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Synergetic effects of potassium and biochar on morphological, physiological, and biochemical attributes of maize crop grown under different levels of drought stress

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Abstract

Global climate change accelerates the challenges of agricultural drought spells, which are alarming for food security and can trigger food scarcity. Therefore, improving soil-water retention capability and crop drought resilience is becoming more important for sustainable agriculture. This study investigates the individual and combined effects of biochar and potassium on soil water retention, crop drought resilience, and related physio-biochemical mechanisms over a 50-day growth period in potted plants. Pine needle biochar (350 g/10 Kg of soil) was used during the soil preparation stage while potassium sulfate (100 mg/L) was applied as a foliar spray at the development (10 days) and vegetative stages (45 days) under three drought stress conditions: control (100% FC), mild (75% FC) and severe (40% FC). The results revealed that the combined application of biochar and potassium significantly increased morphological, physiological, and biochemical attributes of maize plants under drought stress, improving shoot fresh weight by 11%, 6%, and 5%, root fresh weight by 19%, 19%, and 23%, shoot length by 17%, 16%, and 19%, and root length by 21%, 30%, and 29% under control, mild, and severe drought stress conditions, respectively. Similarly, relative water contents (RWC) increased by 12%, 16%, and 20%, water potential (Ψ) increased by 26%, 22%, and 24%, osmotic potential (Ψs) increased by 100%, 59%, and 30%, and turgor potential (Ψp) increased by 28%, 35%, and 51% under combined treatment compared to control, mild, and severe drought stress. Additionally, biochar application with potassium foliar spray also improved membrane stability and integrity, cell wall loosening, membrane lipid peroxidation, and protein denaturing by decreasing electrolytic leakage by 35%, 28%, and 43%, proline by 30%, 27%, and 22%, hydrogen peroxidase by 47%, 45%, and 41%, and malondialdehyde contents by 24%, 20%, and 28% through activation of enzymatic (CAT, POD, SOD) and non-enzymatic (TSS, AsA, GSH) antioxidants. Furthermore, nutrient uptake was enhanced, with N increasing by 47%, 19%, and 45%, P by 64%, 82%, and 52%, and K by 24%, 42%, and 35% in shoots compared to normal, mild, and severe drought stress. These improvements mitigated cell dehydration, reduced transpiration inefficiency and delayed senescence, and ultimately supporting plant growth under drought stress. In conclusion,

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integrating biochar with potassium application effectively improves soil-water retention, alleviates oxidative stress and enhances drought tolerance in maize plants. This strategy can play a crucial role in sustainable agriculture by mitigating the adverse effects of drought stress and improving food security in drought-prone regions.

Keywords Maize, Drought, Potassium, Membrane stability, Cellular damage, Lipid peroxidation, Antioxidants

Introduction

Crops are facing serious threats due to sudden climate changes, including unexpected increased temperatures, irregular rainfall, and uneven precipitation, which intensify drought conditions in semi-arid and arid regions worldwide [1–3]. Several findings demonstrated the increasing spell of droughts, with an estimation presenting that approximately 30% of the worldwide agricultural land will face intensive drought by the 2090s, which is an alarming situation for the global agriculture sector. These challenges are expected to increase agricultural water demand by 10% [1, 3]. Drought stress is an extreme abiotic stress that damages crop growth, survival, and, ultimately, overall crop yield [4–7], which causes a significant risk to global food production, especially considering predictions demonstrating a massive rise in world pollution of 1 billion by 2030 and 2.4 billion by 2050 [8]. Additionally, global food demand is projected to rise by 50–70% by 2050 [1]. Therefore, enhancing plant resistance to drought is a critical challenge in efforts to increase plant yield and meet the growing food needs of a growing world population [9, 10].

Maize (*Zea mays L.*) is the most crucial staple food crop worldwide, with an average yield of more than rice and wheat. It is mostly cultivated in semi-arid regions, and nowadays, it is also cultivated in arid regions, which may be important to solve the imminent food scarcity challenges in areas where drought and increased temperature are prominent issues [11, 12]. Maize has been reported as vulnerable to drought stress, which triggered a substantial decline in growth and production [13–15]. Drought stress mainly damages maize metabolic activities, reduces biomass accumulation, and decreases photosynthetic rate by limiting chlorophyll contents in leaves, leading to stunt growth and yield [3]. Additionally, water stress at the vegetative stage can inhibit the overall growth rate, hindered root elongation, alter carbohydrate translocation and circulation, extend the vegetative growth of maize, extremely restrict the leaf surface area, and inhibit yield [16]. Consequently, a better understanding of drought stress and the role of organic and inorganic nutrients in maize production is essential to the soil-crop management system.

Several soil-crop management practices play a crucial role in increasing soil upholding capacity against drought stress and maintaining nutrient levels for ideal plant growth. In numerous studies, soil amendment with organic additives like biochar has been reported to be beneficial in sequestrating carbon from the environment, increasing water holding capacity, and amending soil health and quality by enhancing cation exchange capacity, organic matter level, and soil fertility by stimulating microbial activities and nutrient retention in soil [4, 11, 16, 17]. Biochar, mainly produced from the decomposition of organic biowaste at relatively high temperatures (400–700 ℃), has appeared as an effective soil supplement for improving crop resistance against drought stress [1]. Because of its high porosity, surface area, and capability to enhance soil-water retention and nutrient accessibility, biochar has been revealed to improve plant growth and development, water-holding and -use capacity, and stress resilience in several crops [18].

Additionally, potassium (K), being a significant mineral nutrient for plant development and growth, plays an important role in numerous physio-chemical and metabolic processes like stomatal regulation, cell elongation, enzymatic and non-enzymatic activities, and osmoregulation [19]. K^+ application in crops grown under drought settings boosts crop resilience to water deficit conditions and improves crop overall growth, dry biomass accumulation, and average yield [1, 19, 20]. K^+ is also crucial to maintaining plant-water interactions through managing ionic equilibrium within cells and accelerating enzymes that participate in several metabolic and biochemical reactions and nutrient uptake, particularly in the absorption and distribution of nitrates from roots to vegetative plant parts [20]. Additionally, potassium is crucial in maintaining crop-water interactions by maintaining ionic balances within cells and trig enzymatic and non-enzymatic to catalyze several metabolic mechanisms and nutrient uptake, particularly the nitrates translocation from roots to the upper parts of plants [21]. Plants facing water deficit conditions need K^+ to regulate CO2 fixation for photosynthesis, protect chlorophyll from reactive oxygen species, maintain stomatal functioning and water relations, and diminish translated damages in carbohydrate metabolisms [22]. Consequently, regulating optimum K^+ levels is essential for creating drought stress tolerance and enhancing crop normal growth under several drought levels.

Recent findings highlight the beneficial effects of potassium and biochar on accelerating drought tolerance in several food crops. For instance, Ali [23] demonstrated that K^+ application alleviated water deficit effects on wheat plants, maintaining crop yield, quality, and other physio-biochemical features. A recent study by Ahmad

[24] reported that K^+ application in *Triticum aestivum* alleviates water deficit conditions by improving leaf surface area, photosynthesis, dry biomass, and overall plant growth by improving antioxidant enzyme activities and metabolic processes, which resulted in improvement in stomatal conductance by 38% and photosynthetic rate by 13%, respectively. However, most literature mainly emphasizes the individual application of K^+ or biochar to mitigate water-deficit stress, leaving a lack of studies revealing their co-application effects on improving physiological, biochemical, and morphological defense mechanisms for improving crop yield under water-deficit conditions. Similar results were reported by Chowdhury [1]. Besides, most studies focus on wheat crops to examine the individual and combined effects of K^+ and biochar, while maize crops have not been studied yet. Thus, it would be imperative to reveal the combined effects of biochar and K^+ on physiological, biochemical, and morphological processes on maize grown under different levels of drought stress. However, limited research has been done to investigate the synergistic impacts of the combined application of K^+ and biochar on maize under different levels of drought, despite its potential benefits in enhancing maize yield, productivity and drought resilience. Given the importance of maize as a fodder crop, this study aimed to investigate the synergistic effects of the combined application of biochar as a soil biological amendment together with the foliar application of K^+. We hypothesized that this combined application would substantially alleviate drought stress, thus improving maize productivity. This study is crucial for advancing sustainable agriculture through strengthening crop resistance to different levels of drought stress, addressing a critical research gap in crop stress agronomy and physiology.

Methodology

Study area and experimental layout

A pot study was conducted in a warehouse at the Department of soil and Environmental Science, University of Agriculture, Faisalabad, Pakistan, and biochar characteristics and biochemical analysis were performed at the College of Chemical and Environmental Engineering, Shenzhen University, Shenzhen, China, from August - October 2023. The experiment was designed to study the biochar and K- induced tolerance against different levels of drought stress in maize cultivars (EV-1089). Figure 1 demonstrates the overall experimental layout. The maize (*Zea mays L.)* cultivar **EV-1089** seeds were obtained from the Maize and Millet Research Institute (MMRI), Yusafwala, Sahiwal, Pakistan. Before being used in the study, these seeds were certified for purity and viability. We used EV-1089 for our experiment due to its high productivity, yield, and resistance to harsh environmental conditions. Seeds were sowed in 36 polyethylene-lined plastic pots filled with 10 kg of air-dried, sieved, grounded, and well-mixed soil collected from the College of Soil and Environmental Sciences, University of Agriculture, Pakistan. The plant materials, including leaves, stems, and roots, were collected after 25 and 50 days of experiment from maize plants grown under controlled experimental conditions at the Research Farm of Soil and Environmental Sciences, Agriculture University Faisalabad. The plants were cultivated following standard agronomic practices, ensuring uniform growth conditions to minimize variability in the experimental results. All plant samples were carefully harvested at the designated growth stage, properly labeled, and stored under appropriate conditions before further analysis.

Soil chemical and physical properties were performed before starting the experiment, as demonstrated in Table 1. Seeds sowing was performed in mid-August using 10 seeds per pot, maintaining an appropriate distance. Before seed sowing, a recommended number of fertilizers were applied. In each pot, basal uniform rates of N, P, and K fertilizers were added respectively @ 120, 90, and 60 mg kg^{-1} soil as urea [$(NH_2)_2CO$], DAP [$(NS_4)_2HPO_4$] and potassium sulfate (K_2SO_4).

Preparation of pine needle biochar

Dried pine needles were collected, cleaned to remove debris, and air-dried for 5–7 days to reduce moisture content. The dried needles were then placed in a stainless-steel crucible and subjected to pyrolysis in a muffle furnace at 500 °C for 2 h under limited oxygen conditions, with a heating rate of 10 °C per minute. After pyrolysis, the biochar was allowed to cool in an inert atmosphere before being ground into a fine powder and sieved to a uniform particle size. The resulting biochar was characterized for pH and cation exchange capacity (CEC), elemental composition, and surface morphology and porosity to assess its suitability for environmental applications, as demonstrated in Table 2. The biochar preparation and characterization process was performed in the College of Chemical and Environmental Engineering, Shenzhen University, Shenzhen, China. We measured biochar surface area and porosity through the BET (Brunauer-Emmett-Teller) strategy, revealing a high porosity that improves biochar's water retention and nutrient absorption. Additionally, the elemental composition of biochar (C, N, P) was measured using ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy), emphasizing its role in soil nutrient enrichment. Moreover, biochar stability and decomposition were analyzed using thermogravimetric analysis (TGA) to examine the biochar's thermal stability and carbon persistence. Finally, CEC was measured through a CEC portable meter to assess its role in nutrient retention and slow-release capability under water deficit conditions. pH was

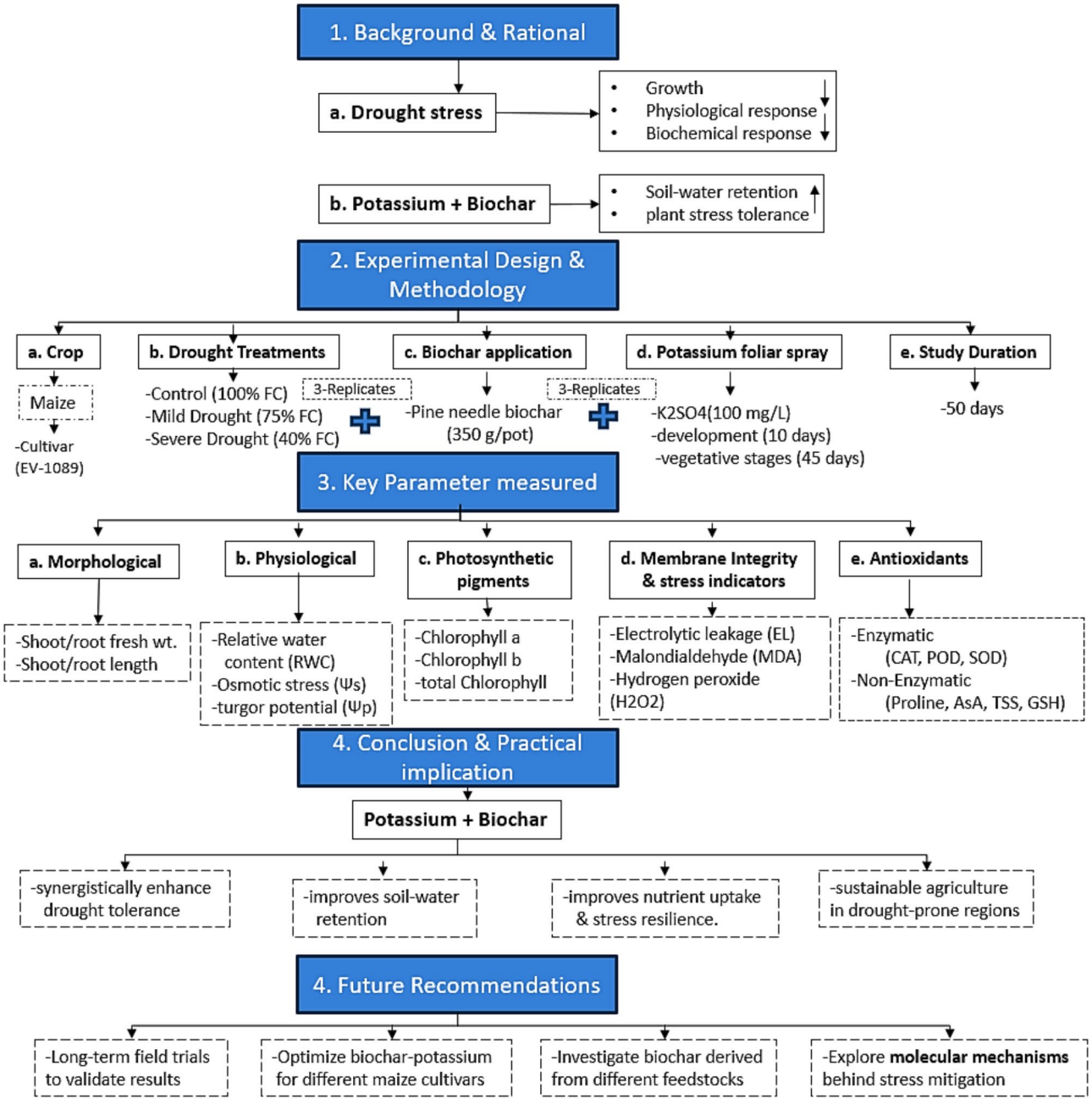

Fig. 1 Graphical representation of experimental layout, methodology, measured parameters, conclusion, and future recommendations

Table 1 Physio-chemical characteristics of experimental soil

Soil properties	Value
Textural Class	Sandy loam
Soil pH	7.8 ± 0.39
Soil EC (dS m^{-1})	13.42 ± 1.21
Bulk density (g cm^{-3})	1.47 ± 0.21
Particle density (g cm^{-3})	2.65 ± 0.67
Organic matter (%)	0.91 ± 0.11
Total nitrogen (%)	0.07 ± 0.003
Available phosphorous (μ g/g soil)	27.66 ± 3.54
Available potassium (meq/100 g soil)	0.21 ± 0.02

Table 2 Physio-chemical properties of pine needle Biochar

Biochar parameters	Units	Value
pH	-	7.11
EC	(dS m^{-1})	2.33
Volatile matter	%	66.39
Ash contents	%	6.19
Fixed carbon	%	53.72
CEC	mmol kg^{-1}	15.9
Total nitrogen	%	0.34
Total phosphorous	%	0.52
Total potassium	%	1.41

measured through a pH meter, and biochar had an alkaline pH, which might neutralize soil acidity and enhance nutrient availability. The prepared biochar was stored in an airtight container for further experimental use.

