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RESEARCH ARTICLE

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Rice Husk Biochar Mitigates Saltwater Intrusion Stress in Rice (*Oryza sativa* L.) by Regulating Symplastic and Apoplastic Sodium Transport

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ABSTRACT

Saltwater intrusion poses a serious environmental threat to rice cultivation by impairing growth and increasing sodium (Na^+) toxicity. In this study, we investigated the effects of rice husk biochar on plant performance, Na^+ accumulation, and the physiological mechanisms involved in salt stress tolerance. Phitsanulok 2 rice seedlings were grown in saline soil with or without 30% (w/w) rice husk biochar and exposed to 6 or 10dS m^{-1} of saltwater for 7 days. The survival and growth of the plant were recorded. Root samples were analyzed to elucidate the expression of Na^+ transport genes (*OsCNGC1* and *OsHAK7*), suberin biosynthesis genes (*CYP86A9* and *CER6*), and suberin content. To evaluate apoplastic (bypass) flow, seedlings were transferred to a tracer solution containing 0.2mM trisodium-8-hydroxy-1,3,6-pyrenetrisulphonic acid (PTS) and 50mM NaCl for 7 days. High saltwater intrusion significantly reduced the survival, shoot height, dry weight, and tiller number, whereas biochar amendment significantly improved these parameters by 7–62%. Na^+ concentrations in shoots increased under salt stress but were significantly reduced in biochar-treated plants. Although *OsCNGC1* and *OsHAK7* were elevated under moderate salinity, their expressions were significantly suppressed with biochar. The *CYP86A9* and *CER6* expressions were significantly elevated in biochar-treated roots under high salinity, while the suberin content increased. Bypass flow of water and Na^+ also increased under high salinity; however, it reduced significantly after biochar application. In conclusion, rice husk biochar mitigates saltwater intrusion damage in rice by significantly reducing Na^+ accumulation in shoots through the suppression of both symplastic Na^+ transport and apoplastic bypass flow. These findings provide mechanistic insights into the role of biochar in enhancing salt tolerance of rice under saltwater intrusion stress.

Keywords: Bypass flow, Food security, Salinity, Seawater, Sodium transporter

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INTRODUCTION

Saltwater intrusion, also known as seawater intrusion, is a significant environmental challenge that threatens global food security. Saltwater intrusion has garnered increasing recognition as a major public concern, driven by sea level rise accelerated by climate change (Bayabil et al., 2020; Mukhopadhyay et al., 2021; Irwandhi et al., 2024; Gudi et al., 2025). Saltwater intrusion currently affects more than 100 countries, causing soil salinity in the agricultural areas of major rice-cultivating nations (Kotera et al., 2008; Cao et al., 2021; Nguyen et al., 2024; Su et al., 2025). For example, Subekti et al. (2020) reported that saltwater intrusion at 10.5dS m^{-1} reduced the shoot height and shoot biomass by 27.4 and 87.9%, respectively, in rice grown in coastal areas. Sudratt & Faiyue (2023) found that

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nearly 95% of rice died after 28 days of exposure to saline soil comprising 6dS m^{-1} of saltwater. Similarly, Sultan et al. (2025) found that exposure to 0.6% saltwater reduced both the shoot height and dry weight of rice plants by 67%. Moreover, saltwater intrusion has significantly reduced rice productivity, with reported yield losses of 20–80, 50–60, 70–90, and 30–100% in Vietnam (Phan & Kamoshita, 2020; Thach et al., 2023), India (Sarangi et al., 2020; Sarkar et al., 2021), Bangladesh (Bhuyan et al., 2023), and Indonesia, respectively (Subekti et al., 2020; Oelviani et al., 2024). Therefore, mitigating the risks of saltwater intrusion in rice production is an urgent priority for building a sustainable global food supply.

Rice is generally classified as a salt-sensitive crop owing to its limited ability to regulate sodium ion (Na^+) influx from saline soils into the roots and shoots, which negatively affects survival, growth, and productivity (Singh & Flowers, 2010; Faiyue et al., 2012; Razzaq et al., 2020; Singh et al., 2021). Na^+ can enter rice plants through both symplastic and apoplastic pathways (Reddy et al., 2017; Keisham et al., 2018; Tong et al., 2024). The symplastic pathway involves the movement of Na^+ across plasma membranes of roots, facilitated by membrane transporter proteins, whereas the apoplastic pathway involves the movement of Na^+ through cell walls and intercellular spaces to the root xylem, bypassing the plasma membrane (Singh & Flowers, 2010; Horie et al., 2012; Lu & Fricke, 2023; Tibesigwa et al., 2025). Notably, the symplastic transport of Na^+ is primarily mediated by non-selective cation channels (NSCCs), including cyclic nucleotide-gated channels (CNGCs) (Keisham et al., 2018; Chen et al., 2021; Balasubramaniam et al., 2023). The *OsCNGC1* expression was downregulated in salt-tolerant rice varieties compared to that in salt-sensitive ones under salinity stress (Senadheera et al., 2009). Moreover, the reduction in Na^+ accumulation and lower Na^+/K^+ ratio in rice treated with apigenin under salinity stress were attributed to the downregulation of *OsCNGC1* expression (Mekawy et al., 2018). High-affinity potassium transporters (HKTs and HAKs) are also involved in the symplastic transport of Na^+ under salt stress. *OsHAK7* expression was significantly lower in salt-tolerant rice varieties than in salt-sensitive ones under salinity stress, suggesting its potential role in regulating Na^+ uptake and salt sensitivity (Senadheera et al., 2009; Nampei et al., 2021, 2024; Mekawy et al., 2024).

