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MITIGATION EFFECT OF BIOCHAR ON SORGHUM SEEDLING GROWTH UNDER SALINITY STRESS

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Abstract

Salinity stress one of the primary abiotic stress affected growth and establishment of crops. The study was conducted to examine if biochar can mitigate the negative effects of salinity on sorghum (*Sorghum bicolor* L) seedlings. The test was a completely randomized design with three replications arranged as a factorial design. The soil was treated with sodium chloride at levels of 0.8, 4.1, and 7.7 dS m^{-1}. The saline soil was treated with four biochar rates of 0, 2.5, 5, and 10% (w/w). Seedling emergence percentage, root length (RL), shoot length (ShL), root fresh weight (RFwt), shoot fresh weight (SFwt), root dry weight (RDwt), shoot dry weight (SDwt), and relative water content (RWC) were significantly affected by salinity stress, biochar and by the interaction between salinity and biochar. However, biochar soil amendment proved to be more useful to alleviate the effect of salinity on seedling growth parameters. Moreover, among all biochar levels applied in this investigation. 5% biochar level has a high impact on mitigating salt stress. These findings evidenced that using biochar in suitable amount could mitigate the impacts of salt stress. However, biochar application at a high level may have negative influences.

Key words: Abiotic stresses, Biochar, Salinity, Seedling growth, Sorghum.

Introduction

Saline soils are one of the critical determinants limiting crop production (Wassmann *et al*., 2009). Presently, around 20% of the whole agricultural areas are influenced by salinity (Anon., 2015). All agricultural soils and irrigation water contain salt. The amount and type of salts present depend on the makeup of both the soil and the irrigation water. The soil not considered saline unless the salt concentration in the root zone is high enough to prevent optimum growth and yield (Anon., 2015). Salinity significantly affects different plant growth stages, the seedling growth stage is recognized as more sensitive growth stage in most plant species (Ibrahim *et al*., 2016a). Seed germination is an essential factor narrowing the establishment of the crop under the saline soil. These conditions may induce significant decreases in the emergence rate and percentage. Many investigations were conducted to examine the effects of salinity on seed germination (Ibrahim *et al*., 2016b, Ibrahim, *et al*., 2016a, Nimir *et al*., 2014).

Many organic materials are applied to mend the saline soils; recently, biochar has much attention as one of the critical soil amendment. Some of the information shows that biochar application is valuable in increasing growth, physiological and biochemical characteristics of the plant exposed to salinity (Akhtar *et al*., 2015, Amini *et al*., 2016).Biochar can change mineral release, holding or immobilization by its surface properties (Blackwell *et al*., 2010). Biochar might be positively affect plant nutrition in various ways such as sorption of nutrients, particularly to the inner facade parts of biochar particles, could block their fixation onto soil colloids, by which nutrients are directly immobilized (Agegnehu *et al*., 2017, Bera *et al*., 2016, Hammer *et al*., 2015).

Various of investigations revealed that biochar amendment has significant impacts on several of the biological processes which happen in plants, before-mentioned as improved germination percentage in several plants under natural conditions (Solaiman *et al*., 2012). Nevertheless, some investigations have also observed an increase in salt concentrations with biochar use at big amount. Furthermore, there is insufficient information on the long-term performance of saline soils treated with biochar treatments (Lashari *et al*., 2015, Thomas *et al*., 2013).

Sorghum (*Sorghum bicolor* L.) is the fifth most important cereal crop grown in the world. Sorghum is grown on approximately 44.0 million hectares in 99 countries (Anon., 2015). Sorghum has potential uses such as food (grain), feed (grain and biomass), fuel (ethanol production), fiber (paper), fermentation (methane production) and fertilizer (utilization of organic byproducts) (Nimir *et al*., 2014). Sorghum is a principal source of energy, protein, vitamins, and minerals for millions of people in the semi-arid regions (Jacob *et al*., 2013).

In this study, we hypothesized that soil treated by the biochar amendment could improve the crop establishment through increase the seedling emergence, and improve seedling growth characteristics of sorghum. The objective of this investigation was limited to examine the impact of biochar application as a possible amendment to enhance the seedling growth of forage sorghum grown in saline area.

