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RESEARCH ARTICLE
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Synergistic Effects of Endophytic Bacteria and Biochar on Morphophysiological Traits, Ionic Homeostasis and Grain Quality of Sorghum under Saline Soil Conditions

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ABSTRACT

Salinity is a major constraint limiting crop productivity in suboptimal lands, necessitating the development of sustainable strategies to improve plant performance. This study evaluated the combined effects of endophytic bacteria and biochar on the morphophysiological traits, ionic homeostasis, and grain quality of sweet sorghum under saline soil conditions (electrical conductivity 5 dS m^{-1}) in North Sumatra, Indonesia. The experiment was conducted using a factorial randomized block design with two factors, namely endophytic bacteria (0, 5, 10, and 15 mL $polybag^{-1}$) and biochar (80 and 120 g $polybag^{-1}$). Unlike previous studies that mainly examined either microbial inoculants or biochar separately, this study provides an integrated evaluation of their synergistic effects across morphological, physiological, anatomical, ionic, and grain biochemical responses under saline conditions. The combined application significantly enhanced plant growth, yield components, chlorophyll content, nutrient uptake, and grain nutritional quality while reducing Na accumulation and improving the K^+/Na^+ ratio, indicating better ionic homeostasis. These improvements were accompanied by increased stomatal density, optimized cuticle thickness, and enhanced carbohydrate, protein, and amylose contents, demonstrating coordinated structural and metabolic adaptation to salinity stress. The synergistic interaction between endophytic bacteria and biochar effectively mitigated the adverse effects of salinity by improving physiological function, nutrient balance, and grain quality. These findings highlight the potential of integrating endophytic bacterial inoculants with biochar as a sustainable management strategy for enhancing sweet sorghum productivity on salt-affected soils and provide a promising approach for climate-resilient agriculture.

Keywords: Endophytic bacteria, Ionic homeostasis, Rice husk biochar, Salinity stress, Sweet sorghum.

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INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is a versatile cereal crop widely recognized for its dual function as a food and bioenergy resource (Ameen et al., 2024). Its grains contain high levels of carbohydrates (68%), protein (9.5%), and crude fiber (2.3%), along with essential minerals such as calcium (0.11%) and amino acids including methionine (0.35%), cysteine (0.35%), and lysine (0.22%) (Rajalakshmi et al., 2021). In addition to its nutritional value as a food source, sweet sorghum is also valued for its high fermentable sugar content in the stalk, which can be utilized for bioethanol production, providing a sustainable alternative to fossil-based energy (Oktem & Oktem, 2024).

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Sorghum has considerable potential for development in Indonesia as an alternative crop. However, its cultivation remains limited, with a total planting area of approximately 8,000 hectares distributed across various regions. Despite its nutritional and economic potential, the average national productivity is still relatively low, at around 2.68 t ha^{-1}, compared to its potential yield of 5–7 t ha^{-1}. In regions such as North Sumatra, efforts to expand sorghum cultivation are increasingly directed toward suboptimal lands (Yusuf et al., 2025), including saline soils. Such expansion requires the development of cultivation technologies that can improve sorghum adaptation and productivity under salinity stress.

Soil salinity refers to the accumulation of soluble salts in the soil, primarily dominated by NaCl, but also NaSO, MgSO, NaHCO, NaCO, CaSO, and CaCO, which collectively determine the electrical conductivity (EC) of the soil solution (Choudhury et al., 2026). High salinity levels impose significant constraints on plant growth through ionic toxicity, osmotic imbalance, and nutrient disruption (Zaque, 2025). Saline soils are often characterized by visible salt crusts, pH values below 5.5, and electrical conductivity exceeding 4 mmhos cm^{-1} at 25°C (Shrivastava & Kumar, 2015). These conditions interfere with water uptake, damage cellular structures, and impair key physiological processes such as photosynthesis and protein synthesis. Consequently, salinity stress affects plants at multiple levels, including reduced morpho-agronomic performance, altered physiological responses such as chlorophyll degradation and proline accumulation, disruption of ionic balance (particularly the K^+/Na^+ ratio), as well as changes in anatomical traits and grain biochemical composition (Balasubramaniam et al., 2023; Barus et al., 2023; Pandit et al., 2024).

A central aspect of plant adaptation to salinity is ionic homeostasis, especially the maintenance of a favorable K^+/Na^+ ratio. Potassium is essential for enzyme activation, stomatal regulation, osmotic adjustment, and photosynthetic metabolism, whereas excessive Na^+ competes with K^+ for uptake and transport, leading to metabolic dysfunction and growth inhibition (Talakayala et al., 2025). Salt-tolerant plants are generally able to restrict Na^+ accumulation while maintaining adequate K^+ levels in their tissues, thereby preserving cellular functions under saline conditions. This regulation is mediated through coordinated ion transport systems, including selective K^+ uptake, Na^+ exclusion from the cytoplasm, vacuolar Na^+ sequestration, and long-distance ion transport between roots and shoots. Efficient operation of these mechanisms maintains membrane integrity, stabilizes enzyme activities, and prevents ionic toxicity in metabolically active tissues (Ma et al., 2026). Thus, plants with superior ionic homeostasis are better able to sustain photosynthetic activity, maintain water relations, and support biomass accumulation and reproductive development under saline environments (Hassan, 2024).

Strategies to mitigate the adverse effects of salinity are increasingly being explored such as organic amendments. The use of plant growth-promoting rhizobacteria (PGPR), particularly endophytic bacteria isolated from saline environments, has received considerable attention as a sustainable strategy to enhance plant tolerance to salinity stress (Jabborova et al., 2025). These microorganisms can improve plant performance through multiple mechanisms, including enhanced nutrient acquisition, phytohormone production, and osmotic adjustment via the accumulation of compatible solutes such as proline (Bhat et al., 2023; Zhang et al., 2025; Fouad et al., 2026). Recent studies have further confirmed that halotolerant PGPR can regulate stress-responsive metabolites, improve antioxidant systems, and enhance nutrient uptake, thereby increasing plant resilience under saline conditions (AbuQamar et al., 2024). In crops, microbial inoculation has been associated with improved chlorophyll retention, proline accumulation, and maintenance of ionic balance under salt stress (Witzel et al., 2026).

Biochar has also been widely recognized as an effective soil amendment due to its high carbon content (Tang, 2025), water-holding capacity (Hien et al., 2021) and cation exchange capacity (Shyam et al., 2025). These properties contribute to improved soil physicochemical characteristics (Adekiya et al., 2025), nutrient retention and microbial interaction (Samaraweera et al., 2023), and soil health (Bera, 2024), thereby supporting plant growth in saline soils. Recent findings indicate that biochar can function as a carrier for beneficial microbes while improving soil quality, organic matter stability, and plant productivity under salinity stress (Abbas et al., 2026; Wang et al., 2026).

Previous studies have shown that both endophytic bacteria and biochar play important roles in alleviating salinity stress (Akhtar et al., 2015; Liu et al., 2025a; Zhao et al., 2025). However, their synergistic effects remain insufficiently explored in sorghum. Most existing studies have focused on either microbial inoculation or biochar application alone, and many have evaluated only a limited set of response variables, primarily growth and yield. Consequently, there is still limited understanding of how the interaction between endophytic bacteria and biochar influences multiple biological levels simultaneously, including morphological performance, physiological regulation, ionic homeostasis, anatomical adaptation, and grain biochemical composition under saline conditions. This knowledge gap is particularly important for sweet sorghum because its value depends not only on biomass production but also on grain quality and physiological resilience in salt-affected soils.

In the present study, the Soper 6 sorghum variety, previously identified as a promising genotype for saline environments (Tarigan et al., 2025), was used to evaluate the integrated application of endophytic bacteria and rice husk biochar. We hypothesized that the combined application of endophytic bacteria and biochar would exert synergistic effects in enhancing salinity tolerance of sweet sorghum by improving growth performance, chlorophyll retention, osmotic adjustment, ionic homeostasis, anatomical stability, and grain biochemical quality under saline soil conditions. This study aimed to evaluate the effects of endophytic bacteria enriched with biochar on growth performance, physiological responses, ionic balance, and grain quality of sweet sorghum under saline soil conditions.

