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Synergistic application of biochar and sodium hydrosulfide enhances maize drought tolerance through improved physiological performance and stress mitigation

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Drought is a major abiotic stress limiting global maize productivity. In this study, we evaluated the combined application of biochar (BC) and sodium hydrosulfide (NaHS) as a potential strategy for enhancing drought resilience in maize (cv. Gohar-19) under greenhouse conditions. The experiment comprised of two factors i.e., drought stress including S_1 (85% ± 5% field capacity), S_2 (55% ± 5% field capacity) and S_3 (35% ± 5% Field capacity) and various treatments of BC and NaHS i.e., T_o (control), T_1 (3% biochar), T_2 (0.05 mmol L^{-1} NaHS), T_3 (0.1 mmol L^{-1} NaHS), T_4 (T_1+T_2) and T_5 (T_1+T_3). Results revealed that drought stress significantly reduced growth traits, photosynthetic pigments, photosynthetic rate, stomatal conductance, and leaf water content, while increasing oxidative damage, osmolyte accumulation, and lipid peroxidation. By contrast, the integrated treatment of biochar and NaHS (0.01 mmol L^{-1}) significantly alleviated these negative effects, improving biomass and growth by ~20%, increasing photosynthetic performance by more than 50%, and enhancing leaf water content by 38%. Furthermore, this combined treatment reduced stress-related biochemical markers, including superoxide dismutase, peroxidase, malondialdehyde, and hydrogen peroxide, by over 30%, and led to a significant decline in osmolyte and secondary metabolite accumulation. Correlation and principal component analyses confirmed strong associations among growth, physiological, and biochemical parameters, highlighting the synergistic protective role of biochar + NaHS treatment. These findings provide experimental evidence that this integrative approach can improve maize drought tolerance and productivity in maize, supporting its potential application in sustainable agriculture. Field-scale trials and molecular investigations are warranted to validate these findings and elucidate underlying mechanisms of this stress-mitigation effect.

Keywords Drought stress, Biochar, NaHS, Growth, Biomass, Physio-chemical attributes

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Drought is considered a major threat to reduce agriculture productivity[1] and globally, crop production is severely threatened by drought stress in the context of climate change[2,3], through impacting the development and growth of the plant[4]. Drought stress inhibits photosynthetic activity in plants (by lowering CO_2 uptake and the activity of the enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase (RUBISCO)[5]. It also decreases the chlorophyll contents[6], hinders cell division and elongation, decreases the rate of photosynthesis, relative water content, and threw off the equilibrium between antioxidants and reactive oxygen species (ROS). Stress from drought causes an overabundance of ROS, which harms proteins, cell membranes, and nucleic acids[7]. For maize crop, if plant has to suffer drought stress during the grain filling stage, it can reduce grain yield by 79–81%[8]. Climate change and hence drought stress will be more severe in future[1]. Therefor developing climate resilient crops is necessary to meet global food security.

Maize (*Zea mays* L.) is a third most important crop after wheat and rice that can be grown in a variety of agro-climatic situations[9]. Farmers prefer this crop because it has the highest grain production potential among all cereals. The crop can be used for grains, fodder, and industrial raw material[10]. During 2024, It was cultivated on 197 Mha and provided 2853 million tons of food[11]. In Pakistan, maize is among most important cash crops[12]. During 2024, Pakistan harvested 1.3 million hectares of maize with estimated production of 11 million tons[9].

Application of H_2S donors external to the plant, such as sodium hydrosulfide (NaHS), has beneficial impacts on plant respond to different environmental stresses[13]. Abiotic stress relief mediated by H_2S is primarily responsible for mitigating oxidative damage by activating antioxidant enzymatic and non-enzymatic mechanisms. The primary enzyme responsible for plants' natural production of H_2S is known as L-cysteine de-sulfhydrase (LCD), which breaks down cysteine into H_2S, pyruvate and ammonium. Researches have proven that H_2S is essential to many biological functions that occur throughout plant life, such as stress reactions, root growth, development and seed germination[14]. Reducing drought effects on plant development through application of biochar (BC) is also highly effective strategy. Biochar, developed via pyrolysis process of organic materials, is distinguished for its higher carbon content and porosity. It serves as a soil conditioner that promotes soil fertility by enhancing water retention, diminishing runoff, and facilitating more effective water absorption by plants during drought conditions[15]. Due to its alkaline nature and high cation exchange capacity, it is a crucial supplement for the reclamation of soils damaged by salinity. By enhancing soil physiochemical, biological, and chemical processes as well as Na leaching, the application of BC lessened the negative effects of drought stress[16]. Application of this technique is becoming a popular way to improve agricultural yields, especially in soils that are stressed by environmental factors like salt, drought, or heavy metal contamination.

Many strategies have been put forth in recent years to minimize the negative impacts of drought, including the use of osmo-protectants (synthetic substances), conservation tillage techniques and the development of drought-tolerant varieties. A study was conducted on the genetic dissection of maize to enhance drought tolerance and support trait improvement[17]. Researchers utilized synthetic bacterial communities derived from xerophytes to strengthen maize resilience under drought stress[1]. Gene editing techniques are actively used to enhance drought tolerance in maize for developing resilient, high-yielding varieties[18]. A previous study investigated the effects of drought on maize and discussed a range of management techniques to alleviate its impact[19]. A previous report highlighted the role of potassium in enhancing drought tolerance in maize[20]. A study addressed the improvement of drought tolerance in maize through foliar application of zinc and phosphorus, which contribute to enhanced nutrient uptake and stress resistance[21]. A study demonstrated that the application of biochar mitigated drought stress in maize by improving soil structure, enhancing water retention, and increasing nutrient availability[22]. Although several approaches such as breeding drought-tolerant varieties, adopting conservation tillage, applying nutrient management, and utilizing gene editing have been developed to enhance maize resilience, the combined application of sodium hydrosulfide (NaHS) and biochar remains largely unexplored. Most existing studies have evaluated these treatments separately, leaving an important gap in understanding their possible synergistic effects. Current study is focused on the combined application of NaHS with biochar to mitigate the adverse effects of drought stress on various traits of maize. The current study aims to: (1) assess the impact of drought stress on maize growth, physio-biochemical properties and quality traits; and (2) evaluate the efficacy of various concentrations of NaHS and biochar in mitigating drought stress in maize.

Materials and methods

A pot experiment was conducted under greenhouse during spring season (April to July, 2024) at research farms of the University of Layyah, Punjab Pakistan. The region is characterized by a hot arid climate, with average maximum summer temperatures approaching 49 °C and average minimum winter temperatures dropping to around 8 °C. Commonly grown local variety Gohar-19 was selected for experimentation. The maize seeds were sown initially in nursery trays and were transplanted to experimental pots after two weeks. To ensure plant availability at each pot, two seedlings per pot were ensured. The pots were filled with sandy loam soil. Each kilogram of soil contained 1.97 g of total nitrogen, 3.38 g of total phosphorous, 13.02 g of total potassium, 93.26 mg alkaline hydro nitrogen contents, 32.14 mg available phosphorous, 51.38 mg available potassium, 13.79 g organic matter and pH = 6.7. The average temperature of greenhouse was in the range of 25–33 °C.

Experimental treatments

The maize plants were treated with three levels of drought stress including S_1 (85% ± 5% Field capacity), S_2 (55% ± 5% Field capacity) and S_3 (35% ± 5% Field capacity). The chemical application included T_o (control), T_1 (3% biochar mixed with pot soil), T_2 (0.05 mmol L^{-1} NaHS application), T_3 (0.1 mmol L^{-1} NaHS application), T_4 (3% biochar mixed with pot soil + 0.05 mmol L^{-1} NaHS application) and T_5 (biochar mixed with pot soil + 0.1 mmol L^{-1} NaHS application). After 45 days of plantation, drought condition was maintained. Moisture meter was used to ensure drought level continuously. There were three replications for each treatment and each replication has

10 pots for precise data collection. Following factors were selected and determined during experimentation to compare the effect of selected treatments.

Growth and biomass attributes

Plants in random were selected and harvested from experimental pots. The cobs were separated and threshed for grains and whole stalk material was used to record plant height (cm), ear length (cm) and ear diameter (cm), following the methods of[20,23].

Physiological/photosynthetic attributes

The leaf chlorophyll fluorescence was recorded before start of pollination[24]. Hand held chlorophyll fluorimeter was used to find plant leaf fluorescence between 11:30 and 14:00 h. Three leaves of each plant, selected from all study treatments, were covered using clips. The fluorimeter was placed on clip after 20 min of darkness and maximum yield of PSII photochemistry $\left({}^{F_v}\!/_{F_m}\right)$ was recorded.