Experimental layout and treatment

A total of 12 treatments were used under a complete randomized design (CRD) with three replications in a two-factor experimental design. We used three levels of drought (D_0: 100% FC, D_1: 75% FC, and D_2: 40% FC) with two levels of K^+ (0 and 100 mg/L) and two levels of biochar (0 and 350 g/10 Kg of soil). In our experiments, the plants belonging to D_0 were irrigated regularly to a maximum amount of soil field capacity and experienced no water stress, and these plants served as control conditions in drought stress. The plants grown under D_1 experienced moderate water stress, and they were irrigated at 75% of soil field capacity, and soil moisture was available at 75%. The plants grown under D_2 experienced severe drought stress, where only 40% of the optimal soil moisture is available. Soil amendments (biochar) and foliar application (potassium; K) were applied as follows: control (T_0: no biochar and K^+), biochar (T_1: 350 g/ 10Kg of soil), potassium (T_2: 100 mg/L), biochar and potassium in combination (T_3: 350 g/10 Kg of soil + 100 mg/L). In this experiment, to improve the accuracy of the physiological and biochemical examination, we applied an optimized amount of biochar as suggested by Zulfiqar [25] and applied foliar application of K^+ by the recommendations of Abdallah [26] for the whole experimental treatment. Additionally, we applied the biochar in soil and mixed it thoroughly during the precipitation stage, while K^+ in the form of K_2SO_4 was sprayed as per the designed treatment on plants during each level of drought at each of the growing stages. To enhance the adhesion of K^+ to the leaves of maize, 0.1% Silwet® Gold was used as per the suggestions of Chowdhury [1] as a wetting or surfactant agent. The K^+ was sprayed in the evening at low temperatures to avoid fast evaporation of the sprayed solution as per the recommendations of Henningsen [27]. Before spraying K, the pots were completely enclosed in plastic files and tissue papers to avoid the contamination of soil as an absorbent. Physiological characteristics of biochar were measured and represented in Table 2.

Morphological attributes

Out of ten plants, six maize plants were uprooted and incorporated into the soil of the same pot, and only four plants were maintained for further study. After 25 days of growth, two plants were harvested out of four, while the remaining two plants were maintained till the end of the experiment. Plant samples were carefully washed with double distilled water and blotted dry with tissue paper to measure physiological and growth-related parameters. Plant fresh weight was measured with digital weight balance; plant height was measured with measuring tape; leaves numbers and number of tillers were counted manually. The harvest plants were separated into root and shoot samples to measure root and shoot fresh and dry weight and root and shoot length. Finally, the plant samples were taken in the lab, placed in a drier, and air-dried at 65 °C to constant weight and measured the dried weight with digital weight balance. The air-dried samples were grounded and preserved in polythene bags for further investigation. The second harvesting was done after 50 days of germination, and two plants per treatment were harvested and preserved in plastic bags for physiological, biochemical, and morphological investigations.

Physiological parameters

Leaf relative water contents (%)

Relative leaf water contents (%) of maize plants were measured by the suggested method of Barrs [28]. For this purpose, three leaf laminas were collected from four plants of each treatment after 50 days of germination, and fresh weight (FW) was measured immediately by digital weight balance. The detached leaf samples were soaked in double distilled water overnight and removed from the water after 24 h; excessive water was removed by blotting tissue paper and measured turgid weight (TW). After that, leaves were incubated in an electronic oven at 80 °C for 48 h until constant weight and dry weight (DW) were recorded. RWC was examined by using the equation recommended by Barrs [28] and expressed in percentage as follows:

$$\boldsymbol{RWC}\ (\%) = \frac{\boldsymbol{FW} - \boldsymbol{DW}}{\boldsymbol{TW} - \boldsymbol{DW}} \times 100 \qquad (1)$$

Assessment of water potential, osmotic potential, and turgid pressure

Water potentials in drought-stressed maize plants were assessed using Boyer's isopiestic strategy [29]. This assessment was instantly followed by assessing osmotic potential in the same leaf tissues using the same strategy after freezing the tissue on solid CO_2 and thawing. Leaf turgid pressure as assessed by subtraction of osmotic potential from water potential. Finally, no association was made for dilution of the protoplast mixture by water swallowed in the cell wall as the cell wall fixed volume was 2.8–7.6% of the total cell volume in the fresh leaf tissue. The cell wall matrix potential was neglected during the measurement of water potential, osmotic potential, and turgid pressure.

Assessment of electrolytic leakage (%)

Electrolytic leakage (EL) was accessed using Lutts' method [30]. Shortly, 1 g of fresh leaves of each treatment

replica was washed in double distilled water and then sliced into very slight parts through a cylindrical bowl with a steel bottom of 1 cm diameter and transferred in test tubes already composed of 20 ml of double deionized water. After that, the plants-occupied test tube was incubated for one day at 25 °C, and first EC1 was measured using an electrical conductivity meter (HI-993,310, Hanna, USA). The test tubes were again incubated to measure the second EC2 at 120 °C for half an hour in the water bath. Lastly, the EL was measured by using the subsequent equation:

$$\textit{Electrical conductivity}\ (\%) = \frac{EC1}{EC2} \times 100 \quad (2)$$

Assessment of hydrogen peroxide

Hydrogen peroxide (H_2O_2) contents were measured from fresh leaf samples using the Velikova method [31]. For this purpose, 0.5 g of fresh leaf samples from each treatment replicate were homogenized with 5 ml of 0.1% trichloroacetic acid (TCA) in an ice bath for half an hour. The mixture was centrifuged at 12,000 rpm for 20 min at 4 °C to collect the clear supernatant. Then, 1 ml of supernatant was thoroughly miscellaneous with 1 ml potassium iodide (KI) and 0.1 M potassium phosphate buffer with pH 7.8. The solution was vortexed and then incubated in an incubator for one hour. The absorption of the solution was accessed at 390 nm through a spectrophotometer (UV-500). The H_2O_2 contents were measured by using a standard curve, and results were expressed in µmol g^{-1} fresh weight.

Assessment of malondialdehyde (MDA) contents

MDA contents were assessed using the Heath strategy [32]. Initially, 0.5 g of fresh leaf samples from each treatment replicate were homogenized with 3 ml of 5% (w/v) trichloroacetic acid (TCA). The supernatant was collected from a homogenized solution after 11,500 rpm centrifugation for 15 min. Then 2 ml of supernatant was thoroughly mixed with 4 ml thiobarbituric acid (TBA) and placed in the water bath for 30 min at 100 °C and cooled down at room temperature, then again centrifuged at 11,500 rpm for 10 min at 4 °C. Finally, the optical density was recorded at 532 nm wavelength and adjusted for non-specific optical density at 600 nm. MDA contents were measured using the extinct factor of 155 mM^{-1} cm^{-1} and articulated the readings in $nmolg^{-1}FW$.

Assessment of proline

Proline is considered a stress-responsive protein and is mainly determined as a catalog of stress in plants. In our study, proline contents were measured after 50 days of germination of maize seedlings from fully expanded uppermost leaves, as suggested by Bates [33]. For this purpose, fresh leaves were homogenized in 5 ml of sulfosalicylic acid using a mortar pestle and ice to lower the temperature. The supernatant was separated after 12,000 rpm centrifugation for 15 min; after that, 2 ml of supernatant was mixed with 2 ml glacial acetate and 2 ml of acid ninhydrin solution. The resulting solution was incubated in a water bath for 1 h at 100 °C, and 4 ml of toluene was added at room temperature. The mixture was vortexed carefully to extract the chromophore. The supernatant of the mixture was transferred into the test tube, and optical density was accessed at 520 nm wavelength through an ultraviolet spectrophotometer. Finally, proline concentration was measured by drawing the value against the standard-curve from identified proline levels (10, 20, 30, 40, 50, 60, and 70 ppm).

Assessment of enzymatic and nonenzymatic antioxidants

Fresh leaf samples (0.2 g) of maize plants were homogenized in phosphate buffer (5 ml) of 50 mM concentration along with neural pH comprising polyvinylpolypyrrolidone (1%) and EDTA (1 mM) to abstract protein. The homogenized samples were passed through the process of centrifugation at 10,000 rpm for 20 min at 4 °C. The transparent supernatant was separated into newly arranged test tubes to examine the subsequent antioxidant enzymes. Bradford [34] method was used to extract and measure protein contents through bovine serum albumin standard with some modifications.

Superoxidase (SOD) activity in the shoot of maize plants was assessed by the method of Beauchamp [35], where 1 SOD unit = 50% reduced amount of SOD in NBT. The assessment of guaiacol oxidation (e = 26.6 mM-1 cm-1) at the wavelength of 470 nm by H_2O_2 indicated the activity of POD in fresh leaves of maize plants. Although CAT contents are based on the consumption of H_2O_2 at the wavelength of 240 nm (e = 39.4 mM^{-1} cm^{-1}) as reported in earlier studies [51].

Ascorbic acid (AsA) concentration was measured by the reduction method of Mukherjee [36], where AsA was reduced in ferric into ferrous ions. In this procedure, initially, fully expended leaves were mashed up in liquid nitrogen (N_2), and the mixture was homogenized with 6% (w/v) trichloroacetic acid, 2% (w/v) dinitrophenylhydrazine mixed with H_2SO_4 (50%), and 10% thiourea in ethanol (70% concentration). The homogenized mixture was attained and put in a water container for 20 min. The mixture was cooled at room temperature and passed through centrifugation (1000×g) for 10 min at 4 °C. The transparent supernatant was transferred to another test tube and was mixed thoroughly with H_2SO_4 (80%). Finally, the light optical density was measured at 530 nm wavelength by spectrophotometer (TU-1810). The standard curve was obtained by pure AsA solution, and readings were articulated in µmol g^{-1} FW.

Additionally, glutathione (GSH) absorbance was measured by the Griffith [37] method, where the fresh leaves were homogenized with 5,5-dithiobis-2-nitrobenzoic acid (DTNB) with neutral pH of sulfhydryl groups. This homogenized mixture was then mixed with 3 ml of 4% sulfosalicylic acid and centrifuged at 1000×g for 15 min. Lately, 500 µL transparent supernatant was mixed with Ellman's reagent. Lastly, the optical density of the mixture was measured at 412 nm wavelength by using a spectrophotometer (TU-1810), and GSH concentration was articulated in µmol g^{-1} FW.

Total soluble sugar (TSS) concentration was assessed by the method of Irigoyen [38], where 0.2 g air-dried leaf samples were homogenized with 5 ml ethyl alcohol (96%: v/v) and then cleaned with 5 ml of ethyl alcohol (70%: v/v). The extract was passed through centrifugation (3500×g) for 10 min by centrifuge (TGL-18 M). The transparent supernatant was cooled at 4 C and mixed with 3 ml of anthrone reagent ([150 mg of anthrone added with 100 ml of H_2SO_4; 72% v/v]) and was heated in a water bath (HWS-28) at 100 °C for 15 min. The mixture was cooled down at room temperature, and the optical density of the mixture was measured at 625 nm wavelength.

Photosynthetic parameters

Assessment of chlorophyll A, B, and carotenoid contents

Chlorophyll pigments (Chla, Chlb, and total chlorophyll) in maize plants were measured by the method of Arnon [39] and modified according to the laboratory setting by the protocol of Rahman [40]. Briefly, 0.2 g of fresh leaves were collected from each treatment replica and placed in colorimetric glass test tubes with 5 ml 80% acetone solution and incubated overnight in dark conditions to determine chlorophyll contents. The colorimetric tubes were centrifuged at 11,500 rpm at 4 °C, and the supernatant was separated. The optical density of the supernatant was assessed at 663 nm, 470 nm, and 645 nm wavelengths using a spectrophotometer (UV-5000) to measure Chl a, carotenoids, and Chl b, correspondingly. The subsequent equations were applied to determine the Chl a and Chl b concentrations:

$$Chlorophyll\ a\ (mg/g) = 12.7(OD663) - 2.69(OD645)\ V/1000(W).$$

$$Chlorophyll\ b(mg/g) = 22.9(OD645) - 4.68(OD663)\ V/1000(W).$$

Here, V stands for final volume, OD stands for wavelength, and W stands for fresh leaf weight.

Finally, total chlorophyll was assessed by totaling the concentrations of Chla and Chlb, which was assessed by the subsequent equation:

$$Total\ Chlorophyll\ (mg/g) = Chl\ a + Chl\ b.$$

Assessment of gaseous parameters (photosynthetic rate, stomatal conductance, and transpiration rate)

Gaseous parameters were measured by using the strategy to assess the rate of CO_2 uptake and release of water vapor (transpiration) through maize leaves. For this purpose, we used the portable gas exchange instrument COR LI-6400. The fresh leaves were enclosed in the chamber where CO_2 concentration and transpiration rate were calculated. This instrument works on the principle of photosynthesis and lowers CO_2 in the leaf chamber as plants fix it into carbohydrates. Using this gas exchange instrument, we measured the Net photosynthetic rate (A), transpiration rate (SD), stomatal conductance (gs), and intracellular CO_2 concentration.

Statistical analysis

The data was analyzed statistically using several R packages (version 4.2.1). All recorded data was observed in two-way ANOVA followed by mean comparison subsequent to Tukey's HSD (honestly significant difference) test using the "Agricolae" statistical bundle. Mean values of the data were articulated as mean ± SD with significant alphabet values and are visualized in a bar graph using the "ggplot2" statistical package. The Tukey pairwise comparison tests were done with a 95% confidence interval and 5% statistically significant level. This post-hoc analysis allowed a detailed examination of specific differences between various treatments and drought levels while maintaining high confidence. Pearson correlation in subjected variables was assessed by using the "corrplot" bundle in R. To recognize the most influencing and contributing variables and the interaction among them, we used principal component analysis (PCA) using "FactoMineR" and "factoextra" statistical packages. For further assessment of association among the subject parameters at different drought levels, biochar, and potassium treatment, a heatmap was made using the "heatmaply" statistical bundle.

