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Biochar Application Improves the Drought Tolerance in Maize Seedlings

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Abstract: Application of biochar to agricultural soils is mostly used to improve soil fertility. Experimental treatments were comprised of two factors: i) drought at two level, i.e., 80% and 40% water holding capacity (WHC) which was maintained on gravimetric basis ii) three levels of biochar i.e., control, 2 t ha^{-1} and 4 t ha^{-1} added to soil. Experimentation was done to examine potential of biochar application to enhance the growth attributes, water relations, photosynthetic pigments and antioxidants activities in maize (*Zea mays* L.) seedlings. Results of study revealed that biochar application increased the growth qualities (total seedlings biomass, dry weight of shoot and root, shoot length and root length). In addition; contents of photosynthetic pigments (chlorophyll *a, b*, *a* + *b* and *a/b*), water relation (relative water contents, turgor potential, osmotic potential and water potential) were improved significantly due to addition of biochar. Addition of 4 t ha^{-1} biochar led to significant rise activity of enzymatic antioxidant catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) in leaf of maize seedling sunder drought as well as well watered circumstances. However, biochar applied at the rate 4 t ha^{-1} improved the all the physiological and biochemical attributes in maize seedlings under drought. From the results it was concluded that biochar application is an efficient way to alleviate adverse effect of drought stress on maize. In drought prone areas, long term impacts of biochar on production of maize and properties of soil could be recommended as upcoming shove.

Keywords: Antioxidants; biochar; drought stress; water relations; chlorophyll contents

1 Introduction

Plants are exposed to numerous abiotic and biotic factors which influence the growth and development [1]. The main limiting factors in crop production are abiotic factors like soil pH, pesticides, salinity, heavy metals, air pollution, extreme temperatures and drought as they influence each function of plant [2-3].The major abiotic factor restricts crop production is drought [4-7]. When the rate of transpiration becomes elevated or when the supply of water to roots significantly reduced then plants suffer drought stress. These two situations frequently concur under water limited conditions, for example, semi-arid and arid conditions [7]. Though, in plants, the morphological, physiological and biochemical processes are eternally or momentarily influenced due to scarcity of water or drought stress.

Productions of reactive oxygen species (ROS) are very crucial which results in lipid peroxidation, protein degradation and membrane injury that finally cause death of cell under water stress condition [8]. Though, certain antioxidant enzymes are released by plants which work as ROS foraging enzymes. These enzymes are glutathione reductase (GR), superoxide dismutase (SOD), peroxidase (POD) and catalase

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(CAT) [9]. Furthermore, these antioxidant enzymes enhance the plant's ability to survive under drought stress and also control the cellular redox status of the plant. To overcome the challenges of drought stress, different water and soil management practice are suggested. Biochar (BC), a black carbon organic compound [10] which can be utilized as fertilizer. It is porous and granular material which is used as soil conditioner to improve the soil's CEC, water holding capacity (WHC) and pH [11-12]. In comparison to organic matter, it sustains for long term because it is less prone to decay due to recalcitrant C [13-14]. Yield of the crops can be effectively increased by using biochar as soil amendment [10]. Crop productivity as well as crop yield under harsh situations like drought and salinity can be enhanced by using BC [15-16]. For example, permanent wilting point was improved by using BC [16-17], though in comparison to water held at permanent wilting point, i.e., increased plant available water, the quantity of water retained at field capacity was improved to a larger extent. Therefore, overall increase in plant available water is an indication that addition of BC in the soil increases WHC of soil [18-19]. A study was conducted on a boreal sandy clay loam, which showed that adding BC in soil at the rate of 10 tons per hectare increased the number of grains in wheat. The reason may be due to increased water holding capacity of soil [20]. In another study, BC addition to a fertile sandy clay loam resulted in increased WHC of soil thus resulted in higher yield [21]. Recently, [22] observed that application of BC enhanced plant growth by enhancing soil-plant water relationships (improved leaf osmotic potential and relative water content) and photosynthesis (stimulated photosynthesis by increasing the electron transport rate of photosystem-II and reduced stomatal resistance) in poor sandy soils under well-watered and drought situations. The BC acts in two ways: direct ways it provides nutrients while in indirect ways it improves structure of soil [23]. Although lot of studies have been done in both controlled environment and field experiments [24-26], but there is no enough information present about actual mechanism of biochar. To date, there has been no study concerning the biochar effect on the physiological and biochemical attributes and antioxidants activities of plants under drought stress. So; the study was done in order to evaluate the impact of biochar in improving the physiological and biochemical basis of drought tolerance in maize seedlings and to also identify the optimized dose of biochar that is better of the growth and development of maize under water deficit conditions.

