Harnessing the Potential of Biostimulants for Promoting Growth and Stress Tolerance in Soybeans

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BACKGROUND AND OBJECTIVES

Biostimulants are increasingly used in farming to improve plant health, nutrient uptake, and crop yields under stress. However, their effect on soybean stress tolerance at different growth stages has been largely overlooked. Successful crop establishment depends on rapid, synchronized seed germination followed by strong early seedling growth. During these crucial stages, seeds encounter numerous environmental factors that can either facilitate or impede growth. Unfavorable conditions, such as extreme temperatures, soil moisture stress, and pH imbalances, frequently result in poor development. Soybean production faces challenges, including low temperatures during seedling emergence and drought or heat stress during the reproductive phase, which reduce yields and profits. Although biostimulants are gaining interest as novel strategies to boost plant health and yield under stress, the wide range of products and their potential for varying responses require careful selection. Additionally, testing the effectiveness of biostimulants in the field is challenging due to the unmanageable growing conditions. This highlights the importance of standard methods and a deeper understanding of how biostimulants impact plants to realize their potential for sustainable farming fully. Therefore, this research aims to investigate how biostimulants affect soybeans at various growth stages under stress. A series of experiments were conducted using the growth chamber, greenhouse, and field-based facilities at the Department of Plant and Soil Sciences, MSU, to evaluate the potential of biostimulants (13 products and two checks) under various stress conditions (Fig. 1).

Objectives:

Objective 1: Assess the influence of commercial biostimulants on germination and emergence
Objective 2: Biostimulants for improving early-season growth and reproductive-stage drought tolerance
Objective 3: Biostimulants for improving heat stress tolerance

REPORT OF PROGRESS RESULTS/OUTCOMES:

Objective 1: Assess the influence of commercial biostimulants on germination and emergence.

To evaluate the effect of commercial biostimulants on germination and emergence under temperature stress conditions, a growth chamber study was conducted at the Department of Plant and Soil Sciences, Mississippi State University. Soybean cultivar (AG45XF3) seeds were treated with three biostimulants (HM-2163, BioWake, and Fertiactyl) and one polymer (BioFriendly), as well as possible combinations (BioWake + BioFriendly, Fertiactyl + BioFriendly). These seeds were subjected to low temperature (15 °C [59 °F], LT) and optimal temperature (25 °C [77 °F], OT) during germination (Fig. 1B). Temperature significantly affected the time to 50% germination and the coefficient of velocity of germination, which increased under LT compared to OT (Figs. 2A, B). Among the biostimulants, BioWake + BioFriendly required less time for 50% of the seeds to germinate under LT. Temperature also significantly influenced radicle length and root weight, both of which declined markedly under LT (Fig. 2C). Radicle biomass and seedling vigor were reduced by 64% under LT compared to OT, with biostimulants showing no significant effect. Of the biostimulants tested, HM-2163 and Fertiactyl yielded the highest seedling vigor under OT conditions. Seeds treated with microbes and fulvic and humic acid compounds exhibited some positive effects on germination traits under LT, but there was no clear impact on seedling vigor.

Objective 2: Biostimulants for improving early-season growth and reproductive-stage drought tolerance.

To investigate the effect of biostimulants on early-season growth and reproductive-stage drought tolerance, two independent experiments were conducted using 15 treatments (Fig. 1C). Four seeds were sown in pots filled with soil composed of sand (1:1), and one seedling was retained at the V2 stage. Drought stress significantly impacted several physiological and morphological traits of the plants (Figs. 3 and 4). Certain biostimulants showed promise (non-significant) in mitigating these negative effects. A significant decline in stomatal conductance and transpiration was observed under DS compared to the control (Fig. 3A, B). Trevo (T14) and a mix of BioSa+BioFriendly+Polymer (T11) boosted stomatal conductance and transpiration (non-significant) compared to untreated check under DS conditions (Fig. 3A). A significant biostimulant effect ($p < 0.05$) was recorded for canopy temperature (Fig. 3C). In fact, the biostimulant showed the opposite impact to what was anticipated. Total root length was decreased by 12.5% under DS compared to the control (Fig. 4). Root biomass decreased significantly ($p<0.01$) by 29% under drought stress compared to the control. Biostimulants showed varying effects under drought stress compared to the untreated check (T1). Root traits, such as total root length, showed a non-significant difference between irrigation treatments. However, traits such as root diameter and root surface area decreased significantly ($p < 0.01$) by 20% and 28%, respectively, under drought stress compared to the control. Overall, no significant differences were noted for any of the above-mentioned root traits among the biostimulant treatments under drought stress (Fig. 4).

The second study investigated the effects of drought stress during the reproductive (R1 flowering) stage, with biostimulants applied as seed treatments, soil amendments, and foliar sprays. (Fig. 1D). Drought led to a decline in pod number (27%) and total seed weight (44.3%) (Fig. 5A, B). HM-2163 (T3) increased pod number by 18%, and the combination of BioWake and BioFriendly (T7) increased by 13% under drought compared to the untreated check. Although there was no significant difference, Trevo (T14) showed an improvement in total seed weight under drought conditions compared to the untreated check (Fig. 5B). Seed protein content increased by 4% under drought stress, ranging from 42% to 43%, compared to 40% to 41% in control (Fig. 6A). A low concentration of Azterknot (T12) further increased seed protein content by an additional 2% under drought (Fig. 6A). Seed oil content decreased by an average of 6% under drought, ranging from 21% to 23% compared to 22% to 24% in control (Fig. 6B). Fertiactyl (T8) increased seed oil content by 5% ($p<0.05$). The combination of BioSa and BioFriendly (T10) increased by 4% under drought conditions compared to the untreated check (Fig. 6B).