Chlorophyll and carotenoids were estimated from 18-day-old maize leaves using 80% acetone extracts, with absorbance measured at 663, 645 and 470 nm wavelength as recommended by[25]. Chlorophyll and carotenoid contents were calculated using Eqs. (1, 2, 3 and 4).

$$Chl_a = \frac{(19.3\ \times\ A663 - 0.86\ \times\ A645)\ \times\ V}{100\ W} \tag{1}$$

$$Chl_b = \frac{(19.3\ \times\ A645 - 3.6\ \times\ A663)\ \times\ V}{100\ W} \tag{2}$$

$$Chl_{total}\ = Chl_a\ + Chl_b \tag{3}$$

$$Carot. = 1000A470 - 1.82\,Chl_a - 85.02\,Chl_b \tag{4}$$

Here, V was volume of purified solution, W was fresh weight, and A663, A645 and A470 were optical absorption wavelengths.

Photosynthetic rates (P_n), stomatal conductance (g_s), and intercellular CO_2 concentration (Ci) of the maize leaves were recorded using portable photosynthesis system between 10:00 and 11:30 on a clear day. The leaf chamber was set at PAR of 1600 mol m^{-2} s^{-1}), leaf temperature of 25 °C and relative humidity was maintained at 70% as per described method of[26].

Water related attribute

Relative water content (RWC) in leaf is an important measure to describe drought stress condition. RWC was calculated using eighteen day old fresh tissue from third leaf. The third leaf from each treatment was cut and immediately placed in sealed plastic bag to avoid any environmental effect. The fresh weight was recorded immediately after leaf transfer to lab. The harvested leaves were soaked in distilled water for 6 to 8 h at ambient temperature and carefully dried with tissue paper. Dry weight was obtained after oven drying at 72 °C for 24 h using weight balance. The RWC was calculated using following Eq. (5)[27].

$$RWC = \frac{Fresh\,Weight - Turgid\,Weight}{Turgid\,Weight - Dry\,Weight}\ \times\ 10 \tag{5}$$

Enzymatic antioxidants activity

The SOD and POD activities were determined utilizing established methodologies documented by[28]. For POD, 0.5 ml enzyme extract was mixed with buffer substrate (guaiacol and Na_3PO_4; pH 6.4) and hydrogen peroxide (24 mM). The absorbance was measured twice at one-minute intervals. The polyphenol oxidase activity was determined by following the methods reported by[29]. The PAL was determined using modified methods suggested by[30].

The phenolic contents were calculated using methods described by[30]. The enzymatic activity of catalase (CAT) was determined following the standard methods given by[31]. Few modifications in these methods were made as suggested by[32]. After enzymatic addition to start the reaction, the absorbance at 240 nm was recorded for 2 min with an interval of 20 s.

APX was determined using standard methods of[33]. The reaction mixture was prepared using enzyme extract (0.1 ml), phosphate buffer (pH 7.0; 50 mM), L-ascorbic acid (0.25 mM), and hydrogen peroxide (1 mM). A spectrometer recorded an increase in absorbance at 290 nm subsequent to ascorbate oxidation. Guaiacol peroxidase was quantified following the approach of[34]. The enzyme assays were conducted by combining 0.5 ml of 0.1 M K-phosphate buffer (pH 7.5), 0.5 ml of 3.4 mM guaiacol, 0.5 ml of H_2O_2, and 0.5 ml of enzyme extract in a glass cuvette. The absorbance at 480 nm was quantified by assessing the quantity of oxidized guaiacol.

To measure H_2O_2, fresh maize leaves (2 g) were ground and mixed into a 4 mL buffer solution (pH 6.8; PBS, 50 mM,), and transferred to a centrifugal tube for centrifugation (10,000× g, 4 °C, 15 min). The supernatant was then added to a mixture of titanium disulfate and sulfuric acid (20% v/v). Absorbance value was recorded at 415 nm and calculate the H_2O_2 content according to the standard curve as per methodology described by[35,36]. O_2 was also recorded using standard methods of[35].

Lipid peroxidation and membrane damage related attributes

Malondialdehyde (MDA) was determined using the methods given by[37]. The frozen maize leaf tissues were homogenized using 0.1% (w/v basis) trichloroacetic acid. Using spectrophotometer, the absorbance was recorded at 535 nm and was corrected at 600 nm for non-specific turbidity. The MDA (nmol g^{-1} FW) was calculated using methods reported by[38]. 200 mg tissue of eighteen day old fresh tissue was used to calculate electrolyte leakage. Tissue was cut in one cm long strip and was placed in screw cap test tube having 8 ml of deionized and distilled water. The electrolyte leakage was calculated using the standard procedures described by[39]. The percentage electrolyte leakage was calculated using Eq. (6) as reported by[27].

$$EL\,(\%) = \frac{EC_1}{EC_2} \times 100 \tag{6}$$

Osmolytes attributes

To measure proline contents in maize leaf, samples (0.5–1 g) from each pot were frozen in liquid nitrogen before determining proline concentration. Afterward, 0.5 g of frozen tissue was homogenized in 10 mL sulfosalicylic acid (3%) and centrifuged for 5 min according to[40]. Ninhydrin buffer (100 µL of 3% sulfosalicylic acid, 200 µL acidic ninhydrin, 200 µL glacial acetic acid,) and acetic acid (100%) were added to 2 mL of supernatant (v/v/v 1:1:1). The tube was incubated for 60 min at 96 °C. After cooling, the sample was extracted by toluene and the optical density of the upper organic phase was read at 520 nm. The proline concentration was determined using a standard L-proline concentration curve.

Maize leaf soluble sugar contents were determined through standard methods suggested by[41]. The standard method of[42] was followed to determine soluble protein content. For 10 min, 0.5 g leaves were ground in 1.0 ml phosphate buffer (pH 7.0) solution. Then 0.1 ml extract was taken and 1.0 ml solution was prepared by adding distilled water and 1.0 ml of standard reagent [0.75 g Na_2CO_3, 0.1 N (NaOH) and 0.37 g Na-K tartrate in 40 ml distilled water. The solution was mixed for 10 min and 0.1 ml foline phenol reagent was added. The solution was in incubator for 30 min. The sample absorbance was determined at 650 nm wavelength.

Quality attributes

The ferulic acid was determined following the procedures suggested by[30]. Soluble sucrose contents were determined following the standard method of[43]. Sucrose contents were determined using glucose and sucrose curves.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) to assess treatment effects, and mean comparisons were performed with Tukey's HSD test at $p \le 0.05$. Statistical analysis were conducted in Minitab (v17), while principal component analysis (PCA), heat maps, and correlation analysis were carried out in R-Studio to visualize multivariate relationships among traits.

Results

Growth and biomass attributes

Drought stress markedly reduced maize growth and biomass, with the greatest decline under severe stress (Fig. 1). Plant height, ear diameter, and ear length decreased compared with controls, whereas NaHS and biochar particularly their combined application (T_5) significantly ($p \le 0.05$) restored these traits under drought conditions. Intense drought stress reduced the height of the plant (2.62%), ear diameter (5.10%), ear length (8.92%), whereas, moderate drought stress decreased the plant height (1.83%), ear diameter (2.36%), ear length (5.37%) as compared to control conditions. Applied biochar + NaHS (0.1 mmol L^{-1}) of showed the maximum plant height (13.07%), ear diameter (14.59%) and ear length (20.23%) as compared to control under drought stressed conditions (Fig. 1).

Physiological/photosynthetic attributes

Drought stress markedly reduced chlorophyll fluorescence, pigments, carotenoids, photosynthetic rate, and stomatal conductance, while elevating int. CO_2 (Fig. 2). These effects were alleviated by biochar and NaHS, with the combined treatment (T_5) showing the greatest improvement in photosynthetic performance (Fig. 2). Severe drought stress proved the maximum decrease in chlorophyll fluorescence (11.65%), chlorophyll a (15.35%), chlorophyll b (24.34%), total chlorophyll (19.44%), photosynthetic rate (10.67%), carotenoids content (16.48%) and stomatal conductance (gs) (12.39%), while, Int. CO_2 (5.71%) was higher in comparison to control circumstances. The best level of applied biochar + NaHS (T_5) enhanced the chlorophyll fluorescence (61.62%), chlorophyll a (50.28%), chlorophyll b (396.22%), total chlorophyll (138.68%), photosynthetic rate (89.74%), carotenoids content (76.62%) stomatal conductance (gs) (115%) and Int. CO_2 (49.40%) as compared to control under drought stressed conditions, respectively.

Water related attribute

Various levels of applied NaHS and biochar significantly improved the leaf water contents in maize over control treatment under the different levels of drought stress (Fig. 3). Severe drought stress proved the maximum decrease in leaf water contents (5.51%) relative to the control group without treatment. Nonetheless, it was observed that applied biochar + NaHS (T_5) treatment had a significant effect on enhancing water-related attributes in maize leaves. The best level of applied biochar + NaHS (T_5) showed higher leaf water contents (37.81%) as compared to control under drought stressed conditions.

