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APPLICATION OF RICE-STRAW BIOCHAR ALLEVIATES OXIDATIVE STRESS IN WATER-STRESSED *Solanum lycopersicum* L.

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ABSTRACT

Excessive production of reactive oxygen species (ROS) in plant tissues capable of cellular damage has been linked to abiotic stress conditions such as drought. However, the role of biochar as a soil amendment in mitigating the impact of oxidative stress has been under-explored. Therefore, this study investigated the role of biochar in mitigating oxidative stress in *Solanum lycopersicum* under drought. The pot experiment was designed in a 3 × 4 factorial with five replicates. Factors included biochar application rates: B0 (no biochar), B1 (7.5 gkg^{-1}) and B2 (30 gkg^{-1}); and water regimes: 100% (field capacity, FC), 75% FC, 50% FC and 25% FC. The use of biochar decreased malondialdehyde (MDA) in tomato leaves and roots by 9.3% and 11.8%, respectively, compared with no biochar. On average, B2 decreased MDA and hydrogen peroxide (H_2O_2) in both leaf and root by 4% and 3.5%, respectively compared with B1. There was a significant interaction between biochar application and water regime in the production of catalase (CAT), ascorbate peroxidase (APx), ascorbic acid (AsA) and glutathione (GSH) in the root of *S. lycopersicum*. AsA, GSH increased by 51.1% and 27.5%, respectively under 25% FC compared with FC. Biochar decreased ROS by promoting the production of antioxidants (CAT, APx, AsA and GSH), especially under 25%FC. Among biochar levels, B2 was considered optimum for the alleviation of ROS and could be recommended for *S. lycopersicum* cultivation under drought for stress adaptation. The findings support biochar as a sustainable soil amendment for drought-prone regions.

Keywords: Drought, Soil amendment, Antioxidant, Reactive Oxygen Species

INTRODUCTION

Drought is one of the critical abiotic stresses that adversely affect soil fertility, water availability and consequently retard crop growth and development (Ghanbary *et al*., 2017; Jafarnia *et al*., 2018). Stresses, especially those of drought and nutrients, could enhance the excessive production of reactive oxygen species (ROS) due to disruption of cellular homeostasis (Meena *et al.*, 2017; Gou *et al*., 2020). ROS are constantly produced at low levels in cell organelles during photosynthesis and respiration, and as by-products of enzymatic reactions during metabolism. Though ROS play important roles in signal transduction and promotion of growth, they are also capable of inducing cellular damage (Du *et al*., 2018; Sachdev *et al.*, 2021), and ultimately resulting in cell death under stress conditions (Bano *et al.*, 2021; Aslam *et al.*, 2022).

To prevent oxidative stress, plants possess an extensive antioxidant defense system consisting of enzymes and metabolites. Major enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT), and peroxidases (POD) (Cuypers *et al*., 2016, Sachdev *et al.*, 2021); however, secondary metabolites, such as polyphenols, flavonoids and terpenoids also participate in the detoxification of ROS under different environmental stresses (Hashim *et al*., 2020; Nadarajah, 2020). Therefore, maintaining an oxidative balance between production and scavenging of ROS is necessary for stress adaptation in plants.

Soil management practices that enhance soil water availability and promote fertility are recommended as drought mitigation tools in irrigated and non-irrigated regions (El-Naggar et al., 2019; Phillips *et al.*, 2020). The application of soil amendment, especially the use of biochar has been identified as an ecofriendly approach towards alleviating the effects of drought stress on crop growth and yield. The use of biochar as a soil amendment has gained more attention recently due to its role in mitigating greenhouse gas emissions (Yang *et al.*, 2020; He *et al.*, 2021; Patikorn *et al.*, 2021), enhancing soil fertility and tolerance of plants to high-temperature stress and arsenic toxicity, promoting yield, water productivity and nutrient-use efficiency of crops (Atif *et al.*, 2021; Rahman *et al.*, 2023, Zhang *et al.*, 2024).

