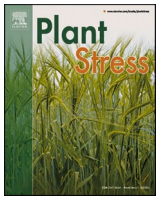
Plant Stress 12 (2024) 100452

Contents lists available at ScienceDirect

Plant Stress

journal homepage: www.sciencedirect.com/journal/plant-stress



# Synergistic effects of biochar and potassium co-application on growth, physiological attributes, and antioxidant defense mechanisms of wheat under water deficit conditions

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ARTICLE INFO

*Keywords:*
Biochar
Foliar K
Drought
Growth stages
Wheat
Antioxidant defense

ABSTRACT

Global wheat production faces a severe threat from drought stress, necessitating innovative strategies for enhanced crop resilience. This study examined the synergistic impact of biochar and potassium co-application on the growth, physiological attributes, and antioxidant defense system of wheat under water deficit conditions at crown root initiation (CRI), anthesis, and grain development stage. Drought-induced reactive oxygen species (ROS) accumulation, particularly pronounced at the CRI stage, adversely affected all growth stages. At CRI, co-application of biochar and foliar potassium delivered significant improvements in growth parameters, including increased plant height (15.4%), spike length (50%), grain yield (43.0%), photosynthetic performance (chlorophyll content 125.8%), and relative water content (11.2%), compared to untreated drought-exposed counterparts. The combined application of biochar and potassium effectively reduced hydrogen peroxide production, electrolyte leakage, proline accumulation, and malondialdehyde generation, while increasing relative water content and glutathione levels under both well-irrigated and drought stress conditions. Furthermore, the combined biochar and potassium treatment was effective in mitigating oxidative stress and enhancing physiological resilience in wheat, particularly during the anthesis stage of drought stress. Specifically, the combined treatment ameliorated the effects of drought by reducing ROS levels through enhanced antioxidant enzyme activities and elevating osmoprotectants levels. The synergistic modulation of tissue osmotic balance and relative water content holds promise for mitigating drought-induced stress, offering an innovative and practical strategy for resilient wheat production in water-limited environments.

## 1. Introduction

The global agricultural sector is facing unprecedented challenges due to climate change, which is expected to result in increased temperatures and uneven precipitation patterns, leading to severe droughts in arid and semi-arid regions of the world (Mehran et al., 2017; Gupta et al., 2020; Bukhari et al., 2021). Recent studies highlighted the greater occurrence of droughts, with projections indicating that up to 30% of the global land surface will experience extreme drought by the 2090s. These heightened risks are expected to cause 10% surge in the demand for irrigation water (Wada et al., 2013; Rajanna et al., 2023). Drought stress is a severe abiotic stressor that directly impacts crop performance and yield (Suzuki et al., 2014; Chai et al., 2016; Iqbal et al., 2020; Kapoor et al., 2020). This abiotic stress presents a substantial threat to worldwide food production, particularly considering projections indicating a population increase exceeding 1 billion by 2030 and 2.4 billion by 2050. Moreover, the collective global food demand is anticipated to surge by 35–56% from 2010 to 2050 (Van Dijk et al., 2021). Consequently, the imperative to bolster plant resilience against drought stress emerges as a pivotal challenge in the quest to enhance food production to meet the

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https://doi.org/10.1016/j.stress.2024.100452
Received 17 January 2024; Received in revised form 1 March 2024; Accepted 27 March 2024
Available online 29 March 2024

demands of a burgeoning global population (Wani and Sah, 2014).

Wheat (*Triticum aestivum* L.) is the most widely cultivated cereal crop globally, with a significant contribution to the world's food supply due to its high carbohydrate (55%) and protein (8–12%) content (Bos et al., 2005; Erenstein et al., 2022). It is a staple food for approximately 40% of the world's population, providing 20% of dietary calories and proteins (Acevedo et al., 2018; Erenstein et al., 2022). As a form of polygenic stress (Suzuki et al., 2014), drought is considered one of the most severe yield-limiting factors in wheat growth and productivity due to its adverse effects on photosynthetic machinery, including degradation of photosynthetic pigments and disruptions in thylakoid electron transport, stomatal conductance, $CO_2$ assimilation, and the Calvin cycle (Rampino et al., 2006; Sharma et al., 2020; Gupta et al., 2020; Ma et al., 2021). However, the severity of drought depends on both the duration and intensity of the water deficit; and also the growth stage when plants are subjected to the exposure, such as the seedling, vegetative, or reproductive stage, all of would have varying biological responses (Blum, 2017). In general, early water stress results in reduced growth, development, and $CO_2$ fixation, while water stress during the reproductive stage leads to reproductive failures such as pollen sterility and lower fertilization, reduced allocation of assimilates to grains, and a shortened grain-filling period, ultimately resulting in smaller grains (Yang et al., 2023; Onyemaobi et al., 2021). However, drought affects differently during the various growth stages of winter wheat (Ning et al., 2023). Furthermore, Abd et al. (2018) reported that recovery at the tillering stage was very pronounced as compared to the jointing or crown root initiation stage under drought. Therefore, it is critical to develop detailed drought restorative management strategies to maintain wheat productivity amidst the challenges posed by water scarcity.

Biochar, a carbonaceous material derived from the pyrolysis of organic biomass at high temperatures (400–700 °C) has emerged as a promising soil amendment for enhancing crop resilience to water stress (Abbott et al., 2018; Ullah et al., 2021; Sani et al., 2023). Due to its high surface area, porous structure, and ability to improve soil water retention and nutrient availability, biochar has been shown to enhance plant growth, water use efficiency, and stress tolerance in various crop species (Abbas et al., 2018; Zulfiqar et al., 2022). Applying biochar in the soil can increase the availability of essential plant nutrients, thus promoting plant growth and biomass production, even under drought stress (Abbott et al., 2018; Hasnain et al., 2023). Several studies have reported that biochar could enhance crop yield by stimulating microbial activity in the rhizosphere, improving soil water holding capacity, and increasing nutrient use efficiency (Sani et al., 2020; Mansoor et al., 2021; Bornø et al., 2022). Additionally, biochar significantly increases soil surface area due to its highly porous structure, resulting in enhanced cation exchange capacity (CEC) and retention of available nutrients in the soil (Jin et al., 2016; Sani et al., 2023). Thus, the application of biochar may contribute not only to alleviating the effects of drought on crops but also to the effective and sustainable management of soil and nutrients.

Similarly, potassium (K) is a crucial inorganic nutrient element for plant growth and development, playing a vital role in multiple physiological processes such as cell expansion, osmoregulation, stomatal regulation, and enzyme activation (Hawkesford et al., 2012; de Bang et al., 2021). Studies have shown that potassium application under moisture stress conditions improves crop tolerance to drought stress and enhances crop growth, dry matter partitioning, and yield (Abdallah et al., 2019). Potassium also plays a crucial role in plant water relations by regulating ionic balances within cells and activating enzymes that catalyze various metabolic processes and nutrient uptake, including the translocation of nitrates from root to aerial parts of plants (Hawkesford et al., 2012; Chowdhury et al., 2020). During drought stress, root growth and the rates of $K^+$ diffusion in the soil towards the roots are restricted, leading to a limited acquisition of potassium. As a result, lower K concentrations can further depress the plant's resistance to drought stress and K absorption, making K-deficient plants more susceptible to drought (Cakmak, 2005). Plants under drought stress have a more significant internal requirement for K, as it is needed for the maintenance of photosynthetic $CO_2$ fixation, protection of chloroplasts from oxidative damage, regulation of stomatal opening and water relations, and impairment of associated disturbances in carbohydrate metabolism (Cakmak, 2005; Wang et al., 2013). The correlation between phytohormones and K has also been studied, as phytohormones interact with one another and other signaling molecules that regulate biochemical processes and metabolism, exerting physiological responses about almost all features of plant growth and development and enhancing stress tolerance (Shabala et al., 2016; de Bang et al., 2021; Singh et al., 2022). Therefore, maintaining adequate plant K levels is critical for developing drought resilience and improving crop productivity under water-deficit conditions.

Recent literature underscores the positive impacts of biochar and potassium application on bolstering drought resilience in food crops. Nawab et al. (2022) demonstrated that foliar potassium spray mitigates water stress effects on wheat plants, regulating crop quality and biochemical characteristics. A recent study demonstrated that potassium supplementation in wheat mitigated water stress effects by enhancing growth, leaf area, dry weight, and chlorophyll content, while increasing antioxidant enzyme production and metabolite levels. This improvement in physiological parameters resulted in enhanced stomatal conductance and photosynthetic rate by 38.4% and 12.8%, respectively, ultimately leading to a significant increase in grain yield by 25.3% (Ahmad et al., 2023). However, existing research predominantly focuses on individual applications of these elements to alleviate drought stress, leaving a dearth of studies exploring their combined influence on enhancing physiological and antioxidant defense mechanisms for heightened wheat productivity under drought conditions. Several research revealed that the combination of biomaterials, as compared to their individual application, has demonstrated the capacity to produce more substantial enhancements in plant growth, yield, and resilience (Abbott et al., 2018; Sani and Yong, 2021). For instance, the synergistic action of salicylic acid and potassium ameliorated drought stress by regulating physiological, biochemical, and antioxidant attributes in wheat (Munsif et al., 2022). Similarly, Sarwar et al. (2023) found that the combined application of K-enriched biochar significantly enhanced chlorophyll content and reduced electrolyte leakage in wheat under both osmotic and non-osmotic stress conditions, indicating its effectiveness in improving drought resilience. The combined application of biochar and water-retaining agent increased maize seedling potassium and soluble sugar contents, enhanced antioxidant enzyme activity, and reduced malondialdehyde content, indicating improved physiological resistance to drought stress in Fluvisols (Dengxiao et al., 2024). However, limited attention has been given to investigating the synergistic effects of co-applying biochar and potassium, despite the potential benefits this combined approach may offer in improving wheat productivity and stress tolerance. Thus, given the centrality of wheat as a staple food crop, this study aimed to investigate the synergistic effects of co-applying biochar as a soil amendment alongside exogenous potassium. We hypothesized that the co-application of biochar and potassium will significantly mitigate drought effects, thereby enhancing wheat productivity. This research assumes the paramount importance in advancing sustainable agricultural practices that fortify crop resilience to drought stress and filling a crucial research gap in the field of crop stress physiology and agronomy.