Results

Synergistic effects on morphological parameters

In the present study, we evaluated the individual as well as synergistic effects of two levels of potassium (0 and 100 mg/L) and biochar (0 and 350 g/10 Kg of soil) on morphological parameters like root and shoot fresh and dry weights, root and shoot length of maize plants grown under different levels of drought stress, as verified in Table 3. Results demonstrated that morphological parameters were negatively affected by increasing drought stress. For instance, shoot weight was decreased by 11% and 38.3% in mild (75 FC) and severe (40 FC) water stress as compared to control (no water stress with

Table 3 The effects of treatment on morphological parameters of maize crop under three levels of drought stress. The different letters along means represents significant variations among treatments at $p < 0.05$ as determined by Tukey test and 95% confidence interval

Drought	Treatment	SFW (g)	SDW(g)	RFW (g)	RDW (g)	SL (cm)	RL (cm)
D0	T_0	81.54 ± 6.34^{c}	39.51 ± 2.87^{d}	30.18 ± 2.44^{c}	11.62 ± 1.01^{cd}	99.15 ± 5.07^{d}	14.35 ± 1.22^{c}
	T_1	85.89 ± 5.41^{b}	45.93 ± 2.29^{b}	33.06 ± 3.01^{b}	13.08 ± 0.87^{b}	104.73 ± 6.54^{c}	16.37 ± 1.19^{b}
	T_2	83.11 ± 6.23^{bc}	42.68 ± 2.43^{c}	31.80 ± 2.60^{b}	12.21 ± 0.67^{c}	109.96 ± 8.01^{b}	15.05 ± 1.09^{c}
	T_3	90.31 ± 7.82^{a}	48.22 ± 3.01^{a}	35.97 ± 2.31^{a}	14.55 ± 1.43^{a}	115.90 ± 5.83^{a}	17.41 ± 1.58^{a}
D1	T_0	73.17 ± 4.35^{e}	29.55 ± 2.88^{g}	22.36 ± 1.89^{f}	7.71 ± 1.07^{f}	79.12 ± 4.56^{h}	11.33 ± 1.37^{ef}
	T_1	75.82 ± 3.87^{de}	35.58 ± 2.67^{e}	24.41 ± 2.02^{e}	9.05 ± 0.77^{e}	82.11 ± 3.82^{g}	13.29 ± 1.42^{d}
	T_2	74.58 ± 4.01^{e}	33.34 ± 1.98^{f}	23.42 ± 1.35^{ef}	8.51 ± 0.91^{e}	88.04 ± 6.01^{f}	12.03 ± 1.77^{e}
	T_3	77.98 ± 5.33^{d}	38.31 ± 1.54^{d}	26.76 ± 1.78^{d}	11.09 ± 0.45^{d}	92.09 ± 2.89^{e}	14.77 ± 0.99^{c}
D2	T_0	58.95 ± 3.09^{i}	19.47 ± 1.19^{i}	17.91 ± 0.89^{h}	4.63 ± 0.22^{i}	66.70 ± 3.50^{j}	8.42 ± 1.01^{g}
	T_1	66.51 ± 2.87^{g}	23.06 ± 2.01^{h}	19.70 ± 1.32^{g}	5.70 ± 0.42^{h}	74.36 ± 4.01^{i}	9.11 ± 0.53^{fg}
	T_2	63.25 ± 3.76^{h}	21.66 ± 1.57^{hi}	18.16 ± 1.45^{h}	4.92 ± 0.19^{i}	74.85 ± 4.27^{i}	8.73 ± 1.28^{g}
	T_3	69.85 ± 4.81^{f}	28.07 ± 1.82^{g}	22.03 ± 2.21^{f}	6.99 ± 0.62^{g}	79.35 ± 3.87^{gh}	10.88 ± 1.71^{f}
CV %		5.94	6.68	4.79	7.07	6.19	5.83

no K and biochar). Similarly, root fresh weight decreased by 35% and 68% in mild and severe water stress compared to control. Contrasting to this, soil amendments were applied individually and in combination (K^+ + biochar), and the deleterious effects of mild and severe water stress were significantly encountered. For instance, K-foliar sprays increased shoot fresh weight by 2%, 2%, and 7%, root fresh weight by 5%, 4%, and 1%, shoot length by 10%, 11%, and 10%, and root length by 8%, 6%, 4% as compared to plants under no, mild, and severe water stress. Similarly, biochar application increased shoot fresh weight by 5%, 4%, and 13%, root fresh weight by 9%, 9%, and 10%, shoot length by 6%, 4%, and 11%, root length by 14%, 17%, and 8%, as compared to plants under no, mild, and severe water stress. In individuals, biochar soil application, compared to K-foliar spray, performed well against no mild or severe water stress. Additionally, our findings emphasized that the combined application of soil amendments (K-foliar spray and biochar soil application) showed more promising results against both mild and severe water stress. For instance, K + biochar application increased shoot fresh weight by 10.8%, 6%, and 5%, root fresh weight by 19%, 19%, and 23%, shoot length by 17%, 16%, and 19%, and root length by 21%, 30%, and 29%, as compared to plants under no, mild, and severe water stress, respectively (Table 3). A similar trend was recorded in root and shoot dry weight.

Synergistic effects on gaseous parameters

We evaluated the individual as well as synergistic effects of potassium (100 mg/L) and biochar (350 g/10Kg of soil) on gaseous parameters like chlorophyll a, b, total chlorophyll, carotenoids, stomatal conductance, photosynthetic rate, and transpiration level of maize plants grown under different levels of water stress, as demonstrated in Figs. 2 and 3. Our findings demonstrated that mild (75FC) and severe (40FC) water stress significantly decreased all measured gaseous parameters as compared to optimal moisture contents (100FC). For instance, a substantial decline of 25% and 71% in Chla contents, 59% and 117% in Chlb contents, 34% and 82% in total Chl contents, 35% and 118% in carotenoids contents, 38% and 149% in photosynthetic rate, 50% and 107% in transpiration rate and 32% and 76% in stomatal conductance was reported in plants grown under mild and severe water stress, respectively as compared to plants grown under optimal moisture level. Contrasting to this, the application of amendments like biochar soil application and K foliar application individually and in combination were reported beneficial in demolishing the deleterious effects of both levels of drought stress. K foliar application in individuals increased all gaseous parameters under all levels of water stress. K individual application increased Chla contents by 2%, 7%, and 9%, Chlb contents by 8%, 13%, and 5%, total Chl contents by 3%, 8%, and 8%, carotenoids contents by 10%, 19%, and 39%, photosynthetic rate by 13%, 10%, and 25%, stomatal conductance by 14%, 8%, and 11%, and transpiration rate by 24%, 23%, and 25%, as compared to plants grown under no, mild and severe water stress conditions. Similarly, biochar individual application increased Chla contents by 6%, 14%, and 17%, Chlb contents by 20%, 26%, and 23%, total Chl contents by 10%, 17%, and 19%, carotenoids contents by 8%, 14%, and 20%, photosynthetic rate by 20%, 15%, and 45%, stomatal conductance by 19%, 16%, and 21%, and transpiration rate by 10%, 12%, and 12%, as compared to plants grown under no, mild, and severe water stresses. Our findings emphasized that biochar individual application was more effective in alleviating mild and severe water stress than K foliar application (Figs. 1 and 2). Additionally, biochar + K combined application gave more promising results in further accelerating gaseous paraments of maize plants under mild and severe water deficit conditions. For instance, biochar + K combined application

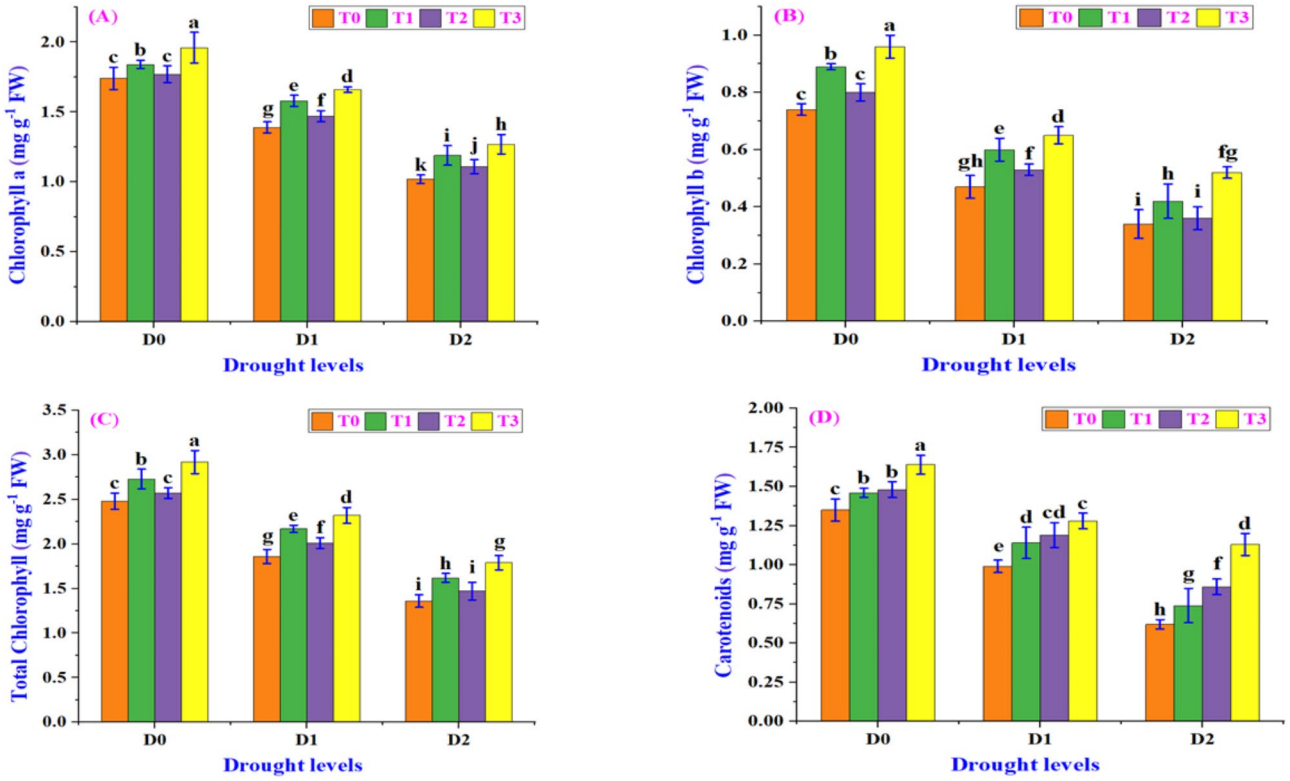

Fig. 2 Individual as well as the combined effect of biochar and potassium (T0: control, T1: 350 g biochar/10 Kg of soil, T2: 100 mg/L K_2SO_4, and T3: 350 g biochar /10 Kg of soil + 100 mg/L K_2SO_4) on chlorophyll a, b, total chlorophyll, and carotenoids of maize crops under different levels of drought stresses (D0: control, D1: mild, and D2: severe). The different letters above bars represents significant variations among treatments at $p < 0.05$ as determined by Tukey test and 95% confidence interval

increased Chla contents by 13%, 20%, and 25%, Chlb contents by 29%, 40, and 54%, total Chl contents by 18%, 25%, ad 32%, carotenoids contents by 22%, 28%, and 83%, photosynthetic rate by 27%, 27%, and 68%, stomatal conductance by 42%, 47%, and 35%, and transpiration rate by 37%, 42%, and 60%, as compared to plants grown under mild and severe water stresses.

Synergistic effects on physiological parameters

Synergistic and individual effects of potassium (100 mg/L) and biochar (350 g/10 Kg of soil) on physiological parameters like relative water contents (RWC), water potential (Ψ), osmotic potential (Ψs), and turgor potential (Ψp) in maize plants grown under different levels of water stress, as demonstrated in Figs. 1 and 2; Table 4. Additionally, synergistic and individual effects of K and biochar were also recorded on reactive oxygen species (ROS) like electrolytic leakage (EL), hydrogen peroxide (H_2O_2) contents, proline contents, and malondialdehyde (MDA) contents. The results showed that water stress significantly affects physiological parameters and ROS. For instance, under mild and severe water stress, RWC was decreased by 27% and 49%, Ψ was increased by 53% and 58%, Ψs was increased by 71% and 82%, and Ψp was decreased by 49% and 141%, as compared to plants grown in optimum moisture contents (100FC). Similarly, under mild and severe water stress, oxidizing agents were increased significantly, which endorsed stressful conditions in plant physiological functions. For instance, under mild and severe water stress, electrolytic leakage contents were increased by 31% and 26%, proline contents were increased by 42% and 26%, MDA contents were increased by 64% and 47%, and H_2O_2 contents were increased by 37% and 27%, respectively, as compared to plants grown under optimum moisture contents. Contrasting to this, the application of amendments individually as well as in combination significantly improved plant physiological parameters by decreasing reactive oxygen species contents. For instance, K foliar spray against water-stressed plants increased RWC by 12%, 7%, and 7%, Ψ by 25%, 9%, and 11%, Ψs by 100%, 12%, and 14%, and Ψp by 28%, 20%, and 32%, and decreased ROS like EL by 36%, 21%, and 12%, H_2O_2 by 47%, 28%, and 27%, MDA by 34%, 16%, and 23%, and proline by 30%, 13%, and 10%, respectively as compared to plants grown under optimum, mild, and severe water stress. Similarly, biochar individual application on water-stress plants significantly improves plant physiological parameters by alleviating ROS contents, and their results were more significant than those of K individual application. For instance, biochar application

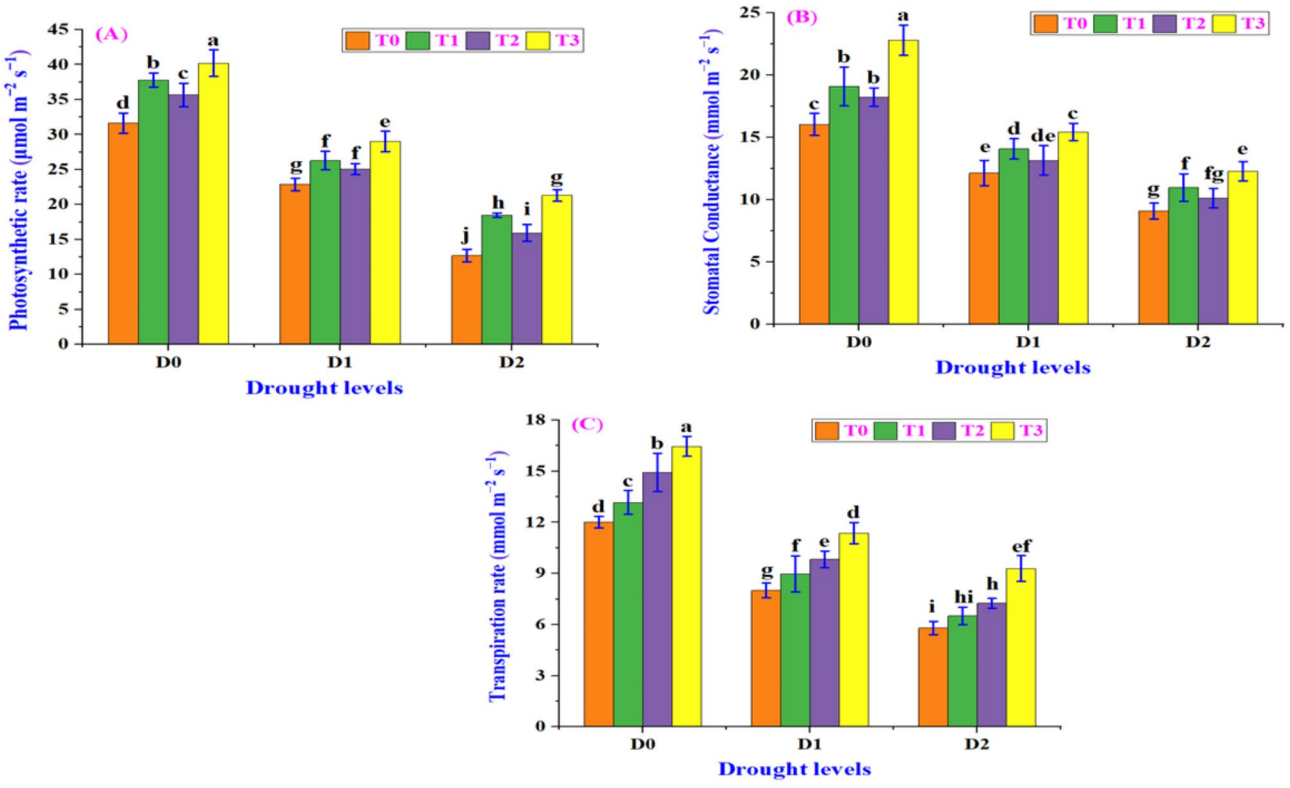

Fig. 3 Individual as well as the combined effect of biochar and potassium (T0: control, T1: 350 g biochar /10 Kg of soil, T2: 100 mg/L K_2SO_4, and T3: 350 g biochar/10 Kg of soil + 100 mg/L K_2SO_4) on photosynthetic rate, stomatal conductance, and transpiration rate of maize crops under different levels of drought stresses (D0: control, D1: mild, and D2: severe). The different letters above bars represents significant variations among treatments at $p < 0.05$ as determined by Tukey test and 95% confidence interval

Table 4 The effects of treatments on biochemical parameters of maize crop under three levels of drought stress. The different letters along means represents significant variations among treatments at $p < 0.05$ as determined by Tukey test and 95% confidence interval

