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EFFECTS OF POTASSIUM-ENRICHED BIOCHAR ON SOYBEAN SEEDLING GROWTH AND ANTIOXIDANT SYSTEM UNDER SALT STRESS

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Abstract

Potassium-enriched biochar (KBC) serves as a novel soil amendment that improves soil fertility and enhances crop stress resistance. This study preliminarily investigated the regulatory effects of different KBC concentrations on soybean (*Glycine max*) seedling growth and the antioxidant system under salt stress through pot experiments. Under normal cultivation conditions, 1% KBC application significantly promoted seedling growth, increasing plant height and root length by 42.19% and 35.89%, respectively. Metabolic optimization was evidenced by reduced soluble sugar (35.33%) and proline (37.46%) levels at 1% and 2% KBC, respectively, indicating improved carbon-nitrogen metabolism. However, under Salt Stress (200 mmol/L NaCl), total phenolic and flavonoid contents were significantly decreased. The 2% KBC treatment comprehensively enhanced key antioxidant enzyme activities: Superoxide dismutase (SOD) showed the most pronounced increase (27.45%), while catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) showed coordinated upregulation. This synergistic enhancement effectively mitigated reactive oxygen species (ROS) accumulation. In conclusion, optimal KBC application (1–2%) simultaneously promotes soybean seedling growth, photosynthetic efficiency, and salt-stress tolerance through reinforced antioxidant capacity. These findings provide a theoretical foundation for saline soil remediation and stress-resistant soybean cultivation.

Key words: Potassium-enriched biochar, Soybean, Growth regulation, Antioxidant System.

Introduction

Soybean (*Glycine max*), as the world's most crucial oilseed and protein crop, plays a pivotal role in global food security and the feed industry (Islam *et al*., 2016). However, modern agricultural practices' overreliance on chemical fertilizers has led to soil structure degradation and ecological imbalance, resulting in compromised soil fertility and severe environmental issues that threaten ecological stability and agricultural sustainability (Mann *et al*., 2009). Compounding this challenge, soil salinization significantly constrains soybean production-approximately 20% of irrigated farmland worldwide is affected by salt stress, causing soybean yield reductions of 30–50% (Xu *et al*., 2020). Salt stress disrupts the plant antioxidant defense system, triggering reactive oxygen species (ROS) bursts that induce membrane lipid peroxidation and metabolic dysfunction, ultimately inhibiting growth and development (Zhao *et al*., 2025). Consequently, developing green and efficient saline soil remediation technologies is strategically vital for ensuring sustainable soybean production.

Potassium (K^+) is frequently a limiting factor in crop production, particularly in arid and saline-alkali regions where plants exhibit high demand for this essential mineral nutrient (Zhang *et al*., 2020). As one of the three primary macronutrients indispensable for plant growth, potassium fulfills multiple physiological functions: Enhancing photosynthetic efficiency and facilitating assimilate translocation; Serving as a cofactor for >60 enzyme systems, including pyridine nucleotide kinase and other antioxidant enzymes(Hafsi *et al*., 2014; Rogiers *et al*., 2017;Kameyama *et al*., 2025); Regulating cellular redox homeostasis by boosting antioxidant enzyme activities (e.g., superoxide dismutase (SOD) and ascorbate peroxidase (APX)), thereby scavenging $O_2^{\cdot -}$ and H_2O_2 to mitigate oxidative damage (Rinklebe, *et al*., 2016).

Biochar, an environmentally benign soil amendment, has garnered extensive attention for its capacity to improve soil physicochemical properties and enhance crop stress resilience (Liang *et al*., 2014; Upadhyay *et al*., 2025; El-Ghamry *et al*., 2024). Produced through pyrolysis of biomass under oxygen-limited conditions, its mineral-rich composition and porous structure enable effective soil remediation and contaminant adsorption (Sohi *et al*., 2010). Potassium-enriched biochar (KBC) synergizes conventional biochar advantages with potassium supplementation, simultaneously replenishing soil nutrients and strengthening plant osmoregulation (Huang *et al*., 2024a). Research confirms its dual role in activating key enzymatic systems and reinforcing antioxidant defenses-specifically by elevating SOD and APX activities to alleviate oxidative injury (Huang *et al*., 2024b).

