Ameliorative effect of Ascorbic acid and biochar on Growth, and antioxidant enzymes on early Seedling of Sorghum under Salinity Conditions

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Abstract

Salt stress is one of the major environmental stresses that limits the growth, antioxidant defense and sustainable crop productivity. A controlled study was done to determine the ameliorative effects of ascorbic acid (ASA) (0, 568, and 850 μM) and biochar (BC) (0, 2 and 4% BC [w/w]) on emergence, growth, and physiological attributes of sorghum grown under three salinity levels (0, 100, and 200 mM NaCl). High salinity stress significantly reduced emergence percentage, emergence rate, shoot length, root length, specific root length, total fresh weight (T.F.W), total dry weight (T.D.W), the activities of peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT), but increased malondialdehyde (MDA) content. At the 200 mM NaCl level, 850 μM ASA with 4% BC enhanced most of the physiological attributes determined. At the 200 mM salinity level, total dry weight of sorghum seedling was increased by 42.7% and 23.1% at 2% and 4% BC levels, respectively as compared with non-BC control. The highest emergence rate at 200 mM NaCl was achieved at 4% BC and 850 μM ASA level. Our study suggested that the combined application of ASA and BC at appropriate amount and concentration on sorghum seedling may be helpful in salt tolerance and getting increase antioxidant enzymes that mitigate harms affected by saline problems worldwide.

Introduction

Saline soil is one of the important determining factors limiting crop production. It is expected that 50% of the world’s arable areas are to be influenced by salt stress by 2050. Sodium chloride (NaCl) stress is one of the typical salt stresses that have tremendous harmful effects on crop growth by osmotic stress and/or specific ion toxicities. Crops can suffer from salt stress at all the growth stages from germination to maturity. However, germination and early seedling growth stages are proved as more salt sensitive in most plant species as compared with other growth stages.

Salinity is known to generate oxidative stress in plants by the extreme production of reactive oxygen species (ROS) including superoxide radical (O2− ), hydroxyl radical (OH− ), hydrogen peroxide (H2O2), and singlet oxygen (O− ), is characteristic of the biochemical changes during abiotic stresses, plants are capable of producing several antioxidant enzymes that defend them against the harmful effects of reactive oxygen species. ROS are greatly affinitive to with several cellular components, including proteins, membrane lipids, nucleic acids, and can alter their structure and functions. The one of the most approach to improving salt tolerance in crops is regulating enzymatic antioxidants such as peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT), which play main roles in eliminating ROS.

Vitamin (C)- ascorbic acid (ASA)- is one of the essential plant growth regulators and considered as an effective tool to mitigate damage in plant stress. Various studied demonstrated the ameliorative effect of ASA on salt stress in wheat (Triticum aestivum) and Radish (Raphanus sativus L.). Moreover ascorbic acid act as regulates physiological and biochemical processes and defenses mechanisms of crops. It can regulate plant responses against biotic stresses by improving early seedling growth, and plant development.

Application of organic soil amendments for salt-affected lands is considering as one of the most effective ways to alleviate salt conditions. Biochar (BC) is gaining increased attention as a critical soil amendment. Many researches indicated that biochar application is valuable to increasing growth and physiological characteristics of crops under saline stress. Application of biochar can also positively affect plant growth in several ways, such as bulk density, hydrological properties, ion exchange capacity, and microbial activity. Biochar amendment may ameliorative salinity effect due to its high salt adsorption capability.

Sorghum (Sorghum bicolor L.) is one of the five most important cereal crops, grown on large area and with large production among cereal crops. The potential uses of sorghum include such as human food, feed (grain and biomass), fermentation (methane production), fertilizer (utilization of organic byproducts), and fuel (ethanol). It is also considered as main source of minerals, vitamins, and protein for millions people in semi-arid regions. However, no information is available about the impact of BC and ASA on alleviating saline condition in early seedlings stage of sorghum.

In the present study, we hypothesized that soaking seeds with ascorbic acid (ASA) and treating soil with biochar (BC) could improve crop establishment through increasing sorghum seedling emergence and growth characteristics. The aim of this examination was to investigate the effect of ascorbic acid (ASA) and biochar (BC) as a possible amendment to improve salt tolerance and increase antioxidant enzymes of sorghum under salinity conditions.

