.42	26.89ab ±2.85
	B11	27.01ab ± 3.50	15.62abc ±1.49	24.64abc ± 4.47	29.39a ±2.89
	SF14	28.06ab ± 3.43	15.81abc ±3.10	27.93ab ± 2.51	28.70ab ±2.22
Fresh root weight	**WI**	10.45abcd ±0.74	3.66d ±1.26	9.64abcd ±1.15	10.39abcd ±1.83
	ACBC1	10.15abcd ±1.42	6.66bcd ±1.66	10.49abcd ±1.03	12.42abc ± 2.55
	B11	9.89abcd ±1.82	4.20cd ±1.10	9.74abcd ±1.65	15.95a ± 3.70
	SF14	11.26abcd ±0.97	6.98bcd ±1.91	11.71abcd ±1.78	14.62ab ± 3.50
Aerial fresh weight	**WI**	37.01abc±2.58	13.01c±2.45	34.17abc±4.08	36.89 abc±5.55
	ACBC1	36.02abc±5.08	23.66bc±3.99	37.18abc±3.62	44.04 ab ±5.05
	B11	35.05abc±4.48	14.90c±3.46	34.17abc±4.08	56.50 a ±6.30
	SF14	39.92abc±3.39	24.73bc±4.79	41.55abc±5.30	51.80 ab ±6.55
Dry weight roots	**WI**	2.57a±0.06	1.01a±0.29	1.20a±0.33	1.42a±0.27
	ACBC1	1.53a±0.38	1.05a±0.35	1.30a±0.36	1.51a±0.33
	B11	1.56a±0.21	1.42a±0.27	1.80a±0.44	2.23a±0.26
	SF14	1.17a±0.19	2.00a±0.29	1.19a±0.33	2.03a±0.51
Air dry weight	**WI**	6.37a±1.02	3.56a±0.81	3.93a±0.61	5.04a±0.97
	ACBC1	5.60a±1.21	3.72a±0.91	6.17a±1.25	5.35a±1.19
	B11	5.53a±0.74	5.04a±1.05	6.40a±1.44	7.90a±1.44
	SF14	4.16a±0.70	4.13a±1.14	4.23a±1.06	4.83a±1.10
Water content	**WI**	89.57a±3.22	68.95cd±3.08	81.22abcd±3.74	85.89a±4.39
	ACBC1	89.12a±3.35	84.97ab±1.78	90.36a±3.71	87.11a±5.55
	B11	84.09ab±1.15	66.62cd±5.12	78.44abcd±3.16	81.53abcd±4.60
	SF14	89.62a±0.91	70.28bcd±5.79	81.97abc±3.74	91.16a±3.27

Table 2. Influence of water stress and bacterial soil inoculation (T0: normal irrigation; T1: severe water stress; T2: moderate water stress; T3: mild water stress. ACBC1: Alcaligenes faecalis bacterial strain, B 11: Bacillus aryabhattai bacterial strain, SF 14: Bacillus amyloliquefaciens bacterial strain, WI: no soil bacterial inoculation) on biochemical parameters (Anthocyanin Content, Flavonoid Content).

		TO	*T1*	*T2*	*T3*
Anthocyanin Content	**WI**	0.45de±0.04	0.79a±0.01	0.43e±0.01	0.45de±0.01
	ACBC1	0.45de±0.03	0.56b±0.03	0.43e±0.02	0.43e±0.07
	B11	0.43e±0.02	0.54bcd±0.01	0.46cde±0.07	0.41e±0.01
	SF14	0.43e±0.01	0.55bc±0.07	0.41e±0.03	0.43e±0.01
Flavonoid Content	**WI**	0.35b±0.03	1.06a±0.16	0.40b±0.06	0.58ab±0.13
	ACBC1	0.37b±0.15	0.86ab±0.10	0.37b±0.14	0.32b±0.07
	B11	0.47ab±0.09	0.82ab±0.26	0.69ab±0.63	0.50ab±0.09
	SF14	0.45ab±0.08	0.72ab±0.23	0.29b±0.06	0.50ab±0.15

of (Koch, 2019), who demonstrated that the number of flower buds decreased even with a slight reduction in soil water content.