This study fabricates potassium-enriched biochar to integrate the benefits of K^+ ions and biochar matrix. We systematically investigate KBC's effects on soybean growth under normal conditions and its regulatory mechanisms on the antioxidant system under salt stress. By elucidating the relationship between KBC and soybean seedling physiology, this work aims to: (1) Optimize science-based KBC application protocols; (2) Provide a theoretical and practical framework for efficient soybean cultivation in saline soils; (3) Uncover novel mechanisms underlying KBC-mediated salt stress mitigation in legumes.

Material and Methods

Preparation of biochar: Biochar was prepared using a muffle furnace. Pine wood was used as the primary feedstock and pyrolyzed at a temperature of 440°C. After cooling, the biochar was ground and passed through a 2 mm sieve. Finally, the biochar was stored in powder form for future use.

Preparation of potassium-enriched biochar (KBC): To prepare potassium-enriched biochar (KBC), 75 g of the aforementioned biochar was weighed. 25 g of potassium (supplied as K_2SO_4) and 25 ml of water were added, and the mixture was stirred evenly using a spatula. The mixture was then incubated in a constant temperature incubator at 25°C for 24 h. After 24 h, the sample was mixed again with the spatula, adding 5 ml of water if necessary. This mixing and incubation process was repeated for 7 days. On the 8th day, the sample was sun-dried, ground, and passed through a 2 mm sieve again. The resulting powder was KBC, ready for use in subsequent experiments.

Experimental Design

Conventional growth conditions: A pot experiment was employed. Four treatment groups were established: CK (control group, no KBC added), T1 (0.50% KBC), T2 (1.00% KBC), T3 (2.00% KBC). Each treatment was replicated 3 times.

Salt stress conditions: Four treatment groups were established: CK: No salt stress, no KBC treatment (irrigated with distilled water only). G1: Salt stress (irrigated with 200 mmol/L NaCl solution) + 0% KBC; G2: Salt stress (irrigated with 200 mmol/L NaCl solution) + 1.0% KBC; G3: Salt stress (irrigated with 200 mmol/L NaCl solution) + 2.0% KBC. Salt stress treatment lasted for 14 days. Each treatment was replicated 3 times.

General cultivation method: Soybean seeds were selected and soaked in distilled water for 8 h to ensure full water absorption. The seeds were then evenly spread on trays lined with moist filter paper, covered with another layer of moist filter paper, and placed in a 25°C constant temperature incubator for germination. When the root length reached approximately 2 cm, uniformly germinated seedlings were selected for sowing. Plastic pots with a diameter of 20 cm and a height of 25 cm were used. Each pot was filled with 2 kg of dry soil. KBC was added according to the requirements of each treatment group and thoroughly mixed with the soil. Five soybean seedlings were transplanted per pot. The salt stress groups were irrigated daily with 200 mmol/L NaCl solution starting from the onset of stress, while the control group was irrigated with distilled water.

Determination of indicators: After 14 days of treatment, entire seedlings were carefully removed, and soil was washed from the roots. Plant height and root length were measured using a ruler, and data were recorded.

Chlorophyll content (Total Chlorophyll, Chlorophyll a, Chlorophyll b): Determined using the ethanol extraction method. Absorbance was measured with a spectrophotometer, and contents were calculated using formulas and recorded (Huang *et al*., 2024).

Soluble sugar content: Determined using the anthrone colorimetric method. Absorbance was measured with a spectrophotometer, and content was calculated and recorded (Xiao *et al*., 2005).

Proline content: Determined using the acidic ninhydrin colorimetric method. Absorbance was measured, and content was calculated and recorded (Xiao *et al*., 2005).

Total phenolic content: Determined using the Folin-Ciocalteu method. An appropriate amount of soybean leaves was weighed, ethanol was added, and the mixture was sonicated, filtered, and made up to volume. Folin-Ciocalteu reagent was added to the extract, mixed well, and allowed to react at room temperature for a period. Sodium carbonate solution was then added for color development. Absorbance was measured at 765 nm, and total phenolic content was calculated using a standard curve.

Total flavonoid content: Determined using the aluminum chloride colorimetric method (referencing the sample preparation for total phenolics). Aluminum chloride solution and an alkaline solution were added to the extract for color development. Absorbance was measured at 510 nm, and total flavonoid content was calculated using a standard curve.