## 2. Materials and methods

### 2.1. Experimental location, plant material, and growing conditions

Wheat (*Triticum aestivum* L.) variety BARI Gom-30 was obtained from Bangladesh Wheat and Maize Research Institute (BWMRI), Dinajpur, Bangladesh. This experiment was conducted (November 2022 to March 2023) in pots (22 cm diameter) using sun-dried, grounded, sieved, and

well-mixed 8 kg soil per pot at the beginning of the experiment derived from the central research farm of Sher-e-Bangla Agricultural University in Dhaka, Bangladesh. The soil texture with different physical and chemical properties is mentioned in Table 1. Seed sowing was conducted in mid-November using ten seeds in each pot to ensure appropriate spacing. Before seed sowing, the pots were prepared with the recommended amount of fertilizer: In each pot, an initial N-P-K fertilization regimen comprised 6 g of Urea, 5 g of triple super phosphate (TSP), and 5 g of muriate of potash (MOP). Subsequently, 35 days post-sowing, a second fertilization application was administered with same amount as initial dozes.

### 2.2. Experimental design and treatments

Two factors experiment consisted of sixteen treatments combined was conducted in a completely randomized design (CRD) with three replications. Plants belonging to control ($D_0$) were irrigated regularly, keeping 80% water holding capacity (WHC), while plants belonging to drought stress were restricted to 40% WHC during crown root initiation (CRI) ($D_1$), anthesis ($D_2$), and grain development stage ($D_3$) following the protocol by Li et al. (2021). Soil amendment and foliar supplement were as follows: control (T0: no input), biochar (T1: 300 gm $pot^{-1}$), potassium (T2: 100 $mgL^{-1}$), and combination of biochar with potassium (T3). In this experiment, the precision of the biochemical investigation was enhanced by employing optimized concentrations of biochar, as recommended by Zulfiqar et al. (2022), and foliar potassium (K) based on the guidelines provided by Abdallah et al. (2019) for the experimental treatments. The biochar was thoroughly mixed with soil during the preparation stage, while potassium (K) was sprayed as per treatment on plants during drought at each of the growth stages. To improve the adhesion of the potassium (K) solution to the leaves, 0.1% Silwet® Gold was used as a wetting ingredient ("surfactant"). Spraying treatments were conducted in the evening while the temperature was becoming low and no sunlight to prevent rapid evaporation of the spray solution (Henningsen et al., 2022). To prevent soil contamination from spray, pots were fully covered with plastic paper and tissue paper as an absorbent. Physiochemical properties of biochar are presented in Table 2.

### 2.3. Morphological parameters

#### 2.3.1. Vegetative growth, plant harvesting and yield

For each of the vegetative and growth-related traits, five plants were randomly chosen from each experimental treatment. Plant height and spike length were measured by using measurement tape; afterward, the average values were used and expressed as cm, and the number of leaves, the number of tillers per plant, and grain per spike counted manually and were recorded before the harvesting. Plant height was measured from the soil surface to the tip of the spike. In each pot, five plants were harvested together, air-dried for a few days to remove excess moisture, and finally dried at oven 72 hr at 60 °C in an electric oven which was used for further nutrient status investigation. The other five plants from each treatment replicates were harvested after the full maturity stage and used for the biochemical and physiological investigation.

**Table 1**
Physio-chemical properties of the initial pot soil.

| Practical size analysis | Value |
|---|---|
| Bulk density (g $cm^{-3}$) | 1.33 |
| Particle density (g $cm^{-3}$) | 2.64 |
| Porosity (%) | 46.4 |
| Textural class | Silty clay loam |
| pH | 6.34 |
| Organic matter (%) | 1.14 |
| Total N (%) | 0.09 |
| Available P (μ g/g soil) | 42.24 |
| Available K (meq/100 g soil) | 0.18 |

**Table 2**
Physiochemical properties of the biochar.

| Biochar | Unit | Value |
|---|---|---|
| pH | – | 7.07 |
| EC | dS m | 0.87 |
| BET (surface area) | $m^2g^{-1}$ | 1035 |
| Volatile matter | % | 30. 20 |
| Ash content | % | 9.80 |
| Fixed carbon | % | 58.40 |
| CEC | mmol $kg^{-1}$ | 16.2 |
| Total N | % | 0.30 |
| Total P | % | 0.44 |
| Total K | % | 1.32 |

#### 2.3.2. Absolute growth rate (AGR) of the plant

Plant height was assessed at two distinct time points: first, 20 days after sowing (DAS), and subsequently, at the time of harvesting. The absolute growth rate (ABGR) was calculated using the formula:

$$ABGR = \frac{\text{Plant height at harvest } -- \text{ Plant height at 20 days after sowing}}{\text{Harvesting Day } -- \text{ 20 days after sowing}}$$

### 2.4. Photosynthetic attributes

#### 2.4.1. Determination of chlorophyll and carotenoid content

To determine the chlorophyll a, chlorophyll *b*, and total chlorophyll contents in wheat leaves, the protocol given by Arnon (1949) was followed with minor modifications (Song et al., 2020). Fresh leaves (0.2 g) were collected, and midribs were removed. Chlorophyll was extracted using an 80% acetone solution in 50 mL colorimetric tube followed by overnight (24 hr) incubation in dark condition for chlorophyll assessment. The supernatant was collected and the absorbance was measured at 663 nm, 645 nm, and 470 nm wavelengths on a spectrophotometer (UV-5000) to estimate chlorophyll a, chlorophyll *b*, and carotenoids respectively. The following equations were used to calculate the chlorophyll a and chlorophyll *b* concentrations:

$$\text{Chlorophylla}(\text{mg}/\text{g}) = 12.7(\text{OD663}) - -2.69(\text{OD645})\text{V}/1000(\text{W})$$

$$\text{Chlorophyllb}(\text{mg}/\text{g}) = 22.9(\text{OD645}) - -4.68(\text{OD663})\text{V}/1000(\text{W})$$

Here, OD = wavelength, V = final volume and W = fresh leaf weight (g)

The total chlorophyll concentration was obtained by adding the concentrations of chlorophyll a and chlorophyll *b*, which was calculated using the following equation:

$$\text{TotalChlorophyll}(\text{mg}/\text{g}) = \text{Chlorophylla} + \text{Chlorophyllb}$$

### 2.7. Physiological attributes

#### 2.7.1. Leaf relative water content (%)

Relative water content (%) of plants was estimated using the method developed by Barrs and Weatherly (1962). Three leaf laminas from five randomly selected plants from each treatment were collected during the maturity stage and fresh weight was recorded (FW). The leaf samples were soaked in distilled water ($dH_2O$) for 24 h followed by removal of excessive water by wiping with blotting paper and thereafter the turgid weight (TW) was measured. The leaves were then placed in the electric oven at 80 °C for 48 h to remove the moisture, and the dry weight (DW) was measured. Relative water content (RWC) was determined using the following formula and expressed in percentage (Barrs and Weatherly 1962)

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

#### 2.7.2. Determination of electrolyte leakage (%)

Electrolyte leakage (EL) in leaves was measured following the methods described in Lutts et al. (1996). Briefly, fresh leaf (1 g) was plucked, washed with deionized water, and then chopped into very small pieces by a steel cylinder in 1 cm diameter. Uniformly chopped fresh leaves were transferred to the test tube containing 20 mL of deionized water. The test tube was incubated at 25 °C for 24 h followed by cooling down at room temperature and electric conductivity EC1 was recorded using an electrical conductivity (EC) meter (HI-993,310, Hanna, USA). The test tube was again incubated in a hot water bath at 120 °C for 20 min and electric conductivity EC2 was recorded. Finally, the EL was calculated by using the following equation.

$$\text{Electrolyteleakage(EL)} = \text{EC1/EC2} \times 100$$

#### 2.7.3. Determination of proline (Pro) content

The proline content in leaf tissues was determined using the method developed by Bates et al. (1973). Fresh leaf tissue (0.25 g) was homogenized in 5 ml of 3% sulfosalicylic acid using mortar and pestle on ice. The clear aliquot supernatant was collected and centrifuged at 1200 rpm for 15 min. An aliquot of 2 ml of the supernatant was mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin solution (prepared by dissolving 1.25 g of ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid) to incorporate the ratio of 1:1:1. The resulting mixture was incubated in a water bath at 100 °C for 1 hour followed by cooling in an ice bath. Then, 4 ml of toluene was added, and the solution was vortexed thoroughly to extract the chromophore. The upper aqueous layer was transferred to a separate test tube, the optical density was measured spectrophotometrically (UV-500) at 520 nm, and the proline content was calculated by plotting the value against using a standard curve from known pro concentrations (10, 20, 40, 60, and 80 ppm).

