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EVALUATION OF POTENTIALLY HIGH YIELDING MODERN WHEAT CULTIVARS FOR DROUGHT TOLERANCE USING KEY PHYSIO-BIOCHEMICAL AND MORPHOLOGICAL INDICATORS

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Abstract

Wheat is the main trading crop, driving food security and the worldwide economy. Severe drought stress is a chief threat to wheat productivity in existing climatic variations. Screening of wheat cultivars based on metabolic strategies to tolerate drought stress is crucial to ensure better yield. Current research work is carried out to evaluate drought-sensitive and tolerant wheat cultivars based on physio-biochemical and morphological stress indices. A pot experiment was organized according to factorial CRD with four replications. Ten latest high-yielding wheat cultivars were selected, and seeds were sown in pots having loamy soil and divided into two treatment portions. One was treated with control (NoDS) conditions, and the other with severe drought conditions (DS). Drought stress caused a significant decline in fresh and dry weight of roots and shoots, length of roots and shoots, leaf area and chlorophyll contents. However, enhanced production of reactive oxygen species (H_2O_2) and lipid peroxidation (MDA) ultimately triggered enzymatic antioxidant (SOD, POD, CAT) activities. All wheat cultivars exhibited osmotic modification by accumulating total soluble proteins (TSP) and free amino acids (FAA). According to our findings, Akber-19 performed better under control (NoDS) conditions among all cultivars. However, it is quite sensitive under severe drought (DS) stress. In contrast, Fakhar-e-Bhakkar significantly maintained osmotic and antioxidative balance under drought stress (DS) and exhibited more tolerant behaviour than other cultivars at the seedling stage.

Key words: Drought stress; Wheat; Total soluble proteins; Amino acids; Antioxidants.

Introduction

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Drought stress, one of the leading abiotic stresses, contributes to a massive loss in crop yield all over the globe. It is a climatological cycle without substantial rainfall (Atta *et al*., 2022). Drought affects plant metabolism directly or indirectly by altering plant physiology and biochemical setup because of oxidative damage that possibly causes cell death (Bijalwan *et al*., 2022). Drought stress reduces crop productivity (Lalarukh *et al*., 2014; Siddiqui *et al*., 2017) as well as seed quality (Parkash & Singh, 2020) because of its high influence on plant's physiology, morphology, and biochemistry (Havrlentova *et al*., 2021). As one of the major ecological factors, drought impacts approximately all stages of plant growth (Hasanuzzaman *et al*., 2018). However, it severely affects the most vital stage, germination (Ullah *et al*., 2019). Drought most frequent and harmful impact on plants is a decline in dry and fresh biomass (Farooq *et al*., 2009). Shrinkage of leaf area, increase in leaf thickness, and root system enlargement all is linked with adaptive responses (Abobatta, 2019). Another photosynthetic parameter is chlorophyll content, which is strongly influenced by drought stress (Gregorova *et al*., 2015; Alghbari & Ihsan, 2018), especially chlorophyll production in leaf and the proportion of chlorophyll a/b are changed under drought.

During osmotic adjustment, plants also accumulate and integrate different compatible solutes, i.e., sugars, proline, and free amino acids (Lalarukh & Shahbaz, 2020b). Different osmotic metabolites, solutes, and free sugars maintain plant growth through their osmolytic compatibility under drought stress (Thalmann & Santelia, 2017). A rise in soluble-protein contents was also detected under drought stress (Mohammadkhani & Heidari, 2008), and this could be credited to the greater production of amino-acids that occurred in response to drought stress (Jin *et al*., 2016; Ozturk *et al*., 2021).

