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Rice husk biochar ameliorates saline-sodic stress in rice through sodium adsorption and improving soil properties

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Abstract

Soil salinity is one of the major problems threatening rice productivity and food security globally. The objectives of this research were to examine the effects of rice husk biochar as a soil amendment on the growth and yield of Khao Dawk Mali 105 (KDML105) rice grown in a saline-sodic soil, and to investigate the mechanism by which rice husk biochar mitigates harmful effects of a saline-sodic soil on rice. KDML105 at 45 days was grown in cement rings containing a saline-sodic soil supplemented with 0%, 0.3%, 0.6%, 1.8%, and 3.0% (w/w) rice husk biochar. The rice plants were harvested at the maturity stage. Yield and yield components were recorded. Flag leaf was determined for Na⁺ and K⁺ accumulations. Soil and biochar were analyzed for their important properties. The results showed that biochar additions to a saline-sodic soil increased survival percentage, shoot and root dry weights, panicle length, number of grains per panicle, grain filling percentage, and grain weight of KDML105 rice, whereas Na⁺ concentration in flag leaf was reduced. Using the Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray Analysis (EDX), it was shown that Na⁺ was detected on the surface of biochar. Biochar applications decreased electrical conductivity (ECe), total Na, extractable Na, and an exchangeable sodium percentage (ESP), but increased OM, total N, and exchangeable K. It can be concluded that rice husk biochar amendment at 3.0% (w/w) alleviated the negative effects of saline-sodic stress and increased KDML105 rice productivity by decreasing Na⁺ availability, increasing K⁺/Na⁺ ratio, and improving soil qualities.

Keywords: Biochar, Climate change, Food security, K⁺/Na⁺ ratio, Rice, Salinity

1. Introduction

Soil salinity is a major problem threatening crop productivity and food security globally [1,2]. It has been estimated that soil salinity already covers 20% of arable lands, and approximately 50% of them would be affected by salts by 2050 [3,4]. Salt-affected areas are increased as a result of anthropogenic activities by using poor quality irrigation water and deforestation, leading to a high level of water tables with salts' concentrations in the root zone of plants, with the natural weathering of saline parental rocks releasing soluble salts, mainly Cl⁻, and SO₄²⁻ of Na⁺ and Mg²⁺, to the soil, as well as saltwater intrusion due to the sea-level rise accelerated by climate change effect [3-5]. Generally, salt-affected soils are classified into saline, sodic, and saline-sodic soils depending on the electrical conductivity (ECe) of a saturated paste extract of soil, an exchangeable sodium percentage (ESP), and pH [6,7]. A saline soil is defined when the ECe is more than 4 deciSiemen/metre (dS/m⁻¹), ESP is less than 15%, and pH is less than 8.5. A sodic soil is characterized when the ECe is less than 4 dS/m⁻¹, ESP is higher than 15%, and pH varies between 8.5 and 10. A saline-sodic soil is measured when the ECe is more than 4 dS/m⁻¹, ESP is more than 15%, and pH may be less or more than 8.5 [7]. Salt-affected lands

globally, are mainly covered by saline soil (60%), followed by sodic soil (26%), and saline-sodic soil (14%), respectively [8]. Although a saline-sodic soil covers a small proportion of salt-affected soils, this type of soil severely inhibits the growth and yield of economic crops in several countries, including China [9,10], Vietnam [11], and Thailand [12-14].

Based on the ability to survive and complete their life cycle in 200 mM NaCl, plants have been classified into two groups: the salt-sensitive glycophytes and the salt-tolerant halophytes [15]. Rice (*Oryza sativa* L.), a staple food crop for more than 50% of the world's population [16], is classified generally as a salt-sensitive glycophyte because it cannot control Na⁺ influx from salt-affected soils to the root xylem, leading to high Na⁺ transport from roots to shoots, and rapid Na⁺ accumulation in cells to the toxic level [7,17]. The osmotic effect, ionic effect, and nutrient deficiency caused by salinity, are the main causes of the reduction in survival, growth, and productivity in rice. Generally, rice is more sensitive to salinity during its seedling stage (1-3 weeks) and early reproductive stages (panicle initiation, anthesis, and fertilization) [7]. Theerakulpisut et al. [1] reported that shoot dry weight of 14-day-old rice cvs. Dang Dawk Kok, Supanburi 2, Luang Ta Moh, KDML105, and IR29 was decreased by approximately 15-37% compared with the controlled plants after salinization with 12 dS/m⁻¹ for seven days. Similarly, Pattanagul and Thitisaksakul [18] found that salinity stress induced a reduction in shoot height and shoot dry weight of 14-day-old rice seedlings cvs. KDML105 and Luang Anan after exposure to 150 mM NaCl for nine days. The rice genotype IR55178 ultimately died within 90 days after exposure to 50 mM NaCl at 14 days [17]. Cha-um and Kirdmanee [19] demonstrated that the growth and yield of rice cv. RD6 was severely affected by a saline paddy field of 8.5 dS/m⁻¹. Also, Thitisaksakul et al. [20] reported that 100-grain and total grain weight of Nipponbare rice were significantly decreased after being treated with 4 dS/m⁻¹ NaCl at the anthesis stage. Therefore, salinity is a worldwide threat to rice production, and it is important to enhance salt-tolerant abilities of rice growing in salt-stressed regions to mitigate the effects of salt stress on rice productivity and supply global food demand [4,21]. Consequently, different approaches alleviate salt stress in rice, such as selection for salt-tolerant varieties [17,22], seed priming [23], transplanting of older seedling [24], and organic amendments including compost, farmyard manure, green manure, and biochar [9-14,19,24].