Materials And Methods

The study was conducted in the Joint International Research Laboratory of Agriculture and Agri-Product Safety, Yangzhou University, Yangzhou, China, in 2019.

Preparation of seeds and potting media and biochar

Abu70, a sorghum variety, provided by Sudan Agricultural Research Corporation, was used in this study. The seeds were less than one-year-old and had been stored in brown paper bags under cold and dry conditions to maintain good germination. Before seed sowing, the seeds were selected for the same shape, color, and size and sterilized with 0.1% HgCl2 solution for 15 min for disinfection, and then rinsed with purified water three times.

The potting soil was a sandy loam collected from the 0-20 cm surface soil in a field nearby. The soil was air-dried and passed through a 5-mm sieve. The biochar use in this study was from wheat straw, which was pyrolyzed at 500°C in a vertical oven built of refractory bricks in Sanli New Energy Company, Henan Province, China. With such technology, 30% of wheat straw dry matter would be extracted to biochar. Before seed sowing, the properties of potting soil and biochar were determined. The soil and BC samples were analyzed for pH (1:1 in water) using a pH meter (Bench Top pH meter U33100 and 1011690, 3B 
Scientific). The determination of soil organic C is determined following the Walkley-Black chromic acid wet oxidation method. Nitrogen, available phosphorus (P), and available potassium (K) were determined following the Kjeldahl method described by, the micro-vanadate-molybdate method, and the neutral ammonium acetate extract method using a flame photometer, respectively. The properties of potting soil and biochar are shown in Table 1.

Preparation of Treatments

The experimental design was split-split-plot randomized complete block with three replications. The study was conducted twice. This study included three salinity levels (0, 100, and 200 mM NaCl) (with an equivalent electrical conductivity (EC) of 0.12, 1.61, 3.22, and 5.78 dS m⁻¹), three BC levels (0, 2 and 4% [w/w], designated as BC 0%, BC 2% and BC 4% respectively, and three levels of ascorbic acid (0, 568.0, and 850.0 μM). Before seed sowing, 30 g of seeds were soaked for 12 h at 25°C in 500 mL of one of the exogenous ASA solutions. The solution was decanted off at the end of seed priming and the seeds were re-dried near to their original weight for 48 h. Unprimed seeds were kept without hormone as the control. Plastic pots (9.5 cm in diameter by 8.5 cm in depth, without holes at the bottom to prevent drainage and leaching) were prepared and each pot was filled with 300 g dry soil. Soil sample (100-g) was collected and oven-dried at 75°C to constant weight, and the moisture content was calculated. BC was weighed on the basis of 0, 2 and 4% [w/w]), each BC levels were prepared and added into the soils before seeding. Without applying BC to the soil was kept as the control. The saline soils in the salted treatments were made by incorporating NaCl solution at different levels to the pots. The unsalted control treatment was made by adding the same amount of tap water (with EC of 0.12 dS m⁻¹). In total, 81 pots were used in this study. Ten seeds were sown in each pot at a seeding depth of 1.5 cm. After seeding, all the pots were placed in growth chambers (Model PYX-300G-B, Yangzhou Yiwei Automatic Instrument Co. Ltd, Jiangsu, China) for 20 days at 25°C with a photoperiod of 16 h and 500 mmol m⁻²s⁻¹ illumination through supplemented fluorescent light. The relative humidity was maintained at 60–70% and evaporation rates were 7.6, 9 and, 12.5 mm d⁻¹.

Observations and measurements

Emergence percentage (EP)

The number of emerged seedlings of each replicate of each treatment was recorded on a daily basis. Seedlings were considered emerged when coleoptiles were visible above the substratum surface. After ten days of seeding, EP was calculated with the following equation:

\[
\text{Emergence Percentage} = \left( \frac{\text{Number of germination seeds}}{\text{Number of total seeds}} \right) \times 100
\]

Emergence rate (ER)

The number of emerged seedlings in every pot was counted every 24 h. A further 10 mL of saline solution or water was added to each pot every 2 d. The emergence rate was calculated according to:

\[
\text{ER} = \left( \frac{\text{Number of germinated seeds}}{\text{Day of the first count}} \right) + \left( \frac{\text{Number of germinated seeds}}{\text{Day of the second count}} \right) + \left( \frac{\text{Number of germinated seeds}}{\text{Day of the final count}} \right)
\]

Specific root length (SRL)