Objective 3: Biostimulants for improving heat stress tolerance

In the final experiment, the effect of biostimulants on reproductive stages under heat stress was assessed using heat tents. A 10-foot row was planted with AG45XF3, a soybean cultivar with a spacing of 2 inches between the seedlings, and three replications were maintained (Fig.1E). Until the reproductive stage, plants were grown under optimum environmental conditions. Heat stress was imposed during the reproductive phase, from flowering to physiological maturity. A 4°C temperature increase over control negatively impacted physiological, pigment, yield, and seed quality parameters. Significant difference for treatment ($p<0.05$), biostimulants ($p<0.01$), and treatment × biostimulants interaction ($p<0.05$) noted for stomatal conductance, transpiration, total pods weight, and seed yield (Fig. 7 – 9). Total seed weight decreased by 32% under heat stress compared to the control (Fig. 8C). The combination of biostimulants BioWake and Biofriendly (T7) increased total seed weight as well as oil under heat stress compared to the untreated check (T1) (Fig. 8C and 9).

In conclusion, drought and heat stress significantly reduced physiological, morphological, yield, and quality attributes of soybean across both vegetative and reproductive stages. Commercial biostimulants demonstrated minimal and non-significant effects under the tested conditions. Future research will focus on identifying suitable application methods and formulations tailored to specific environmental conditions, thereby enhancing the effectiveness of biostimulants.

IMPACTS AND BENEFITS TO MISSISSIPPI SOYBEAN PRODUCERS

This project aims to assist approximately 3,000 soybean farms in Mississippi, which cover an area of 2.25 million acres. Our findings suggest that treating soybeans with certain biostimulants improves (non-significant) seedling vigor in cold weather and enhances their ability to withstand drought and heat stress during the vegetative stage and grain filling. In the next phase of the project, we are testing how seed priming with biostimulants improves stress tolerance during various growth stages. These findings enable farmers to select the most suitable biostimulants for seed or soil/foliar treatment, tailored to their cultivation practices and regional weather conditions. This could help farmers increase their revenue by at least 5% under resource-limited and stressful situations. Next-generation scientists (graduate and undergraduate students) trained in soybean stress physiology. Graduate students supported by the MSPB published *seven* abstracts and presented their findings at regional and national conferences, including the Mississippi Academy of Sciences, the MSU Graduate Research Symposium, and the ASA-CSSA-SSSA Annual Meeting.

END PRODUCTS

Publications

1. Sivarathri, B. S., Kodadinne Narayana, N., Bryant, C.J., Dhillon, J., Reddy, K.R. and Bheemanahalli, R. (2025). Influence of seed-applied biostimulants on soybean germination and early seedling growth under low and high temperature stress. *Plant Physiology Reports*. https://doi.org/10.1007/s40502-024-00834-z
2. Thingujam, D., Majeed, A., Sivarathri, B.S., Narayana, N.K., Bista, M.K., Cowart, K.E., Knight, A.J., Pajerowska-Mukhtar, K.M., Bheemanahalli, R. and Mukhtar, M.S., 2025. The impact of soybean genotypes on rhizosphere microbial dynamics and nodulation efficiency. *International Journal of Molecular Sciences*, *26*(7), 2878; https://doi.org/10.3390/ijms26072878
3. Sankarapillai, L. V., Adhikari, B., Bista, M. K., Shrestha, A., Stetina, S. R., Reddy, K. R., & Bheemanahalli, R. (2025). High night temperature disrupts the assimilate utilization and yield potential in soybean. *Plant Stress*, *16*, 100826. https://doi.org/10.1016/j.stress.2025.100826
4. Sivarathri, B. S., Kodadinne Narayana, N., Bista, K. B., Vellaichamy Gandhimeyyan, R., Wijewardan, N. K., & Bheemanahalli, R. (2025). Impact of water deficit on nodulation and rooting potential in soybean. *Farming Systems. (review).*

