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RESEARCH ARTICLE

Effect of drought stress on physiological, biochemical and antioxidant activity of wheat genotypes at booting stage

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Abstract

Drought is a major abiotic stress affecting wheat (*Triticum aestivum* L.) cultivation, particularly in arid and semi-arid regions. This study was conducted on three wheat genotypes PBW644, WH1080 and PBW175 under controlled conditions at the Division of Plant Physiology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu. The experiment involved withholding irrigation for 10 days during the booting stage, with sampling carried out at the onset of wilting and leaf rolling symptoms. The objective was to investigate drought-induced changes in physiological, biochemical and antioxidant responses and their impact on yield-related traits. PBW644 exhibited superior drought tolerance, maintaining higher chlorophyll stability, water use efficiency and osmotic adjustment (proline and sugar accumulation). Antioxidant activity (SOD and CAT) was significantly upregulated, with PBW644 showing the strongest ROS scavenging capacity. Principal component analysis (PCA) identified key physiological and biochemical traits, including chlorophyll stability index, proline accumulation and antioxidant enzyme activities, as significant contributors to drought tolerance. Strong correlations between these parameters and grain yield highlight the critical role of oxidative stress mitigation and osmotic adjustment in maintaining stability of yield under drought scenarios. These findings emphasize the importance of physiological, biochemical and antioxidant mechanisms in enhancing drought resilience and provide valuable insights for breeding drought-tolerant wheat genotypes to ensure global food security in the face of climate change.

Keywords: antioxidant enzymes**;** drought tolerance; proline; water use efficiency; wheat

Introduction

Wheat (*Triticum aestivum* L.) is a globally essential and strategic cereal crop, serving as the primary staple food for millions of people worldwide. In India, after rice it is the second most important crop, playing a vital role in ensuring food security. Significant strides in wheat productivity have contributed to the country's self-sufficiency in food production (1). As a member of the Poaceae family, wheat is cultivated over 225 million hectares globally, producing approximately 772.64 million tons. In India, wheat cultivation spans 31.5 million hectares, yielding about 108 million tons at a productivity rate of 3.4 t/ha (2).

However, wheat production faces growing challenges due to water scarcity, especially in regions prone to drought. With competing demands for water from industrial, urban and environmental sectors, the availability of water for agricultural purposes is projected to decrease in the coming decades. Furthermore, climate change forecasts predict increasingly erratic rainfall patterns, leading to more frequent and severe droughts (3). Water supply restrictions have an immediate negative impact on the plant's water efficiency. Plants activity is disturbed by drought stress in terms of antioxidant production, protein production, osmotic adjustment, root depth, hormone composition, stomatal movement, cuticle thickness, inhibition of photosynthesis, decrease in chlorophyll content, change in osmotic balance in plant organs reduction in transpiration and growth inhibition. Drought also results in in pollen sterility, grain loss, deposition of abscisic acid in spikes.

Drought activates transcription factors that leads to the abscisic acid production in anthers (4, 5). The involvement of reactive oxygen species (ROS) has been established in several biochemical investigations. Drought stress leads to optimization of ROS, which amends the cell's oxidative balance (6). When ROS levels rise, abscisic acid (ABA) is produced, which acts as a general drought signal and can therefore influence expression of antioxidant gene by forming superoxide dismutase (SOD) and catalase (CAT). As a result, there is a significant economic loss in wheat production all over the world.

It's critical to understand the physiological response of wheat in drought scenarios if you want to improve yield. Under this situation, by understanding the physiological and biochemical responses of wheat, genotypes which are drought

tolerant are to be identified. Understanding these complex responses is essential for developing wheat varieties with enhanced drought resilience. By elucidating the physiological and biochemical strategies employed by wheat under water deficit conditions, researchers can inform breeding programs aimed at improving drought tolerance, safeguarding food security in the face of climate uncertainty.

Materials and Methods

Experimental setup

The root growth and water uptake pattern in different wheat genotypes were studied using large acrylic pipes. The research was executed in the winter growing season of 2021 at the Division of Plant Physiology, Faculty of Basic Sciences, SKUAST Jammu, Chatha, India. Acrylic pipes (62 cm deep, 10 cm wide and 0.3 cm thick) were constructed to simulate a controlled environment for root growth. All pipes were loaded with a soil mixture comprised of soil, composted cattle manure and coarse sand in a 50:30:20 volume ratio. The soil used in the experiment was sourced from a local agricultural field and it was characterized by a loamy texture.

Prior to planting, the soil was saturated with water to ensure sufficient nutrient supply and was allowed to drain for 3 days to prevent waterlogging. This drainage period ensured that the soil reached optimal moisture content conducive to root growth. The area was exposed to natural variations in temperature, humidity and light, with ambient temperature ranging from 20 °C to 32 °C during the experimental period. The natural light cycle was not supplemented and the experiment relied on natural rainfall and environmental factors for the irrigation schedule.