SOD (Superoxide Dismutase) activity: Determined using the nitroblue tetrazolium (NBT) photoreduction method. An appropriate amount of soybean leaves was weighed, extraction buffer was added, and the mixture was ground and centrifuged to obtain the supernatant. SOD enzyme extract, NBT, riboflavin, and phosphate buffer were added to the reaction system. After reaction under specific light intensity for a period, absorbance was measured at 560 nm. SOD activity was reflected by calculating the inhibition rate (Huang *et al*., 2024).

POD (Peroxidase), CAT (Catalase), APX (Ascorbate Peroxidase) activity: POD: Determined using the guaiacol method. POD enzyme extract, guaiacol, and hydrogen peroxide were added to the reaction system. The change in absorbance rate was measured at 470 nm after reaction. CAT: Determined using the hydrogen peroxide method. CAT enzyme extract and hydrogen peroxide were added to the reaction system. The change in absorbance rate was measured at 240 nm after reaction. APX: Determined using the ascorbate method. APX enzyme extract, ascorbic acid, and hydrogen peroxide were added to the reaction system. The change in absorbance rate was measured at 290 nm after reaction (Huang *et al*., 2024).

Data analysis: Data analysis was performed using SPSS software. One-way analysis of variance (ANOVA) was used to test the significance of differences between different treatment groups.

Results

Effect of KBC on growth indicators of soybean seedlings: As shown (Fig. 1) compared to the control group (CK), application of KBC significantly promoted soybean seedling height growth ($p<0.05$). Plant height showed an increasing trend with increasing KBC application rate, reaching its maximum at the 1.00% KBC (T2) treatment, after which the increase rate slowed (T3). The plant heights of T1, T2, and T3 groups increased by 31.20%, 42.19%, and 16.54% respectively compared to CK. KBC provided soybean

seedlings with abundant potassium and other nutrients, promoting cell elongation and division, thereby increasing plant height. Concurrently, biochar improved soil structure and aeration, facilitating root growth and nutrient absorption, indirectly promoting above-ground growth. Compared to CK, the root length of soybean seedlings in KBC-treated groups increased significantly (p<0.05). The promoting effect of different KBC application rates on root length varied, with the 1.00% KBC (T2) treatment showing the best effect. The root lengths of T1, T2, and T3 groups increased by 24.67%, 35.89%, and 6.20% respectively compared to CK. KBC can regulate soil structure, creating a favorable environment for root growth. Potassium is crucial for root growth and development; potassium ions provided by KBC stimulated the growth and differentiation of root cells, enabling roots to extend deeper into the soil to absorb more water and nutrients.

Effect of KBC on physiological indicators of soybean seedlings: As shown (Fig. 2) compared to CK, the addition of appropriate amounts of KBC (T1: 0.50%, T2: 1.00%) significantly increased chlorophyll a, chlorophyll b, and total chlorophyll content in soybean seedlings (p<0.05). Under T1 and T2 treatments, chlorophyll a content increased by an average of 0.41 mg/g and 0.23 mg/g, respectively, compared to CK; chlorophyll b content increased by an average of 0.10 mg/g and 0.15 mg/g, respectively; total chlorophyll content increased by an average of 0.50 mg/g and 0.37 mg/g, respectively. KBC significantly increased total chlorophyll content by promoting chlorophyll synthesis. However, upon adding 2.00% KBC (T3), chlorophyll content showed a decreasing trend compared to CK, with total chlorophyll content decreasing by an average of 0.11 mg/g. This indicates that excessive KBC application inhibited chlorophyll synthesis in soybean seedlings to some extent, potentially affecting photosynthetic efficiency.