#### 2.7.4. Determination of hydrogen peroxide ($H_2O_2$)

A fresh leaf of 0.5 g was homogenized, ground, and mixed with 1.6 mL of 0.1% trichloroacetic acid (TCA) in an ice bath for 30 min, followed by centrifugation at 12,000 × *g* for 20 min at 4 °C. After centrifugation, 0.5 mL clear supernatant was collected and mixed with 1 mL potassium iodide (1 M KI) and 0.1 M potassium phosphate buffer (K-P buffer; pH 7.8). The mixture was vortexed for short period and incubated in the dark for 1 hour. The absorbance of the solution was measured at 390 nm using a spectrophotometer (UV-500). Using the standard curve, $H_2O_2$ was quantified Velikova et al. (2000), and the quantity was displayed as μmol $g^{-1}$ fresh weight.

#### 2.7.5. Malondialdehyde (MDA) content quantification

Leaf malondialdehyde (MDA) content was estimated using the method developed by Heath and Packer (1968). First, we randomly sampled from each treatment replicate 0.5 g of fresh leaves and homogenized them with 3 mL of 5% (w/v) trichloroacetic acid (TCA). The homogenized mixture was then centrifuged at 11,500 × *g* for 15 min, and a clear aliquot was obtained. Next, 1 mL of the supernatant was fused with 4 mL of thiobarbituric acid (TBA) reagent (0.5% TBA in 20% TCA) and heated in a water bath for 30 min at 95 °C followed by cooling on ice bath. The mixture was then centrifuged at 11,500 × *g* for 10 min at 4 °C temperature. We then measured the absorbance of the colored chromophore spectrophotometrically (UV-500) detected at 532 nm and corrected for non-specific absorbance at 600 nm. Finally, we calculated the MDA content using an extinction coefficient of 155 $mM^{-1}cm^{-1}$ and expressed the results as nmol $g^{-1}$ FW.

#### 2.7.6. Reduced glutathione (GR) content (nmol $g^{-1}$) extraction and measurement

The activity of glutathione reductase (GR, EC: 1.6.4.2) was determined to assess the reduced glutathione (GSH) content (nmol $g^{-1}$) using the method described by Anderson (1985). Briefly, a reaction mixture was prepared by adding 0.1 M potassium phosphate buffer (pH 7.0), 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM oxidized glutathione (GSSG), 0.2 mM nicotinamide adenine dinucleotide phosphate (NADPH), and plant extract to make a final volume of 1 mL. The mixture was incubated for 1 min, and the absorbance at 340 nm was continuously recorded with a spectrophotometer (UV-5000). The activity of GR was determined using an extinction coefficient of 6.2 $mM^{-1}cm^{-1}$.

### 2.8. Determination of macronutrient content in shoots

Macronutrients in shoot such as nitrogen (N), phosphorus (P), and potassium (K) was determined following the multistep Kjeldahl's, colorimetric, and flame photometer method respectively and with some modifications (Shi et al., 2019; Song et al., 2019). First, the oven-dried (0.25 g) samples were transferred to a clean 10 mL flask, digested each replicated treatment was with 5 mL sulfuric acid ($H_2SO_4$); after that, 2 mL of 50% hydrogen peroxide ($H_2O_2$) was added in each flask. Then, the flasks were heated three times to digest the sample clearly at 285 °C for 45 min and subsequently cool. and then made up the volume up to 100 mL with filtered (Whatman no. 1) distilled water. Then, it was sorted into plastic vials at 4 °C for N, P, and K analysis. Finally N and P was analyzed by auto analyzer (Snartchem 200) the absorbance at 420 nm to 880 nm following the Kjeldahl method (Schuurman et al.,1971). For potassium determination, the digested stored sample were used and then assessed on a flame spectrophotometer (FP 6410, INESA Scientific Instrument CO., Ltd., Shanghai, China) following the protocol described by Asch et al. (2022).

### 2.9. Statistical analysis

The experimental data from the pot experiment were statistically analyzed and visualized using different packages in R (version 4.2.1). All data were subjected to two-way analysis of variance (ANOVA) followed by mean comparison following Tukey's honest significant difference (Tukey HSD) test using "Agricolae" package. Mean values are expressed as mean ±SE with significant alphabet and are visualized in bar charts using "ggplot2" package. "corrplot" package in R was used to estimate the Pearson correlation among the response variables. To identify the most contributing responses variables and the association among them, principal component analysis (PCA) was carried out using "FactoMineR" and "factoextra" packages. To further reveal the relationship among the response variables at specific drought level and biochar treatment, heatmap was prepared using the "heatmaply" package.

## 3. Results

### 3.1. Interaction effects on morphological characters

The combined influence of drought and applied treatments delivered statistically different changes on parameters such as the number of leaves per plant, spike length, number of grains per spike, and grain yield per plant. In contrast, no significant impact was observed on plant height, number of tillers per plant, average growth rate, and days to 50% flowering (**Supplementary Table 1**). Drought stress adversely affected all the morphological characters of wheat (Table 3).

The data represent the mean values ± standard deviation, ($n = 3$). Different letters denote significant differences at $p \leq 0.05$ by TuekyHSD test. D0, D1, D2, and D3 indicate the control, drought stress at CRI, anthesis, and grain development stage and T0, T1, T2, and T3 indicate the control (no input), 300 g biochar, foliar 100 $mgL^{-1}$ $K_2SO_4$ and 300 g biochar + 100 $mgL^{-1}$ $K_2SO_4$ treatment, respectively.

Drought stress at CRI stage had the highest negative effects on all the characters compared to control. Drought stress at grain developmental stage has minimal effect on plant height, number of leaves per plant, number of tillers per plant, average growth rate, days to 50% flowering

**Table 3**
The effects of biochar and potassium application on growth and yield attributes of wheat under drought stress at various growth stages.

| Drought | Treatment | Plant height (cm) | Leaf number/ plant | Tiller number/ plant | Absolute growth rate (%) | Days to 50% flowering | Spike length (cm) | Grains number/ spike | Grain yield (ton/ha) |
|---|---|---|---|---|---|---|---|---|---|
| D0 | T0 | 73.17±7.72a | 3.63±0.47abc | 2.00±0.45a | 56.33±3.75a | 48.00±6.00a | 10.33 ±2.02abc | 42.17±4.92a-d | 2.78±0.62bcd |
| | T1 | 74.81±6.16a | 3.92±0.55ab | 2.10±0.85a | 59.33±4.01a | 50.00±6.56a | 15.90 ±2.95ab | 45.42±5.08ab | 3.50±0.5abc |
| | T2 | 73.88±6.60a | 3.82±0.24abc | 2.20±0.75a | 57.33±2.91a | 48.33±5.43a | 14.23 ±1.96abc | 42.4 ± 3.95abc | 3.64±0.31abc |
| | T3 | 76.13±4.99a | 4.07±0.50a | 2.30±0.75a | 60.33±4.75a | 50.67±3.01a | 16.53±2.54a | 48.03±5.00a | 4.00±0.36a |
| D1 | T0 | 55.47 ±10.21a | 2.27±0.67c | 0.88±0.16a | 45.00±5.27a | 38.00±6.00a | 9.33±1.81c | 26.70±6.19e | 2.11±0.30d |
| | T1 | 63.43±7.84a | 3.00±1.00 abc | 1.20±0.30a | 45.30±5.25a | 39.57±4.75a | 12.00 ±1.80abc | 33.4 ± 2.91b-e | 2.80±0.20a-d |
| | T2 | 62.41±7.23a | 2.43±0.60bc | 1.00±0.50a | 45.47±5.05a | 39.00±4.00a | 12.00 ±2.60abc | 30.73±4.38cde | 2.50±0.50cd |
| | T3 | 64.00 ±14.15a | 3.20±0.60abc | 1.33±0.67a | 46.37±6.58a | 40.00±4.00a | 14.00 ±2.50abc | 35.30±5.10a-e | 3.01±0.4a-d |
| D2 | T0 | 60.07±9.77a | 2.83±0.29abc | 1.33±0.45a | 45.33±4.95a | 41.00±3.61a | 10.00 ±1.80bc | 27.80±4.81de | 3.00±0.40a-d |
| | T1 | 66.69±8.31a | 3.30±0.46abc | 1.47±0.45a | 49.33±3.90a | 42.00±5.57a | 13.00 ±2.62abc | 31.27±5.90b-e | 3.30±0.36a-d |
| | T2 | 65.46 ±10.31a | 3.25±0.25abc | 1.50±0.46a | 49.10±7.12a | 42.00±6.24a | 11.87 ±1.50abc | 30.33±3.91cde | 3.20±0.31a-d |
| | T3 | 69.00±8.59a | 3.4 ± 0.36abc | 1.80±0.40a | 50.33±4.90a | 43.00±4.58a | 13.57 ±2.97abc | 33.23±4.95b-e | 3.50±0.51abc |
| D3 | T0 | 64.40 ±10.23a | 3.43±0.6abc | 1.70±0.35a | 52.33±5.49a | 44.0 ± 4.00a | 9.67±1.24bc | 32.53±3.72b-e | 3.07±0.20a-d |
| | T1 | 70.33±9.55a | 3.64±0.39abc | 1.93±0.55a | 54.20±6.05a | 46.00±5.27a | 13.00 ±1.30abc | 39.40±3.95a-e | 3.57±0.35abc |
| | T2 | 69.49±9.26a | 3.29±0.47abc | 1.93±0.85a | 53.23±4.90a | 45.00±3.00a | 12.00 ±1.40abc | 35.43±4.95a-e | 3.40±0.40abc |
| | T3 | 70.57 ±10.13a | 3.70±0.60abc | 2.00±0.50a | 55.00±5.50a | 47.00±5.50a | 15.00 ±1.50abc | 42.13±6.11a-d | 3.94±0.42ab |
| | CV% | 11.64 | 15.17 | 34.74 | 10.38 | 9.77 | 16.23 | 12.90 | 12.47 |