MATERIALS & METHODS

Study Area

Sorghum cultivation was carried out in the experimental field of the Faculty of Agriculture, Universitas Muhammadiyah Sumatera Utara, on Tuar Street Medan, North Sumatra, Indonesia (3°32'09.0" N, 98°42'40.0" E) (Fig. 1). The experimental site is located at an altitude of approximately 27 m above sea level and is characterized by a tropical humid climate. The soil used in this study was classified as an Inceptisol with saline characteristics (electrical conductivity of 5 dS m^{-1}). The soil had a pH of 6.9, organic C of 3.07%, total N of 0.32%, total P of 726.76 mg kg^{-1}, and exchangeable K of 0.90 cmol kg^{-1}. The research was conducted from June to September 2024. Physiological analysis was conducted in the Analysis laboratory, Faculty of Agriculture, Universitas Muhammadiyah Sumatera Utara, Indonesia. Anatomical analysis was conducted in the Plant Physiology laboratory, Faculty of Mathematics and Natural Sciences, and plant nutrient uptake analysis was conducted in the Research and Technology laboratory, Faculty of Agriculture, Universitas Sumatera Utara, Indonesia. Biochemical analysis and biochar content were conducted in the Soil, Plant, Fertilizer, and Water Laboratory of BSIP (Badan Standardisasi Instrumen Pertanian), Medan, North Sumatra, Indonesia.

Materials and Tools

The materials used were sorghum seeds of the Soper 6 variety obtained from the Maros Cereal Research Institute, Makassar, Indonesia, saline soil, rice husks, NPK fertilizer, endophytic bacteria, insecticides with the active ingredient emamectin benzoate and fungicides with the active ingredient tebuconazole. The tools used were tape measure, hoe, scissors, analytical balance, research nameplate, treatment label, envelope, handspreader, cutter knife, 40 x 50 cm polybag, microscope, spectrophotometer, and several other tools used for laboratory analysis.

Method

The study was conducted using a factorial Randomized Block Design (RBD). The first factor was endophytic bacteria (M), consisting of 4 levels, namely 0, 5, 10, and 15 mL/polybag. The second factor was biochar (B), consisting of 2 levels, namely 80 and 120 g/polybag. This resulted in 8 treatment combinations (M_0B_1: 0 mL/polybag endophytic bacteria + 80 g/polybag biochar; M_0B_2: 0 mL/polybag endophytic bacteria + 120 g/polybag biochar; M_1B_1: 5 mL/polybag endophytic bacteria + 80 g/polybag biochar; M_1B_2: 5 mL/polybag endophytic bacteria + 120 g/polybag biochar; M_2B_1: 10 mL/polybag endophytic bacteria + 80 g/polybag biochar; M_2B_2: 10 mL/polybag endophytic bacteria + 120 g/polybag biochar; M_3B_1: 15 mL/polybag endophytic bacteria + 80 g/polybag biochar; M_3B_2: 15 mL/polybag endophytic bacteria + 120 g/polybag biochar), each repeated 3 times. Each plot measured 1.5 m x 1.5 m, and each treatment plot consisted of 4 polybags.

Procedures

Biochar is produced using the pyrolysis method using a retort kiln (pyrolysis drum) that utilizes rice husks as the raw material. Dried rice husks with a moisture content of <15% are placed in a drum or kiln and then sealed with limited ventilation to control oxygen. Heating is carried out at a temperature of 400–600°C for 2–5 hours. After the carbonization process is complete, the drum is cooled with a tightly closed lid, deprived of oxygen, to prevent combustion from turning to ash. Next, the biochar nutrient content is analyzed (Table 1).

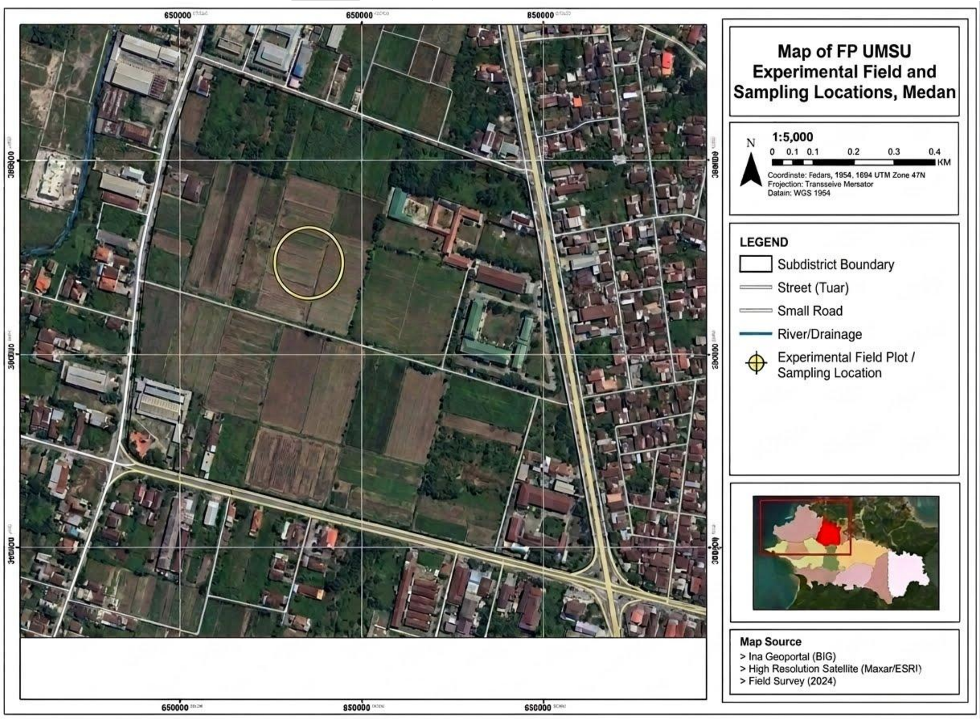


Fig. 1: Map of study area.

Table 1: Nutrient content in rice husk biochar

Nutrient content	Value	Test Method
C-organic (%)	17.82	Spectrophotometry
N-total (%)	1.59	IK 0.3.14.0 (Kjeldahl)
P_2O_5 (%)	0.28	IK 0.3.15.0 (Spectrophotometry)
K_2O (%)	0.75	IK 0.3.16.0 (A.A.S)
pH	7.5	IK. 0.3.12.0 (Electrometry)

The endophytic bacteria used in this study was obtained from our previous study (Tarigan et al., 2023). The isolates were originally recovered from sorghum plants growing under saline conditions in North Sumatra, Indonesia, and were identified using morphological. Based on the previous characterization, the selected isolates exhibited multiple plant growth-promoting traits, including phosphate solubilization, nitrogen fixation, and tolerance to saline conditions.

Saline soil weighing 10 kg was placed in polybags. Biochar was added to the saline soil medium two weeks before planting, mixed evenly into the medium. Sorghum seeds were planted 1-2 cm deep from the soil surface, with 2 seeds sown in each planting hole. After the plants reached 2 weeks after planting, thinning was carried out, leaving 1 plant per polybag, and an initial application of NPK fertilizer of 2.5 g/polybag was applied. Further NPK fertilization was carried out at 6 and 10 weeks after planting at 3.5 g/polybag. The endophytic bacteria application was carried out twice, at 40 days after planting and 60 days after planting, with doses according to the treatment, by watering around the roots. Plant maintenance was carried out manually and chemically, depending on the condition of the plants in the field.

The parameters observed included morpho-agronomic characters (plant height, number of leaves, stem diameter, panicle length, panicle weight, number of seeds/panicle, seed weight/panicle, seed weight/plant, weight of 100 seeds), anatomical characters (number of stomata, stomata density, cuticle thickness), physiological characters (chlorophyll content), proline content, and nutrient absorption of N, P, K, Na, K/Na ratio and biochemical characters (water content, protein, fat, ash, amylose and carbohydrates).

Data Analysis

The morpho-agronomical data were statistically analyzed using analysis of variance (ANOVA). Prior to analysis, the data were tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test. The significant mean differences were further compared and separated with Duncan's Multiple Range Test (DMRT) at the 5%. Statistical analyses were performed using IBM SPSS Statistics version 28.0.

RESULTS & DISCUSSION

Morpho-Agronomical Traits

Salinity stress significantly influences plant growth and yield formation by disrupting water uptake, nutrient balance, and metabolic activities. The combined application of endophytic bacteria and biochar affected several morpho-agronomic traits of sweet sorghum grown under saline soil conditions. Significant differences among treatments were observed for plant height, stem diameter, panicle length, panicle weight, number of seeds per panicle, seed weight per panicle, and 100-seed weight (Table 2).