Biochar, a biomass residue pyrolyzed under low/no oxygen conditions, has been broadly discussed as an effective strategy to sequester carbon, improve soil properties, and mitigate environmental risks, including salinity [25,26]. For instance, Akhtar et al. [27] showed that adding 5% (w/w) biochar, produced from a mixture of hardwood and softwood to pots containing sandy loam soil irrigated with 25 and 50 mM NaCl solutions decreased the negative effects of salinity on growth and yield of potato (*Solanum tuberosum*). Jin et al. [9] found that 3% (w/w) of peanut shell biochar decreased Na⁺ accumulation and increased the yield of rice cv. G9 growing in a saline-sodic soil (ECe = 26 dS/m⁻¹). Nguyen et al. [11] reported that rice husk biochar at 2.5% (w/w) increased biomass of shoot and root of rice variety OM6162 grown in a saline-sodic soil with ECe of 5.7 dS/m⁻¹ by increasing soil cation exchangeable capacity (CEC) and soil nutrients. Phuong et al. [28] showed that rice husk biochar amendment at 1 kg/m² (equivalent to 0.6% w/w) increased biomass of rice varieties OM6162, OM5451, and Tep Hanh growing in salt-affected soils with ECe of 4.10-4.50 dS/m⁻¹. Also, Ran et al. [10] reported that peanut shell biochar at 6.75 kg/m² enhanced ion concentrations, growth, and yield of rice variety Changbai-9 grown in a saline-sodic soil with ECe of 24.08 dS/m⁻¹. Parkash and Singh [29] reported that hardwood and softwood biochar at 5% (w/w) reduced the salinity stress of 4 dS/m⁻¹ on eggplant (*Solanum melongena* L.) by improving stomatal conductance, increasing photosynthetic rate, reducing leaf temperature, and decreasing electrolyte leakage in leaf tissue, resulting in better root and shoot growth, and fruit yield of eggplant compared with non-biochar treatment. Zhang et al. [30] revealed that an addition of 1% (w/w) wheat straw biochar reduced the negative effects of soil salinity (10.6 dS/m⁻¹) on soybean (*Glycine max*) biomass and grain yield by increasing the water use efficiency. Soothar et al. [31] found that wood biochar at 4.5% (w/w) increased plant height, the number of leaves, fresh and dry weight of shoot and root of maize (*Zea mays* L.) seedlings under salinity stress by increasing nutrient concentrations such as N, P in shoot and root, but decreasing Na⁺ accumulation. Karabay et al. [32] found that applying olive pruning biochar at 2.5% (w/w) increased shoot length and pod number in common bean (*Phaseolus vulgaris* L.) under salinity stress of 50 mM NaCl by decreasing hydrogen peroxide (H₂O₂) content and ion leakage in leaves.

Although biochar has been documented as an alleviating substance in salt-affected soils, more studies are needed to shed more light on the effects of biochar on the growth and yield of rice under a saline-sodic soil as the positive effects of biochar depend on the type and rate of application [10,11,28,33]. Therefore, the objectives of this research were (1) to examine the effects of rice husk biochar as a soil amendment on growth, yield, and yield components of KDML105 rice grown in a saline-sodic soil, and (2) to investigate the mechanisms by which rice husk biochar mitigates harmful effects of a saline-sodic soil in KDML105 rice.

2. Materials and methods

2.1 Rice materials and treatments

Rice cv. KDML105 was used in this research as it is the same variety widely cultivated by local farmers in the areas of study [12-14]. The experiment was conducted in-season between August and December 2019 under natural light and temperature at Kham Thale So, Nakhon Ratchasima, Thailand. The average temperatures ranged from 23°C to 32°C with 55%-75% relative humidity.

Approximately 2,000 kg of soil was randomly collected at a depth of 0-30 cm from saline paddy fields located in Kham Thale So, Nakhon Ratchasima, Thailand. Then, the soil was air-dried, passed through a 2 mm sieve, and analyzed for important physicochemical properties based on the standard procedure of a soil survey laboratory methods manual [12,34,35], including soil texture (hydrometer method), organic matter (OM) content (Walkley and Black method), CEC (ammonium acetate method), total N (Kjeldahl method), total P (vanadomolybdophosphoric acid colorimetric method), exchangeable K (ammonium acetate method), total Na, extractable Na (ammonium acetate method), ECE (ammonium acetate method), ESP, and pH (soil:water = 1:1). From the physicochemical properties, the soil was classified as a saline-sodic soil (Table 1).

Biochar was produced from rice husk using a slow pyrolysis process at 400°C-500°C at the Huaysai Royal Development Study Center, Petchaburi, Thailand [36], and analyzed for its basic properties of pH, OM (Walkley and Black method), CEC (ammonium acetate method), total N (Kjeldahl method), total P (vanadomolybdophosphoric acid colorimetric method), total K (K_2O ; atomic absorbance spectrometry), EC (biochar : water = 1:5), and total Na, as shown in Table 1.

Table 1 The physicochemical properties of soil and rice husk biochar.

Characteristic	Soil	Rice husk biochar
Silt content (%)	4	
Clay content (%)	2	
Exchangeable K (mg/kg)	11	
Sand content (%)	94	
OM (%)	0.08	9.40
CEC (cmol _c /kg)	1.10	30.84
pH	10.20	7.24
ECe (dS/m)	12.64	
ESP (%)	464.90	
Extractable Na (mg/kg)	1,023	
EC (dS/m)		1.12
Total K (%)		1.06
Total N (%)	0.01	0.75
Total P (%)	0.06	0.74
Total Na (%)	0.16	0.01

Data represent an average instrumental measurement of a composite sample.

Saline-sodic soil was added with 0.3% cow manure (w/w) as an organic fertilizer, mixed, and filled in a cement ring (80 cm diameter×40 cm height) for 100 kg/cement ring. Rice husk biochar was added to cement rings at rates of 0 (control), 0.3, 0.6, 1.8, and 3.0% (w/w), which are equivalent to 0, 0.5, 1, 3, 5 kg/m², respectively. All planting media in cement rings were thoroughly mixed, filled with water (EC of 0.53 dS/m⁻¹, pH 7.48), and incubated for 14 days. KDML105 rice at the age of 45 days was transplanted into each cement ring (five hills per ring, three seedlings per hill). The distance between hills was 20 cm. Water levels in cement rings were maintained at 5-10 cm above the soil surface until one week before harvesting.

2.2 Measurements of survival, growth, yield, and yield components

At the maturity stage (117 days after sowing), the number of hills with rice plants in each cement ring was counted and calculated for survival percentage. The number of tillers per hill was counted. Then, rice plants were measured using a ruler from the soil surface to the flag leaf tip for shoot height before being removed from the soil. The rice plants were divided into the shoot and root parts, oven-dried at 80°C for three days and recorded for the shoot and root dry weights. Grain yield and yield components were determined from panicle length, the number of grains/panicles, grain filling percentage, grain weight/panicle, and 5-grain weight (as it was the maximum number of filled grains produced from the control without biochar adding).

2.3 Determination of Na^+ and K^+

Flag leaf of rice was measured for dry weight, cut into small pieces with scissors, and put in a test tube filled with 5 mL 70% HNO_3 . The test tube was incubated in a microwave digester for 30 min. Na^+ and K^+ in the extracts were determined using atomic absorption spectroscopy (ZEEnit 700 P, Analytik Jena, Germany).