Three wheat genotypes were studied, namely WH1080, PBW175 and PBW644. The seeds of these genotypes were sourced from the Division of Plant Breeding and Genetics, SKUAST, Jammu. Five seeds of each respective wheat genotypes were sown in individual acrylic pipe and the practice of cultural and farming activities were homogenously practised. The experiment was carried out following a two factorial completely randomized design (CRD) with three biological replications. Seed germination occurred approximately after 14 days post-sowing, the three supreme vigorous and spatially aligned seedlings were preserved per container, while the rest were carefully removed to maintain uniformity in plant density.

A total of 18 pots were used in the experiment, comprising 9 pots maintained under well-watered conditions (control) and 9 subjected to drought stress treatment. Drought stress was imposed at the booting stage by withholding water for 10 consecutive days to simulate water-deficit conditions. The pipes were weighed periodically to ensure no additional water was applied during the drought period. Samples were collected after the drought stress exposure at the booting stage for further analysis of physiological and biochemical parameters of wheat genotypes.

Physiological parameters

Water use efficiency (WUE)

Water use efficiency (WUE) can be explained as the ratio of the crop yield to total available water used by the crop (7). It may be defined as the ratio between marketable yield and the total water received by the crop. The WUE Was calculated using the formula of (8).

$$\text{WUE (GperlitreFW)} = \frac{\text{Total grain yield produced by the plants (g)}}{\text{Total water consumed by the plants (L)}}$$

Chlorophyll stability index (CSI)

Chlorophyll extraction was employed by the non-destructive approach, using dimethyl sulfoxide (DMSO) (9). A total of 30 mg of fresh, fully developed young leaf, 3rd from top was taken in a test tube comprising 3 mL of DMSO to determine photosynthetic pigments. These tubes were maintained at room temperature until the tissue became chlorophyll free and become decolourized (12-16 hr). Another batch comprise 30 mg of the above-mentioned leaves were immersed in test tubes containing 10 mL of de-ionized water and heated at 65 °C for 30 min in water bath. Cooled leaves were taken out from the water, dried using blotting paper and immersed in test tubes with 3 mL of DMSO, where they were treated for pigment extraction as described above. After incubation, the extract was transferred to a graduated tube and absorbance was measured at 665, 645 and 454 nm using a computer-aided spectrophotometer (Systronic India Spectrophotometer 117) with a multiple wavelength programme (10). DMSO was used as blank.

Calculations for distinct pigments were determined according to the formulae (11):

Chlorophyll a (mg/g FW) = $(11.75 \times A_{665} - 2.35 \times A_{645}) \times 3/30$

Chlorophyll b (mg/g FW) = $(18.61 \times A_{645} - 3.96 \times A_{665}) \times 3/30$

Carotenoid (mg/g FW) = $[(1000 \times A_{454}) - (2.27 \times \text{chl a}) - (81.4 \times \text{Chl-b})/227] \times 3/30$

Quantities of these pigments were calculated in mg g^{1} tissue fresh weight.

$$\text{Chlorophyll stability index (\%)} = \frac{\text{Total chlorophyll of non heated sample}}{\text{Total chlorophyll of heated sample}} \text{ X } 100$$

Biochemical estimation

Proline content

The concentration of proline was estimated by the method determined by (12). A total of 250 mg of leaf sample was taken and homogenized in the test tube along with 2 mL of glacial acetic acid 2 mL of ninhydrin solution and boiled at 100 °C in a water bath for about 1 hr. The test tube was then placed to an ice bath to stop the reaction. The test tubes were then filled with 4 mL of toluene and homogenised using 10 mL of 2 % sulphosalicylic acid. The 2 mL of extract was separately taken and shift to the separating funnel, where the pink-coloured solution was retrieved from the top layer. Reading of the pink colour solution was taken with spectrophotometer at 520 nm. Simultaneously, the blank was also prepared excluding the leaf extract and the proline content was assessed on fresh weight basis by a method:

µ moles per g tissue = µg proline/mL × mL toluene/115.5 × 5/g sample

Where, 115.5 is the molecular weight of proline.