2 Materials and Methods

2.1 Growth Conditions and Treatments

A pot study was conducted at College of Agriculture, BZU, Bahadur Campus Layyah, to evaluate the potential of BC in enhancing the physiological and biochemical attributes of drought tolerance in maize seedling. Maize hybrid 3412 was used as test variety/hybrid which was a gift from ICI Seed Company Limited. In earthen pots (15.5 cm in diameter, 60 cm in height), the seeds were sown, each pot had 12 kg of fine and well ground loam. In Tab. 1, the physiochemical properties of soil are presented. Growth of seedling was maintained by applying P_2O_5 90 mg kg^{-1} as DAP (0-46-0), K_2O 60 mg kg^{-1} of soil as potassium sulphate and the basal dose of N at the rate of 100 mg kg^{-1} as urea (46-0-0).

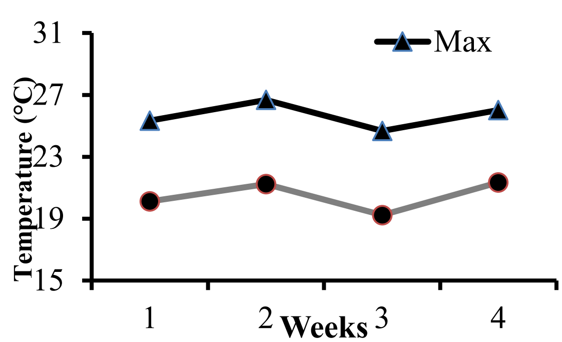

Figure 1: Maximum and minimum temperature during the experimental duration

Table 1: Physio-chemical properties of soil used in the experiment

Soil Analysis	Value
Mechanical Analysis	
Sand (%)	52
Silt (%)	22
Clay (%)	27
Textural class	Sandy loam
Chemical analysis	
Soil pH	8.5
EC (dSm^{-2})	2.32
Cations exchangeable capacity (dSm^{-2})	2.01
Organic matter (%)	0.78
Calcium carbonate (%)	2.96
Nitrogen (%)	0.16
Phosphures (mg kg^{-1})	22
Potassium (mg kg^{-1})	58

The earthen pots were set in completely randomized design (CRD) with factorial arrangements with four replications. Factors include i) drought at two level, i.e., 80% and 40% water holding capacity (WHC), following gravimetric method [27] and ii) biochar at three levels i.e., control, 2 t ha^{-1} and 4 t ha^{-1}. In each pot, ten (10) seeds were sown and 5 plants per pot were maintained. Drought stress was imposed after two weeks of sowing (two leave stages) and was applied for 15 days continuously. Plants samples for morphological and biochemical parameters were taken after 30 days old seedlings.

2.2 Preparation of Biochar

Biochar was prepared from cotton sticks according to the method elaborated by [28]. The burned material was crushed and to ensure the uniform mixing in the soil, it is passed through a 2 mm sieve.

2.3 Growth Characteristics

To collect root-shoot data, 30 days old seedling along with soil were removed from pots and soil was washed out with tap water and then washed by distilled water. Shoot and root were separated to measure the length and fresh weights. Oven drying at 72ºC of the root-shoot samples was done until a constant dry weight of shoot and root. On dry weight basis shoot root ratio was determined. Shoot and root dry weights were summed up and overall dry biomass weight was determined.

2.4 Water Relations

Fresh leaves (0.5 g) were dipped in water to make the weight of leaves became constant for measuring relative water contents (RWC). Again the saturated leaves were balanced (W_s) and then dried for 24 h at 80°C to determine the dry weight (W_d). The RWC were determined following the method of [29].

$$RWC\ (\%) = (W_f - W_d)/(W_S - W_d) \times 100$$

By use of pressure bomb, water potential (Ψ_w) of fresh leaf was calculated (Santa Barbara, CA, USA). Same leaf was centrifuged at 5000 × g, sap expressed, thawed, frozen and osmotic potential (Ψ_s)

was calculated by using osmometer (Digital Osmometer, Wescor, Logan, UT, USA). As a difference of Ψ_w and Ψ_s, pressure potential of leaf (Ψ_p) was determined.

The method elaborated by [30] was used to determine the chlorophylls *a* and *b*. Extraction of new leaves (0.5 g) was done overnight with 5 mL 80% acetone at 0-4°C. The centrifugation of extracts was done at 10,000 × g for 5 min. Using a spectrophotometer, absorbance of the supernatant was read at 645 and 663 nm (Hitachi-U2001, Tokyo, Japan).