The combined application of endophytic bacteria and biochar generally improved vegetative growth under saline conditions, as reflected by increased plant height, leaf number, and stem diameter, with the highest in M_2B_2. Enhanced growth may be associated with improved nutrient availability, water uptake, and physiological activity in the rhizosphere. Endophytic bacteria are known to stimulate plant growth through phytohormone production, particularly indole-3-acetic acid (IAA), and improved nutrient acquisition under stress conditions (Eid et al., 2021), whereas biochar contributes through improved soil structure, aeration, water retention, and cation exchange capacity (Tang, 2025). The relatively stable leaf production across treatments also suggests that the Soper 6 genotype possesses adaptive characteristics that enable maintenance of vegetative development under saline environments, likely supported by improved osmotic adjustment and water-use efficiency (Dehnavi et al., 2024). In addition, the greater stem diameter observed in combined treatments indicates enhanced vascular development and assimilate transport capacity, which are essential for sustaining biomass accumulation and plant productivity under salinity stress (Pandit et al., 2024).

Yield-related traits, including panicle length, panicle weight, number of seeds per panicle, and seed weight per panicle, were markedly improved by the combined application of endophytic bacteria and biochar. The M_2B_2 treatment consistently produced the highest yield components, indicating that the integrated treatments effectively maintained reproductive development under saline conditions. Salinity stress commonly disrupts flowering and grain filling through reduced photosynthetic activity and assimilate partitioning (Aninbon et al., 2023).

Table 2: Morpho-agronomical traits

Treatment	Plant height (cm)	Number of leaves (leave)	Stem diameter (mm)	Panicle lenght (cm)	Panicle weight (g)	Number of seeds/ panicle (seed)	Seed weight/ panicle (g)	Weight 100 seeds (g)
M_0B_1	169 e	9 b	31 c	21.6 d	15.7 e	84 e	17.5 f	19.6 e
M_0B_2	170 d	9 b	33 bc	22.6 c	17.1 d	90 d	18.3 ef	21.2 d
M_1B_1	173 d	9 b	34 b	22.9 c	18.3 c	93 d	19.5 de	21.7 d
M_1B_2	175 cd	9 b	34 b	24.4 b	20.2 b	99 bc	21.0 cd	22.6 bc
M_2B_1	173 d	9 b	34 b	23.4 c	20.0 b	98 c	20.9 d	22.5 c
M_2B_2	193 a	10 a	37 a	25.6 a	22.6 a	134 a	25.6 a	24.7 a
M_3B_1	177 bc	9 b	36 a	25.0 a	20.3 b	103 b	22.4 bc	23.1 bc
M_3B_2	179 b	9 b	36 a	25.5 a	21.9 a	131 a	23.8 b	23.4 b

Note: Numbers followed by different letters in the same column and row indicate significant differences according to Duncan's Multiple Range Test's at α = 5%.

The improved reproductive performance observed in treated plants suggests that enhanced physiological stability, nutrient balance, and photosynthetic efficiency supported more effective grain development. Increased potassium availability under biochar-amended conditions likely contributed to assimilate transport and starch synthesis, while reduced Na^+ toxicity helped maintain pollen viability and reproductive tissue development (Xiu et al., 2025). Similar findings were reported by Nehela et al. (2021), who demonstrated that biochar and beneficial microbial applications improved grain yield and reproductive performance under abiotic stress conditions.

The 100-seed weight showed a similar trend, with M_2B_2 producing the highest value that significantly higher than the other treatment. Seed weight is an important indicator of grain filling efficiency and overall plant metabolic performance (Cao et al., 2025). Increased seed weight suggests that plants treated with endophytic bacteria and biochar maintained more efficient photosynthetic activity and nutrient assimilation during the reproductive phase. This finding is consistent with reports by Liu et al. (2025b), who showed that biochar-based soil amendments improved grain filling and yield stability in cereal crops grown under saline environments.

Anatomical Traits

Leaf anatomical traits are closely associated with plant adaptation to salinity stress because they directly influence transpiration, gas exchange, and water conservation. The combined application of endophytic bacteria and biochar affected the anatomical traits of sweet sorghum grown under saline conditions. Variations were observed in the number of stomata, stomata density, and cuticle thickness among treatments, as presented in Fig. 2.

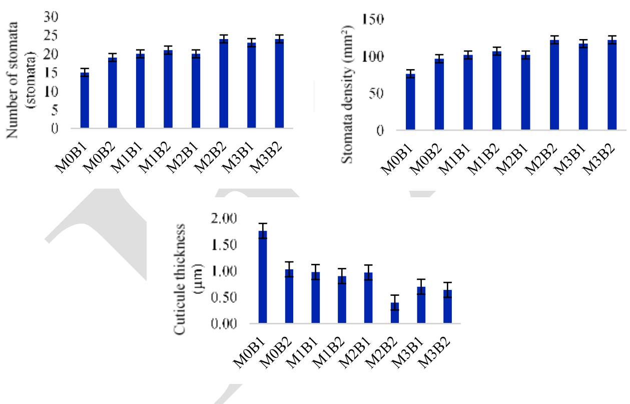


Fig. 2: Anatomical traits of sweet sorghum under different endophytic bacteria and biochar treatments in saline soil conditions.

The number of stomata and stomata density increased markedly following the application of endophytic bacteria and biochar. The highest stomata density was recorded in M_2B_2 and M_3B_2, whereas the M_0B_1 showed the lowest value (Fig. 2). Stomata play a central role in regulating CO_2 diffusion and transpiration, and their density is highly responsive to environmental stress (Haworth et al., 2021). Under saline conditions, excessive salt accumulation often reduces stomata development as a protective mechanism to minimize water loss (Bawa et al., 2023). The improved stomata characteristics were closely associated with enhanced ionic balance, particularly increased K uptake and higher K^+/Na^+ ratio in plants treated with endophytic bacteria and biochar. The combined treatment likely improved ionic homeostasis through greater Na^+ adsorption and cation exchange capacity provided by biochar, while endophytic bacteria enhanced nutrient mobilization and stress adaptation in the rhizosphere. Similar findings were reported by Chen et al. (2023), who showed that biochar-assisted microbial amendments improved stomatal conductance and maintained leaf structural integrity in salt-stressed plants.

Cuticle thickness showed an opposite trend, where combined treatments reduced cuticle thickness compared to the control. According to Srivastava (2022), plants frequently develop thicker cuticles in saline environments as a structural defense mechanism to reduce excessive transpiration and water loss. Although thicker cuticles can protect leaf tissues under stress, they may also limit gas exchange and reduce photosynthetic efficiency. The thinner cuticle observed under combined treatments indicates that plants experienced lower stress intensity and maintained better physiological activity. This response suggests that endophytic bacteria and biochar effectively alleviated osmotic and ionic stress, thereby reducing the need for excessive structural protection. Similar observations were reported by El-Tarabily et al. (2021), who found that beneficial rhizobacteria improved leaf anatomical flexibility and reduced stress-induced structural abnormalities under saline conditions.

The anatomical observations of leaf stomata further confirmed the effects of endophytic bacteria and biochar on the adaptive responses of sweet sorghum to salinity stress (Fig. 3). Clear differences in stomata distribution and density were observed among treatments. Plants grown under the control treatment (M_0B_1) exhibited fewer stomata with relatively wider spacing between epidermal cells, indicating restricted stomata development under saline conditions. In contrast, treatments receiving combined applications of endophytic bacteria and biochar, particularly M_2B_2 and M_3B_2, showed a greater number of stomata distributed more uniformly across the leaf surface. This response may indicate improved epidermal cell stability and water balance within leaf tissues. Enhanced stomata organization under saline conditions is important for maintaining transpiration efficiency and regulating gas exchange without excessive water loss (Hasanuzzaman et al., 2023). Previous studies have shown that plants capable of maintaining stomata functionality under salinity stress exhibit better photosynthetic efficiency and higher biomass production (Mohamed et al., 2020).

Microscopic observations of leaf cuticle structure revealed clear anatomical differences among treatments under saline conditions (Fig. 4). Control plants exhibited thicker and more irregular cuticle layers, whereas plants treated with endophytic bacteria and biochar showed thinner, more organized, and compact epidermal tissues. These anatomical improvements were likely associated with enhanced water relations, ionic regulation and reduced oxidative damage under saline stress.

