Black Sea Journal of Agriculture

doi: 10.47115/bsagriculture.1946326

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 9 - Issue 4 : 641-651 / July 2026

COMPARATIVE EFFICACY OF SEED AND SOIL INOCULATION IN SOYBEAN UNVEILING THE DOMINANT ROLE OF SOIL MICROBIAL ACTIVITY IN DROUGHT MITIGATION

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Abstract: Drought stress severely negatively affects the growth and yield of soybean (*Glycine max* L.) by causing oxidative damage and suppressing physiological functions. While plant growth-promoting rhizobium alleviates these effects, the comparative effectiveness of different inoculation techniques has not been adequately investigated. This study investigated the physiological, biochemical, and soil microbiological responses of drought-stressed soybean (50% field capacity) to two *Bradyrhizobium japonicum* inoculation methods (conventional seed inoculation (I1) versus direct soil inoculation (I2)). Drought significantly reduced plant biomass, relative water content (RWC), and chlorophyll levels while aggravating markers of oxidative stress (H_2O_2, MDA, and electrolyte leakage). Both inoculation methods reduced drought-induced damage; however, soil inoculation (I2) showed significant superiority. Compared to uninoculated stressed plants, I2 treatment increased fresh and dry weight by 91.7% and 104.3%, respectively, while maximally suppressing lipid peroxidation and preserving cell membrane integrity. Moreover, soil inoculation profoundly increased rhizosphere microbial activity, increasing soil respiration (CO_2) and dehydrogenase activity (DHA) by 110.6% and 86.7%, respectively. In particular, Random Forest machine learning analysis identified soil DHA as the most critical determinant in predicting plant biomass under stress, far outweighing internal oxidative stress indicators. These findings suggest that direct soil inoculation optimizes rhizosphere enzymatic activity and offers a more robust agronomic strategy than seed inoculation for sustainable soybean cultivation in drought-prone regions.

Keywords: *Bradyrhizobium japonicum*, Soybean, Rhizosphere engineering, Oxidative stress alleviation, Random Forest modeling, Drought tolerance

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Received: May 07, 2026
Accepted: July 07, 2026
Published: July 15, 2026

Cite as: Tuncer, E. N., & Sarıoğlu, A. (2026). Comparative efficacy of seed and soil inoculation in soybean unveiling the dominant role of soil microbial activity in drought mitigation. *Black Sea Journal of Agriculture*, 9(4), 641-651.

1. Introduction

Increasing drought stress as a result of global climate change has become one of the most important abiotic stress factors threatening agricultural production worldwide. Drought causes increased water loss in plants and a decrease in cellular water potential, leading to serious impairments in basic vital functions such as photosynthesis, respiration and nutrient uptake (Farooq et al., 2009). In this process, although plants develop a strategy of closing their stomata to minimize water loss, this limits the carbon dioxide intake required for photosynthesis and, as a result, causes a significant decrease in photosynthetic efficiency (Lawson and Vialet-Chabrand, 2019). Soybean (*Glycine max* L.), one of the world's most important sources of protein and oil, although it is a mesophyte plant, is very sensitive to water deficit, especially during critical development periods such as flowering and pod formation. Under drought stress, significant decreases occur in the growth parameters, biomass production and final grain yield of the soybean plant (Karakaya and Ödemiş, 2019; Çirka et al., 2022).

One of the most prominent cellular responses that occurs in plants under water stress conditions is the overproduction of reactive oxygen species (ROS). Although ROS compounds such as hydrogen peroxide (H_2O_2) and superoxide radicals are byproducts of normal cellular metabolism, they accumulate in stress situations such as drought, causing oxidative stress that damages vital cellular components such as cell membranes, proteins, and DNA (Apel and Hirt, 2004; Gill and Tuteja, 2010). To determine the extent of this oxidative damage, electrical leakage (EL%), an indicator of cell membrane integrity, and malondialdehyde (MDA) levels, a product of lipid peroxidation, are frequently used (Siddique et al., 2012; Prasad et al., 2020). Plants activate enzymatic and non-enzymatic complex antioxidant defense mechanisms to cope with this oxidative damage, but in cases of severe or long-term drought, these defense systems may be inadequate (Singh et al., 2021).

In recent years, the use of plant growth-promoting rhizobium has become increasingly important as a sustainable and environmentally friendly approach to increase the tolerance of plants to abiotic stresses. These beneficial microorganisms, naturally found in the soil, expand the root system of plants, ensuring more effective uptake of water and nutrients from the soil, thus increasing the resistance of plants to water stress (Grover et al., 2011; Vurukonda et al., 2016). It has been shown that inoculation of *Bradyrhizobium japonicum*, a specific symbiotic nitrogen fixer for soybean plant, can not only increase nitrogen fixation but also improve plant adaptation to drought stress (Sarıoğlu et al., 2017; Sheteiwy et al., 2021). It has been reported in various studies that microbial applications alleviate oxidative stress by strengthening antioxidant defense mechanisms in plants, protect cell membrane integrity, and regulate the expression of stress response genes (Marulanda et al., 2007; Sandhya et al., 2009; Zahedi and Abbasi, 2015).

However, the effectiveness of microbial treatments can vary greatly depending on the bacterial strain used, environmental conditions, and especially the inoculation method. Although there are studies in the literature examining the effects of bacterial applications to alleviate drought stress in soybean plants (Martins et al., 2018; Dubey et al., 2021; Moretti et al., 2021), studies directly comparing different inoculation techniques are quite limited. In particular, a comparative analysis of the specific effects of direct soil bacterial applications by seed inoculation on plant physiological responses and soil microbial activity (soil respiration and dehydrogenase activity) is lacking (İşler and Coşkan, 2009). While seed inoculation ensures direct contact of bacteria with the germinating root, soil application can affect the general microbial population in the rhizoster and soil health in different ways (Chauhan et al., 2023; Panek et al., 2026).

