Role of biochar and rhizobacterial strains in improving drought and lead stress tolerance in maize (*Zea mays* L.)

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Abstract

The limited availability of water and increasing lead (Pb) pollution are posing serious challenges to agricultural crop production. Both drought and Pb stress can impede plant growth by causing severe oxidative damage via the production of reactive oxygen species. However, plants possess enzymatic and non-enzymatic antioxidants that can act as potential mechanisms for survival under such stress conditions. The use of rhizobacteria and biochar for the regulation of antioxidants is gaining importance in this regard, but the literature on the use of deashed biochar with rhizobacteria for the alleviation of drought and Pb stress is limited. To address this gap in knowledge, the current study aimed to investigate the impact of Deashed biochar (BC) and pre-screened *Agrobacterium fabrum* on maize growth and biochemical attributes. Two levels of BC (0 and 0.5%) were applied with and without *A. fabrum*. *The results revealed that the BC+A. Fabrum have* greatest increase of 53.33% in germination, 102.64% in shoot length, 11.76% in root length, and 32.36% in total soluble protein in maize over the control at 400 mg Pb/kg soil. The effectiveness of BC+*A. The ability of fabrum* to alleviate drought stress was further validated by improvements in chlorophyll a (55.02%), chlorophyll b (50.61%), and total chlorophyll (53.37%) compared to the control. In conclusion, the use of BC+*A. fabrum* can be an effective approach for enhancing maize growth and biochemical attributes under drought and Pb stress conditions.

Keywords: Maize, Growth, Lead stress, Biochar, *Agrobacterium fabrum*

Introduction

Zea mays, also known as corn, is a member of the grass family of plants (Poaceae). It is grown everywhere in the world. It is one of the most significant cereal crops grown worldwide (1). By the end of 2020, the maize yield per acre is projected to increase to 2.36 tonnes from its present level of 1.7 tonnes in order to meet increased demand. Oil from the wet milling of maize, which is used in cooking and salad dressing, makes approximately 45–50% of its weight (2). In Pakistan, maize is the fourth-ranked cash crop. In Punjab and NWFP, maize is produced in 98% of Pakistan, and greater seedling establishment is a significant barrier for higher crop yield (3). In many countries around the world, it is regarded as a staple food. 967 million metric tonnes of maize were produced globally (MMT) (4)

Crop development and growth, as well as agricultural yields, are impacted by drought. Food production has dramatically decreased as a result of droughts occurring more frequently and with greater intensity year after year. Over the previous 50 years, drought stress has been predicted to result in a 10% drop in grain production, with predictions of productivity lossesin more than 50% of arable land by 2050 (5). Most of the major agricultural commodities grown around the world, such as wheat, corn, and rice, have experienced significant reductions, according to the International Food Policy Research Institute (IFPRI). Water scarcity has caused reports from 1980 -2015 that show that wheat yields have decreased by 21% and that corn production has decreased by 40% globally (6, 7). When the amount of water transpired that transpires exceeds the amount of water absorbed by the roots, a water deficit results because the plant's needs for water cannot be fully met. Numerous morphological, physiological, biochemical, ecological, and molecular aspects and functions of plants are impacted by this phenomenon. A lack of water is a primary source of stress for plants; it hinders their overalldevelopment by reducing their capacity for photosynthetic activity, the production of hormones, the integrity of their membranes, etc. (8). Researchers are movingtoward the development of a number of strategies, such as the development of drought -resistant cultivars, molecular breeding, genetic engineering, and the use of nanoparticles, filmculture, super-absorbent hydrogels, and biochar.