Drought stress alters the concentration of reactive oxygen species (ROS), hence, the activity of peroxidase, superoxide dismutase, and catalase in wheat both in laboratory and field environments (Caverzan *et al*., 2016; Jiang *et al*., 2019). In drought stress, plants ' most familiar and functional characteristic is the elevation in ROS scavenging enzymes (CAT, SOD, and others) due to changes in cellular metabolism and oxidative injury (Havrlentova *et al*., 2021). Drought stress activates ROS production and disturbs the balance between ROS production and the scavenging system. ROS accumulation depends on duration, stress intensity, and species type. A genetically tolerant plant species rapidly executes its antioxidant defence system and drought-specific genes (Atta *et al*., 2022). The superoxide dismutase (SOD) enzyme is considered a significant component of the plant defence system under oxidative stress because it controls superoxide and hydrogen-peroxide concentration in cells (Sharma *et al*., 2012). The action of peroxidase and superoxide-dismutase enhanced drought stress (Hassanpour & Niknam, 2014). Increases in root shoot ratio, biochemical dynamics of stomatal closure, osmotic modification, solute buildup, and antioxidant defence strategy are general methods that help drought resistance (Lopez-Galiano *et al*., 2019). Under drought, morphological characters like plant height, root length (RL), shoot length (SL), their fresh (FW) and dry weight (DW), chlorophyll *a* & *b*, carotenoids, and total chlorophyll declined. In contrast, wheat's values of proline, peroxidase, superoxide dismutase, catalase, and malondialdehyde increased (Adl *et al*., 2020).

Screening wheat cultivars for drought tolerance is essential to enhance wheat output, and that can only be achieved by exploring the latest wheat cultivars. Information about associated seedling traits under drought is also essential to understand those factors that limit wheat yield. So, the current study was planned to screen the highly tolerant cultivar under drought among largely cultivated wheat cultivars.

Material and Methods

Experimental area and seeds collection: Research was arranged in the years 2020 and 2021 in the experimental area of the Department of Botany at Government College Women University Faisalabad to assess the impact of drought stress on wheat. Seeds of ten wheat cultivars (i.e., Fsd-08, Ujala-16, Anaj-17, Akber-19, Ghazi-19, Dilkash-20, Subhani-21, MH-21, Arooj-22 and Fakhar-e-Bhakkar) were collected from Wheat Research Institute (AARI).

Experimental layout and field capacity determination: Plastic pots having 1 kg soil capacity were selected and filled with nutrient-rich soil. All pots were organized in a completely randomized fashion with four repetitions. After that soil was thoroughly watered and left for 48 hours, to determine field capacity. After 48 hours, three soil samples were taken from three different pots separately. We weighed the fresh soil samples and kept them in the oven at 105°C for 48 hours to let the soil completely dry and lose all moisture contents. After 48 hours, 100g dry weight of soil was taken from each sample collectively. Then distilled water was added gradually to the oven-dried soil sample to make a smooth, saturated paste, and saturation percentage was noted. The field capacity was calculated using the formula (Nachabe, 1998). Two levels of field capacity (FC) were selected as treatments, the first with 100% FC labeled as control and the second with 35% FC labeled as drought stress.

Seed sowing and sample collection: After determining field capacity, seeds of each wheat cultivar were planted in the pots (10 seeds per pot). Drought stress (100% FC and 35% FC) was maintained from the beginning of the experiment till the end. After 20 days of germination, seedling samples were collected. Four seedlings of equal size were taken from all pots for the morphological parameters and preserved at low temperatures for physiological attributes.

Morphological attributes determination: Shoot and root fresh weights (g) were determined through electric balance after uprooting plant samples carefully. The samples were placed in paper envelopes for dry weights of shoot and root and kept in an oven at 70°C for 48 hours (Al-Karaki, 2000). Shoot and root lengths (cm) were noted manually by scale, number of leaves was counted, and leaf area (m^2) was computed (Aldesuquy *et al*., 2014).

Chlorophyll pigments determination: Chlorophyll contents (Chl. *a*, and Chl. *b*) were analyzed by Arnon (1949) technique. For that objective, 0.1g fresh leaf samples were chopped and extracted in 5 mL acetone (80%) overnight, then optical density was noted at 480, 663, and 645 nm operating a UV-VIS double beam spectrophotometer (Halo DB-20).

Biochemical analysis: For antioxidant enzyme activity determination, enzyme extract was prepared for each replicate by grinding 0.1g plant leaf in 2mL 50mM phosphate buffer (7.8 pH) at low temperature (4°C) in prechilled pestle and mortar. After that centrifuged the homogenized grinded mixture for 10 minutes at 13000rpm. The further resultant supernatant was preserved in separate eppendorf at -20°C to determine the different activities of antioxidant enzymes (POD, CAT, and SOD). This evaluation was done on a protein basis.