The apoplastic pathway, also known as "bypass flow," has been identified as a significant route for Na^+ uptake into rice under salinity stress (Yeo et al., 1987; Yeo et al., 1999; Faiyue et al., 2012). Under low to moderate salinity, the contribution of bypass flow is relatively small, accounting for approximately 1–5%, but bypass flow increases substantially under high salt concentrations (Yeo et al., 1987; Anil et al., 2005; Flam-Shepherd et al., 2018; Lu & Fricke, 2023). The magnitude of bypass flow can be reduced by the deposition of the biopolymer suberin in the Casparian bands and suberin lamellae of the root exodermis and endodermis (Krishnamurthy et al., 2009, 2011; Faiyue et al., 2010a, b; Cui et al., 2021). Suberin is a complex biopolymer comprising both aromatic and aliphatic components. The aromatic domain primarily comprises ferulic acid, whereas the aliphatic portion includes various compounds including ω-hydroxy acids, 2-hydroxy acids, and various alcohols (Bernards, 2002; Schreiber et al., 2005; Krishnamurthy et al., 2009; Ranathunge et al., 2011). Genes involved in suberin biosynthesis in rice have also been reported, such as Cytochrome P450 monooxygenase (*CYP86A9*) and elongase (*CER6*) (Krishnamurthy et al., 2009; Ranathunge et al., 2016; Nishiuchi et al., 2021). Suberin formation is more extensive in salt-tolerant rice varieties than in salt-sensitive ones, resulting in lower Na^+ accumulation in the shoots and greater salt tolerance (Krishnamurthy et al., 2009, 2011; Jayabalan et al., 2022). Consistently, impaired or delayed development of suberin in root tissues has been linked to increased Na^+ accumulation and heightened sensitivity to salt stress (Krishnamurthy et al., 2021; Shukla et al., 2021; Tong et al., 2024).

Biochar, a carbon-rich material produced through the thermochemical conversion of biomass, has recently garnered attention for enhancing rice yield performance in salt-affected soils (Jin et al., 2018, 2024; Liu et al., 2023; Wang et al., 2023; Chi et al., 2024). For instance, Sudratt & Faiyue (2023) demonstrated that applying rice husk biochar significantly improved the growth and yield of Phitsanulok 2 rice grown in saline soil affected by saltwater intrusion. Similarly, Zhang et al. (2024) reported that the application of mixed biochar derived from maize, wheat, and peanut shell residues significantly increased the dry weights of leaves, roots, and grains in Tianlongyou 619 rice cultivated in coastal saline soil. Sultan et al. (2025) found that the application of rice straw biochar significantly enhanced shoot height and dry weight in Jing Liang You 534 rice subjected to saltwater stress. Moreover, biochar amendment can alleviate ion toxicity in rice under salinity stress by reducing Na^+ accumulation. For example, the application of peanut shell biochar significantly reduced Na^+ concentrations and the Na^+/K^+ ratio in the leaves, stems, and panicles of Changbai 9 rice under salinity stress (Ran et al., 2020; Piao et al., 2023). A reduction in leaf Na^+ concentration after amendment with rice straw biochar has also been reported in the Jing Liang You 534 and Xiang Liang You 900 rice varieties under salinity stress (Sultan et al., 2025). Similarly, rice husk biochar amendment has been shown to alleviate the effects of moderate to high saltwater intrusion in Phitsanulok 2 rice by reducing Na^+ accumulation and the Na^+/K^+ ratio, indicating a potential role of biochar in modulating Na^+ transport in rice (Sudratt & Faiyue, 2023; Faiyue, 2025).

Although biochar is widely recognized for its effectiveness in alleviating salinity stress in rice, the mechanisms through which it regulates Na^+ transport at both the cellular and gene expression levels remain largely unexplored (Mehmood et al., 2020; Liu et al., 2023; Soliman et al., 2023; Wu et al., 2023). In particular, the specific roles of symplastic and apoplastic Na^+ transport pathways under biochar amendment are not clear. Understanding these pathways is critical as they directly control ion homeostasis and plant performance under salinity stress. Understanding how biochar modulates these transport routes is essential for guiding the development of targeted biochar formulations or application strategies to enhance salt tolerance in rice through specific physiological interventions. We hypothesized that rice husk biochar mitigates saltwater intrusion stress in rice by modulating both symplastic and apoplastic Na^+ transport pathways. Therefore, this study aims to 1) evaluate the effects of rice husk biochar on Na^+ accumulation, growth and growth performance of rice under saltwater intrusion stress, and 2) investigate the physiological and molecular mechanisms underlying biochar-mediated salt tolerance in rice by focusing on symplastic and apoplastic Na^+ transport.

MATERIALS & METHODS

Experimental Site

The pot experiment was conducted in a glasshouse at Suranaree University of Technology, Nakhon Ratchasima, Thailand (14°51′39.8″N, 102°01′58.4″E) during November–December 2024. The average high and low temperatures were 29.7 and 19.7°C, respectively, with an average relative humidity of 68.5%.

Soil Physicochemical Characteristics

Samples of saline soil were randomly collected from the upper 15cm of paddy fields impacted by seawater intrusion in Bang Kha, Chachoengsao, Thailand (13°46′07.1″N, 101°13′39.2″E). The soil was classified as clay, comprising 19, 25 and 56% sand, silt, and clay, respectively. It had a pH of 4.14 and an organic matter content of 2.5%. The total nitrogen (N), phosphorus (P), and potassium (K) contents were 1.34, 0.21 and 9.2gkg^{-1}, respectively. The electrical conductivity of the saturated soil extract (ECe) was 5.09dSm^{-1}, and the cation exchange capacity (CEC) was 28.4cmolkg^{-1} (Sudratt & Faiyue, 2023).

Biochar Physicochemical Characteristics

Rice husk biochar was produced through slow pyrolysis (400°C, 8 h) and had an average particle size of 4mm, pH of 7.55, organic matter content of 16.0%, 0.68% N, 0.62% P, 1.03% K, 0.08% Na, CEC of 26.8cmolkg^{-1}, EC of 1.36dSm^{-1} (Sudratt & Faiyue, 2023) and BET surface area of 2.21m^{2}g^{-1}.

