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Effects of Biochar Amendments on Rice Growth and Metabolic Response to Salinity Stress in Salt-Affected Soils

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Abstract
Exploring cheaper and sustainable strategies for managing salt-affected soils remains crucial in irrigated areas. Many researchers recommended using gypsum as material for reclamation salt-affected soils, which are costly and unavailable for most farmers. In this study, we used biochar derived from the common materials found in the irrigated areas that are locally available and less utilized as a substitute for gypsum for sustainable reclamation of salt-affected soils. Salt-affected soils (saline, saline-sodic and sodic) and unaffected were collected from the Watari Irrigation project, Kano State Nigeria using farmers' perceptions about the salinity situations of the irrigation scheme and existing legacy map data. The biochar used for this study were made using the Typha grass, rice straw, and rice bran samples collected from the same area. Thirty-six (36) containers (plastic pots) with a diameter of 27.5cm and height of 45cm each were filled with eight-kilogram dried soils, including non-saline, saline, and saline-sodic. We measured plant height, and normalized difference vegetation index (NDVI) at interval of 14 days. Number of tillers per plant were also measured during tillering stage at interval of 14 days. Stover dry weight, grain dry weight, electrolytic leakage and proline contents were measured after the end of the experiment. The results revealed a high significant effect ($p<0.01$) of biochar amendments in remediating different forms of salt affected soils. Saline sodic soil was effectively managed when using Typha biochar with a mean value very close to non-saline soil. Rice straw and rice bran reduces the salinity levels particularly in sodic soil. Therefore, biochar from different source could be used in addressing salt-specific problems rather than using a uniform treatment.

Keyword: Biochar, Growth, Salinity, Soil, Response, Rice,

INTRODUCTION
One of the major agricultural problem worldwide is caused by soil salinization, mainly in arid and semi-arid regions, which is adversely affecting soil properties and plant physiology (Rajput *et al.,* 2015). It is a significant constraint limiting agricultural productivity in nearly 20% of the cultivated and irrigated area worldwide (Zheng *et al.,* 2001). Similarly, it has been projected that by 2050, the world population will cross 9 billion marks, an increase of 57% in food production would be required which (Wild, 2003; FAO, 2011). Therefore, soil salinity

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significantly decreases agricultural productivity and challenges the agricultural capacity to sustain an increasingly growing population (Kopittke *et al.,* 2019). Consequently, putting a significant food security risk and making the way challenging to accomplish United Nations Sustainable Development Goals by 2030. These challenges have led researchers to investigate more ways to channelize sustainable techniques to meet future demands.
On a global scale, salinization is causing the loss of arable land area of about 2000 ha per day, contributing 1 to 2% agricultural soil losses every year worldwide (Zaman *et al.,* 2018). Therefore salt-affected areas will increase in the future due to salinization, such as increasing water tables, over-irrigation, flooding, silting, and seepage (Suhani *et al.,* 2020). Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield (Khan and Panda 2008). Also high exogenous salt concentrations affect seed germination, water deficit, ion imbalance of the cellular ions resulting in ion toxicity and osmotic stress. As previously reported, salt stress caused an inhibition of growth and development, reduce photosynthesis, respiration, and protein synthesis in many crop species (Meloni *et al.,* 2003; Pal *et al.,* 2004).

Photosynthesis is the most critical process that is affected in plants growing under saline conditions. Reduced photosynthesis under salinity is attributed to stomata closure leading to a reduction in intercellular CO_2 concentration and non-stomata factors. There is strong evidence that salt affects photosynthetic enzymes, chlorophylls, and carotenoids (Stepien and Klobus, 2006). Moreover, soil salinity affects osmotic and leaf water potential, transpiration rate, leaf temperature, and relative leaf water content in rice (Ashraf and Foolad, 2007). Early studies conducted under controlled conditions revealed that salt injury in rice plants is caused by osmotic imbalance and chloride ions accumulation (Clarkson and Hanson, 1980). Recent studies indicated that elevated concentrations of salts in the soil are harmful to the soil ecosystem, which adversely affects plant physiology, microflora, and soil-dwelling organisms (Skarkova *et al.,* 2016). Therefore this study is aimed to explore biochar derived from available organic materials in irrigated areas for treating salt-affected areas.