2.4 X-ray microanalysis

Samples of rice husk biochar (the control) and those separated manually from the saline-sodic soil were examined under the magnification of 150x and 500x, respectively, for element compositions using a scanning electron microscope equipped with an energy dispersive X-ray spectrometer (SEM-EDX, JEOL, JSM-IT500HR, Japan). The accelerating voltage was 15 kV.

2.5 Soil sample analysis

Soil samples from each treatment in cement rings were randomly collected from topsoil (0-15 cm), air-dried, crushed, sieved through a 2 mm size mesh, and used for laboratory analyses for pH, OM, CEC, total N, total P, exchangeable K, extractable Na, ECe, and ESP, on the basis of the standard procedure of soil survey laboratory methods manual [12,34,35].

2.6 Statistical analysis

The experiment was conducted with a randomized complete block design of three replicates. Data were expressed as mean \pm standard error of the mean (SEM). Significant differences between treatments were examined with a one-way analysis of variance using Duncan's multiple range test (DMRT) by the SPSS software (v16.0) at the 5% level of significance.

3. Results

3.1 Survival and growth of rice

KDML105 rice grown in a saline-sodic soil without biochar additions survived only approximately 6%, and the survival rate significantly increased to 77%, 80%, and 100% when biochar was added to the soil at 0.6%, 1.8%, and 3.0% (w/w), respectively (Figure 1).

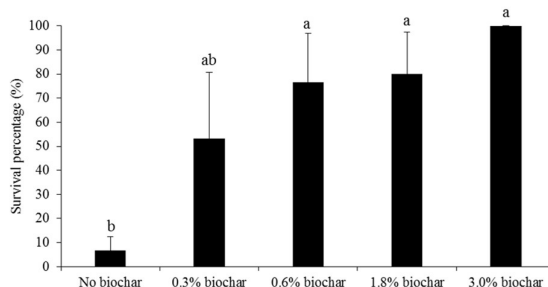


Figure 1 Survival percentages of KDML105 rice grown in saline-sodic soil with different rates of biochar amendment. Data are mean \pm SEM (n = 3). Bars with different letters are significantly different at $p < 0.05$ according to DMRT.

The rice plants grown in saline-sodic soil produced approximately two tillers per hill and adding biochar at rates of 0.3-3.0% (w/w) did not significantly change the number of tillers (Figure 2A). The height of the controlled rice was about 30 cm, and it increased significantly to an average of 60 cm when 0.3-3.0% (w/w) biochar was added to a saline-sodic soil (Figure 2B). The shoot dry weight of rice grown in a saline-sodic soil was about 1.2 g/hill, and it increased significantly approximately to 4 g/hill when 1.8% or 3.0% (w/w) biochar was added to the soil (Figure 2C). Root dry weight was approximately 0.4 g/hill for rice without biochar, and it increased significantly to an average of 1.2 g/hill after adding 0.6-3.0% (w/w) biochar to the soil (Figure 2D).

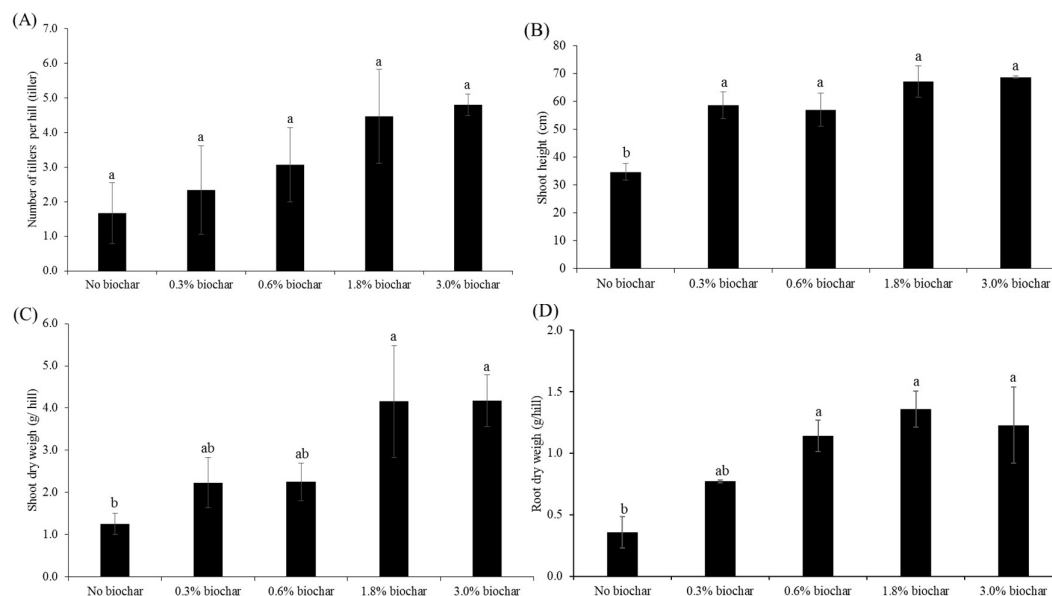


Figure 2 Number of tillers per hill (A), shoot height (B), shoot dry weight (C), and root dry weight (D) of KDML105 rice grown in saline-sodic soil with different rates of biochar amendment. Data are mean \pm SEM ($n = 3$). Bars with different letters are significantly different at $p < 0.05$ according to DMRT.

3.2 Yield and yield components of rice

KDML105 rice grown in saline-sodic soil without biochar had a panicle length of approximately 14 cm (Table 2). It produced approximately 25 grains/panicle with 37% grain filling in a panicle, and grain weight/panicle was approximately 0.2 g with an average weight of 5 grains of 0.09 g (Table 2). The biochar application at 0.3% (w/w) did not improve the yield and yield components of rice compared with the control (Table 2). The biochar amendment at 0.6% (w/w) did not increase panicle length and the number of grains/panicles compared with the control, but significantly increased grain filling percentage, grain weight/panicle, and 5-grain weight by 25%, 0.38 g, 0.022 g, respectively (Table 2). The biochar amendment at 1.8% (w/w) significantly increased panicle length, the number of grains/panicle, grain filling percentage, grain weight/panicle, and 5-grain weight by 3.4 cm, 20.9 grains, 30.7%, 0.49 g, 0.022 g, respectively, compared with the control, whereas adding biochar at 3.0% (w/w) significantly increased panicle length, the number of grains/panicle, grain filling percentage, grain weight/panicle, and 5-grain weight by 3.7 cm, 25.3 grains, 47.4%, 0.68 g, 0.03 g, respectively (Table 2). Among biochar treatments, the results revealed that the yield and yield components of KDML105 rice did not differ significantly between 0.3% and 0.6% (w/w) biochar amendments, whereas biochar amendments at 1.8% and 3.0% (w/w) significantly increased the panicle length compared with 0.3% and 0.6% (w/w) biochar (Table 2). The number of grains/panicles, grain filling percentage, grain weight/panicle, and 5 grain weight was significantly increased by 3.0% (w/w) biochar amendment compared with those of 0.3% (w/w) biochar (Table 2). Although there was no significant difference in the yield and yield components of KDML 105 rice between 1.8% and 3.0% (w/w) biochar amendments, only the application rate of 3.0% (w/w) significantly increased grain filling percentage and 5 grain weight of rice compared with 0.3% and 0.6% (w/w) biochar amendments (Table 2).