Tomato (*Solanum lycopersicum* L.), an important vegetable with a short life cycle is prone to environmental challenges including stress (Cuypers *et al*., 2016). Meena *et al.* (2017) reported that both enzymatic and non-enzymatic antioxidants are involved in ROS detoxification to ensure the acclimation of plants to stress. However, the adaptive mechanisms of *S. lycopersicum* to varying degrees of water regimes and the role of biochar in alleviating oxidative stress have not been well studied.

There are several mechanisms through which biochar as soil amendment might influence ROS balance. The ability of biochar to retain water and improve soil structure can indirectly enhance the management of ROS production. Biochar's surface has been reported to possess electron-exchanging properties that can interact with and neutralize ROS (Malik *et al.*, 2022). Biochar addition to soil can enhance the synthesis of metabolites in plants that aid the removal of

ROS, maintain cell homeostasis and promote absorption of N by plants (Yang *et al.,* 2020; Su *et al.,* 2023).

Therefore, this study aimed to determine the response of *S. lycopersicum* to varying degrees of water regime and to determine if the use of biochar as soil amendment could reduce the effect of drought on *S. lycopersicum* via exploring the maintenance of ROS and antioxidant production in its root and leaf.

MATERIALS AND METHODS

Study area

The experiment was conducted at the screen house of the Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, Nigeria (latitude7°30'N and longitude3°54'E) from April 2, 2023 to August 15, 2023. This is to investigate the effect of biochar application, water regime, and their interaction on ROS and antioxidants production in the roots and leaves of *S. lycopersicum.*

Sample collection and experimental design

Sandy-loam soil samples were collected from the experimental field of the Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta (latitude 7°30'N and longitude 3°54'E). Rice straw was sourced from rice farmers of Alabata community, air-dried and pyrolyzed at 400°C for 2 hours in a combustion chamber described by Sadaka *et al.* (2014) and modified by Fawibe *et al.* (2023). The biochar morphology and surface composition were examined using Scanning Electron Microscope-Energy Dispersive X-ray Spectroscopy (SEM-EDX) (Figure 1). Table 1 shows the physicochemical properties of the soil and rice-straw biochar.

The experiment was conducted using pots (height of 30 cm and an average diameter of 24 cm), each containing 5 kg of soil. Tomato seeds (Roma VF) obtained from the Institute of Agricultural Research and Training, Ibadan, Nigeria were raised in the nursery for three weeks before transplanting. One healthy seedling of *S. lycopersicum* was transplanted into each pot. The experiment was designed as a 3 × 4 factorial combination of biochar levels - control (B0), B1, and B2, and water regimes: 100% (field capacity, FC), 75% FC, 50% FC and 25% FC.

Biochar treatments were applied following the procedure of Pokovai *et al.* (2020). The control (B0) had no biochar, B1 and B2 contained 7.5 and 30 gkg^{-1} biochar in the soil, respectively. The 7.5 gkg^{-1} and 30 gkg^{-1} biochar applications indicate low and relatively high biochar treatments, respectively. Experimental pot surfaces were covered with foil after transplanting of seedlings to reduce direct evaporation. In FC, irrigation was supplied daily in the evening (17:00 hr to 19:00 hr) at a rate equal to the average evapotranspiration of the crop calculated by weighing the pots daily with an electronic balance. However, 75% FC, 50% FC and 25% FC received 75%, 50%, and 25% of the irrigation water supplied to FC, respectively.

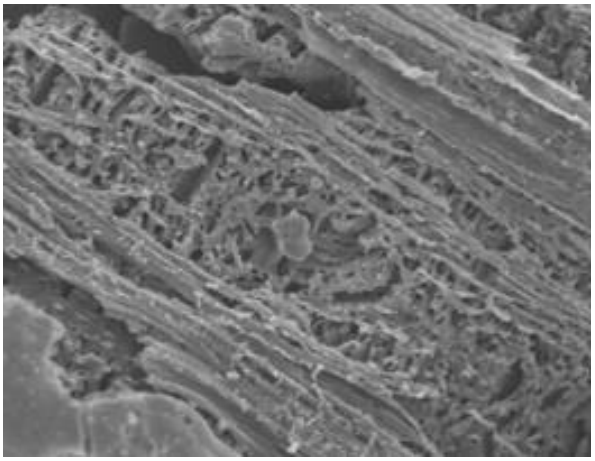

Figure 1: SEM image of rice-straw biochar at x500 magnification with a scale of 100 µm