MATERIALS AND METHODS

Pre-Transplanting Activities

Salt-affected soils (saline, saline-sodic and sodic) and unaffected soil were collected from the Watari Irrigation project, Kano State Nigeria using farmers' perceptions about the salinity situations of the irrigation scheme and legacy data from Bashir *et al.* (2019). The soils were air-dried, crushed gently, sieved to remove impurities, and store for later use. The biochar used for this study were made using the Typha grass, rice straw, and rice bran samples collected from the same area.

Experimental Design

Thirty-six (36) containers (plastic pots) with a diameter of 27.5cm and height of 45cm were filled with eight-kilogram dried soils, including non-saline, saline, and saline-sodic. Each pot was filled with 20 grams of the biochar produced from the Typha, rice-straw, and rice-brand in a completely randomized design (CRD) replicated three times. Phosphate and potash fertilizer were used as the basal fertilizers following the recommendation for rice. Urea was applied in three equal splits i.e., at planting (basal), at 21 and 42 days after transplanting. The dosages of N, P and K were 0.85 g/pot (N), 0.43 g/pot (P_2O_5) and 0.26 g/pot (K_2O), respectively. The pots were watered to their field capacity and left for two days to equilibrate before transplanting. One seedling of FARO-44 raised in nursery was transplanted to each pot and placed in a screen house.

Measurement of Morphological Parameters
The seedlings were for each pot were used to measure plant height, and normalized difference vegetation index (NDVI) at interval of 14 days. Number of tillers per plant were also measured during tillering stage at interval of 14 days. Stover dry weight and grain dry weight from each replication were measured after drying the harvested samples.

Determination of Electrolytic Leakage
From each pot fresh leaf of the rice at harvest were cut and washed with deionized water three times to remove surface-adhered electrolytes. The Leaves were placed in closed tubes containing 5 cm^3 of deionized water and incubated at 10°C for 24 h. Subsequently, the initial electrical conductivity of the solution (EC1) was measured using electrical conductivity meter. The samples were then incubated in a water bath at 95°C for 20 min to release all electrolytes, and allowed to cool 25°C and their final electrical conductivity (EC2) was measured. The electrolyte leakage (EL) was calculated from EL = (EC1/EC2) × 100 (%).

Determination of Proline Content
The proline content was determined as described by Bates *et al*. (1973). Briefly: One gram (1.0 g) of each fresh radical and plumule were homogenized using 10 cm^3 of 3% aqueous sulphosalicylic acid. Then it was filtered through Whatman No. 2 filter paper. Two ml of filtrate was poured into a test tube, and mixed with a solution containing 2 ml of acid ninhydrin and 2 $cm^3$of glacial acetic acid. The mixture was vortexed for 5 seconds and autoclave at 100°C. Then after 1 hour the samples were removed, and the reaction was terminated on ice. To the solution Four cm^3 of toluene was added and vortexed for 15–20 seconds. The chromophore containing free proline was aspirated from the aqueous phase in a test tube and warmed to room temperature. The absorbance was measured at 520 nm using 96 wall plate microplate reader. The actual proline content (concentration) was calculated using Standard curve.

Statistical Analysis
A statistical evaluation of the experiment was made using the analysis of variance (ANOVA) and the values obtained were compared in further detail, using an LSD (least significant difference) test at the significance level $P < 0.05$. Statistical analyses were performed using JPM statistical package.

RESULTS

Influence Biochar Amendments on Rice Performance

Plant Height
Effects of Soil Salinity Level and Biochar Sources on Plant Height at 4 WAT was presented in Table 1 and the results shows that there is no significant effect ($p>0.05$) of the salinity level, biochar amendments, and their interactions on plant height at four weeks after transplanting (4 WAT). Compared to non-saline soils (17.83 cm), the application of Typha-based amendment in saline-sodic soil produced the tallest plant that is very close to the control. However, using rice straw and rice bran biochar has not improved the plant height compared to control with a mean value of 9.50 and 6.67, respectively.

Surprisingly, at five weeks after transplanting (Table 1), a significant effect ($p<0.050$) was observed in salinity level with no significant effects ($p>0.05$) on biochar types and their interaction ($p>0.05$). Regardless of the biochar type, application of the biochar produced

statistically similar rice height (20.8 cm) with control (non-saline) soil. Though, biochar application did not influence the height of the rice after application with a mean value of 13.0 and 13.4 for sodic and saline soil respectively.