Experimental Design

Eight plastic pots (internal diameter: 19.5cm, height: 14cm) with drainage holes at the bottom were each filled with 3.5kg of saline paddy soil. For the treatment group, soil mixed with 30% (w/w) rice husk biochar has been previously identified as the most effective way for enhancing salt tolerance in rice (Sudratt & Faiyue, 2023). Another set of eight pots containing only saline paddy soil served as the control. All pots were placed in trays (40cm (width) × 50cm (length) × 11.5cm (height)), with four pots per tray, and submerged in Yoshida solution (EC: 1.10dSm^{-1}, pH: 5.0) for 14 days. Subsequently, 15 germinated Phitsanulok 2 rice seeds were sown in each pot. Fourteen days after sowing, the Yoshida solution was replaced with saltwater-amended Yoshida solution to simulate saltwater intrusion at two salinity levels. The ECs of the solution were adjusted to 6 and 10dSm^{-1} for medium and high salinity, respectively and applied to both biochar-amended and control treatments. After 7 days of exposure to salinity, seedlings from each pot were harvested to assess the survival rate, growth performance, ion accumulation, gene expression, and bypass flow.

Measurements of Survival, Growth, and Ion Accumulation

The survival of rice plants was determined by counting individuals with green leaves. The shoot height was measured from the base of the plant to the tip of the longest leaf using a ruler. Subsequently, the plants were separated into shoot and root components. The shoots were oven-dried at 80°C for 3 days and weighed to determine their dry weight. Dried shoots were subsequently digested in 100mM HNO_3 at 90°C for 2h. The resulting extracts were analyzed for Na^+ and K^+ concentrations using an atomic absorption spectrometer (ZEEnit 700 P, Analytik Jena, Jena, Germany).

Determination of Symplastic Pathway- and Bypass Flow-Related Genes

Rice roots from each treatment were washed with tap water, excess water was removed, and samples were immediately frozen in liquid nitrogen. Prior to RNA extraction, the frozen samples were ground into a fine powder. Total RNA was extracted using the FavorPrep™ Plant Total RNA Mini Kit (Favorgen Biotech Corp., Ping Tung, Taiwan) according to the manufacturer's instructions. RNA integrity was assessed using 1.0% (w/v) agarose gel electrophoresis. RNA concentration and purity (A260/280 ratio) were determined using a NanoDrop™ 2000 Spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA). A total of 0.5µg of RNA was reverse transcribed into cDNA using the iScript™ Reverse Transcription Supermix for RT-qPCR (Bio-Rad, Hercules, California, USA) with the SimpliAmp™ Thermal Cycler (Thermo Scientific, Waltham, Massachusetts, USA). Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) was conducted using the THUNDERBIRD SYBR qPCR Mix (TOYOBO, Osaka, Japan) on a CFX Opus 96 Real-Time PCR System (Bio-Rad, Hercules, California, USA). The PCR conditions were as follows: initial denaturation at 95°C for 3min; followed by 40 cycles at 95°C for 10s and 60°C for 30s; and a melt curve analysis from 60 to 90°C with increments of 1.5°C every 5s. Gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001), with Actin as the reference gene. All reactions were conducted in triplicate. Primer sequences for all analyzed genes are presented in Table 1.

Table 1: Gene-specific primer sequences used for quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) analysis

Gene name	Direction	Gene primer sequence
Actin	F	CCTCTTCCAGCCTTCCTTCAT
	R	ACGGCGATAACAGCTCCTCTT
OsCNGC1	F	ACCACCTCCGAGATTTGAGGAC
	R	AGCATTATGCCTCCGCAGTGAC
OsHAK7	F	TTGGCGCATGCATGGTGATAGG
	R	GCAATGAACACCACCCAACCATC
CYP86A9	F	CGCCTCACCTTCGATAACAT
	R	CACTCGCAGTCCATTCTTCA
CER6	F	TCGTAATCTTCTCCGCCATC
	R	GATGTAGGCGAGCTCGTACC

Determination of Suberin

Suberin extraction and analysis were conducted according to the method of Sano et al. (2022), with some modifications to suit the experimental conditions. Dried rice roots from each treatment were initially heated in isopropanol at 85°C for 30min. Following solvent removal, the remaining residues were subjected to sequential extractions with chloroform-methanol mixtures in ratios of 2:1, 1:1, 1:2, and 0:1 (v/v), each shaken at 120rpm for 24h. All extract solutions, including those from the steps involving initial isopropanol and chloroform-methanol, were pooled, evaporated to dryness, and redissolved in a 1:1 chloroform-methanol mixture to a final volume of 5mL prior to analysis. Suberin monomers were quantified using high-performance liquid chromatography coupled with evaporative light scattering detection (HPLC-ELSD, Agilent 1260 Infinity II). The mobile phase comprised deionized water and acetonitrile, applied in a gradient elution beginning at a ratio (v/v) of 95:5 during the initial 3min, gradually transitioning to 100% acetonitrile over the next 18 min, and maintained at this composition for 30min. The flow rate was maintained at 1.0mLmin^{-1}, with separation conducted on an Inertsil ODS-3 column (150mm) at 40°C and an injection volume of 10µL. ELSD was conducted with a nebulizer temperature of 40°C and nitrogen gas as the carrier at a flow rate of 1.6L min^{-1}. Calibration curves were generated using ferulic acid and 16-hydroxyhexadecanoic acid standards, prepared in 99.9% HPLC-grade methanol.