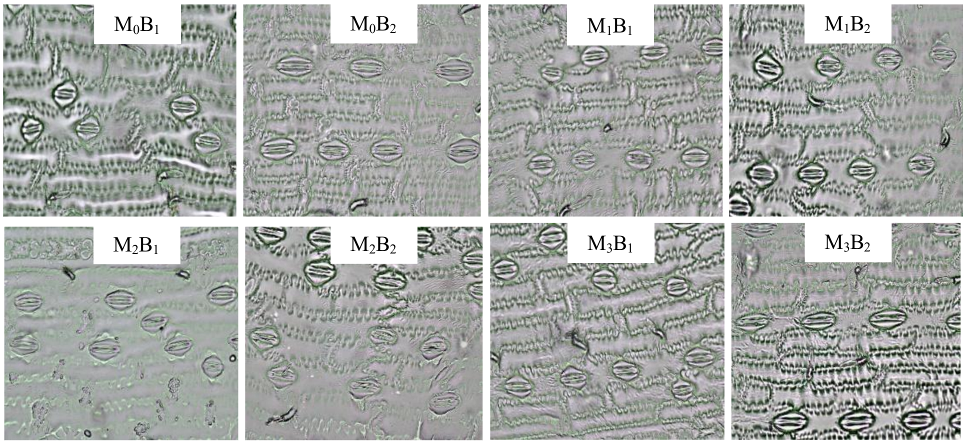


Fig. 3: Stomata structure and density of sweet sorghum leaves under different endophytic bacteria and biochar treatments in saline soil conditions.

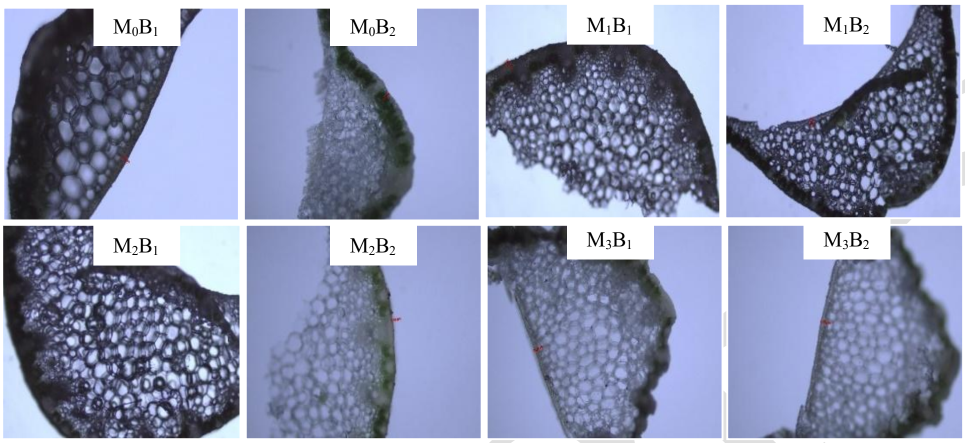


Fig. 4: Leaf cuticle structure of sweet sorghum under different endophytic bacteria and biochar treatments in saline soil conditions.

Endophytic bacteria may have contributed through osmotic adjustment and antioxidant activity (Ali et al., 2022), while biochar improved soil moisture retention, Na^+ adsorption, and cation exchange capacity, thereby reducing physiological drought stress (Dey et al., 2023). The improved cuticle organization observed in treated plants was also consistent with higher chlorophyll content and improved K^+/Na^+ ratio, indicating a close relationship between anatomical adaptation and physiological stability under saline conditions.

Visual examination of the microscopic also suggests that plants treated with higher biochar doses possessed more organized and compact epidermal tissues. This response may indicate improved cellular integrity and tissue hydration under saline stress. Previous studies have shown that improved epidermal organization under stress conditions is often associated with enhanced nutrient status and better maintenance of photosynthetic activity (Yavas et al., 2024). In the present study, treatments exhibiting thinner cuticles were also associated with higher chlorophyll content and improved K^+/Na^+ ratio, indicating that anatomical adaptation was closely linked to physiological performance.

Physiological Traits

Physiological performance is one of the most sensitive indicators of plant adaptation to salinity stress because it directly reflects the ability of plants to maintain photosynthetic activity, osmotic balance, and nutrient homeostasis under stress environments. The combined application of endophytic bacteria and biochar markedly influenced chlorophyll content (Fig. 5), proline accumulation (Fig. 6), and nutrient uptake (Fig. 7) in sweet sorghum grown under saline soil conditions.

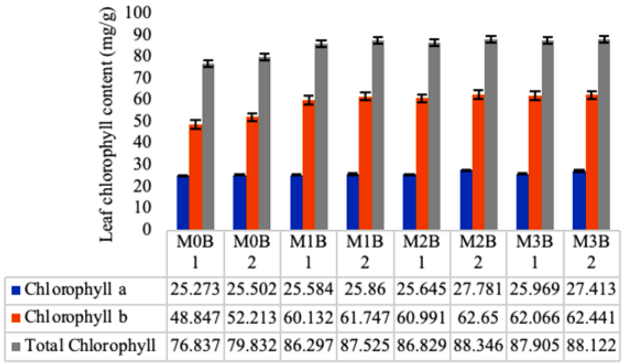


Fig. 5: Chlorophyll content of sweet sorghum under different endophytic bacteria and biochar treatments in saline soil conditions.

Chlorophyll content increased markedly following the combined application of endophytic bacteria and biochar. Total chlorophyll increased, largely due to greater chlorophyll b accumulation relative to chlorophyll a across treatments (Fig. 5). Chlorophyll retention is considered a key indicator of salinity tolerance because excessive Na^+ accumulation and oxidative stress commonly accelerate chlorophyll degradation through lipid peroxidation, chloroplast membrane disruption, and activation of chlorophyll catabolic enzymes (Nounjan et al., 2020; Wang et al., 2024). The higher chlorophyll content observed in the present study suggests that the integrated treatments alleviated these detrimental effects by preserving chloroplast integrity and reducing oxidative damage. Endophytic bacteria likely contributed by producing phytohormones and stimulating antioxidant defense systems that scavenge reactive oxygen species (ROS), thereby protecting photosystem II and thylakoid membranes from oxidative injury (Linh & Duc, 2025). Meanwhile, biochar may have enhanced chlorophyll biosynthesis by improving the availability of nitrogen and magnesium, the principal structural elements required for chlorophyll formation, while simultaneously enhancing nutrient retention and reducing Na^+ toxicity (Murtaza et al., 2024).

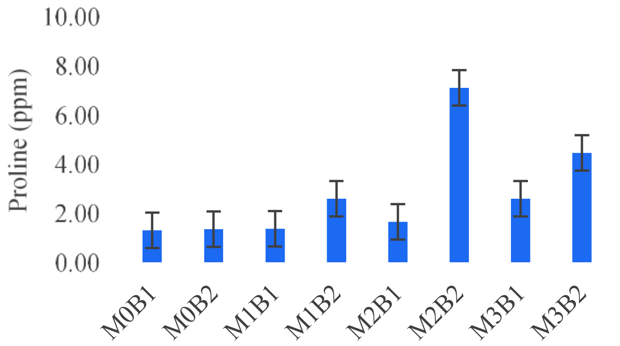
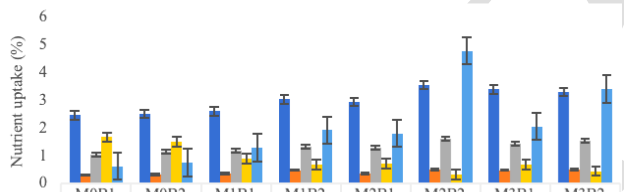


Fig. 6: Proline content of sweet sorghum under different endophytic bacteria and biochar treatments in saline soil conditions.



	M0B1	M0B2	M1B1	M1B2	M2B1	M2B2	M3B1	M3B2
N	2.47	2.53	2.61	3.04	2.96	3.55	3.4	3.32
P	0.31	0.33	0.36	0.49	0.36	0.51	0.49	0.5
K	1.05	1.15	1.18	1.35	1.31	1.63	1.43	1.54
Na	1.68	1.52	0.9	0.7	0.72	0.34	0.69	0.45
K/Na	0.63	0.76	1.31	1.93	1.82	4.79	2.07	3.42

Fig. 7: Nutrient uptake of sweet sorghum under different endophytic bacteria and biochar treatments in saline soil conditions.

The improved K^+/Na^+ ratio observed in this study may have further supported chlorophyll stability by maintaining ionic homeostasis and reducing ion-induced chloroplast damage. Similar findings were reported by AbuQamar et al. (2024) and Bagnazari et al. (2025), who demonstrated that microbial inoculation enhanced chlorophyll retention and photosynthetic activity under salinity stress. Moreover, the predominance of chlorophyll b may represent an adaptive adjustment that expands light-harvesting capacity and stabilizes light-harvesting complex proteins, thereby sustaining photosynthetic efficiency under osmotic and ionic stress (Tamiaki & Kichishima, 2025).