Number of Tillers

Table 1 shows that there is a highly significant effect ($p<0.01$) of salinity level, biochar amendment, and their interaction on the number of tillers per plant at 4WAP, application of rice bran in non-saline soil tend to produce a greater number of tillers followed by rice straw and then Typha. Saline soil, these were found to be statistically with rice bran in saline-sodic, rice straw in saline-sodic and Typha in saline-sodic, and its statistically different intern of the number of tillers with rice bran in saline soil, rice straw in saline soil, and Typha in saline soil followed by Typha in sodic soil, rice straw in sodic soil and rice bran in sodic soil.

Significant effects of salinity level, biochar amendment, and interaction were observed for the number of tillers at five weeks after transplanting (Table 1). The largest number of tillers was produced when using rice bran for non-saline soil (57.03). Application of Typha in sodic soil tends to produce the number of tillers close to that of control (39.87), and plant with the least number of tillers was found when rice bran (2.83) and rice straw (4.17) were applied on saline soil. And saline-sodic soil also produces the number of tillers close to that of non-saline (38.87).

Normalized difference vegetation index (NDVI).

The result presented in Table 2, shows a significant effect of salinity level on NDVI at 4WAP, here saline- sodic tend to have NDVI very close to control/ non- saline followed by sodic soil and then saline soil. And also, there is no significant effect of Biochar amendment and Biochar amendment interaction with salinity level. The result presented in Table 3b shows a significant effect of salinity level on NDVI at 5WAP; saline-sodic also tends to have NDVI very close to non- saline-filled by sodic soil and then saline soil. And there is no significant effect of Biochar amendment and Biochar amendment and salinity level interaction.

Influence Biochar Amendments on Dry Seed and Straw Weights

Table 2 shows a significant effect of salinity level, biochar amendment, and their interaction on seed weight per plant. The plant with a large amount of seed weight was produced when using Typha for non-saline (7.53g) and application of Typha in both sodic (6.93g). Similarly, saline-sodic (6.67g) tend to produce seed weight close to that of non-saline (7.53g), plants with a small amount of seed weight were found when rice bran was applied in saline-sodic (1.77g) and saline soil (1.10g).

The result presented in Table 3 shows a significant effect of salinity level, biochar amendment, and their interaction on dry straw weight per plant. The plant with a large quantity of straw was produced when using rice bran for non-saline (57.03g), and application of Typha on both sodic and (39.87g) and saline-sodic (38.87g) produce straw almost close to that of non-saline, and low quantity of straw was found when rice bran is applied to saline soil (4.17g).

Influence Biochar Amendments on Metabolic Responses to Salinity Stress

The result shows a highly significant effect of salinity level, biochar amendment, and their interaction on electrolytic leakage (Table 3). The plant with little quantity of electrolytic leakage was produced when using Typha for non-saline (36.77), and application of Typha (99.71) and rice straw (98.62) both in sodic soil will give the largest quantity of electrolytic

leakage. Saline-sodic soil produced electrolytic leakage close to that of sodic soil and then followed by saline soil.

Regarding proline content, there is a significant effect ($p<0.01$) of salinity level, biochar amendment, and their interaction on proline content (Table 4). The plant with a low quantity of proline was produced when using Typha for non-saline (25.62), and a large quantity of proline was found when rice bran was applied in both saline-sodic (128.07) and saline soil (112.28). Sodic soil tends to have a quantity almost similar to that of control (41.37).

Table 1: Effects of Soil Salinity Level and Biochar Sources on plant height

Parameters	Plant Height at 4 WAT				Plant Height at 5 WAT			
Salinity Level / Biochar	Rice Bran	Rice Straw	Typha	Salinity Mean	Rice Bran	Rice Straw	Typha	Salinity Mean
Non-Saline	16.33	17.5	17.83	17.2	20.5	20.5	22.67	21.2a
Saline	15.33	11.33	10.33	12.3	16.73	12.1	11.4	13.4b
Saline Sodic	16.67	17.57	17.83	17.4	21.5	20.9	20	20.8a
Sodic	9.5	6.67	14.83	10.3	8.5	10.07	20.43	13.0b
Biochar Mean	14.5	13.3	15.2		16.8	15.9	18.6	21.2a
LSD salinity	2.28^{ns}				3.10*	20.5	22.67	
LSD biochar	2.63 ^{ns}				3.58 ^{ns}			
LSD biochar x salinity	4.56 ^{ns}				6.19 ^{ns}			

LSD means least significant difference; ns means not significant at 5 % probability level; * means significant at 5 % probability level; WAT means weeks after transplanting.