Abstracts published or presentations

1. Sivarathri, B.S., Hosahalli, V., Bryant, C., Dhillon J.S., Reddy K.R., Bheemanahalli, R. (2025). Investigating the impact of biostimulants on physiology and seed yield under heat stress. Spring Graduate Research Symposium, Mississippi State, MS, Feb 15, 2025. (Poster).
2. Sivarathri, B.S., Kodadinne Narayana, N., Corey, J.B., Reddy, K.R. & Bheemanahalli, R. (2025). Priming soybean seeds with biostimulants and gibberellic acid: a strategy to enhance cold tolerance. Southern Branch of American Society of Agronomy, Irving, TX, Feb 2-4, 2025 (Poster).
3. Sivarathri, B.S., Kodadinne Narayana, N., Corey, J.B., Reddy, K.R. & Bheemanahalli, R. (2025). Priming soybean seeds with biostimulants and gibberellic acid: a strategy to enhance cold tolerance. 89th annual meeting, Mississippi Academy of Sciences, Biloxi, MS, March 20-21, 2025 (Poster, I place).
4. Sivarathri, B. S., Kodadinne Narayana, N., Bryant, C. J., Reddy, K. R., & Bheemanahalli, R. (2024). effects of temperature stress and biostimulants on the root and shoot parameters of soybean. ASA, CSSA, SSSA International Annual Meeting, San Antonio, TX (*5M rapid Oral and Poster, II place*)
5. Hosahalli, V., Poudel, S., Sivarathri, B. S., Bryant, C. J., Reddy, K. R., & Bheemanahalli, R. (2024). Do biostimulants help to alleviate reproductive stage drought stress in soybean? ASA, CSSA, SSSA International Annual Meeting, San Antonio, TX. (*Oral*)
6. Sivarathri, B.S., Nisarga, K.N., Corey, J.B., Reddy, K.R. & Bheemanahalli, R. (2024). Effects of temperature stress and biostimulants on root and shoot parameters of soybean. Fall Graduate Research Symposium, Mississippi State, MS. (*Poster, II place*).
7. Sivarathri, B. S., Kodadinne Narayana, N., Bista, M. K., Corey, J.B., Reddy, K. R. & Bheemanahalli, R. (2024). Can seed priming with biostimulants boost soybean germination? 6th MAS Summer Research Symposium, Mississippi State, MS. (*Poster, Honorable mention*).

TABLE AND GRAPHICS

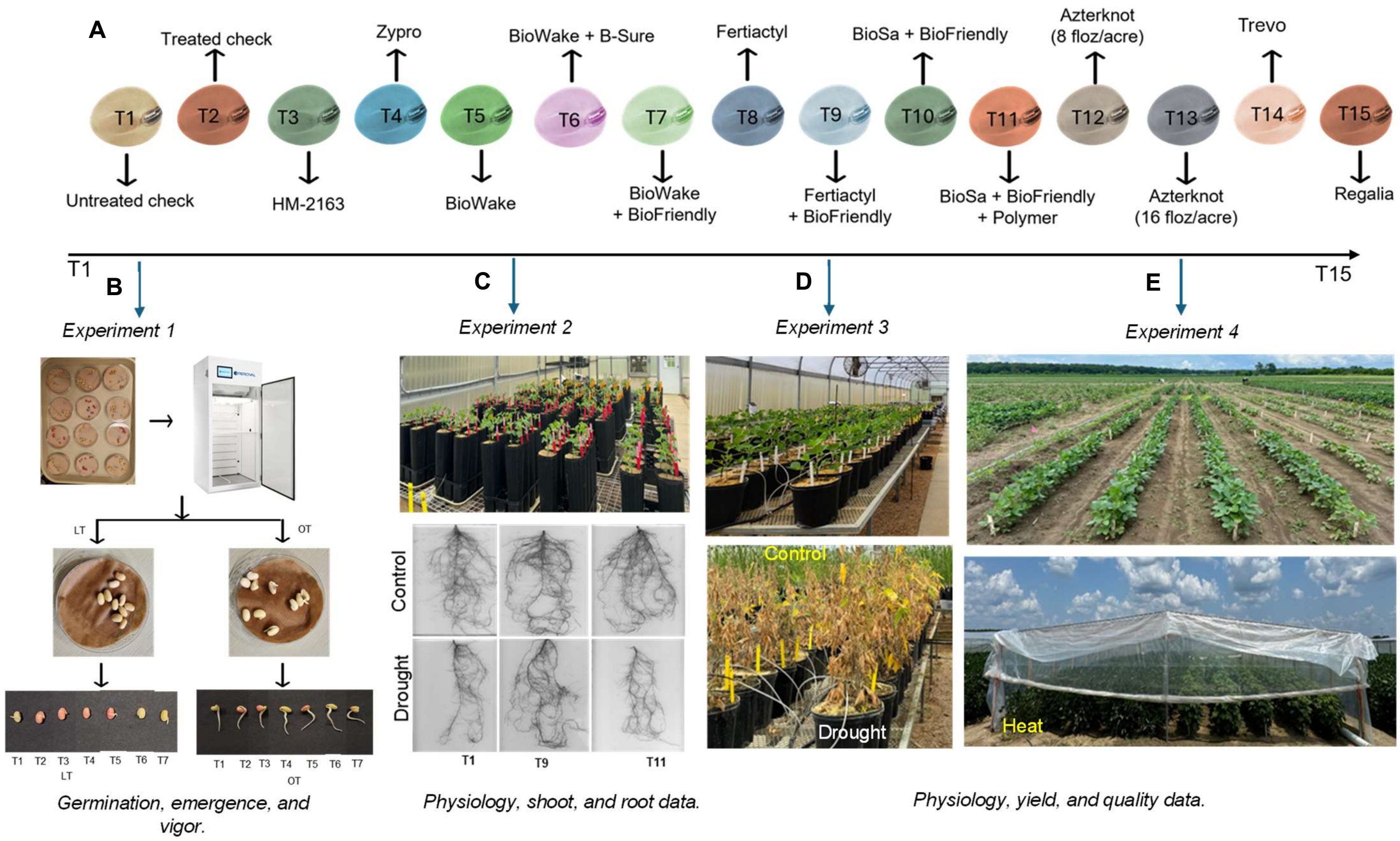

Figure 1. Overview of experiments. List of biostimulants used in the study (A). Various phenotyping methods were employed to assess the role of biostimulants on germination (Experiment 1, B), drought stress at the vegetative stage (Experiment 2, C), and reproductive stage (Experiment 3, D), as well as heat stress during the reproductive stage (Experiment 4, E).