Total soluble carbohydrates (TSC)

Total soluble sugars were determined by the sulfuric acid-UV method (13). This method is based on the dehydration of carbohydrates with concentrated sulphuric acid, which give furfural derivatives absorbing strongly at 315 nm in the UV region, which obviates the use of hazardous reagents such as phenol and anthrone and colour developing steps. In this method, 1.0 mL of the carbohydrate containing sample is mixed with 3.0 mL of concentrated sulfuric acid, vortexed for 30 sec, cooled in an ice bath for 2 min, the absorbance is read at 315 nm and measured using UV spectrophotometer. A calibration curve is prepared using standard sugar solutions (e.g., glucose) and sample concentrations are derived accordingly. Compared to conventional colorimetric assays, this UV-based method is more sensitive ($R^2 > 0.998$), yields lower error (± 2.9 %), is quicker (≤ 5 min/sample) and avoids hazardous chemicals, making it especially suitable for plant, soil or microbial carbohydrate analyses in high-throughput workflows.

Antioxidant defence system (ADS)

Enzymes assay

Extraction: High temperature plants along with 500 mg of leaves from control were excised, washed chilled distilled water and homogenised in 2 mL of 0.1 M, pH 7.0 extraction buffer (potassium phosphate) with a chilled pestle and mortar. The extract was then centrifuged for 20 min at 10000 X g at 4 °C. The supernatant was carefully decanted and utilised to estimate the following enzymes.

Superoxide dismutase (EC 1.15.1.1): Superoxide dismutase was examined by calculating its ability to block the photochemical reduction of NBT (14). Experimental mixture contained 0, 10, 20, 30 and 40 µL of enzyme extraction in different batches, to which 0.25 mL of NBT, methionine and EDTA were added, for a total volume of 3.0 mL in each set. Each set received 0.25 mL of riboflavin in the last set. The tubes were gently agitated before being positioned 30 cm far from the light source, which consisted of two 15 W fluorescent lamps (Phillips, India). The reaction was terminated after 20 min by switching off the light source. Once the reaction was finished, the tubes were enclosed in black cloth to defend them from light. A non -irradiated experimental solution that did not develop colour, served as the blank. The experimental mixture without enzyme extract produced the optimum colour and its absorbance diminished as the volume of extract rises. The absorbance was monitored at 560 nm. However, in the presence of SOD, the reaction got blocked and the degree of inhibition was utilised to quantify the enzyme. Log A_{560} was plotted as a function of the volume of enzyme extract employed in the reaction mixture. The volume of enzyme extract equivalent to 50 % inhibition of the photochemical reaction was determined from the resulting graph and regarded to be one enzyme unit. The enzyme activity was estimated as [units mg^{-1} (protein) min^{-1}].

Catalase: The activity of catalase was assessed by the UV method (15). In the concluding volume of 3 mL, the reaction mixture composed of 50 µL of cell enzyme extract, 0.1 M phosphate buffer and 10 mM hydrogen peroxide. The reaction was commenced with the addition of hydrogen peroxide and the action of enzyme activity was evaluated by following the deterioration of hydrogen peroxide degradation for 2 min at 240 nm. The enzyme activity was determined by utilizing the extinction coefficient of 39.4 Mm^{-1} cm^{-1}. One nmol of hydrogen peroxide used during the process equivalent to one unit of enzyme activity and estimated as [units mg^{-1} (protein) min^{-1}].

Statistical analysis

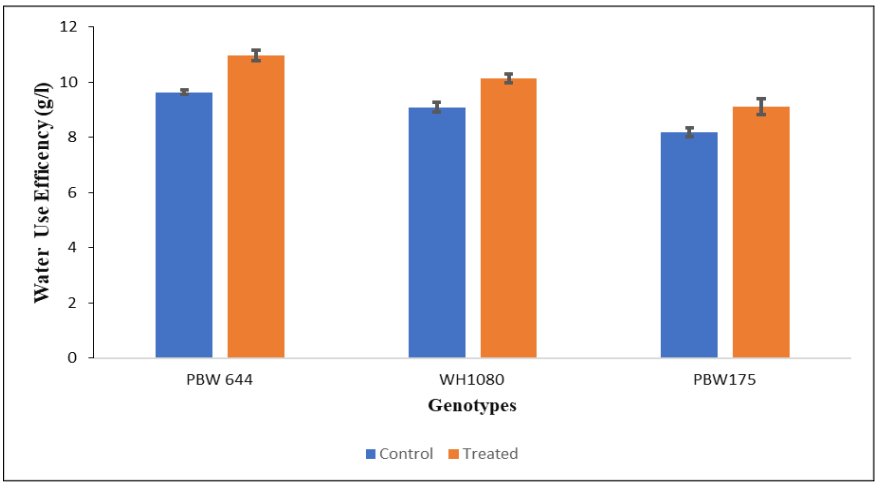
The data collected from the experiments were analyzed using R software (GGplot2) for ANOVA and plotting. Analysis of variance (ANOVA) assessed differences among treatments for root length, grain yield and biomass. Post-*hoc* pairwise evaluation was executed with Tukey's honestly significant difference (HSD) test at a 5 % significance level ($p < 0.05$). Principal component analysis (PCA) was employed to identify key traits contributing to drought tolerance. PCA identified key drought-tolerance traits, while Pearson's correlation analysis assessed relationships between physiological and biochemical traits. Graphs were generated using Microsoft Excel Office 2019.