Chance & Maehly (1955) method was applied to find out the activities of catalase and peroxidase enzymes. For catalase, first, one mL H_2O_2 was added in a cuvette; after that, 1.9 mL phosphate buffer (5.9mM; 7.8 pH) was combined with it; for the starting reaction 0.1 mL enzyme extract was added in the end. The optical density of the above combination was noted every 20 seconds at 240nm to calculate catalase activity. For peroxidase, first prepared a mixture of 100µL H_2O_2 (40mM), 100µL guaiacol (20mM), 750µL phosphate buffer in a cuvette, then 100µL enzyme extract was introduced to begin the reaction. Changes in optical density were noted at 470nm every 30 seconds for 3 minutes to calculate peroxidase activity. 0.01 units/minute change in optical density was equivalent to 1 unit of peroxidase and catalase activity. The superoxide-dismutase activity was estimated by the protocol of Giannopolitis & Ries (1977). Reaction blends for SOD confined 100µL H_2O_2, 250µL phosphate buffer, 100µL L-methionine, 100µL triton-X, 50µL NBT, 50µL riboflavin and 50µL enzyme extract. Cuvettes containing the reaction mixture were arranged below a white beam (fluorescent lamp) for 15 minutes. After that optical density of the mixture was seen at 560nm. One unit SOD activity was equal to the quantity of enzyme that caused 50% inhibition in photoreduction of NBT.

Protocol by Velikova *et al*., (2000) was applied for the estimation of hydrogen peroxide (H_2O_2). Initially, 0.5g freshly ground leaf was mixed with 5mL Trichloroacetic acid (0.1% w/v) and centrifugated at 12000rpm for fifteen minutes. Later 0.5mL phosphate buffer (pH 7.8) and 1mL KI were mixed with 0.5mL supernatant in a test tube, the mixture was vortexed, and absorbance was checked at 390nm.

The protocol was followed to determine leaf malondialdehyde content (Carmak & Horst, 1991). After homogenizing 10 mL (0.1% w/v) TCA and 0.5g fresh leaf, the mixture was centrifuged at 12000rpm for ten minutes. Thiobarbituric acid (0.5%) was prepared in 20% TCA, 4mL of it was introduced in 1mL supernatant and put in a water bath for 30 minutes at 95°C. Optical density was noted at 532nm and 600nm after cooling in ice.

Total soluble proteins were measured by Bradford's (1976) protocol. Once homogenizing fresh plant leaf (0.1g) with 2mL phosphate buffer (7.8 pH) in pestle mortar, the supernatant was collected after centrifuging the above mixture for 10 minutes at 13000rpm. Further, 0.1 mL supernatant and 5mL Bradford's reagent were mixed in test tubes, vortexed for a few seconds, and rested for 30 minutes. Reading was noted at 595nm using a spectrophotometer.

Total amino acids were quantified by (Van-Slyke *et al*., 1943) approach. Material of fresh plant leaf was extracted in (7.8pH) phosphate buffer. After that 3mL solution was prepared by combining 1mL extract, 1mL pyridine (10%), and 1mL (2%) ninhydrin and heated in a steaming water bath for 30 minutes. 50mL purified water was included to retain the quantity. The optical density of the colored solution was noted with the help of a spectrophotometer at 570nm.

Statistical Analysis

The final data outcome was analyzed for statistical analysis by OriginPro 2021 software. The variance evaluation was done to evaluate the impacts of drought, cultivar, and their interaction on different traits. Each factor's significance was described, corresponding to P-values. ***, ** and * presented significance levels corresponding to $p<0.001$, $p<0.01$ and $p<0.05$. The least significant difference (LSD) analysis ($p<0.05$) was applied for means comparison.