In this context, the main purpose of the presented study is to comparatively examine the effects of two different *Bradyrhizobium japonicum* inoculation techniques (seed and soil) applied on soybean plants grown under drought stress. Within the scope of the research, the effects of these two different inoculation methods on plant growth parameters, water use efficiency, chlorophyll content and oxidative stress indicators (H_2O_2, MDA, EL%), as well as their effects on soil microbial activity parameters such as soil respiration (CO_2) and dehydrogenase activity (DHA), will be evaluated. The findings from this study aim to make significant contributions to determining the most appropriate bacterial inoculation strategy in soybean production under drought conditions and to the development of sustainable agricultural practices.

2. Materials and Methods

2.1. Research Area and Materials

This study was carried out under controlled conditions in the glass greenhouse of Harran University, Department of Soil Science and Plant Nutrition. The experiment was carried out in soil environment and in pots with a capacity of 3 liters. The soil material to be used in the research was obtained from the trial plots on the university campus; soil type (Clay Loam), pH (7.8), organic matter content (2.0%), available P (17.2 mg kg^{-1}), and electrical conductivity (1.17 dS m^{-1}).

As plant material, "GAPSOY 16" soybean (*Glycine max* L.) variety, which was bred specifically for the region's ecology by the GAP Agricultural Research Institute, was used. For microbial inoculation, *Bradyrhizobium japonicum* bacterial strain, specific to soybeans, obtained from Ankara Soil, Fertilizer and Water Resources Central Research Institute, was used.

The experiment was set up with 3 replications according to the randomized block trial design. The study is based on two main factors (irrigation level and inoculation method). The irrigation factor consists of two levels: full irrigation (control, 100% of potted field capacity) and water stress (50% of potted field capacity). The vaccination factor is; It includes three levels: ungrafted (control), seed inoculation (I1) and soil inoculation (I2).

Before planting, DAP (Diammonium phosphate) fertilizer was applied to the pot soil as base fertilizer at a rate of 15-20 kg da^{-1}, and no top fertilization was made afterwards (Masrahi et al., 2023). 5 seeds were planted in each pot, and after germination, thinning was made to leave 3 homogeneously developing plants in each pot.

Bacterial inoculation was carried out using two different techniques:

İnoculation from Seed (I1): İnoculation material is directly applied to the seed surface. A 4-5% sugar solution was used to ensure that bacteria adhere to the seed better. After the application, the seeds were planted in a semi-bright environment away from sunlight, after which they were allowed to absorb moisture.

Soil İnoculation (I2): The inoculation material was applied directly to the soil on the seed bed to be planted by the pulverization (spraying) method, and then the seed was planted.

Drought stress application was started when the plants germinated and reached the stage of 3-5 leaves. Irrigation operations were carried out on the basis of weighing the pots regularly and completing the lost water amount to the targeted field capacity (100% or 50%).

2.2. Parameters Examined

Physiological and biochemical measurements were carried out when the plants reached the flowering period and the effects of drought stress began to become evident.

Growth Parameters and Chlorophyll Content

Before harvest, the chlorophyll content in the leaves of the plants was measured using the SPAD-502 (Konica Minolta) chlorophyllometer device, which is a non-destructive method. Additionally, chlorophyll a and b contents were determined by Witham et al. (1971). It was determined spectrophotometrically according to the method of 1971. In this method, 0.25 g of leaf sample was homogenized in 80% acetone, the extract was read at

663 nm and 645 nm wavelengths and the chlorophyll amounts were calculated. During the harvesting process, the root and shoot (above-root) lengths of the plants were measured.

After the fresh weights of the plants were weighed, the samples were dried in an oven set at 70°C until they reached a constant weight and their dry weights were determined.