Plant growth-promoting rhizobacteria (GPR) bacterial inoculants offer a viable alternative to enhance plant growth and tolerance under drought conditions. The employment of beneficial microorganisms can increase a plant's tolerance to abiotic conditions such as drought, salinity, nutrient insufficiency, and metal contamination, as various studies have demonstrated in recent years. Using a bacterial inoculant to improve food security under drought conditions offers an alternative and practical economic solution (8, 9). The bacterial inoculant may improve root systems by stimulating plant growth, increasing plant nutrient uptake by fixing atmospheric nitrogen, solubilizing insoluble soil phosphate, and shielding plants from soil-borne diseases through antimicrobial activity (10). Additionally, their use in crop production could decrease the need for chemical pesticides and fertilizers (11). Legumes are known as the most effective mechanism for biological nitrogen fixation because of their symbiotic connections with rhizobia. However, a number of abiotic variables, including dryness, salinity, and high or low temperatures,have a significant negative impact on bacterial survival and their interaction with plants (12, 13). The combination of different carrier substrates with microorganisms increases their capacity for colonization and impacts plant growth and development (14). For years, the rhizobium inoculant carrier material peat has been ineffective against the stressors of water and temperature (12, 13). Compost is also thought to be a good carrier for good bacteria associated with roots. A beneficial rhizobacterial inoculation strategy was established to overcome this issue. Microbial inoculants can increase the tolerance of plants to multiple stressors, such as drought and heavy metals, by providing essential nutrients, improving their water-holding capacity (15), and increasing the availability of essential micronutrients. The effects of the coapplication of biochar and plant growth-promoting rhizobacteria (PGPR) on soil quality and agronomic productivity under both normal andstressed conditions have been investigated in numerous studies (16). Biochar can increase nutrient availability, thus providing a suitable environment for PGPR to flourishand enhance their beneficial activities (e.g., phytohormone production and nutrient solubilization) (17).

As heavy metals, lead interferes with the uptake of essential nutrients such as calcium, magnesium, and potassium, which can affect plant growth and development. Its widespread use has resulted in significant environmental pollution and health issues in many parts of the world (18, 19). Because of this, the concentration in soil, plants, and animals increases as the food chain moves up (20, 21). The current study aimed to explore the beneficial impacts of growth-promoting rhizobacteria and biochar on maize cultivated under osmotic and Pb stresses independently.

Materials and methods

In the Botany Department #39;s botanical garden at Islamia University Bahawalpur, an experiment was carried out to study the role of biochar and rhizobacterial strains in improving tolerance to drought and lead stress in maize (Zea mays L.).

Soil Testing and Characterization

Soil samples were taken from a region typified by grimy calcareous soil using minimal natural material with high abundance. The experiments included shadow-parching, scraping, and sieving to a size of 2 mm, and touching metallic particles was established via the technique of (22) by mixing 10 mL of nitric acid with 1 g of dried soil and incubating it overnight. A 1-gram sample of dried soil was placed in an Erlenmeyer flask, to which 10 milliliters of nitric acid were added (23). Following incubation, the flask was heated to 200°C and chilled. Approximately 1 mL of hydrogen nitrate and 4 mL of $HClO4$ were transferred into the bottle after it had cooled. After that, the container was heated at 280°C until it was removed from the hot plate when fumes from the $HClO$ 4 became visible. Approximately 10 mL of the hydrochloric acid solution was added once the solution had cooled, while the Erlenmeyer flask that had been used was then heated to 70°C (24) for one hour before allowing it to cool again. The filtered solution of the flask was then titrated with 50 mL of 1% hydrochloric acid, and the soil pH was measured in the soil-saturated paste via a pH meter. The potassium dichromate oxidation technique was used to quantify the amount of organic material (OM) in the substrate (25). A 1:10 ratio of soil and deionized water was prepared, and an extract was acquired for the purpose of analyzing the electrical conductivity of the soil with an EC meter (26). The Olsen method was employed to analyze soil phosphorus (27). However, a flame photometer was used for the analysis of K from soil extracts taken with ammonium acetate (Pratt, 2016). The pH of the soil employed in the research study was 8.04, the electrical conductivity (EC) was 2.14 dS/m-1, the proportion of organic matter was 0.40%, the overall N content was 0.038 g/kg, the accessible P content was 4.56 mg/kg, the accessible K content was 159 mg/kg, and the overall Pb content was 0.105 mg/kg.

Lead Toxicity

The desiccated farm instead of loam (5 kg per pot) was amended with Sigma–Aldrich lead nitrate (Pb (NO 3) 2 99%, based on trace metal analysis = product number: 203580-BULK; batch number: 0000192271; color: white; form: crystal) at 0 and 400 mg Pb kg −1 soil) in 27 cm×20 cm × 24 cm pots.

Seed collection and seed sterilization

Hybrid seeds of FH-1036 were purchased from a local market. In this study, the sample was treated with 5% sodium hypochlorite, followed by three consecutive washes with ethanol. (95%) was used to sterilize the seeds. The method involved soaking the seeds in sodium hypochlorite solution for 30 minutes, followed by three washes with 95% ethanol (28).