Enzymatic antioxidants activity

Drought stress elevated antioxidant enzyme activities and ROS markers (H_2O_2, O_2^-) (Fig. 4), while the combined biochar + NaHS treatment moderated these responses, indicating reduced oxidative stress. Induced

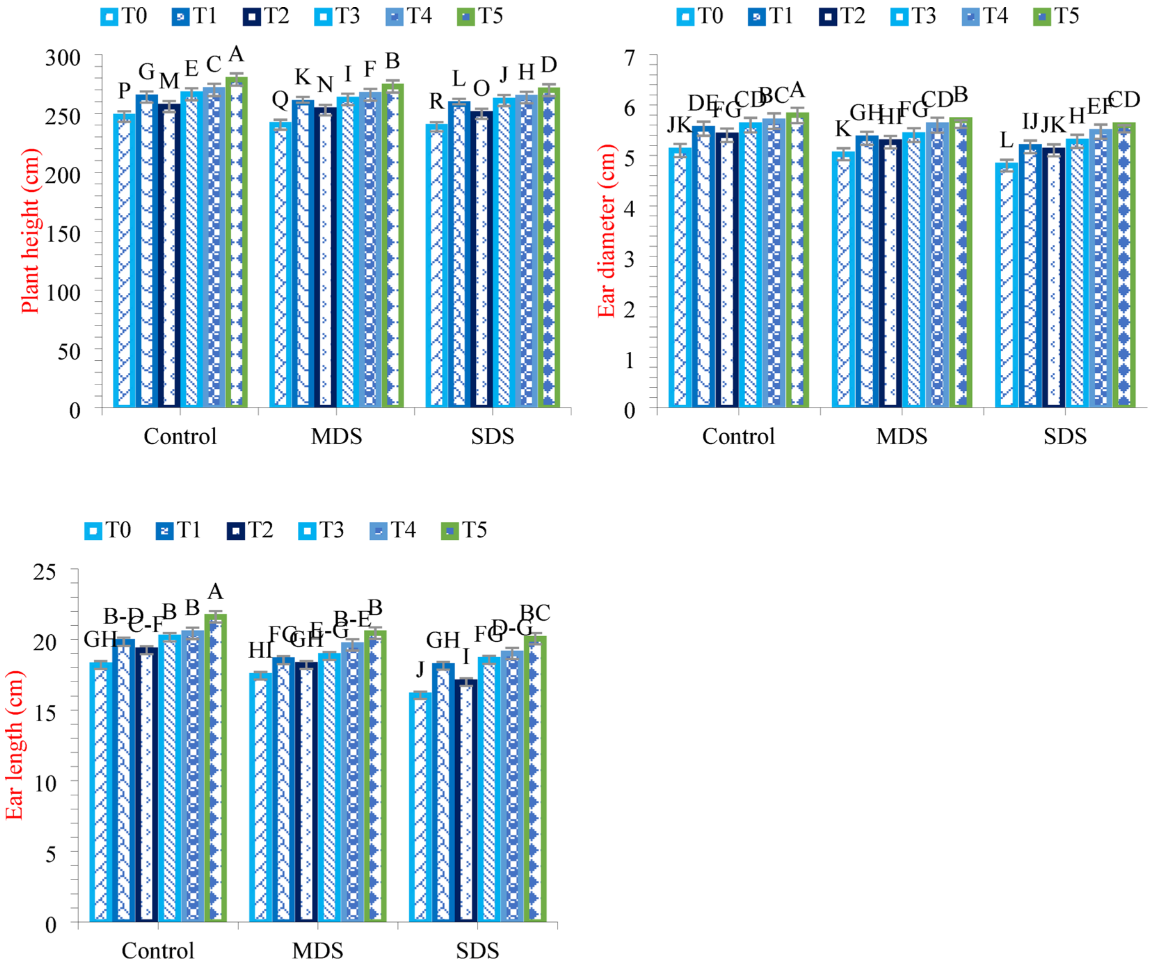

Fig. 1. Effect of biochar and sodium hydrosulfide (NaHS) on maize growth and biomass under drought stress. Treatments: T_0 = control, T_1 = 3% biochar mixed with pot soil, T_2 = 0.05 mmol L^{-1} NaHS, T_3 = 0.1 mmol L^{-1} NaHS, T_4 = biochar + 0.05 mmol L^{-1} NaHS, T_5 = biochar + 0.1 mmol L^{-1} NaHS. Stress levels: MDS = moderate drought stress, SDS = severe drought stress. Traits: plant height, ear diameter, and ear length.

severe drought stress increased the SOD activity (17.25%), POD activity (8.71%), PPO activity (53.86%), PAL activity (56.42%), phenolics activity (62.57%), catalase activity (15.55%), APX activity (14.36%), GPX activity (12.91%), H_2O_2 activity (13.85%) and O_2^- activity (2.37%) relative to control circumstances. The optimal degree of biochar + NaHS (T_5) treatment decreased the SOD activity (54.20%), POD activity (37.84%), PPO activity (56.23%), PAL activity (57.69%), phenolics activity (62.44%), catalase activity (60.58%), APX activity (26.42%), GPX activity (26.41%), H_2O_2 activity (37.29%) and O_2^- activity (16.50%) relative to the control group under drought environment.

Lipid peroxidation and membrane damage related attributes

Data on lipid per oxidation and membrane damage attributes of maize plants shown in Fig. 5 exhibited that drought stress increased the lipid per oxidation and membrane damage attributes of maize. Severe drought stress increased the MDA contents (3.54%) and electrolyte leakage (9.34%) as compared to control. However, the addition of NaHS along with biochar (T_5) also decreased the MDA contents (32.82%) and electrolyte leakage (57.21%) in comparison with control where no treatment were applied under drought stressed conditions.

Osmolytes attributes

The osmolyte characteristics of the drought-stressed maize plants increased linearly (Fig. 6). Under severe drought stress, there was an increased formation of osmolyte characteristics, such as proline (24.01%), soluble sugar (24.26%) and soluble protein (17.77%), compared to control scenarios. The tested level of NaHS (0.1 mmol L^{-1}) + biochar (T_5) decreased the proline (66.26%), soluble sugar (55.94%) and soluble protein (61.57%) levels in maize plants relative to the control group without treatment.

Quality attributes

Figure 7's data on maize plant quality attributes demonstrated that drought stress improved the plants' quality attributes. Severe drought stress increased the feraulic acid (64.50%) and sucrose (18.16%) as compared to