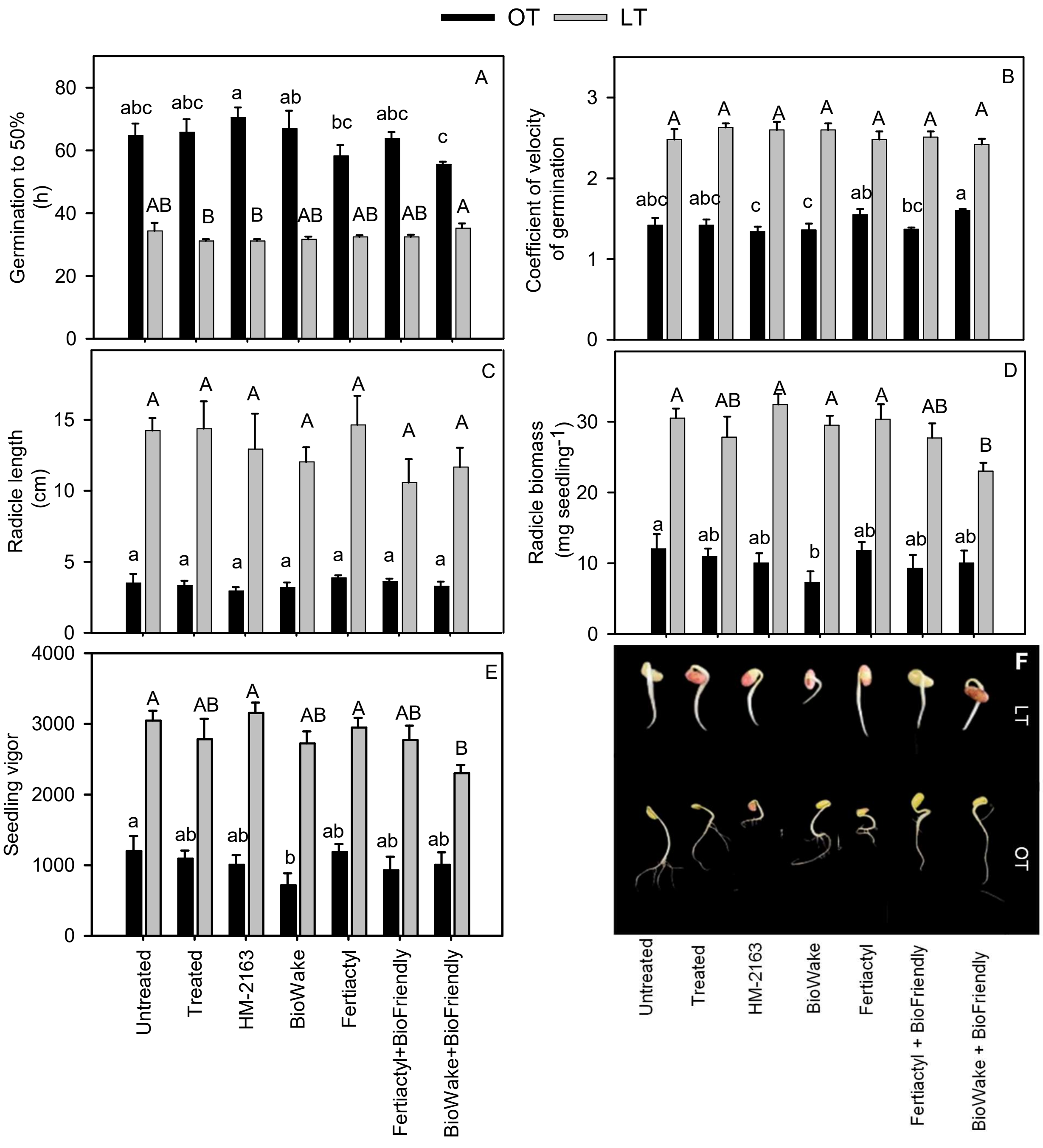

Figure 2. Effect of seed-applied biostimulants on time to 50% germination (A), coefficient of velocity of germination (B), radicle length (C), radicle biomass (D), and seedling vigor (E and F) under low (LT) and optimum temperature (OT) in experiment 1. Different lowercase and uppercase letters represent statistically significant ($p<0.05$) differences within low and optimum temperature treatments, respectively. Bars represent mean ± standard error (n=4).