Higher proline contents were consistently observed in treatments receiving combined applications compared with the control. The highest proline accumulation was recorded in M_2B_2, followed by M_3B_2, while the M_0B_1 treatment exhibited the lowest proline content (Fig. 6). Increased proline accumulation in treated plants indicates that the combined application of endophytic bacteria and biochar enhanced the osmotic adjustment capacity of sweet sorghum. Endophytic bacteria likely promoted this response by regulating stress-responsive signaling pathways and phytohormone-mediated activation of proline biosynthesis (Sharaya et al., 2023), whereas biochar improved soil water availability and root-zone moisture, reducing the severity of osmotic stress while supporting osmolyte production (Ali et al., 2025). This coordinated response likely contributed to the improved physiological performance observed in treated plants, including higher chlorophyll content, better ionic homeostasis, and enhanced growth under saline conditions. Similar mechanisms have been reported by Abulfaraj & Jalal (2021) reported that use of plant growth-promoting bacteria under salinity stress enhanced proline biosynthesis and antioxidant defense, thereby improving plant tolerance to salinity stress.

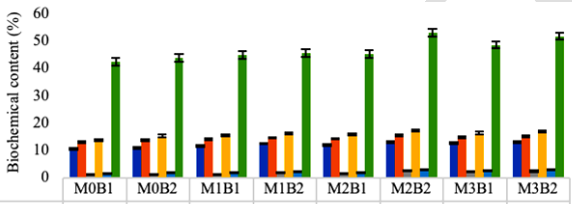
The combined treatments also markedly improved nutrient uptake, particularly nitrogen, phosphorus, and potassium (Fig. 7). The highest uptake values were recorded in M_2B_2, whereas Na accumulation was reduced, significantly lower than the control treatment. The improved nutrient status suggests that the combined application of endophytic bacteria and biochar enhanced rhizosphere conditions and nutrient availability. Endophytic bacteria may facilitate nutrient mobilization through nitrogen fixation and phosphate solubilization (Sen et al., 2026). In addition, based on the analysis results, the rice husk biochar used in this study contained appreciable levels of organic carbon, nitrogen, potassium, and alkaline pH, which likely contributed to reduced Na toxicity and improved nutrient dynamics in the root zone. Sultan et al. (2024) reported that biochar amendments improved nutrient uptake and reduced ionic toxicity under saline conditions.

Salinity stress frequently disrupts nutrient acquisition because excessive Na^+ competes with essential nutrients for uptake and transport processes. Under saline conditions, elevated Na^+ concentrations can inhibit K^+ absorption by roots, resulting in ionic imbalance and metabolic dysfunction in plant tissues (Rauf et al., 2024). This effect was clearly reflected in the K^+/Na^+ ratio, which increased substantially (Fig. 7). According to Assaha et al. (2017), maintenance of a high K^+/Na^+ ratio is widely recognized as one of the most important physiological indicators of salinity tolerance because potassium is required for enzyme activation, stomata regulation, osmotic adjustment, and photosynthetic metabolism. Excessive Na^+ accumulation disrupts these functions by displacing K^+ from cellular binding sites and damaging membrane stability. The improved K^+/Na^+ ratio observed following the combined application of endophytic bacteria and biochar suggests that the treatments effectively limited Na^+ accumulation while enhancing potassium uptake and retention. Similar findings have been reported in wheat treated with biochar and PGPR under salinity stress, where improved ionic homeostasis was closely associated with enhanced stress tolerance and yield stability (Malik et al., 2024).

The maintenance of ionic homeostasis may also involve coordinated regulation of ion transport and compartmentalization within plant cells (Balasubramaniam et al., 2023). Under saline conditions, salt-tolerant plants minimize cytosolic Na^+ toxicity by restricting Na^+ influx at the root surface, actively extruding Na^+ through plasma membrane Na^+/H^+ antiporters, and sequestering excess Na^+ into vacuoles via tonoplast transport systems. Simultaneously, selective K^+ transporters facilitate preferential potassium uptake and retention, ensuring adequate cytosolic K^+ concentrations required for enzymatic activity and cellular metabolism (Mansour, 2022; Zhou et al., 2024). Although the expression of these ion transport systems was not examined in the present study, the marked increase in the K^+/Na^+ ratio strongly suggests that the combined application of endophytic bacteria and biochar promoted more efficient ionic regulation.

Biochemical Traits

Salinity stress not only affects plant growth and physiological processes during vegetative development, but also assimilate accumulation and grain quality at the reproductive stage. The combined application of endophytic bacteria and biochar not only improved plant growth and physiological performance, but also markedly influenced grain biochemical composition under saline soil conditions (Fig. 8).



	M0B1	M0B2	M1B1	M1B2	M2B1	M2B2	M3B1	M3B2
Water	10.78	10.97	11.80	12.65	12.20	13.14	12.95	13.12
Protein	13.15	13.85	14.13	14.78	14.45	15.75	14.88	15.40
Fat	1.19	1.27	1.43	1.97	1.65	2.80	2.35	2.57
Ash	13.86	15.48	15.73	16.26	15.95	17.38	16.55	16.97
Amylose	1.63	1.87	1.96	2.35	2.18	3.07	2.74	2.97
Carbohydrate	42.64	43.96	45.18	45.89	45.36	53.31	48.77	51.94

Fig. 8: Biochemical composition of sweet sorghum grains under different endophytic bacteria and biochar treatments in saline soil conditions.

Water content in sorghum grains responded to the combined application of endophytic bacteria and biochar, although the magnitude of change was relatively smaller compared with other biochemical parameters. A consistent trend was observed between biochar levels, where treatments receiving the higher biochar dose (B_2) generally showed greater grain water content than the corresponding B_1 treatments at the same bacteria level. This pattern suggests that increased biochar application improved soil moisture retention and water availability under saline conditions, thereby supporting better grain filling and assimilate deposition during seed development. According to Schaffert et al. (2022), biochar possesses a porous structure and strong water-holding capacity, which enable it to retain soil moisture and improve water availability in saline environments. In addition, biochar can enhance soil aggregation and reduce osmotic stress by adsorbing excess Na^+ ions, thereby improving root water uptake and maintaining plant water balance (Arkian et al., 2025). Enhanced proline accumulation and improved stomata regulation observed in previous sections further support the role of integrated treatments in maintaining cellular hydration under saline conditions.

The combined treatments influenced the accumulation of nitrogen-derived and mineral-associated biochemical components in sorghum grains, particularly protein, fat, and ash contents. The components increased consistently in response to the combined treatments, with the highest values consistently recorded in M_2B_2. These findings suggest that the combined application of endophytic bacteria and biochar improved nutrient acquisition, assimilation, and metabolic efficiency under saline conditions. Enhanced nitrogen uptake likely contributed to greater protein accumulation, while improved potassium availability and ionic balance may have supported enzymatic activities associated with lipid metabolism. The increased ash content further indicates enhanced mineral accumulation in grains, which was likely associated with improved nutrient retention and cation exchange capacity provided by rice husk biochar, as well as microbial-mediated nutrient mobilization in the rhizosphere. According to Vacheron et al. (2013), PGPR inoculation can stimulate nitrogen metabolism and amino acid synthesis through enhanced nutrient acquisition and phytohormone-mediated root activity, while biochar improves nutrient availability and enzymatic processes involved in carbon and lipid metabolism under saline conditions (Gao et al., 2024).

Carbohydrate and amylose contents at treatments receiving combined applications, particularly M_1B_2, M_2B_2, and M_3B_2, consistently higher compared with the control. These findings indicate that the integrated treatments improved photosynthate production and starch biosynthesis during grain filling. Salinity stress commonly suppresses carbohydrate accumulation through impaired photosynthesis, ionic toxicity, and restricted assimilate translocation. The higher carbohydrate and amylose contents observed in treated plants were consistent with the increased chlorophyll content and improved K^+/Na^+ ratio recorded in physiological observations, suggesting that better ionic regulation and photosynthetic efficiency supported more effective carbon metabolism. Endophytic bacteria inoculation and biochar application can enhance carbohydrate metabolism by improving leaf photosynthetic capacity, stimulating carbon assimilation enzymes, and maintaining source–sink translocation under abiotic stress conditions (Che et al., 2024; Su et al., 2024). Similar responses have been reported in rice (Hafez et al., 2019) and barley (Gul et al., 2023).