Table 2 Yield and yield components of KDML105 rice.

Treatment	Panicle length (cm)	Number of grains/panicle (grain)	Grain filling (%)	Grain weight/panicle (g)	5-grain weight (g)
No biochar	13.7 \pm 0.25 ^b	25.5 \pm 0.5 ^c	36.9 \pm 1.6 ^c	0.218 \pm 0.08 ^c	0.095 \pm 0.004 ^d
0.3% biochar	14.1 \pm 0.30 ^b	30.8 \pm 4.7 ^{bc}	57.1 \pm 3.9 ^{bc}	0.447 \pm 0.09 ^{bc}	0.102 \pm 0.006 ^{cd}
0.6% biochar	14.8 \pm 0.11 ^b	38.7 \pm 0.9 ^{abc}	61.9 \pm 1.6 ^b	0.602 \pm 0.03 ^{ab}	0.113 \pm 0.006 ^{bc}
1.8% biochar	17.1 \pm 0.10 ^a	46.4 \pm 7.5 ^{ab}	67.6 \pm 2.1 ^{ab}	0.712 \pm 0.14 ^{ab}	0.118 \pm 0.003 ^{ab}
3.0% biochar	17.4 \pm 0.24 ^a	50.8 \pm 3.1 ^a	84.3 \pm 0.2 ^a	0.893 \pm 0.05 ^a	0.126 \pm 0.001 ^a

Data are mean \pm SEM ($n = 3$). Values within a column of a parameter followed by different letters are significantly different at $p < 0.05$ according to DMRT.

3.3 Ion accumulation

Na^+ concentration in the flag leaf of rice grown in a saline-sodic soil was approximately 500 mg/Gross Dry Weight (gDW), and the Na^+ concentration was significantly reduced to approximately 150 mg/gDW, when 0.3–3.0% (w/w) biochar was added to the soil (Figure 3A). Na^+ concentration did not differ significantly in flag leaf among different rates of biochar amendment (Figure 3A). Biochar applications also significantly decreased K^+ concentration in flag leaf (Figure 3B). The value of the K^+/Na^+ ratio in the flag leaf of the control was approximately 1.9, and it was significantly increased after a 3.0% (w/w) biochar addition to the soil (Figure 3C).

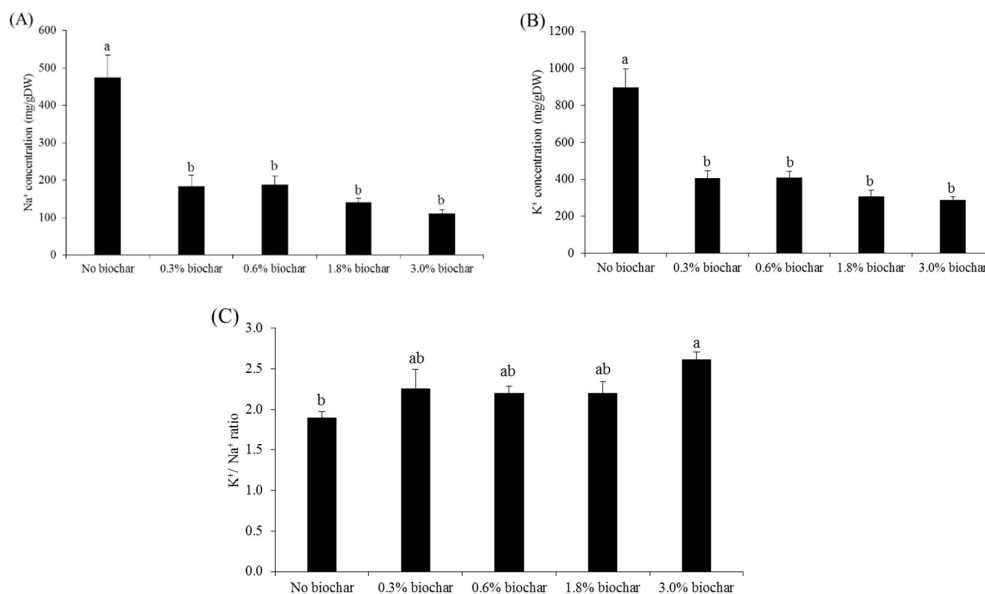


Figure 3 Na^+ concentration (A), K^+ concentration (B), and K^+/Na^+ ratio (C) in the flag leaf of KDML 105 grown in a saline-sodic soil with different rates of biochar amendment. Data are mean \pm SEM ($n = 3$). Bars with different letters are significantly different at $p < 0.05$ according to DMRT.

3.4 X-ray microanalysis

The SEM-EDX was used to quantify element compositions in rice husk biochar. The result revealed that the main elements in rice husk biochar were C, O, P, K, and Ca (Figure 4). Na^+ was detected only on biochar added to the saline-sodic soil (Figure 5). The SEM-EDX map showed that Na^+ was evenly distributed on the surface of biochar (Figure 6).