After the root was dried, SRL was calculated according to the following equation described by:

\[
\text{SRL} \% = \left( \frac{\text{seedling root length}}{\text{seedling root dry weight}} \right) \times 100
\]

Seedling growth characteristics

Three weeks after seed sowing, growth parameters, including seedling emergence percentage, Emergence rate, shoot length, root length, Specific root length, and total fresh weight, were determined using five seedlings selected from each replicate of each treatment randomly. On the same day, 5 g sample of leaves of seedlings from each replicate of each treatment was sampled and carefully washed with tap water, immersed in liquid nitrogen for 15 min, and stored in a low-temperature freezer (-80°C) for the determination of the activity of SOD, CAT, POD, and MDA. The activity of POD was assayed according to the method described by. The activity of SOD and CAT was determined following the method described by. The malondialdehyde (MDA) content was determined following the method described by. The shoot length and root length were determined using a ruler. Total dry weight was determined for five plants from each pot after oven drying at 80°C for 2 d to constant weight.

Data analysis

In the present study, the data were analyzed using a statistical package of MSTATC according to a factorial experiment arranged in a completely randomized design. The data of each variable were subject to analysis of variance (ANOVA) and separated by LSD test (P ≤ 0.05) when 'F' values were significant.
Results

The results of analysis of variance indicated that salt, ascorbic acid, biochar and their interactions affected most emergence and growth observations and physiological attributes (Table 1).

**Emergence percentage (EP)**

Emergence percentage was significantly influenced by all trial factors and their interactions (Table 1). At 200 mM salinity concentration, the lowest emergence percentage was measured in the 0% BC with 0 μM ASA treatment, and the highest emergence percentage value was determined at 4% BC with 850 μM ASA. Moreover, at the 100 mM salinity level, emergence percentage was increased by 11.9 % at interaction between 4% BC and 850 μM ASA as compared with relative control (0 μM and 0%). ASA improved emergence percentage, for example, the treatments (850 μM ASA with 4% BC) at the 200 mM salinity level increased emergence percentage by 43.3% as compared with control (0 μM with 0%). The emergence percentage gradually was decreased with increased salinity level (Table 2).

**Emergence rate (ER)**

Emergence rate was as significantly affected by all the experimental factors and their interactions except interaction among three experimental factors (Table 1). In interaction between salinity (S) and (BC), at the high salinity level with 2% BC application had the lowest emergence rate while 4% BC level had the highest emergence rate. Moreover, at the 100 mM salinity level, emergence rate was increased by 39% at 4% BC as compared with control (0% BC). Emergence rate was decreased with the increased salinity level (Fig. 1a). In interaction between salinity (S) and (ASA), at the high salinity concentration, 850 μM ASA enhanced emergence rate by 37.1% as compared with 0 μM (Fig. 1b). In the interaction between ASA and BC, the highest emergence rate value was achieved at 4% BC with 850 μM ASA (Table 3).

**Shoot length (SH-L)**

Shoot length was significantly influenced by all the trial factors and their interactions on most occasions (Table 1). In the interaction between salinity (200 mM NaCl concentration), ASA (0μM), and biochar (4%) treatments, had the lowest shoot length, while 850 μM with 2% BC at same salinity level had the highest shoot length. At the 100 mM salinity level, shoot length was increased by 50.8% at 850 μM with 2% BC as compared with non-hormone and biochar application (0 μM ASA and 0% BC), at the same level of salinity the shoot length was increased by 50.0% at 4% BC with 850 μM treatment, shoot length was decreased with the increased in salinity level (Table 2).

**Root length (R-L)**

Results revealed that root length was significantly influenced by all experimental factors and their interactions except hormone treatment (Table 1). In the interactions between (S X ASA X BC), at the 200 mM NaCl concentration, with 0% BC and non-hormone treatment, the lowest root length was achieved. Moreover, at the same salinity level, the highest root length value was recorded at 4% BC with 850 μM treatment. The root length was significantly decreased with increased salinity level. However, the root length was increased with ASA and BC treatment, for example, the treatments (4% with 850 μM) at the 200 mM salinity level increased root length by 52.6% as compared with control (0% with 0 μM) (Table 2).