Table 1: Physicochemical properties of soil and rice-straw biochar

	pH	Organic C (%)	Organic matter (%)	Exch. Anion (mEq/100g)	Nitrogen (%)	Available phosphorus (mg/kg)
Soil	6.6	0.6	1.1	0.2	0.2	17.8
Rice-straw biochar	9.2	56.8	97.9	1.2	0.7	6.4

(Adopted from Fawibe *et al*., 2023)

Biochemical Analysis of *Solanum lycopersicum* Leaves and Roots

At the flowering stage, leaves and roots of *S. lycopersicum* were collected and analyzed for the determination of reactive oxygen species (ROS), oxidative stress and antioxidant indices.

Determination of Reactive Oxygen Species and Oxidative Damage Indices

Malondialdehyde (MDA) level, an index for lipid peroxidation was used to assess the extent of oxidative damage in the root and leaf tissues as described by Buege and Aust (1978). Briefly, 0.5 mL of the 10% leaves or roots homogenates (prepared by homogenizing 1g of sample in 10 mL of 0.05M potassium phosphate buffer at pH 7.4) was mixed with the MDA working reagent containing 0.35% TBA, 25 mM HCl, and 15% TCA. Subsequently, the mixture was boiled for 15 minutes at 95ºC, and was cooled on the ice before centrifugation at 3500 rpm for 10 minutes. The absorbance of the supernatant was measured at 532 nm.

The level of hydrogen peroxide (H_2O_2), in the leaves and roots was measured based on the reaction between potassium iodide

(KI) and unreacted peroxides as described by Junglee *et al*. (2014). To initiate the reaction, equal volumes of the homogenate and 1M KI were mixed on the ice and centrifuged for 15 minutes at 5000 rpm. The sample was kept away from sunlight to avoid degradation, and the supernatant of the centrifuged sample was read at 390 nm on the spectrophotometer.

Determination of Enzymatic Antioxidant

To evaluate the enzymatic antioxidant, fresh leaves or roots extract was prepared by crushing 1g of the sample and was homogenized in a buffer containing 50 mM potassium phosphate buffer (pH 7.0), 1 mM ascorbic acid, 5 mM β-mercaptoethanol, 100 mM KCl, and 10% (w/v) glycerol, and then centrifuged at 5000 rpm for 15 minutes. The leaves and roots extracts were used for enzymatic activity assays including catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APx).

Catalase activity was measured in the extract as described by Hadwan and Abed (2015). Briefly, 0.2 mL of the extract was mixed with the catalase working reagent containing 0.001% hydrogen peroxide prepared in 0.05M potassium phosphate buffer, pH 7.0 on the ice. The reaction was terminated sequentially using 0.32 mM ammonium molybdate and the absorbance was read at 405 nm.

Superoxide dismutase was measured as described by Marklund and Marklund (1974). The principle was based on the auto degradation of pyrogallol in an alkaline pH. Briefly, 0.1mL of the extract was mixed with the SOD buffer containing 100 mM EDTA, 50 mM sodium phosphate buffer (pH 7.8). The reaction was initiated by adding 0.1mL 10 mM pyrogallol and the absorbance was read at 420 nm.

To measure the ascorbate peroxidase, 0.05 mL of the extract was mixed with the reaction mixture containing 0.5 mM ascorbate, 0.1M sodium phosphate buffer (pH 7), and 0.25 mL of EDTA. The reaction was initiated by adding 0.1 mM H_2O_2 and the absorbance was measured at 290 nm as described by Nakano and Asada (1981).

Determination of non-enzymatic antioxidant

The concentration of ascorbic acid (AsA) was measured according to Law *et al*. (1983). The same homogenates used for enzymatic antioxidants was used for the non-enzymatic antioxidants. The assay mixture (5.0 mL) containing 1.0 mL 5% TCA, 1.0 mL absolute ethanol, 0.5 mL 0.4% H_3PO4 – ethanol, 1.0 mL 0.5% phenanthroline – ethanol, 0.5 mL 0.03% $FeCl_3$ – ethanol and 1.0 mL enzyme extract. The assay was based on the reduction of Fe^{3+} to Fe^{2+} by AsA; Fe^{2+} was quantified spectrophotometrically at 534 nm for 90 min at 30ºC.