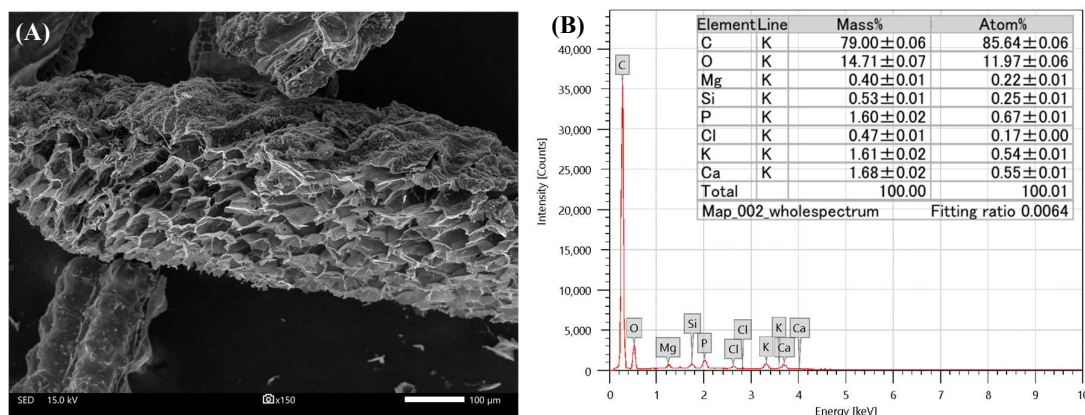


Figure 4 Scanning electron microscopy (SEM) image (A), and X-ray spectroscopy (EDX) spectra (B) of rice husk biochar at a magnification of 150x before being added to a saline-sodic soil.

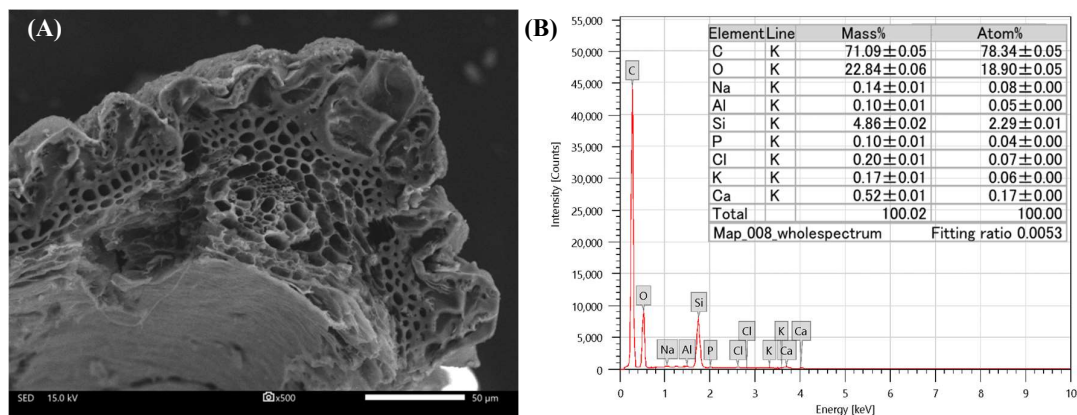


Figure 5 Scanning electron microscopy (SEM) image (A) and X-ray spectroscopy (EDX) spectra (B) of rice husk biochar at a magnification of 500x after being added to a saline-sodic soil.

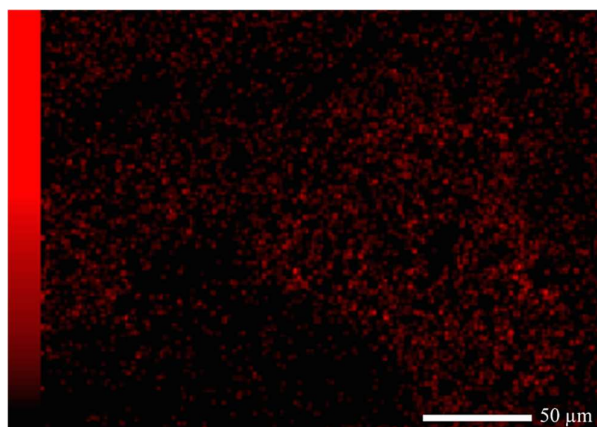


Figure 6 Scanning electron microscopy (SEM) image and X-ray spectroscopy (EDX) map of Na showing in red dots.

3.5 Physicochemical properties of a saline-sodic soil

The pH value was approximately 10 for the saline-sodic soil without adding biochar, and it was slightly reduced to 9.03-9.28 after biochar amendment at rates of 1.8% and 3.0% (w/w) (Table 3). These two rates of biochar amendment clearly increased OM and CEC values of the soil (Table 3). Total N and exchangeable K were also substantially increased compared to those of soil without biochar (Table 3). The values of soil salinity indicators, including extractable Na, ECE, and ESP, were clearly decreased after biochar amendment (Table 3).

Table 3 Physicochemical properties of the saline-sodic soil with and without biochar.

Treatment	pH	OM (%)	CEC (cmol/kg)	Total N (%)	Total P (%)	Exchangeable K (mg/kg)	Extractable Na (mg/kg)	ECe (dS/m ⁻¹)	ESP (%)
No biochar	10.4	0.12	1.10	0.009	0.007	73	609	8.34	354.66
0.3% biochar	10.5	0.11	1.20	0.009	0.007	67	365	4.72	185.04
0.6% biochar	10.0	0.13	1.30	0.013	0.008	85	269	3.06	123.95
1.8% biochar	9.28	0.30	1.50	0.018	0.010	101	218	2.49	88.30
3.0% biochar	9.03	0.34	1.90	0.022	0.009	211	142	1.92	42.56

Data represent an average instrumental measurement of a composite sample.

4. Discussion

KDML105 is the most popular aromatic rice variety in Thailand, and the demand is increasing for domestic and international markets [37]. The main region for cultivating this rice variety is in the northeastern region of the country; however, the growth and yield of rice were significantly reduced by salt-affected soils [1,18,24]. This study showed that under a saline-sodic soil, KDML105 rice survived by only 6% (Figure 1), indicating that

KDML105 rice is sensitive to salinity. The result agrees with the work of Nishimura et al. [38], who reported that the KDML105 survival rate was approximately 58% after 28-day-old seedlings being treated with 100 mM NaCl for 14 days. Similarly, Kanawapee et al. [39] found that KDML105 survived by about 76% when 14-day-old seedlings were salinized with NaCl solutions with an EC of 6 dS/m⁻¹ for three days following an EC of 12 dS/m⁻¹ for seven days. The salinity effect on rice depends on rice growth stages, salt concentrations, and duration of stress [7,39]. The low survival percentage of KDML105 in this study compared with that of Nishimura et al. [38] and Kanawapee et al. [39] could be explained by the differences in salt concentrations and duration of stress. In this study, KDML105 rice was exposed to a saline-sodic soil with an E_{ce} of 12.64 dS/m⁻¹, which is classified a severely salt-affected soil [24], at the age of 45 days until the harvesting stage. Consequently, a low survival percentage of KDML105 was reported because of the high salt concentration in soil and the long exposure period of the plant. Figure 2 showed that the saline-sodic soil also decreased shoot height, shoot and root dry weight of KDML105 rice. These results are compatible with the work of Theerakulpisut et al. [1], and Pattanagul and Thitisaksakul [18], who reported a significant decrease in KDML105 growth under salinity.