In this study, the bacterial strains employed consistently exhibited a positive impact on several key morphological parameters, encompassing the number of leaves, flowers, growth rate, root length, and stem diameter. The data presented earlier supports these observations, revealing distinct trends in response to bacterial inoculation under different water stress levels. Firstly, with regard to the number of leaves, the introduction of bacterial strains, particularly ACBC1, B11, and SF14, consistently resulted in higher leaf counts compared to non-inoculated plants. This suggests that the beneficial bacteria promoted leaf development, potentially enhancing photosynthetic capacity and overall plant vitality. Similarly, the positive influence of bacterial inoculation was evident in the increased number of flowers. ACBC1, B11, and SF14 exhibited a notable impact, fostering greater floral abundance compared to non-inoculated plants. This suggests a potential role of these bacterial strains in promoting reproductive processes and enhancing the plant's reproductive capacity. The positive impact on growth rate further supports the beneficial effects of bacterial inoculation. Plants inoculated with ACBC1, B11, and SF14 consistently displayed higher growth rates, indicating that these bacteria may contribute to overall plant vigor and development. Moreover, the positive effects extended to root length, with bacterial strains enhancing the development of root systems. This is crucial for nutrient uptake and water absorption, which are especially important under water stress conditions. The observed increase in root length suggests that the bacterial strains play a role in improving the plant's ability to withstand and adapt to water stress. Finally, the positive impact on stem diameter highlights the potential role of bacterial strains in enhancing stem development. A thicker stem diameter is often associated with increased structural support and nutrient transport efficiency, contributing to overall plant robustness many studies confirm our results as (Castillo et al., 2013; Chakraborty et al., 2013; Curá et al., 2017; Heidari and Golpayegani, 2012; Kaur et al., 2022; Seleiman et al., 2019).

Our findings reveal a noteworthy decrease in chlorophyll content under severe water stress, aligning with the observations made by (Kirnak et al., 2001). Conversely, mild or moderate water stress did not elicit a significant impact on chlorophyll content, consistent with studies by (Dbara S, Ouni R, Fezai N, 2016) in pear and Messaoudi et al in citrus, where reduced water requirements did not affect chlorophyll levels. The reduction in chlorophyll content under severe stress is likely attributed to enzymatic degradation prompted by stomatal closure and limited water availability. This leads to a decrease in CO_2 diffusion conductance, biochemically restricting the chloroplast's capacity to fix CO_2. Severe water stress significantly decreased both aerial and root fresh weight, consistent with (Mahpara et al., 2018) findings. Mild and moderate water stress had no impact on fresh weight, aligning with (Mahpara et al., 2018) demonstration that a 50% reduction in water requirements for tomatoes did not affect plant fresh weight. Water content serves as a crucial parameter for assessing a plant's water status. In our experiment, a substantial reduction in the water content of tomato plants was observed under conditions of severe water stress. This outcome aligns with the research findings of (Nemeskéri and Helyes, 2019), who similarly reported a decline in plant moisture content during prolonged water stress. Conversely, under mild to moderate water stress, the moisture content

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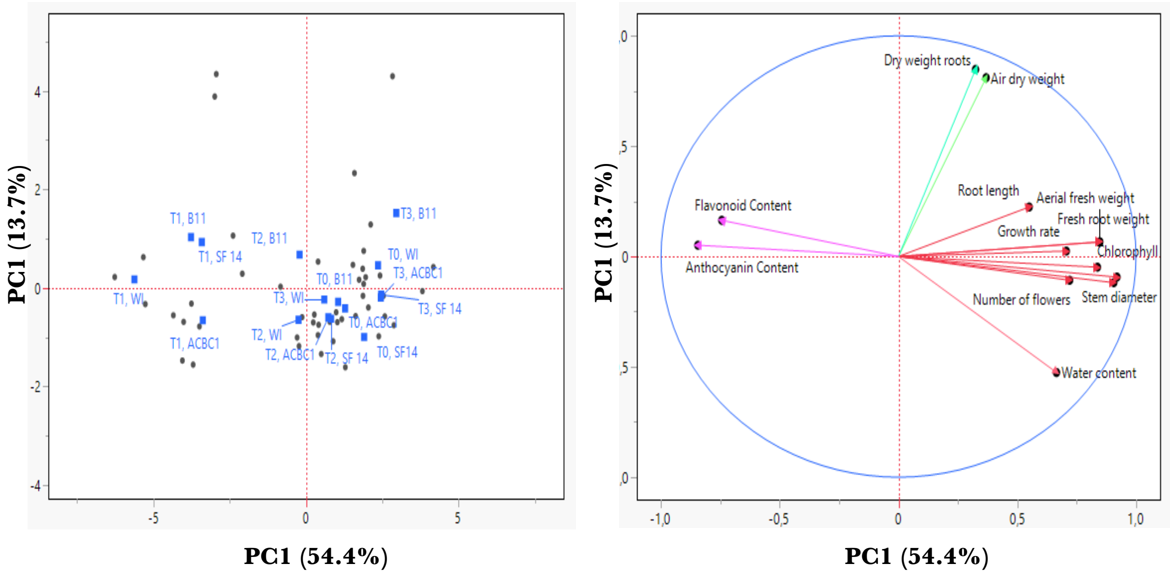