Results and Discussion

Physiological parameter influenced by drought stress

Water Use efficiency (WUE)

Fig. 1 shows that the mean WUE (g/L) in three wheat genotypes significantly declined from 7.44 to 6.22 with increasing the drought stress at booting stage from control to treated condition. In control condition, the maximum water use efficiency was observed in



CD at 5 %	Genotypes	= 0.42
	Treatment	= 0.34
	Genotype x Treatment	= 0.59

Fig. 1. Changes in water use efficiency (g/L) in wheat genotypes on exposure to drought stress at booting stage.

PBW644 (9.63 g/L) followed by WH1080 (9.10 g/L) and minimum was observed in PBW175 (8.19 g/L). Similarly, in treated conditions, the maximum water use efficiency was observed in PBW644 (10.46 g/L) followed by WH1080 (10.14 g/L) and minimum was observed in PBW175 (9.12 g/L).WUE increased significantly under drought conditions, in agreement with previous findings, suggesting that drought-stressed plants regulate transpiration and photosynthetic rates to minimize water loss (16).

Chlorophyll stability index

Chlorophyll stability index (%) decreased under drought conditions at booting stage in three wheat variants and the values varied from control to treated condition (82.30 % to 61.11 %) as presented in Table 1. The maximum CSI percentage was observed in PBW644 (78.54 %), followed by WH1080 (71.87 %) and minimum was recorded in PBW75 (64.71 %) genotype. Our results align that demonstrated the dehydration stress negatively affects membrane stability, while osmo protectants such as proline help maintain chlorophyll content (17).

Biochemical parameters influenced by drought stress

Total carotenoids

Total carotenoid of the wheat genotypes is depicted in Fig. 2. The stressed leaves showed decline in total carotenoid content over control in wheat leaves. The mean total carotenoid of the wheat genotypes under control was 0.36 mg/g FW and was reduced to 0.26 mg/g FW under drought stress. The maximum mean total carotenoid was observed in PBW644 (0.34 mg/g FW) followed by WH1080 (0.32 mg/g FW) and minimum was found in PBW75 (0.26 mg/g FW) genotype. This reduction is linked to decreased photosynthetic efficiency and stomatal conductance as reported by (18, 19).

Proline

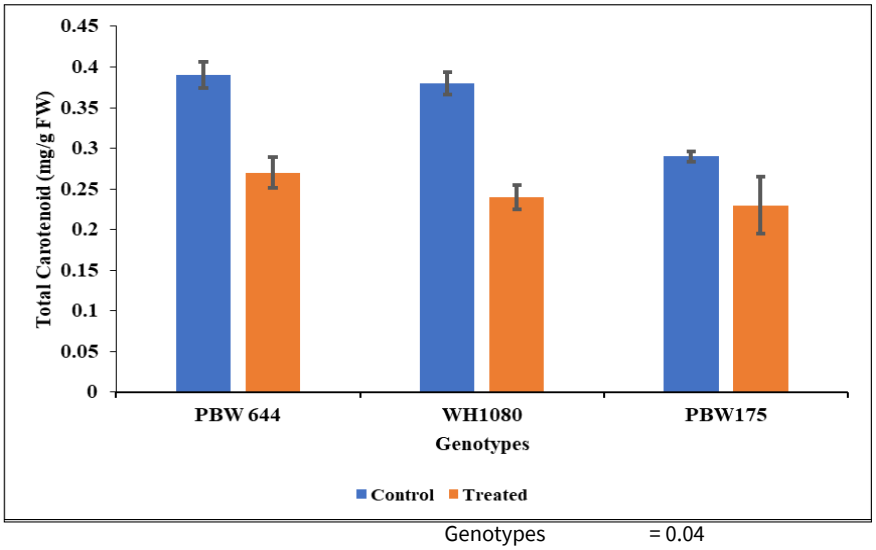
The changes in proline content (µmole/g FW) at booting stage under drought stress in leaves are represented in Fig. 3. The data revealed significant differences in mean proline level of leaves and it escalated from control to treated condition such as, 7.5 µmole/g FW to 16.6 µmole/g FW, respectively. In control condition, the higher level of proline was observed in PBW644 (8.99 µmole/g FW), followed by WH1080 (7.91 µmole/g FW) and lowest mean proline content was observed in PBW175 (7.32 µmole/g FW). Similarly, in treated conditions, the maximum proline content was observed in PBW644 (18.53 µmole/g FW), followed by WH1080 (16.88 µmole/g FW) and lowest mean proline content was observed in PBW175 (15.49 µmole/g FW). Proline accumulation was significantly higher under stress, with PBW644 exhibiting the maximum increase, supporting its role as a key osmolyte for drought adaptation (20, 21).