Table 2: Effects of Soil Salinity Level and Biochar Sources on number of tillers

Parameters	Number of Tillers at 4 WAT				Number of Tillers at 5 WAT			
Salinity Level / Biochar	Rice Bran	Rice Straw	Typha	Salinity Mean	Rice Bran	Rice Straw	Typha	Salinity Mean
Non-Saline	42.0	32.67	25.33	33.3	57.03	43	38.67	57.03
Saline	13.67	6.33	5.00	8.3	2.83	4.17	0.00	2.83
Saline Sodic	25.33	21.33	23.0	23.2	35.07	26.6	38.87	35.07
Sodic	3.33	2.67	22.33	9.4	7.70	10	39.87	7.7
Biochar Mean	21.1	15.8	18.9		25.7	20.9	29.4	25.7
LSD salinity	4.36**				5.37*			
LSD biochar	5.04*				10.82*			
LSD biochar x salinity	8.73**				18.74**			

LSD means least significant difference; * means significant at 5 % probability level; ** means significant at 1 % probability level; WAT means weeks after transplanting.

Table 3: Effects of Soil Salinity Level and Biochar Sources on normalized difference vegetation index (NDVI)

Parameters	NDVI 4 WAT	NDVI at 5 WAT

Salinity Level / Biochar	Rice Bran	Rice Straw	Typha	Salinity Mean	Rice Bran	Rice Straw	Typha	Salinity Mean
Non-Saline	0.71	0.62	0.67	0.71	0.73	0.73	0.67	0.73
Saline	0.30	0.29	0.22	0.30	0.39	0.26	0.20	0.39
Saline Sodic	0.49	0.62	0.7	0.49	0.67	0.71	0.69	0.67
Sodic	0.37	0.20	0.55	0.37	0.29	0.31	0.60	0.29
Biochar Mean	0.47	0.43	0.54	0.47	0.52	0.50	0.54	
LSD salinity	0.08*				0.09*			
LSD biochar	0.09 ns				0.10 ns			
LSD biochar x salinity	0.16 ns				0.17 ns			

LSD means least significant difference; ns means not significant at 5 % probability level; * means significant at 5 % probability level; WAT means weeks after transplanting.

Table 4: Effects of Soil Salinity Level and Biochar Sources on dry seed and stover weights

Parameters	Dry seed weight (gram)				Dry stover weight (gram)			
Salinity Level / Biochar	Rice Bran	Rice Straw	Typha	Salinity Mean	Rice Bran	Rice Straw	Typha	Salinity Mean
Non-Saline	5.47	6.13	7.53	5.47	57.03	43	38.67	46.23
Saline	1.10	PD	PD	1.10	4.17	PD	PD	4.17
Saline Sodic	1.77	3.13	6.77	1.77	35.07	26.6	38.87	33.51
Sodic	2.07	2.63	6.93	2.07	7.7	10	39.87	19.19
Biochar Mean	2.60	3.96	7.08		25.99	26.53	39.14	
LSD salinity	0.97*				5.37*			
LSD biochar	1.12*				10.82*			
LSD biochar x salinity	1.94**				18.74**			

LSD means least significant difference; * means significant at 5 % probability level; ** means significant at 1 % probability level; PD means plant died.

Table 5: Effects of Soil Salinity Level and Biochar Sources on electrolytic leakage and proline content

Parameters	Electrolytic Leakage				Proline Content (µmol/gram)			
Salinity Level / Biochar	Rice Bran	Rice Straw	Typha	Salinity Mean	Rice Bran	Rice Straw	Typha	Salinity Mean
Non-Saline	57.84	84.6	36.77	59.74	26.17	34.83	25.62	28.87
Saline	90.32	PD	PD	90.32	112.28	PD	PD	112.28
Saline Sodic	93.54	90.51	93.76	92.60	128.07	68.11	58.36	84.85
Sodic	97.08	98.62	99.71	98.47	44.9	108.44	41.37	64.90
Biochar Mean	84.70	91.24	76.75		77.86	70.46	41.78	
LSD salinity	1.27**				9.70*			
LSD biochar	1.47*				11.61*			
LSD biochar x salinity	2.55**				19.40*			

LSD means least significant difference; * means significant at 5 % probability level; ** means significant at 1 % probability level; PD means plant died.