2.5 Biochemical Analysis

0.5 g of fresh plant material (leaves) was crushed by adding 1 mL phosphate buffer saline (PBS) containing 1.37 mM NaCl, 2.7 mM KCl, 2 mM KH_2PO_4 and 10 mM Na_2HPO_4 with pH 7.2 for the extraction of total soluble proteins, in a concentration of 1 µM were added to the buffer, before extraction of proteins, cocktail protease inhibitors. The ground leaf material was centrifuged at 12000 × g for 5 min. Supernatant was preserved in a separate centrifuge tube for the analysis of soluble proteins. Total soluble proteins were determined by following the Bradford assay [31].

Extraction of 0.5 g fresh leaf sample was done in 5 ml of 50 mM phosphate buffer (pH 7.8) for enzymatic antioxidants determination. The supernatant was used for more examination of enzymatic antioxidants, after the centrifugation at 15000 × g for 20 min.

2.6 Superoxide Dismutase

The photochemical reduction of nitrobluetetrazolium (NBT) at 560 nm was repressed due to SOD activity. SOD activity was assayed by monitoring of this repression. The reaction mixture was made by taking 50 µL enzyme extract and adding 500 µL EDTA (75 mM), 1 mL riboflavin (1.3 µM), 950 µL (50 mM) phosphate, buffer 500 µL methionine (13 mM) and 1 mL NBT (50 µM). 30 W fluorescent lamp illuminations and turning the fluorescent lamp on was used to start the reaction. After 5 minutes lamp was turned off to stop the reaction. Blue formazane was produced by NBT photo reduction. Increase in absorbance at 560 nm was calculated by the Blue fornazane. As a blank, the similar reaction mixtures with no enzyme extract was used. The activity of SOD was observed and stated as SOD IU min^{-1} mg^{-1} protein [32].

2.7 Catalase

After every 30 s for 5 min at 240 nm using a UV- visible spectrophotometer, the CAT activity examined by the decay of H_2O_2 and change in absorbance due to H_2O_2 was detected. Reaction mixture consisted of 2 mL phosphate buffer (50 mM) and 900 µL H_2O_2 (5.9 mM) for CAT. By addition of 100 µL enzyme extract to the reaction mixture, reaction was started. CAT activity was stated as µmol of H_2O_2 min^{-1} mg $protein^{-1}$ [33].

2.8 Peroxidase

Guaiacol oxidation was used to examine the POD activity and defined as 0.01 absorbance change min^{-1} mg^{-1} protein. By adding 2 mL phosphate (50 mM), 500 µL H_2O_2 (40 mM) and 400 µL guaiacol (20 mM) in 100 µL enzyme extract, the reaction mixture was made. After every 20 s up to 5 min, the alteration in absorbance at 470 nm of the reaction mixture was detected.

All enzymatic activities were expressed as respective enzymatic unit (U) $mint^{-1}mg^{-1}$ protein.

2.9 Statistical Analysis

Fisher's Analysis of Variance technique was used to analyze data statistically of all parameters and treatment means difference was calculated LSD test at 5% probability level [34] using statistical software Statistics 8.1.

3 Results

3.1 Growth Characteristics

BC application significantly influenced growth characteristics of maize seedlings at 80% WHC. With 80% WHC, 2 and 4 t ha^{-1} BC application surpassed all other treatments with maximum increase in root dry weight, shoot dry weight, total seedling biomass, shoot length and root length. At 40% WHC, addition of 4 t ha^{-1} BC, improved the growth performance of maize seedlings, being superior to the seedlings without BC application (Tab. 2).

3.2 Photosynthetic Pigments

Chlorophyll contents (a & b) and its components responded significantly with BC application being maximum at 4 t ha-1 under 80% WHC, followed by 2 t ha^{-1}. Under water stress conditions of 40% WHC, 4 t ha^{-1} BC enhanced the chlorophyll contents, followed by 2 t ha-1(Tab. 3).

3.3 Water Relations

Under drought conditions, relative water contents of maize seedlings were decreased. Under normal conditions, 4 t ha^{-1} BC application increased RWC by 19.38%, followed by 2 t ha^{-1} as indicated by an increase of 14.25%. Under stressed conditions, BC application of 4 t ha^{-1} maintained RWC with a surge of 28.58%, with respect to control. Water potential increased under the influence of BC application under normal and stressed conditions. At 40% WHC, BC application of 2 and 4 t BC ha^{-1} enhanced the water potential by 13.33 and 19.44%, respectively (Tab. 4). Osmotic potential also experienced to be hanced up to 42.59%, with 4 t ha^{-1} biocharat 80% WHC. With 40% WHC, the increase in osmotic potential due to BC application of 4 t ha^{-1}, was found to be 16.02%. Turgor potential increased with application of BC, being more pronounced as 27.58%, with application of 4 t ha^{-1} at 80% WHC (Tab. 4). Under stress conditions, same application of BC maintained turgor potential of 33.33%, as compared to control (no biochar).