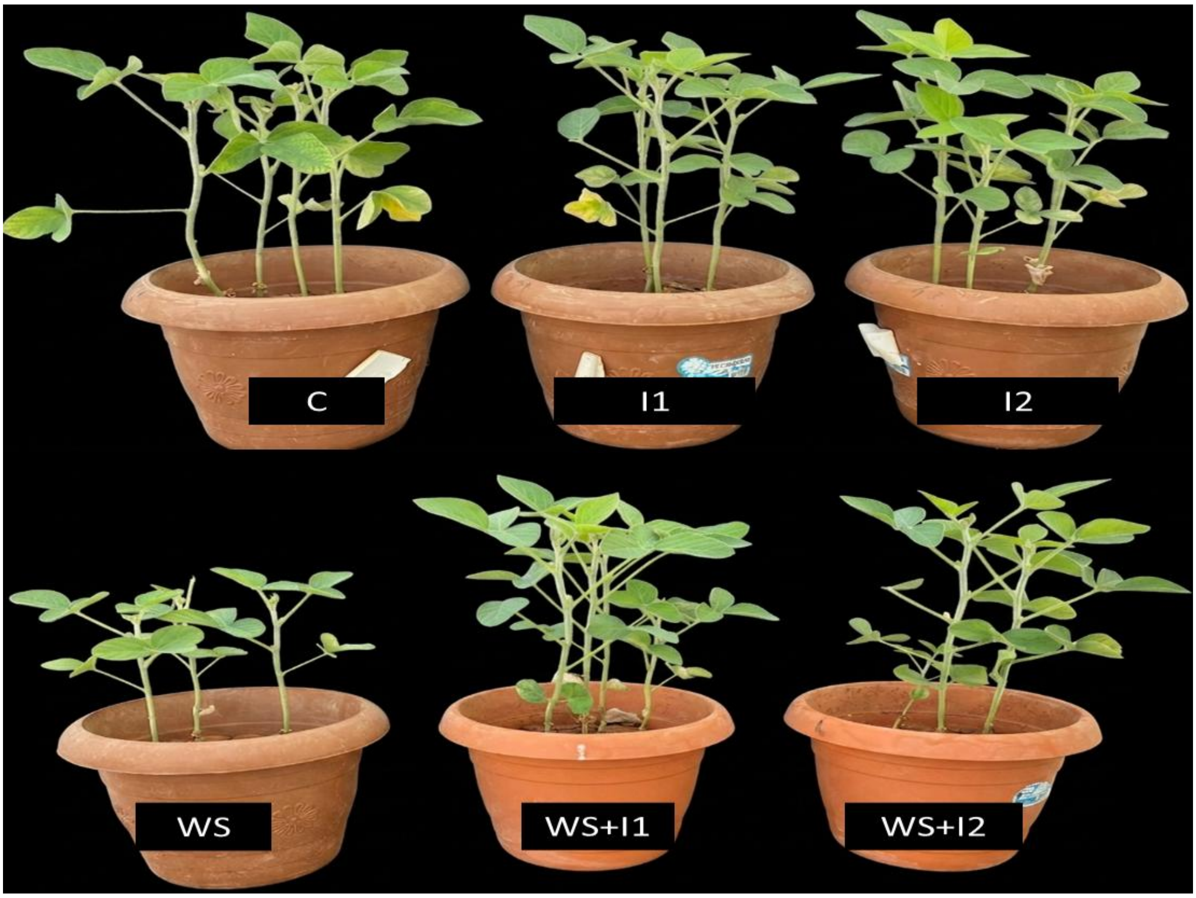

Figure 1. Effect of drought stress and bacterial inoculation applications on the visual appearance of soybean plants.

2.3. Plant Water Content (RWC%)

Leaf relative water content (RWC) was determined according to the method reported by Bertamini et al., (2006). Fresh weights (FW) of randomly selected top (flag) leaves were weighed, then turgor weights (TW) were measured by keeping the leaves in pure water at 4-5 °C in the dark for 24 hours. Finally, RWC% was calculated by taking the dry weight (DW) of the leaves dried at 70 °C for 24 hours.

2.4. Oxidative Stress Indicators

Electrical leakage (EL%), an indicator of cell membrane damage, was determined according to the method of Dionisio-Sese and Tobita (1998). Leaf samples were shaken in pure water at 25 °C for 24 hours, the first electrical conductivity (EC1) was measured, and then the second measurement (EC2) was made after the samples were autoclaved at 120 °C for 20 minutes and cooled. Electrical leakage is calculated by the formula (EC1/EC2) × 100.

Hydrogen peroxide (H_2O_2) content was measured spectrophotometrically at 390 nm according to the method of Loreto and Velikova, (2001). The amount of malondialdehyde (MDA), which is an indicator of lipid peroxidation, was determined by Weisany et al. (2012). It was determined by reading the absorbance values at 532 nm using the thiobarbituric acid (TBA) reaction according to the method of 2012.

2.5. Soil Microbiological Analyzes

Soil respiration (CO_2 release), as an indicator of soil microbial activity, was determined by incubation and titration technique according to the closed jar method of Isermeyer (1952). Soil dehydrogenase activity (DHA) was determined according to the method of Thalmann (1968) by incubating soil samples with TTC (triphenyltetrazolium chloride) and measuring the formed formazan spectrophotometrically at 546 nm.

2.6. Data Analysis

All data obtained were subjected to analysis of variance (ANOVA) in accordance with the randomized block trial design. The significance of the differences between applications was evaluated at the $P<0.05$ significance level using Duncan. In addition to conventional statistical analyses, a machine learning approach (Random Forest) was applied as a data-driven method to assess the relative importance of variables (Sevinç and Yılmaz, 2026).

3. Results

3.1. Growth Parameters and Relative Water Content (RWC%)

According to the research results, it was determined that drought stress (WS) and bacterial inoculation applications had statistically significant effects on fresh weight (FW), dry weight (DW) and RWC% of soybean plants (Figure 2). WS application caused a significant decrease in fresh weight by 52.7% and dry weight by 56.6% compared to control (non-appl.) plants. On the other hand, seed bacterial inoculation (WS+I1) applied under drought stress increased fresh weight by 26.9% and dry weight by 12.7% compared to WS application. Bacterial inoculation from soil (WS+I2) was found to be much more effective and provided a significant increase of 91.7% in fresh weight and 104.3% in dry weight compared to WS application. These results were found to be statistically significant ($P<0.05$).

When the RWC% was evaluated (Figure 2C), it was found that WS reduced the water content in the leaves by 48.9% compared to the control group. While WS+I1 application under stress conditions increased the RWC% value by 9.0% compared to WS, WS+I2 application increased it by 29.9%. These results were found to be statistically significant ($p<0.05$).

WS application significantly reduced the height of soybean plants (Figure 2D) by 36.8% compared to the control group (C). However, bacterial inoculations applied under drought stress were very effective in recovering plant height. While seed inoculation increased plant height by 45.4% compared to WS, soil inoculation increased by 51.7%. These results were found to be statistically significant ($P<0.05$).

3.2. Chlorophyll Content and SPAD Values

When SPAD, chlorophyll a and chlorophyll b values, which reflect the photosynthetic capacity of plants, were examined, it was seen that drought stress and bacterial applications were effective at different levels (Figure 3). WS caused a 23.0% decrease in SPAD compared to the control group. However, bacterial inoculations administered under stress provided significant improvements in SPAD values. While WS+I1 application increased the SPAD value by 40.9% compared to WS, WS+I2 application increased it by 37.2%. These results were found to be statistically significant ($P<0.05$).

When the chlorophyll a and chlorophyll b contents were evaluated (Figure 3A-B), it was determined that drought stress caused a slight decrease of 5.7% in chlorophyll a and 6.5% in chlorophyll b. The difference between treatments in chlorophyll a values was not found to be statistically significant. While WS+I1 application showed a 22.6% decrease in chlorophyll b content compared to WS, WS+I2 application increased by 9.1%.