**Specific root length (SRL)**

The highest specific root length was recorded at the high salinity level, with 2% BC and 586 μM ASA while the treatment of 4% BC with similar μM ASA had the lowest specific root length value. Moreover, at the 100 mM salinity level specific root length was increased by 25.6 % at 2% BC with 586 μM ASA relative to control (0 μM and 0% BC treatment). The specific root length was decreased with the increase in salinity level. ASA and BC enhanced the specific root length (Table 2).

**Total fresh weight (T.F.W)**

Total fresh weight was significantly affected by all experimental factors and their interactions except interactions between three factors (Table 1). In terms of interaction between SXASA, the (T.F.W) was increased with ASA level increased. At the high level, 568 and 850 μM concentrations increased (T.F.W) by 7.5% and 3.1 %, respectively as compared with non-ASA treatment. The (T.F.W) was reduced with increased salinity (Fig.1a and b). In the interaction between SXBC, the highest (T.F.W) value was observed at 2% and 4% BC application increased (T.F.W) by 30.2% and 30.5%, respectively as relative to control 0% BC. In the interaction between ASAXBC, the highest (T.F.W) value was achieved at 850 μM in 2% BC, while the lowest (T.F.W) value was noted for the 0 % BC and 586 μM treatment (Table 3).

**Total dry weight (T.D.W)**
The data analysis showed that, total dry weight was significantly impacted by all experimental factors except (BC treatment) and their interactions (Table 1). In the interactions between (SXASA), total dry weight was increased by 42.7% and 23.1% at 2% and 4% BC, respectively as compared with 0% BC (Fig. 2a). Moreover, at the 100 mM salinity level, total dry weight was increased by 16% at 4% BC compared with non-BC treatment. In the interactions between (SXBC), at the 200 mM salinity level, the treatment 586 μM improved total dry weight by 27.3%, while 850 μM increased total dry weight by 2% as compared with 0 μM at 200 mM salinity (Fig. 2b). However, at the 100 mM salinity level, total dry weight was increased by 14.0% at 850 μM hormone application relative to non-hormones treatment. The total dry weight was decreased with the increase in salinity level (Fig. 2 a and b).

**Peroxidase activity (POD)**

At 200 mM NaCl and 850 μM ASA with 4% BC level was recorded the highest POD activity (52.50 U g−1 min−1). However, at the same salinity level and 2% BC application with the 0 μM treatment observed the lowest POD activity (33.63 U g−1 min−1). Peroxidase activity was decreased with salinity level increase, and at 100 mM NaCl treatment, the highest POD activity value was recorded in the 586 μM and 4% BC levels (Table 4).

**Catalase Activity(CAT)**

The activity of catalase was increased by ASA treatments at all salinity levels. At 200 mM NaCl, the treatment 0 μM ASA and 0% BC had the lowest catalase activity value (3.96 U g−1 min−1). While the 850 μM with 4% BC application, had the highest CAT activity (5.96 U g−1 min−1) at the same salinity level. Catalase activity was decreased with salinity levels increase (Table 4).

**superoxide dismutase (SOD)**

In the interaction between 200 mM NaCl, 850 μM ASA treatment with 4% BC level had the highest SOD activity (10.27 U g−1 min−1). However, at the same salinity level, 0% BC application with the 0 μM treatment observed the lowest SOD activity (8.20 U g−1 min−1). superoxide dismutase (SOD) was decreased with salinity level increased, and at 100 mM NaCl treatment, the highest SOD activity value was recorded in the 586 μM and 4% BC levels (Table 4).

**Malondialdehyde Content (MDA)**

At high salinity level, the lowest MDA content (21.77 U g−1 min−1) was measured in the 0 μM and 0% BC application, while the highest MDA content (65.23 U g−1 min−1) was recorded in the interaction between 850 μM with 4% BC application in non-saline treatments. However, the treatment (586μM + 2% and 4% BC levels) at the highest salinity level increased MDA content by 25.3% and 92.3% respectively, compared with the non-salinity treatment. In the 200 mM NaCl treatment, BC and ASA application significantly increased MDA content as compared with the non-BC and ASA treatment (0% and 0 μM). Malondialdehyde content was increased with increase salinity levels. (Table 4).