Results

Shoot fresh weight: Under NoDS conditions, the varieties performed as follows in terms of shoot fresh weight (from highest to lowest mean value): Akber-19 (0.338 g/plant), MH-21 (0.295 g/plant), Fakhar-e-Bhakkar (0.305 g/plant), Ujala-16 (0.240 g/plant), Urooj-22 (0.268 g/plant), FSD-08 (0.268 g/plant), Anaj-17 (0.265 g/plant), Subhani-21 (0.278 g/plant), Ghazi-19 (0.185 g/plant), and Dilkash-20 (0.155 g/plant). However, the shoot fresh weight decreased significantly for all varieties under DS conditions compared to the NoDS condition. The percentage changes for shoot fresh weight (compared to NoDS) were as follows (from highest decrease to lowest): Akber-19 (-48.15%), Ujala-16 (-42.71%), Anaj-17 (-38.68%), MH-21 (-38.14%), Ghazi-19 (-40.54%), Urooj-22 (-40.19%), Dilkash-20 (-35.48%), Subhani-21 (-29.73%), Fakhar-e-Bhakkar (-24.59%), and FSD-08 (-31.78%). Based on these results, the variety Fakhar-e-Bhakkar exhibited the least decrease in shoot fresh weight under DS conditions (-24.59%), indicating better tolerance to drought stress. On the other hand, the variety Akber-19 showed the highest decrease in shoot fresh weight (-48.15%), indicating poor tolerance to drought stress (Fig. 1-A).

Shoot dry weight: Results showed that the varieties exhibited differences in shoot dry weight, with Akber-19 (0.048 g/plant) displaying the highest mean value, followed by Fakhar-e-Bhakkar (0.044 g/plant), Anaj-17 (0.039 g/plant), Subhani-21 (0.039 g/plant), Urooj-22 (0.038 g/plant), Ujala-16 (0.032 g/plant), Ghazi-19 (0.032 g/plant), FSD-08 (0.030 g/plant), Dilkash-20 (0.029 g/plant), and MH-21 (0.027 g/plant) under optimal NoDS conditions. However, when subjected to DS conditions, all varieties experienced a reduction in shoot dry weight compared to the NoDS condition. Varieties exhibiting the highest reduction in shoot dry weight under DS conditions were Akber-19 (-49.21%), Urooj-22 (-46.67%), FSD-08 (-37.50%), Ujala-16 (-37.50%), and Fakhar-e-Bhakkar (-35.03%). Varieties with relatively lower reductions were Subhani-21 (-31.82%), Anaj-17 (-29.68%), Dilkash-20 (-24.79%), Ghazi-19 (-20.93%), and MH-21 (-2.75%). The results indicate that drought stress negatively affected all varieties, as evidenced by decreased shoot dry weights. Akber-19 exhibited the most pronounced reduction among the varieties, signifying its limited ability to tolerate drought stress. Conversely, MH-21 demonstrated the least reduction in shoot dry weight, suggesting greater resilience to drought stress (Fig. 1-B).

Root fresh weight: In case of NoDS conditions, the varieties exhibited varying levels of root fresh weight, with Akber-19 (0.039 g/plant) having the highest mean value, followed by Anaj-17 (0.033 g/plant), Fakhar-e-Bhakkar (0.023 g/plant), Ghazi-19 (0.028 g/plant), Subhani-21 (0.026 g/plant), FSD-08 (0.027 g/plant), Ujala-16 (0.031 g/plant), Dilkash-20 (0.025 g/plant), Urooj-22 (0.023 g/plant), and MH-21 (0.023 g/plant). However, when exposed to DS conditions, there were variations in the response of the varieties regarding root fresh weight. Some varieties showed an increase in root fresh weight under DS conditions compared to NoDS, while others exhibited a decrease. Notably, Fakhar-e-Bhakkar demonstrated the most significant increase in root fresh weight (+58.70%), indicating its ability to adapt well to drought stress. On the other hand, Akber-19 exhibited a notable decrease in root fresh weight (-25.00%), suggesting its susceptibility to drought stress. These findings underscore the importance of selecting appropriate cultivated varieties under DS conditions. Varieties such as Fakhar-e-Bhakkar, which can maintain or increase root fresh weight, may prove valuable in mitigating the adverse effects of drought stress on crop productivity. Conversely, caution should be exercised when considering cultivating varieties like Akber-19, which showed reduced root fresh weight under DS conditions (Fig. 1-C).