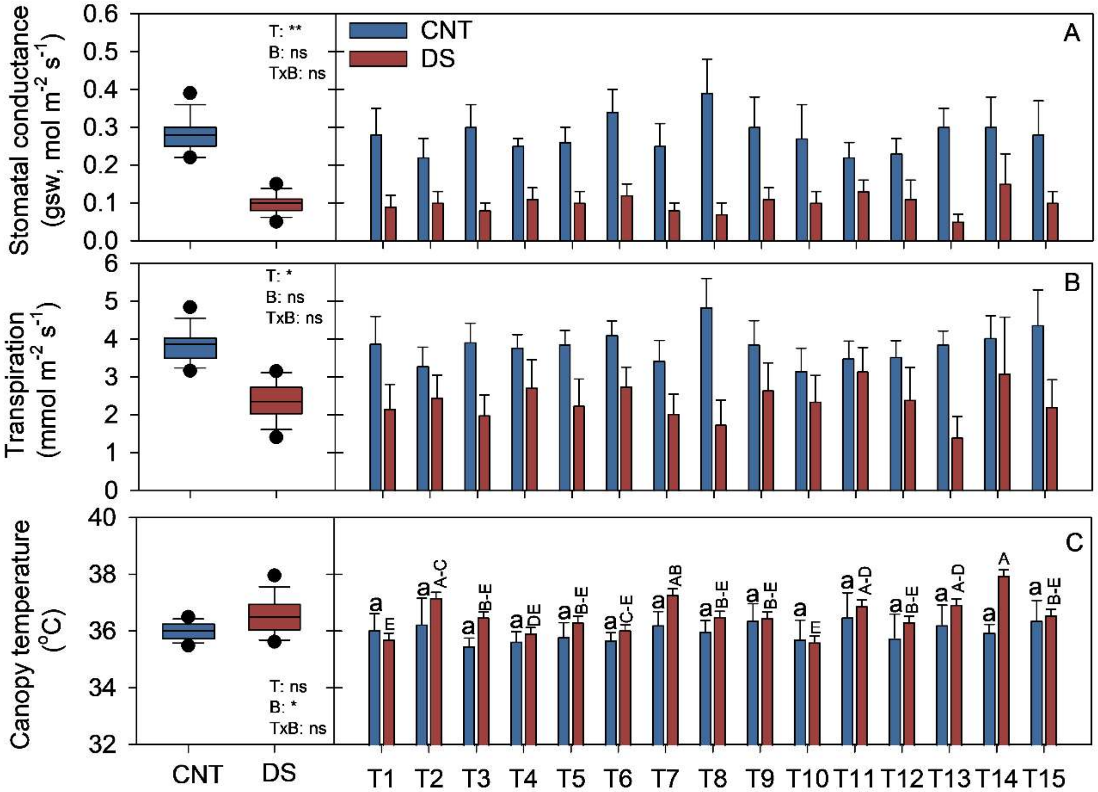

Figure 3. Influence of biostimulants on A) stomatal conductance (gsw), B) transpiration rate (E), and C) canopy temperature (°C) under control (CNT) and drought stress (DS) in experiment 2.

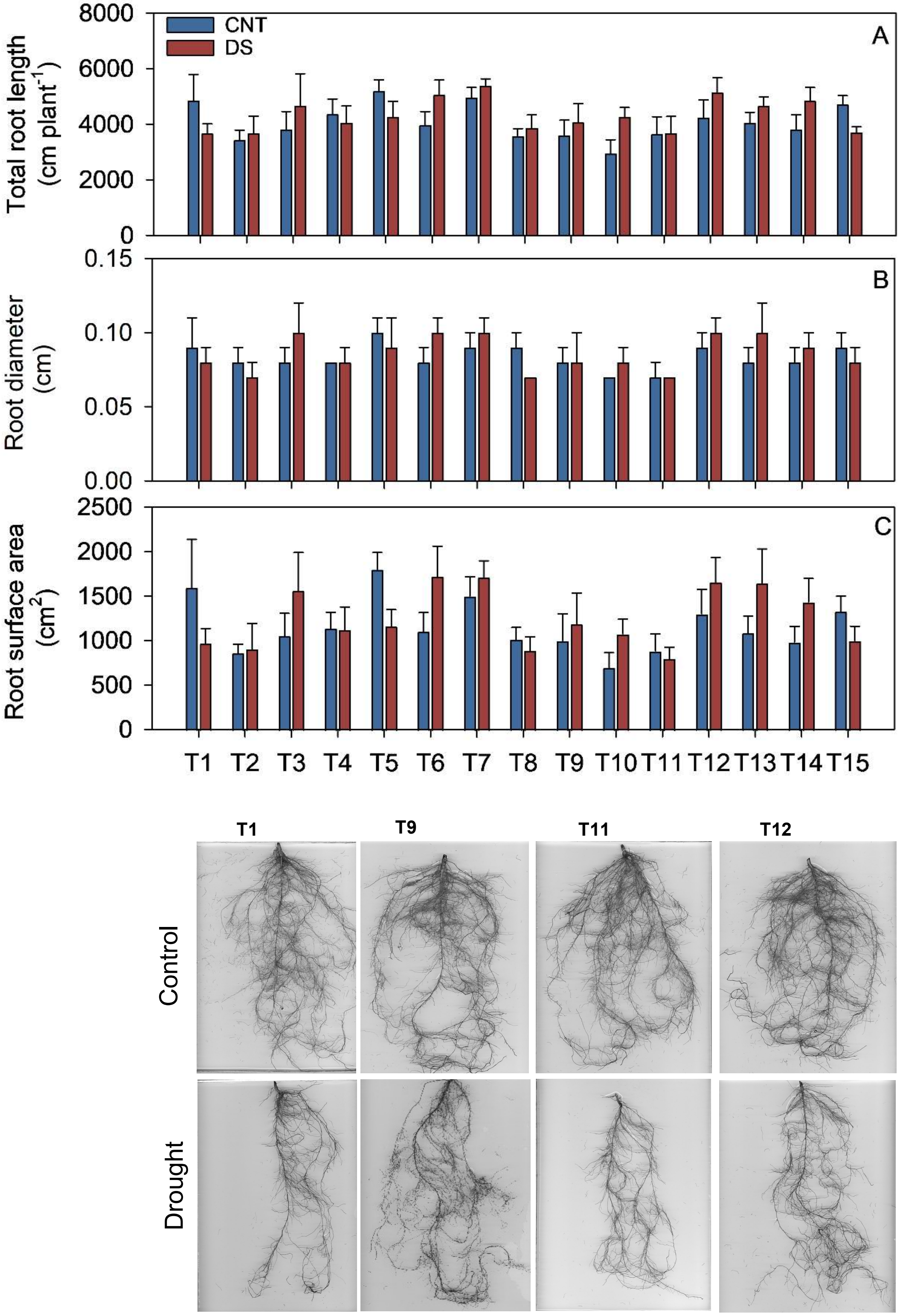

Figure 4. Effects of biostimulants on A) shoot biomass (g plant^{-1}), B) root biomass (g plant^{-1}), C) root to shoot ratio, and root morphology under control (CNT) and drought stress (DS) in experiment 1.