and number of grains per spike while drought stress at anthesis had minimum effect on spike length and grain yield. Under control conditions, application of biochar and potassium treatment alone or in mixtures, improved the agronomic responses (Table 3). The combined effects of biochar and potassium showed greater improvements on all the characters compared to their individual effect. Under control conditions, biochar application delivered higher positive effect on number of leaves per plant, average growth rate, days to 50% flowering, spike length, and number of grains per spike compared to potassium application. Whereas potassium application showed maximum positive effect on number of tillers per plant and grain yield. Both biochar and potassium alone or in mixtures alleviated the effect of the drought stress at CRI, anthesis and grain development stage (Table 3). However, the drought stress at all the stages were alleviated by individual biochar treatment compared to the individual alleviation by potassium.

### 3.2. Effects on photosynthesis

Interactive effects of drought and treatment showed significant effect on carotenoids, chlorophyll-a, chlorophyll-b and total chlorophyll content (Fig. 1, **Supplementary Table 1**). Drought stress at CRI stage showed minimum value for all the characters followed by drought stress at anthesis stage (Fig. 1). On the other hand, drought stress at grain developmental stage showed the maximum value. Under the well irrigated systems, application of potassium, biochar and combined potassium and biochar showed the maximum pigment concentration compared to the untreated plant. In all the cases, the individual effect of biochar application showed higher value compared to the individual effect of potassium. Biochar, potassium, and their combination alleviated the drought stress by reducing the photosynthetic degradation (Fig. 1). However, co-application of biochar and potassium resulted in higher concentration of chlorophyll a (Fig. 1A), chlorophyll *b* (Fig. 1B), total chlorophyll (Fig. 1C), and carotenoids (Fig. 1D) compared to their individual effect.

### 3.3. Effects on physiological attributes

Drought and treatment significantly affected the hydrogen peroxide generation, electrolyte leakage, elative water content, proline content, glutathione, and malondialdehyde content in wheat plants (Fig. 2, **Supplementary Table 1**). Maximum and minimum electrolyte leakage (Fig. 2B), proline content (Fig. 2C), and hydrogen peroxide (Fig. 2D) were observed during the drought stress at CRI and grain development stage respectively. Drought stress at CRI stage showed minimum relative water content followed by anthesis stage (Fig. 2A) and similar pattern was observed for glutathione content (Fig. 2F).

Based on individual effect of biochar and potassium treatment under well irrigated system, both potassium and biochar individually or in mixtures reduced the production of hydrogen peroxide, occurrence of electrolyte leakage, proline accumulation and malondialdehyde generation (Fig. 2). On the contrast, potassium, and biochar individually or in mixtures increased relative water content and glutathione content. Biochar alone increased the relative water content, glutathione and malondialdehyde generation compared to potassium alone. However, potassium application suppressed the hydrogen peroxide generation and lowers the electrolyte leakage.

The combined effect of co-application of potassium and biochar was maximum compared to biochar and potassium alone. Based on the interactive effect of treatment and drought stress, the combined biochar and potassium showed minimum hydrogen peroxide, electrolyte leakage, proline content, Malondialdehyde and maximum relative water content and glutathione content under all the three drought stresses. The combined effect of biochar and potassium showed lowest hydrogen peroxide, electrolyte leakage, proline content, malondialdehyde under drought stress at anthesis stage whereas highest under drought stress at CRI and pollen development stages. Biochar and potassium increased

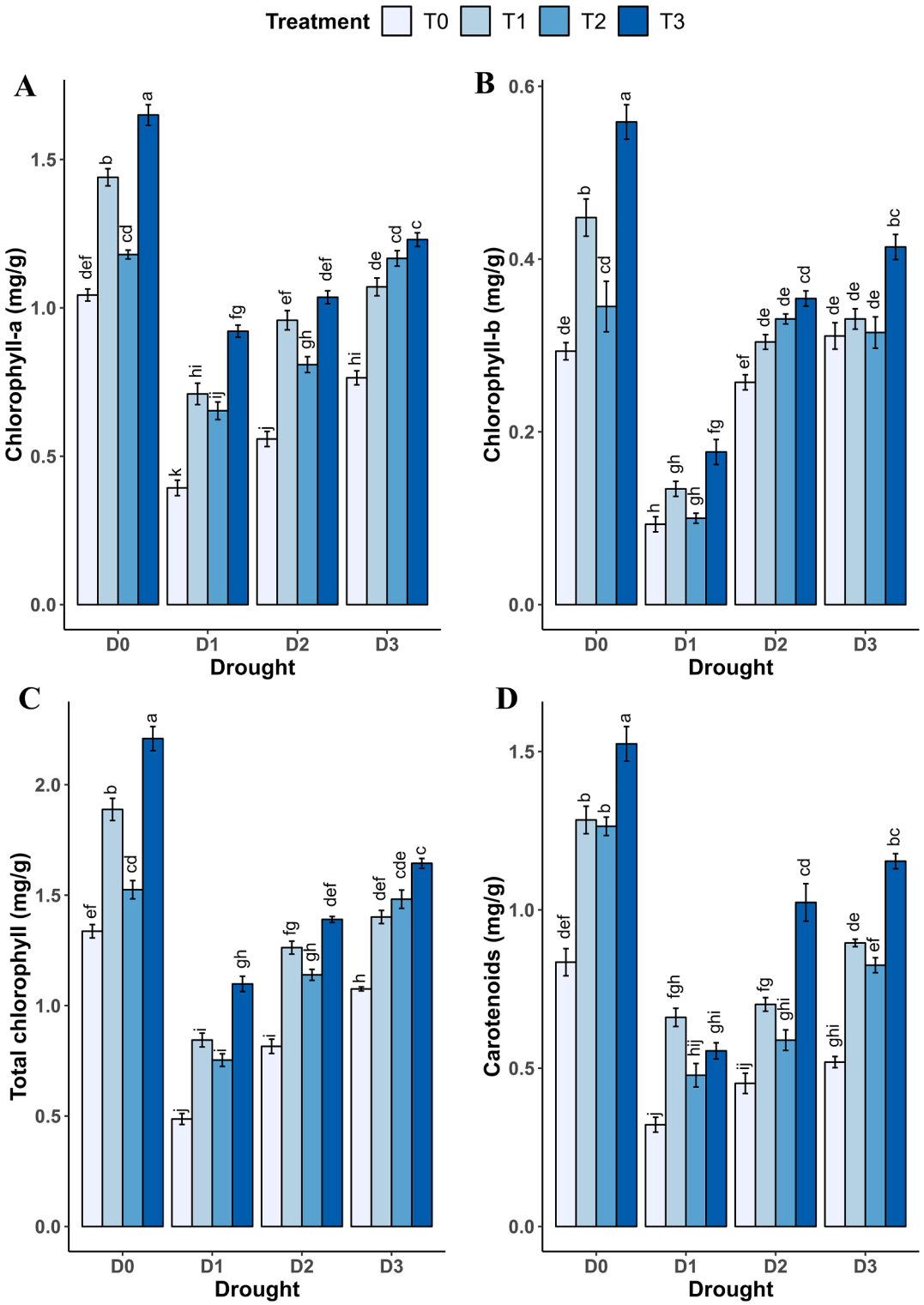


**Fig. 1.** The effects of treatment on photosynthetic pigments of wheat under different drought stresses. D0, D1, D2, and D3 indicate the control, drought stress at CRI, anthesis, and grain development stage. T0, T1, T2, and T3 indicate the control, 300 g biochar, foliar 100 $mgL^{-1}$ $K_2SO_4$ and 300 g biochar with 100 $mgL^{-1}$ of $K_2SO_4$. The bar chart, error bar, and letter represent the mean value, standard error, and significant differences ($n = 3$).

the relative water content, and glutathione for all three stages compared to the untreated plants whereas decreased the hydrogen peroxide, electrolyte leakage, proline content and malondialdehyde content.