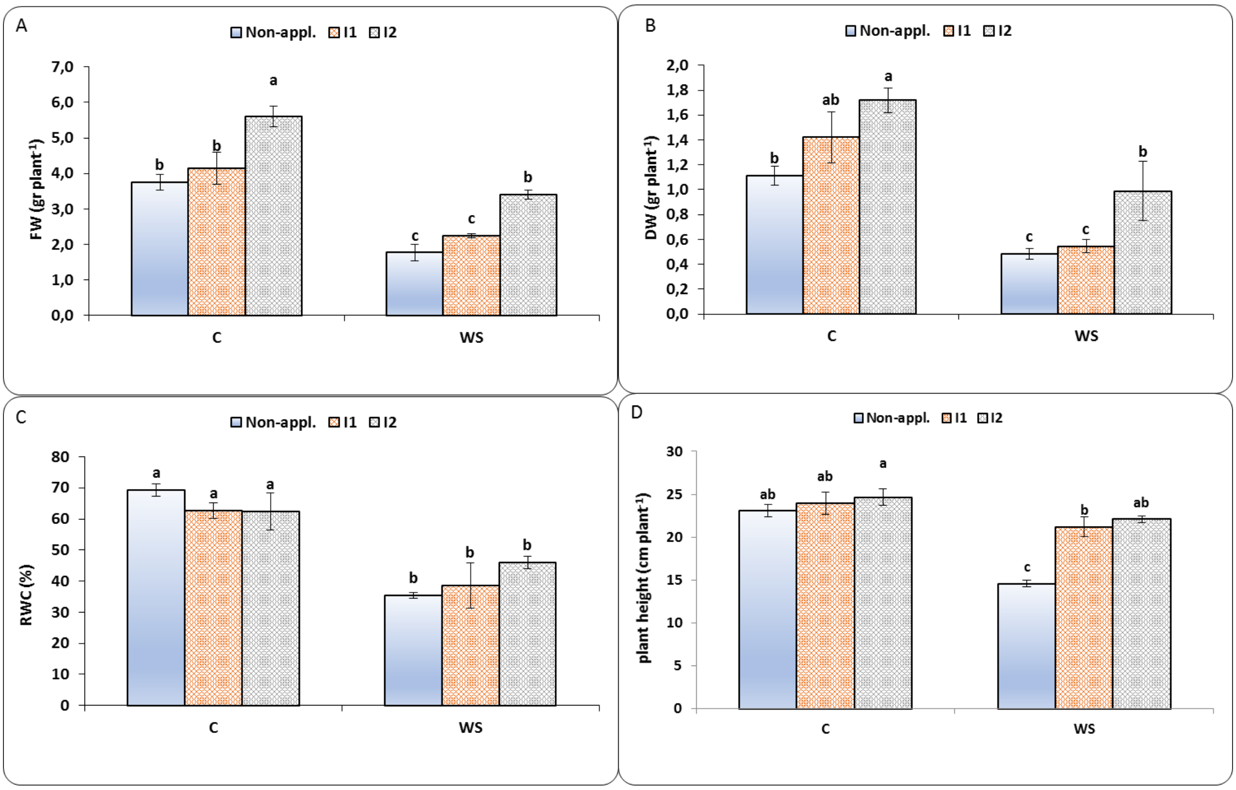

Figure 2. Shoot fresh(A), dry (B) weight, RWC% (C) and plant height (D) Soybean plant under water stress (WS) of not inoculation (Non- appl.), inoculation from seed (I1) and inoculation from soil (I2) application (Mean ± S.E.), Different alphabets on bars exhibit significant difference between means within each variable. C= treatment with water alone, WS= irrigation 50%.

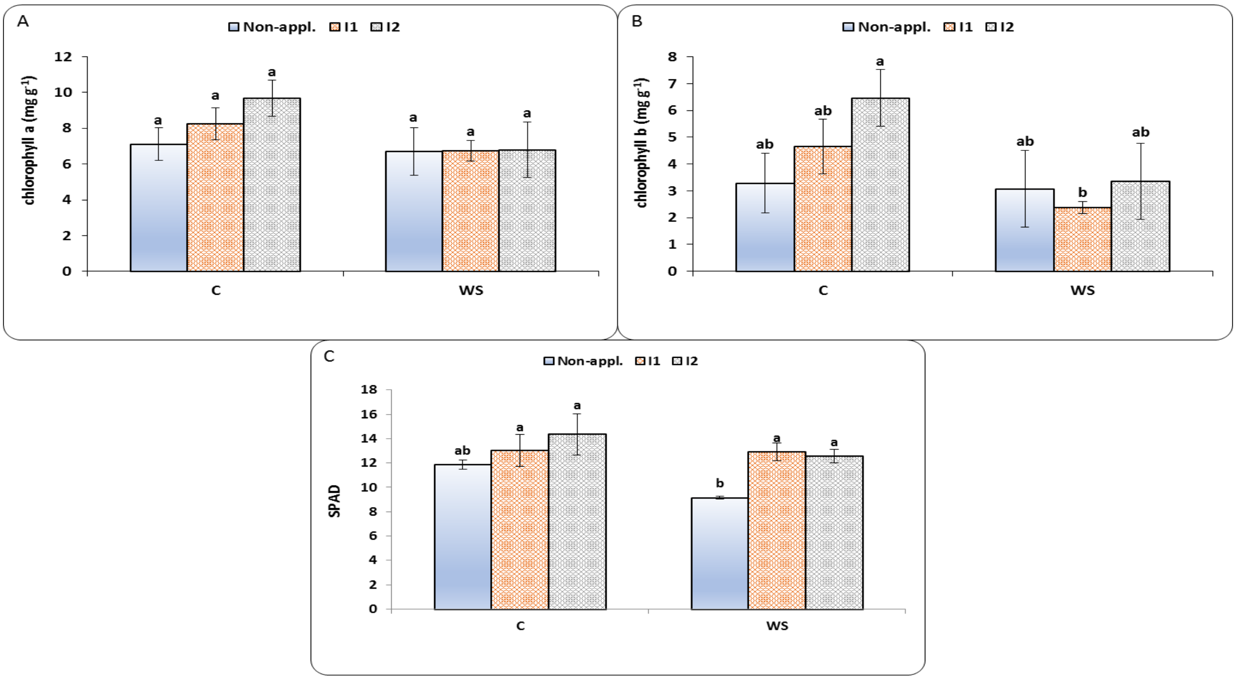

Figure 3. Chlorophyll a (A), chlorophyll b (B) weight and SPAD (C) Soybean plant under water stress (WS) of not inoculation (non-appl.), inoculation from seed (I1) and inoculation from soil (I2) application (Mean ± S.E.), Different alphabets on bars exhibit significant difference between means within each variable. C= treatment with water alone, WS= irrigation 50%.