**Discussion**

Our current study investigated the impact of a wide range of ASA concentrations combined with biochar on emergence, and early seedling growth and physiological attributes of sorghum grown under salinity stress. We found that the combined application of ASA and BC at appropriate concentration increased salt tolerance by enhancing emergence, seedling growth and physiological attributes of sorghum grown under salt stress. Root and shoot growth are often expressed with the vigorous growth of seedlings, which usually lays a solid foundation for a proper crop density, especially under saline stress 31. Poor seedling emergence is one of the main reasons limiting crop establishment under saline stress 32.

**Emergence percentage(EP) and emergence rate(ER)**

In our study, emergence and early seedling growth were decreased by increasing salinity level. Those findings are in agreement with previous reports in sweet sorghum (Sorghum bicolor) 33, who founded that, The reduction in emergence and seedling growth under saline stress might be due to the combined effects of osmotic pressure 34 and /or due to the effects of added chloride ion that gave rise to osmotic stress 35.

We observed that BC amendment successfully promoted emergence and early seedling growth. Similar results have also been observed by 36 who reported that BC promoted the growth of soybean (Glycine max) 37. This may be due to the application of to soils providing additional nutrients that facilitate plant growth. However, there are some studies in which BC was observed having negative effects on emergence of rice (Oryza sativa) 38, the addition of some biochars to agricultural soils may cause a spike in microbial activity resulting in net N immobilization, in some cases reducing plant-available N and plant yields.

In the present study, we observed that ASA caused positive influences on emergence under salt stress. Seed priming with appropriate plant growth regulators can increase metabolic and enzymes activities, which can facilitate the preparation of seeds for rapid germination 33. Similar finding was also remarked in Sunflower (Helianthus annuus L.) by 39 who observed that, improved in emergence percentage and rate in seeds soaked with ASA may be due to improved oxygen absorption, increased α-amylase activity, and increased transfer of nutrients from cotyledons to embryos.
Seedling growth parameters

The growth of seedlings, such as shoot and root length, are most important parameters for salinity stress, because roots are the plant part that contact with the soil and absorb water from the soil and provide it to other plant parts \(^{40}\). Root length and shoot provide important evidence for plant response to salinity stress \(^{41}\). In the present study, salinity stress decreased seedling growth (Table 2). Our study is agreement with \(^{40}\), who noticed that, a negative correlation between salinity and seedling growth (Root length, shoot length, total fresh and total dry weight) may be caused by the lethal influence of NaCl as well as by a deficiency of the nutrient amount \(^{23}\).

In this investigation, BC application increased seedling growth (shoot length, root length, specific root length, total fresh and total dry weight) and alleviated adverse effects of salinity. Similar results were found by \(^{42}\), was indicated that, BC had a positive effect on seedling growth by reduced transient sodium ions by adsorption and released mineral nutrients such as potassium, calcium, and magnesium into the soil solution under saline condition.

Seed soaking with proper plant growth regulators can ameliorate the negative effects of salt stress on the seedling growth parameters \(^{43}\). In this investigation, exogenous ASA significantly increased the seedling growth (root length, shoot length, total fresh and total dry weight) and ameliorative the effects of salinity (Table 2) and (Fig 3). This is in support of earlier studies by \(^{44}\), who mention that treatment with ASA significantly improved root length, shoot length, total fresh and total dry weight. The Improvement of seedling growth might be due to increased cell division within the apical meristem of seedling growth due to enhanced exogenous hormone level in the plant tissues.

Antioxidant enzymes

Superoxide dismutase (SOD) is a major scavenger of O2 – and its enzymatic action results in the production of H2O2 and O2. Then, H2O2 is scavenged by a variety of peroxidases (POD) or directly broken into water and oxygen by catalases (CAT) \(^{45}\).

In our study, salt stress caused a significant reduction in the POD, SOD and CAT activities. A similar result was substantiated with the findings by \(^{46}\), who noticed that POD, SOD and CAT activities were reduced under salt stress. Reduced enzymes activity in the stressed plants might have stimulated H2O2 accumulation in plant cells under stress, which are finally lead to damage biological systems \(^{47}\). A dissimilar results has been reported by \(^{48,49}\), they showed that salt stress increased POD, SOD and CAT activity. An increase in the anti-oxidative enzymes under salt stress could be suggestive of an increased result of ROS and improvement of a protective mechanism to decrease oxidative harm triggered by stress in plants. At 200 mM NaCl The highest activity of POD, SOD and CAT were observed in the high level of ASA, and the lowest POD, SOD and CAT were recorded in 0 μM level of ASA.