Rhizobacteria inoculation

Microbial inoculation of Agrobacterium fabrum was performed on the seeds. A mixture of sterilized seeds and 10% glucose with an optical density of 0.5 at 535 nm was created in the form of a solution by combining 10 ml of inoculum with 100 g of sterilized seeds and 10% sugar. The mixture was thoroughly mixed, and once that, the seeds received the top layer of moss as well as clay soil (3:1 ratio) (29)

Biochar

To produce biochar, fruit and vegetable waste material was collected from a local market located at 30°11'29.8"N 71°28'48.8"E. Initially, the waste was sun-dried and then cut into small pieces. Pyrolysis was carried out under aerobic conditions at 325±5°C.
The deashed biochar was achieved by washing the biochar with tap water. After the ash content was removed, the biochar was rinsed thoroughly with deionized water to eliminate any remaining residue. The biochar was then dried in a well-ventilated area until it was completely dry. Finally, the deashed biochar was stored.

Investigational plan and treatment strategy

A completely randomized design was used for the experiments. There were 2 levels of irrigation (no drought stress = 65% and drought stress 40% field capacity) and Pb (0 and 400 mg Pb/kg soil). The levels of biochar used were 0 and 0.5%, which were applied to the soil on a weight basis with and without A. fabrum. All the treatments were applied in 3 replicates. A total of 48 pots were used in this experiment. Each pot was filled with 10 kg of soil. Soil samples were collected from 0 to 15 cm depth.

Different rtreatments i.e., T1 Control (No drought stress = 65% Field Capacity), T2 0.5%BC (No drought stress), T3 Rhizobacteria inoculation (No drought stress), T4 BC+RB (No drought stress), T5 Control (Drought Stress 40% Field Capacity), T6 0.5%BC (Drought Stress 40% Field Capacity), T7 Rhizobacteria inoculation (Drought Stress), T8 BC+RB (Drought Stress), T9 Control (No Pb Stress), T10 0.5%BC, T11 Rhizobacteria inoculation (No Pb Stress), T12 BC+RB, T13 Control (400 Pb mg/kg soil), T14 0.5%BC, T15 Rhizobacteria inoculation (400 Pb mg/kg soil), T16 BC+RB were used during the study.

Fertilizer application

For macronutrients, the recommended fertilizer application rates of 200--150--100 kg NPK ha -1 were applied (I. Naz et al., 2013). The sources of fertilizers used for N, P, and K were urea, single superphosphate, and sulfate, respectively.

Harvesting of maize plants

Maize plants were harvested at the V10 Zadok growth scale. Samples of shoot and roots were collected for data collection. The data on the root distance, sprout distance, shoot fresh mass, and dry mass were collected quickly after collection. To dry the shoot and root samples, an oven was used (65°C for 72 h). Maize harvesting can occur at different times, ranging from 70 to 120 days or more, depending on the variety and environmental conditions.

Morphological and growth parameters

Different morphological and growth parameters were assessed during the study, including i) root and shoot length (cm) (measured by a long meter stick) and ii) germination (%) (germination percentage measurement involves assessing the number of seeds that have successfully sprouted and comparing it to the total number of seeds planted). The following formula was used: germination percentage = (number of germinated seeds/total number of seeds) × 100; iii) fresh root and shoot weight (g) (an electronic balance was used to weight the fresh roots and shoots); and iv) dry root and shoot weight (g) (roots and shoots were placed in paper bags and left in the oven at 65°C for 72 hours, and the dry weight was recorded via an electronic balance).

Physiological parameters1Different physiological parameters were assessed during the study, including the following:

i) Chlorophyll contents

The samples were homogenized in a mortar and pestle with 80% acetone. Following filtration, the test tubes were centrifuged to eliminate any insoluble material, and then a new test tube was used to transfer the waste product. The amount of chlorophyll in the plant extract was then measured via an ultraviolet (UV) spectrophotometer at 663 nm and 645 nm (Arnon, 1949).

$$\text{Chlorophyll a ((mg/g)} = \frac{12.7 \times A663) - (2.69 \times A645) \times V}{1000 \times W}$$

$$\text{Chlorophyll b ((mg/g)} = \frac{(22.9 \times A645) - (4.68 \times A645) \times V}{1000 \times W}$$