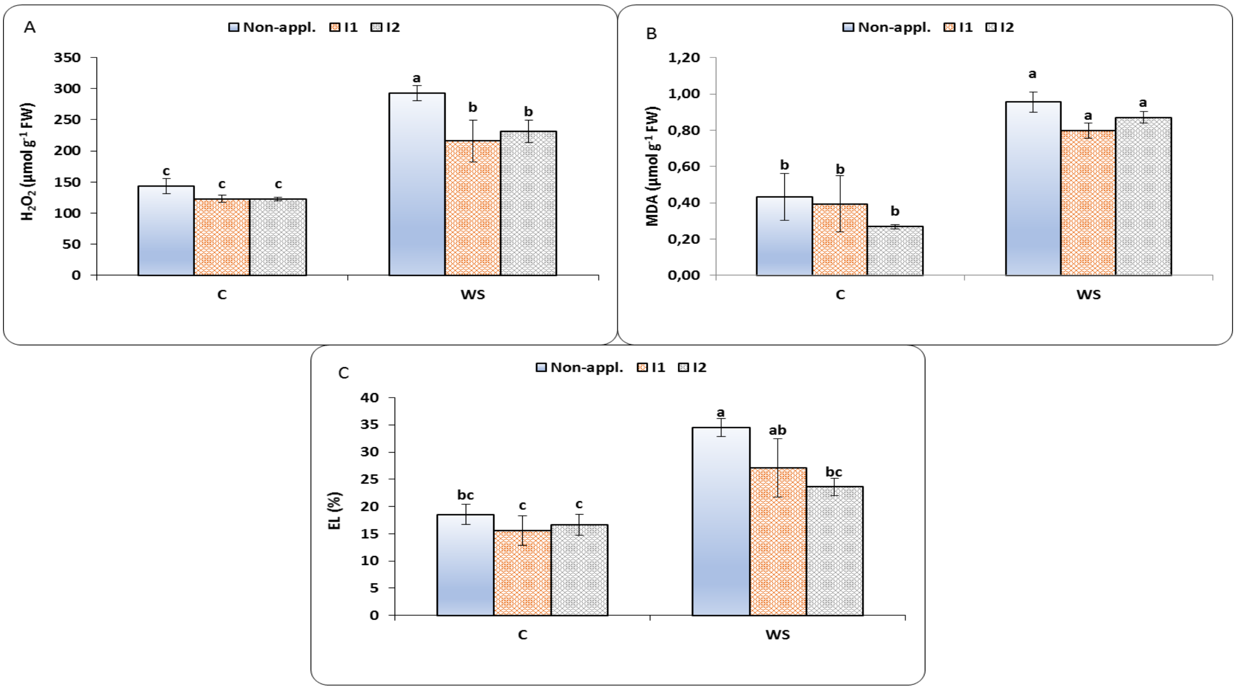

Figure 4. Hydrogen peroxide [H_2O_2 (A)], malondialdehyde [MDA (B)] and Electrolyte leakage [EL% (C)] Soybean plant under water stress (WS) of not inoculation (Non- appl.), inoculation from seed (I1) and inoculation from soil (I2) application (Mean ± S.E.), Different alphabets on bars exhibit significant difference between means within each variable. C= treatment with water alone, WS= irrigation 50%.

3.3. Oxidative Stress Indicators (H_2O_2, MDA and EL%)

Cellular damage and oxidative stress levels caused by drought stress in plants were evaluated with H_2O_2, MDA and EL% parameters (Figure 4). WS application triggered oxidative stress by increasing the amount of H_2O_2 by 103.8% compared to control plants. However, bacterial vaccinations have been effective in suppressing this increase. WS+I1 application reduced the amount of H_2O_2 by 26.1% compared to WS, while WS+I2 application reduced it by 20.9%. These results were found to be statistically significant ($P<0.05$).

When MDA content, an indicator of cell membrane lipid peroxidation, was examined (Figure 4B), WS application caused a dramatic increase of 120.8% compared to the control. Bacterial inoculations limited MDA accumulation; WS+I1 application resulted in a 16.4% decrease compared to WS, and WS+I2 application provided an 8.7% decrease. Similarly, the EL% (Figure 4C) value, which expresses the disruption of cell membrane integrity, increased by 85.9% in WS application compared to the control. Under stress conditions, bacterial inoculations had a protective effect on cell membrane integrity, and WS+I1 application reduced the EL% value by 21.3% compared to WS, and WS+I2 application decreased by 31.5%. These results were found to be statistically significant ($P<0.05$).

3.4. Soil Respiration (CO_2) and Soil Dehydrogenase Activity (DHA)

WS significantly reduced soil microbial activity by 53.3% in CO_2 output and 51.7% in DHA compared to the control group. These findings indicate that drought stress suppresses soil microbiological activity. However, bacterial inoculations applied under stress conditions were quite effective in improving soil microbial activity (Figure 5). WS+I1 application increased CO_2 output by 97.0% compared to WS and increased DHA by 63.0%. WS+I2 application was found to be more effective, increasing CO_2 output by 110.6% and DHA by 86.7%. These results were found to be statistically significant ($P<0.05$).

Strong relationships were determined between the parameters examined in the study (Figure 6). A very strong positive correlation ($r=0.985$, $P<0.01$) was detected between FW and DW, showing that both parameters are affected in the same direction. A very strong positive correlation ($r=0.970$, $P<0.01$) was also found between plant height and DHA, showing that soil microbial activity plays a critical role in plant physical development. Very strong positive correlations were also observed between chlorophyll a and chlorophyll b contents ($r=0.977$, $P<0.01$) and between SPAD value and plant height ($r=0.920$, $P<0.01$), revealing that photosynthetic capacity and plant growth are closely related. Among oxidative stress indicators, very strong positive correlations were determined between H_2O_2 and MDA ($r=0.967$, $p<0.01$), H_2O_2 and EL% ($r=0.968$, $P<0.01$), and MDA and EL% ($r=0.901$, $P<0.01$), showing that oxidative damage is related to each other. Strong negative correlations were detected between growth parameters and oxidative stress indicators; Strong negative correlations were observed between FW and H_2O_2 ($r=-0.861$, $P<0.05$), DW and MDA ($r=-0.897$, $P<0.05$), and especially RWC and EL% ($r=-0.911$, $P<0.01$), showing that cellular damage under stress inhibits plant growth. Strong positive correlations were also found between soil microbial activity and growth parameters; Very strong positive relationships were detected between FW-CO_2 ($r=0.920$, $P<0.01$), DW-DHA ($r=0.926$, $P<0.01$) and plant height-DHA ($r=0.970$, $P<0.01$). The most striking finding was the very strong negative correlation ($r=-0.978$, $P<0.01$) between DHA and EL%; this demonstrated that increasing soil activity through bacterial inoculation is very effective in maintaining cell membrane integrity.