Glutathione (GSH) was quantified according to Hissin and Hilf (1976). Fresh roots and leaves (0.5 g) were ground in a mixture of 1 mL of 25% H_3PO4 and 3 mL of 0.1M sodium phosphate-EDTA buffer (pH 8.0). The homogenate was centrifuged at 10000×g for 20 min, and the supernatant was further diluted fivefold with sodium phosphate – EDTA buffer (pH 8.0). The final assay mixture (2.0 mL) contained 100 µL of the diluted supernatant, 1.8 mL of phosphate-EDTA buffer, and 100 µL of O-phthalaldehyde (1 mg mL^{-1}). The solution was observed at 420 nm.

For the determination of total soluble sugar (TSS), 500 mg of the plant sample was homogenized in 80% EDTA and centrifuged at 2000 rpm for 20 minutes. 1 mL of the supernatant was added to 1 mL of 5% phenol and mixed. Then, 5 mL of 96% sulphuric acid was added and allowed to stand in water for 20 mins at 26 - 30ºC. The absorbance was read at 490 nm.

Statistical Analysis

Two-way analysis of variance was performed using SPSS version 26.0 (IBM SPSS). The statistical model included sources of variation due to replication, Biochar (B), Water regime (W), and their interaction (B×W). Means were separated using Duncan's multiple range test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Effect of biochar application on production of reactive oxygen species (ROS) in leaves and roots of *S. lycopersicumn* under varying water regimes

MDA production in the leaves and roots of *S. lycopersicum* under varying applications of biochar and water regimes was not significantly different. However, the application of biochar decreased the quantity of MDA produced in the leaves and roots by 9.3% and 11.8%, respectively compared with no biochar (Table 2).

The application of biochar had no significant influence on the production of H_2O_2 in the roots of *S. lycopersicum*; nonetheless, B1 and B2 markedly decreased the H_2O_2 production in the leaves of *S. lycopersicum* by 15.5% and 19.9%, respectively compared with the control. The production of H_2O_2 significantly increased under high water stress condition (W25) compared with the field capacity (W100) (Table 2). Notably, the increase in biochar level further decreased the production of ROS (MDA and H_2O_2) in both the leaf and root of *S. lycopersicum*. On average, B2 decreased MDA and H_2O_2 by 4% and 3.5%, respectively compared with B1.

Table 2: Effect of biochar application on production of reactive oxygen species (ROS) in leaves and roots of *S. lycopersicum* under varying water regimes

Biochar (gkg^{-1})	Water regime	Reactive oxygen species			
		Leaf (nmol/g)		Root (nmol/g)	
		MDA	H_2O_2	MDA	H_2O_2
B0	25% FC	19.7	37.9a	17.6	23.8a
	50% FC	16.7	36.1b	17.6	21.6a
	75% FC	17.5	33.7bc	17.3	14.9b
	FC	18.9	29.1c	15.4	17.4b
B1	25% FC	16.9	29.5a	17.0	22.3a
	50% FC	15.4	31.7a	16.1	16.0ab
	75% FC	16.0	30.6a	15.8	13.4b
	FC	17.9	23.7b	13.3	15.8ab
B2	25% FC	15.6	30.3a	16.1	19.2a
	50% FC	16.0	25.0b	14.8	18.1a
	75% FC	16.5	29.9a	14.2	15.6a
	FC	17.5	24.5b	12.7	14.1b

Biochar (B)	ns	*	ns	ns
Water regime (W)	ns	*	ns	*
B×W	ns	ns	ns	ns

Means followed by different lowercase letters within a column for each biochar level indicate a significant difference at $p<0.05$ among water regimes. B0: no biochar, B1 and B2 with 7.5 and 30 gkg^{-1} soil, respectively. FC indicates field capacity. * indicates a significant difference at $p<0.05$ while ns means no significant difference. MDA and H_2O_2 are malondialdehyde and hydrogen peroxide, respectively.