Root dry weight: The results revealed distinct variations in root dry weight among the tested varieties under NoDS conditions. Akber-19 exhibited the highest mean root dry weight (0.024 g/plant), followed by Fakhar-e-Bhakkar (0.018 g/plant), MH-21 (0.017 g/plant), Urooj-22 (0.015 g/plant), Ujala-16 (0.016 g/plant), FSD-08 (0.014 g/plant), Anaj-17 (0.013 g/plant), Subhani-21 (0.012 g/plant), Dilkash-20 (0.012 g/plant), and Ghazi-19 (0.009 g/plant). Under DS conditions, the varieties displayed varied responses in root dry weight compared to NoDS conditions. Notably, some varieties showed an increase in root dry weight, with Ghazi-19 exhibiting the most significant increase of 89.19%. Subhani-21 (60.87%), Dilkash-20 (58.70%), Anaj-17 (41.18%), and Fakhar-e-Bhakkar (37.14%) also experienced notable increases in root dry weight under DS. Conversely, Akber-19 (-31.96%), Urooj-22 (-23.73%), and Ujala-16 (-12.31%) demonstrated reductions in root dry weight (Fig. 1-D).

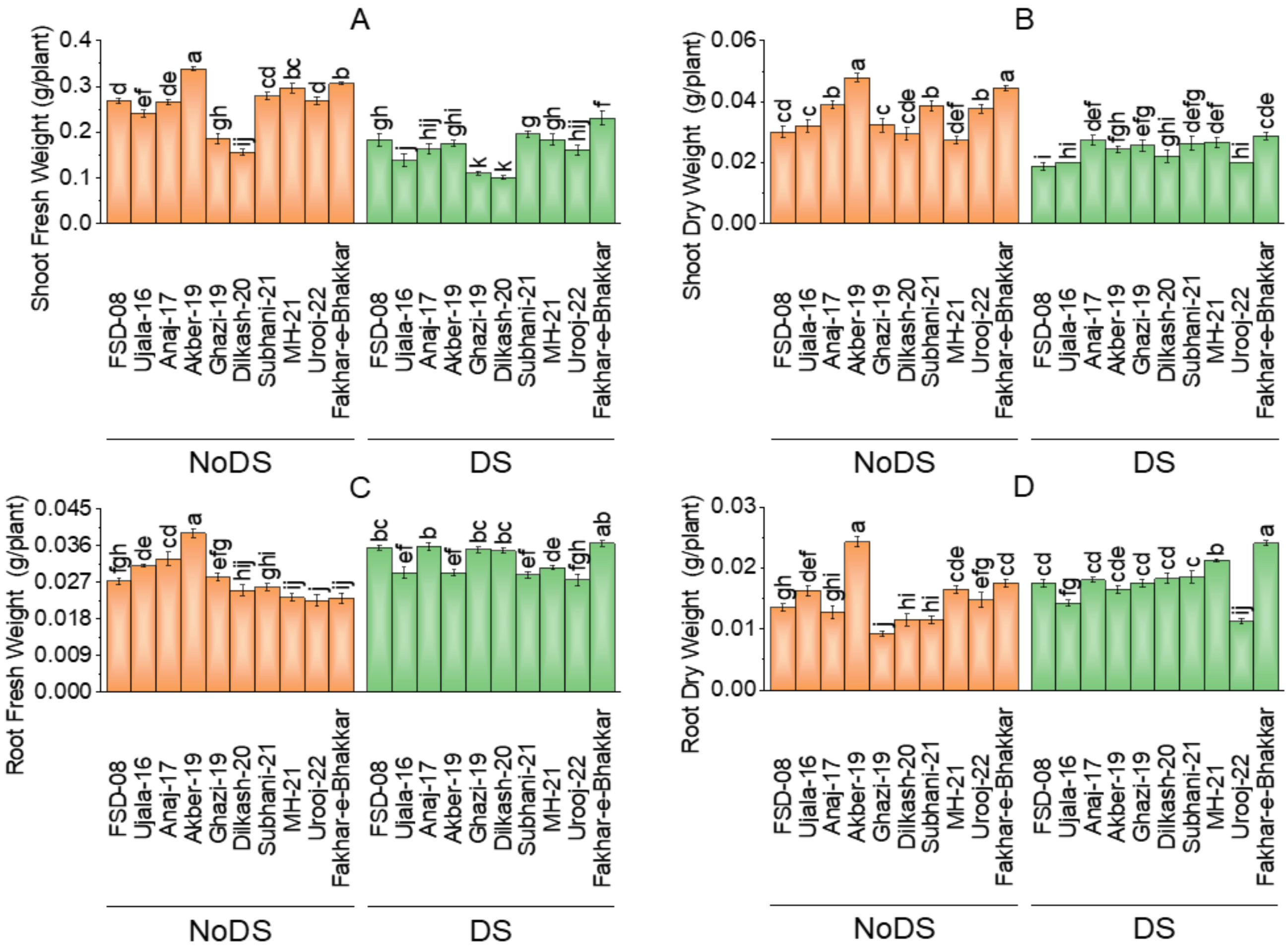

Fig. 1. Influence of control (NoDS) and drought stress (DS) on shoot fresh weight (A), shoot dry weight (B), root fresh weight (C) and root dry weight (D) of 10 wheat cultivars. Bars indicate mean ± SE (n=4); compared using Fisher's LSD at significance $p \leq 0.05$.

Shoot length: Under NoDS conditions, the varieties performed as follows in terms of shoot length (from highest to lowest mean value): Akber-19 (33.75 cm), Ghazi-19 (28.75 cm), Subhani-21 (28.38 cm), Anaaj-17 (28.12 cm), MH-21 (28 cm), Urooj-22 (27.75 cm), Fakhar e Bhakkar (26.25 cm), Dilkash-20 (25.75 cm), Ujala-16 (25.45 cm), and FSD-08 (23.75 cm). However, the shoot length decreased significantly for all varieties under DS conditions compared to the NoDS condition. The percentage changes for shoot length (compared to