Biochar application under saline soil can improve seedling growth, physiological and biochemical process by hanged nutrient release, holding, or immobilization by its surface properties (cation exchange capacity and anion exchange capacity) \(^{11}\). Our results showed that the application of BC increased the activities of antioxidant enzymes such as CAT, SOD and POD in the sorghum seedlings. Similar result were finding by many investigators that the BC application was improving stress tolerance by increased activity of POD, SOD and CAT \(^{50,51}\). The BC application can regulate the synthesis of antioxidant enzymes in plants and thereby ameliorate salt stress in plants. Nevertheless, there are some researches in which observed that application of BC reduced the activity of the POD, POD and CAT enzymes under salt condition \(^{49,52}\). The reduction on antioxidant enzyme activities can be associated to the concentration of heavy metals immobilization effect of BC followed by decreased metal translocation into plant tissues. The highest POD, SOD and CAT activity was observed in the 4% BC application.

The activity of antioxidant enzymes was increased with increasing of ASA when exposed to salinity stress. Our study revealed that exogenous ASA could improve POD, SOD and CAT activity under NaCl. A similar result observed that ASA improved POD, SOD, and CAT activities, which are significance for antioxidative defense required for the plants under saline condition \(^{53-55}\). The improvement of the activity of antioxidant enzymes could be due to the regenerative nature of ascorbate which plays a key role in quenching intermediate/excited reactive forms of molecular oxygen either directly or through enzymatic catalysis \(^{56}\).

Malondialdehyde enzyme

Malondialdehyde is a naturally and the end product occurring of membrane lipid peroxidation, its considered to be indicator of oxidative damage from stress. MDA content increased under salinity in sweet sorghum (Sorghum bicolor. L) \(^{33}\). When NaCl was applied, the MDA content was substantially higher than the control in sweet sorghum \(^{57}\). Similar results were reported by \(^{58}\), who demonstrated that MDA content was increased by an increase in salinity in sunflower.

BC has the positive significant effect on the MDA content when sorghum plants exposed to salinity stress. The maximum content of MDA was appeared under highly salt-stressed at 4% BC application (Table 4). Similar report was observed by \(^{59}\), who showed that MDA content under NaCl-stressed plants was increased significantly by applying a high level of BC.

In this study, the MDA content improved by ASA treatment. Our result disagreement with \(^{50,61}\), they mentioned that MDA content had significant decreased when the ASA Appling on sorghum plant. It was shown that exogenous application of AsA modified antioxidant enzymes and none nzymatic antioxidants in plants under different stress conditions and induced various defensive signaling mechanisms by interacting/stimulating the signaling of different plant hormones \(^{62}\). Seed soaking by appropriate concentration of ASA improved seedling growth of sorghum seedling under salt stress. Under salt stress, mixing soil
with 4% biochar alone or combine with ASA could also enhanced the antioxidant enzyme system, and improved photosynthesis by increased chlorophyll content. Farmers can use this technique to achieved strong seedling growth, good crop establishment and high yielding of dry matter accumulation.

**Conclusions**

We examined the effects of ASA and BC on germination, growth and physiological responses of sorghum exposed to salinity. Emergence, seedling growth, and antioxidant enzymes were inhibited by high salinity, however the application of ASA and BC mitigated the adverse impacts of salinity. The findings from this study revealed that treating saline soils with an appropriate concentration of ASA and BC could improve salinity tolerance on sorghum plant. Therefore, soaking seeds with ASA and treating soil with BC amendment can be used in saline soils. The 850 μM ASA and 4% BC application may be more effectively used in salt-affected soil to improving seedling growth and productivity of dry matter. Further research is needed to investigate the impact of different concentrations ASA and different sources of BC in more sorghum varieties under salinity conditions.

**Abbreviations**

S, salinity; biochar; BC, ascorbic acid; ASA; POD, peroxide; SOD, superoxide dismutase; CAT, catalase; MDA, Malondialdehyde; ROS, reactive oxygen species.