Table 2: Influence of biochar application on growth characteristics of maize seedlings under drought conditions

Drought levels	Biochar levels (t ha^{-1})	Shoot dry weight (g)	Root dry weight (g)	Total seedlings biomass (g)	Shoot length (cm)	Root length (cm)
Well-watered (80% WHC)	0	13.55 ± 0.55 b	7.17 ± 0.41 b	20.72 ± 0.31 b	40.34 ± 0.86 c	8.81 ± 0.42 c
	2	16.07 ± 0.66 a	8.71 ± 0.27 a	24.79 ± 0.74 a	45.56 ± 0.43 b	11.18 ± 0.35 b
	4	16.59 ± 0.48 a	9.48 ± 0.30 a	26.07 ± 0.79 a	48.45 ± 0.62 a	13.08 ± 0.39 a
Drought stress (40% WHC)	0	8.43 ± 0.26 e	3.13 ± 0.33 e	11.75 ± 0.53 e	30.32 ± 1.27 f	5.42 ± 0.39 e
	2	11.59 ± 0.49 d	5.44 ± 0.28 d	17.04 ± 0.76 d	34.42 ± 0.46 e	7.13 ± 0.37 d
	4	12.92 ± 0.47 c	6.99 ± 0.16 c	19.92 ± 0.43 c	37.52 ± 0.72 d	7.41 ± 0.20 d

WHC; Water holding capacity, values represent mean ± SE (standard error) (n = 3).

Table 3: Influence of biochar application on photosynthetic pigments of maize seedlings under drought conditions

Drought levels	Biochar levels (t ha^{-1})	Relative water contents (%)	Water potential (-MPa)	Osmotic potential (-MPa)	Turgor Potential (MPa)
Well-watered (80% WHC)	0	78.14 ± 1.46 c	0.83 ± 0.029 d	0.54 ± 0.020 d	0.29 ± 0.020 ab
	2	89.28 ± 1.84 b	0.76 ± 0.023 de	0.42 ± 0.020 e	0.33 ± 0.023 a

	4	93.29 ± 1.18 a	0.68 ± 0.012 e	0.31 ± 0.014 f	0.37 ± 0.014 a
Drought stress (40% WHC)	0	57.34 ± 1.26 f	1.80 ± 0.044 a	1.56 ± 0.067 a	0.24 ± 0.067 b
	2	67.07 ± 1.43 e	1.56 ± 0.044 b	1.32 ± 0.039 b	0.25 ± 0.039 b
	4	73.73 ± 0.93 d	1.45 ± 0.017 c	1.31 ± 0.029 c	0.32 ± 0.029 ab

WHC; Water holding capacity, values represent mean ± SE (standard error) (n = 3).

Table 4: Influence of biochar application on water relations of maize seedlings under drought conditions

Drought levels	Biochar levels (t ha^{-1})	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a + b*	Chlorophyll *a/b*
Well-watered (80% WHC)	0	2.10 ± 0.021c	0.74 ± 0.041 bc	2.85 ± 0.051 c	2.83 ± 0.160
	2	2.65 ± 0.066 b	0.82 ± 0.018 b	3.48 ± 0.047 b	3.22 ± 0.156
	4	2.98 ± 0.073 a	0.93 ± 0.030 a	3.91 ± 0.055 a	3.19 ± 0.174
Drought stress (40% WHC)	0	1.60 ± 0.096 f	0.42 ± 0.031 e	2.02 ± 0.066 f	3.88 ± 0.521
	2	1.96 ± 0.064 e	0.62 ± 0.026 d	2.58 ± 0.081 e	3.15 ± 0.126
	4	2.01 ± 0.053 d	0.70 ± 0.020 d	2.72 ± 0.066 d	2.99 ± 0.078

WHC; Water holding capacity, values represent mean ± SE (standard error) (n = 3).