### 3.4. Effects on shoot nitrogen, phosphorus, and potassium

Drought and treatment showed significant effect on nitrogen, phosphorus, and potassium content in shoot of wheat plant (Table 4, **Supplementary Table 1**). Drought stress at CRI stage reduced the N, P and K content in shoot having the lowest (1.23±0.03, 0.12±0.03, 0.96 ±0.03 respectively) content compared to the drought stress at anthesis and grain developmental stage followed by drought stress at anthesis stage (Table 4). Drought stress at grain development stage showed minimum effect on N, P and K.

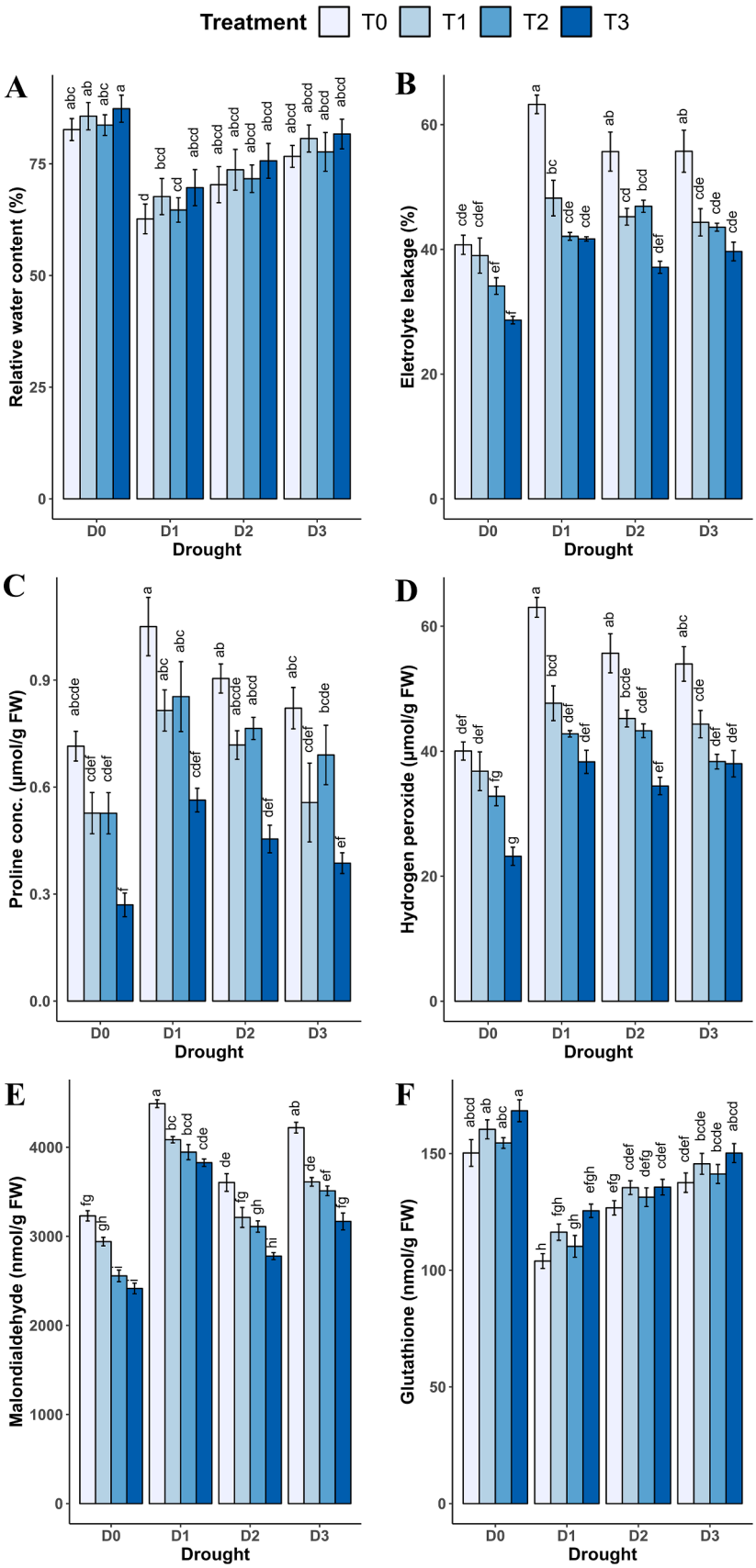


**Fig. 2.** The effects of treatment on physiological responses of wheat under different drought stresses. D0, D1, D2, and D3 indicate the control, and drought stress at CRI, anthesis, and grain development stages, respectively. T0, T1, T2, and T3 indicate the control, 300 g biochar, foliar 100 $mgL^{-1}$ $K_2SO_4$ and 300 g biochar + 100 $mgL^{-1}$ $K_2SO_4$ treatment. The bar charts, error bar, and letter represents the mean value, standard error, and significant differences ($n = 3$).

**Table 4**
Effects of treatment on nitrogen, phosphorus, and potassium content in the shoot of the wheat plants under different drought stresses.

| Drought | Treatment | Shoot nitrogen (%) | Shoot phosphorus (%) | Shoot potassium (%) |
|---|---|---|---|---|
| D0 | T0 | 1.67±0.06ef | 0.29±0.04def | 2.03±0.03d |
| | T1 | 2.28±0.03b | 0.45±0.04b | 2.19±0.02c |
| | T2 | 2.12±0.03c | 0.39±0.03bc | 2.39±0.03b |
| | T3 | 2.66±0.04a | 0.61±0.06a | 2.68±0.03a |
| D1 | T0 | 1.23±0.03i | 0.12±0.03i | 0.96±0.03j |
| | T1 | 1.38±0.03h | 0.2 ± 0.02 ghi | 1.14±0.04h |
| | T2 | 1.26±0.05i | 0.17±0.02hi | 1.24±0.03fg |
| | T3 | 1.65±0.04ef | 0.31±0.03cde | 1.30±0.01ef |
| D2 | T0 | 1.31±0.03hi | 0.2 ± 0.02 ghi | 1.05±0.04i |
| | T1 | 1.58±0.03fg | 0.26±0.02efg | 1.20±0.02 gh |
| | T2 | 1.53±0.04 g | 0.22±0.02fgh | 1.31±0.02ef |
| | T3 | 1.84±0.04d | 0.36±0.03cd | 1.36±0.02e |
| D3 | T0 | 1.31±0.03hi | 0.28±0.03defg | 1.15±0.02h |
| | T1 | 1.67±0.04ef | 0.34±0.01cd | 1.20±0.02 gh |
| | T2 | 1.70±0.03e | 0.31±0.01cde | 1.27±0.03fg |
| | T3 | 1.93±0.04d | 0.46±0.02b | 1.36±0.03e |
| | CV% | 2.03 | 9.14 | 1.78 |

Under well irrigated conditions, biochar, potassium and combined potassium and biochar increased the N, P and K content compared to control plant. In all the cases, combined biochar and potassium treatment showed the highest N, P and K content (2.66±0.04, 0.61±0.06 and 2.68±0.03 respectively). However, biochar showed higher N and P content compared to potassium treatment except higher potassium content treated with potassium.

Application of biochar, potassium, and combined biochar and potassium alleviated the drought stress by increasing the shoot nitrogen, phosphorus, and potassium content (Table 4). Combined potassium and biochar increased the N, P and K content compared to untreated plants under drought stress in all three stages. Biochar showed a higher increase in N and P content under drought stress at CRI and anthesis stage compared to the potassium treatment, whereas potassium showed a higher increase in potassium content at each of the tree drought stresses as expected.

The data represent the mean values ± standard deviation, ($n = 3$). Different letters denote significant differences at $p \leq 0.05$ by TuekyHSD test. D0, D1, D2, and D3 indicate the control, drought stress at CRI, anthesis, and grain development stage and T0, T1, T2, and T3 indicate the control (no input), 300 g biochar, foliar 100 $mgL^{-1}$ $K_2SO_4$ and 300 g biochar + 100 $mgL^{-1}$ $K_2SO_4$ treatment, respectively.