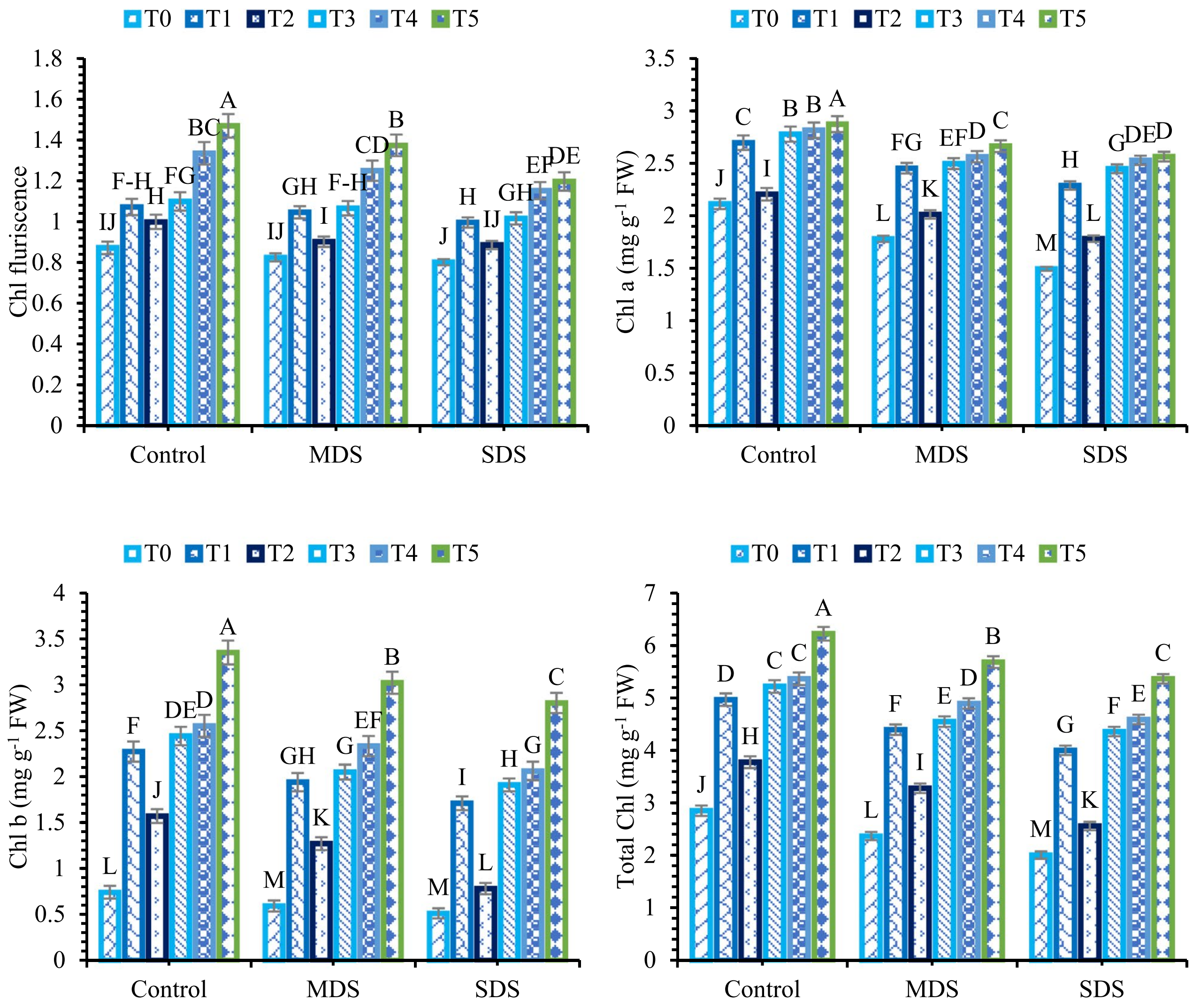

Fig. 2. Effect of biochar and various levels of NaHS on the physiological/photosynthetic traits of maize grown under drought stress. MDS = moderate drought stress; SDS = severe drought stress; To = control, $T_1 = 3\%$ biochar mixed with pot soil, $T_2 = 0.05$ mmol L^{-1} NaHS application, $T_3 = 0.1$ mmol L^{-1} NaHS application, T_4 = biochar mixed with pot soil + 0.05 mmol L^{-1} NaHS application and T_5 = biochar mixed with pot soil + 0.1 mmol L^{-1} NaHS application.

control condition. The best level of NaHS (0.1 mmol L^{-1}) + biochar (T_5) decreased the feraulic acid (42.72%) and sucrose (70.20%) levels in maize plants compared to the control group, which received no treatment.

Correlation analysis

An evident association was observed across all growth, biochemical, lipid peroxidation, reactive oxygen species (ROS) related, enzymatic, and quality-related parameters of maize plants. Physiological characteristics such as chlorophyll and carotenoid contents, water-related attributes, and growth attributes demonstrated negative correlations with enzymatic activities, oxidation of lipids, proline accumulation, and quality traits. Similarly, other attributes showed positive correlation with each other (Fig. 8).

Principal component analysis

The principal component analysis (PCA) of the 29 measured variables accounts for 96.1% variability, where Dim1 exhibits 92.2% and Dim2 3.9% contributions for the maize plants. A clear trend of the control and drought-induced stress responses are observed (Fig. 9). The PCA analysis demonstrates clear clusters of variables. Phenolics, FA and PAL have a strong correlation and considerably affect Dim1, but Chl a, Chl b, and Car show a moderate correlation and contribute to the variation in Dim1. Sucrose, O_2^-, and CAT have a moderate correlation and contribute to the variance in Dim2. Int. CO_2, LWC, and SC exert a diminished impact on overall variation.

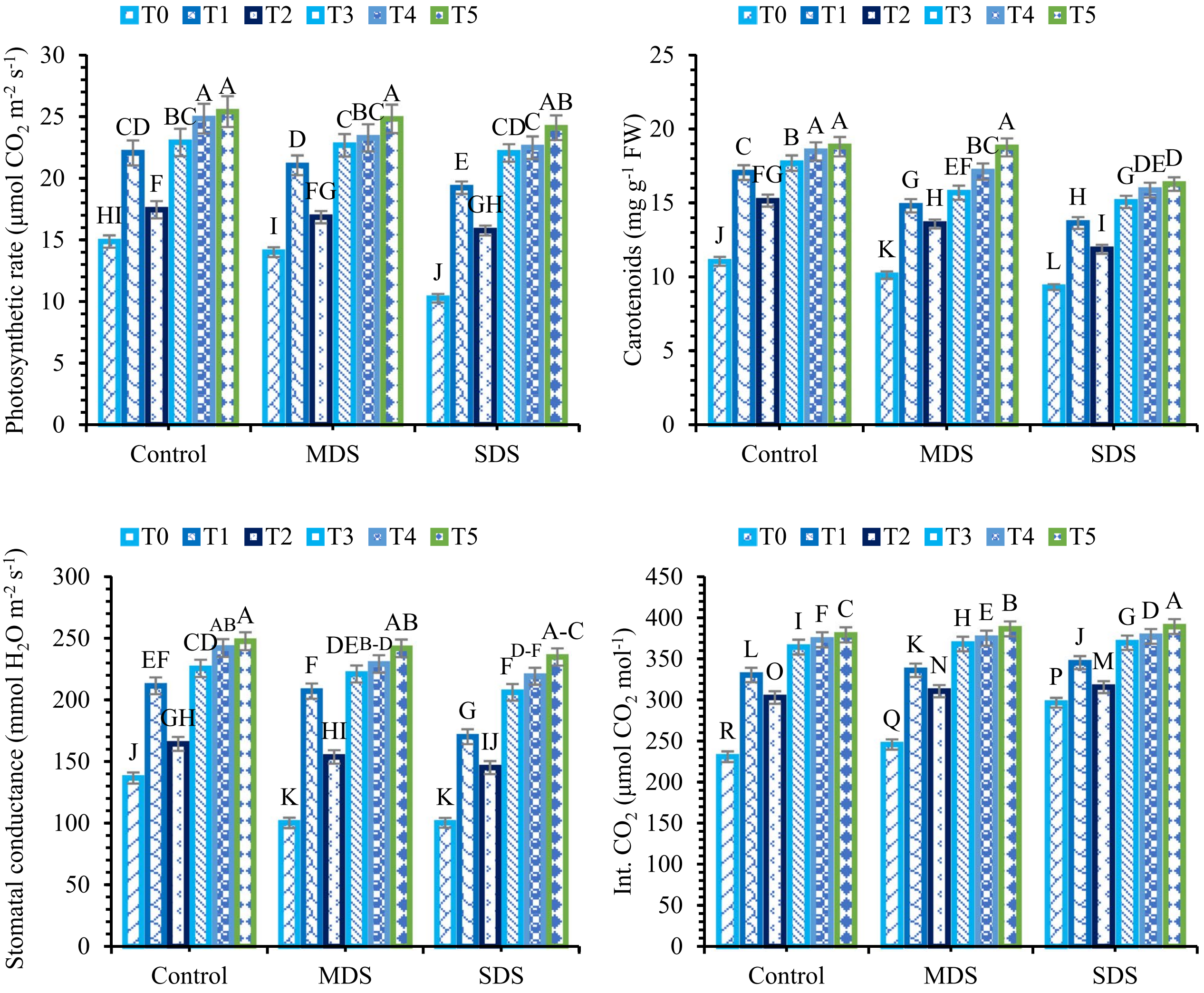

Fig. 2. (continued)

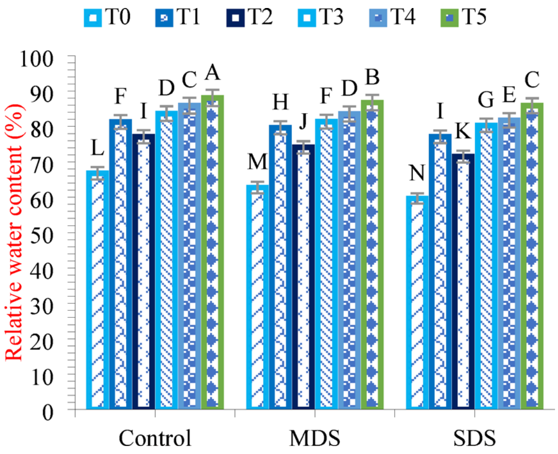

Fig. 3. Effect of biochar and various levels of NaHS on the water related traits of maize grown under drought stress. MDS = moderate drought stress; SDS = severe drought stress; To = control, T_1 = 3% biochar mixed with pot soil, T_2 = 0.05 mmol L^{-1} NaHS application, T_3 = 0.1 mmol L^{-1} NaHS application, T_4 = biochar mixed with pot soil + 0.05 mmol L^{-1} NaHS application and T_5 = biochar mixed with pot soil + 0.1 mmol L^{-1} NaHS application.