As shown (Fig. 3) the soluble sugar content in the control group (CK) was significantly higher than that in the T1, T2, and T3 treatment groups (p<0.05). Soluble sugar content showed a trend of first decreasing (T1, T2) and then increasing (T3) with increasing KBC concentration. The lowest soluble sugar content was observed at the 1.00% KBC (T2) treatment, which was 35.33% lower than CK. This suggests that KBC, by promoting photosynthesis and carbohydrate metabolism, likely accelerated the utilization of soluble sugars, leading to a reduction in their content. The proline content in KBC-treated groups (T1, T2, T3) was significantly lower than that in the control group (CK) (p<0.05). The decrease in proline content was most significant at the 2.00% KBC (T3) treatment, with a reduction of 37.46%. KBC improved the soil environment (e.g., moisture status), alleviating potential osmotic stress faced by the plants, thereby reducing proline synthesis and accumulation. This also reflects that KBC helps enhance the growth homeostasis of soybean seedlings under conventional conditions.

Effect of KBC on total phenolic and flavonoid content in soybean under salt stress: As shown (Fig. 4) under salt stress (G1 group), the total phenolic and flavonoid content in soybean leaves significantly decreased compared to the no-salt, no-KBC control group (CK), indicating that salt stress inhibited the synthesis of total phenolics and flavonoids. Compared to salt stress alone (G1), the application of KBC (G2: 1.0% KBC, G3: 2.0% KBC) resulted in varying degrees of increase in total phenolic and flavonoid content in soybean. For total phenolic content, the G3 (2.0% KBC) treatment showed the most significant increase; whereas for total flavonoid content, the G2 (1.0% KBC) treatment was the most effective (G3 < G2). This indicates that the effect of KBC on secondary metabolites (total phenolics, flavonoids) in soybean under salt stress varies significantly depending on the treatment concentration.

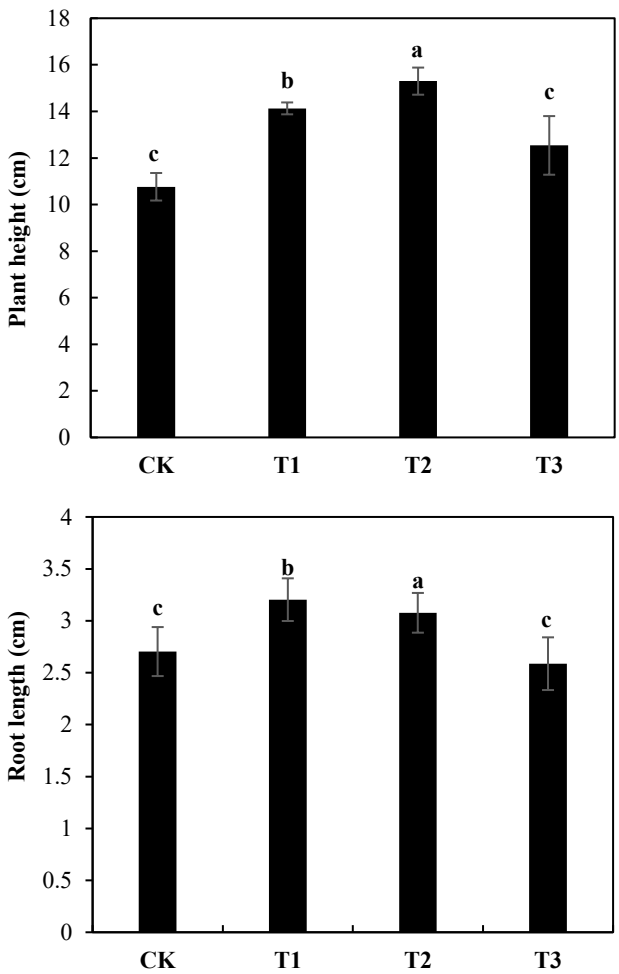

Fig. 1. Effect of KBC on the plant height and root length of Soybean Seedlings.

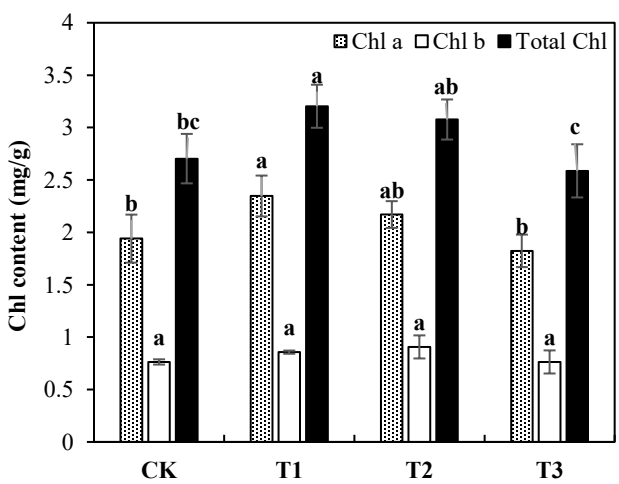

Fig. 2. Effect of KBC on chlorophyll contents of soybean seedlings.