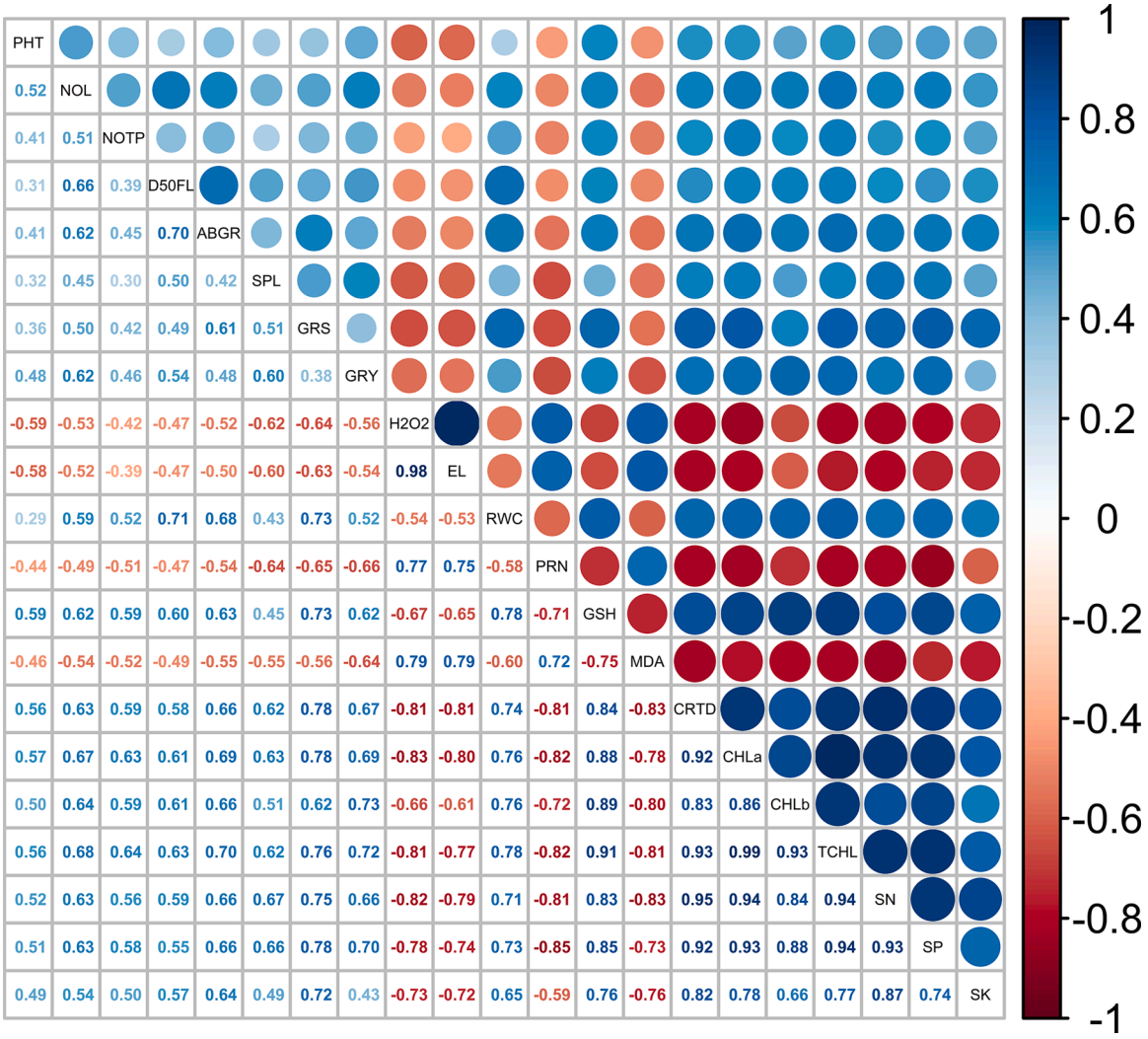


**Fig. 3.** Correlation analysis among the wheat plants' morphological, physiological, and biochemical characteristics. Here, $H_2O_2$ (hydrogen peroxide), PRN (proline), MDA (malondialdehyde), PHT (plant height), SPL (spike length), SN (shoot nitrogen), carotenoid, SP (shoot phosphorus), SK (shoot potassium), CHL-a (chlorophyll-a), GRS (grains per spike), TCHL (total chlorophyll), GSH (glutathione), GRY (grain yield), CHL-b (chlorophyll-b), NOTP (number of tiller per plant), RWC (relative water content), NOL (number of leaves), AVGR (average growth rate), and D50FL (days to 50% flowering).

### 3.5. Pearson correlation and principal component analysis

To assess the general relationships among the response variables, Pearson correlation and PCA biplot was prepared (Fig. 3 and 4). Morphological characters showed highest correlation among them and the similia pattern was observed for N, P and K content in shoot (Fig. 3). Electrolyte leakage, hydrogen peroxide, proline content, and monoaldehyde showed strong correlation among them while strong negative correlation with most of the morphological traits. Leaf electrolyte leakage showed highest positive correlation (0.98) with leaf hydrogen peroxide content whereas highest negative correlation (−0.81) with carotenoids. Hydrogen peroxide showed highest negative correlation with shoot nitrogen (−0.82). Yield contributing characters showed strong and positive correlation among them. Grain yield per plant displayed strong positive correlation with shoot nitrogen, shoot phosphorus and leaf chlorophyll *b* contents (0.66, 0.70, and 0.73 respectively). However, grain yield per plant displayed negative correlation with electrolyte leakage (−0.54), hydrogen peroxide (−0.56), proline content (−0.66), and monoaldehyde (−0.64).

PCA biplot displayed the relationships among the response variables (Fig. 4). PC1 and PC2 explained 68% and 6% variations respectively. Photosynthetic pigments (total chlorophyll content, chlorophyll a, carotenoids, chlorophyll *b*), shoot nitrogen and phosphorus, and glutathione displayed higher contribution to explain the first major PC1 (Fig. 4A). Days to 50% flowering, electrolyte leakage, hydrogen peroxide, relative water content, absolute growth rate and number of leaves per plant contributed most for explaining the PC2 (Fig. 4B). PCA biplot displayed the association among the response variables (Fig. 4C). Photosynthetic pigments, and shoot mineral content displayed strong association with the yield contributing traits whereas hydrogen peroxide, monoaldehyde, proline, hydrogen peroxide, and electrolyte leakage displayed negative association with leaf pigments, yield contributing traits, and shoot mineral contents (Fig. 4C) which is supported by their correlation value in Fig. 3.

### 3.6. Two-way hierarchical clustering analysis (HCA)

Two-way hierarchical clustering and heatmap displayed the effect of drought, treatment, and their interaction on the relationships among the response variables (Fig. 5). HCA associated dendrogram among the treatment and drought showed that all treatments and drought were clustered separately in four distinct clusters. First cluster included the D1T0 (drought at CRI + control), D2T0 (drought at anthesis + control), D1T2 (drought at CRI + 100 mg/L $K_2SO_4$) and D1T1 (drought at CRI + 300 g biochar). Second cluster included the D1T3 (drought at CRI + 100 mg/L $K_2SO_4$ + 300 g biochar), D2T1 (drought at anthesis + 300 g biochar), D2T2 (drought at anthesis+ 100 mg/L $K_2SO_4$) and D3T0 (drought at grain development + control). Cluster 3 included the D0T0 (control + control), D3T1 (drought at grain development + 300 g biochar), D3T2 (drought at grain development + 100 mg/L $K_2SO_4$), and D2T3 (drought at anthesis + 100 mg/L $K_2SO_4$ + 300 g biochar). The last cluster included the D3T3 (drought at grain development +100 mg/L $K_2SO_4$ + 300 g biochar), D0T2 (control + 100 mg/L $K_2SO_4$), D0T1 (control + 300 g biochar), and D0T3 (control + 100 mg/L $K_2SO_4$ + 300 g biochar). In agreement with our PCA and correlations, the response variables showed similar relationships and clustered among them. The response variables were clearly clustered into four distinct clusters. Yield and yield contributing characters along with the mechanisms related to drought stress alleviation (relative water content, glutathione, membrane stability index) were grouped together. Similarly, the Malondialdehyde, Proline, hydrogen peroxide and electrolyte leakage clustered together. N and K were clustered together whereas shoot P clustered with photosynthetic pigments.

## 4. Discussion

Drought, as a severe abiotic stressor, detrimentally impacts crops throughout critical developmental stages, including seedling, vegetative, and reproductive phases, thus leading to yield losses (Yang et al., 2023; Khan et al., 2021a; Onyemaobi et al., 2021; Jafarnia et al., 2018). Our current study noted significant decrease in physiological and yield contributing traits of wheat in all growth stages where the impacts were more pronounced in the crown root initiation (CRI) stage (Table 3, Fig. 1-2). Khan et al., 2020 reported that the demand for water is most critical during the CRI and dough stages for wheat cultivation. Similarly, Wan et al., 2022 concluded that the lack of moisture at this stage leads to significant yield loss, up to 60% for wheat. In addition, heat stress lowered up to 46% of wheat yield (Pandey et al., 2018; Bhandari et al., 2024). Drought imposed at the crown root initiation stage totally disturbed the root system development; this stage confirms that anthesis is coming (Aziz et al., 2016; Figueroa-Bustos et al., 2018; 2020). The principal factors contributing to the reduction in yield and associated traits under stress conditions encompass pollen abortion (Ji et al., 2010), diminished food stem reserves (Sinclair and Jamieson, 2006), and the generation of sterile tillers (Duggan et al., 2005). Furthermore, the extent of trait reduction is contingent upon the intensity and duration of the stress (Barnabás et al., 2008). Moreover, the manifestation of drought stress can induce the generation of reactive oxygen species (ROS) within plant cells. These ROS encompass highly reactive molecules such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals (Sachdev et al., 2023) and possess the capacity to adversely affect cellular structures, including lipids, proteins, and DNA. Their inherent reactivity may result in detrimental consequences, notably cellular oxidative damage (Hosain and Dietz, 2016; Hasanuzzaman et al., 2023).