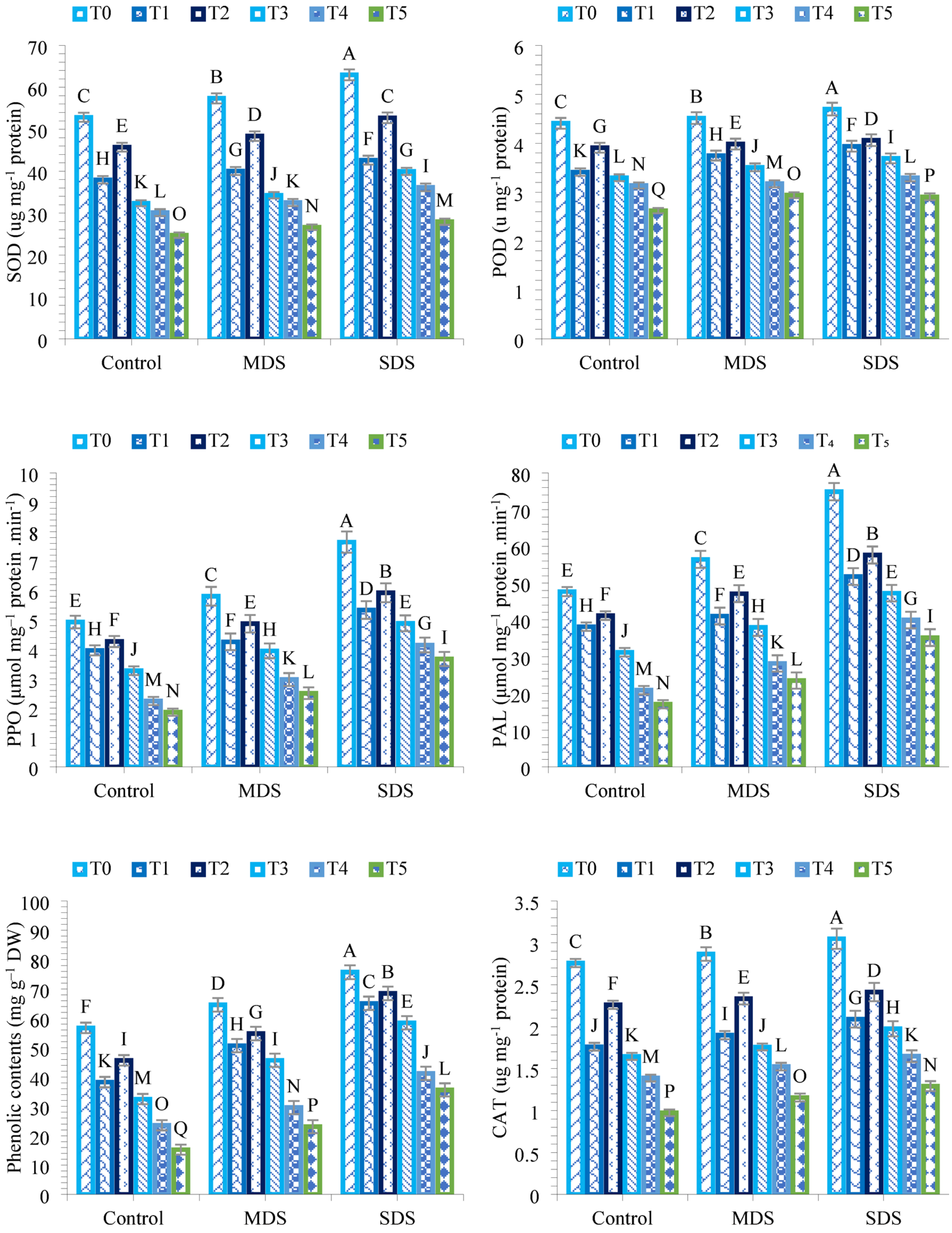

Fig. 4. Effect of biochar and various levels of NaHS on the enzymatic antioxidants activity of maize grown under drought stress. MDS = moderate drought stress; SDS = severe drought stress; To = control, $T_1 = 3\%$ biochar mixed with pot soil, $T_2 = 0.05$ mmol L^{-1} NaHS application, $T_3 = 0.1$ mmol L^{-1} NaHS application, T_4 = biochar mixed with pot soil + 0.05 mmol L^{-1} NaHS application and T_5 = biochar mixed with pot soil + 0.1 mmol L^{-1} NaHS application.

Heat map

The heatmap illustrates clear grouping patterns among the variables, indicating possible linkages and functional categories. Variables such as Chl a, Chl b and Car display analogous expression patterns, suggesting their possible role in photosynthetic activities. Likewise, H_2O_2, CAT, and APX exhibit synchronized expression, indicating their involvement in antioxidant defense systems. Phenolics, PAL and FA form a unique cluster, likely linked to

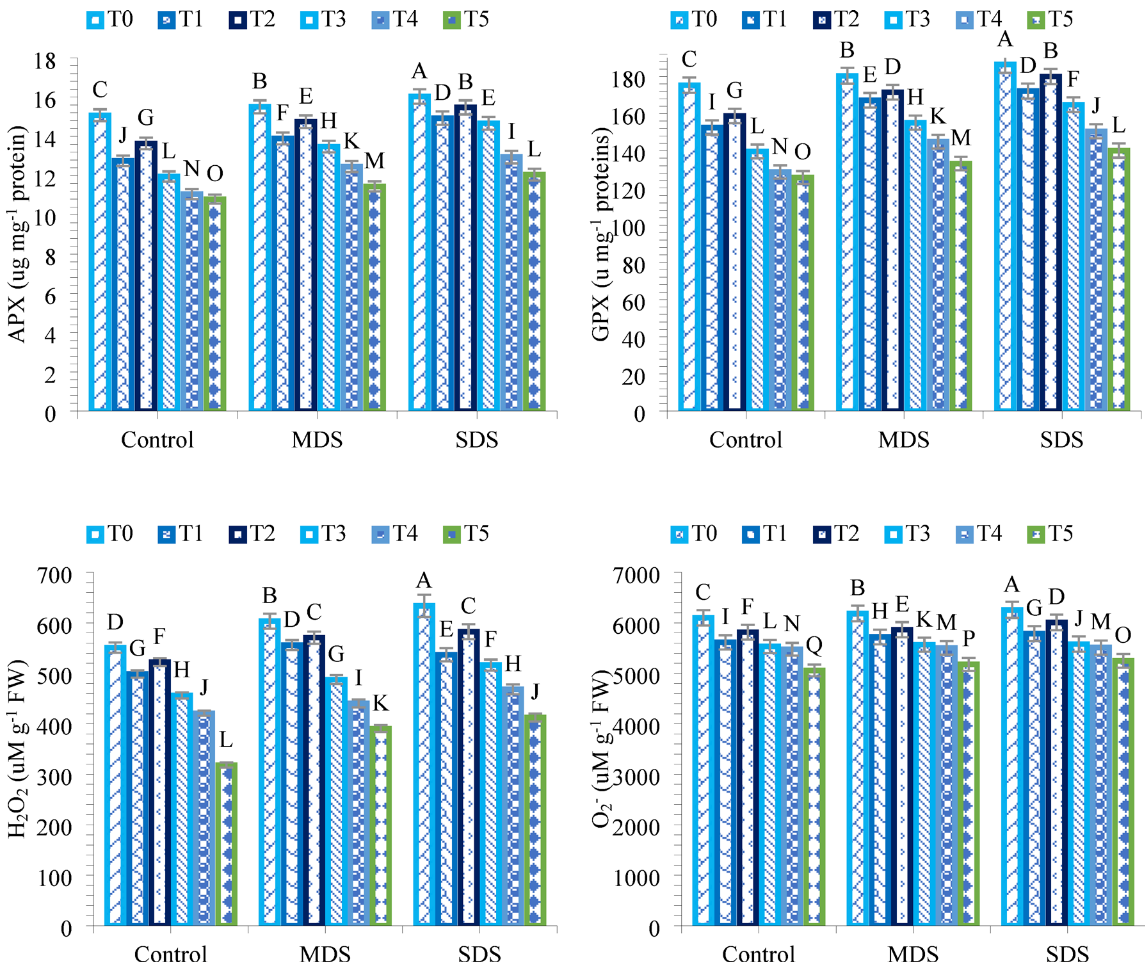

Fig. 4. (continued)

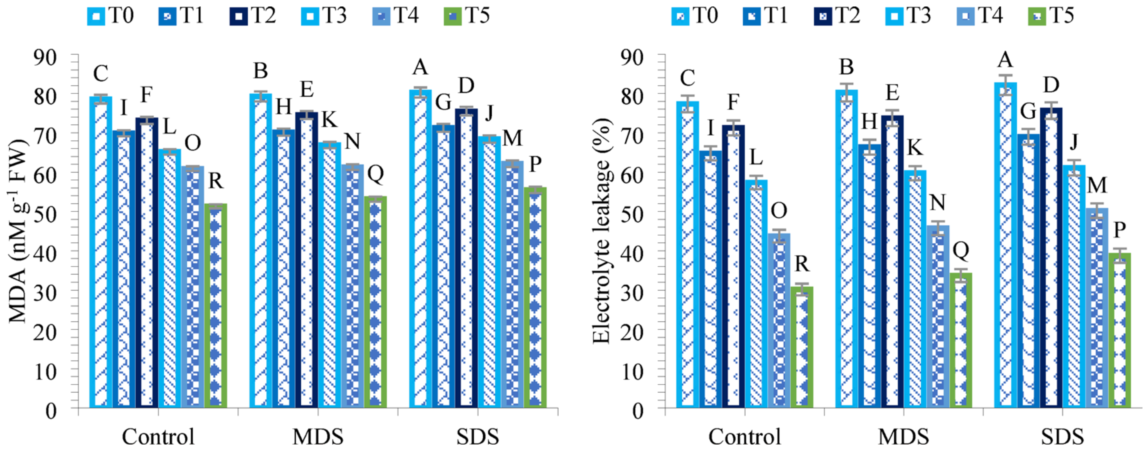

Fig. 5. Effect of biochar and various levels of NaHS on the lipid peroxidation and membrane damage related traits of maize grown under drought stress. MDS = moderate drought stress; SDS = severe drought stress; To = control, T_1 = 3% biochar mixed with pot soil, T_2 = 0.05 mmol L^{-1} NaHS application, T_3 = 0.1 mmol L^{-1} NaHS application, T_4 = biochar mixed with pot soil + 0.05 mmol L^{-1} NaHS application and T_5 = biochar mixed with pot soil + 0.1 mmol L^{-1} NaHS application.