**Declarations**

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Tables

Table 1
The ANOVA table for effects of salinity (S), ascorbic acid (ASA), biochar (BC) and their interaction on emergence percentage, emergence rate, shoot length, root length, specific root length, total fresh weight, and total dry weight at early seedling growth of sorghum

| Source | Source Effect | F value | Emergence percentage | Emergence rate | Shoot length | Root length | Specific root length | Total fresh weight | Total dry weight | POD | SOD | CAT | MDA |
|--------|---------------|---------|-----------------------|----------------|--------------|-------------|----------------------|-------------------|-----------------|-----|-----|-----|-----|
|        | Salinity (S)  | 367.2***| 1705.3***             | 74.13***       | 37.3**       | 43.2**      | 10.20*               | 8.01*             | 22.8**          | 26.5** | 383.4*** | 8.37* |
|        | Ascorbic acid (ASA) | 9.1** | 10.2** | 15.7*** | 0.76ns | 5.02* | 10.26** | 4.03* | 12.55** | 15.7*** | 10.6** | 0.37ns |
|        | Biochar (BC)  | 18.0*** | 36.6*** | 126.4*** | 16.27*** | 2.4ns | 5.82** | 0.82ns | 1.38ns | 4.3* | 56.30*** | 2.19ns |
|        | S × ASA       | 107.0*** | 6.35** | 2.3ns | 4.2* | 77.82*** | 7.63*** | 3.55** | 57.66*** | 3.6* | 8.7** | 6.91** |
|        | S × BC        | 32.9*** | 10.4*** | 3.1* | 2.3* | 4.18** | 42.93*** | 30.14*** | 3.1* | 15.7*** | 8.0*** | 5.66** |
|        | ASA × BC      | 39.5*** | 2.2* | 0.73ns | 4.2** | 1.01ns | 5.91*** | 2.67* | 3.42* | 1.2ns | 2.4* | 2.63* |
|        | S × ASA × BC  | 9.5*** | 1.2ns | 14.8*** | 3.7** | 7.46*** | 1.82ns | 0.74ns | 5.12*** | 4.30*** | 4.8** | 8.00*** |

Ns, not significant. *, ** and *** indicate statistical difference at $P \leq 0.05$, 0.01 and 0.001, respectively.
Table 2
Effects of salt (S), ascorbic acid (ASA) and biochar (BC) on emergence seedling percentage, emergence seedling rate, shoot length, root length and specific root length of seedling growth of sorghum.

| Salinity (mM NaCl) | Biochar (%) | Emergence percentage (%) | Shoot length (cm) | Root length (cm) | Specific root length |
|-------------------|-------------|--------------------------|-------------------|------------------|---------------------|
| 0                 | 0           | 94.1a 85.0fg 84.8g 26.0t 27.8q 47.7c 26.7a 23.3cd 23.1cd 44.7g 60.7b 65.3a |
| 0                 | 2           | 82.2J 91.7b 88.2d 32.6l 44.4d 53.3a 23.5cd 23.6c 22.1e 44.3h 55.6c 55.5c |
| 0                 | 4           | 85.2fg 82.4ij 93.6a 34.9j 38.7g 43.7f 19.7g 25.2b 26.6a 38.1m 51.4d 55.3c |
| 100               | 0           | 83.7h 82.8ij 85.0fg 25.0v 30.0o 30.8n 18.1j 20.4f 20.9f 44.1hi 44.4gh 43.7i |
| 100               | 2           | 83.0hi 85.4efg 91.7bc 30.7p 34.7k 37.7h 21.9e 21.8e 21.8e 30.9q 55.4c 40.3j |
| 100               | 4           | 82.3j 85.9e 93.7a 25.7u 22.6w 37.5i 18.9hi 21.7e 17.4k 30.6q 33.6o 51.3d |
| 200               | 0           | 63.5n 82.3j 85.6ef 17.5y 27.2s 31.9m 15.0m 18.3ij 18.9hi 40.6j 39.4k 32.8p |
| 200               | 2           | 72.9k 88.2d 82.6ij 19.2x 25.1v 48.2b 16.7l 18.1j 14.6m 38.7l 50.5e 29.3j |
| 200               | 4           | 72.03l 71.3m 91.0c 15.6z 27.4r 44.0e 19.0h 19.6g 22.9d 35.6n 28.1s 47.6f |

† Within the same column means followed by different letters are statistically different at the 0.05 probability level.