Under stressful conditions, plants are unable to detoxify ROS, especially leading to oxidative stress in plants (Abbas et al., 2018; Hosain and Dietz, 2016). The application of biochar and foliar potassium significantly improved the photosynthesis and activities of osmolytes and antioxidants and, thereby, the wheat growth and yield under drought stress (Fig. 1-2 and Table 3-4). Similarly, higher cellular ROS were reported under heat and drought stress compared to well-watered conditions by Caverzan et al. (2016). To alleviate such cellular damage, the co-application of potassium with organic amendment acts as a stress-ameliorating agent, thus alleviating the negative effects of abiotic stress by regulating physiological and biochemical processes (Sarwar et al., 2023). This includes enhancing root growth, cell turgor pressure, and osmotic balance, while maintaining a stable equilibrium between antioxidant enzymes and reactive oxygen species (Ahanger and Agarwal, 2017; Lotfi et al., 2022). Biochar application positively influences soil health by improving soil structure, augmenting water-holding capacity, and enhancing soil aeration resulting in enhanced drought resilience (Haider et al., 2020). Therefore, in the present research, the potential synergistic interaction between biochar and potassium was shown to promote drought resistance by upregulating the antioxidant profile, thereby improving potassium use efficiency and subsequently increasing wheat yield.

Drought stress in wheat at various growth stages increased the hydrogen peroxide ($H_2O_2$) concentration and malondialdehyde (MDA) (Fig. 2). Biochar amendments improve antioxidants by improving plant metabolic functions, and cell growth, reducing ROS production, and better soil-plant and water relations (Ghazouani et al., 2023; Zulfiqar et al., 2022; Dusenge et al., 2019). Similarly, results obtained by Suliman et al., 2017 biochar application increase the presence of oxygen as a functional group and porous structure, thus robustly the antioxidant defense mechanism from ROS. However, Kapoor et al., 2024 observed that biochar amendment helps plants cope with arsenic-induced ROS by improving the antioxidant and glyoxalase systems. In contrast with biochar, the activities of antioxidant enzymes were enhanced by improving the K content in wheat plants, which is involved in

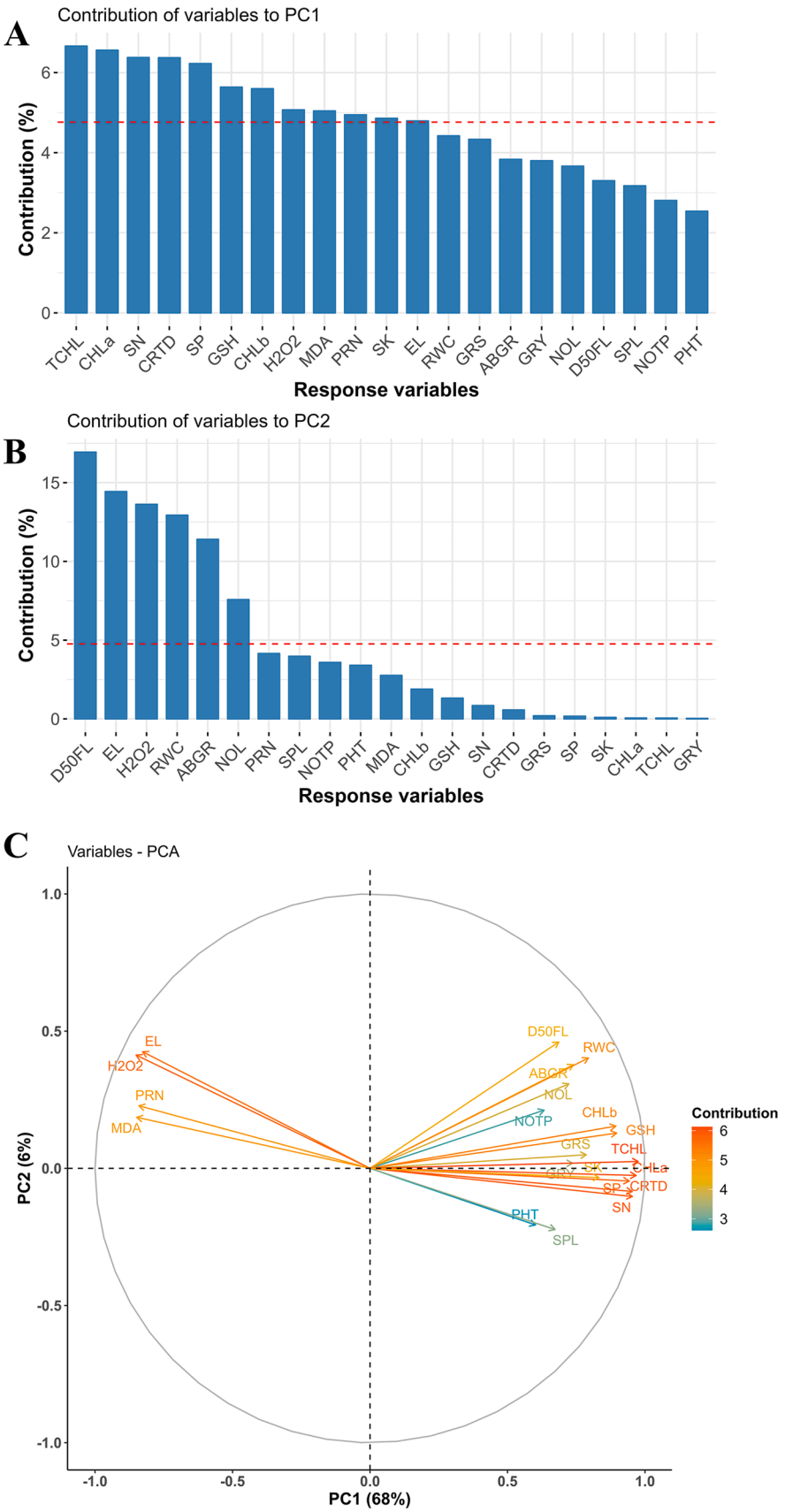


**Fig. 4.** PCA biplot highlighted the contribution and relationship among the individual variables. Here, $H_2O_2$ (hydrogen peroxide), PRN (proline), MDA (malondialdehyde), PHT (plant height), SPL (spike length), SN (shoot nitrogen), carotenoid, SP (shoot phosphorus), SK (shoot potassium), CHL-a (chlorophyll-a), GRS (grains per spike), TCHL (total chlorophyll), GSH (glutathione), GRY (grain yield), CHL-b (chlorophyll-b), NOTP (number of tiller per plant), RWC (relative water content), NOL (number of leaves, AVGR (average growth rate), and D50FL (days to 50% flowering).

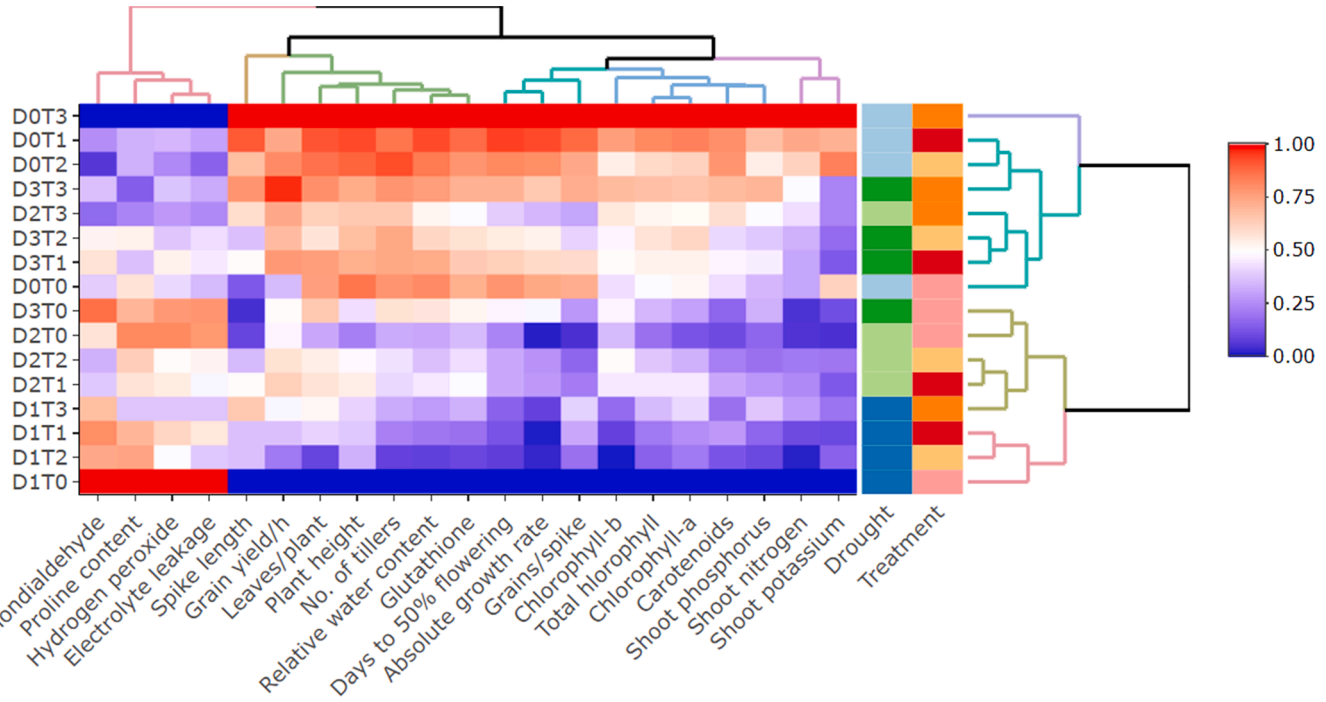


**Fig. 5.** Two-way hierarchical cluster analysis (HCA) and heatmap of individual response variables in wheat plants under four drought stresses with four treatments of biochar and potassium. Rows correspond to different combinations of drought and treatment, whereas columns correspond to response variables. Blue and red colours are the corresponding high and low numeric values. Here, D0, D1, D2, and D3 indicate the control, drought stress at CRI, anthesis, and grain development stage. T0, T1, T2, and T3 indicate the control, 300 g biochar, foliar 100 $mgL^{-1}$ $K_2SO_4$, and 300 g + foliar 100 $mgL^{-1}$ $K_2SO_4$ treatment.