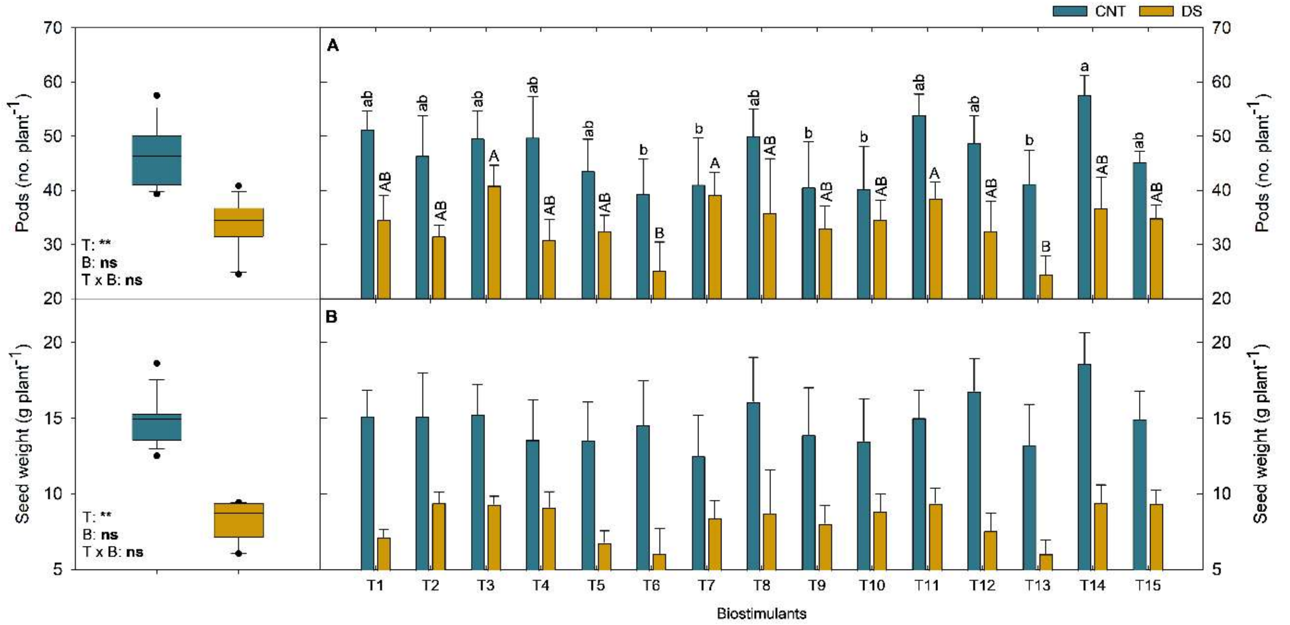

Figure 5. Effect of biostimulants on A) pods (no. plant^{-1}) and B) seed weight (g plant^{-1}) under control (CNT) and drought stress (DS) in experiment 3. Bars indicate means ± standard error (n=6). Different lowercase (CNT) and uppercase (DS) letters above error bars indicate statistically significant differences within the irrigation treatment ($p<0.05$). T, B, and T × B represent treatment, biostimulants, and the interaction between treatment and biostimulants.