Total soluble carbohydrates

The variations in the amount of TSC (mg g^{-1}FW) with increase in drought stress in leaves of wheat genotypes are shown in Fig. 4. The data showed significant differences in mean TSC of leaves and it increased from control to treated conditions, 15.22 mg/g FW to 22.57 mg/g FW, respectively. The highest mean TSC was observed in PBW644 (22.11 mg/g FW), followed by WH1080 (18.82 mg/g FW) and lowest mean total soluble carbohydrate was observed in PBW175 (15.77 mg/g FW). The overall interaction of genotypes and drought was found significant. This observation is consistent with studies indicating that sugar accumulation enhances osmotic adjustment and cellular stability under water deficit (22, 23).

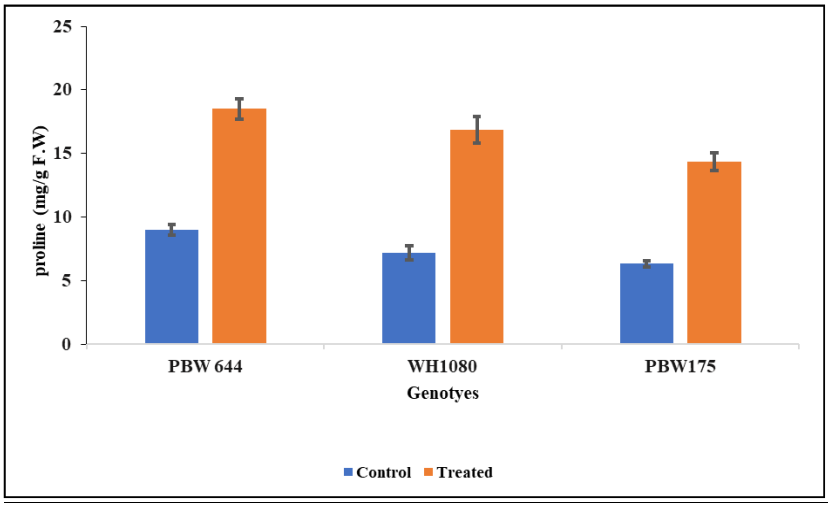
Table 1. Changes in chlorophyll stability index (%) in wheat genotypes on exposure to drought stress at booting stage

	Chlorophyll stability index (%)		
Genotypes	**Control**	**Treated**	**Mean**
PBW 644	88.95 ± 1.92[a]	68.14 ± 2.02[d]	78.54
WH1080	81.84 ± 2.03[b]	61.90 ± 1.53[e]	71.87
PBW175	76.12 ± 2.17[c]	53.31 ± 0.85[f]	64.71
Mean	82.30	61.11	
CD at 5 %		Genotypes = 3.94 Treatment = 3.21 Genotype x Treatment = 5.57	



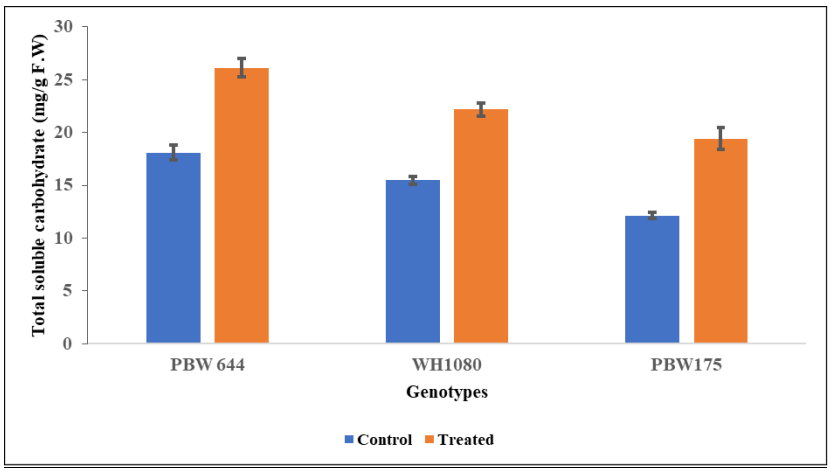
CD at 5 %	Genotypes = 0.04 Treatment = 0.03 Genotype x Treatment = 0.05	

Fig. 2. Changes in total carotenoid (mg/g FW) in wheat genotypes on exposure to drought stress at booting stage.