3.4 Biochemical Analysis

At 80% WHC, BC application rate showed non-significant effects on SOD activities, however, in stressed conditions, higher SOD activity was observed at 4 t ha^{-1} followed by 2 t ha^{-1} and showed an increase of 30.77% and 50.58% over control (Tab. 5). CAT enzyme activity also experienced an increment of 22.69 and 41.53% under 80% WHC and 24.40 and 30.68% under 40% WHC, with application of 2 and 4 t BC ha^{-1}; respectively. A percentage increase of 71.25 and 84.43% in POD was noted with application of 2 and 4 t ha^{-1} under well-watered conditions, whereas, 28.59% and 47.03% increase in POD activity was recorded under water stress conditions, respectively. In well-watered conditions, Ascorbate peroxidase enzyme activity was found to be increased by 60% and 73.3% with application of 2 and 4 t ha^{-1} BC, respectively (Tab. 5). Under 40% WHC, the increase was observed to be 29.33 and 41.33%, under the influence of 2 and 4 t ha^{-1}, biochar application; respectively.

4 Discussion

Drought stress hampered the growth of maize seedlings which might be attributed to modifications of various biochemical and physiological functions of plant at cell level, including the ionic disruption, and inhibition of photo-assimilation, nutrient absorption and respiration [35-36]. However, biochar application improved the growth attributes, chlorophyll contents, water relations and enzymatic activities of maize seedlings under drought conditions. Indeed, biochar is a useful organic soil amendment which improves the soil fertility level. Improvement in the growth of maize seedlings due to biochar application might also be attributed to increased soil porosity and carbon sequestration which leads towards increased retention of soil moisture [37], thus sustaining maize growth under drought stress. Biochar has the capacity to improve the carbon assimilation [38] cation exchange capacity, essential ionic uptake (N, K, P, Ca and Mg), which support the plant physiologically to sustain its growth under drought [39-40], as was observed in this study. Our findings are according to results of [41,22,42], who found that biochar application improved the growth of okra, maize and soybean seedlings under drought stress, respectively.

Drought stress leads towards the oxidative stress which negatively affects the stay green [43-44], similar results were observed in the present study. However, with the addition of BC, the chlorophyll contents were increased significantly, which consequently improved the photosynthetic performance of the maize seedlings. The improvement in chlorophyll contents in our study are quite in line with results of [45] in tomato seedlings with BC application.

Table 5: Influence of biochar application on enzymatic antioxidants of maize seedlings under drought conditions

Drought levels	**Biochar levels ($t\ ha^{-1}$)**	**Superoxide dismutase** (unit mg^{-1} of protein)	**Catalase** (unit mg^{-1} of protein)	**Peroxidase** (unit mg^{-1} of protein)	**Ascorbate peroxidase** (unit mg^{-1} of protein)
Well-watered (80% WHC)	0	50.06 ± 3.95 d	2.60 ± 0.34 c	1.67 ± 0.129 e	0.15 ± 0.024 c
	2	57.31 ± 6.69 d	3.19 ± 0.30 c	2.86 ± 0.235 d	0.24 ± 0.015 c
	4	62.11 ± 3.84 d	3.68 ± 0.11 c	3.08 ± 0.294 d	0.26 ± 0.032 c
Drought stress (40% WHC)	0	141.56 ± 12.72 c	7.17 ± 0.41 b	6.40 ± 0.493 c	0.75 ± 0.050 b
	2	185.12 ± 3.78 b	8.92 ± 0.30 a	8.23 ± 0.620 b	0.97 ± 0.031 a
	4	213.17 ± 5.87 a	9.37 ± 0.62 a	9.41 ± 0.350 a	1.06 ± 0.088 a

WHC; Water holding capacity, values represent mean ± SE (standard error) (n = 3).

Water relations directly affect photosynthesis rates and thus cause growth modification under stress conditions. In this study, biochar application improved the water relation of maize seedlings. Indeed, biochar increases expands the permanent wilting point of the plants [17] by improving the water retention in soil [46] which might have also positively affected the water relations in maize seedlings. BC also increases the soil porosity thus facilitating the movement of air and water through transmission pores and its retention through storage pores [38] which improves the crop growth under water stress as observed in the present study. BC application also enhanced the water potential of drought stressed maize plants. Indeed, BC improve the nutrient uptake particularly potassium [47]. Potassium regulation due to BC application helps in maintaining the osmotic potential of the plant cell [48] attributed by its more cation contents, which adjust the water relation of the plant under stressed conditions. Reduction in transpiration rate is also reported by [49] in biochar supplemented quinoa plants, which can be a reason for improved turgor potential and relative water contents of biochar treated maize seedlings.

The enzymatic antioxidant activity (SOD, CAT, POD and APX) of maize seedlings were enhanced with BC amendment under drought conditions. The increase of antioxidant enzyme activity with soil amendments including the BC has have been previously reported [50-51]. The reasons behind the improvement in antioxidant enzymes due to biochar application still needs to be investigated in future studies. In conclusion, biochar application improved the water relation, antioxidant activities and growth of maize seedlings under drought stress.

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