As a result of principal component analysis (Figure 7), PC1 and PC2 explained 92.70% of the total variance, showing that the information of 12 parameters could be effectively represented. PC1 (84.87% variance) was the main axis separating the stress state and the health state, while growth parameters (FW, DW) and soil activity (CO_2, DHA) were on the positive side, oxidative stress indicators (H_2O_2, MDA, EL%) were on the negative side. When the PC1 scores of the applications were examined, soil inoculation (I2) represented the healthiest plants with the highest score (+4.58), seed inoculation (I1) ranked second with a score of +2.71, the control group (C) showed the normal situation with a score of +0.98, and water stress (WS) represented the most negative situation with a score of -5.11.

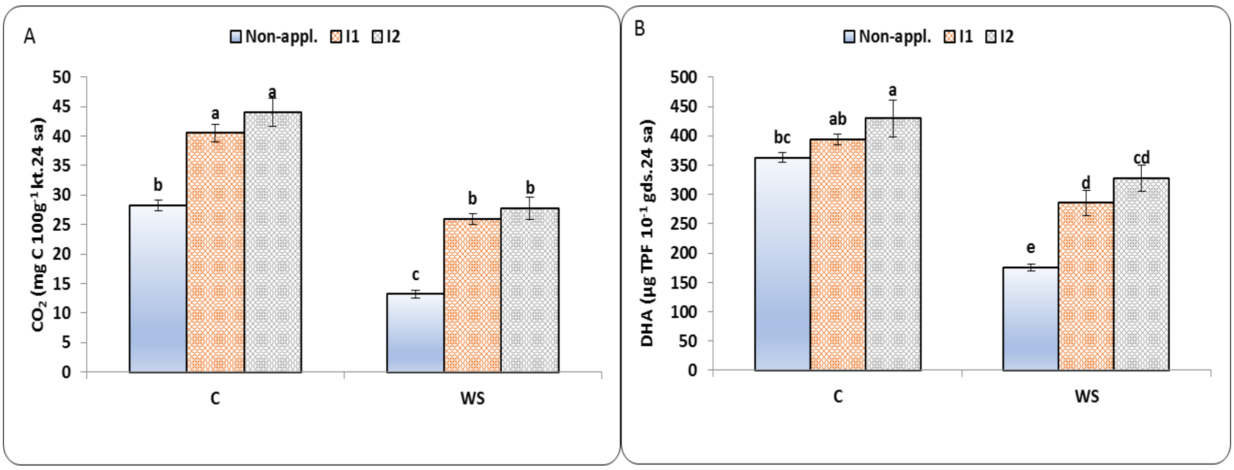

Figure 5. Soil respiration [CO_2 (A)] and Dehydrogenase enzyme activity [DHA (B)] Soybean plant under water stress (WS) of not inoculation (non-appl.), inoculation from seed (I1) and inoculation from soil (I2) application (Mean ± S.E.), Different alphabets on bars exhibit significant difference between means within each variable. C= treatment with water alone, WS= irrigation 50%.