	Genotypes	= 1.489
CD at 5 %	Treatment	= 1.215
	Genotype x Treatment	= 2.105

Fig. 3. Changes in proline (µmole/g FW) in wheat genotypes on exposure to drought stress at booting stage.



	Genotypes	= 1.53
CD at 5 %	Treatment	= 1.25
	Genotype x Treatment	= 2.17

Fig. 4. Changes in total soluble carbohydrates (mg/g FW) in wheat genotypes on exposure to drought stress at booting stage.

Antioxidant enzyme activity influenced by drought stress

Superoxide dismutase

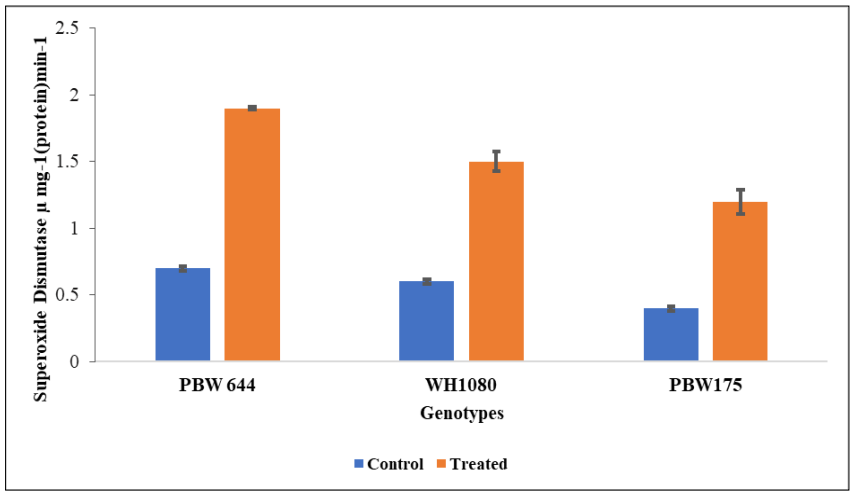
The specific activity of SOD [unit's min^{-1} mg^{-1} (protein) min^{-1}] was found to be elevate significantly under drought stress at booting stage in all three wheat variants. The activity of SOD increased from 0.56 to 1.53 from control to treated condition respectively. Fig. 5 shows that the mean maximum value of SOD activity observed in PBW644 (1.30), followed by WH1080 (1.05) and lowest was found in PBW175 (0.80). However, SOD activity in genotypes and drought stress were statistically significant.

Catalase

The effect of drought stress on catalase activity [unit's mg^{-1} (protein) min^{-1}] is presented in Fig. 6. Drought stress results in the high level of catalase activity in leaves of all three wheat genotypes and the activity values varied from 1.43 to 3.09 from control to treated conditions. PBW644 (2.66) showed maximum activity, followed by WH1080 (2.28) and minimum was observed in PBW175 (1.85) genotypes. The values of catalase between genotypes and drought stress were statistically significant. Enhanced SOD and CAT activities have been reported as key indicators of stress tolerance, as they help neutralize ROS and protect cellular components (24). The ability of wheat genotypes to sustain high antioxidant enzyme activities correlates with their resilience to oxidative stress, further supporting their role in drought tolerance (25).

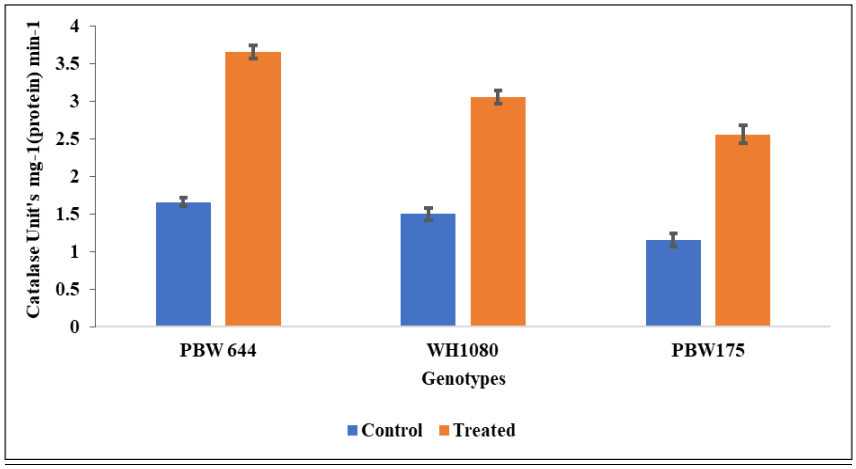
Principal component analysis (PCA)

In this study, PCA analysis revealed significant variation among wheat genotypes under control and drought stress, with PC1 (82.29 %) and PC2 (12.95 %) explaining 95.24 % of the total variance (Fig. 7). Key traits influencing genotype separation included WUE, proline, antioxidant enzymes (SOD, catalase) and carotenoids. Control samples clustered together, while treated samples shifted, indicating metabolic changes. PBW644 clusters separately, suggesting superior drought adaptation through enhanced osmotic adjustment and antioxidant defense. Proline, catalase and SOD play a significant role in stress tolerance, consistent with previous studies (26). The tighter grouping of control samples suggests metabolic stability in unstressed conditions. WH1080 and PBW175 show intermediate shifts, indicating moderate drought resilience. The strong influence of WUE on PC1 highlights its importance in stress adaptation (27).