The effects of salinity on rice are related to osmotic effect, ionic effect, and nutrient deficiency [7,10]. The osmotic effect occurs when salt concentrations in salt-affected soils lower the water potential and reduce the water uptake, reducing cell elongation [5,40]. The over-accumulation of Na⁺ within the cell cytoplasm of roots and shoots has an ionic effect by inhibiting enzyme activities, resulting in protein synthesis inhibition [41]. Soil salinity causes nutrient deficiency by inhibiting root growth and interfering with root ion transporters [41,42]. Due to high Na⁺ concentration in salt-affected soils, Na⁺ can penetrate the root xylem along with the water movement via a symplastic and an apoplastic pathway [7,17,40,43]. Rice is generally classified as a salt-sensitive crop because it cannot control Na⁺ influx from soil salinity to the root and shoot, reducing survival, growth and productivity [7,17]. This study showed that applying rice husk biochar at rates of 1.8% and 3.0% (w/w) significantly increased the survival, shoot height, shoot and root dry weight of KDML105 rice under a saline-sodic soil (Figure 2). Therefore, a biochar addition could alleviate the negative effects of a saline-sodic stress on survival and growth of the rice plant. The results agree with that of Jin et al. [9] and Nguyen et al. [11], who discovered that growth of rice cvs. G9 and OM6162 were improved by adding biochar to the saline-sodic soil. Also, Ran et al. [10] reported that the shoot height of rice variety Changbai-9 was increased with biochar amendment.

The yield and yield components of KDML105 were significantly affected by the saline-sodic soil (Table 2), indicating harmful effects of saline-sodic soil on rice productivity. These results agree with previous reports [12,24], confirming that KDML105 is sensitive to salt stress. The reduction in rice's yield and components is possibly attributed to decreased pollen viability and stigmatic receptibility induced by salinity. Khatun and Flowers [44] reported that the pollen viability was 63%, 39%, and 0% when rice cv. IR36 was treated with 10, 25, and 50 mM NaCl, respectively, from one month after germination until the main tiller flowered, whereas the percentage of seed set decreased by 38%, 72%, and 100%, when the female plants were treated with 10, 25, and 50 mM NaCl, respectively. Rice husk biochar additions at 1.8% and 3.0% (w/w) significantly increased the panicle length, the number of grains/panicles, grain filling percentage, grain weight/panicle, and 5-grain weight with an average of 26.4%, 90.7%, 105.7%, 267.5%, and 27.0%, respectively, as compared to those of the controlled rice grown in the saline-sodic soil without biochar additions (Table 2). These results indicated that the negative effects of a saline-sodic soil on the yield and yield components of KDML105 rice could be reduced by rice husk biochar amendment. It is possible that 1.8% and 3.0% (w/w) biochar amendments could increase pollen viability and stigmatic receptibility in KDML105 rice. These results are consistent with that of Jin et al. [9], who found that adding 3% (w/w) peanut shell biochar to a saline-sodic soil increased the yield and yield components of rice cv. G9. Also, Ran et al. [10] reported that peanut shell biochar at 6.75 kg/m² improved yields of rice variety Changbai-9 grown in a saline-sodic soil.

A high Na⁺ concentration in the flag leaf of KDML105 rice under the saline-sodic soil was observed in the rice plant without biochar amendment (Figure 3). This result is compatible with the work of Suriya-arunroj et al. [22], who reported that high Na⁺ accumulation was found in young leaves, old leaves, and stems of KDML105 rice under salinity, and the Na⁺ concentration was increased with increasing salinity levels. A possible explanation for this is that Na⁺ in salt-affected soils entered the root xylem through a symplastic and an apoplastic pathway and was transported to the flag leaf of rice via the transpiration stream. High Na⁺ accumulation in the shoot was also observed in other rice varieties under salinity, such as G9 and Chanbai-9 [9,10]. These results correlate with previous findings that rice is a salt-sensitive species as it cannot efficiently control Na⁺ influx across the root, leading to rapid toxic Na⁺ concentration in the shoot [7,17]. Consequently, the decrease in Na⁺ accumulation could increase salt-tolerant abilities in rice.

Biochar is a carbon-rich material produced by the thermal decomposition of biomass under limited or absent oxygen conditions [25,26,45,46]. It is a high-value product from biorefineries using thermochemical technologies, including pyrolysis, gasification, torrefaction, and hydrothermal carbonization [25]. During heating processes, the chemical bonds of hemicelluloses, cellulose, lignin, and other OM in the biomass were

decomposed and rearranged, forming new functional groups [46]. The dominance of carboxylate and phenolate functional groups contributed to biochar's negative surface charge, enhancing its ability to hold and exchange nutrients [45,46]. These results showed that applying rice husk biochar at the rates of 0.3%-3.0% (w/w) significantly reduced Na^+ concentrations in the flag leaf of KDML105 rice under a saline-sodic soil (Figure 3A). The average Na^+ reduction induced by biochar amendment was approximately 67%, compared with the control. A possible explanation is that biochar adsorbed Na^+ in a saline-sodic soil due to the negative charges on its structure, thus reducing the amount of Na^+ availability in the rhizosphere. This was evident by SEM-EDX images (Figure 4). It was shown that Na^+ was detected only on rice husk biochar surface after being applied to a saline-sodic soil (Figure 4). Thus, adding 0.3%-3.0% (w/w) rice husk biochar could alleviate the ionic effect of salt stress in KDML105 rice via Na^+ adsorption abilities of biochar. Similar results were reported on rice genotypes Changbai-9, G9, OM6162, OM5451, Tep Hanh [9,10,28], and other crops such as potato [27]. Moreover, the high ability of Na^+ adsorption of rice husk biochar also improved the characteristics of the saline-sodic soil. This study showed that the values of extractable Na, ECe, and ESP of biochar-added soil were obviously reduced compared with those of a saline-sodic soil without biochar additions (Table 3). Note that aluminum (Al) was detected using SEM-EDX on the surface of rice husk biochar collected from the saline-sodic soil (Figure 5). Thus, the negative charge on biochar structure could play a crucial role in Al adsorption and reducing Al toxicity of the soil [47].