Materials and Methods

In this study, Kambal, a sorghum variety, kindly provided by Sudan Agricultural Research Corporation, was used. The seeds were less than 18-months old and kept in brown paper bags to maintain good germination. Before the study, the seeds of uniform color, size, and shape were selected and sterilized with 2.5% sodium hypochlorite for 4-5 min, then flushed with distilled water three times.

The soil used in this study was the 0- to 20-cm layer of a Typic fluvaquents Entisols collected from the surface of sandy loam soil (0–20 cm) of the Experimental Farm of Yangzhou University (32,30° N, 119,43° E+), Jiangsu Province, China. The soil was dried and sieved with a 5 mm sieve. Then the soil was spread at a thickness of about 50 mm over a piece of polyethylene sheet. Soil and biochar suspensions were prepared in deionized water at a ratio of 1:2 (soil: water). The suspension was moved and allowed to stand overnight. After that, the electrical conductivity (EC) of the supernatant solutions were 0.26 and 1.4 dSm^{-1} in the soil and biochar respectively measured with an EC meter (TZS-EC-I, Zhejiang Top Instrument Co., Ltd., Hangzhou, China). The soil and biochar samples were analyzed for pH (1:1 in water) using a pH meter (Bench Top pH-meter by 3B Scientific - U33100 - 1011690 - pH meter), The determination of soil organic carbon is based on the (Walkley & Black, 1934) chromic acid wet oxidation method (Nelson & Sommers, 1996), nitrogen was estimated following the Kjeldahl method described by (Labconco, 1998), available phosphorus (P) following Micro-Vanadate-Molybdate method (Olson, 1954), available potassium (K) following neutral ammonium acetate extract method determined by flame photometer (Chapman & Pratt, 1962). The soil contained 1.22% organic matter, 0.96 g kg^{-1} total N, 8.3 mg kg^{-1} P, and 72.4 mg kg^{-1} soil test K with pH 7.4. The biochar contained 39.84% organic matter, 7.43 g kg^{-1} total N, 13.88 mg kg^{-1} P, and 130.6 mg kg^{-1} soil test K.

The experiment was conducted twice in a controlled environment at Yangzhou University, Jiangsu Province, China, in the summer season of 2017/2018. The study was designed as a factorial experiment arranged in a completely randomized design with three replications for each experimental unit. The treatments were included four different levels of biochar [0, 2.5, 5, and 10% (w/w)] designed as Bc0, Bc1, Bc2, Bc3 respectively and three levels of salinity (0.8, 4.1, and 7.7 dS m^{-1}) designed as S1, S2, S3 respectively. The saline soils were made by incorporating sodium chloride into the non-saline soils. Wheat straw biochar was applied, which was pyrolyzed at 500°C in a vertical oven produced of refractory bricks in Sanli New Energy Company, Henan Province, China. With such a technology, 30% of wheat straw dry matter would be extracted to be transformed to biochar. Each pot (9.5 cm in diameter × 8.5 cm in depth) was filled with 300 g dry soil. Ten seeds were sown at the seeding depth of 1.5 cm. All the pots were placed in the growth chamber (Model PYX-300G-B, Yangzhou Yiwei Automatic Instrument Co. Ltd, Jiangsu, China) for two weeks at 30 / 25ºC day/ night. The relative humidity was maintained at 55-60% and 14/10 h day/ night under a photoactive radiation (PAR) of 500 W m^{-2}. The Hoagland solution was added once every five days.

Observations and measurements

Seedling emergence (%): Seedling emergence percentage were calculated after seven days as:

$$\text{Emergence (\%)} = \frac{\text{Emerged seedlings}}{\text{Total number of seeds}} \times 100$$

Seedling growth measurements: Two weeks after sowing, seedlings were collected, and the following measures were examined:

Root length (RL) and shoot length (ShL): After three weeks from planting, five plants randomly harvested. Root and shoot were separated. The lengths were measured using measuring ruler.

Root (RFwt) and shoot (SFwt) fresh weight: SFwt and RFwt were measured from 5 plants from every pot.

Root (RDwt) and shoot (SDwt) dry weight: SDwt and RDwt were measured from 5 plants from every pot after drying at 80°C for two days to fixed weight.