Drought	Treatment	TSS (mg g^{-1} DW)	Proline (μmol g^{-1} FW)	SOD (unit mg^{-1} protein)	POD (unit mg^{-1} protein)	CAT (unit mg^{-1} protein)	AsA (μmol g^{-1} DW)	Protein (mg g^{-1} FW)	GSH (μmol g^{-1} DW)
D0	T_0	30.62 ± 0.51^{j}	0.64 ± 0.04^{h}	97.74 ± 3.56^{j}	3.58 ± 0.09^{i}	7.38 ± 0.05^{h}	1.54 ± 0.07^{i}	23.87 ± 0.19^{a}	0.73 ± 0.02^{d}
	T_1	35.24 ± 0.33^{i}	0.58 ± 0.08^{i}	113.21 ± 6.75^{h}	4.16 ± 0.13^{gh}	8.61 ± 0.12^{fg}	1.71 ± 0.05^{h}	24.90 ± 0.23^{bc}	0.76 ± 0.01^{c}
	T_2	34.00 ± 0.72^{i}	0.61 ± 0.06^{i}	105.84 ± 9.11^{i}	3.91 ± 0.11^{hi}	8.13 ± 0.15^{gh}	1.63 ± 0.06^{hi}	26.68 ± 0.11^{ab}	0.84 ± 0.03^{b}
	T_3	38.04 ± 0.46^{h}	0.45 ± 0.03^{j}	126.71 ± 4.32^{g}	4.52 ± 0.07^{g}	9.70 ± 0.09^{e}	1.82 ± 0.09^{g}	28.14 ± 0.31^{c}	0.90 ± 0.04^{a}
D1	T_0	40.90 ± 0.80^{g}	1.11 ± 0.09^{a}	143.20 ± 5.82^{f}	6.57 ± 0.22^{f}	9.51 ± 0.07^{ef}	1.90 ± 0.03^{g}	14.26 ± 0.26^{fg}	0.54 ± 0.02^{g}
	T_1	46.18 ± 0.91^{f}	0.90 ± 0.02^{c}	161.19 ± 7.43^{d}	7.46 ± 0.31^{de}	11.43 ± 0.17^{d}	2.55 ± 0.06^{ef}	15.41 ± 0.35^{f}	0.59 ± 0.02^{f}
	T_2	44.22 ± 0.49^{f}	0.97 ± 0.06^{b}	156.37 ± 2.89^{e}	6.97 ± 0.18^{ef}	10.93 ± 0.13^{d}	2.44 ± 0.04^{f}	18.06 ± 0.51^{e}	0.67 ± 0.03^{e}
	T_3	51.08 ± 0.22^{e}	0.81 ± 0.03^{e}	176.22 ± 1.98^{c}	7.99 ± 0.15^{c}	14.17 ± 0.10^{c}	2.86 ± 0.07^{d}	20.89 ± 0.22^{d}	0.74 ± 0.06^{d}
D2	T_0	54.70 ± 0.74^{d}	0.87 ± 0.08^{d}	158.25 ± 3.56^{de}	7.55 ± 0.08^{cd}	11.77 ± 0.11^{d}	2.64 ± 0.03^{e}	8.21 ± 0.09^{j}	0.31 ± 0.01^{j}
	T_1	62.25 ± 0.39^{b}	0.73 ± 0.11^{f}	183.86 ± 3.09^{b}	8.91 ± 0.17^{b}	16.44 ± 0.09^{b}	3.25 ± 0.06^{b}	9.01 ± 0.16^{ij}	0.44 ± 0.02^{i}
	T_2	57.74 ± 0.56^{c}	0.79 ± 0.03^{e}	180.61 ± 2.88^{bc}	8.49 ± 0.20^{b}	15.62 ± 0.12^{b}	3.12 ± 0.10^{c}	10.73 ± 0.21^{hi}	0.51 ± 0.02^{f}
	T_3	65.89 ± 0.87^{a}	0.68 ± 0.06^{g}	194.07 ± 3.85^{a}	9.73 ± 0.13^{a}	18.74 ± 0.18^{a}	3.51 ± 0.08^{a}	12.67 ± 0.07^{gh}	0.58 ± 0.03^{h}
CV%		3.44	4.09	2.87	5.01	3.82	2.91	6.09	4.52

in water stress plants increased RWC by 7%, 13%, and 11%, Ψ by 0.7%, 1%, and 2%, Ψs by 42%, 24%, and 17%, and Ψp by 13%, 10%, and 17%, and decreased ROS like EL by 10%, 14%, and 20%, H_2O_2 by 6%, 24%, and 20%, MDA by 14%, 8%, and 9%, and proline by 9%, 19%, and 16%, respectively as compared to plants grown under optimum, mild, and severe water stress. Our results showed that the co-application of K and biochar against different levels of water stress showed a more promising effect on improving plant physiological effects and alleviating ROS than individual application of K and biochar (Figs. 3 and 4; Table 4). For instance, K^+ + biochar

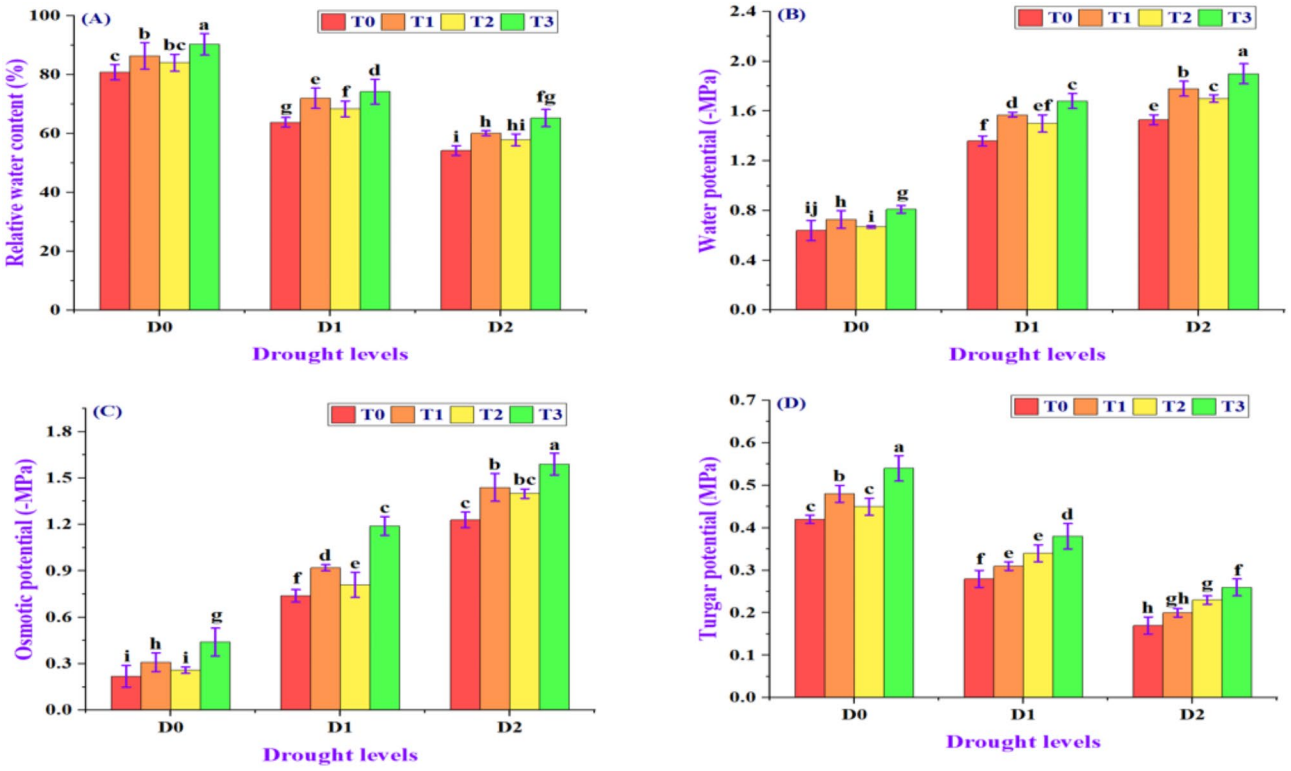

Fig. 4 Individual as well as the combined effect of biochar and potassium (T0: control, T1: 350 g biochar/10 Kg of soil, T2: 100 mg/L K_2SO_4, and T3: 350 g biochar /10 Kg of soil + 100 mg/L K_2SO_4) on relative water content, water potential, osmotic potential, and turgor potential of maize crops under different levels of drought stresses. The different letters above bars represents significant variations among treatments at $p < 0.05$ as determined by Tukey test and 95% confidence interval

application in water-stressed plants increased RWC by 12%, 16%, and 20%, Ψ by 26%, 22%, and 24%, Ψs by 200%, 59%, and 29%, and Ψp by 28%, 35%, and 51%, and decreased ROS like EL by 48%, 69%, and 64%, H_2O_2 by 47%, 45%, and 41%, MDA by 33%, 21%, and 28%, and proline by 30%, 27%, and 22%, correspondingly as parallel to plants grown under optimum, mild, and severe water stress.

Synergistic effects on enzymatic and non-enzymatic antioxidants

Potassium (100 mg/L) and biochar (350 g/10Kg of soil) as individual or in combination imposed significant effects on enzymatic (Catalase; CAT, Superoxide Dismutase: SOD, and Peroxidase: POD) and non-enzymatic (total soluble sugar: TSS, Ascorbic Acid: ASA, Glutathione: GSH, and protein) in maize plants grown under moderate, mild, and severe water stress, as verified in Table 4. Our results demonstrated that mild and severe water stress compared to optimum water contents significantly enhances enzymatic and non-enzymatic antioxidants, which were further boosted by individual application of K and biochar and attained the peak values under the combined application of K and biochar. For instance, mild and severe water stress up-regulated both enzymatic and non-enzymatic antioxidants like CAT contents by 22% and 37%, SOD contents by 32% and 38%, POD contents by 45% and 52%, TSS contents by 25% and 44%, ASA contents by 19% and 41%, GSH contents by 35% and 135%, and protein contents by 67% and 190%, respectively, as compared to plants grown under moderate water contents. K foliar application in individual to moderate, mild, and severe water-stressed plants further increased CAT contents by 31%, 15%, and 33%, SOD contents by 27%, 9%, 14%, POD contents by 26%, 6%, and 12%, TSS contents by 24%, 8%, and 6%, ASA contents by 18%, 29%, and 18%, GSH contents by 22%, 23%, and 62%, and protein contents by 18%, 27%, and 31%, correspondingly as compared to plants under alone moderate, mild and severe water stress. Similarly, biochar alone application also increased CAT contents by 17%, 20%, and 39%, SOD contents by 16%, 12%, and 16%, POD contents by 16%, 13%, and 18%, TSS contents by 15%, 13%, and 14%, ASA contents by 10%, 34%, and 23%, GSH contents by 5%, 10%, and 42%, and protein contents by 4%, 8%, and 10%, as compared to plants under alone moderate, mild, and severe water stress. Biochar individual application was more effective than K^+ individual foliar spray in alleviating water stress. Additionally, K^+ + biochar combined application further up-regulated CAT by 31%, 49%, and

59%, SOD by 30%, 23%, and 22%, POD by 26%, 21%, and 29%, TSS by 24%, 25%, and 20%, ASA by 18%, 50%, and 33%, GSH by 22%, 36%, and 87%, and protein by 18%, 46%, and 54%, as parallel to plants under alone moderate, mild, and severe water stress. Maximum upregulation in enzymatic and non-enzymatic antioxidants was recorded in combined applications, compared to individual applications of K and biochar (Table 4).

Synergistic effects on shoot macronutrients

Potassium (100 mg/L) and biochar (350 g/10 Kg of soil) as individual or in combination imposed significant effects on the uptake of macronutrients (Nitrogen: N, Phosphorus: P, and Potassium) in the shoots of maize plants grown under moderate, mild, and severe water stress, as demonstrated in Table 5. Our results demonstrated that mild and severe water stress significantly affects the uptake of N, P, and K in maize plants. For instance, under mild and severe water stress, N concentration in the shoot was decreased by 26% and 35%, P concentration decreased by 66% and 33%, and K concentration decreased by 104% and 87%, respectively, as compared to plants grown under moderate moisture contents. Contrasting to this, individual as well as combined application of amendments significantly enhanced N: P: K concentration in the shoot of water-stressed maize plants. For instance, K alone application improved N concentration by 47%, 3%, and 22%, P concentration by 63%, 10%, and 16%, and K concentration by 24%, 28%, and 25%, as compared to plants under alone moderate, mild, and severe water stress. Similarly, biochar soil application improved N concentration in shoot by 25%, 10%, and 34%, P concentration by 45%, 34%, and 23%, and K concentration by 6%, 17%, and 13%, correspondingly, as compared to plants grown under alone moderate, mild, and severe water stress. Overall, the K^+ application performed better in improving NPK concentration than biochar. The combined application of K and biochar provided more promising results. For instance, K + biochar application improved shoot-N by 47%, 19%, and 45%, shoot-P by 64%, 82%, and 52%, and shoot-K by 25%, 42%, and 35%, correspondingly, as compared to shoots of plants under alone moderate, mild, and severe water stress (Table 5).

Table 5 Effects of treatments on the accumulation of shoot-nitrogen (N), -phosphorus (P), and -potassium (K) of the maize crop three drought levels. The different letters along means represents significant variations among treatments at $p < 0.05$ as determined by Tukey test and 95% confidence interval

Drought	Treatment	Shoot *N* (%)	Shoot *P* (%)	Shoot K (%)
D0	T_0	1.79 ± 0.04^{e}	0.39 ± 0.02^{ef}	2.18 ± 0.04^{c}
	T_1	2.24 ± 0.06^{b}	0.57 ± 0.04^{b}	2.32 ± 0.02^{bc}
	T_2	2.09 ± 0.05^{c}	0.49 ± 0.03^{c}	2.44 ± 0.06^{b}
	T_3	2.63 ± 0.07^{a}	0.64 ± 0.05^{a}	2.72 ± 0.03^{a}
D1	T_0	1.42 ± 0.03^{h}	0.23 ± 0.01^{i}	1.07 ± 0.02^{i}
	T_1	1.56 ± 0.04^{g}	0.31 ± 0.03^{gh}	1.26 ± 0.01^{g}
	T_2	1.37 ± 0.01^{i}	0.26 ± 0.02^{h}	1.37 ± 0.03^{f}
	T_3	1.69 ± 0.02^{f}	0.43 ± 0.04^{de}	1.52 ± 0.05^{de}
D3	T_0	1.33 ± 0.03^{j}	0.30 ± 0.02^{gh}	1.16 ± 0.03^{h}
	T_1	1.78 ± 0.04^{e}	0.37 ± 0.03^{f}	1.31 ± 0.02^{fg}
	T_2	1.63 ± 0.02^{fg}	0.34 ± 0.03^{g}	1.45 ± 0.04^{e}
	T_3	1.92 ± 0.06^{d}	0.45 ± 0.04^{cd}	1.57 ± 0.03^{d}
CV%		1.98	3.32	2.29

Principal component analysis (PCA) and pearson correlation

To evaluate the general relationships among the identical variables, principal component analysis (PCA) and person correlation analysis were performed (Figs. 5 and 6). PCA of recorded physiological, morphological, and biochemical attributes indicated by arrows across different treatment groups demonstrated by the green asterisk-marked points (*D0T0, *D0T1, *DOT2, etc.). The two principal components (PC1 and PC2) accounted for 75.09% and 18.34% of the variance, correspondingly summing to 93.43% of the total variance, which showed that these two components contain most of the variation in the data (Fig. 6). All of the 12 treatments were disseminated effectively by the first two principal components. The dissemination trend of treatments provided a clear sign that K and biochar supplementation under water-stressed plants had a dominant positive effect on various physiological attributes of maize compared to D0T0 (control). D1T0 (mild water stress alone), D2T0 (severe water stress alone), D1T1(mild water stress with biochar), and D1T2 (mild water stress with K) were displaced from all other treatments, indicating that mild and severe stress caused deleterious effects on the physiological and morphological parameters of maize plants. The variables parallel with PC1 were significantly positively associated with each other, like SFW, RL, TChl, SL, RFW, carotenoids, Shoot K: P: N, and SC, contrasting to this, a significantly negative correlation of variables on PC1 was recorded with variables located on PC2: CAT, TSS, SOD, POD, WP, OSMP, proline, EL, and H_2O_2 (Fig. 6).