DISCUSSION

Variability in response observed between the different salt-affected soils in terms of rice performance and other physiological responses could be due to the differences in

composition between the biochar materials. Saline soil was found to have the least performance with high metabolic response to salinity stress compared to other soil types. Though, the problem become worst when using either rice straw or rice bran derived biochar for reclamation of the soil. This agrees with the findings of several scholars who reported that salinity affects plant growth and development by interrupting its metabolic processes, leads to decreased productivity. Salinity in the soil causes osmotic stress (primary effect), and later this results in cellular ions imbalance triggering ion toxicity (secondary effect) (Saberali and Moradi, 2019). The high concentrations of certain salts, including Na^+, Cl^- and HCO_3^- are toxic to many plant species (Safdar *et al.* (2019).

The crops require appropriate conditions for the augmented yield to create economic advantages in agroecosystems (Srivastava *et al.,* 2017). Salts abundance in the root zone affect plant growth and development. The severity of salinity is different for sensitive plants and salt-tolerant plants. At the lower salinity levels, the plant yield remains unaffected (Maggio *et al.,* 2001), whereas higher salinity levels highly affect and decline plant growth (Lakhdar *et al.,* 2009). Yadav *et al.* (2019) found that the elevated concentrations of cations and their salts in soil results in the generation of external osmotic potential that reduces the influx of water into the plant root cells, leading to declined growth development.

Using Typha biochar proven to be promising in managing saline sodic soils than other biochar sources with a yield very close to control. This confirmed the finding of Rezaie *et al. (*2019) who reported biochar has the potential which imparts salt tolerance to plants by improving physical (soil structure, porosity, pH, EC, water holding capacity, hydraulic conductivity, SOC), chemical (Na^+ absorption, cation-anion exchange, nutrients, enzymatic activities), and biological (MBC, N2-fix-ation, CFU) properties of soil. Other Studies (Kim *et al.,* 2016; Amini *et al.,* 2016) reported that the use of biochar amendments in saline soils reveled a conforming improvement in the water retention capacity, percentage of water, and hydraulic conductivity, with a considerable increase in Ca content.

According to Akhtar *et al*. (2015a), biochar was able to ameliorate saline soil by absorbing Na^+ from the soil and imparting tolerance to the plant by increasing K^+ and decreasing the Na^+/K^+ ratio in plant xylem. It was also found that the incorporation of biochar in saline soil increased photosynthetic rate, root length and volume, shoot biomass, tuber yield midday leaf water potential, stomatal conductance, and decreased Absecic acid (ABA) concentration in the leaf and xylem sap of potato plant as compared with unamended control. Similarly, studies on saline soils amended with biochar showed mitigating effect like increased MBC content; enhanced activities of urease, invertase, and phosphatase in rhizospheric soils; enhances soil microbiota; soil organic matter; CEC and inhibits exchangeable Na (Rizwan *et al.,* 2016; Kanwal *et al.,* 2018). Biochar application (2.5%w/w) can alleviate salt stress with its high salt sorption capacity and increase the K^+/Na^+ ratio in the soil (Rezaie *et al.,* 2019).

Further studies have shown that the salt stress alleviating capacity can be aggravated by supplementing biochar with other inoculants and additives. Biochar application, in combination with suitable microbial inoculants like plant growth-promoting rhizobacteria (PGPR), is more efficient for the growth of plants as compared to single biochar amendments in saline conditions (Akhtar *et al.,* 2015b, 2015c). The combined application of biochar with AMF inoculation resulted in increased plant biomass as reported by Hammer *et al*. (2015). In another study conducted by Iwai and Kruapukee (2017), the application of earthworms in saline soil supplemented with biochar (1% of biochar per 1 kg of dry soil) showed augmented crop yield.

CONCLUSION

In conclusion, rice growth and yield could be enhanced in salt-affected soils when using biochar particularly Typha based biochar for saline sodic soils. Therefore, appropriate biochar will help in addressing specific salinity problems in most of the irrigated areas.

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