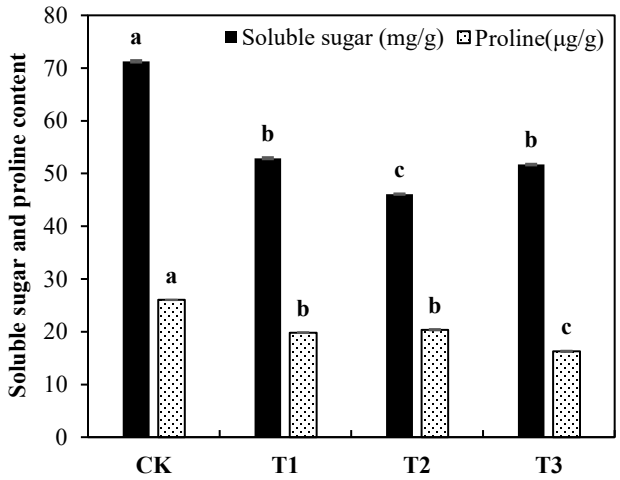

Fig. 3. Effect of KBC on soluble sugar proline contents of soybean seedlings.

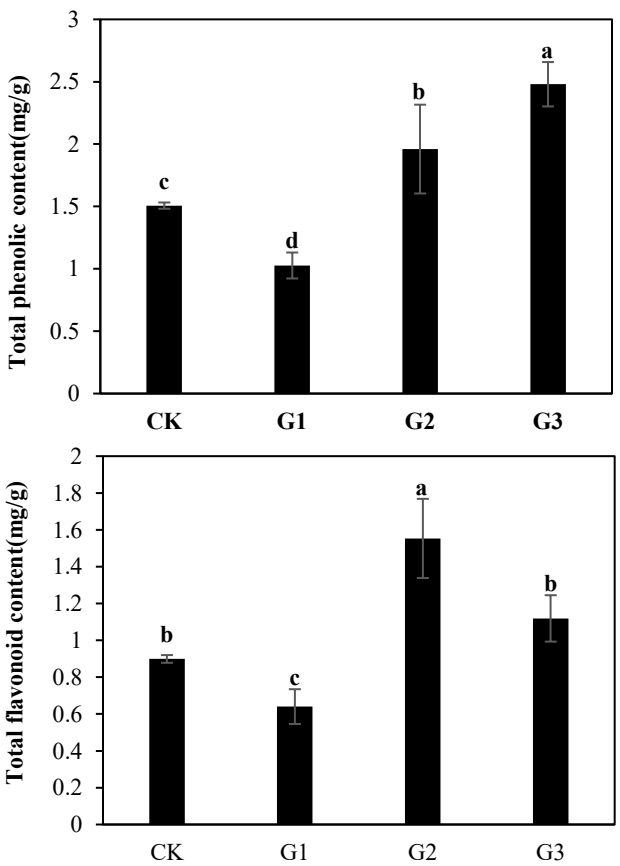

Fig. 4. Effect of KBC on total phenolic and flavonoid content in soybean under salt stress.

Effect of KBC on antioxidant enzyme activities in soybean under salt stress: Antioxidant enzymes (POD, SOD, CAT, APX) in soybean are crucial components of the plant reactive oxygen species (ROS) scavenging system. As shown (Fig. 5) under salt stress conditions (G1 group), compared to CK, the activities of POD, CAT, and APX in soybean increased significantly (p<0.05), while SOD activity did not change significantly. Specifically, the activities of POD, SOD, CAT, and APX in the G1 group (200 mmol/L NaCl) increased by 19.53%, 13.30%, 25.52%, and 24.45%, respectively, compared to CK. Adding 1.0% KBC (G2 group) further increased POD, CAT, and APX activities, but SOD activity decreased. Adding 2.0% KBC (G3 group) resulted in decreased POD, CAT, and APX activities compared to G2, but SOD activity increased significantly (by 27.45% compared to G1).