Determination of Bypass Flow

Rice seedlings from each pot were transplanted into individual black-painted plastic cups (450mL) containing Yoshida solution. After the recovery period for 24h, seedlings were subjected to bypass flow analysis following the method described in previous studies (Faiyue et al., 2010a, b; 2012). In particular, each plastic cup was replaced with 400mL of a solution containing 0.2mM trisodium-8-hydroxy-1,3,6-pyrenetrisulphonic acid (PTS) and 50mM NaCl. The seedlings were exposed to this solution for 7 days to enable the uptake of Na$^+$ and PTS. Plant transpiration was quantified by weighing the cups, and the values were corrected for evaporation using control cups without plants. Following the treatment, the shoots were harvested and oven-dried at 80°C for 3 days. Dried samples were digested in 100mM HNO_3 at 90°C for 2h, and the Na$^+$ concentration in the extract was determined using an atomic absorption spectrometer (ZEEnit 700 P, Analytik Jena, Jena, Germany). The fluorescence intensity of PTS in the extracted solutions was measured using a spectrofluorometer (Jasco FP-8200, Tokyo, Japan) at excitation and emission wavelengths of 403 and 510nm, respectively. Bypass flow of water (J_{VB}) was calculated using the following equation:

$$J_{VB}\ (\%) = (PTS_{[xyl]} / PTS_{[ext]}) \times 7.57 \times 100$$

where $PTS_{[xyl]}$ is the apparent PTS concentration in the xylem, calculated by dividing the PTS content by transpired water (J_V); $PTS_{[ext]}$ is the PTS concentration in the external medium or salt solution; 7.57 is an empirical correction factor accounting for the differential mobility of PTS dye compared with that of water (Yeo et al., 1987). Bypass Na$^+$ delivery was calculated by multiplying J_{VB} (bypass flow of water) with J_V (transpired water) and the external Na$^+$ concentration ($Na_{[ext]}$), and its proportion relative to the total shoot Na$^+$ content was used to determine the percentage of bypass flow of Na$^+$.

Statistical Analysis

The experiment was conducted using a completely randomized design with three independent experimental replicates. Data are expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS software (version 28.0; IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were used to determine differences among the mean values. $p<0.05$ was considered statistically significant.

RESULTS

Impact of Biochar Amendment on Rice Growth and Survival under Saltwater Intrusion

The survival rate of Phitsanulok 2 rice seedlings grown in saline paddy soil without biochar amendment was approximately 83% under medium saltwater intrusion (6dSm^{-1}) for 7 days, and significantly declined to 60% under high saltwater intrusion (10dSm^{-1}) (Fig. 1A). Amendment with 30% (w/w) rice husk biochar maintained the survival rate of rice seedlings at approximately 86%, which was significantly higher than that observed under high saltwater intrusion without biochar amendment (Fig. 1A). The shoot height of rice grown in saline soil without biochar was approximately 36cm under medium saltwater intrusion, but significantly declined to approximately 32cm under high saltwater intrusion (Fig. 1B). In contrast, the addition of biochar significantly enhanced the shoot height under high saltwater intrusion conditions (Fig. 1B). The shoot dry weight of rice grown in saline soil under medium saltwater intrusion was approximately 0.23g, which decreased to 0.18g under high saltwater intrusion (Fig. 1C). Biochar amendment significantly improved the shoot dry weight under high saltwater intrusion compared with the control without biochar (Fig. 1C). Rice plants grown in saline soil under saltwater conditions produced approximately two tillers per plant; however, biochar amendment significantly increased this number to approximately three tillers per plant (Fig. 1D).

Effect of Biochar Amendment on Na$^+$ and K$^+$ Accumulation in Rice under Saltwater Intrusion

Under medium saltwater intrusion, the Na$^+$ concentration in the shoots of rice grown in saline soil without biochar amendment was 14.2mg gDW^{-1}, whereas biochar amendment significantly reduced it to approximately 7.0mg gDW^{-1} (Fig. 2A). Under high saltwater intrusion, shoot Na$^+$ concentration increased to 15.7mg gDW^{-1} in the absence of biochar but significantly decreased to 8.8mg gDW^{-1} with biochar amendment (Fig. 2A). The shoot K$^+$ concentrations were not significantly influenced by saltwater intrusion or biochar amendment, with an average value of approximately 43mg gDW^{-1} across

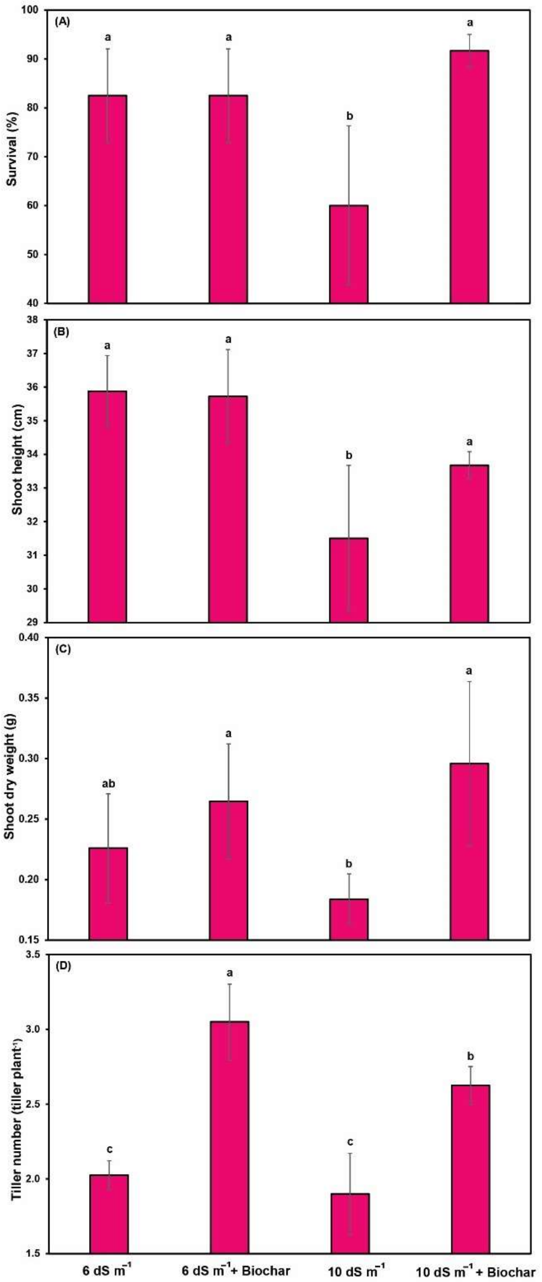

Fig. 1: Impact of biochar amendment on the survival (A), shoot height (B), shoot dry weight (C), and tiller number per plant (D) of Phitsanulok 2 rice under medium (6dS m^{-1}) and high (10dS m^{-1}) saltwater intrusion conditions. Data are presented as mean±SD (n = 3). Different letters indicate significant differences at $P<0.05$ by Duncan's multiple range test (DMRT).

all treatments (Fig. 2B). The shoot Na^+/K^+ ratio in rice grown without biochar amendment was approximately 0.35 under both medium and high saltwater conditions, whereas biochar amendment significantly reduced these ratios to 0.07 and 0.20 under medium and high salinity, respectively (Fig. 2C).