The correlation matrix showed that growth and biomass attributes like SFW, SDW, RFW, and RDW and photosynthetic pigments like Chla, Chlb, TChl, and carotenoids showed a strong correlation with each other (r close to +1) and strongly negative correlation with stress-responsive indicators associated with cellular damage and cellular damage like MDA, H_2O_2, and proline (r close to -1), indicating an inverse correlation among stress and plant growth. Total chlorophyll contents showed the highest negative correlation (-0.81) with osmotic pressure and the highest positive correlation (0.99) with turgor pressure. H_2O_2 showed a highly

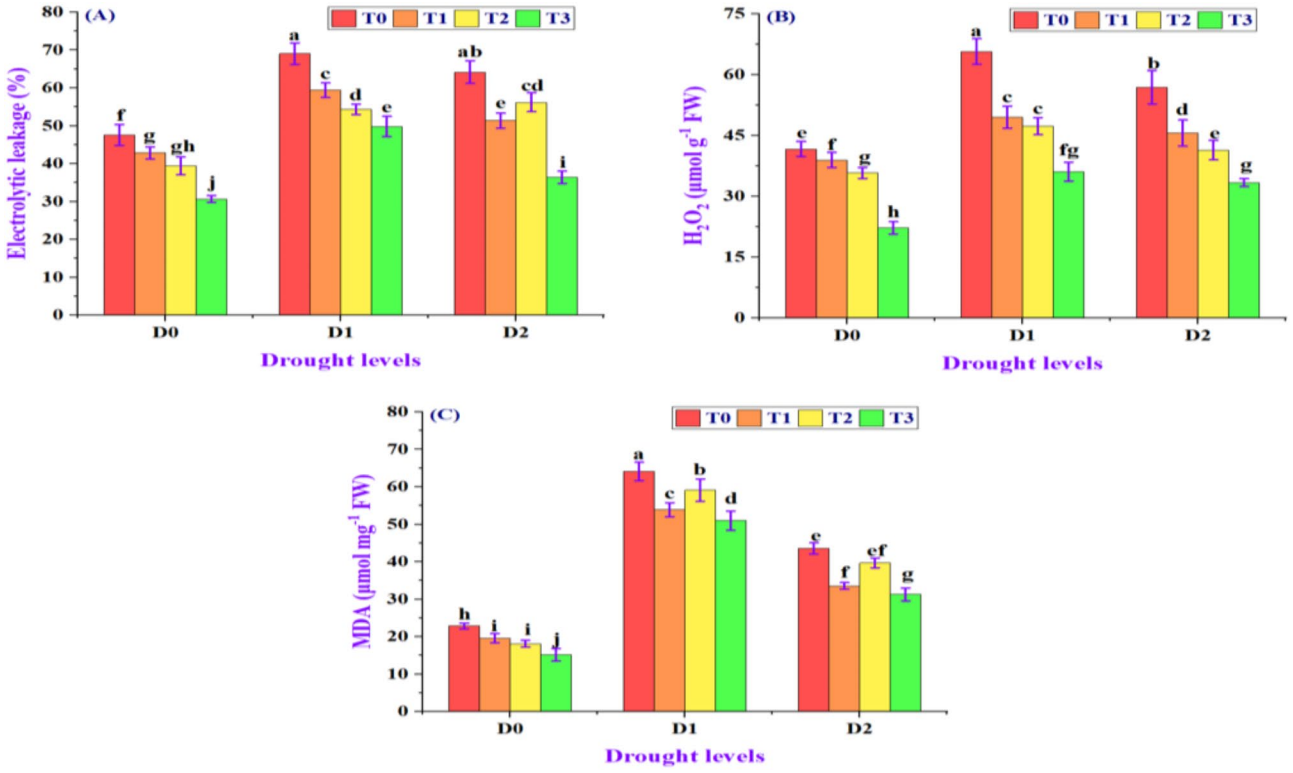

Fig. 5 Individual as well as the combined effect of biochar and potassium (T0: control, T1: 350 g biochar/10 Kg of soil, T2: 100 mg/L K_2SO_4, and T3: 350 g biochar /10 Kg of soil + 100 mg/L K_2SO_4) on electrolytic leakage (EL), hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) of maize crops under different levels of drought stresses (D0: control, D1: mild, and D2: severe). The different letters above bars represents significant variations among treatments at $p < 0.05$ as determined by Tukey test and 95% confidence interval

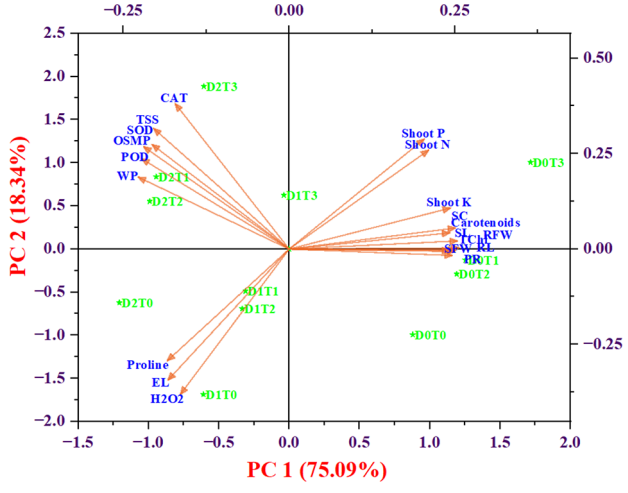

Fig. 6 A biplot graphical presentation of principal component analysis (PCA) score and loadings of several observed parameters of roots and leaves of water-stressed maize plants supplemented individually or in combination with K and biochar. The score plot demonstrates the dispersion of all 12 treatments around two principal components. The abbreviations of variables are as followings: PR: photosynthetic rate, SFW: shoot fresh weight, RL: root length, SC: stomatal conductance, SL: shoot length, TChl: total chlorophyll, CAT: catalase, TSS: total soluble sugar, SOD: superoxidase, OSMP: osmotic potential, POD: peroxidase, WP: water pressure, EL: electrolytic leakage, H_2O_2: hydrogen peroxide, Shoot P: shoot phosphorus, Shoot K: shoot potassium, Shoot N: shoot nitrogen

negative correlation (-0.87) with shoot P and a strongly positive correlation (0.94) with EL. Similarly, MDA showed a strongly positive correlation (0.81) with EL and strongly negative correlation (-0.88) with shoot K. Proline showed a strongly positive correlation (0.94) with MDA and strongly negative (-0.92) with shoot P. Enzymatic (CAT, SOD, POD) and non-enzymatic (TSS, protein) showed moderate positive correlations with each other and negative correlation with growth parameters tend to enhance under water stress conditions (Fig. 7).

Two-way hierarchical clustering analysis (HCA)

Two-way hierarchical clustering and heatmap demonstrated the potential impact of drought, treatment, and their collaboration on the correlation among the subjected variables. HCA interrelated dendrogram between the drought and treatment demonstrated that all treatments and drought were grouped distinctly in four different clusters. Grouping of D1T0, D1T1, and D1T2 represented that these treatments under mild drought conditions may produce similar physiological responses. D0T1, D0T1, D0T2, and D0T3 clustered separately, presenting that drought treatments (D1 and D2) mainly lead to distinctive changes compared to control. The enzymatic antioxidants (SOD, POD, CAT) clustered together,

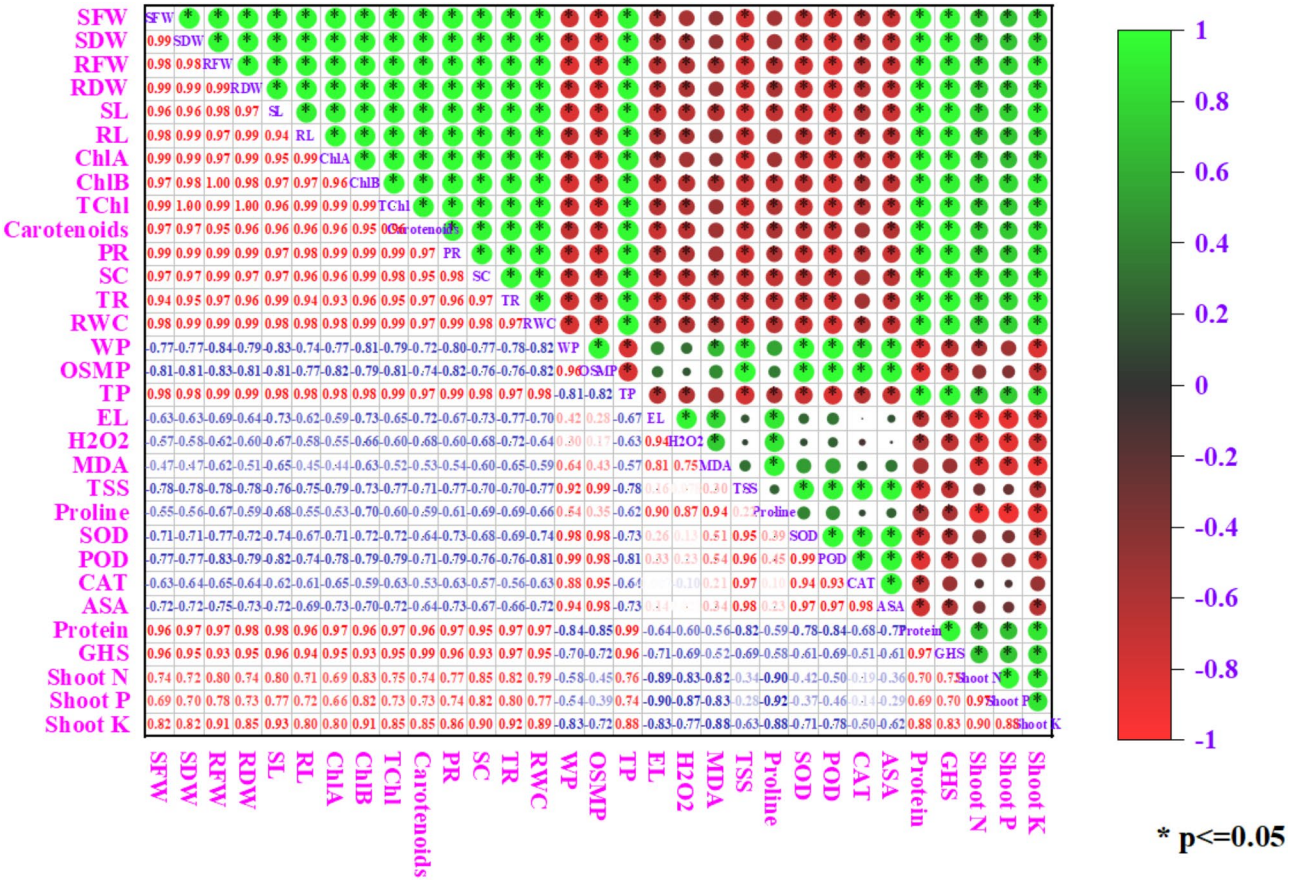

Fig. 7 Correlation analysis between the physiological, morphological, and biochemical attributes of maize plants. Where the abbreviations are stands for SFW: shoot fresh weight, SDW: shoot dry weight, RFW: root fresh weight, RDW: root dry weight, SL: shoot length, RL: root length, Chla: chlorophyll a, Chlb: chlorophyll b, TChl: total chlorophyll, PR: photosynthetic rate, SC: stomatal conductance, TR: transpiration rate, RWC: relative water content, WP: water potential, EL: electrolytic rate, H_2O_2: hydrogen peroxide, MDA: malondialdehyde, TSS: total soluble sugar, SOD: superoxidase, POD: peroxidase, CAT: catalase, AsA: ascorbic acid, and GHS: Glutathione

indicating their similar response under different drought and treatment conditions, and showed increased (darker blue hues) under severe drought (D2), especially in treatments like D2 with potassium (T2) and D2 with potassium + biochar (T3). Non-enzymatic antioxidants (TSS, ASA, GSH, and protein) clustered separately with enzymatic antioxidants, highlighting a different response pattern. These antioxidants showed higher values (blue hues) under mild and severe drought with biochar (T1) and combined potassium and biochar (T3), which indicates combined treatments accelerated the accumulation of non-enzymatic antioxidants, helping the maize plants cope with oxidative stress under water stress. Growth parameters (SFW, SDW, RFW, RDW, SL, RL) are primarily in red, presenting lower values under water stress. In contrast, treatments, especially potassium (T2) with severe water stress (D2) and combined biochar and potassium (T3) with D2, show slight alleviation but are still affected by water stress. Finally, photosynthetic pigments (Chla, Chlb, TChl) show reduced values (red hues) in water stress, particularly under severe drought (D2). Biochar (T2) and combined potassium and biochar (T3) under severe water stress (D2) provide a protective effect on chlorophyll stability.

Pearson and mental correlation analysis

In Pearson correlation, growth parameters (SFW, SDW, RFW, RDW, SL, RL) and photosynthetic parameters (Chla, Chlb, TChl) showed positive correlations, which reflected the plants' overall health and productivity. Similarly, a strong positive association was detected between enzymatic and non-enzymatic antioxidants, indicating that plants triggered several defense mechanisms in response to water stress. Contrasting to this, a negative correlation (blue cells) was reported between growth parameters and reactive oxygen species (MDA and H_2O_2), indicating water stress intensifies oxidative damage and inhibits growth and productivity. Stress indicators (MDA and H_2O_2) showed a positive correlation with each and with electrolytic leakage (EL), indicating oxidative damage to membranes is intensified under water stress. The mental correlation showed that D1 and

D2 with combined treatment (T3) significantly affected antioxidant levels and growth parameters, forming correlations with each other treatment under water stress. The clustering of D2 with treatments (T1, T2, T3) presents that applying potassium, biochar, or combined treatments modifies the biochemical and physiological profile but does not fully demolish the deleterious effects of severe water conditions.

Discussion

Previous studies have reported that abiotic stresses like high or low temperature, salinity, drought or flooding, metal toxicity, or nutrient deficiency impose significantly deleterious effects on plants like growth impairment, nutrient imbalance, cellular organelle dysfunction, homeostatic balance disruption, stress adaptation incompatibility, photosynthesis, and respiration disruption, and ultimately reduced crop productivity [3, 15, 41]. Contrasting to this, the use of several soil organic amendments like biochar application, farmyard manure, vermicompost, and green manure has been documented to alleviate these depressing effects of abiotic stressors by firstly, modifying soil physical and chemical properties like soil bulk density, total porosity, particle size distribution, soil texture, ECE, N, P, K, and soil pH and secondly, by activating several enzymatic and non-enzymatic defensive mechanisms in plants to up-regulate the resilience of plants against stress-mediated oxidative and -osmotic stress [42–44]. The present study was planned to observe the protective role of soil organic amendments like biochar and growth supplements like potassium (K^+) on the physiological, morphological, and biochemical attributes of maize plants grown under different levels of drought stress (Figs. 1, 2, 3 and 4; Tables 1, 2, 3 and 4). For this purpose, biochar was applied directly in the soil during soil preparation, while K^+ was sprayed on the establishment and vegetative stages, and drought stress was applied to the 100%, 75%, and 40% field capacity of the soil. The results of our study demonstrated that mild (75% FC) and severe (40%) levels of water stress significantly affect the morphological parameters like reduced overall biomass and length of maize plants (Fig. 2), which might be due to water stress triggering metabolic alterations including glycolysis, transamination, and tricarboxylic acid which directly involved to regulate growth in maize plants. Similar results were stated in several findings, where drought stress substantially inhibited maize growth and development by triggering alteration in multiple metabolic reactions and by root proliferation, early senescence, and reduced stem elongation [15, 45, 46]. Biochar soil application and K^+ foliar spray separately to water-stressed maize plants significantly mitigated the deleterious effects on morphological parameters, and these beneficial effects were more obvious in the combined application of K^+ and biochar (Fig. 2). Similar results were reported by Sarwar [47] where potassium-enriched biochar with or without growth supplement (gibberellic acid) enhanced germination (9%), shoot fresh weight (14%), root fresh weight (33%), shoot dry weight (68%), root dry weight (29%), root length (22%), and shoot length (29%) in drought-stressed wheat plants. Biochar has been stated to improve soil water-holding capacity and nutrient uptake, maintain nutrient homeostasis, improve water use efficiency, and ultimately enhance plant growth parameters under water-stressed conditions [1, 17]. Similarly, K^+ application in several findings was observed to demolish water-stressed conditions on plant morphological parameters by improving root anatomy, maintaining cell turgor pressure, and improving plant water use efficiency [13, 17, 47]. Biochar and K^+ application in combination has been observed to give more promising results against mitigating water stress in several crops like wheat [48], maize [3], corn [49], barley [50], and rice [51], which is aligned with our findings (Fig. 2).