Figure 6. Principal Component Analysis (PCA) of Studied Variables Across Diverse Treatments (A); Two-Component Double Projection Diagram (B).

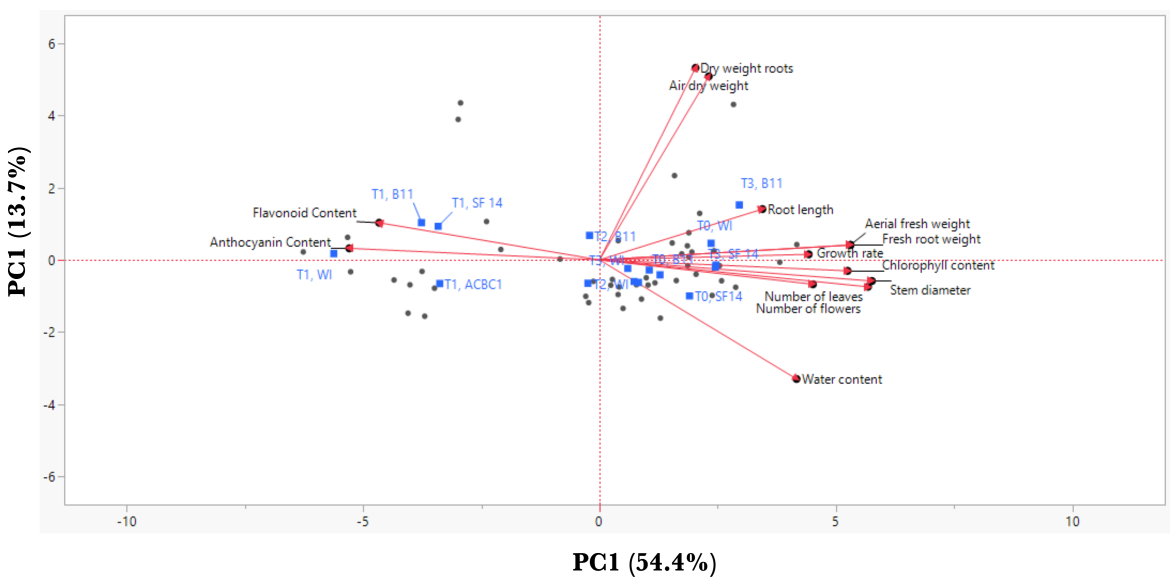

Figure 7. Biplot represents the projection of the individuals and variables on the PC1 and PC2. Projection of the variables according to the different treatments used.

Table 3. Times New Roman (Headings CS).