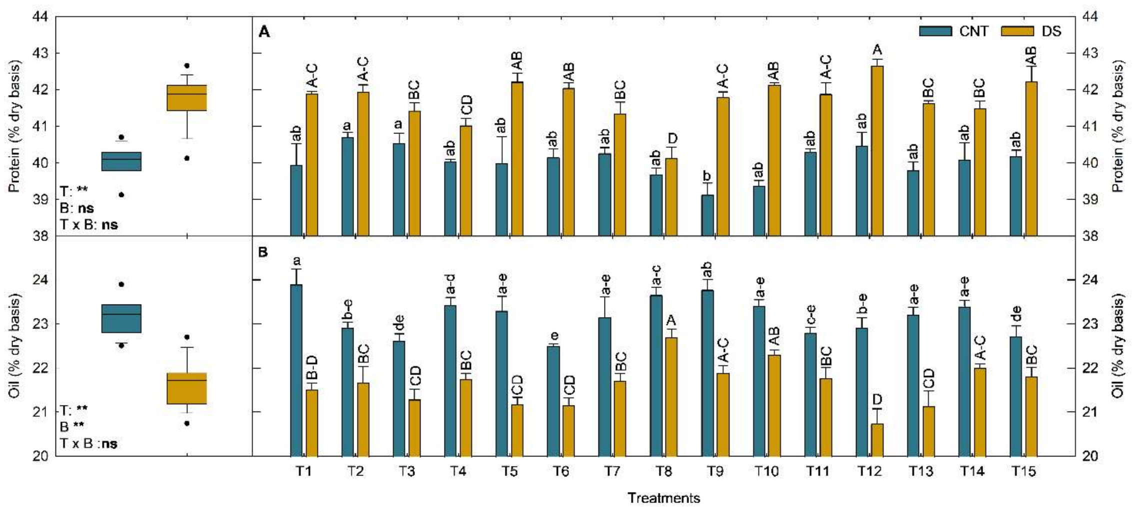

Figure 6. Effect of biostimulants on A) protein (% dry basis) and B) oil (% dry basis) under control (CNT) and drought stress (DS) conditions in experiment 3. Bars indicate means ± standard error (n=6). Different lowercase (CNT) and uppercase (DS) letters above error bars indicate statistically significant differences within the irrigation treatment ($p<0.05$). T, B, and T × B represent treatment, biostimulants, and the interaction between treatment and biostimulants.

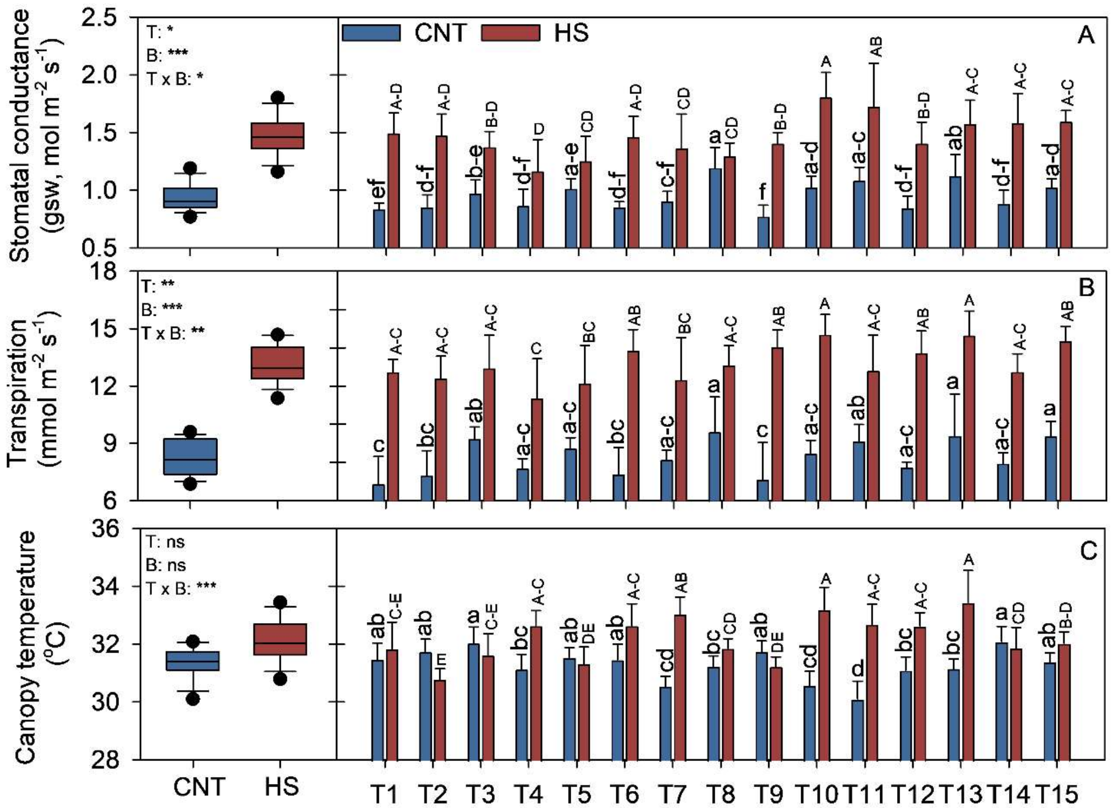

Figure 7. Effect of biostimulants on A) stomatal conductance (gsw), B) canopy temperature (°C), and C) transpiration (E) under control (CNT) and heat stress (HS) conditions in experiment 4. Bars indicate means ± standard error (n=3). Different lowercase (CNT) and uppercase (HS) letters above error bars under control and heat stress indicate statistically significant differences ($p<0.05$). 'T', 'B', and 'T×B' represent treatment, biostimulants, and the interaction between treatment and biostimulants.