Conclusion

The combined application of endophytic bacteria and rice husk biochar effectively improved morphophysiological traits, ionic homeostasis, and grain biochemical quality of sweet sorghum cultivated under saline soil conditions. Treatments receiving integrated applications, particularly M_2B_2, consistently showed superior responses in morpho-agronomic traits, chlorophyll content, proline accumulation, K^+ uptake and K^+/Na^+ ratio, stomata development, and grain nutritional composition, including increased protein, carbohydrate, amylose, fat, and mineral contents. These improvements indicate that the synergistic interaction between endophytic bacteria and biochar enhanced plant tolerance to salinity through better osmotic adjustment, ionic homeostasis, nutrient acquisition, and photosynthetic efficiency. The findings highlight the potential of integrated endophytic bacteria with rice husk biochar as sustainable approaches for improving sorghum productivity and grain quality on salt-affected lands. However, this study was conducted under a single salinity level and on one sorghum variety. Therefore, future research should evaluate the long-term effectiveness of this integrated approach across different salinity levels, sorghum genotypes, soil types, and agroecological environments, while further investigating the underlying molecular and microbiome-mediated mechanisms of salinity tolerance.

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REFERENCES

Abbas, H.M.M., Liu, Z., Khan, M.N., Sultan, H., Shah, A., Rasul, F., Tahir, A., Nafees-Ur-Rehman, M., & Nie, L. (2026). Microbial-inoculated biochar combined with nitrogen mitigates salinity stress in rice by reducing salt accumulation and enhancing soil–plant interactions. *Frontiers in Plant Science, 17*, 1751156. https://doi.org/10.3389/fpls.2026.1751156

Abulfaraj, A.A., & Jalal, R.S. (2021). Use of plant growth-promoting bacteria to enhance salinity stress tolerance in soybean (*Glycine max* L.) plants. *Saudi Journal of Biological Sciences, 28*(7), 3823–3834. https://doi.org/10.1016/j.sjbs.2021.03.053

AbuQamar, S.F., El-Saadony, M.T., Saad, A.M., Desoky, E.M., Elrys, A.S., El-Mageed, T.A.A., Semida, W.M., Abdelkhalik, A., Mosa, W.F.A., Al Kafaas, S.S., Naser, S., Ibrahim, E.H., Alshamsi, F.M.K., Mathew, B.T., & El-Tarabily, K.A. (2024). Halotolerant plant growth-promoting rhizobacteria improve soil fertility and plant salinity tolerance for sustainable agriculture: A review. *Plant Stress, 12*, 100482. https://doi.org/10.1016/j.stress.2024.100482

Adekiya, A.O., Ogunbode, T.O., Esan, V.I., Adedokun, O., Olatubi, I.V., & Ayegboyin, M.H. (2025). Short-term effects of biochar on soil chemical properties, growth, yield, quality, and shelf life of tomato. *Scientific Reports, 15*(1), 24965. https://doi.org/10.1038/s41598-025-10411-5

Akhtar, S.S., Andersen, M.N., Naveed, M., Zahir, Z.A., & Liu, F. (2015). Interactive effect of biochar and plant growth-promoting bacterial endophytes on ameliorating salinity stress in maize. *Functional Plant Biology, 42*(8), 770–781. https://doi.org/10.1071/FP15054

Ali, A., Jabeen, N., Chachar, Z., Chachar, S., Ahmed, S., Ahmed, N., Laghari, A.A., Sahito, Z.A., Farruhbek, R., & Yang, Z. (2025). The role of biochar in enhancing soil health and interactions with rhizosphere properties and enzyme activities in organic fertilizer substitution. *Frontiers in Plant Science, 16*, 1595208. https://doi.org/10.3389/fpls.2025.1595208

Ali, B., Hafeez, A., Javed, M.A., Afridi, M.S., Abbasi, H.A., Qayyum, A., Batool, T., Ullah, A., Marc, R.A., Al Jaouni, S.K., Alkhalifah, D.H.M., & Selim, S. (2022). Role of endophytic bacteria in salinity stress amelioration through physiological and molecular defense mechanisms: A comprehensive review. *South African Journal of Botany, 151*, 33–46. https://doi.org/10.1016/j.sajb.2022.09.036

Ameen, M., Mahmood, A., Shahzad, A.N., Zia, M.A., & Javaid, M.M. (2024). Sorghum's potential unleashed: A comprehensive exploration of bio-energy production strategies and innovations. *Bioresource Technology Reports, 27*, 101906. https://doi.org/10.1016/j.biteb.2024.101906

Aninbon, C., Ruttanaprasert, R., Pinta, W., Rodnuch, N., Boonsom, M., Thabthimtho, T., & Phakamas, N. (2026). Impact of salinity stress during the reproductive stage on physiological responses and yield of landrace rice genotypes. *BMC Plant Biology, 26*, 550. https://doi.org/10.1186/s12870-026-08355-7

Arkian, Z., Bijanzadeh, E., & Boostani, H.R. (2025). Biochar mitigates salt stress by regulating phytochemical and physiological traits of triticale. *Discover Sustainability, 6*, 1308. https://doi.org/10.1007/s43621-025-02182-w

Assaha, D.V.M., Ueda, A., Saneoka, H., Al-Yahyai, R., & Yaish, M.W. (2017). The role of Na^+ and K^+ transporters in salt stress adaptation in glycophytes. *Frontiers in Physiology, 8*, 509. https://doi.org/10.3389/fphys.2017.00509

Bagnazari, M., Alymanesh, M.R., Ghanbari, F., & Azizi, A. (2025). Isolated endophytic bacteria promoted growth, essential oil content, and antioxidant activity in basil (*Ocimum basilicum* L.). *Scientific Reports, 15*, 36205. https://doi.org/10.1038/s41598-025-20218-z

Balasubramaniam, T., Shen, G., Esmaeili, N., & Zhang, H. (2023). Plants' response mechanisms to salinity stress. *Plants, 12*(12), 2253. https://doi.org/10.3390/plants12122253

Barus, W.A., Sulistiani, R., Utami, S., Tarigan, D.M., & Lestami, A. (2023). Black soybean response to antioxidant application for growth and yield under salinity stress conditions. *SABRAO Journal of Breeding and Genetics, 55*(2), 417–426. https://doi.org/10.54910/sabrao2023.55.2.14

Bawa, G., Yu, X., Liu, Z., Zhou, Y., & Sun, X. (2023). Surviving the enemies: Regulatory mechanisms of stomatal function in response to drought and salt stress. *Environmental and Experimental Botany, 209*, 105291. https://doi.org/10.1016/j.envexpbot.2023.105291

Bera, B. (2024). Biomass-derived biochar and its role in carbon sequestration and soil health improvement. *Plant Science Review, 5*(1), 1–7. https://doi.org/10.51470/PSR.2024.05.01.01

Bhat, M.A., Mishra, A.K., Jan, S., Bhat, M.A., Kamal, M.A., Rahman, S., Shah, A.A., & Jan, A.T. (2023). Plant growth-promoting rhizobacteria in plant