	Aerial fresh weight	Root length	Stem diameter	Growt h rate	Number of leaves	Number of flowers	Chlorophyll content	Fresh root weight	Air dry weight	Dry weight roots	Water content	Anthocyanin Content	Flavonoid Content
Aerial fresh weight	1.00	0.56	0.71	0.53	0.68	0.44	0.64	1.00	0.29	0.27	0.52	-0.65	-0.53
Root length	0.55	1.00	0.47	0.47	0.33	0.27	0.36	0.56	0.23	0.20	0.11	-0.42	-0.33
Stem diameter	0.70	0.47	1.00	0.65	0.87	0.69	0.77	0.71	0.29	0.21	0.65	-0.75	-0.67
Growth rate	0.53	0.47	0.65	1.00	0.57	0.52	0.54	0.53	0.29	0.20	0.52	-0.49	-0.34
Number of leaves	0.68	0.34	0.87	0.58	1.00	0.72	0.77	0.69	0.27	0.25	0.68	-0.76	-0.68
Number of flowers	0.44	0.27	0.68	0.52	0.72	1.00	0.61	0.44	0.25	0.19	0.57	-0.50	-0.48
Chlorophyll content	0.64	0.36	0.77	0.54	0.77	0.61	1.00	0.64	0.25	0.24	0.47	-0.72	-0.68
Fresh root weight	1.00	0.56	0.71	0.53	0.69	0.44	0.64	1.00	0.29	0.27	0.52	-0.65	-0.53
Air dry weight	0.29	0.23	0.29	0.29	0.27	0.25	0.25	0.29	1.00	0.74	-0.06	-0.26	-0.12
Dry weight roots	0.27	0.20	0.21	0.20	0.25	0.19	0.24	0.27	0.74	1.00	-0.17	-0.25	-0.15
Water content	0.52	0.11	0.65	0.51	0.68	0.57	0.46	0.52	-0.06	-0.17	1.00	-0.54	-0.44
Anthocyanin Content	-0.65	-0.42	-0.75	-0.48	-0.76	-0.49	-0.72	-0.65	-0.26	-0.25	-0.54	1.00	0.77
Flavonoid Content	-0.53	-0.33	-0.67	-0.34	-0.68	-0.48	-0.67	-0.53	-0.12	-0.15	-0.44	0.77	1.00

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of stressed plants closely approximated that of unstressed plants. These results highlight the sensitivity of plant water content to the severity of water stress, providing valuable insights into the plant's adaptive responses to varying levels of water availability.

In terms of adaptation and resilience to water stress, (Bouzidi, 2005), notes that plants develop adaptive characteristics in their structures or molecules. These adaptations often involve the accumulation of secondary metabolites such as anthocyanins and flavonoids, crucial for various defense processes. In our study, severe water stress triggered an increase in anthocyanin levels, while mild to moderate stress resulted in levels comparable to those in unstressed plants. Similar conclusions were drawn by other researchers, including (KHOUILDI and DERAOUI, 2016) in their investigations on durum wheat, where the plant exhibited stress indicators through anthocyanin production or chlorophyll degradation. Likewise, the soil inoculation with the three bacteria employed in our experiment led to a decrease in the average anthocyanin levels under severe water stress. This unexpected outcome prompts further exploration into the interactions between soil bacterial inoculation and the plant's response to severe water stress, as it contradicts the common trend observed in stress-induced anthocyanin accumulation a number of studies conducted on several plants confirm our results (Curá et al., 2017; Heidari and Golpayegani, 2012; Toscano et al., 2019). The reduction in anthocyanin levels may suggest a complex interplay between the introduced bacteria and the plant's physiological responses. It raises questions about potential regulatory mechanisms or signaling pathways influenced by bacterial inoculation that may counteract the usual anthocyanin accumulation triggered by severe water stress. To fully comprehend this phenomenon, additional research is warranted to unveil the underlying mechanisms and interactions between the bacterial strains and the plant's stress responses. It is essential to explore whether this reduction in anthocyanin levels correlates with any alterations in the plant's overall stress tolerance or if it represents a unique aspect of the interplay between bacterial inoculation and severe water stress. The consistency in results observed with flavonoid content under severe water stress following bacterial inoculation prompts a similar need for investigation.

Conclusion

In conclusion, this study delved into the intricate interplay between water stress and soil inoculation with plant growth-promoting bacterial strains, examining their collective impact on various morphological, physiological, and biochemical parameters in tomato plants. The research aimed to unravel the nuanced responses of plants to water stress while specifically assessing the influence of PGPR bacterial strains on diverse aspects of plant performance. Morphological features, physiological indicators, and biochemical contents were thoroughly examined, providing a comprehensive understanding of plant adaptation mechanisms to water stress and the potential benefits of bacterial strains. Intriguingly, the introduction of bacterial strains, specifically ACBC1, B11, and SF14, consistently demonstrated positive impacts on various morphological parameters, including the number of leaves, flowers, growth rate, root length, and stem diameter. These findings suggest that these beneficial bacteria play a crucial role in promoting plant development, potentially enhancing overall plant vitality and reproductive capacity. The positive effects extended to physiological and biochemical aspects, emphasizing the potential of bacterial strains to mitigate the adverse effects of water stress.

Conflicts of Interest: The author declare no conflict of interest.

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