Total Chlorophyll (mg/g) = Chlorophyll a + Chlorophyll b

i) Electrolyte leakage

Three leaves were randomly selected from each plant, and the midrib of each leaf was cut with a sharp blade. The foliage was thoroughly cleaned with deionized water by wiping. The weight of each leaf was recorded as the dry weight before being immersed in 10 mL of deionized water. The tubes containing the leaves were then placed in a waving incubator at 25°C for two hours to reach equilibrium. The initial electrical conductivity (C1) was ascertained via a conductivity meter. Thereafter, the leaf samples were exposed to a waterbath at 121°C for 20 minutes to denature the cells and liberate the electrolytes. Following cooling to room temperature, the final (30).

$$\text{Electrolyte leakage (\%)} = (C2 - C1)/C1 \times 100$$

ii) Total soluble protein

Leaf tissue was whipped in removal buffer (50 mM Tris-HCl, pH 7.5; 10 mM EDTA; 2% (w/v) SDS; and 2% (v/v) β-mercaptoethanol) after being ground in liquid nitrogen. Centrifugation was performed at 12000 rpm and 4°C for 15 minutes. Finally, the supernatant was stored. The protein concentration was determined through the addition of 100 µL of the supernatant to 1 ml of Bradford reagent (Bio-Rad) and was subsequently quantified via spectrophotometry at 595 nm (31).

Statistical Analysis

Average statistical analysis was performed for the collected data (32). The paired comparison analysis was conducted via OriginPro 2021 software (OriginLab Corporation, 2021), and changes were computed at $p \leq 0.05$. To analyze the correlation of the studied attributes, principal component analysis (PCA) was carried out via the software OriginPro 2021.

Results

Germination

Without drought stress, 0.5% BC increased germination by 8.33%, whereas A. fabrum increased it by 30.36%. Combining BC and A. fabrum resulted in the highest increase of 42.26%. Under drought stress, 0.5% BC improved germination by 12.61%, and A. fabrum improved germination by 21.01%, with their combination leading to a 27.73% increase. At 0Pb, 0.5% BC and A. fabrum increased germination by 8.79% and 17.03%, respectively, while their combination resulted in a 36.26% increase. Compared with the control, 0.5% BC increased germination by 20.00%, A. fabrum increased germination by 27.62%, and their combination increased germination by 53.33% (Figure 1).

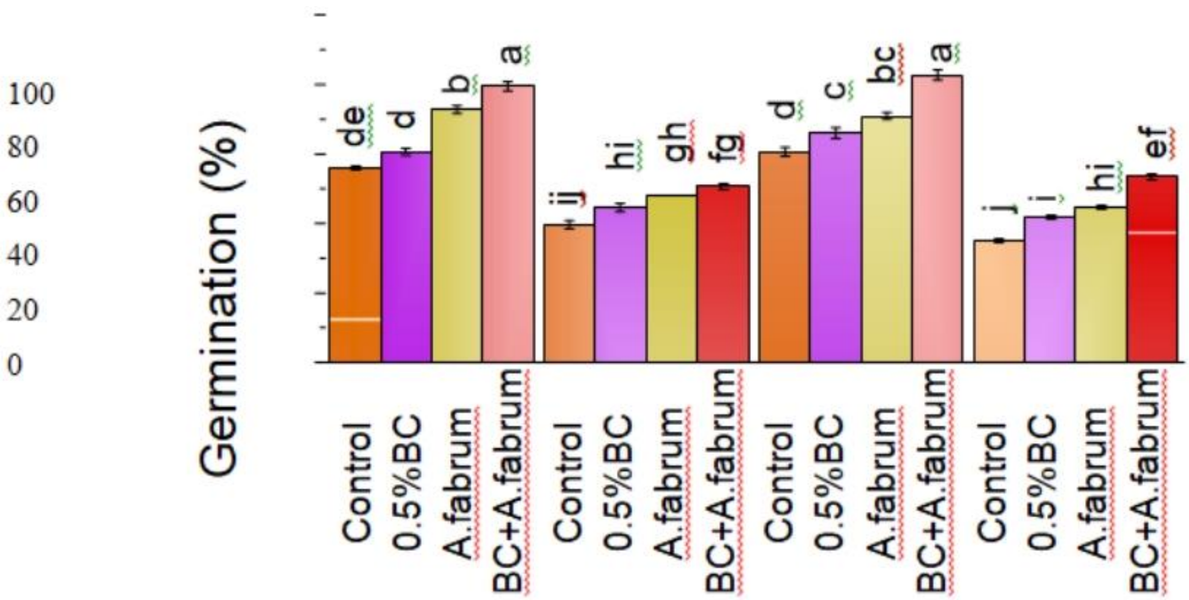

Figure 1: Effects of the combination of rhizobacteria and biochar on the germination of maize cultivated under drought stress and lead toxicity.