K^+ is an essential macronutrient for enzyme activities, cell homeostasis, and cell signaling [42,48]. Plants' ability to maintain a high K^+/Na^+ ratio is considered one of the salt-tolerant characteristics of rice [49,50]. This study showed that the K^+/Na^+ ratio in the flag leaf of KDML105 rice was significantly increased with 3.0% (w/w) biochar addition (Figure 3C). This could be explained by high K^+ availability to the plant from rice husk biochar (Table 1 and Figure 4-6). It is evident that the value of exchangeable K in the saline-sodic soil increased by 2.9 times after 3.0% (w/w) rice husk biochar amendment (Table 3). These results agree with that of Nguyen et al. [11], who reported that rice husk biochar at 2.5% (w/w) increased biomass of OM6162 rice under a saline-sodic soil by increasing soil nutrients such as K^+ and P.

Rice husk biochar amendment also positively improved the physicochemical properties of the saline-sodic soil. These results showed that the addition of 3.0% (w/w) biochar obviously increased OM and CEC values as well as the total N content of a saline-sodic soil (Table 3), indicating that biochar increased soil fertility of a saline-sodic soil. These results are consistent with the work of Nguyen et al. [11], who reported that rice husk biochar increased CEC and soil nutrients. The increased soil quality could stimulate root growth and facilitate the nutrient uptake of rice. Consequently, the negative effect of plant nutrient deficiency in KDML105 caused by a saline-sodic soil could be mitigated by biochar amendment.

5. Conclusion

The saline-sodic soil significantly reduced survival, shoot height, dry weight, and yield of KDML105 rice. Applications of rice husk biochar adsorbed Na^+ in the soil, reduced Na^+ accumulation in the shoot, increased K^+/Na^+ ratio, and improved soil fertility. Rice husk biochar amendment at the rate of 3.0% (w/w) potentially alleviated the negative effects of a saline-sodic stress, and increased KDML105 rice productivity.

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7. References

- [1] Theerakulpisut P, Bunnag S, Kong-ngern K. Genetic diversity, salinity tolerance and physiological responses to NaCl of six rice (*Oryza sativa* L.) cultivars. Asian J Plant Sci. 2005;4(6):562-573.
- [2] Anshori MF, Purwoko BS, Dewi IS, Ardie SW, Suwarno WB. A new approach to select doubled haploid rice lines under salinity stress using indirect selection index. Rice Sci. 2021;28(4):368-378.
- [3] Butcher K, Wick AF, DeSutter T, Chatterjee A, Harmon J. Soil salinity: a threat to global food security. Agron J. 2016;108:2189-2200.
- [4] Mukhopadhyay R, Sarkar B, Jat HS, Sharma PC, Bolan NS. Soil salinity under climate change: challenges for sustainable agriculture and food security. J Environ Manag. 2020;280:111736.
- [5] Munns R, Tester M. Mechanisms of salinity tolerance. Annu Rev Plant Biol. 2008;59:651-681.
- [6] Eynard A, Lal R, Wiebe K. Crop response in salt-affected soils. J Sustain Agr. 2005;27(1):5-50.

- [7] Singh RK, Flowers TJ. The physiology and molecular biology of the effects of salinity on rice. In: Pessarakli M, editor. Handbook of plant and crop stress. 3rd ed. Oxfordshire: Taylor and Francis Group; 2010. p. 899-939.
- [8] Armada RN, Yazlle MF, Irazusta VP, Rajal VB, Moraga NB. Potential of bioremediation and PGP traits in *Streptomyces* as strategies for bio-reclamation of salt-affected soils for agriculture. Pathogens. 2020;9:1-28.
- [9] Jin F, Ran C, Anwari Q, Geng YQ, Guo LY, Li JB, et al. Effects of biochar on sodium ion accumulation, yield and quality of rice in saline-sodic soil of the west of Songnen plain, Northeast China. Plant Soil Environ. 2018;64:612-618.
- [10] Ran C, Gulaqa A, Zhu J, Wang X, Zhang S, Geng Y, et al. Benefits of biochar for improving ion contents, cell membrane permeability, leaf water status and yield of rice under saline-sodic paddy field condition. J Plant Growth Regul. 2020;39:370-377.
- [11] Nguyen BT, Trinh NN, Le CMT, Nguyen TT, Tran TV, Thai BV, et al. The interactive effects of biochar and cow manure on rice growth and selected properties of salt-affected soil. Arch Agron Soil Sci. 2018;64:1744-1758.
- [12] Jedrum S, Thanachit S, Anusontpornperm S, Wiriyakitnatekul W. Soil amendments effect on yield and quality of jasmine rice grown on typic Natraqualfs, Northeast Thailand. Int J Soil Sci. 2014;9:37-54.
- [13] Wijitkosum S. Applying rice husk biochar to revitalize saline sodic soil in Khorat Plateau area - a case study for food security purposes. In: Singh JS, Singh C, editors. Biochar applications in agriculture and environment. 1st ed. New York: Springer; 2020, p. 1-31.
- [14] Prasertsuk S, Wijitkosum S. Innovative use of rice husk biochar for rice cultivation in salt-affected soils with alternated wetting and drying irrigation. Eng J. 2021;25(9):19-32.
- [15] Flowers TJ, Colmer TD. Salinity tolerance in halophytes. New Phytol. 2008;179:945-963.
- [16] Roy SC, Shil P. Assessment of genetic heritability in rice breeding lines based on morphological traits and caryopsis ultrastructure. Sci Rep. 2020;10(1):7830.
- [17] Faiyue B, Al-Azzawi MJ, Flowers TJ. A new screening technique for salinity resistance in rice (*Oryza sativa* L.) seedlings using bypass flow. Plant Cell Environ. 2012;35:1099-1108.
- [18] Pattanagul W, Thitisaksakul M. Effect of salinity stress on growth and carbohydrate metabolism in three rice (*Oryza sativa* L.) cultivars differing in salinity tolerance. Indian J Exp Biol. 2008;46:736-742.
- [19] Cha-um S, Kirdmanee C. Remediation of salt-affected soil by the addition of organic matter - an investigation into improving glutinous rice productivity. Sci Agric. 2011;68(4):406-410.
- [20] Thitisaksakul M, Tananuwong K, Shoemaker CF, Chun A, Tanadul O, Labavitch JM, et al. Effects of timing and severity of salinity stress on rice (*Oryza sativa* L.) yield, grain composition, and starch functionality. J Agric Food Chem. 2015;63:2296-2304.
- [21] Quan R, Wang J, Hui J, Bai H, Lyu X, Zhu Y, et al. Improvement of salt tolerance using wild rice genes. Front Plant Sci. 2018;8:2269.
- [22] Suriya-arunroj D, Supapoj N, Vanavichit A, Toojinda T. Screening and selection for physiological characters contributing to salinity tolerance in rice. ANRES. 2005;39(2):174-185.
- [23] Chunthaburee S, Sanitchon J, Pattanagul W, Theerakulpisut P. Alleviation of salt stress in seedlings of black glutinous rice by seed priming with spermidine and gibberellic acid. Not Bot Horti Agrobo. 2014;42(2):405-413.
- [24] Arunin S, Pongwichian P. Salt-affected soils and management in Thailand. Bull Soc Sea Water Sci Jpn. 2015;69:319-325.
- [25] Wijitkosum S., Jiwnok P. Elemental composition of biochar obtained from agricultural waste for soil amendment and carbon sequestration. Appl Sci. 2019;9:3980.
- [26] Bis Z, Kobylecki R, Scislowska M, Zarzycki R. Biochar-Potential tool to combat climate change and drought. Ecohydrol Hydrobiol. 2018;18:441-453.
- [27] Akhtar SS, Andersen MN, Liu F. Biochar mitigates salinity stress in potato. J Agron Crop Sci. 2015;201(5):368-378.
- [28] Phuong NTK, Khoi CM, Ritz K, Linh TB, Minh DD, Duc TA, et al. Influence of rice husk biochar and compost amendments on salt contents and hydraulic properties of soil and rice yield in salt-affected fields. Agronomy. 2020;10(8):1-23.
- [29] Parkash V, Singh S. Potential of biochar application to mitigate salinity stress in eggplant. Hort Sci. 2020;55(12):1946-1955.
- [30] Zhang Y, Ding J, Wang H, Su L, Zhao C. Biochar addition alleviate the negative effects of drought and salinity stress on soybean productivity and water use efficiency. BMC Plant Biol. 2020;20:1-11.
- [31] Soothar MK, Hamani AKM, Sootahar MK, Sun J, Yang G, Bhatti SM, et al. Assessment of acidic biochar on the growth, physiology and nutrients uptake of maize (*Zea mays* L.) seedlings under salinity stress. Sustainability. 2021;13:3150.