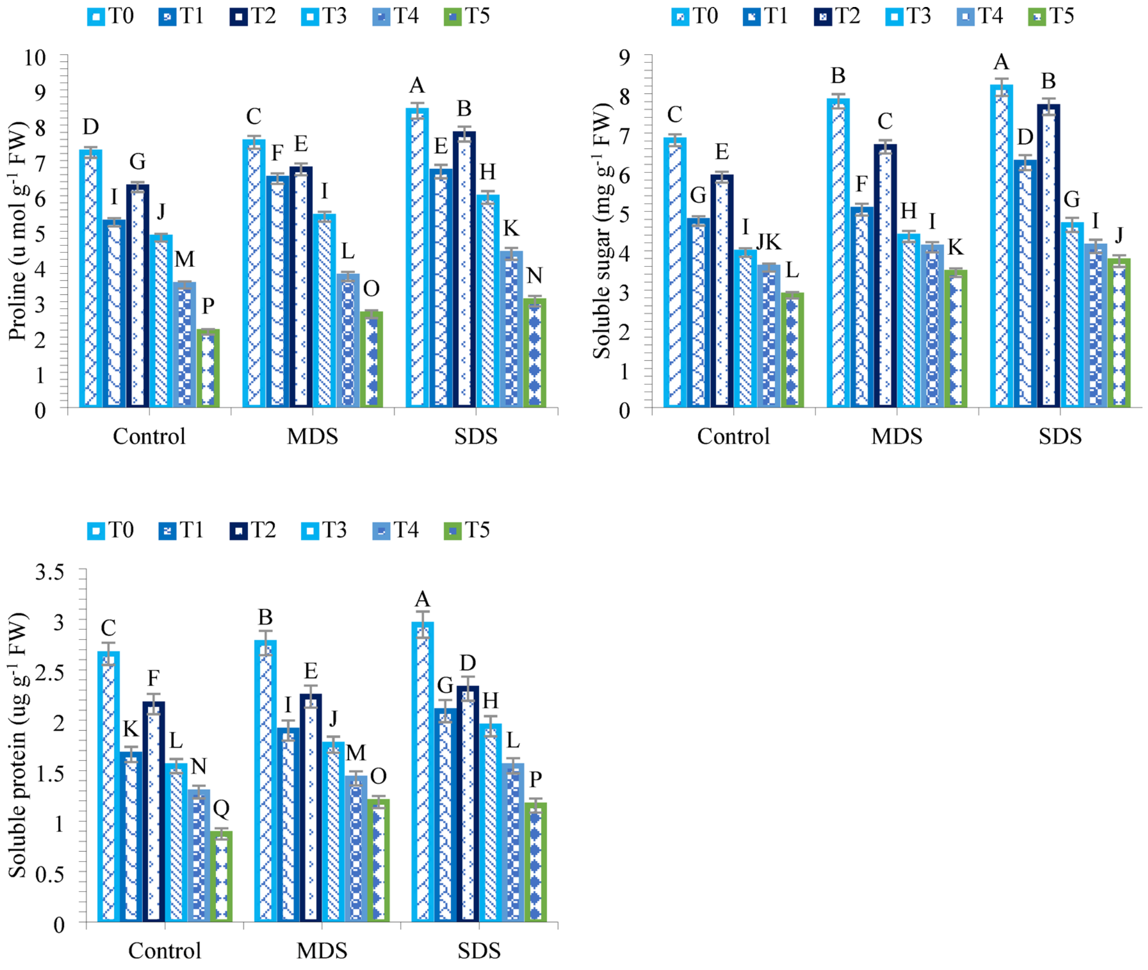

Fig. 6. Effect of biochar and various levels of NaHS on the osmolyte traits of maize grown under drought stress. MDS = moderate drought stress; SDS = severe drought stress; To = control, $T_1 = 3\%$ biochar mixed with pot soil, $T_2 = 0.05$ mmol L^{-1} NaHS application, $T_3 = 0.1$ mmol L^{-1} NaHS application, T_4 = biochar mixed with pot soil + 0.05 mmol L^{-1} NaHS application and T_5 = biochar mixed with pot soil + 0.1 mmol L^{-1} NaHS application.

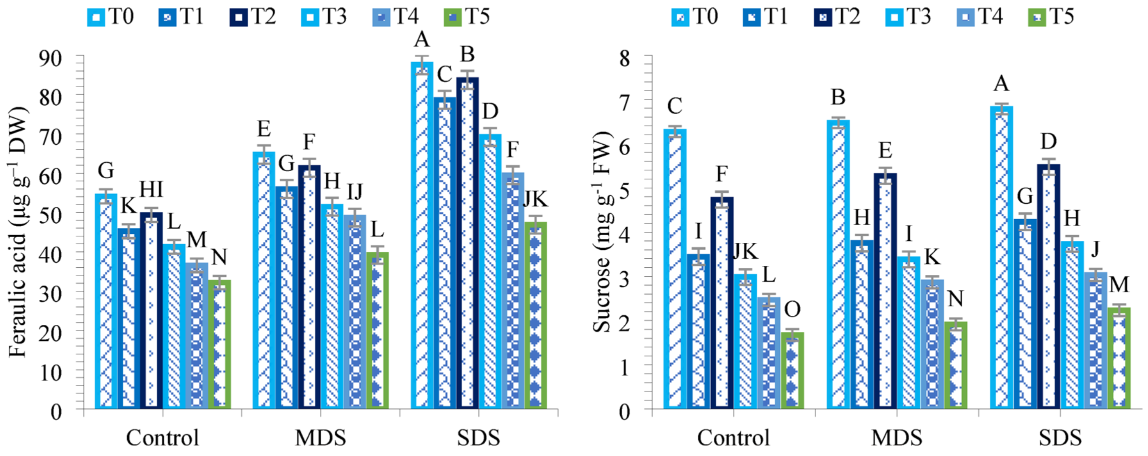

Fig. 7. Effect of biochar and various levels of NaHS on the quality traits of maize grown under drought stress. MDS = moderate drought stress; SDS = severe drought stress; To = control, $T_1 = 3\%$ biochar mixed with pot soil, $T_2 = 0.05$ mmol L^{-1} NaHS application, $T_3 = 0.1$ mmol L^{-1} NaHS application, T_4 = biochar mixed with pot soil + 0.05 mmol L^{-1} NaHS application and T_5 = biochar mixed with pot soil + 0.1 mmol L^{-1} NaHS application.