Shoot and root length with fresh and dry weight

All factors significantly affected shoot length, with stress and treatments having P values less than 0.0001 and the interaction term having a P value of 0.02197. The stress had a high F value of 275.29223, the treatment had 99.75254, and the interaction had a lower F value of 2.61007. The overall model was significant ($P < 0.0001$), indicating that the factors accounted for substantial variability in shoot length. For root length, stress and treatment had P values less than 0.0001, whereas the interaction term was not significant ($P = 0.1264$). The stress factor had an F value of 89.9293, the treatment factor had 87.62132, and the interaction factor had a lower F value of 1.71413. The model was significant ($P < 0.0001$), indicating that substantial variability in root length was present.

Shoot fresh weight was significantly affected by stress and Treatmentstreatment ($P < 0.0001$), with the interaction term being nonsignificant ($P = 0.13216$). The stress factor had an F value of 203.84404, the treatment factor had an F value of 54.07818, and the interaction factor had a lower F value of 1.69105. The Model was significant ($P < 0.0001$), indicating significant variability in shoot fresh weight. For shoot dry weight, stress and treatment had P values less than 0.0001, whereas the interaction term was not significant ($P = 0.30516$). The stress had an F value of 315.54511, the treatment had 112.88624, and the interaction had a lower F value of 1.24249. The model was significant ($P < 0.0001$), indicating that substantial variability in shoot dry weight was present (Table 2).

Root and shoot fresh weight with total soluble protein

The ANOVA results for root fresh weight revealed significant effects of stress, treatment, and their interaction. Stress had a highly significant effect ($p < 0.0001$, $F = 275.23063$), as did treatment ($p < 0.0001$, $F = 80.46043$). The interaction was also significant ($p = 0.03932$, $F = 2.31134$). The model was highly significant ($p < 0.0001$, $F = 72.52502$), indicating that substantial variability was present. For root dry weight, both stress and treatment had significant effects ($p < 0.0001$), whereas the interaction effect was not significant ($p = 0.9863$). The model also had a significant effect ($p < 0.0001$).

The total soluble protein levels were significantly affected by stress and treatment ($p < 0.0001$), and the interaction effect was also significant ($p = 0.02674$). The overall model was significant ($p < 0.0001$, $F = 104.15598$), demonstrating a good fit for the data. These results highlight that stressors such as drought and Pb poisoning, along with treatments such as rhizobacteria and biochar, significantly impact the total soluble protein content. The combination of these factors and their interactions strongly influence protein accumulation in maize (Table 1).

Table 1: Effects of drought and Pb stress on maize

Effect on maize under drought and Pb stress			
		Shoot length	**Root length**

Attributes	Df	Sum of Squares	Mean Square	F Value	P Value	Sum of Squares	Mean Square	F Value	P Value
Stress	3	426.83	142.277	275.292	<0.0001	13.6181	4.53937	89.9293	<0.0001
Treatments	3	154.663	51.5542	99.7525	<0.0001	13.2686	4.42287	87.6213	<0.0001
Interaction	9	12.1405	1.34894	2.61007	0.02197	0.77872	0.08652	1.71413	0.1264
Model	15	593.633	39.5756	76.575	<0.0001	27.6654	1.84436	36.5386	<0.0001
Error	32	16.5383	0.51682			1.61527	0.05048		
Corrected Total	47	610.172				29.2807			
		Shoot fresh weight				**Shoot dry weight**			
Stress	3	333.091	111.03	203.844	<0.0001	1.22312	0.40771	315.545	<0.0001
Treatments	3	88.3665	29.4555	54.0782	<0.0001	0.43757	0.14586	112.886	<0.0001
Interaction	9	8.28977	0.92109	1.69105	0.13216	0.01445	0.00161	1.24249	0.30516
Model	15	429.748	28.6498	52.5991	<0.0001	1.67514	0.11168	86.4318	<0.0001
Error	32	17.4299	0.54468			0.04135	0.00129		
Corrected Total	47	447.177				1.71649			
		Root fresh weight				**Root fresh weight**			
Stress	3	1.61603	0.53868	275.231	<0.0001	0.21323	0.07108	189.093	<0.0001
Treatments	3	0.47243	0.15748	80.4604	<0.0001	0.06824	0.02275	60.511	<0.0001
Interaction	9	0.04071	0.00452	2.31134	0.03932	7.99E-04	8.88E-05	0.23622	0.9863
Model	15	2.12917	0.14194	72.525	<0.0001	0.28227	0.01882	50.0626	<0.0001
Error	32	0.06263	0.00196			0.01203	3.76E-04		
Corrected Total	47	2.1918				0.29429			
		Total soluble protein							