	Genotypes	=0.10
CD at 5 %	Treatment	= 0.08
	Genotype x Treatment	= 0.15

Fig. 5. Changes in superoxide dismutase [U mg^{-1} (protein) min^{-1}] in wheat genotypes on exposure to drought stress at booting stage.



	Genotypes	=0.19
CD at 5 %	Treatment	= 0.15
	Genotype x Treatment	= 0.27

Fig. 6. Changes in catalase [U mg^{-1}(protein) min^{-1}] in wheat genotypes on exposure to drought stress at booting stage.

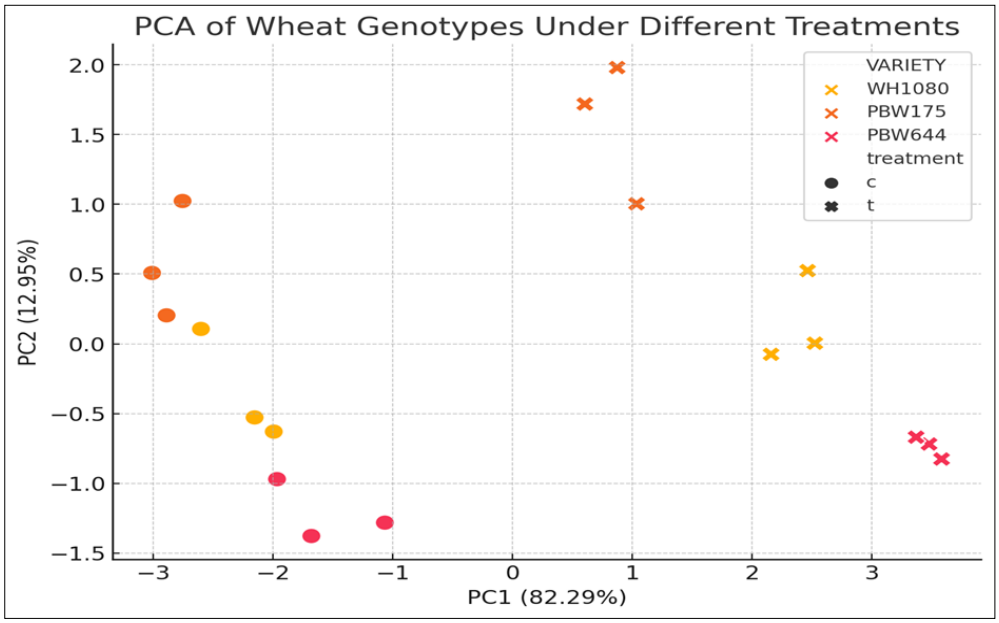


Fig. 7. Principal component analysis (PCA) biplot illustrating the clustering of wheat genotypes under control (c) and drought-stressed (t) conditions.

[PC1 and PC2 together explain 95.24 % of the total variance. Different colours represent wheat genotypes (WH1080, PBW175 and PBW644), while marker style indicates treatment conditions.]

Antioxidant enzyme activity contributes significantly to genotype differentiation, reinforcing its role in drought mitigation (28). These findings align with previous reports that emphasize the role of osmolytes and ROS scavengers in drought tolerance (29).

Pearson correlation heatmap

Pearson correlation analysis revealed strong positive associations among biochemical traits under drought stress. TSC correlated highly with catalase (r = 0.96) and SOD (r = 0.95), linking carbohydrate accumulation with oxidative stress regulation. SOD and catalase (r = 0.99) showed a close antioxidant defence relationship. WUE correlated with carotenoids (r = 0.82) and TSC (r = 0.80), highlighting its role in biochemical responses. Proline was strongly linked to SOD (r = 0.95) and catalase (r = 0.94), indicating its role in stress tolerance. CSI showed a negative correlation with TSC (r = -0.61), proline (r = -0.73) and carotenoids (r = -0.43), suggesting that reduced chlorophyll stability triggers protective biochemical accumulation. The Pearson correlation matrix highlights key biochemical interactions under drought stress, emphasizing their roles in stress adaptation (Fig. 8). WUE shows a strong positive correlation with SOD (r = 0.80) and catalase (r = 0.81), indicating its link to oxidative stress mitigation (30, 31).