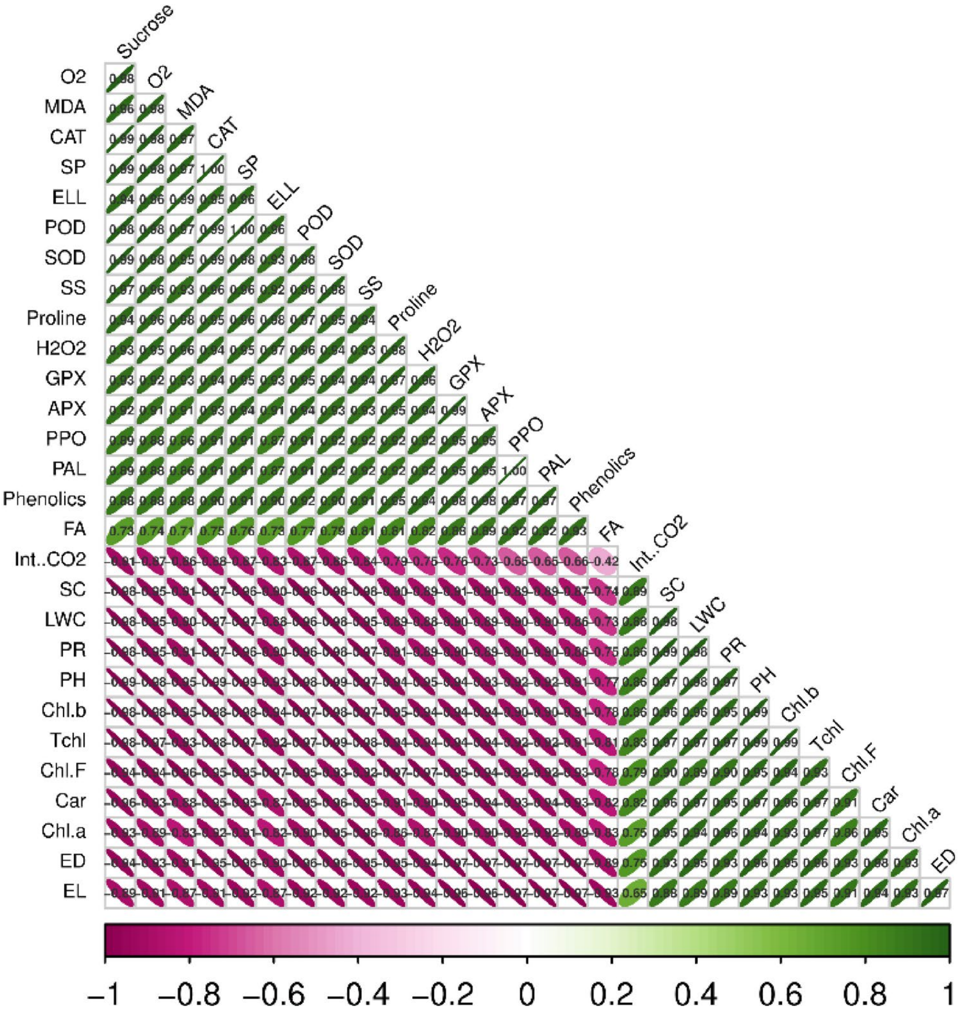

Fig. 8. Correlation matrix of various measured attributes of maize by the use of various treatments under drought stress. PH = plant height; ER = ear diameter; EL = ear length; LWC = leaf water contents; SS = soluble sugar; Chl. f = chlorophyll fluorescence; PR = photosynthetic rate; Chl a = chlorophyll a; Chl b = chlorophyll b; Tchl = total chlorophyll; ELL = electrolyte leakage; MDA = malonaldehyde; POD = peroxidase activity; SOD = superoxide dismutase activity; APX = ascorbate activity; CAT = catalase; sucrose; proline; Car = carotenoids; O_2- activity; H_2O_2; SP = soluble protein; GPX; SC = stomatal conductance; Int. CO_2; PPO = polyphenol Oxidase; PAL = phenylalanine ammonia-lyase; phenolics; FA = ferulic acid.

secondary metabolism. The color gradient (often red to blue) signifies the comparative expression level of each variable.

Red often signifies strong expression, whereas blue denotes low expression. White or gray signifies a neutral or intermediate expression level (Fig. 10).

Discussion

Abiotic stress markedly impairs plant growth by disrupting essential physiological processes, resulting in diminished overall growth[44]. This study explores the potential benefits of using BC and NaHS for the cultivation of maize crop under limited water regimes without affecting their quality. The results of the present study suggested the application of drought stress markedly affected the growth and biomass attributes of the maize crop depending upon the severity of water stress. The application of BC and NaHS, individually or cumulatively, enhanced the growth and development of the maize crop (Fig. 1). Root and shoot biomass, and growth rates show a notable decline in plants in response to inadequate water availability[45]. Several factors may contribute to this reduction, including decreased water uptake due to diminished soil moisture, oxidative stress-induced damage to root and shoot cells, reallocation of resources to prioritize essential survival over root and shoot improvement, and impaired nutrient absorption[46,47]. Application of BC and NaHS in a synergistic way alleviates drought induced stress and enhance plant growth and biomass in maize. Biochar alleviates the physical water stress, and NaHS improves internal plant defense mechanism and leads to osmotic adjustment by the accumulation of proline and synthesis of soluble sugars. The possible reason for the improved growth and biomass of maize might be due to the dual action of these treatments that reduces the degradation of photosynthetic pigments,

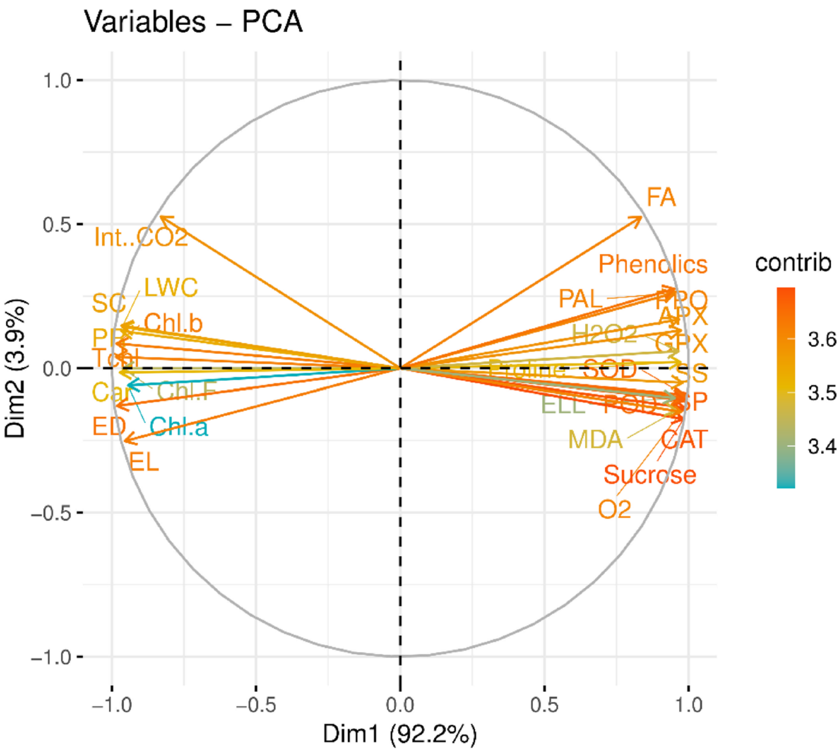

Fig. 9. Principal component analysis figure illustrating the loadings of assessed traits and the contributions of the two principal components (PC1 and PC2). PH = plant height; ER = ear diameter; EL = ear length; LWC = leaf water contents; SS = soluble sugar; Chl.f = chlorophyll fluorescence; PR = photosynthetic rate; Chl a = chlorophyll a; Chl b = chlorophyll b; Tchl = total chlorophyll; ELL = electrolyte leakage; MDA = malonaldehyde; POD = peroxidase activity; SOD = superoxide dismutase activity; APX = ascorbate activity; CAT = catalase; sucrose; proline; Car = carotenoids; O_2- activity; H_2O_2; SP = soluble protein; GPX; SC = stomatal conductance; Int. CO_2; PPO = polyphenol Oxidase; PAL = phenylalanine ammonia-lyase; phenolics; FA = ferulic acid.

maintains the photosynthetic efficiency that ultimately results in increases the biomass accumulation and plant vigor under abiotic stress[48,49]. Application of BC increases soil water retention, porosity and cation exchange capacity, thereby assuring sustained water and nutrient availability to plants under drought conditions[50]. It also helps to improve root growth and stimulate activity of the microbes, involved in nutrient cycling[51]. As a signaling molecule, NaHS helps to protect the plant from drought stress induced oxidative damage by enhancing plant's antioxidative defense system[52]. The sole application of BC makes soil more favorable, while NaHS used together enhances maize physiological resilience by manipulating stress response pathways[53,54]. That might be the possible reason for improving the drought tolerance in the maize crop. This integrated approach results in better water uptake, more favorable utilization of nutrients and sustained metabolic activity, thereby improving plant growth and biomass even under water limited conditions.