Stress	3	0.45448	0.15149	425.764	<0.0001				
Treatments	3	0.09339	0.03113	87.4894	<0.0001				
Interaction	9	0.00803	8.93E-04	2.50884	0.02674				
Model	15	0.55591	0.03706	104.156	<0.0001				
Error	32	0.01139	3.56E-04						
Corrected Total	47	0.56729							

Under DS conditions, the application of 0.5% BC and *A. fabrum* resulted in 5.56% and 18.19% increases in total soluble protein, respectively. Compared with the control, the combined application of BC and *A. fabrum* resulted in a 35.81% increase in total soluble protein. These results suggest that under drought stress conditions, the combined application of *A. fabrum* and BC can significantly increase the protein content of maize seedlings. At 0Pb, the application of0.5% BC and *A. fabrum* resulted in 9.92% and 20.30% increases in total soluble protein, respectively. The greatest increase (28.69%) was observed with the combined application of BC and *A. fabrum*. These results suggest that the combined application of BC and *A. fabrum* can effectively increase the protein content of maize seedlings under lead toxicity conditions. Compared with the control, the application of 0.5% BC, *A. fabrum*, and the combined application of BC and *A. fabrum* resulted in 20.62%, 25.15%, and 32.36% increases in total soluble protein, respectively. These results suggest that under high lead toxicity conditions, the combined application of BC and *A. fabrum* can effectively increase the protein content of maize seedlings (Figure 2).

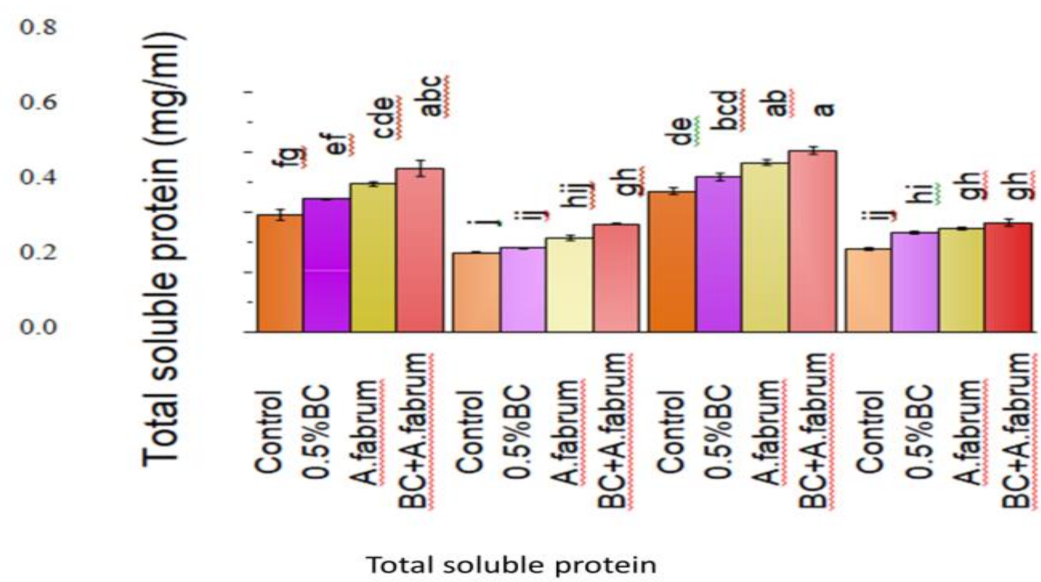

Figure 2: Effects of the combination of rhizobacteria and biochar on total soluble protein.