Additionally, water stress with the preference of 40% FC (severe) significantly impacts the gaseous parameters like chlorophyll a, b, total chlorophyll, carotenoids, stomatal conductance, photosynthetic rate, and transpiration level in maize plants (Figs. 2 and 3). There was a significant decline in all recorded gaseous parameters under both levels of drought stress (mild and severe), which might be due to increased anthocyanins, osmoprotectant metabolites, lipid peroxidation, stomatal closure, leaf rolling, and reduced leaf size. Similar results were stated in earlier findings where drought stress substantially reduced photosynthesis rate, stomatal conductance, transpiration rate, rubisco activity, irregular turgor pressure, poor root-absorption function, and impaired metabolisms [4, 7, 52]. Bornø [53] and Sarwar [47] reported that drought stress causes stomatal closing to reduce water losses, which limits carbon oxide supply to leaves, resulting in a reduction in photosynthesis, and under severe water stress, wilting is caused by loss of leaf turgor pressure, which is aligned to our results (Fig. 3). Biochar soil application combined with K^+ foliar spray significantly improved maize gaseous parameters to uphold plant resilience against drought stress (Figs. 2 and 3). Aboelsoud [54] and Yang [55] reported the same conclusion, where biochar increased plant gaseous parameters in water-stressed plants by up-regulating stress-mitigating mechanisms like escaping, avoidance, tolerance, osmotic adjustment, hormonal regulation, histone acetylation, stomatal responses, mobile signaling like hydraulic pressure, phytohormones, and peptides to communicate water stress resilience throughout the plant. Similarly, K^+ with or without biochar-maintained stress-mediated damage by regulating nutrient imbalance and increasing K^+ supply to leaves to

demolish stomatal dysfunction; these regulatory effects multiplied under biochar soil application (Figs. 2 and 3; Table 5). Aslam [56] reported potassium's potential role in mitigating drought stress in *Zea mays* by improving osmotic potential in the leaf and regulating stomatal conductance. The same conclusion was drawn by Zahoor [19] in drought-stressed cotton plants, Aksu [57] in sugar beet, and Bahrami-Rad [58] in tobacco plants. Moreover, two-way hierarchical clustering analysis (HCA) and Pearson and mental correlation analysis in the present study supported this conclusion where growth parameters (SFW, SDW, RFW, RDW, SL, RL) and photosynthetic parameters (Chla, Chlb, TChl) were positively correlated with each other, which reflected the plants' overall health and productivity (Fig. 8). Additionally, HCA emphasized that photosynthetic pigments (Chla, Chlb, TChl) show reduced values (red hues) in water stress, particularly under severe drought (D2), while biochar individually (T2) and combined with K^+ (T3) under severe water stress (D2) provide a protective effect on chlorophyll stability (Figs. 7 and 8). Our results were aligned with previous findings [1, 11, 14].

Our results showed that drought stress with the preference of 40% FC (severe) substantially disturbed the relative water contents (RWC), water potential (Ψ), osmotic potential (Ψs), and turgor potential (Ψp) in maize plants, which might be due to reduced biosynthesis of osmoprotectants like glycinbetain, sugars, and proline contents which are responsible for maintaining high water status to regulate osmotic potential under water deficit conditions (Fig. 4; Table 4). A similar conclusion has been drawn by Zhang [59], where biochar application improved water contents and leaf water contents by 24% and 10% in drought and salinity-stressed tomato seedlings. Additionally, several findings revealed that drought stress decreased Ψ, Ψs, Ψp, and RWC to trigger cell Plasmolysis, reduced cellular functions, senescence, reduced transpiration efficiency, and ultimately stunted growth [15, 60, 61]. Biochar soil application and K^+ foliar spray substantially regulate the water-related parameters [62] by regulating osmotic balance, improving water holding capacity and water use efficiency, increasing leaf water potential, and improving antioxidant activities [25]. Previous researchers have drawn the same conclusion where biochar [25] and K^+ [23] individually or in combination [1] improved RWC, Ψ, Ψs, and Ψp by alleviating drought-mediated water imbalance. Moussaoui [63] reported that biochar improved soil-water holding capacity by increasing soil porosity, which allows more water to be retained in the soil around the rhizospheric zone and is easily available to Alfalfa (*Medicago sativa*) to sustain RWC, Ψ, Ψs, and Ψp under water deficit conditions. Similar beneficial results of biochar against drought stress were reported in *Triticum eastivum* [1, 25], *Zea mays* [64], and in *Brassica napus* [65]. In addition to biochar, K^+ is actively involved in osmotic adjustment by lowering the cell's osmotic potential and allowing water absorption even under drought conditions [19]. In combination, K^+ and biochar collectively alleviate water stress more efficiently by K^+-regulating osmotic potential in plants and

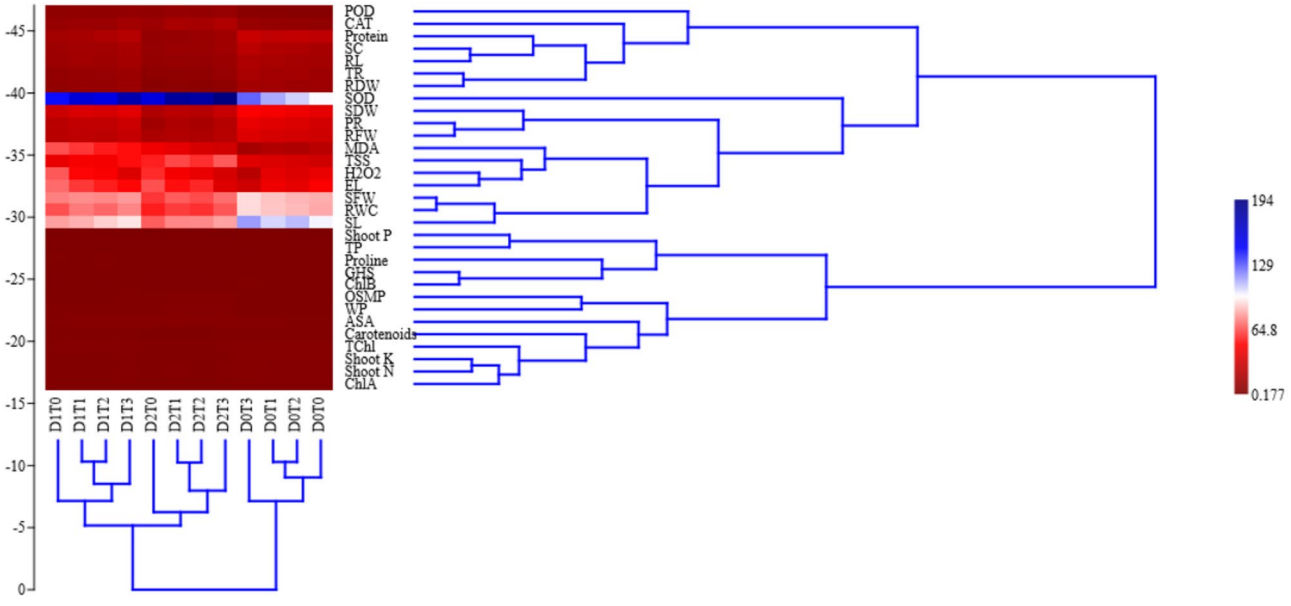

Fig. 8 Graphical presentation of two-way hierarchical cluster analysis (HCA) and heatmap of all recoded parameters in maize plants grown under three levels of drought stress with two levels of biochar and two levels of potassium. Rows represent several combinations of treatments and drought stress, while columns represent the response variables. Red and blue colors represented the high and low numeric values. Here, D0, D1, and D2 showed control, mild and severe stress, while T0, T1, T2, and T3 represented control, biochar (350 g/ 10Kg of soil), potassium (K_2SO_4: 100 mg/L), and biochar + potassium

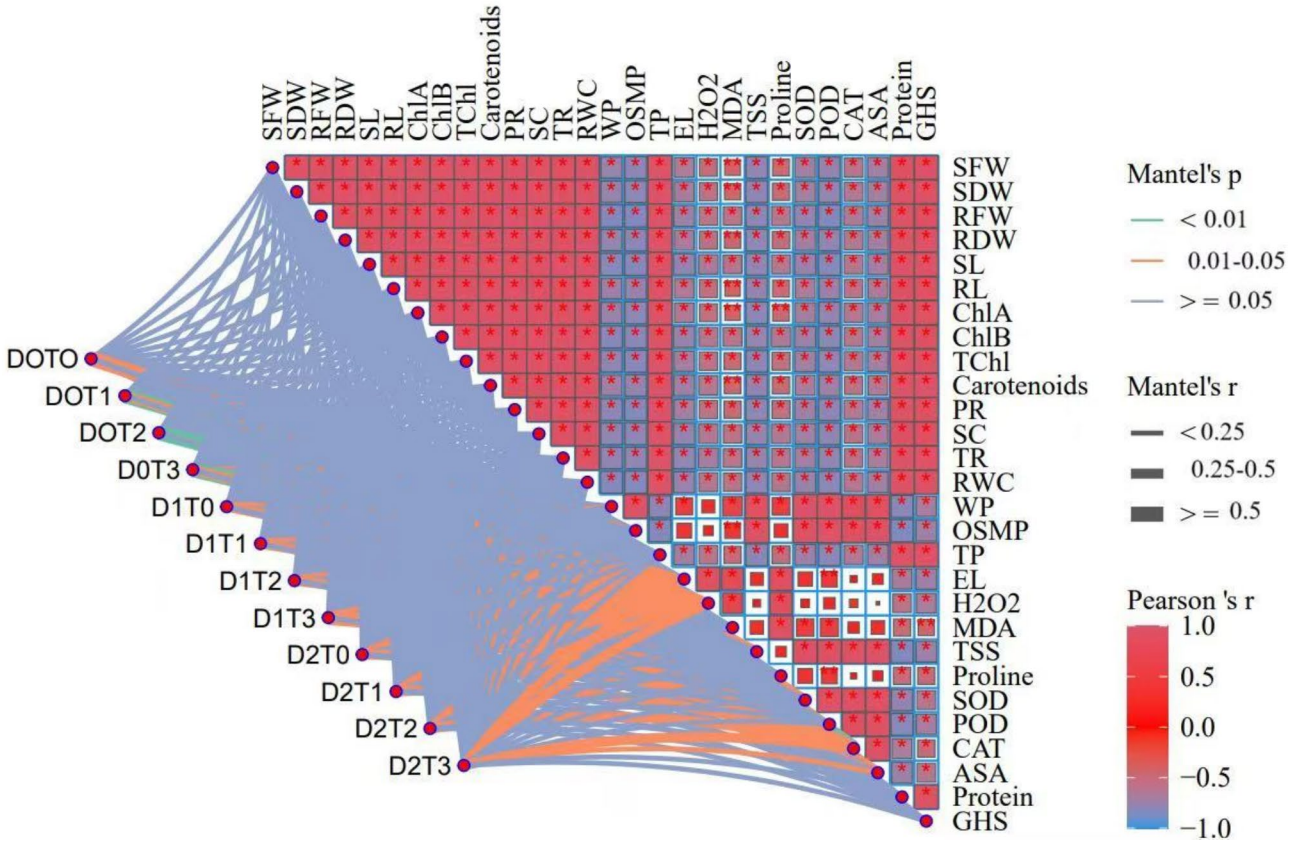

Fig. 9 Mental and Pearson correlation analysis between pigments (Chlorophyll a, b, total chlorophyll, and carotenoids), physiological parameters (SFW: shoot fresh weight, SDW: shoot dry weight, RFW: root fresh weight, RDW: root dry weight, SL: shoot length, RL: root length; SC: stomatal conductance, PR: photosynthetic rate, TR: transpiration rate, RWC: relative water content, WP: water potential, OSMP: osmotic pressure, TP: turgor pressure), stress-indicating parameters (EL: electrolytic leakage, H_2O_2: hydrogen peroxide, MDA: malondialdehyde, proline), enzymatic enzymes (CAT: catalase, POD: peroxidase, SOD: dismutase), non-enzymatic antioxidants (TSS: total soluble sugar, ASA: ascorbic acid, GHS: Glutathione) and treatments where D0, D1, D2 indicates control, mild, and severe drought and T0, T1, T2, and T3 indicate control, 350 g/10 kg of soil biochar,, 100 mg/L K_2SO_4, and 350 g/ 10 Kg of soil biochar + 100 mg/L K_2SO_4

by biochar-retaining water in the soil simultaneously [1]. Our results supported the same conclusion, where K^+ and biochar combined application was more effective in regulating drought-mediated water and osmotic potential (Fig. 4).

In our present study, drought stress significantly influenced the biosynthesis of reactive oxygen species (ROS) like electrolytic leakage (EL), hydrogen peroxide (H_2O_2) contents, proline contents, and malondialdehyde (MDA) contents (Fig. 5; Table 4). Our results demonstrated that ROS were up-regulated under mild water stress (75% FC) and attained the threshold level at severe (40% FC) water stress, resulting in membrane damage and protein denaturing, oxidative burst, cell damage, and uncontrolled oxidative cascades, which was reflected in plant-reduced morphological, physiological, and biochemical attributes (Figs. 2, 3, 4 and 5; Tables 1, 2, 3 and 4). Pearson and mental correlation emphasized that a negative correlation (blue cells) was reported between growth parameters and reactive oxygen species (MDA, EL, and H_2O_2), indicating water stress intensifies oxidative damage and inhibits the growth and productivity of maize (Fig. 9). The intensive amount of H_2O_2 can trigger cell wall loosening by oxidizing cell wall polysaccharides, leading to cell injury [40, 66]. Overproduction of ROS in terms of H_2O_2 is one of the main damaging effects of drought stress in maize. Additionally, numerous studies have used EL as an indicator of stress-mediated damage of plant tissues and 'an indicator' of plant stress resilience [40, 67]. The increased concentration of EL up-regulates linolenic acid and down-regulates linoleic acid, which are membrane-related fatty acids that trigger membrane injury, leading to membrane damage [68]. Several studies have reported the upregulation of EL in drought-stressed plants, which triggered several irregularities in metabolic activities and ultimately caused membrane damage [20, 56, 69]. Recently, Chowdhury [1] observed drought stress in *Triticum aestivum*, and results demonstrated that EL production was increased with increasing water stress and demolished metabolic activities by triggering

uncontrolled cascades and damaging cellular membranes. Similar results have been reported by Wang [70], Aksu [57], and Qi [71], where the intensity of EL production was positively correlated with levels of drought stress, which was aligned with our findings (Fig. 5; Table 4). Alternatively, overproduction of MDA triggers membrane peroxidation and protein denaturing under drought stress [56, 72], resulting in functional and structural damage to the plasma membrane [1]. In our findings, drought stress up-regulated MDA contents, which triggered lipid peroxidation, causing the logging of maize (Figs. 2, 3 and 4). Based on current findings, lipid peroxidation of cellular membranes can be predicted as one of the key damaging effects of drought stress in maize. Thus, biochar application with or without K^+ foliar spray, in our study, demolished the overproduction of ROS to regulate membrane integrity and permeability and cellular damage (Fig. 5; Table 4), but results were more promising in the combined application of biochar with K^+. Khan [73] reported the same results where biochar application (30 t ha^{-1}) demolished the deleterious effects of drought stress by reducing MDA, EL, and H_2O_2 contents in drought-stressed rapeseed. Nasiri [5] applied biochar (0.25%) and 50 µmol per liter of methyl jasmonate against drought-stressed *Hordeum vulgare*, and results demonstrated that biochar foliar application with or without jasmonate significantly reduced MDA, H_2O_2, and EL contents to alleviate drought-mediated osmotic stress. Hafez [74] stated that biochar (10 t ha^{-1}) with the combination of glycine betaine (50 mM) reduced MDA, EL, and H_2O_2 contents to mitigate the harmful effects of drought on rice plants. Seham [21] applied biochar with potassium silicate against drought-stressed borage plants in two-year field settings, and the results showed a significant decline in ROS. Based on our findings and previously reported results, we can emphasize that biochar with the combination of different supplements significantly demolished ROS contents to minimize the deleterious effects of drought stress.