Effect of biochar application on production of enzymatic antioxidants in leaves and roots of *S. lycopersicum* under varying water regimes

Variation exists in the production of enzymatic antioxidants under varying water regimes as influenced by the biochar treatment. There was a significant interaction between biochar and water regime in the production of SOD in the leaves and roots of *S. lycopersicum*. However, the interaction between the water regime and biochar was only significant in the production of CAT and APx in the root of *S. lycopersicum*. On average, B1 significantly increased SOD in the leaf and root by 102% and 60.9%, respectively, while APx was increased in the root by 1% compared with B0. Although CAT produced in the leaf under varying water regimes and biochar application was not significant, the application of biochar (B1 and B2) significantly increased its production in the root by 14.1% and 101.1%, respectively (Table 3).

B2 increased the production of SOD by 11.2% in the leaf and that of CAT by 76.3% in the root compared with B1. Enzymatic antioxidant production was higher in *S. lycopersicum* under 25 % water stress compared with those under field capacity.

Effect of biochar application on production of non-enzymatic antioxidants in leaves and roots of *S. lycopersicum* under varying water regimes

The variation in the response of *S. lycopersicum* roots to the production of non-enzymatic antioxidants under varying biochar applications and water regimes was significant. Biochar application increased the production of non-enzymatic antioxidants. On average, B1 significantly increased AsA and GSH in both the leaf and root of *S. lycopersicum* by 2.6% and 2.9%, respectively compared with B0. Moreover, the increase in biochar application in B2 further increased the production of AsA and GSH by 13% and 16.2% compared with B1 (Table 4).

Also, there was significant interaction between biochar application and water regime in relation to AsA and GSH production in the root of *S. lycopersicum*. TSS in the root of *S. lycopersicum* was not significantly influenced by the variation in both biochar and water level treatments; however, the influence of the interaction between biochar and water level was significant on the production of TSS in *S. lycopersicum* leaf (Table 4).

Irrespective of the biochar treatment, non-enzymatic antioxidant production increased in the root and leaf of *S. lycopersicum* as the stress level increased. On average AsA, GSH, and TSS increased by 51.1%, 27.5%, and 40.5%, respectively under severe (25% water regime) compared with the field capacity (100%).

Table 3: Effect of biochar application on production of enzymatic antioxidants in leaves and roots of *S. lycopersicum*under varying water regimes

Biochar (t/ha)	Water regime	Enzymatic antioxidants					
		Leaf			Root		
		CAT (kU/mg tissue)	SOD (U/mg)	APx (U/mg)	CAT (kU/mg tissue)	SOD (U/mg)	APx (U/mg)
B0	25% FC	1490	9.5a	0.43a	1035a	9.5a	0.33a
	50% FC	1291	4.6b	0.41b	903a	4.3b	0.30b
	75% FC	1125	1.8c	0.41b	244b	2.9b	0.26c
	FC	1075	1.6c	0.40b	185b	1.5b	0.23c
B1	25% FC	1477	16.0a	0.45a	1238a	13.2a	0.38a
	50% FC	1621	10.2b	0.41ab	641b	6.0b	0.28b
	75% FC	1631	3.9c	0.41ab	230c	5.1b	0.25c
	FC	1260	5.3bc	0.38b	592b	5.2b	0.25c
B2	25% FC	1928	24.6a	0.43a	1834a	9.3a	0.35a
	50% FC	1680	10.4b	0.43a	1856a	8.3a	0.35a
	75% FC	1315	3.1c	0.40b	845b	4.4b	0.25b
	FC	1305	1.3c	0.38b	226b	2.2c	0.20b
Biochar (B)		ns	*	ns	*	*	ns
Water regime (W)		ns	*	*	*	*	*
B × W		ns	*	ns	*	*	*

Means followed by different lowercase letters within a column for each biochar level indicate a significant difference at $p<0.05$ among water regimes. B0: no biochar, B1 and B2 with 7.5 and 30 gkg^{-1} soil, respectively. FC indicates field capacity. * indicates a significant difference at $p<0.05$ while ns means no significant difference. CAT, SOD, and APx are catalase, sodium dismutase and ascorbate peroxidase, respectively.