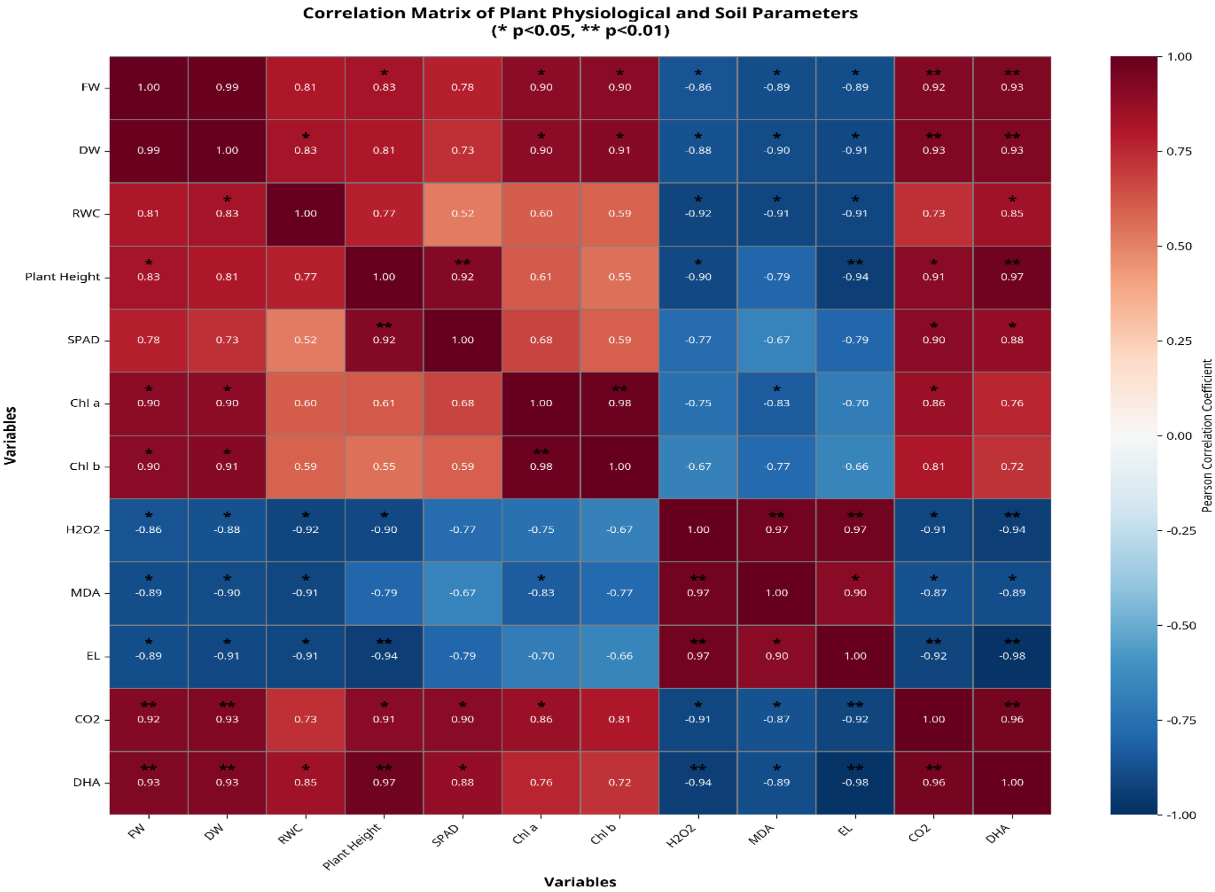

Figure 6. Correlation matrix.

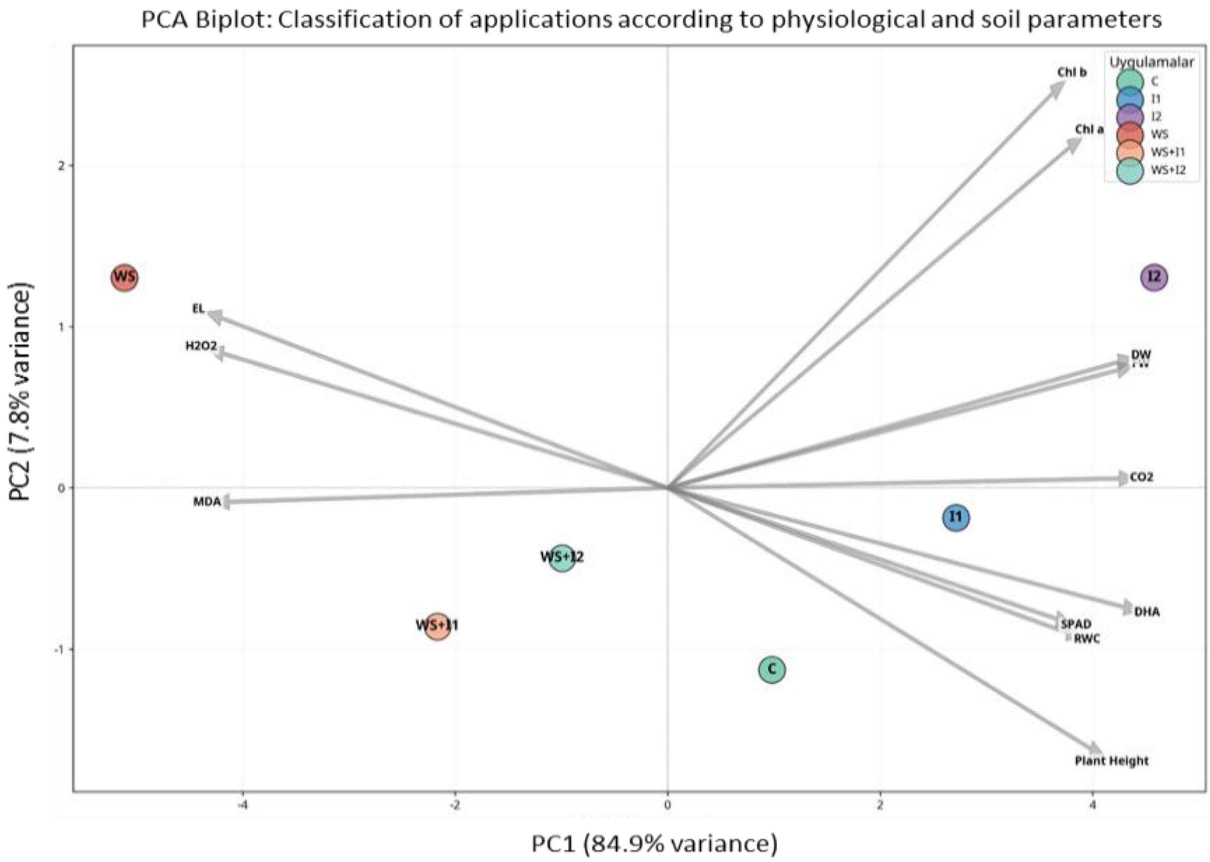

Figure 7. PCA analysis of the results.

İnoculation applied under stress was able to partially eliminate the stress effect with WS+I2 (-0.99) and WS+I1 (-2.16) scores, and especially soil inoculation reduced the stress effect to a level close to the control group. PC2 (7.82% variance) represented the photosynthetic capacity axis, with chlorophyll a and b contents on the positive side, and plant height and relative water content on the negative side. In the biplot display, the applications are clearly separated; healthy plants (C, I1, I2) are positioned on the right, stressed plants (WS) on the left, and rescued plants (WS+I1, WS+I2) in the middle. These results showed that soil inoculation is more effective than seed inoculation, bacterial inoculations strengthen the resistance of plants to oxidative stress by increasing soil microbial activity, and inoculations play an important role in saving plants even under water stress.

Random Forest analysis (Figure 8) was performed to evaluate the relative contribution of soil microbial and oxidative stress parameters in predicting plant fresh weight. As shown in Figure 7, DHA had the highest importance, followed by H_2O_2 and MDA, whereas CO_2 showed the lowest contribution.