Effect of Biochar Amendment on the Expression of Genes Involved in Symplastic Transport and Bypass Flow

The transcriptional responses of Na^+ transporter-related genes, including *OsCNGC1* (cyclic nucleotide-gated channel) and *OsHAK7* (high-affinity potassium transporter), were analyzed in the roots of rice seedlings subjected to medium and high saltwater levels, with and without biochar amendment. The relative expression of *OsCNGC1* was significantly suppressed in rice grown under medium saltwater conditions with biochar amendment (Fig. 3A). Under high saltwater conditions, *OsCNGC1* expression was downregulated compared with that under medium saltwater conditions and showed no significant difference between treatments with and without biochar, and remained comparable to that observed under medium salinity conditions with biochar amendment (Fig. 3A). Similarly, *OsHAK7* expression was markedly reduced with biochar amendment under medium saltwater conditions (Fig. 3B).

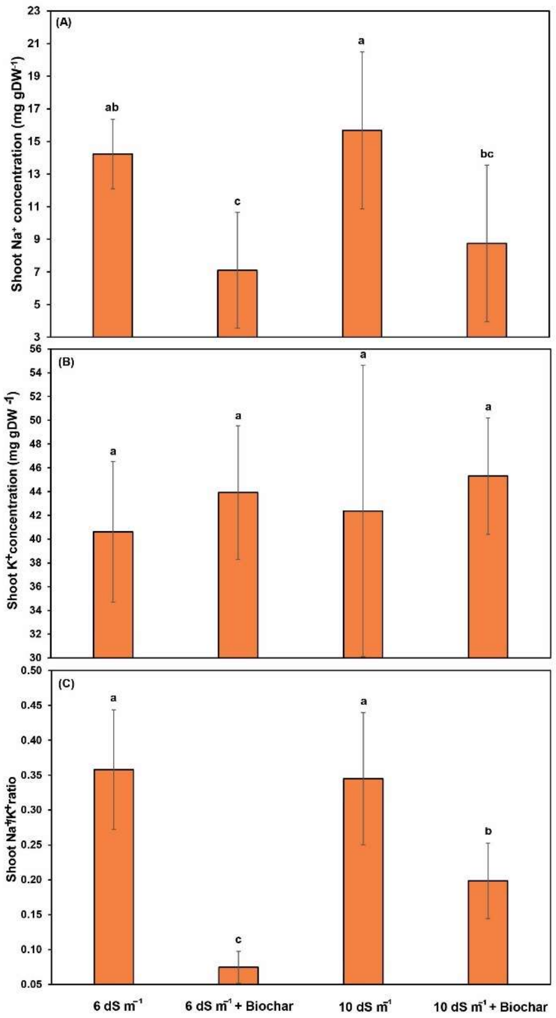

Fig. 2: Effect of biochar amendment on shoot Na^+ concentrations (A), shoot K^+ concentrations (B), and shoot Na^+/K^+ ratio (C) in Phitsanulok 2 rice under medium (6dS m^{-1}) and high (10dS m^{-1}) saltwater intrusion conditions. Data are presented as mean±SD (n = 3). Different letters indicate significant differences at $P<0.05$ by Duncan's multiple range test (DMRT).

Under high saltwater conditions, *OsHAK7* expression showed no significant difference between treatments with and without biochar but was 3.7-fold lower than that in rice exposed to medium saltwater intrusion without biochar amendment (Fig. 3B). The transcriptional levels of suberin biosynthesis-related genes, including *CYP86A9* and *CER6*, remained low under medium saltwater conditions both with and without biochar, as well as under high saltwater intrusion without biochar amendment (Fig. 3C, D). Notably, biochar amendment significantly upregulated the expression of *CYP86A9* and *CER6* under high saltwater conditions (Fig. 3C, D).

Effect of Biochar Amendment on Bypass Flow

During the experimental period, rice seedlings transpired approximately 34mL of water under both medium- and high-saltwater conditions. This value was not significantly affected by the presence or absence of biochar (Fig. 4A). Bypass flow of water was 1.1–1.3% in rice seedlings under medium saltwater intrusion (6dSm^{-1}), regardless of biochar treatment. The flow under high saltwater intrusion (10dS m^{-1}) significantly increased to 3.68% without biochar amendment but that with biochar amendment was significantly reduced to 2.0% (Fig. 4B). Similarly, the bypass flow of Na^+ under medium saltwater intrusion was approximately 18.5% for both treatments with and without biochar. Upon exposure to high salinity, this value significantly increased to 46.9% without biochar. Still, it significantly reduced to 14.2% with biochar amendment (Fig. 4C). A positive correlation was observed between Na^+ bypass flow and Na^+ accumulation in rice shoots (Fig. 4D).

Effect of Biochar Amendment on Suberin Accumulation in Rice Roots

The amount of aromatic suberin in rice roots, represented by ferulic acid, did not differ significantly among treatments, with an overall average of 57mg gDW^{-1} (Fig. 5A). In contrast, the aliphatic suberin content, indicated by hexadecanoic acid, varied significantly across treatments (Fig. 5B). Under high-salinity saltwater intrusion, rice plants treated with biochar showed the highest levels of hexadecanoic acid, averaging at 9.30mg gDW^{-1}. This was followed by rice grown under medium salinity with and without biochar, with average values of 7.17 and 6.80mg gDW^{-1}, respectively. The lowest aliphatic suberin content was found in rice grown under high salinity without biochar, averaging only 4.35mg gDW^{-1} (Fig. 5B). Root suberin content, indicated by hexadecanoic acid, was negatively correlated with Na^+ bypass flow and shoot Na^+ accumulation in Phitsanulok 2 rice under saltwater intrusion conditions (Fig. 5C, D).