Chlorophyll a, b and total chlorophyll

The ANOVA results indicate that both stress and treatment significantly affect chlorophyll a levels in maize, with p values < 0.0001 for both factors and an interaction p value of 0.004. The model is highly significant (p < 0.0001), indicating that it fits the data well and explains a large proportion of the variability in chlorophyll a levels. For chlorophyll b levels, stress and treatment were significant (p < 0.0001), but their interaction was not significant (p = 0.33614). The model is significant (p < 0.0001), indicating that it adequately explains the variability in chlorophyll b levels. The total chlorophyll content was significantly influenced by stress and treatment (p < 0.0001), but the interaction effect was not significant (p = 0.95884). The model fits the data well (p < 0.0001), accounting for a substantial amount of variability in total chlorophyll levels (Table 2). Electrolyte leakage was also significantly affected by stress and treatment (p < 0.0001), although the interaction effect was not significant (p = 0.15752). The model is significant (p < 0.0001), effectively explaining the variability in electrolyte leakage levels (Table 2).

Table 2: Chlorophyll a, b and total chlorophyll contents under drought and Pb stress

		Chlorophyll a				Chlorophyll b			
Attributes	**Df**	**Sum of Squares**	**Mean Square**	**F Value**	**P Value**	**Sum of Squares**	**Mean Square**	**F Value**	**P Value**
Stress	3	1.4246	0.47487	289.057	<0.0001	1.21466	0.40489	165.98	<0.0001
Treatments	3	0.40832	0.13611	82.8507	<0.0001	0.51433	0.17144	70.2817	<0.0001
Interaction	9	0.05195	0.00577	3.51333	0.004	0.02607	0.0029	1.18748	0.33614
Model	15	1.88487	0.12566	76.4895	<0.0001	1.75507	0.117	47.9648	<0.0001
Error	32	0.05257	0.00164			0.07806	0.00244		
Corrected Total	47	1.93744				1.83313			
		Total chlorophyll a				**Electrolyte leakage**			
Stress	3	3	4.89007	1.63002	228.293	4811.97	1603.99069	487.662	<0.0001
Treatments	3	3	1.93653	0.64551	90.4067	1557.73	519.2 4326	157.866	<0.0001
Interaction	9	9	0.06162	0.00685	0.95884	47.3521	5. 26135	1.59961	0.15752
Model	15	15	6.88822	0.45921	64.3152	6417.05	42 7.8036	130.065	<0.0001
Error	32	32	0.22848	0.00714		105.253	3. 28914		
Corrected Total	47	47	7.1167			6522.31			

Discussion

The cultivation of *Zea mays* (maize) presents various challenges, including heavy metal toxicity from lead (Pb) and drought stress. These issues can significantly hinder plant development and agricultural yields. One potential approach to increase plant resilience against these stresses is the use of ameliorative substances such as biochar and rhizobacteria (RH). This discussion explores how biochar and rhizobacteria can improve *Zea* mays tolerance to both drought and lead stress (33). Both drought and Pb stress can generate reactive oxygen species (ROS) within plant cells (34). ROS, including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, are highly reactive molecules that can damage cellular structures such as lipids, proteins, and DNA (34). A major effect of drought and Pb stress on plants is a decrease in chlorophyll content, which is essential for photosynthesis (33, 35). Reduced chlorophyll levels can lead to diminished photosynthesis rates, lower energy production, and, consequently, reduced plant growth and productivity (36). Drought stress and Pb pollution can lead to chlorophyll damage through oxidative stress, membrane damage, and ion toxicity. Research has indicated that both drought and Pb stress significantly affect electrolyte leakage in maize plants. Drought stress disrupts the water balance within cells, leading to physiological responses such as stomatal closure, reduced photosynthesis, and the accumulation of Osmo protectants. These responses increase membrane permeability, allowing ions and other solutes to leak out and potentially cause cellular damage. In contrast, Pb stress results in elevated electrolyte leakage from maize plants. This leakage occurs because Pb disrupts the stability and fluidity of cell membranes. Additionally, lead can generate reactive oxygen species (ROS) through processes such as lipid peroxidation, further damaging biological components, including membranes.