- [32] Karabay U, Toptas A, Yanik J, Aktas L. Does biochar alleviate salt stress impact on growth of salt-sensitive crop common bean. *Commun. Soil Sci Plant Anal.* 2021;52(5):456-469.
- [33] Ali S, Rizwan M, Qayyum MF, Ok YS, Ibrahim M, Riaz M, et al. Biochar soil amendment on alleviation of drought and salt stress in plants: a critical review. *Environ Sci Pollut. Res.* 2017;24:12700-12712.
- [34] U.S. Salinity Laboratory Staff. Determination of the properties of saline and alkali soils. In: Richards LA, editor. *Diagnosis and improvement of saline and alkali soils*. 1st ed. Washington: U.S. Department of Agriculture; 1954. p. 7-33.
- [35] Phetmak K, Anusontpornperm S, Kheoruenromne I, Thanachit S. Effects of chicken manure, perlite and rate of chemical fertilizer on virgin cane grown in a coarse-texture soil. *Khon Kaen Agr J.* 2019;47:1-14.
- [36] Pituya P, Sriburi T, Wijitkosum S. Properties of biochar prepared from acacia wood and coconut shell for soil amendment. *EJ.* 2017;21(3):63-76.
- [37] Yoshihashi T, Nguyen TTH, Kabaki N. Area dependency of 2-acetyl-1-pyrroline content in an aromatic rice variety, Khao Dawk Mali 105. *JARQ.* 2004;38(2):105-109.
- [38] Nishimura T, Cha-um S, Takagaki M, Ohyama K, Kirdmanee C. Survival percentage, photosynthetic abilities and growth characters of two indica rice (*Oryza sativa* L. spp. *indica*) cultivars in response to iso-osmotic stress. *Span J Agric Res.* 2011;9(1):262-270.
- [39] Kanawapee N, Sanitchon J, Lontom W, Theerakulpisut P. Evaluation of salt tolerance at the seedling stage in rice genotypes by growth performance, ion accumulation, proline and chlorophyll content. *Plant Soil.* 2012;358:235-249.
- [40] Horie T, Karahara I, Katsuhara M. Salinity tolerance mechanisms in glycophytes: an overview with the central focus on rice plants. *Rice.* 2012;5:11.
- [41] Tester M, Davenport R. Na⁺ tolerance and Na⁺ transport in higher plants. *Ann Bot.* 2003;91:503-527.
- [42] Shabala S, Cuin TA. Potassium transport and plant salt tolerance. *Physiol Plant.* 2008;133:651-669.
- [43] Faiyue B, Al-Azzawi MJ, Flowers TJ. The role of lateral roots in bypass flow in rice (*Oryza sativa* L.). *Plant Cell Environ.* 2010;33:702-716.
- [44] Khatun S, Flowers TJ. Effects of salinity on seed set in rice. *Plant Cell Environ.* 1995;18:61-67.
- [45] Mukherjee A, Zimmerman AR, Harris W. Surface chemistry variations among a series of laboratory-produced biochars. *Geoderma.* 2011;163(3-4):247-255.
- [46] Tomczyk A, Sokołowska Z, Boguta P. Biochar physicochemical properties: pyrolysis temperature and feedstock kind effects. *Rev Environ Sci Biotechnol.* 2020;19:191-215.
- [47] Xia H, Riaz M, Zhang M, Liu B, El-Desouki Z, Jiang C. Biochar increases nitrogen use efficiency of maize by relieving aluminum toxicity and improving soil quality in acidic soil. *Ecotoxicol. Environ. Saf.* 2020;196:110531.
- [48] Wu H, Zhang X, Giraldo JP, Shabala S. It is not all about sodium: revealing tissue specificity and signalling roles of potassium in plant responses to salt stress. *Plant Soil.* 2018;431:1-17.
- [49] Morales SG, Trejo TLI, Merino FCG, Caldana C, Espinosa VD, Cabrera BEH. Growth, photosynthetic activity, and potassium and sodium concentration in rice plants under salt stress. *Acta Sci Agron.* 2012;34:317-324.
- [50] Madee P, Chunthaburee S, Sanitchon J, Theerakulpisut P. Alleviation of salt stress effects on physiology of leaves in two cultivars of pigmented rice by application of spermidine. *Asia Pac J Sci Technol.* 2018;23(2):1-13.