In ROS-alleviating strategies, enzymatic (CAT, SOD, POD) and non-enzymatic (TSS, protein, AsA, GSH) antioxidants are presumed as the first line of defense, where SOD neutralized O^{2-} while POD and CAT neutralized H_2O_2 [1, 40]. GSH has been observed to detoxify drought-mediated reactive intermediate byproducts of xenobiotics metabolism and protect lipid peroxidation in the membrane [75]. Ascorbic acid (AsA) contributes to GSH in the ascorbate-glutathione cycle to demolish ROS [76]. Additionally, upregulation of AsA prevents organelles and cells from the harmful effect of ROS, which over-produce due to stress-mediated oxidative damage [77, 78]. Total soluble sugar (TSS) contents feed NADPH biosynthesis metabolism to activate antioxidant processes. Additionally, TSS activated ROS-scavengers and repair enzymes through signaling under abiotic stressors [79]. In the current study, water-deficit stress in maize plants triggered both the antioxidative (enzymatic and non-enzymatic) defense mechanism to scavenge ROS, which was further up-regulated by biochar amendments with and without potassium (Table 4). Biochar significantly enhances antioxidant activities with the preference of PODs by accelerating plant metabolic relations and cell development, alleviating ROS overproduction, and improving soil-plant and water interactions [1, 25]. PODs (peroxidases) have been reported to build up physiological barriers against abiotic stressors by utilizing H_2O_2 through several physiological and biochemical processes and contributing to the lignin biosynthesis process [40, 80]. Similar findings were reported by Suliman [81], where biochar amendments accelerated the existence of oxygen molecules as a functional group and porous structure, resulting in the upregulation of CAT, SOD, POD, AsA, GSH, and TSS to encounter ROS. However, Kapoor [82] revealed that biochar supplementation enables plants to cope with arsenic-mediated ROS by up-regulating antioxidants and glyoxalase defense systems. Contrasting to biochar, K^+ foliar application also improved the activities of both enzymatic and non-enzymatic antioxidants by sustaining turgor pressure and normalizing ROS [1, 83].

Biochar's relatively higher absorption capacity increases the accessibility and retention rate of N, P, and K, resulting in nitrogen immobilization and cation-anion interactions in biochar-supplemented pots [73]. Similar to our findings (Table 4), biochar application with the combination of K^+ foliar spray significantly enhances the plant nutrient availability, particularly at the vegetative stage, where drought-mediated deleterious effects are more prominent. Potassium, considered a stress regulator plant essential nutrient, enhances nutrient uptake [2], minimizes water losses from the plant [84], delays senescence and leaf chlorosis [85], and alleviates the harmful effects of drought stress by normalizing physio-chemical mechanisms [86], like optimum nutrient availability, activating enzymes, membrane transport, and osmoregulation in *Oryza sativa* [85], *Gossypium herbaceum* [19], *Zea Mays* [12], and *Brassica napus* [87]. Generally, under drought stress, osmolytes like proline mediate nitrogen metabolic activities by removing water to sustain the energy level of the stressed plants, therefore ensuring stability to the main plant skeleton [88]. In the present findings herein, the maximum amount of proline was recorded in drought-stressed maize plants with the presence of potassium and biochar (Table 4). A similar conclusion was drawn by previous researchers where abiotic stressors were demolished by the application of biochar [1, 89]. However, in this context, Bilias [90], Islam [91], and Nie [92] reported that biochar amendments to volcanic soil

enable a higher amount of exchangeable K^+ and translocation of a higher amount of K^+ to plants. Therefore, the beneficial effects of K^+ application were reflected through proline at the root development and elongation stage of drought-stressed maize crops (Table 4). In several findings, biochar application in water stressed-plants alleviated soil N_2O emissions [93], restrained NH_3 volatilization rate in soil [18], and accelerated soil N retention by minimizing the fertilizer requirement [94–96]; these findings are parallel to our results (Table 5). Additionally, several recent findings have emphasized the beneficial role of biochar in improving N, P, and K uptake by plants under abiotic stress [97, 98]. However, some findings are opposing to our results where they observed the negative correlation of biochar with increased N leaching because of deterioration of soil structure [99, 100], enhanced soil N_2O emissions by increased nitrification [101], and augmented soil NH_3 volatilization due to increased soil pH. These opposing results emphasized that the effects of biochar are still ambiguous, which might be due to biochar characteristics, soil type, and other environmental conditions.

Limitations

However, our present study provided comprehensive and deep knowledge about the synergistic effects of biochar and K^+ application against different levels of drought stress in maize plants, but some limitations should be acknowledged:

1. The present study was conducted in a pot-based controlled environment, which may not fully address the complex interactions in field conditions. Several factors, such as microbial interactions, soil heterogeneity, and fluctuating environmental conditions, were not addressed.
2. The present experiment was conducted for 50 days, covering only maize's growth and vegetative stages. There is an intensive need to address the long-term effects of biochar and K^+ on flowering, grain filling, and final yield.
3. Drought stress was simulated using fixed field capacity (FC) levels (100%, 75%, and 40%), which may not fully address drought stress's unpredictable and gradual nature in natural environmental conditions. Field trials will help to validate these findings under real drought conditions.
4. The present finding provides a fundamental understanding, but more in-depth research is needed to optimize K^+ and biochar application rates for long-scale farming. Economic feasibility, practical field implementation strategies, and economic cost-benefit analysis must be conducted to emphasize these strategies' effectiveness.

Conclusion

The present study addressed an essential research gap by evaluating the combined effects of biochar soil application with potassium foliar sprayto improve maize growth and development under different levels of drought stress (100% FC, 70% FC, and 40% FC). Biochar @ 350 g/10 Kg of soil was applied during soil preparation, while potassium @ 100 mg/L was sprayed during the development and vegetative stages of maize cultivar EV-1089. Our results highlight the beneficial effects of potassium and biochar, both individually and in combination, in alleviating drought-mediated osmotic and oxidative stress, particularly during development and vegetative stages of maize.

The combination of biochar and potassium foliar spray proved most prominent in mitigating drought stress by improving morphological, physiological, and biochemical attributes in drought-stressed maize plants. Two-way hierarchical clustering analysis (HCA) and Pearson and mental correlation analysis confirmed our hypothesis that growth parameters (SFW, SDW, RFW, RDW, SL, RL) and photosynthetic parameters (Chla, Chlb, TChl) were positively correlated with each other, reflecting the plants' overall health and productivity. Additionally, HCA showed those photosynthetic pigments (Chla, Chlb, TChl) reduced under drought stress (75% FC and 40% FC), especially under severe drought (40% FC). However, biochar individually and combined with K^+ under 40% FC provided a protective effect on chlorophyll stability.

Biochar soil amendment with potassium exogenous application was actively involved in increasing plant gaseous parameters in water-stressed plants by up-regulating stress-mitigating mechanisms like osmotic adjustment via osmolytes (proline), hormonal regulation via antioxidants (enzymatic and non-enzymatic), stomatal responses via stomatal conductance, mobile signaling like hydraulic pressure via relative water content (RWC), water potential (Ψ), osmotic potential (Ψs), and turgor potential (Ψp), phytohormones via photo-pigments (Chla, Chlb, and total Chl), and peptides via MDA regulation to communicate water stress resilience throughout the plants. Biochar with potassium foliar spray regulated the overproduction of ROS (MDA, H_2O_2, EL) by activating enzymatic (SOD, POD, CAT) and non-enzymatic (TSS, AsA, GSH, protein) antioxidants to mitigate drought-mediated oxidative stress. These mechanisms contributed to improved water stress resistance across plants. Finally, the combined application of biochar and potassium increased nutrient uptake (N, P, K) in drought-stressed maize plants. Overall, soil application of biochar with potassium foliar spray emerged as an effective strategy to enhance maize resistance, supporting growth and development in drought-prone areas and contributing to addressing global food security challenges.

Based on present findings, future research should focus on exploring the long-term effects of potassium and biochar on soil health and crop productivity in varying environmental conditions. Further studies should also investigate the optimal concentrations and application timing of biochar and potassium, as these factors could influence their efficacy under different stress conditions. Additionally, research into the molecular mechanisms underlying the biochar and potassium-mediated drought tolerance could provide deeper insights into their roles in plant stress response. Moreover, field trials are necessary to assess the effectiveness of biochar and potassium foliar applications under real-world agricultural conditions and evaluate their economic viability for farmers in drought-prone regions. These future directions could contribute significantly to enhancing sustainable agricultural practices and improving food security in the face of increasing climate variability.

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Author contributions
S.U.R.: Conceptualization, methodology design, data collection, formal analysis, interpretation of results, and manuscript drafting. Project administration, supervision. J.-C.H.: Supervision, resources provision, review, and editing of the manuscript. G.Y.: Data analysis, visualization, and critical revision of the manuscript. M.T.I.: Laboratory work, statistical analysis, and manuscript formatting. X.Z.: Fieldwork assistance, data curation, and validation of results. S.A.A.: Funding acquisition, resource support, and manuscript review. S.A.: Project administration, funding support, and final approval of the manuscript. A.A.A. drafted the revised manuscript, performed revisions and extra statistical analysis, and helped finalize the revised manuscript.

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Data availability
The data and materials used in this study are available from the corresponding author upon reasonable request. Our data did not involve any clinical study, so there is no need of declaring registration details.

Declarations

Ethical approval
This study does not involve any experiments on humans or animals; therefore, no ethical approval was required. [If your study does involve ethical considerations, provide details here.]

Consent to participate
Not applicable.

Consent for publication
All authors have reviewed the manuscript and consent to its publication.

Competing interests
The authors declare no competing interests.

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References

1. Chowdhury MSN, Sani MNH, Siddique AB, Hossain MS, Yong JWH. Synergistic effects of Biochar and potassium co-application on growth, physiological attributes, and antioxidant defense mechanisms of wheat under water deficit conditions. Plant Stress. 2024;12:100452.
2. Waraich EA, Ahmad R, Ashraf M. Role of mineral nutrition in alleviation of drought stress in plants. Aust J Crop Sci. 2011;5(6):764–77.
3. Naeem MB, Jahan S, Rashid A, Shah AA, Raja V, El-Sheikh MA. Improving maize yield and drought tolerance in field conditions through activated Biochar application. Sci Rep. 2024;14(1):25000.
4. Romdhane L, Awad YM, Radhouane L, Dal Cortivo C, Barion G, Panozzo A, Vamerali T. Wood Biochar produces different rates of root growth and transpiration in two maize hybrids (Zea Mays L.) under drought stress. Arch Agron Soil Sci. 2019;65(6):846–66.
5. Nasiri S, Andalibi B, Tavakoli A, Delavar MA, El-Keblawy A, Van Zwieten L. Using Biochar and foliar application of Methyl jasmonate mitigates destructive effects of drought stress against some biochemical characteristics and yield of barley (Hordeum vulgare L). Gesunde Pflanzen. 2023;75(5):1689–703.
6. Qureshi MK, Munir S, Shahzad AN, Rasul S, Nouman W, Aslam K. Role of reactive oxygen species and contribution of new players in defense mechanism under drought stress in rice. Int J Agric Biol. 2018;20(6):1339–52.
7. Shah MN, Wright DL, Hussain S, Koutroubas SD, Seepaul R, George S, Ali S, Naveed M, Khan M, Altaf MT. Organic fertilizer sources improve the yield and quality attributes of maize (Zea Mays L.) hybrids by improving soil properties and nutrient uptake under drought stress. J King Saud University-Science. 2023;35(4):102570.
8. Van Dijk M, Morley T, Rau ML, Saghai Y. A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. Nat Food. 2021;2(7):494–501.
9. Fita A, Rodríguez-Burruezo A, Boscaiu M, Prohens J, Vicente O. Breeding and domesticating crops adapted to drought and salinity: a new paradigm for increasing food production. Front Plant Sci. 2015;6:978.
10. Taft HL. Water scarcity: global challenges for agriculture. Food, energy, and water. Elsevier; 2015. pp. 395–429.
11. Aslam M, Zamir M, Afzal I, Amin M. Role of potassium in physiological functions of spring maize (Zea Mays L.) grown under drought stress. JAPS: J Anim Plant Sci 2014, 24(5).
12. Matłok N, Piechowiak T, Królikowski K, Balawejder M. Mechanism of reduction of drought-induced oxidative stress in maize plants by fertilizer seed coating. Agriculture. 2022;12(5):662.
13. Haider FU, Khan I, Farooq M, Cai L, Li Y. Co-application of Biochar and plant growth regulators improves maize growth and decreases cd accumulation in cadmium-contaminated soil. J Clean Prod. 2024;440:140515.
14. Dengxiao Z, Hongbin J, Wenjing Z, Qingsong Y, Zhihang M, Haizhong W, Wei R, Shiliang L, Daichang W. Combined Biochar and water-retaining agent application increased soil water retention capacity and maize seedling drought resistance in fluvisols. Sci Total Environ. 2024;907:167885.