In summary, POD, CAT, and APX activities were most significantly enhanced at the 1.0% KBC application (G2 group), while SOD activity was most effectively boosted at the 2.0% KBC application (G3 group). This demonstrates that appropriate concentrations of KBC can effectively enhance the antioxidant enzyme activities in soybean under salt stress.

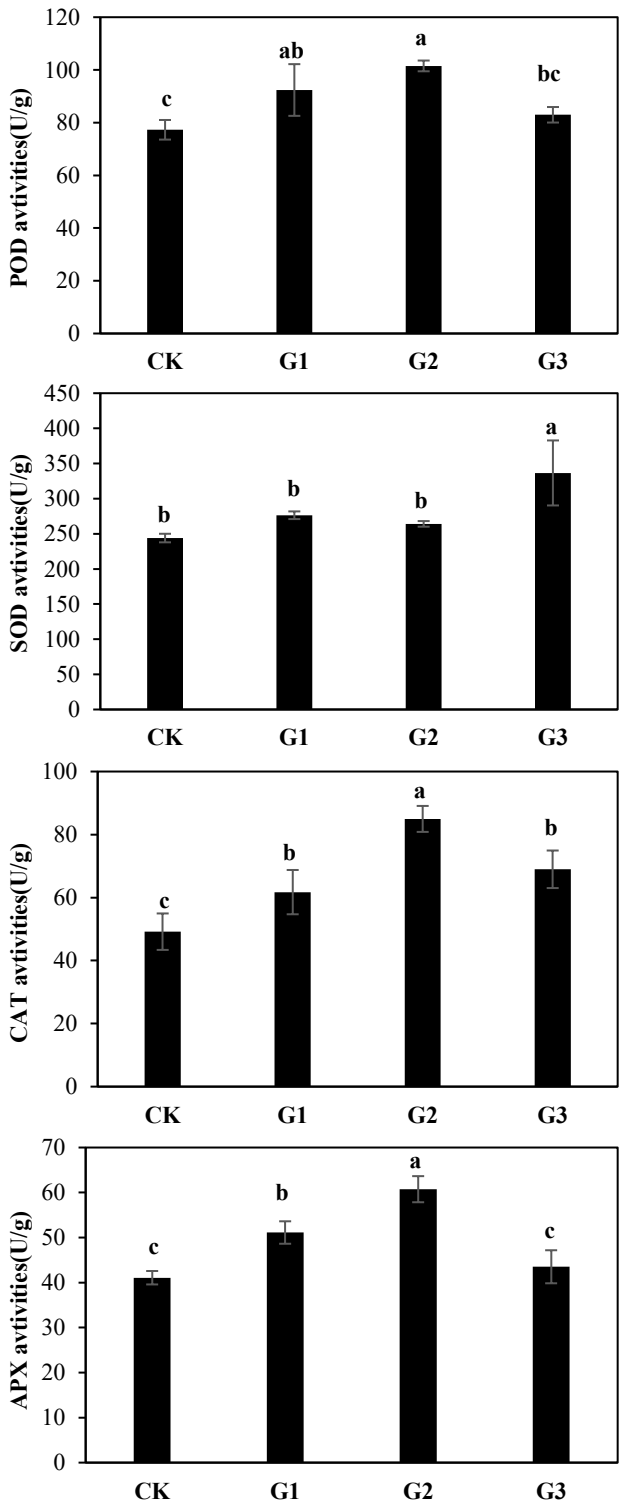

Fig. 5. Effect of KBC on antioxidant enzyme activities in soybean under salt stress.