However, further research involving larger sample sizes and a broader range of stress levels might reveal more intricate interactions. The present study revealed no statistically significant effect of the interaction between drought stress and Pb stress on electrolyte leakage. These findings underscore the vulnerability of plants to different stresses and emphasize the importance of understanding both their individual and combined effects for effective crop management and environmental protection. Recent studies have indicated that plant growth-promoting rhizobacteria (PGPRs) can help mitigate the adverse effects of various abiotic stresses on plants (37, 38). Specifically, the presence of ACC deaminase, IAA, and siderophores in PGPR has been found to be particularly advantageous (39, 40). In our study, we observed that the application of A. fabrum notably increased plant growth under drought and Pb stress. This beneficial effect is likely attributed to the production of ACC deaminase, IAA, and siderophores by A. fabrum. ACC deaminase, an enzyme produced by various PGPR, helps mitigate the adverse impacts of ethylene, which can accumulate during drought and heavy metal stress (41). There was no statistically significant effect on the growth rate from the combination of stress, rhizobacterial, and biochar treatments. This suggests that the individual effects of each element are stronger than their combined influence. These findings underscore the importance of considering stressors such as drought and lead poisoning in agricultural settings, as they can severely impair plant development. Ameliorative treatments such as rhizobacteria and biochar have substantial effects, demonstrating their potential to increase growth under stressful conditions. Beneficial rhizobacteria can support plant development by increasing nutrient uptake, hormone production, and systemic resistance. On the other hand, biochar can improve soil structure, water retention, and nutrient availability, thereby promoting plant growth.

A. fabrum, through the production of ACC deaminase, can help reduce ethylene accumulation, which supports root growth and delays senescence, leading to increased plant growth (42). In addition to ACC deaminase, A. fabrum also produces IAA, a plant hormone that stimulates root growth and development. During drought and heavy metal stress, plant growth is often hindered by reduced root development. By producing IAA, A. fabrum can increase root growth and development, thus improving overall plant growth and stress tolerance (43). Additionally, A. fabrum produces siderophores, which can further mitigate the adverse effects of drought and heavy metal stress. Siderophores are iron-chelating molecules that increase the availability of iron to plants (44). Several studies have shown that applying biochar can mitigate the negative effects of both drought and Pb stress on plants (41, 45). For example, the use of biochar has been associated with increased plant growth, increased chlorophyll content, and improved photosynthetic activity under Pb stress conditions (46). One proposed mechanism is the enhancement of soil physical and chemical properties, such as water-holding capacity, soil pH, and nutrient availability (47). This improvement can increase water and nutrient availability to plants, thereby increasing their stress tolerance. Additionally, the high surface area of biochar provides a habitat for beneficial microorganisms, which can support plant growth and provide protection against stress. Furthermore, rhizobacteria can help decompose biochar, releasing stored nutrients and making them more accessible to plants (48). Additionally, rhizobacteria can increase the effectiveness of biochar by increasing the availability of nutrients and growth-promoting substances for plants (49).

Rhizobacteria can produce plant growth hormones such as indole acetic acid (IAA) and gibberellins, which stimulate plant growth and development (50). They also generate enzymes such as phosphatase and nitrogenase, which help release nutrients from biochar (51, 52). Additionally, biochar has been found to increase antioxidant activity in plants. Antioxidants, including ascorbic acid and glutathione, are crucial for protecting plants from oxidative stress caused by drought and heavy metal toxicity (53). The application of biochar has been reported to increase the activity of antioxidant enzymes such as catalase and peroxidase, which assist in scavenging reactive oxygen species (ROS) and mitigating oxidative damage to plant cells (54).

Conclusion

In conclusion, maize was inoculated with A. fabrum, and 0.5% biochar (BC+A. fabrum) can increase growth and chlorophyll content and reduce electrolyte leakage under drought and Pb stress. The combination of BC and A. fabrum was more effective than the control. The use of BC+A is recommended for growers. fabrum to improve maize growth under these stresses. This study provides valuable insights into the potential of combining deashed biochar (BC) with Agrobacterium fabrum to alleviate drought and lead (Pb) stress in maize. Further research on different crops and climates is needed to establish BC+A. fabrum as the optimal treatment for mitigating drought and Pb stress.

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Author's contribution

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Conflict of interest

None

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