DISCUSSION

Biochar soil amendment is an innovative approach for remediating the effects of soil salinity from saltwater intrusion on the growth, development, and productivity of plants. Its potential mechanisms have been reported, including the improvement of soil physicochemical properties, modification of the microbial community, and regulation of plant metabolism (Diatta et al., 2020; Huang et al., 2022; Helaoui et al., 2023; Gautam et al., 2024; Wang et al., 2024). Although biochar application has been shown to reduce Na^+ accumulation in rice under salinity (Jin et al., 2018; Ran et al., 2020; Piao et al., 2023; Sudratt & Faiyue, 2023; Faiyue, 2025; Sultan et al., 2025), its effects on Na^+ transport have not yet been completely elucidated. In this study, we investigated how rice husk biochar affects the symplastic and apoplastic pathways of Na^+ transport in rice exposed to saltwater intrusion, aiming to better understand how biochar helps alleviate salt-induced stress in rice plants.

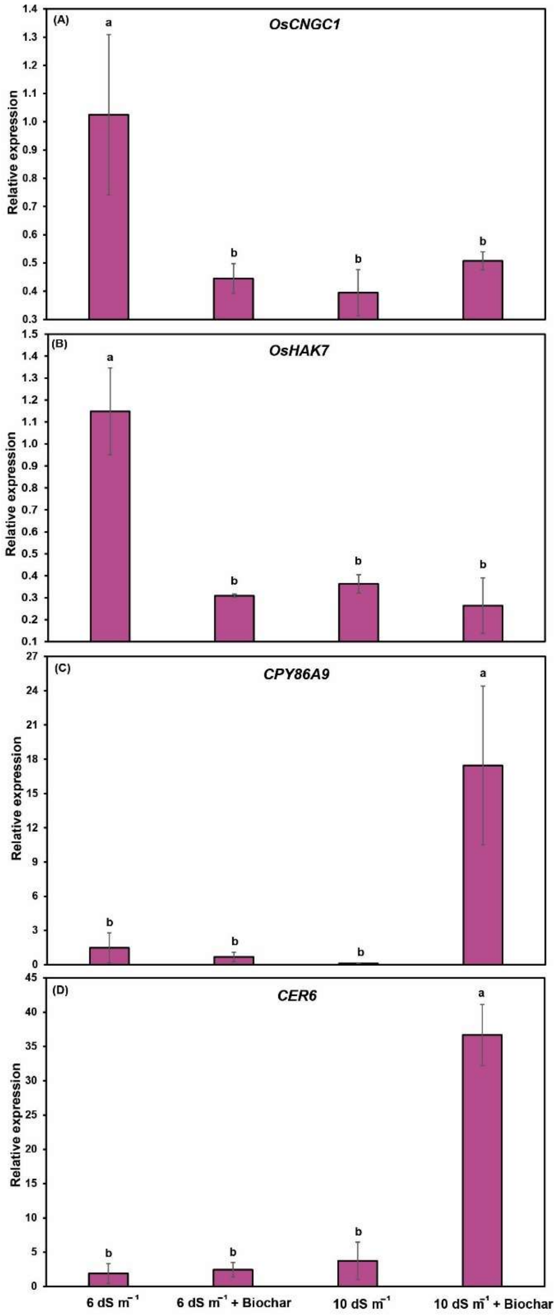

Fig. 3: Effect of biochar amendment on the expressions of *OsCNGC1* (A), *OsHAK7* (B), *CYP86A9* (C), and *CER6* (D) genes in Phitsanulok 2 rice seedlings exposed to medium (6dS m^{-1}) and high (10dS m^{-1}) saltwater intrusion conditions. Data are presented as mean±SD (n = 3). Different letters indicate significant differences at $P<0.05$ by Duncan's multiple range test (DMRT).

Our results showed that the survival and growth of Phitsanulok 2 rice seedlings grown in saline paddy soil without biochar significantly decreased by 12–27% after 7 days of exposure to high saltwater intrusion (10dS m^{-1}) compared with those of seedlings under medium saltwater intrusion (6dS m^{-1}) (Fig. 1). This finding highlights the detrimental effect of saltwater intrusion on rice yield performance.