Relative water content (RWC): RWC was determined according to(Mäkelä *et al*., 1998). Leaflet samples were collected from the studied plants. The fresh weight (FW) was measured, and the samples were kept in water for four h for the saturation weight (FWsat) determination. The samples were after that dried at 75°C for 60 h for dry weight (DW) and. RWC was calculated as follows:

$$\text{RWC \%} = [(\text{FW} - \text{DW}) / (\text{FWsat} - \text{DW})] \times 100$$

Data analysis

The study was performed in two times, and results of statistical analysis showed there were no significant differences in all factors among the two times. So, the mean of the two times of each variable was applied for statistical analysis. The data were analyzed following the method of (Gomez & Gomez, 1984) using the statistical package of MSTAT-C (Freed *et al*., 1991). Means compared by the Tukey's range test when F values were significant ($p \leq 0.05$) by an ANOVA-protected test.

Results

Seedling emergence percentage: Seedling emergence percentage was significantly influenced by salinity and biochar (Table 1). Salinity decreased seedling emergence % gradually from 96.7, 90. And 78.5% at S1, S2, and S3 salinity levels respectively (Table 2). While biochar treatment increased seedling emergence %. At different biochar treatments, Bc1 biochar level had the highest seedling emergence percentage (94.4%), following by Bc2 (91.1%), Bc0 (88.2%), and Bc03 (85.6%) (Table 3). At the high salt concentration of 7.7 dSm^{-1}, Bc1 increased seedling emergence percentage by 40 % relative to control treatment (Table 4).

Seedling growth characteristics

Shoot length (ShL): Salinity stress significantly reduced the ShL from 38.7 cm at the control to 25.5 cm at the high salinity level (Table 2). At different biochar levels, the Bc2 recorded the highest shoot (Table 3). At the high salinity level (7.7 dSm^{-1}), the Bc2 biochar treatment increased ShL by 80% compared to control (Table 4).

Shoot fresh weight (SFwt) and shoot dry weight (SDwt): Both salinity and biochar and their interactions significantly influenced shoot fresh and dry weight (Table 2). The shoot fresh weight was decreased from 0.7, 0.6, and 0.4 g $Plant^{-1}$ at salinity level of S0, S1, and S2 (Table 2). Moreover, biochar amendment was positively affected the SFwt. The highest value of SFwt was 0.75 g $Plant^{-1}$ at was measured at Bc2. While the lowest value of SFwt was measured at Bc3 was 0.4 g $Plant^{-1}$ (Table 3). At the 7.7 dSm^{-1} salinity treatment, the Bc2 biochar level increased shoot fresh weight by 106.4% relative to control (Table 4).

The SDwt was dramatically decreased with increasing salinity level. At the high salinity level, the SDwt decreased by 57.9% compared with the control (S0) (Table 2). The Bc2 biochar treatment had the highest SDwt (90.67.3 mg $plant^{-1}$) (Table 3). At and the high (7.7 dSm^{-1}) salinity treatment, Bc2 biochar treatment increased SDwt by 67.6% compared with the control (Table 4).

Root length (RL): The RL affected by salinity, biochar, and the interaction between salinity and biochar and decreased with increasing salinity concentration (Table 2). Biochar treatments increased root length from 14.1 cm to 16.3 and 17.9 at Bc1 and Bc2 respectively (Table 3). At the high salinity level (7.7 dSm^{-1}), Bc2 increased the RL by 44.1% relative to control (Table 4).

Table 1. Summary of analysis of variance (ANOVA) for effects of Salt (S), Biochar (B), and their interaction (AB) on emergence percentage (EM %), shoot length (SHL), shoot fresh weight (SFwt), shoot dry weight (SDwt), root length (RL), root fresh weight (RFwt), root dry weight (RDwt), relative water content (RWC) of sorghum seedling.

Dependent variable	Independent variable			
	Salinity (A)	Biochar (B)	AB	CV (%)
Em%	**	*	**	7.10
SHL	**	**	***	5.43
SFwt	***	***	***	16.83
SDwt	***	***	**	13.02
RL	**	***	***	12.9
R.Fwt	***	**	***	12.9
R.Dwt	**	***	***	15.99
RWC	*	ns	**	17.8

Ns = Insignificant difference. *Significant the difference at $p \leq 0.05$. **Significant difference at $p \leq 0.01$

Table 2. Effect of salinity (0.8, 4.1, and 7.7 dS m^{-1} as S1, S2, S3 respectively) on emergence percentage (EM %), shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight (RDwt), relative water content (RWC) of sorghum seedling.