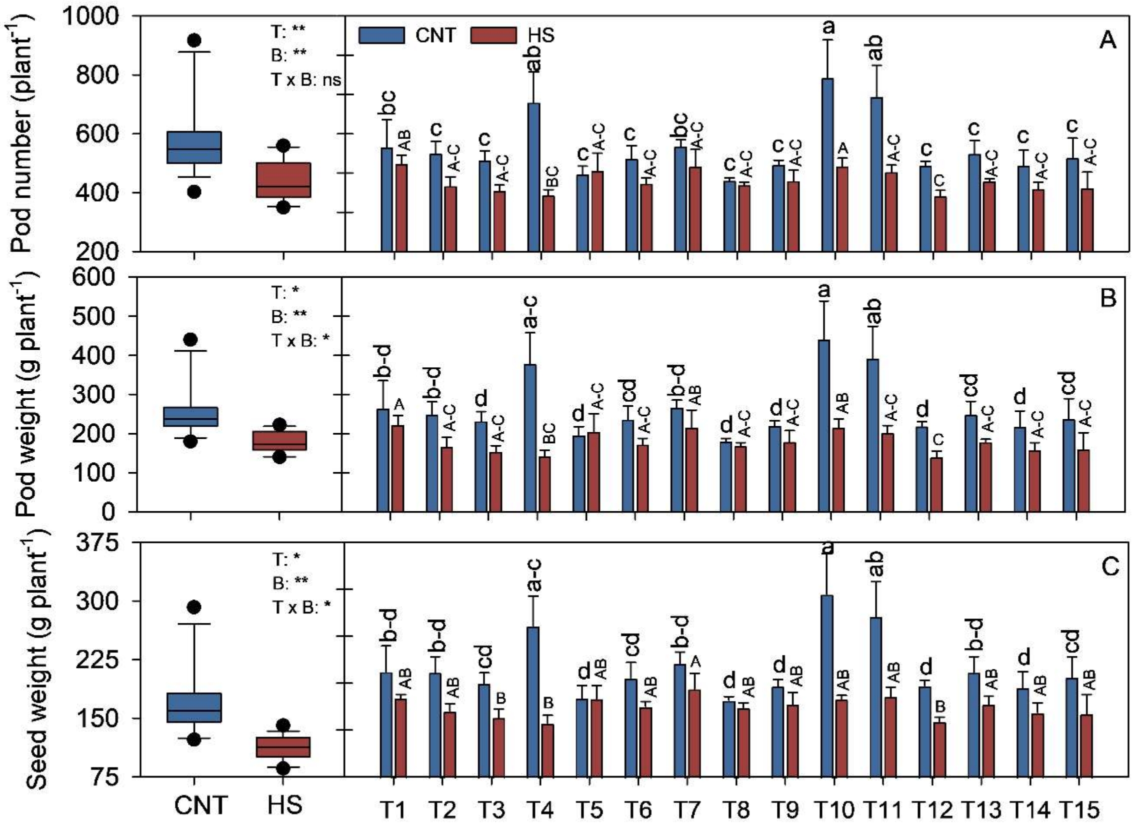

Figure 8. Effects of biostimulants on A) pod number (plant^{-1}), B) pod weight (g plant^{-1}), and C) seed weight (g plant^{-1}) under control (CNT) and heat stress (HS) conditions in experiment 4. Bars represent means ± standard error (n=3). Different lowercase (CNT) and uppercase (HS) letters above the error bars indicate statistically significant differences ($p<0.05$) under control and heat stress conditions. T, B', and 'T × B' denote treatment, biostimulants, and the interaction between treatment and biostimulants.

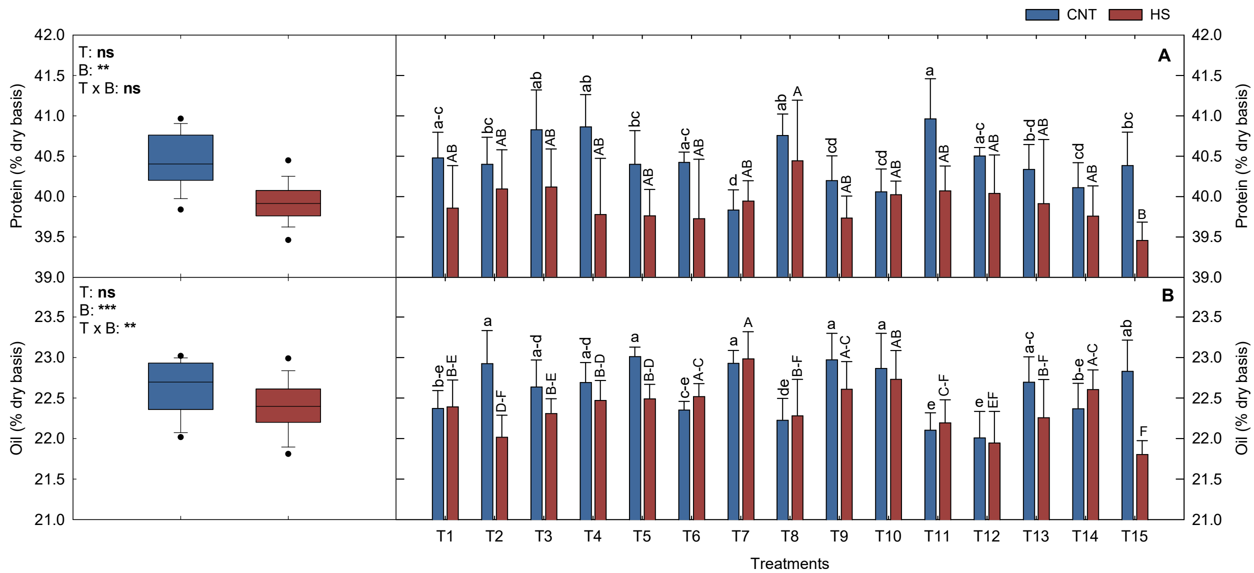

Figure 9. Effects of biostimulants on A) protein (% dry basis), and B) oil (% dry basis) under control (CNT) and heat stress (HS) conditions in experiment 4. Bars represent means ± standard error (n=3). Different lowercase (CNT) and uppercase (HS) letters above the error bars indicate statistically significant differences ($p<0.05$) under control and heat stress conditions. T, B', and 'T × B' denote treatment, biostimulants, and the interaction between treatment and biostimulants.