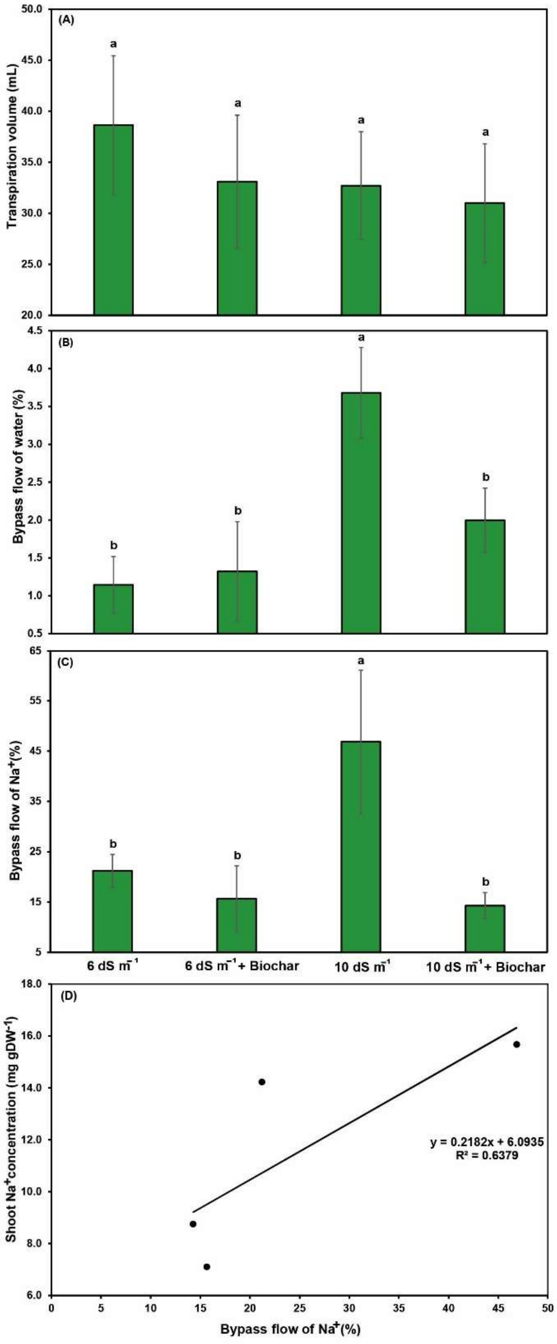

Fig. 4: Effect of biochar amendment on plant transpiration (A), bypass flow of water (B), bypass flow of Na^+ (C), and the relationship between bypass flow of Na^+ and shoot Na^+ concentration (D) in Phitsanulok 2 rice grown under medium (6dS m^{-1}) and high (10dS m^{-1}) saltwater intrusion conditions. Data are presented as mean±SD (n = 3). Different letters indicate significant differences at P<0.05 by Duncan's multiple range test (DMRT).

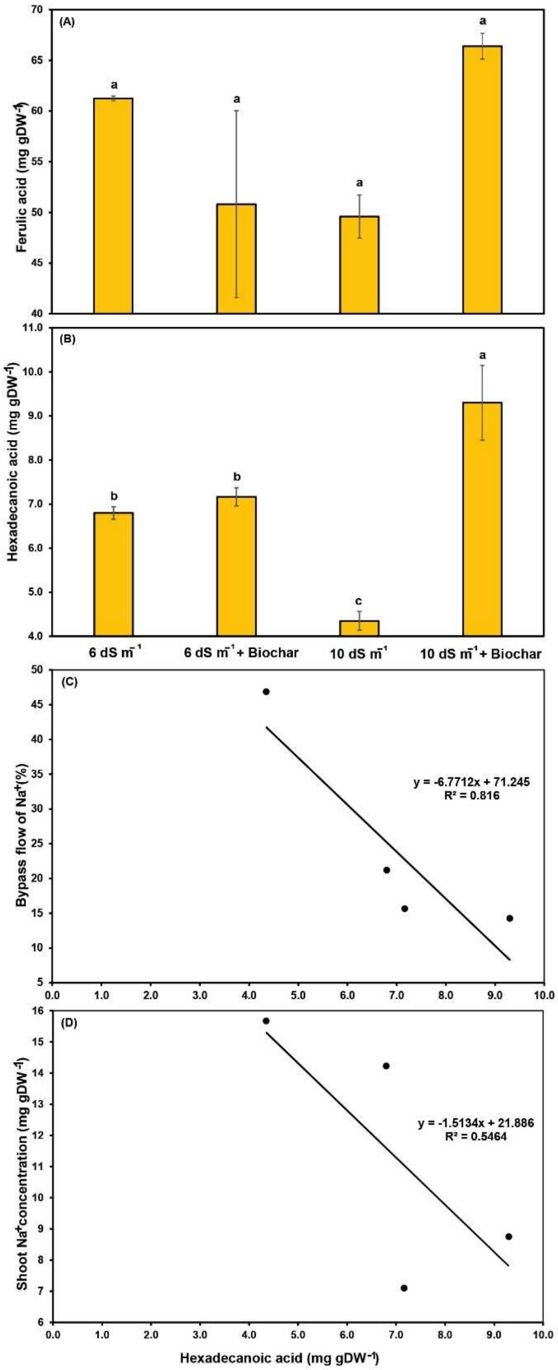

Fig. 5: Effect of biochar amendment on the concentrations of ferulic acid (A) and hexadecenoic acid (B) in roots, and their relationships with bypass flow of Na^+ (C) and shoot Na^+ concentration (D) in Phitsanulok 2 rice grown under medium (6dSm^{-1}) and high (10dS m^{-1}) saltwater intrusion conditions. Data are presented as mean±SD (n = 3). Different letters indicate significant differences at P<0.05 by Duncan's multiple range test (DMRT).

Overall, our results are consistent with those of previous studies reporting that rice grown under saltwater intrusion stress undergoes significant reductions in seedling survival, shoot height, and biomass, ranging from 27.4 to 95% (Subekti et al., 2020; Sudratt & Faiyue, 2023; Sultan et al., 2025). The application of rice husk biochar significantly enhanced the survival rate, shoot height, and shoot dry weight of Phitsanulok 2 rice grown under high saltwater intrusion conditions (Fig. 1). It also increased the number of tillers under both medium and high salinity conditions compared with that in plants grown without biochar (Fig. 1), highlighting its effectiveness in reducing the impact of salt stress. These findings are consistent with those of previous studies (Sudratt & Faiyue, 2023; Zhang et al., 2024; Sultan et al., 2025).

In this study, we demonstrated that the application of rice husk biochar significantly reduced shoot Na^+ concentrations, while alleviating Na^+/K^+ ratios by 40–80% without affecting K^+ accumulation (Fig. 2). These findings are consistent with those of previous studies (Ran et al., 2020; Piao et al., 2023; Sudratt & Faiyue, 2023; Faiyue, 2025; Sultan et al., 2025). Notably, the negative charges of the hydroxyl and carboxyl functional groups on the surface of rice husk biochar may absorb Na^+ in saline soils, thereby reducing Na^+ availability in the rhizosphere, and limiting its transport to the shoots (Sudratt & Faiyue, 2023). The mechanisms underlying salinity tolerance in rice are complex and involve several coordinated responses, including limiting the entry of Na^+ into the roots, reducing its transport to the shoots, regulating Na^+ distribution among leaves, and compartmentalizing Na^+ within and between cells (Yeo et al., 1990; Singh & Flowers, 2010; Singh et al., 2021; Liu et al., 2022). Among all the salt-tolerance strategies in rice, reducing the entry of Na^+ into the root xylem is the most effective. When less Na^+ is absorbed, other mechanisms are less necessary (Zhang et al., 2010; Cui et al., 2021).