EM%		Shoot length	Shoot fresh weight	Shoot dry weight	Root length	Root fresh weight	Root dry weight	RWC
S0	97.5a	39.6a	0.7a	96.6a	18.4a	223.6a	33.9a	59.1a
S1	90b	38.0a	0.6b	71.7b	14.5b	145.1b	27.4b	49.2b
S2	82c	33.2b	0.4c	48.0c	13.8b	115.8c	21.7c	45.5b

Different letters in the same column show significant differences at the $p \leq 0.05$ level

Table 3. Effect of biochar [0, 2.5, 5, and 10 % (w/w) as Bc0, Bc1, Bc2, Bc3 respectively] on emergence percentage (EM %), shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight (RDwt), relative water content (RWC) of sorghum seedling.

EM%		Shoot length	Shoot fresh weight	Shoot dry weight	Root length	Root fresh weight	Root dry weight
Bc0	88.2bc	30.8c	0.47c	66.6c	14.1c	129.5	18.4d
Bc1	94.4a	38.1b	0.59b	76.8b	16.3b	145	27.2b
Bc2	91.1b	45.0a	0.75a	87.3a	17.8a	244.4	43.1a
Bc3	85.6c	33.8c	0.40d	57.9d	14.0c	127.1	21.9c

Different letters in the same column show significant differences at the $p \leq 0.05$ level

Table 4. Effect of the interaction between salinity (0.8, 4.1, and 7.7 dS m^{-1} as S1, S2, S3 respectively) and biochar [0, 2.5, 5, and 10 % (w/w) as Bc0, Bc1, Bc2, Bc3 respectively] on emergence percentage (EM %), shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight (RDwt), relative water content (RWC) of sorghum seedling.

	EM%		Shoot length	Shoot fresh weight	Shoot dry weight	Root length	Root fresh weight	Root dry weight	RWC
S0	Bc0	100.0a	36.2e	0.57d	90.8c	16.5c	0.21c	25.3e	62.8a
	Bc1	100.0a	38.9d	0.69c	95.1b	19.7b	0.21c	32.1c	63.7a
	Bc2	93.33c	48.0a	0.96a	119.5a	21.43a	0.31a	49.8a	59.1b
	Bc3	96.67b	35.2e	0.57d	81.0d	18.8d	0.16e	28.2d	50.7de
S1	Bc0	90.00d	32.7f	0.49f	72.3f	13.33f	0.10h	17.5g	49.1e
	Bc1	90.00d	39.5d	0.70c	72.9f	14.73e	0.12g	24.7e	52.8cd
	Bc2	96.67b	44.4b	0.75b	76.7e	16.37c	0.24b	46.5b	54.3c
	Bc3	83.33e	35.5e	0.40g	65.0g	13.47f	0.13f	21.0f	40.6g
S2	Bc0	66.67g	23.6h	0.26h	36.7i	12.4g	0.08j	12.3h	40.5f
	Bc1	93.33c	36.0e	0.38g	62.3h	14.43e	0.10h	24.8e	45.2f
	Bc2	83.33e	42.5c	0.54e	65.6g	15.87d	0.20d	31.0c	53.3cd
	Bc3	76.67f	30.5g	0.23i	27.6j	12.67g	0.09i	16.4g	38.9g

Different letters in the same column show significant differences at the $p \leq 0.05$ level

Root fresh and dry weight: Root fresh and dry weights were significantly influenced by all the trial factors and their interactions (Table 2). The 7.7 dS m^{-1} salinity level decreased root fresh weight by 48.2% as compared with the control. (Table 2). Biochar application had a positive effect on RFwt. The highest value of RFwt was 244.4 mg Plant $^{-1}$ recorded in Bc2 biochar treatment (Table 3). At high salinity level, Bc2 increased RFwt by 153.6% compared to the control (Table 4).

The Salinity stress decreased RDwt from 30.

7 mg Plant $^{-1}$ at the control salinity treatment to 18.5 mg Plant $^{-1}$ S2 salinity levels (Table 2). The biochar treatment increased RDwt at Bc1 and Bc2 and then decreased at Bc3. Bc2 increased RDwt by 134.4% relative to Bc0 biochar treatment (Table 3). At the 7.3 dSm^{-1} salinity level, Bc2 increased RDwt by 151.2% (Table 4).