health: A perspective study of the underground interaction. *Plants, 12*(3), 629. https://doi.org/10.3390/plants12030629
Cao, S.-K., Ye, N., Teng, Z., Shaw, B. P., Liu, R., & Zhang, J. (2025). From genes to grains: Advancements in understanding seed development and grain filling. *Frontiers in Plant Science, 16*, 1701948. https://doi.org/10.3389/fpls.2025.1701948
Che, W., Li, X., Piao, J., Zhang, Y., Miao, S., Wang, H., Xie, L., & Jin, F. (2024). Biochar improves yield by reducing saline–alkaline stress and enhancing the grain-filling rate of rice in soda saline–alkaline paddy fields. *Plants, 13*(16), 2237. https://doi.org/10.3390/plants13162237
Chen, R., Zheng, L., Zhao, J., Ma, J., & Li, X. (2023). Biochar application maintains photosynthesis of cabbage by regulating stomatal parameters in salt-stressed soil. *Sustainability, 15*(5), 4206. https://doi.org/10.3390/su15054206
Choudhury, R.K., Palit, A., Ali, T., Pati, S., Ghosh, A., Mondal, S., & Bhattacharyya, K. (2026). Impact of salinity stress on field crops and cropping systems. In D. Moulick, S. Sarkar, & K. Brahmachari (Eds.), *Salinity Stress and Remediation in Agro-Ecosystems* (Chap. 7). Springer. https://doi.org/10.1007/978-3-032-24001-9_7
Dehnavi, A.R., Zahedi, M., & Piernik, A. (2024). Understanding salinity stress responses in sorghum: Exploring genotype variability and salt tolerance mechanisms. *Frontiers in Plant Science, 14*, 1296286. https://doi.org/10.3389/fpls.2023.1296286
Dey, S., Purakayastha, T.J., Sarkar, B., Rinklebe, J., Kumar, S., Chakraborty, R., Datta, A., Lal, K., & Shivay, Y.S. (2023). Enhancing cation and anion exchange capacity of rice straw biochar by chemical modification for increased plant nutrient retention. *Science of the Total Environment, 886*, 163681. https://doi.org/10.1016/j.scitotenv.2023.163681
Eid, A.M., Fouda, A., Abdel-Rahman, M.A., Salem, S.S., Elsaied, A., Oelmüller, R., Hijri, M., Bhowmik, A., Elkelish, A., & Hassan, S.E. (2021). Harnessing bacterial endophytes for promotion of plant growth and biotechnological applications: An overview. *Plants, 10*(5), 935. https://doi.org/10.3390/plants10050935
El-Tarabily, K.A., Sham, A., Elbadawi, A.A., Hassan, A.H., Alhosani, B.K.K., El-Esawi, M.A., AlKhajeh, A.S., & AbuQamar, S.F. (2021). A consortium of rhizosphere-competent actinobacteria exhibiting multiple plant growth-promoting traits improves the growth of *Avicennia marina* in the United Arab Emirates. *Frontiers in Marine Science, 8*, 715123. https://doi.org/10.3389/fmars.2021.715123
Fouad, N., Amr, D., El-Zayat, E., Abd-Elhalim, H.M., Radwan, K.H., Hamwieh, A., Tadesse, W., Tawkaz, S., Al-Shamaa, K., Youssef, A., Ahmed, H., & Baum, M. (2026). Harnessing the plant-associated microbiome: Rhizosphere and endophyte plant growth-promoting bacteria (PGPB) to mitigate salt stress in Egyptian wheat. *Biologia, 81*, 66. https://doi.org/10.1007/s11756-025-02077-1
Gao, Z.-W., Ding, J., Ali, B., Nawaz, M., Hassan, M.U., Ali, A., Rasheed, A., Khan, M.N., Ozdemir, F.A., Iqbal, R., Çiğ, A., Ercisli, S., & El Sabagh, A. (2024). Putting biochar in action: A black gold for efficient mitigation of salinity stress in plants—Review and future directions. *ACS Omega, 9*(29), 31237–31253. https://doi.org/10.1021/acsomega.3c07921
Gul, F., Khan, I.U., Rutherford, S., Dai, Z.-C., Li, G., & Du, D.-L. (2023). Plant growth-promoting rhizobacteria and biochar produced from *Parthenium hysterophorus* enhance seed germination and productivity in barley under drought stress. *Frontiers in Plant Science, 14*, 1175097. https://doi.org/10.3389/fpls.2023.1175097
Hafez, E.M., Alsohim, A.S., Farig, M., Omara, A. E.-D., Rashwan, E., & Kamara, M.M. (2019). Synergistic effect of biochar and plant growth-promoting rhizobacteria on alleviation of water deficit in rice plants under salt-affected soil. *Agronomy, 9*(12), 847. https://doi.org/10.3390/agronomy9120847
Hassan, N.E. (2024). Salinity stress in plants: Growth, photosynthesis, and adaptation review. *GSC Advanced Research and Reviews, 20*(2), 231–243. https://doi.org/10.30574/gscarr.2024.20.2.0304
Hasanuzzaman, M., Zhou, M., & Shabala, S. (2023). How do stomatal density and residual transpiration contribute to osmotic stress tolerance? *Plants, 12*(3), 494. https://doi.org/10.3390/plants12030494
Haworth, M., Marino, G., Loreto, F., & Centritto, M. (2021). Integrating stomatal physiology and morphology: Evolution of stomatal control and development of future crops. *Oecologia, 197*(4), 867–883. https://doi.org/10.1007/s00442-021-04857-3
Hien, T.T.T., Tsubota, T., Taniguchi, T., & Shinogi, Y. (2021). Enhancing soil water-holding capacity and providing a potassium source through optimization of bamboo biochar pyrolysis. *Biochar, 3*, 51–61. https://doi.org/10.1007/s42773-020-00071-1
Jabborova, D., Jabbarov, Z., Abdrakhmanov, T., Fayzullaev, O., Saharan, B.S., Perveen, K., Zaka, S.M., Mastinu, A., & Sayyed, R. (2025). Assessing the synergistic effects of biochar, hydrogel, and biofertilizer on growth and physiological traits of wheat in saline environments. *Functional Plant Biology, 52*, FP24277. https://doi.org/10.1071/FP24277
Linh, T.C., & Duc, C.K.T. (2025). Analytical methods for antioxidant screening of endophytic bacteria: A comparative review. *Journal of Microbiological Methods, 237*, 107224. https://doi.org/10.1016/j.mimet.2025.107224
Liu, B., Zhang, S., Zhang, J., Yang, X., Zhang, W., & Guo, X. (2025a). Biochar and *Chlorella* synergistically enhance grain yield in saline soil. *Agronomy, 15*(11), 2508. https://doi.org/10.3390/agronomy15112508
Liu, H., Dou, W., Zhang, W., Li, Y., Shi, G., Fu, L., & Wang, L. (2025b). Analysis of the synergistic effect of endophytic bacteria and metabolites on soybean yield under biochar application with nitrogen fertilization. *Agronomy, 15*(1), 177. https://doi.org/10.3390/agronomy15010177
Ma, L., Li, J., Li, J., Huo, Y., Yang, Y., Jiang, C., & Guo, Y. (2026). Plant salt-tolerance mechanisms: Classic signaling pathways, emerging frontiers, and future perspectives. *Molecular Plant, 19*(3), 538–570. https://doi.org/10.1016/j.molp.2025.12.009
Malik, L., Sanaullah, M., Mahmood, F., Hussain, S., & Shahzad, T. (2024). Co-application of biochar and salt-tolerant PGPR to improve soil quality and wheat production in naturally saline soil. *Rhizosphere, 29*, 100849. https://doi.org/10.1016/j.rhisph.2024.100849
Mansour, M.M.F. (2022). Role of vacuolar membrane transport systems in plant salinity tolerance. *Journal of Plant Growth Regulation, 42*(3), 1364–1401. https://doi.org/10.1007/s00344-022-10655-9
Mohamed, I.A.A., Shalby, N., Bai, C., Qin, M., Agami, R.A., Jie, K., Wang, B., & Zhou, G. (2020). Stomatal and photosynthetic traits associated with sodium chloride tolerance in *Brassica napus* L. cultivars. *Plants, 9*(1), 62. https://doi.org/10.3390/plants9010062
Murtaza, G., Rizwan, M., Usman, M., Hyder, S., Akram, M.I., Deeb, M., Alkahtani, J., AlMunqedhi, B.M., Hendy, A.S., Ali, M.R., Iqbal, R., Harsonowati, W., Habib Ur Rahman, M., & Rizwan, M. (2024). Biochar enhances the growth and physiological characteristics of *Medicago sativa*, *Amaranthus caudatus*, and *Zea mays* in saline soils. *BMC Plant Biology, 24*(1), 304. https://doi.org/10.1186/s12870-024-04957-1
Nehela, Y., Mazrou, Y.S.A., Alshaal, T., Rady, A.M.S., El-Sherif, A.M.A., Omara, A.E.-D., Abd El-Monem, A.M., & Hafez, E.M. (2021). Integrated amendment of sodic-saline soils using biochar and plant growth-promoting rhizobacteria enhances maize (*Zea mays* L.) resilience to water salinity. *Plants, 10*(9), 1960. https://doi.org/10.3390/plants10091960
Nounjan, N., Mahakham, W., Siangliw, J.L., Toojinda, T., & Theerakulpisut, P. (2020). Chlorophyll retention and high photosynthetic performance contribute to salinity tolerance in rice carrying drought tolerance quantitative trait loci (QTLs). *Agriculture, 10*(12), 620. https://doi.org/10.3390/agriculture10120620
Oktem, A., & Oktem, A.G. (2024). Bioethanol obtained from soluble sugars in sweet sorghum: Influencing properties and their interrelationships. *Industrial Crops and Products, 221*, 119351. https://doi.org/10.1016/j.indcrop.2024.119351
Pandit, K., Chandni, Kaur, S., Kumar, M., Bhardwaj, R., & Kaur, S. (2024). Salinity stress: Impact on plant growth. *Advances in Food Security and Sustainability, 9*, 145–160. https://doi.org/10.1016/bs.af2s.2024.07.002
Rajalakshmi, G., Gnanalakshmi, K.S., Baskaran, D., & Reiyaz, M.A. (2021). Proximate composition of sorghum grain and pearl millet. *International Journal of Agricultural Science and Research, 11*(1), 97–102.
Rauf, A., Barus, W.A., Munar, A., & Lestami, A. (2024). Physiological characteristics of red rice with the application of ascorbic acid under salinity stress conditions. *SABRAO Journal of Breeding and Genetics, 56*(4), 1437–1445. https://doi.org/10.54910/sabrao2024.56.4.10
Samaraweera, H., Palansooriya, K.N., Dissanayaka, P.D., Khan, A.H., Sillanpää, M., & mLsna, T. (2023). Sustainable phosphate removal using Mg/Ca-modified biochar hybrids: Current trends and future outlooks. *Case Studies in Chemical and Environmental Engineering, 8*, 100528. https://doi.org/10.1016/j.cscee.2023.100528
Schaffert, E., Lukac, M., Percival, G., & Rose, G. (2022). The influence of biochar soil amendment on tree growth and soil quality: A review for the arboricultural industry. *Arboriculture & Urban Forestry, 48*(3), 176–202. https://doi.org/10.48044/jauf.2022.014
Sen, A., Saji, J., Faseela, P., Zhang, C., Mohanan, S., & Xia, Y. (2026). Exploring the functional roles of endophytic bacteria in plant stress tolerance for sustainable agriculture: Diversity, mechanisms, applications, and challenges. *Plants, 15*(2), 206. https://doi.org/10.3390/plants15020206
Sharaya, R., Gill, R., Kalwan, G., Naeem, M., Tuteja, N., & Gill, S.S. (2023). Plant–microbe interaction-mediated salinity stress tolerance for sustainable crop production. *South African Journal of Botany, 161*, 454–471. https://doi.org/10.1016/j.sajb.2023.08.043
Shrivastava, P., & Kumar, R. (2015). Soil salinity: A serious environmental issue and plant growth-promoting bacteria as one of the tools for its