maintaining turgor pressure and reducing oxidative stress (Hemavathi et al., 2011; Rasuli et al., 2022). Cation-anion interactions and nitrogen immobilization due to the adsorption capacity of biochar are responsible for the increased rates, retention, and accessibility of nitrogen, phosphorus, and potassium in biochar-modified pots (Clough and Condron, 2010; Khan et al., 2021b). Consistent with our current investigation (Table 4), biochar with foliar K improves drought-affected plant nutrition, especially at the CRI stage, where destructiveness is more pronounced. Under stress conditions, osmolytes such as proline induce nitrogen metabolic developments by replacing water to maintain the energy level of the stressed plant, thus providing stability to the main structures (Meena et al., 2019; Ou et al., 2018). In this study, a higher level of proline content was observed in wheat plants applied without potassium and biochar under drought stress (Fig. 2). Our investigation was in line with the previous studies that demonstrated that abiotic stresses were reduced with the addition of biochar (Zhu et al., 2022; Zoghi et al., 2019). However, in this regard, Nie et al. (2016) and Bilias et al. (2023) found that applying biochar to volcanic soil gave higher soil exchangeable K and delivered high K levels in wheat tissues. Thus, the positive effects of K addition were acting through osmolytes (acting as a protective agent) at the crown root initiation stage (CRI) of wheat under water-limited conditions. Furthermore, an increased level of proline content in plants under stress conditions helps to maintain osmotic potential-inducing tolerance, as evidenced by its correlation with relative water content (RWC) (Figs. 2-5). Besides, the biochar can absorb more water to reduce the negative effect of drought stress by providing sufficient water to plants during drought stress conditions to decrease the proline content (Fig. 2). To induce tolerance against drought, adjustment of osmolytes like proline and sugar through increasing osmoprotectants to maintain turgor and water content of the cell (Farhangi-Abriz and Torabian, 2017), thus strongly supported by our present investigation. Low soil moisture availability to plants increases proline accumulation under drought stress (Mafakheri et al., 2010), causes a reduction of photosynthetic efficiency by enhancing chlorophyll degradation, and increasing lipid peroxidation (Khayatnezhad and Gholamin, 2021; Liu et al., 2019; Tränkner et al., 2018). Plants that possess the ability to retain higher chlorophyll concentration under drought stress can be more resistant to moisture stress and maintain higher growth and biomass production (Khayatnezhad and Gholamin, 2021; Li et al., 2018). Our results show that the combined application of potassium and biochar enhanced the total chlorophyll content and relative water content of the stressed wheat plants compared to control plants (Figs. 1 and 2). Similarly, the mixtures of both sources, biochar and potassium, potentially mitigated the negative effect of osmotic stress by upholding the physiological performance found by Choudhary et al., 2021 and Munsif et al., 2022 potassium has an amelioration role in inhabiting osmotic stress that causes membrane damage. Furthermore, Li et al. (2018) found a strong relationship between plant growth and total chlorophyll concentration. This is also evidenced in the present study, one of the crucial parameters for photosynthetic efficiency, which is chlorophyll content in wheat leaves varied significantly. In contrast, the combination of biochar and foliar potassium significantly improved photosynthetic pigments under drought stress (Fig. 1). However, osmotic stress reduces in chlorophyll a, chlorophyll *b*, and total chlorophyll levels (Zafar-ul-Hye et al., 2019; Faizan et al., 2024). Increased soil water retention is another mechanism by which biochar and foliar K mitigate osmotic stress. During dry periods, biochar preserved the moisture content and porous structure of the soil thus preventing rapid desiccation and compaction. These improved rhizospheric attributes were likely to minmize anaerobic respiration of the root system (Jabborova et al., 2023). In order to maintain adequate hydration levels in plant cells, this increased water availability is critical. By promoting soil moisture retention and providing a stable environment for plant roots, in this investigation, biochar and potassium together indirectly reduce electrolyte leakage (Fig 2b.). This, in turn, contributes to the maintenance of cell membrane integrity and the reduction of electrolyte loss from plant cells. Similar to the observation of the study, biochar first improves soil properties and then positively increases plants' antioxidant enzyme activities (Amami et al., 2022; Semid et al., 2019).

Furthermore, drought stress reduced the availability and transport of

essential plant nutrients resulting in distinctive lower physiological performance of plants (Ge et al., 2012; de Bang et al., 2021; Begum et al., 2022). In the present study, drought stress severely affected nutrient uptake and assimilation when the wheat plants were exposed to drought stress conditions at various growth stages (Table 4). Biochar possesses the capacity to provide readily available nutrients, including potassium (K), in forms that are easily assimilated by plants, thereby enabling direct nutrient delivery for plant utilization (Allohverdi et al., 2021). Furthermore, as proposed by Martineau et al. (2017), potassium supplementation emerges as an innovative drought management approach for potassium-deficient soils. Moreover, the application of potassium (K) fertilizer was effective in delivering positive effects on soil parameters, including pH, available N, phosphorus (P), and potassium (K) content, as well as the diversity index of soil microbes under conditions of drought stress (Xu et al., 2021; de Bang et al., 2021). Several studies demonstrated that a foliar spray of K on leaves is more effective than the basal dose (Hasanuzzaman et al., 2018). Interestingly, K is an effective plant nutrient that alleviates the harmful effects of drought by regulating different physiological and biochemical processes in relation to water and nutritional balance of plants, leading to improvement in root growth (Bilias et al., 2023; Xu et al., 2021; Keske et al., 2020; Kochanek et al., 2016). It controls stomatal opening under drought conditions and assists plants in synthesis of protein, carbohydrate metabolism, and enzyme activation under water stress conditions (Aksu and Altay, 2020; Pathak et al., 2020; Kumar et al., 2020). Similarly, Sarwer et al., 2023 also reported that mitigating osmotic stress using potassium-enrich biochar impacts synergistically. Therefore, the synergistic effect of biochar with K-based nutrition could provide an effective alternative drought management strategy (Bilias et al., 2023).

Biochar, increasingly valued as soil amendment, enhances agricultural soils, and offers environmental, economic benefits, potentially impacting carbon credit systems, ultimately amplifying crop yields (Abbott et al., 2018; Allohverdi et al., 2021; Bilias et al., 2023; Sani et al., 2023; Hasnain et al., 2023). Our investigation highlighted the efficacy of biochar and foliar potassium application in improving plant performance, particularly under drought stress at the crown root initiation stage in wheat. This soil amendment enhances drought tolerance by enhancing chlorophyll content, modifying gas exchange, improving plant water status, and augmenting mineral uptake, net photosynthesis, growth, biomass, and ultimately wheat yield. Biochar amendments stimulate oxidation–reduction reactions, enhancing soil quality. Concurrently, foliar potassium synergistically further supported the physiological and biochemical processes, collectively alleviating the detrimental effects of drought stress in wheat plants.

## 5. Conclusion

This study addressed a critical research gap by investigating the synergistic effects of co-applying biochar as a soil amendment alongside exogenous potassium to sustain wheat productivity under drought conditions. Our findings demonstrated the effectiveness of this combined approach in mitigating drought's adverse effects, particularly during the crown root initiation stage. By strengthening photosynthetic processes and reinforcing oxidative defense mechanisms, the biochar-foliar potassium treatments significantly improved wheat plants' resilience to drought stress. Notably, the application of biochar with foliar potassium significantly enhanced wheat plants' nutritional value, macronutrient levels (N, P, K), and grain yield. The collective action of potassium, an essential nutrient, and biochar, known for enhancing soil physiochemical properties, plausibly modulated various biochemical and physiological processes across different growth stages negatively affected by drought-induced reactive oxygen species accumulation. Throughout various wheat growth stages affected by drought-induced reactive oxygen species (ROS) accumulation, the biochar-foliar potassium treatments substantially reduced $H_2O_2$, MDA, and electrolytic leakage. These reduction of metabolites occurred via the effective scavenging competence facilitated by enhanced antioxidant enzyme activities and increased osmoprotectants content. These actions contributed towards improving the osmotic potential and relative water content of wheat plants, thereby bolstering their drought resilience. Overall, the utilization of biochar and foliar potassium was proven as an effective strategy to enhance wheat resilience and continual grain production in drought-prone regions, addressing the fundamental concerns related to global food security.

## Funding

This work was supported by the Ministry of Science and Technology (MOST), Bangladesh.

## CRediT authorship contribution statement

**Md. Shah Newaz Chowdhury:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Md. Nasir Hossain Sani:** Writing – review & editing, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Abu Bakar Siddique:** Data curation, Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Md. Sazzad Hossain:** Writing – review & editing. **Jean Wan Hong Yong:** Validation, Supervision, Writing – review & editing.

## Declaration of competing interest

The authors declared that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2024.100452.

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