Available Na^+ in the rhizosphere of salt-affected soils can enter the rice plants through the symplastic and apoplastic pathways or bypass flow (Singh & Flowers, 2010; Horie et al., 2012; Reddy et al., 2017; Keisham et al., 2018; Lu & Fricke, 2023; Tong et al., 2024; Tibesigwa et al., 2025). The symplastic transport of Na^+ across plasma membranes of rice roots is facilitated by NSCCs, including OsCNGC1 (cyclic nucleotide-gated channel), as well as by high-affinity potassium transporters (HKTs and HAKs), such as *OsHAK7* (Senadheera et al., 2009; Keisham et al., 2018; Chen et al., 2021; Balasubramaniam et al, 2023). Our results showed that under medium saltwater intrusion, the relative expression levels of *OsCNGC1* and *OsHAK7* were significantly suppressed by biochar amendment (Fig. 3A, B). In contrast, under high saltwater conditions, their expression levels were low and did not significantly differ between treatments with and without biochar (Fig. 3A, B). These results are consistent with the findings of Mekawy et al. (2018), who reported that the reduced Na^+ accumulation and lower Na^+/K^+ ratio in rice grown under salinity stress and treated with apigenin were associated with the downregulation of *OsCNGC1* expression. This highlights the potential role of biochar amendment in regulating the symplastic pathway of Na^+ uptake under medium saltwater intrusion. Salt-tolerant rice varieties have also been reported to exhibit the downregulation of *OsCNGC1* and *OsHAK7* (Senadheera et al., 2009; Nampei et al., 2021, 2024; Mekawy et al., 2024).

Our results showed that amendment with rice husk biochar did not affect the transpiration volume of rice under either medium or high saltwater conditions (Fig. 4A), suggesting that the reduction of shoot Na^+ accumulation is not solely attributed to decreased transpiration flow. This finding aligns with that of Gong et al. (2006), who reported that the application of exogenous silicon (Si) to saline solutions significantly reduced the Na^+ concentration in rice shoots without affecting the transpirational volume flow. The lack of correlation between Na^+ accumulation and transpirational water loss has also been reported in other plants under salt stress, such as potato (Mondal et al., 2024). Under medium saltwater intrusion, the percentage of water bypass flow in rice seedlings was 1.1%, contributing to 21.2% of Na^+ accumulation in the shoots (Fig. 4B, C), and the percentages of bypass flow of water and Na^+ increased significantly to 3.7 and 46.9%, respectively, when exposed to high saltwater intrusion (Fig. 4B, C). These results are consistent with those of previous studies (Yeo et al., 1987, Anil et al., 2005; Flam-Shepherd et al., 2018; Lu & Fricke, 2023), which demonstrate that bypass flow substantially increases under high salt concentrations. Although biochar amendment did not affect the bypass flow of water and Na^+ under medium saltwater intrusion, it significantly reduced the bypass flow of both water and Na^+ by 45–70% in rice exposed to high saltwater intrusion (Fig. 4B, C); this suggests a negative correlation between biochar amendment and bypass flow under high salinity stress. The transcriptional levels of suberin biosynthesis-related genes, namely, *CYP86A9* and *CER6*, were significantly upregulated in rice roots under high saltwater intrusion with biochar amendment (Fig. 3C, D). Consistently, the highest suberin content was found in roots of rice grown under high salinity conditions with biochar application (Fig. 5A, B). These findings suggest that the biochar-induced reduction in bypass flow of water may be attributed to the upregulation of genes involved in suberin biosynthesis, thereby enhancing suberin deposition in roots. This can restrict the bypass flow of Na^+ and its accumulation in the shoots. Consistently, the suberin content in roots was negatively correlated with Na^+ bypass flow and shoot Na^+ accumulation in Phitsanulok 2 rice under saltwater intrusion (Fig. 5C, D). These findings are consistent with previous studies reporting that a more developed bypass flow barrier is associated with reduced Na^+ accumulation in shoots and enhanced salt tolerance in rice (Krishnamurthy et al., 2009, 2011; Jayabalan et al., 2022; Tong et al., 2024). Biochar application has been shown to enhance salt tolerance in plants by increasing the expression of antioxidant defense genes in soybean (Mehmood et al., 2020) and water transporter genes in wheat (Soliman et al., 2023; Wu et al., 2023). Based on our findings, we propose that rice husk biochar helps alleviate the harmful effects of saltwater intrusion in rice by reducing shoot Na^+ accumulation and through the downregulation of symplastic Na^+ transporters and a decrease in bypass flow.

Although our results indicate that rice husk biochar can help rice plants cope with saltwater intrusion stress, there are some limitations to consider. The experiment was conducted in a greenhouse over a short period, which may not completely reflect conditions in real rice fields. Environmental factors, such as rainfall, soil type, and microbial activity, could affect how biochar works in the long term. To confirm its effectiveness, further studies on field conditions and over longer periods are required. Nevertheless, our findings highlight the potential of biochar to support the development of salt-tolerant rice varieties by enhancing root suberization and reducing Na^+ transport to shoots. The simplicity and cost-effectiveness of this approach offer promising implications for sustainable rice cultivation in salinity-affected areas.

Conclusion

In this study, we demonstrated that amendment with rice husk biochar mitigates the detrimental effects of saltwater intrusion on the growth and survival of rice by reducing shoot Na$^+$ accumulation. Under medium saltwater intrusion (6dS m^{-1}), biochar suppressed the symplastic uptake of Na$^+$ by downregulating ion transporters, such as *OsCNGC* and *OsHAK7*. In contrast, under high saltwater intrusion (10dS m^{-1}), biochar reduced Na$^+$ bypass flow by enhancing suberin biosynthesis in roots. Overall, these findings highlight the mechanisms through which biochar alleviates salt stress in rice through the regulation of both symplastic and apoplastic Na$^+$ transport pathways.

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