alleviation. *Saudi Journal of Biological Sciences, 22*(2), 123–131. https://doi.org/10.1016/j.sjbs.2014.12.001

Shyam, S., Ahmed, S., Joshi, S.J., & Sarma, H. (2025). Biochar as a soil amendment: Implications for soil health, carbon sequestration, and climate resilience. *Discover Soil, 2*, 18. https://doi.org/10.1007/s44378-025-00041-8

Srivastava, S. (2022). Morpho-anatomical adaptation against salinity. In *Plant Defense Mechanisms*. IntechOpen. https://doi.org/10.5772/intechopen.101681

Su, F., Zhao, B., Dhondt-Cordelier, S., & Vaillant-Gaveau, N. (2024). Plant growth-promoting rhizobacteria modulate carbohydrate metabolism in connection with host plant defense mechanisms. *International Journal of Molecular Sciences, 25*(3), 1465. https://doi.org/10.3390/ijms25031465

Sultan, H., Li, Y., Ahmed, W., Yixue, M., Shah, A., Faizan, M., Ahmad, A., Abbas, H.M.M., Nie, L., & Khan, M.N. (2024). Biochar and nano-biochar: Enhancing salt resilience in plants and soil while mitigating greenhouse gas emissions—A comprehensive review. *Journal of Environmental Management, 355*, 120448. https://doi.org/10.1016/j.jenvman.2024.120448

Talakayala, A., Jupally, Y., Asinti, S., Mekala, G.P., Kirti, P.B., & Sharma, I. (2025). Differences in the regulation of ion imbalance in response to high Na^+ load hint at differential strategies for salt tolerance in mungbean genotypes (*Vigna radiata* L.). *Plant Growth Regulation, 105*, 89–109. https://doi.org/10.1007/s10725-024-01257-4

Tamiaki, H., & Kichishima, S. (2025). Chlorophyll pigments and their synthetic analogs. *Plant and Cell Physiology, 66*(2), 153–167. https://doi.org/10.1093/pcp/pcae094

Tang, K.H.D. (2025). Biochar amendments for soil restoration: Impacts on nutrient dynamics and microbial activity. *Environments, 12*(11), 425. https://doi.org/10.3390/environments12110425

Tarigan, D.M., Barus, W.A., Munar, A., & Lestami, A. (2023). Exploration and morphological characterization of phosphate-solubilizing and nitrogen-fixing bacteria in saline soil. *SABRAO Journal of Breeding and Genetics, 55*(2), 550–563. https://doi.org/10.54910/sabrao2023.55.2.27

Tarigan, D.M., Lestami, A., Nawar, M.K., Barus, W.A., & Munar, A. (2025). Screening sorghum varieties for salinity tolerance based on morpho-physiological and biochemical traits. *Biodiversitas, 26*(10), 5226–5237. https://doi.org/10.13057/biodiv/d261036

Vacheron, J., Desbrosses, G., Bouffaud, M.L., Touraine, B., Moënne-Loccoz, Y., Muller, D., Legendre, L., Wisniewski-Dyé, F., & Prigent-Combaret, C. (2013). Plant growth-promoting rhizobacteria and root system functioning. *Frontiers in Plant Science, 4*, 356. https://doi.org/10.3389/fpls.2013.00356

Wang, R., Cui, Q., Wang, Z., Yang, H., Bai, Y., & Meng, L. (2026). Biochar integrated with beneficial microorganisms boosts soil organic fractions by increasing carbon-related enzymes and microbial activities in coastal saline–alkali land. *Microorganisms, 14*(1), 115. https://doi.org/10.3390/microorganisms14010115

Wang, X., Chen, Z., & Sui, N. (2024). Sensitivity and responses of chloroplasts to salt stress in plants. *Frontiers in Plant Science, 15*, 1374086. https://doi.org/10.3389/fpls.2024.1374086

Witzel, K., Motos, J.R.A., Atay, E., Çiçek, N., Mistríková, V., Oney-Birol, S., Soto, S.R., Solymosi, K., Yücedağ, C., & Papenbrock, J. (2026). Leveraging microorganisms and biostimulants: Mitigating salinity stress in crops with agricultural biologicals. *Plant and Soil, 523*, 851–873. https://doi.org/10.1007/s11104-025-07578-1

Xiu, L., Sun, Y., & Han, X. (2025). Effects of biochar amendment on potassium supply capacity and potassium accumulation in soybean across diverse soils. *Plants, 14*(13), 1959. https://doi.org/10.3390/plants14131959

Yavas, I., Jamal, M.A., Ul Din, K., Ali, S., Hussain, S., & Farooq, M. (2024). Drought-induced changes in leaf morphology and anatomy: Overview, implications, and perspectives. *Polish Journal of Environmental Studies, 33*(2), 1517–1530. https://doi.org/10.15244/pjoes/174476

Yusuf, M., Roestawita, D., & Tarigan, D.M. (2025). Identification of sorghum distribution and soil nutrient status in local sorghum genotype growing locations in North Sumatera. *International Journal of Science and Environment, 5*(2), 58–62. https://doi.org/10.51601/ijse.v5i2.151

Zaque, S. (2025). Understanding the impact of salt stress on plants and adaptive survival approaches. *Journal of Plant Biochemistry & Physiology, 13*(336), 1–2. https://doi.org/10.35248/2329-9029.25.13.336

Zhang, Y.Y., Liu, J.L., Wang, X., Cao, X., Liu, K.H., Luo, Y. T., Chen, J.Y., Zhang, J., & Fan, Y.H. (2025). A review of the regulatory role of plant growth-promoting rhizobacteria in alfalfa under stress conditions. *Plants, 14*(21), 3248. https://doi.org/10.3390/plants14213248

Zhao, X., Sun, P., Wang, L., Liu, J., Lu, P., Liu, L., & Chen, Y. (2025). Regulatory pathways of biochar and endophytic bacteria in Na^+ translocation and salt stress alleviation in the soil–plant system. *Plant Growth Regulation, 105*, 375–387. https://doi.org/10.1007/s10725-025-01276-9

Zhou, H., Shi, H., Yang, Y., Feng, X., Chen, X., Xiao, F., Lin, H., & Guo, Y. (2024). Insights into plant salt stress signaling and tolerance. *Journal of Genetics and Genomics, 51*(1), 16–34. https://doi.org/10.1016/j.jgg.2023.08.007