Relative water content (RWC): RWC was decreased with increase salinity level. The S3 salinity level was reduced RWC by 34.9% as compared with the control (Table 2). At the highest value of RWC recorded was 53.3 was recorded at Bc2 biochar treatment. The Bc2 increased RWC by 31.6% at the high salinity level when compare with Bc0 (Table 3).

Discussion

Among abiotic stress, salinity stresses especially critical in agriculture because it can influence significantly decrease crop productivity which is one of the most countable impacts on crop cultivation.

In this study, the reduction in emergence percentage was seen by increasing salt concentration in the soil. Our findings are in agreement with similar results in wheat (Ibrahim, *et al*., 2016b). The osmotic impediment due to salt concentration impacted water imbibition (Atak *et al*., 2006). (Ibrahim, *et al*., 2016a). The application of biochar was significantly improved seedling growth under salinity stress. Others have reported similar findings (Hafeez *et al*., 2017, Zhu *et al*., 2018).

In this investigation, salinity stress affected the seedling growth, and leaf RWC. The decrease in the RL and ShL might be caused by the toxic influence of sodium chloride as well as to a deficiency of the nutrient amount. Our findings are in agreement with whom published that a negative correlation was identified between increasing salinity and vegetative growth measurements, also related observing has been reached by (Ibrahim *et al*., 2016b, Ibrahim *et al*., 2019).

Biochar has useful absorptive features due to the large surface area, cation exchange capacity, and high porosity. In this study biochar application significantly increased the seedling growth and alleviated adverse effects of salinity. Furthermore, this is in support of earlier studies in soybean (*Glycine max*) (Hafeez *et al*., 2017, Zhu *et al*., 2018), tomato (*Lycopersicum esculentum*) (Akhtar *et al*., 2014, She *et al*., 2018) and paper (*Capsicum annuum* L.) plants (Graber *et al*., 2010) who showed that applied biochar soil amendment significantly increased seedling growth.

The characteristics of biochar seem to be mainly responsible for most situations in which biochar works to alleviate the adverse impacts of abiotic stress, both by decreasing exposure of plants to stress or by enhancing the plant stress resistance (Beesley *et al*., 2011, Buss *et al*., 2012). It is probable that biochar alleviated adverse impacts of salinity in sorghum plants by three main mechanisms: reducing transient N^+ by adsorption; releasing mineral nutrients and decreasing osmotic stress by improving the soil water availability (Akhtar *et al*., 2015c). (Novak *et al*., 2012) reported that biochar has strong absorptive properties binding refers to its high porosity, surface area, and cation exchange capacity. By adsorbing toxic ions, or by releasing more beneficial ions, biochar can, consequently, decrease the adverse impacts of salinity on plants, either by decreasing the exposure of plants to stress agents or by alleviating the stress responses of plants (Akhtar *et al*., 2015a, Akhtar *et al*., 2015b, Akhtar *et al*., 2015c, Akhtar *et al*., 2014, Novak *et al*., 2012).

These results shown that biochar is a stable organic amendment and has effect in alleviating salinity stress in sorghum. Some investigations summarized that biochar enhanced responses plant growth under salinity conditions (Agbna *et al*., 2017, Hammer *et al*., 2015, She *et al*., 2018, Zainul *et al*., 2017). Plants growth in amended soil with biochar are identified to reduce much water waste through stomatal closing and transpiration this encourages keeping water balance and leaf turgidity when plants are grown in saline soil (Akhtar *et al*., 2014, Zainul *et al*., 2017).

Conclusions

Our study tested the effect of biochar on growth of sorghum seedling under salinity. The results showed that seedling emergence, seedling growth and antioxidant enzymes were significantly inhibited by high salinity and that application of biochar mitigated the adverse impacts of salinity on seedling growth and antioxidant defense system. The findings from this study revealed that treating saline soils by appropriate amount of biochar exerts a positive and effective influence on salt stress tolerance. Therefore, biochar amendment management is needed in the salt-affected area to sustain provide growth and yield of crops and to decrease the degradation of soil. Crop development is severely reduced by salt stress. This study reported that biochar application improved the seedling emergence and seedling growth. Overall, this investigation could provide a better conclusion on the effect of biochar in plants under salinity stress. Nevertheless, further research is required to investigate the impact of biochar from different sources in more species under environmental conditions including salinity stress. Therefore, biochar amendment management is needed in the salt-affected area to provide growth and yield of crops and to decrease the degradation of soil.

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