Table 4: Effect of biochar application on production of non-enzymatic antioxidants in leaves and roots of *S. lycopersicum* under varying water regimes

Biochar (t/ha)	Water regime	Non-enzymatic antioxidants					
		Leaf (µmol/g)			Root (µmol/g)		
		AsA	GSH	TSS	AsA	GSH	TSS
B0	25% FC	147.1a	5.8a	17.9a	114.6a	7.4a	18.5
	50% FC	125.6ab	5.4a	13.0b	109.6ab	4.2b	16.5
	75% FC	127.1ab	4.9a	15.4ab	98.5b	4.3b	15.9
	FC	115.2b	4.7a	13.0b	94.9b	4.2b	13.7
B1	25% FC	161.7a	6.0a	20.2a	127.1a	6.0a	19.5
	50% FC	129.9ab	5.4a	15.8b	102.4b	4.7a	21.9
	75% FC	125.6ab	5.4a	15.2b	97.8b	4.4a	16.9
	FC	114.9b	5.8a	14.4b	97.8b	3.9b	15.7
B2	25% FC	175.6a	7.3a	31.8a	213.9a	6.4a	22.5
	50% FC	147.4a	7.8a	14.3b	102.8c	5.4b	17.8
	75% FC	169.9a	5.2b	17.9b	168.5b	4.9b	19.4
	FC	98.5b	6.9a	17.8b	101.0c	5.0b	18.2
Biochar (B)		*	*	*	*	*	ns
Water regime (W)		*	ns	*	*	*	ns
B×W		ns	ns	*	*	*	ns

Means followed by different lowercase letters within a column for each biochar level indicate a significant difference at $p<0.05$ among water regimes B0: no biochar, B1 and B2 with 7.5 and 30 gkg^{-1} soil, respectively. FC indicates field capacity. * means significant difference at $p<0.05$ and ns means not significant by ANOVA. ASA, GSH, and TSS are ascorbic acid, glutathione, and total soluble sugar, respectively.

Discussion

Drought affects various physiological and metabolic activities in plants resulting in a rapid production of reactive oxygen species (ROS) thereby causing irreversible cellular damage through their strong oxidative properties. The increased production of ROS in both the leaf and root of *S. lycopersicum* under water-stressed conditions compared with field capacity could be attributed to the possible increase in photorespiration due to partial or total stomata closure- a common adaptive strategy developed by plants under water stress conditions. The mechanism of photorespiration in plants accounts for over 70% of total hydrogen peroxide production under drought stress (Farooq *et al.* 2009). This observation is in line with previous studies that reported increased accumulation of ROS in different plant parts due to water stress (Gilroy *et al.,* 2016; Swati *et al.,* 2021).

However, the application of biochar decreased MDA and H_2O_2 content in both the leaf and root of *S. lycopersicum* cultivated under severe water stress conditions. MDA is a product of ROS-mediated membrane lipid peroxidation, used as a biomarker of membrane damage caused by oxidative stress in plants. The reduction of H_2O_2 in the leaf and MDA in both the leaf and root of *S. lycopersicum* under biochar-amended soil suggested a coordinated activation of protective enzymes capable of scavenging excess ROS production; hence, avert oxidative damage in the tissues of the leaves and roots of plant (Lyu *et al*., 2016; Rondon *et al*., 2017). This finding corroborates the report of previous studies where biochar-amended soil decreased the MDA content of *Phragmites karka* and *Brassica oleracea* (cabbage seedling) under drought stress conditions (Abideen *et al*., 2020; Yildirim *et al*., 2021). However, our result of 9.3% and 11.8% decrease in MDA of *S. lycopersicum* leaves and roots, respectively with biochar application was in contrast with the report of Murtaza et al. (2024), who reported 40% reduction in MDA of *Zea mays* shoot after the application of 40 gkg^{-1} Acacia-derived biochar. The discrepancies in these observations could be related to the biochar composition, the quantity applied, and the sensitivity of the crop type to biochar treatments.