15. Ruan R, Lambers H, Wang Y. Comparative responses of two maize genotypes with contrasting drought tolerance to Biochar application. Biochar. 2024;6(1):66.
16. Terzi H, Yıldız M. Proteomic analysis reveals the role of exogenous cysteine in alleviating chromium stress in maize seedlings. Ecotoxicol Environ Saf. 2021;209:111784.
17. Danish S, Hasnain Z, Dawar K, Fahad S, Shah AN, Salmen SH, Ansari MJ. Enhancing maize resilience to drought stress: the synergistic impact of Deashed Biochar and carboxymethyl cellulose amendment. BMC Plant Biol. 2024;24(1):139.
18. Taghizadeh-Toosi A, Clough TJ, Sherlock RR, Condron LM. A wood based low-temperature Biochar captures NH 3-N generated from ruminant urine-N, retaining its bioavailability. Plant Soil. 2012;353:73–84.
19. Zahoor R, Dong H, Abid M, Zhao W, Wang Y, Zhou Z. Potassium fertilizer improves drought stress alleviation potential in cotton by enhancing photosynthesis and carbohydrate metabolism. Environ Exp Bot. 2017;137:73–83.
20. Ahmad Z, Anjum S, Waraich EA, Ayub MA, Ahmad T, Tariq RMS, Ahmad R, Iqbal MA. Growth, physiology, and biochemical activities of plant responses with foliar potassium application under drought stress–a review. J Plant Nutr. 2018;41(13):1734–43.
21. Seham M, El-Din WMS, Farouk S, Mokhtar N. Integrated effects of Biochar and potassium silicate on borage plant under different irrigation regimes in sandy soil. J Hortic Sci Ornam Plants. 2021;13(1):60–76.
22. Jordan-Meille L, Martineau E, Bornot Y, Lavres J, Abreu-Junior CH, Domec J-C. How does water-stressed corn respond to potassium nutrition? A shoot-root scale approach study under controlled conditions. Agriculture. 2018;8(11):180.
23. Ali. Unraveling wheat grain quality, physiological indices, dry matter accumulation, and attenuating water stress adverse effect via foliar potassium application at different growth stages. Gesunde Pflanzen. 2022;74(1):41–52.
24. Ahmad M, Waraich EA, Shahid H, Ahmad Z, Zulfiqar U, Mahmood N, Al-Ashkar I, Ditta A, El Sabagh A. Exogenously applied potassium enhanced Morpho-Physiological growth and drought tolerance of wheat by alleviating osmotic imbalance and oxidative damage. Pol J Environ Stud 2023, 32(5).
25. Zulfiqar B, Raza MAS, Saleem MF, Aslam MU, Iqbal R, Muhammad F, Amin J, Ibrahim MA, Khan IH. Biochar enhances wheat crop productivity by mitigating the effects of drought: insights into physiological and antioxidant defense mechanisms. PLoS ONE. 2022;17(4):e0267819.
26. Abdallah MMS, El-Bassiouny HMS, AbouSeeda MA. Potential role of Kaolin or potassium sulfate as anti-transpirant on improving physiological, biochemical aspects and yield of wheat plants under different watering regimes. Bull Natl Res Centre. 2019;43:1–12.
27. Henningsen JN, Görlach BM, Fernández V, Dölger JL, Buhk A, Mühling KH. Foliar P application cannot fully restore photosynthetic capacity, P nutrient status, and growth of P deficient maize (Zea Mays L). Plants. 2022;11(21):2986.
28. Barrs H, Weatherley P. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian J Biol Sci. 1962;15(3):413–28.
29. Boyer JS, Knipling EB. Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. Proc Natl Acad Sci USA. 1965;54(4):1044.
30. Lutts S, Kinet J, Bouharmont J. NaCl-induced senescence in leaves of rice (Oryza sativaL.) cultivars differing in salinity resistance. Ann Botany. 1996;78(3):389–98.
31. Velikova V, Yordanov I, Edreva A. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci. 2000;151(1):59–66.
32. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys. 1968;125(1):189–98.
33. Bates LS, Waldren R, Teare I. Rapid determination of free proline for water-stress studies. Plant Soil. 1973;39:205–7.
34. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72(1–2):248–54.
35. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem. 1971;44(1):276–87.
36. Mukherjee S, Choudhuri M. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in vigna seedlings. Physiol Plant. 1983;58(2):166–70.
37. Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem. 1980;106(1):207–12.
38. Irigoyen J, Einerich D, Sánchez-Díaz M. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (Medicago sativa) plants. Physiol Plant. 1992;84(1):55–60.
39. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in beta vulgaris. Plant Physiol. 1949;24(1):1.
40. Rahman SU, Xuebin Q, Kamran M, Yasin G, Cheng H, Rehim A, Riaz L, Rizwan M, Ali S, Alsahli AA. Silicon elevated cadmium tolerance in wheat (Triticum aestivum L.) by endorsing nutrients uptake and antioxidative defense mechanisms in the leaves. Plant Physiol Biochem. 2021;166:148–59.
41. Wan H, Hou J, Wei Z, Liu F. Contrasting maize responses to soil phosphorus and potassium availability driven by Biochar under reduced irrigation. Plant Soil 2024:1–24.
42. Imran S, Sarker P, Hoque MN, Paul NC, Mahamud MA, Chakrobortty J, Tahjib-Ul-Arif M, Latef AAHA, Hasanuzzaman M, Rhaman MS. Biochar actions for the mitigation of plant abiotic stress. Crop Pasture Sci 2022.
43. Hasnain M, Munir N, Abideen Z, Zulfiqar F, Koyro HW, El-Naggar A, Caçador I, Duarte B, Rinklebe J, Yong JWH. Biochar-plant interaction and detoxification strategies under abiotic stresses for achieving agricultural resilience: A critical review. Ecotoxicol Environ Saf. 2023;249:114408.
44. Kumari K, Khalid Z, Alam SN, Sweta, Singh B, Guldhe A, Shahi D, Bauddh K. Biochar amendment in agricultural soil for mitigation of abiotic stress. Ecol Practical Appl Sustainable Agric 2020:305–44.
45. Munir N, Anwar T, Qureshi H, Soysal S, Siddiqi EH, Ullah N, Iqbal R, Ercisli S, Alarfaj AA, Ansari MJ. Enhancing maize growth under drought and lead stress with Deashed Biochar and growth-Promoting rhizobacteria. Plant Stress 2024:100643.
46. Danish S, Zafar-ul-Hye M, Mohsin F, Hussain M. ACC-deaminase producing plant growth promoting rhizobacteria and Biochar mitigate adverse effects of drought stress on maize growth. PLoS ONE. 2020;15(4):e0230615.
47. Sarwar G, Anwar T, Malik M, Rehman H, Danish S, Alahmadi TA, Ansari MJ. Evaluation of potassium-enriched Biochar and GA3 effectiveness for improving wheat growth under drought stress. BMC Plant Biol. 2023;23(1):615.
48. Choudhury TR, Acter T, Uddin N, Kamal M, Chowdhury AS, Rahman MS. Heavy metals contamination of river water and sediments in the Mangrove forest ecosystems in Bangladesh: A consequence of oil spill incident. Environ Nanatechnol Monit Manage. 2021;16:100484.
49. Park J-H, Yun J-J, Kim S-H, Park J-H, Acharya BS, Cho J-S, Kang S-W. Biochar improves soil properties and corn productivity under drought conditions in South Korea. Biochar. 2023;5(1):66.
50. Abdelaal K, Alamrey S, Attia KA, Elrobh M, Elnahhas N, ABOU EL-YAZIED A, Ibrahim MA. The pivotal role of Biochar in enhancement soil properties, morphophysiological and yield characters of barley plants under drought stress. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2022;50(2):12710–12710.
51. Adhikari A, Khan MA, Imran M, Lee K-E, Kang S-M, Shin JY, Joo G-J, Khan M, Yun B-W, Lee I-J. The combined inoculation of curvularia Lunata AR11 and Biochar stimulates synthetic silicon and potassium phosphate use efficiency, and mitigates salt and drought stresses in rice. Front Plant Sci. 2022;13:816858.
52. Alharbi OM, Khattab RA, Ali I, Binnaser YS, Aqeel A. Assessment of heavy metals contamination in the sediments and mangroves (Avicennia marina) at Yanbu Coast, red Sea, Saudi Arabia. Mar Pollut Bull. 2019;149:110669.
53. Bornø ML, Müller-Stöver DS, Liu F. Biochar modifies the content of primary metabolites in the rhizosphere of well-watered and drought-stressed Zea Mays L.(maize). Biol Fertil Soils. 2022;58(6):633–47.
54. Aboelsoud HM, Ahmed AA. Effect of Biochar, vermicompost and polymer on wheat and maize productivity in sandy soils under drought stress. Environ Biodivers Soil Secur. 2020;4(2020):85–102.
55. Yang A, Akhtar SS, Li L, Fu Q, Li Q, Naeem MA, He X, Zhang Z, Jacobsen S-E. Biochar mitigates combined effects of drought and salinity stress in Quinoa. Agronomy. 2020;10(6):912.
56. Aslam M, Zamir M, Afzal I, Yaseen M, Mubeen M, Shoaib A. Drought stress, its effect on maize production and development of drought tolerance through potassium application. 2012.
57. Aksu G, Altay H. The effects of potassium applications on drought stress in sugar beet. Sugar Tech. 2020;22(6):1092–102.
58. Bahrami-Rad S, Hajiboland R. Effect of potassium application in drought-stressed tobacco (Nicotiana rustica L.) plants: comparison of root with foliar application. Annals Agricultural Sci. 2017;62(2):121–30.
59. Zhang W, Wei J, Guo L, Fang H, Liu X, Liang K, Niu W, Liu F, Siddique KH. Effects of two Biochar types on mitigating drought and salt stress in tomato seedlings. Agronomy. 2023;13(4):1039.

60. Gharred J, Derbali W, Derbali I, Badri M, Abdelly C, Slama I, Koyro H-W. Impact of Biochar application at water shortage on biochemical and physiological processes in medicago ciliaris. Plants. 2022;11(18):2411.
61. Zaheer MS, Ali HH, Soufan W, Iqbal R, Habib-ur-Rahman M, Iqbal J, Israr M, El Sabagh A. Potential effects of Biochar application for improving wheat (Triticum aestivum L.) growth and soil biochemical properties under drought stress conditions. Land. 2021;10(11):1125.
62. Hazrati S, Rostami N, Mohammadi H, Ebadi M-T. Improving the growth parameters, yield, and oil quality of camelina in rainfed farming due to the combined use of Biochar and supplementary irrigation. J Agric Food Res. 2024;16:101160.
63. El Moussaoui H, Idardare Z, Bouqbis L. Effect of integrated soil fertility management on water retention, productivity and physiological parameters of alfalfa (Medicago sativa) without and under deficit irrigation. Sci Hort. 2024;327:112816.
64. Mannan MA, Shashi MA. Amelioration of drought tolerance in maize using rice husk biochar. In: *Maize-Production and Use.* IntechOpen; 2019: 1–19.
65. Ullah S, Shah S, Jamal A, Saeed MF, Mihoub A, Zia A, Ahmed I, Seleiman MF, Mancinelli R, Radicetti E. Combined effect of Biochar and plant Growth-Promoting rhizbacteria on physiological responses of Canola (Brassica Napus L.) subjected to drought stress. J Plant Growth Regul 2024:1–19.
66. Loix C, Huybrechts M, Vangronsveld J, Gielen M, Keunen E, Cuypers A. Reciprocal interactions between cadmium-induced cell wall responses and oxidative stress in plants. Front Plant Sci. 2017;8:1867.
67. Rahman SU, Han J-C, Ahmad M, Gao S, Khan KA, Li B, Zhou Y, Zhao X, Huang Y. Toxic effects of lead (Pb), cadmium (Cd) and Tetracycline (TC) on the growth and development of triticum aestivum: A meta-analysis. Sci Total Environ 2023:166677.
68. Kim Y-H, Khan A, Waqas M, Shahzad R, Lee I-J. Silicon-mediated mitigation of wounding stress acts by up-regulating the rice antioxidant system. Cereal Res Commun. 2016;44(1):111–21.
69. Cruz de Carvalho MH. Drought stress and reactive oxygen species: production, scavenging and signaling. Plant Signal Behav. 2008;3(3):156–65.
70. Wang X, Liu H, Yu F, Hu B, Jia Y, Sha H, Zhao H. Differential activity of the antioxidant defence system and alterations in the accumulation of osmolyte and reactive oxygen species under drought stress and recovery in rice (Oryza sativa L.) tillering. Sci Rep. 2019;9(1):8543.
71. Qi J, Song CP, Wang B, Zhou J, Kangasjärvi J, Zhu JK, Gong Z. Reactive oxygen species signaling and stomatal movement in plant responses to drought stress and pathogen attack. J Integr Plant Biol. 2018;60(9):805–26.
72. Kaya C, Akram NA, Ashraf M, Alyemeni MN, Ahmad P. Exogenously supplied silicon (Si) improves cadmium tolerance in pepper (Capsicum annuum L.) by up-regulating the synthesis of nitric oxide and hydrogen sulfide. J Biotechnol. 2020;316:35–45.
73. Khan Z, Khan MN, Zhang K, Luo T, Zhu K, Hu L. The application of Biochar alleviated the adverse effects of drought on the growth, physiology, yield and quality of rapeseed through regulation of soil status and nutrients availability. Ind Crops Prod. 2021;171:113878.
74. Hafez EM, Gowayed SM, Nehela Y, Sakran RM, Rady AM, Awadalla A, Omara AE-D, Alowaiesh BF. Incorporated biochar-based soil amendment and exogenous Glycine betaine foliar application ameliorate rice (Oryza sativa L.) tolerance and resilience to osmotic stress. Plants. 2021;10(9):1930.
75. Raihan MRH, Rahman M, Rastogi A, Fujita M, Hasanuzzaman M. Exogenous Allantoin confers rapeseed (Brassica campestris) tolerance to simulated drought by improving antioxidant metabolism and physiology. Antioxidants. 2023;12(8):1508.
76. Mukhtar A, Akram N, Ashraf M, Shafiq S, Aisha R. Foliar-applied ascorbic acid enhances antioxidative potential and drought tolerance in cauliflower (Brassica Oleracea L. Var. Botrytis). Agrochimica: Int J Plant Chem Soil Sci Plant Nutr Univ Pisa. 2016;60(2):2016–100.
77. Akram NA, Shafiq F, Ashraf M. Ascorbic acid-a potential oxidant scavenger and its role in plant development and abiotic stress tolerance. Front Plant Sci. 2017;8:613.
78. Latif M, Akram N, Ashraf M. Regulation of some biochemical attributes in drought-stressed cauliflower (Brassica Oleracea L.) by seed pre-treatment with ascorbic acid. J Hortic Sci Biotechnol. 2016;91(2):129–37.
79. Das A, Mukhopadhyay M, Sarkar B, Saha D, Mondal TK. Influence of drought stress on cellular ultrastructure and antioxidant system in tea cultivars with different drought sensitivities. J Environ Biol. 2015;36(4):875–82.
80. Riaz M, Kamran M, Fang Y, Wang Q, Cao H, Yang G, Deng L, Wang Y, Zhou Y, Anastopoulos I. Arbuscular mycorrhizal fungi-induced mitigation of heavy metal phytotoxicity in metal contaminated soils: A critical review. J Hazard Mater. 2021;402:123919.
81. Suliman W, Harsh JB, Abu-Lail NI, Fortuna A-M, Dallmeyer I, Garcia-Pérez M. The role of Biochar porosity and surface functionality in augmenting hydrologic properties of a sandy soil. Sci Total Environ. 2017;574:139–47.
82. Kapoor RT, Hasanuzzaman M. Unlocking the potential of co-application of steel slag and Biochar in mitigation of arsenic-induced oxidative stress by modulating antioxidant and glyoxalase system in Abelmoschus esculentus L. Chemosphere. 2024;351:141232.
83. Rasuli F, Owliaie H, Najafi-Ghiri M, Adhami E. Effect of Biochar on potassium fractions and plant-available P, Fe, Zn, Mn and Cu concentrations of calcareous soils. Arid Land Res Manage. 2022;36(1):1–26.
84. Farahani S, Shahsavari N, Mohammadi Arasteh M. Effect of potassium sulfate on the physiological characteristics of Canola cultivars in late season drought stress conditions. J Plant Nutr. 2020;43(9):1217–28.
85. Weng XY, Zheng CJ, Xu HX, Sun JY. Characteristics of photosynthesis and functions of the water–water cycle in rice (Oryza sativa) leaves in response to potassium deficiency. Physiol Plant. 2007;131(4):614–21.
86. Hafsi C, Debez A, Abdelly C. Potassium deficiency in plants: effects and signaling cascades. Acta Physiol Plant. 2014;36(5):1055–70.
87. Farahani S, Majidi Heravan E, Shirani Rad AH, Noormohammadi G. Effect of potassium sulfate on quantitative and qualitative characteristics of Canola cultivars upon late-season drought stress conditions. J Plant Nutr. 2019;42(13):1543–55.
88. Meena M, Divyanshu K, Kumar S, Swapnil P, Zehra A, Shukla V, Yadav M, Upadhyay R. Regulation of L-proline biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable environmental conditions. Heliyon 2019, 5(12).
89. Zoghi Z, Hosseini SM, Kouchaksaraei MT, Kooch Y, Guidi L. The effect of Biochar amendment on the growth, morphology and physiology of Quercus castaneifolia seedlings under water-deficit stress. Eur J for Res. 2019;138:967–79.
90. Bilias F, Kalderis D, Richardson C, Barbayiannis N, Gasparatos D. Biochar application as a soil potassium management strategy: A review. Sci Total Environ. 2023;858:159782.
91. Islam MS, Magid ASIA, Chen Y, Weng L, Arafat MY, Khan ZH, Ma J, Li Y. Arsenic and cadmium load in rice tissues cultivated in calcium enriched Biochar amended paddy soil. Chemosphere. 2021;283:131102.
92. Nie X, Li Z, Zhang R-h, Wang X, Gu C, Liu Y, Zhang G, Chen F. Effects of Biochar and its combined application with chemical fertilizers on physical and chemical properties and microbial quantity of fluvo-aquic soil and winter wheat yield. Chin Agric Sci Bull. 2016;32(9):27–32.
93. Razzaghi F, Obour PB, Arthur E. Does Biochar improve soil water retention? A systematic review and meta-analysis. Geoderma. 2020;361:114055.
94. Laird D, Fleming P, Wang B, Horton R, Karlen D. Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma. 2010;158(3–4):436–42.
95. Güereña D, Lehmann J, Hanley K, Enders A, Hyland C, Riha S. Nitrogen dynamics following field application of Biochar in a temperate North American maize-based production system. Plant Soil. 2013;365:239–54.
96. Sun H, Min J, Zhang H, Feng Y, Lu K, Shi W, Yu M, Li X. Biochar application mode influences nitrogen leaching and NH3 volatilization losses in a rice paddy soil irrigated with N-rich wastewater. Environ Technol. 2018;39(16):2090–6.
97. Kannan P, Paramasivan M, Marimuthu S, Swaminathan C, Bose J. Applying both Biochar and phosphobacteria enhances vigna mungo L. growth and yield in acid soils by increasing soil pH, moisture content, microbial growth and P availability. Agric Ecosyst Environ. 2021;308:107258.
98. Azeem M, Hayat R, Hussain Q, Ahmed M, Pan G, Tahir MI, Imran M, Irfan M. Biochar improves soil quality and N2-fixation and reduces net ecosystem CO2 exchange in a dryland legume-cereal cropping system. Soil Tillage Res. 2019;186:172–82.
99. Singh BP, Hatton BJ, Singh B, Cowie AL, Kathuria A. Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrasting soils. J Environ Qual. 2010;39(4):1224–35.
100. Mukherjee A, Lal R. The Biochar dilemma. Soil Res. 2014;52(3):217–30.

101. Sánchez-García M, Roig A, Sánchez-Monedero MA, Cayuela ML. Biochar increases soil N2O emissions produced by nitrification-mediated pathways. Front Environ Sci. 2014;2:25.

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