Conclusion

This study highlights the physiological and biochemical adaptations of wheat genotypes under drought stress, providing valuable insights into drought tolerance mechanisms. Increased WUE and CSI percentage tolerant genotypes suggest efficient water conservation and membrane stability under stress. The decline in carotenoid content and the significant accumulation of proline and TSC further indicate adaptive responses that enhance osmotic adjustment and cellular protection. The upregulation of antioxidant enzymes, particularly SOD and CAT, underscores their critical role in mitigating oxidative damage and improving drought resilience. Among the genotypes, PBW644 exhibited superior drought tolerance due to its higher proline accumulation, TSC content and antioxidant enzyme activities. Overall, our findings emphasize the implication of biochemical and physiological traits in contributing drought tolerance in wheat. These traits can serve as key selection criteria for breeding drought-resistant cultivars, ensuring sustainable wheat production under water-limited conditions.

Authors' contributions

The field research was executed and the manuscript prepared by MK, while GC formulated the research proposal and contributed to reviewing and manuscript editing. DS provided guidance on agronomic aspects for conducting the research and BKS contributed by outlining the research article and reviewing it. SD guided on picture representation, whereas CT assisted with checking and English editing. FK and MS provided guidance on checking, with MS also contributing to reviewing. JK carried out the data analysis and NT was responsible for the final checking and drafting of the paper.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

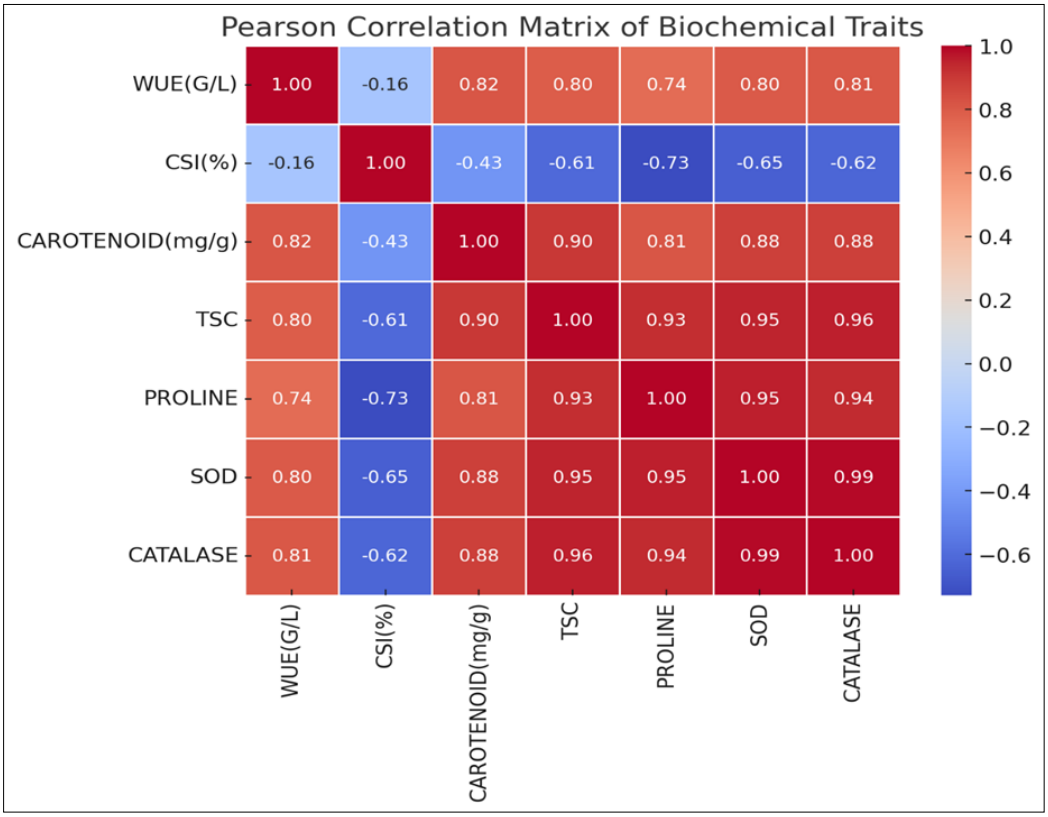


Fig. 8. Pearson correlation matrix heatmap depicting the relationships between biochemical and physiological and antioxidant traits in wheat genotypes under control and drought stress conditions.

[Positive correlations are shown in red and negative correlations are in blue, with colour intensity indicating correlation strength.]

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