Moreover, our results denote a significant increase in the activities of enzymatic antioxidants such as CAT, SOD, and APx in *S. lycopersicum* under a low water regime compared with the field capacity. This outcome could be ascribed to the activation of ROS scavenging system for the protection of cellular components such as chloroplasts, mitochondria, and peroxisomes against oxidative burst that could be caused by transient ROS generation (Khan et al., 2023).

The presence of porous structure and oxygen functional groups capable of enhancing water-use efficiency and stabilization of plasma membrane activity have been previously reported on biochar particles (Suliman *et al*., 2017). The particles offset ROS accumulation by enhancing antioxidant enzymes activities in cells of *S. lycopersicum* under biochar-amended soil compared with no biochar. CAT possibly disproportionated H_2O_2 into H_2O and O_2, hence, controlling the concentration of H_2O_2 in cells (Malik *et al.,* 2022; Khan *et al.,* 2023). Also, SOD aligns with CAT and other enzymes to stop the production of toxic OH by both H_2O_2 and O_2 (Khan et al., 2023).

Biochar application improved the activities of protective enzymes to scavenge the over-accumulation of ROS under stress conditions thereby stabilizing the impact on photosynthetic apparatus and in turn preventing oxidative stress (Lyu *et al.,* 2016; Hafez *et al*., 2020). The non-enzymatic defense system of the AsA-GSH cycle in the chloroplast is a vital defense system to regulate the over-accumulation of ROS (Cuypers *et al*., 2016). The significant increase in AsA and GSH in both the leaf and root of *S. lycopersicum* under biochar-amended soil compared with no biochar indicates that the application of biochar as soil amendments can mitigate drought stress in plants.

The increased production of osmolytes such as soluble sugar has been reported as an indicator of reduced stress conditions (Zhu *et al*., 2019; Zoghi *et al*., 2019). Consequently, the significant increase in total soluble sugar in the leaf *S. lycopersicum* under biochar-amended soil with a low water regime indicates the ability of biochar to alleviate stress by managing osmotic pressure at the cellular level (Zhu *et al.,* 2019).

Batool *et al*. (2015) reported biochar as a drought stress mitigation approach for different plants. The integration of biochar into the soil improves the binding capacity of ions, elevates photosynthesis, transpiration, water potential, and stomatal conductance through improving soil quality, fertility, and water availability and consequently decreases the susceptibility of plants to drought conditions (Hafeez *et al*., 2017; Abideen *et al*., 2020). The influence of biochar on soil water management and nutrient dynamics depends on the soil type, source of biochar, quantity of biochar supplied, and pyrolysis conditions of biochar preparation (Al-Wabel *et al*., 2017). In this study, the increase in the quantity of biochar (B2) applied as an amendment further reduced the concentration of reactive oxygen species (MDA, H_2O_2) produced in both the leaf and root of *S. lycopersicum.* This is attributable to the maintenance of high enzymatic and non-enzymatic antioxidants compared with B1 which probably prompted the efficient scavenging of the toxic ROS, especially MDA and H_2O_2; hence, ensuring better tolerance of *S. lycopersicum* to both mild and severe water stress conditions.

The use of a single soil type and short-term experimental evaluation were considered limitations to this study, as they remain insufficient to provide information on the biochemical adaptation responses of *S. lycopersicum* under different soil types over a long period. Future research should establish long-term field trials using other crops to evaluate the varying influence of biochar concentrations on water stress adaptation.

CONCLUSION

The study shows that water stress increased the production of ROS in the leaves and roots of *S. lycopersicum*. However, the use of biochar as soil amendment decreased reactive oxygen species by promoting the production of both enzymatic and non-enzymatic antioxidants capable of scavenging the excess ROS, especially under severe water stress conditions.

Moreover, the increased level of biochar (30 gkg^{-1}) further alleviated the influence of the water stress; therefore, could be recommended as an appropriate quantity for soil amendment for the cultivation of *S. lycopersicum* where there is a paucity of water supply. These findings support biochar as a low-cost strategy for sustainable agriculture in water-limited regions.

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