Findings of the current study revealed that all the physiological and photosynthetic attributes of maize were significantly affected by drought stress and overall productivity is reduced (Fig. 2). Limited water supply caused the closure of stomata that hinder the availability of carbon dioxide and ultimately disrupts the Calvin cycle which is important process of photosynthesis[55]. Limited water regimes further reduces the CO_2 assimilation with the accumulation of reactive oxygen species (ROS) that damage chlorophyll molecules, thylakoid membranes and photosynthetic enzymes[56]. Under drought stress, the efficiency of electron transport rate decrease result in reduction of the net photosynthetic rate[57]. Adverse effects on other physiological processes, for example transpiration, and chlorophyll fluorescence, also cause a reduction in overall decrease in the photosynthetic traits that reduces the plant vigor[58]. From the findings it is obvious that soil addition of BC and foliar applied NaHS application could improve the physiological and photosynthetic of maize under limited water supply. By enhancing soil water retention and availability of nutrients, BC assures a sufficient supply of water and essential nutrients to the plants, and hence stomatal conductance, and photosynthetic activity[59]. Moreover, addition of BC stimulates the soil microbial functioning and stimulates production of plant growth promoting substances and stress alleviating metabolites[60] that might be the possible reason of these improvements. As a supplier of hydrogen sulfide, NaHS is a critical activator for plants' antioxidative defense mechanisms[61]. Expression of stress responsive mechanisms also modulated by hydrogen sulfide which leads to gradual improvement of chlorophyll stability and photosynthetic capacity that might be the possible reason for improved photosynthetic and physiological attributes of maize under limited water supply. Cumulative effect of biochar and NaHS enhances the antioxidative defence that in turn suppress oxidative damage to cellular membranes and chloroplasts which

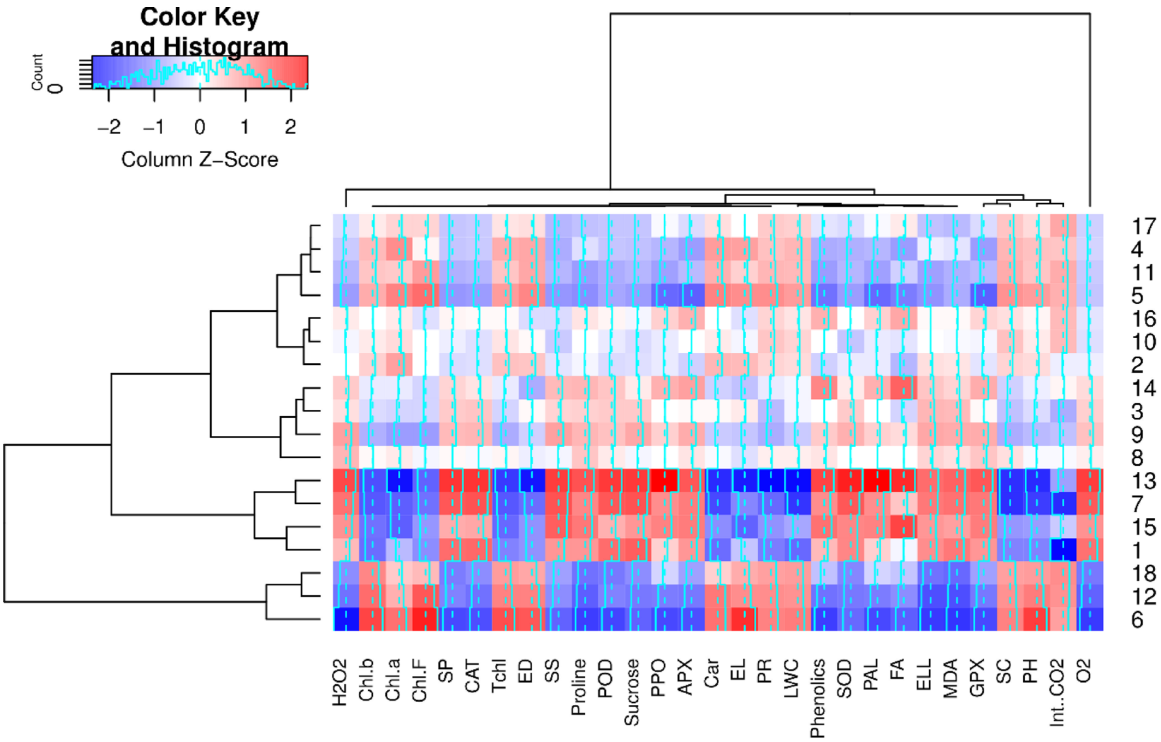

Fig. 10. Heatmap presenting response of maize plants treated with various treatments under drought stress. PH = plant height; ER = ear diameter; EL = ear length; LWC = leaf water contents; SS = soluble sugar; Chl.f = chlorophyll fluorescence; PR = photosynthetic rate; Chl a = chlorophyll a; Chl b = chlorophyll b; Tchl = total chlorophyll; ELL = electrolyte leakage; MDA = malonaldehyde; POD = peroxidase activity; SOD = superoxide dismutase activity; APX = ascorbate activity; CAT = catalase; sucrose; proline; Car = carotenoids; O2- activity; H2O2; SP = soluble protein; GPX; SC = stomatal conductance; Int. CO_2; PPO = polyphenol Oxidase; PAL = phenylalanine ammonia-lyase; phenolics; FA = ferulic acid.

ultimately results in improved photosynthetic traits[62,63]. Together, BC provides suitable environment that is stable, whereas NaHS improves the cellular capacity of the plant to tolerate and recover from stress, that might be the possible reason of improvement of photosynthetic and physiological attributes of maize under drought stress.

Limited water supply caused significant changes in the levels of enzymatic antioxidants, lipid peroxidation, osmolyte and quality variables in maize crop (Figs. 3, 4, 5, 6 and 7). Maize plants under drought stress experience a major metabolic modulation which results in oxidative damage and poor productivity[64]. Drought stress affect the plant's antioxidative defense system by the excessive production of ROS, thus limiting the activity of enzymatic antioxidants[65]. Osmotic imbalances induced by drought stress, and osmoprotective compatible solutes such as proline and soluble sugars are reduced for maintaining cellular turgor and osmoprotection[66]. When these biochemical disruptions are combined together, there is a decline in the yield and quality attribute of maize. Based on N nutrient concentration, further reduction in photosynthates and impaired translocation of assimilates exacerbate the stress induced decline in productivity and grain quality[67]. Combined application of BC and NaHS treatment provide a long-term solution to mitigate the negative impacts of drought in maize. Application of BC caused an improvement of water retention and nutrient availability in soil, which results in improved physiological performance owing to a reduction in oxidative stress, via improving soil quality[68]. In addition, it stimulates accumulation of osmolytes, including proline and soluble sugars, which contribute to osmotic adjustment and stress tolerance[69]. The application of NaHS enhances the activity of enzymatic antioxidants (SOD, CAT, POD), reduces the level of ROS and maintains the level of lipid peroxidation[70]. A reduction of MDA levels in maize plants treated with NaHS under drought stress demonstrates similar observations[71]. Moreover, NaHS has been shown to modulate the expression of stress responsive mechanism including osmolyte biosynthesis that protect cellular structures[72], that might be the possible reason for the modulation of enzymatic activities under drought stress. The combined application of BC and NaHS significantly enhance the maize drought stress tolerance by the multiple cumulative mechanisms. The possible reason behind these mechanisms is that BC improves soil physical properties, increases water retention capacity and improves root aeration creating a more favorable rhizospheric environment for nutrient uptake[73]. At the same time, NaHS is an H_2S donor, a key gaseous signaling molecule that is involved in the modulation of stress-dependent pathways[74]. The resultant decrease in oxidative stress results in lower lipid peroxidation as reflected by lower MDA levels and ensures membrane integrity. So, co-application promotes the accumulation of osmolytes that help maintain cellular turgor during water deficit[75,76]. Together, biochar and NaHS not only modulate the antioxidative defense system, but they also increase yield and quality attributes by increasing photosynthate production and partitioning mechanism. Improved enzymatic antioxidant activity, reduced lipid peroxidation, and higher yield is also correlated in maize under combined treatments. Significant implications for agriculture and environmental sustainability are provided by the findings of this study. The combined action of BC and NaHS not only mitigates the negative impacts of limited water related effects but also enhances plant growth, resulting in improved overall plant health and increased resistance to limited water supply.

Conclusion

This study demonstrates that drought stress severely compromises maize growth by reducing photosynthetic pigments and leaf water content, while intensifying oxidative damage and osmolyte accumulation. The results demonstrated that combined application of biochar and sodium hydrosulfide (NaHS) at 0.1 mmol L^{-1} alleviated these negative impacts, leading to improvements in growth traits, photosynthetic activity, and water conservation, along with reductions in excessive antioxidant enzyme activity, lipid peroxidation, and membrane injury. Overall, the findings highlight that integrating biochar with NaHS could serve as an effective approach to strengthen drought tolerance and enhance maize productivity, particularly in water-limited regions. Further investigations at the field level and molecular scale are recommended to validate and deepen the understanding of this synergistic strategy.

Data availability

The data will be provided by the corresponding author on suitable statement.

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Author contributions

Conceptualization, M.Z.M, K.A., A.E, W.A, Q.Z.; methodology, K.I.; validation, H.M.A.; formal analysis, T.A. A.E, W.A, H.R, M.A.; investigation, M.Z.M., A.E, W.A, H.R, M.A, Ch.S.H.; resources, A.E, M.A.A.; data curation, Ch.S.H.; writing: original draft preparation, M.Z.M., K.I., A.E, W.A, H.R, M.A K.A., Q.Z.; writing: review and editing, M.Z.M., A.E, W.A, H.R, M.A K.A.; funding acquisition,A.E Q.Z. All authors have read and agreed to the published version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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