Discussion

This study demonstrates that potassium-enriched biochar (KBC) has multifaceted significant effects on the growth and development of soybean seedlings. In terms of growth, an appropriate amount of KBC (especially 1.00%) significantly promoted increases in plant height and root length. Biochar effectively improves soil physicochemical properties (e.g., structure, aeration) and influences microbial communities (Ennis *et al*., 2012; Shuaikun *et al*., 2024; Chen *et al*., 2025), providing a more favorable environment for seedling growth. The potassium element abundant in KBC enhances potassium availability in the soil after application (Sofyan *et al*., 2025), promoting potassium uptake by the crop, thereby fostering root growth and development and above-ground growth. Regarding physiological metabolism, appropriate KBC (0.50%-1.00%) significantly increased chlorophyll content, indicating its promotion of chlorophyll synthesis and enhancement of photosynthetic capacity in soybean seedlings, providing more energy and material basis for plant growth. Biochar amends the soil and slowly releases nutrients like potassium, enabling sustained promotion of plant growth (Schulz *et al*., 2012; Verma *et al*., 2020). The decrease in soluble sugar content (especially at 1.00% KBC) may reflect KBC's promotion of photosynthetic product metabolism and utilization. Under conventional conditions, KBC treatment reduced proline content, indicating it improved the soil environment (especially moisture conditions), alleviated osmotic stress, and eliminated the need for plants to synthesize large amounts of proline for regulation. This also reflects that KBC enhances the stress resistance potential of soybean seedlings under conventional conditions.

Under salt stress, KBC application demonstrated a mitigation effect. Salt stress reduced total phenolic and flavonoid content in soybean, while KBC treatment (especially 1.0%-2.0%) effectively increased their content, enhancing the plant's antioxidant capacity. More importantly, KBC significantly activated the soybean antioxidant enzyme system (POD, CAT, APX, SOD). An appropriate concentration of KBC (1.0%) most significantly enhanced POD, CAT, and APX activities, while a higher concentration of KBC (2.0%) was more effective in boosting SOD activity. This activation helps scavenge excess reactive oxygen species (ROS) produced under salt stress, protecting cell membrane structure and function, thereby improving the salt tolerance of soybean seedlings.

Salt stress causes severe damage to plant growth, primarily including osmotic stress leading to cell dehydration and ion toxicity caused by high Na^+ concentration (Verslues *et al*., 2006; Chaves *et al*., 2009). These stresses inhibit plant growth, cause metabolic disorders (e.g., reduced dry matter accumulation) (Aşkım *et al*., 2010), disrupt oxidative metabolic balance (producing ROS), limit photosynthesis, and hinder material transport (Suriyan *et al*., 2009). Soybean seedlings are sensitive to salt stress (Chen, 1997). Although chemical fertilizers are the primary means to increase yield, their marginal contribution is declining, and excessive application leads to problems such as soil compaction, groundwater nitrate pollution, and significant greenhouse gas emissions (Herawati *et al*., 2019). As a novel soil amendment, biochar plays a significant role in soil improvement and crop stress resistance and yield stabilization (Hernandez-Soriano *et al*., 2016; Ma *et al*., 2025). The potassium-enriched biochar (KBC) used in this study is rich in water-soluble K^+, effectively supplementing potassium in the soil and plants. Potassium acts as an activator for numerous enzymes, participates in photosynthesis, sugar transport, and protein synthesis, and is crucial for improving crop yield and quality (Rubio *et al*., 2011; Dreyer *et al*., 2011). KBC also possesses characteristics such as a large specific surface area, high porosity, and strong ion exchange capacity. Its application can enhance soil microbial activity (Liao *et al*., 2024), provide microbial nutrition, improve soil quality (e.g., water holding capacity, aeration), and promote root development (Liao *et al*., 2024), making it a high-quality soil amendment.

Conclusion

Potassium-enriched biochar (KBC) significantly promotes the growth and development of soybean seedlings. KBC not only provides potassium but also improves soil fertility and structure through its inherent biochar properties. An appropriate proportion of KBC (e.g., 1.00%) can promote the growth and development of soybean seedlings through multiple pathways, including improving the soil environment, promoting nutrient absorption (especially potassium), enhancing photosynthesis, and regulating osmotic substances (as reflected by reduced proline and soluble sugar content indicating homeostasis). Simultaneously, under salt stress, the appropriate addition of KBC (1.0%-2.0%) can increase the total phenolic and flavonoid content in soybean seedlings, significantly activate their antioxidant enzyme system (POD, CAT, APX, SOD), enhance antioxidant capacity, and effectively protect plant cells from oxidative damage caused by salt stress. This study provides strong theoretical support for utilizing potassium-enriched biochar to enhance soybean growth potential, ameliorate saline-alkali soils, and improve soybean salt tolerance.

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