NoDS) were as follows (from highest decrease to lowest): Akber-19 (-35.78%), Ghazi-19 (-31.91%), Subhani-21 (-22.48%), MH-21 (-22.05%), Anaj-17 (-20.8%), Urooj-22 (-19.37%), Ujala-16 (-16.79%), FSD-08 (-15.79%), Dilkash-20 (-12.04%), Fakhar-e-Bhakkar (-6.67%). Based on these results, the variety Fakhar-e-Bhakkar exhibited the least decrease in shoot length under DS conditions (-6.67%), indicating better tolerance to drought stress. On the other hand, the variety Akber-19 showed the highest decrease in shoot length (-35.78%), indicating poor tolerance to drought stress (Fig. 2-A).

Root length: Results showed that the varieties exhibited differences in root length, with Akber-19 (31.12 cm) displaying the highest mean value, followed by Fakhar-e-Bhakkar (30 cm), Subhani-21 (28.5 cm), Anaj-17 (27.87 cm), MH-21 (27.75 cm), Urooj-22 (27.12 cm), Dilkash-20 (26.12 cm), Ujala-16 (25.75 cm), FSD-08 (25.57 cm) and Ghazi-19 (24.75 cm) under optimal NoDS conditions. However, when subjected to DS conditions, all varieties experienced a reduction in root length compared to the NoDS condition. Varieties exhibiting the highest reduction in root length under DS conditions were Akber-19 (-28.91%), FSD-08 (-21.79%), MH-21 (-19.82%) Ghazi-19 (-16.97%), Ujala-16 (-13.59%) and Urooj-22 (-13.36%). Varieties with relatively lower reductions were Subhani-21 (-12.28%), Anaj-17 (-11.21%), Dilkash-20 (-10.05%) and Fakhar-e-Bhakkar (-7.08%). The results indicate that drought stress negatively affected all varieties, as evidenced by decreased root length. Akber-19 exhibited the most pronounced reduction among the varieties, signifying its limited ability to tolerate drought stress. Conversely, Fakhar-e-Bhakkar demonstrated the least reduction in root length, suggesting greater resilience to drought stress (Fig. 2-B).