Table 3
Effects of interaction between ascorbic acid and biochar application (BC) on emergence rate and total fresh weight on seedling of sorghum.

| Biochar (%) | Ascorbic acid (μM) | Emergence rate | Total fresh weight (g plant⁻¹) |
|-------------|---------------------|----------------|--------------------------------|
| 0           | 0                   | 12.5d          | 4.3g                           |
| 0           | 586                 | 11.1e          | 3.7i                           |
| 0           | 850                 | 10.4f          | 4.5f                           |
| 2           | 0                   | 12.3d          | 4.0h                           |
| 2           | 586                 | 13.3c          | 5.0d                           |
| 2           | 850                 | 14.0b          | 6.2a                           |
| 4           | 0                   | 14.2b          | 4.6e                           |
| 4           | 586                 | 14.3ab         | 5.7c                           |
| 4           | 850                 | 14.6a          | 6.0b                           |

† Within the same column means followed by different letters are statistically different at the 0.05 probability level.
Table 4
Effects of interaction between salt (S) and biochar (BC) and ascorbic acid (ASA) on the activity of peroxidase (POD), superoxide dismutase (SOD), and catalas (CAT), and malondialdehyde (MDA) content of at early seedling growth stage of sorghum

| Salinity (mMNaCl) | Biochar (%) | peroxidase (POD) | superoxide dismutase (SOD) | catalase (CAT) | malondialdehyde (MDA) |
|-------------------|-------------|------------------|-----------------------------|----------------|------------------------|
|                   |             | 0                | 586                         | 850            | 0                      | 586         | 850         | 0           | 586         | 850         |
| 0                 | 0           | 68.43i           | 72.57g                      | 76.60e         | 12.97de                | 13.00de     | 13.97c      | 12.03g      | 12.73e      | 12.43f      | 6.93w       | 8.13v       | 8.70u       |
|                   | 2           | 71.37h           | 75.57f                      | 92.77c         | 12.70ef                | 13.17d      | 13.83c      | 11.97g      | 12.43f      | 13.00d      |                     |             |             |
| 4                 | 90.50d      | 93.43b           | 95.40a                      | 13.27d         | 16.83b                 | 19.77a      | 9.10t       | 9.56s       | 10.87qr     |                     |             |             |
| 100               | 0           | 52.63p           | 55.23o                      | 61.03k         | 10.70j                 | 11.30i      | 12.07gh     | 8.73i       | 9.00h       | 11.83g       | 11.03q       | 12.87o       | 14.07m       |
|                   | 2           | 64.50k           | 60.77l                      | 57.50m         | 10.27jk                | 12.30fg     | 12.13gh     | 7.46l       | 7.70k       | 7.93j        | 11.50p       | 14.40m       | 14.87l       |
| 4                 | 56.67n      | 66.50j           | 57.37m                      | 11.30i         | 12.37fg                | 11.77h      | 6.13mn      | 6.33m       | 7.4l        | 13.30n       | 15.33k       | 20.20j       |                     |
| 200               | 0           | 38.30v           | 39.70u                      | 46.57r         | 8.20m                  | 8.96l       | 9.93k       | 3.96s       | 4.53r       | 4.80q        | 21.77l       | 26.97d       | 25.33e       |
|                   | 2           | 33.63w           | 45.93s                      | 49.57q         | 9.10l                  | 9.36l       | 10.00k      | 5.40p       | 5.86o       | 5.40p        | 23.47h       | 27.47c       | 24.40f       |
| 4                 | 42.17t      | 45.43s           | 52.50p                      | 8.36m          | 10.03j                 | 10.27jk     | 5.56p       | 5.60p       | 5.96no      | 23.97g       | 41.87b       | 65.23a       |

The data followed with different letters are statistically different at the 0.05 probability level.

Figures

Figure 1
Effects on emergence rate of early seedling growth of sorghum of: (a) interaction between salt (S) and biochar (BC); and (b) interaction between salt (S) and ascorbic acid (ASA). Columns marked with different letters are statistically different at the 0.05 probability level.
Figure 2

The effect on the total fresh weight of early seedling growth of sorghum of (a) interaction between salt (S) and Ascorbic acid (ASA); and (b) interaction between Salt(S) and biochar (BC). Columns with different letters are statistically different at the 0.05 probability level.

Figure 3

The effects on total dry weight (g Plant⁻¹) of early seedling growth of sorghum of: (a) interaction between salt(S) and Biochar (BC); and (b) interaction between salt(S) and Ascorbic acid (ASA). Columns with different letters are statistically different at the 0.05 probability level.

Supplementary Files

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