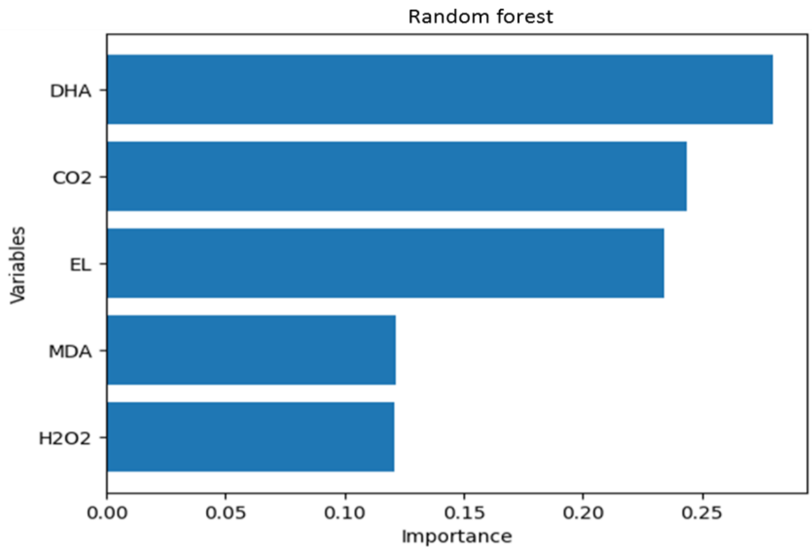

Figure 8. Feature importance of soil microbial and stress-related variables for predicting plant fresh weight using a Random Forest model. Values represent normalized importance scores derived from the Random Forest model.

4. Discussion

Drought stress significantly limits plant biomass production by reducing cell turgor pressure and suppressing physiological functions. Indeed, in our findings, it was observed that water limitation caused significant decreases in the fresh and dry weights of soybean plants, as well as in the RWC%; This is consistent with general drought stress mechanisms in the literature (Farooq et al., 2009; Seleiman et al., 2021). It was also confirmed in our study that water stress reduces photosynthetic efficiency (SPAD) by disrupting chlorophyll synthesis (Ashraf and Harris, 2013). However, what is most striking is the success of microbial inoculations in alleviating this abiotic stress and the striking difference in this success depending on the inoculation method.

Drought leads to excessive production of reactive oxygen species (ROS) at the cellular level, triggering MDA and EL%. Plants activate antioxidant defense systems to cope with this oxidative damage (Gill and Tuteja, 2010). However, this process is a "reactive" defense mechanism that creates a serious carbon and energy cost for the plant. In our study, it was determined that bacterial inoculation practices, especially soil inoculation, significantly reduced H_2O_2 and MDA levels. This not only confirms the protective effect of microbial inoculation on cellular membrane integrity (Khan et al., 2017) but also shows that it allows the plant to direct its energy to growth and development rather than ROS scavenging.

Soil microbial activity is central to plant-microorganism symbiosis and soil health. The most critical finding in our study is that CO_2 and DHA are suppressed under drought, but increase dramatically with inoculations, especially soil application. DHA is an indicator of not only microbial viability but also the active carbon cycle, cellular respiration and microbial biomass in the soil (Daunoras et al., 2024). High DHA levels; indicate that the microbial population in the rhizosphere actively synthesizes phytohormones that promote root growth, such as indole acetic acid (IAA), and maximizes exopolysaccharide (EPS) production, which increases the water retention capacity of the soil against drought stress (Linda et al., 2024). Therefore, this active microbial network created by bacteria inoculated from the soil buffers the devastating effects of drought by stimulating plant growth at the cellular level.

These biochemical dynamics exactly coincide with the results of our machine learning (Random Forest) and PCA analyses. Our data-based analyzes proved that the parameter with the highest feature importance in predicting plant biomass is soil DHA, not the plant's own internal stress indicators (MDA, H_2O_2). This finding has a philosophical dimension: The plant's capacity to survive and grow under stress depends on the establishment of microbial health in the root zone (proactive defense) rather than on its own cellular stress tolerance (reactive defense). Improving microbial processes in the soil creates a much stronger and more decisive agronomic effect than reducing oxidative stress within the plant (Bogati and Walczak, 2022).

Finally, why soil inoculation (I2) showed a much superior performance than seed inoculation (I1) in this study should be explained by physical and ecological mechanisms. In seed inoculation, bacteria are in direct contact with the seed surface (seed coat). In drought or insufficient moisture conditions, the thin biofilm layer on the seed surface may evaporate rapidly, causing the inoculated bacteria to die due to desiccation (drying) stress (O'Callaghan, 2016). On the other hand, the inoculum material applied directly to the soil by the pulverized method settles into micro-aggregates in the soil pores. These micro-habitats serve as a physical shelter for bacteria, protecting them from environmental shocks and allowing them to survive longer (Vessey, 2003). In addition, soil application increases the overall success of inoculation by supporting the faster integration of bacteria into the local microbial community and the readiness of the rhizosphere for colonization even before root germination.

5. Conclusion

This study revealed that *Bradyrhizobium japonicum* inoculation is an effective agronomic strategy in mitigating the damage caused by water shortage in soybean cultivation under drought stress. According to the findings comparing two different inoculation techniques, the soil inoculation (I2) method exhibited a significantly superior performance in both protecting plant physiology and promoting microbial activity in the rhizosphere compared to the traditional seed inoculation (I1) method. It has been determined that soil application suppresses oxidative stress (H_2O_2 and MDA) and EL% more effectively and protects photosynthetic capacity (SPAD).

One of the most striking outcomes of the study is that soil microbial activity indicators (DHA) are much more predictive than cellular stress parameters in predicting plant biomass development. This situation, supported by machine learning (Random Forest) and PCA, proves that the plant's tolerance to drought is directly linked to the improvement in soil microbial dynamics, not only to its own antioxidant or cellular defense mechanisms.

As a result, microbial inoculation directly into the soil for soybean production in regions with high drought risk stands out as a sustainable approach that focuses on soil health. However, since the findings from this research are limited to controlled greenhouse conditions, the effectiveness, competitiveness and economic feasibility of soil inoculation in complex soil flora need to be confirmed by large-scale and long-term field trials.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	E.N.T.	A.S.
C	40	60
D	40	60
S	70	30
DCP	70	30
DAI	30	70
L	40	60
W	40	60
CR	40	60
SR	30	70
PM	40	60

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

Acknowledgments

This work was supported by TUBİTAK under Grant No: 1919B012400260. The authors would like to thank TUBİTAK (2209) for their valuable support and contributions to this study.

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