Leaf area: In case of NoDS conditions, the varieties exhibited varying levels of leaf area, with Akber-19 (6.58 mm^2) having the highest mean value, followed by Fakhar-e-Bhakkar (6.57 mm^2), Subhani-21 (6.16 mm^2), MH-21 (5.77 mm^2), Anaj-17 (5.62 mm^2), Urooj-22 (5.60 mm^2), Ghazi-19 (5.29 mm^2), Dilkash-20 (4.86 mm^2), FSD-08 (4.05 mm^2) and Ujala-16 (3.85 mm^2). However, when subjected to DS conditions, all varieties experienced a reduction in leaf area compared to the NoDS condition. Varieties exhibiting the highest reduction in leaf area under DS conditions were Akber-19 (-40.32%), FSD-08 (-39.45%), Ghazi-19 (-37.81%), MH-21 (-34.67%) Urooj-22

(-30.49%). Anaj-17 (-29.14%), Subhani-21 (-28.21%), Ujala-16 (-27.82%) Dilkash-20 (-20.46%) and Fakhar-e-Bhakkar (-8.81%). The results indicate that drought stress negatively affected all varieties, as evidenced by decreased leaf area values. Akber-19 exhibited the most pronounced reduction among the varieties, signifying its limited ability to tolerate drought stress. Conversely, Fakhar-e-Bhakkar demonstrated the least reduction in leaf area, suggesting greater resilience to drought stress (Fig. 2-C).

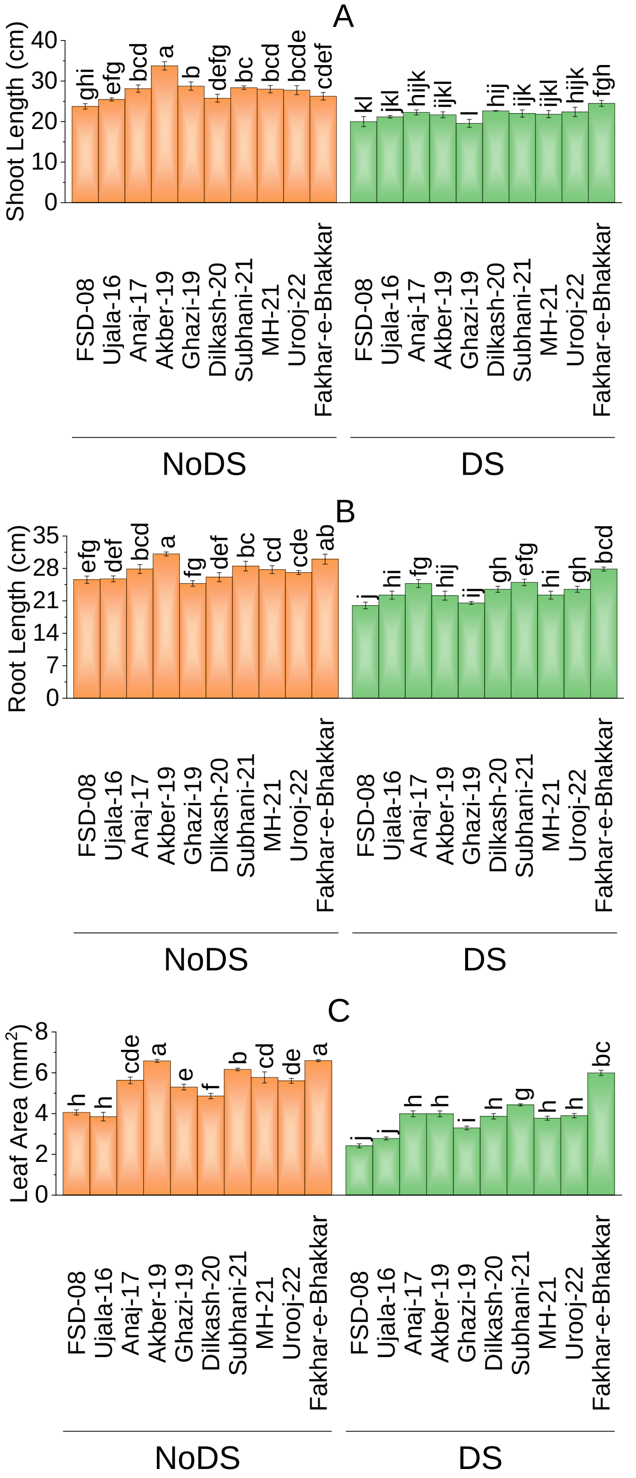

Fig. 2. Influence of control (NoDS) and drought stress (DS) on shoot length (A), root length (B), leaf area (C) of 10 wheat cultivars. Bars indicate mean ± SE (n=4); compared using Fisher's LSD at significance $p \leq 0.05$.

Chlorophyll a: The results revealed distinct variations in chlorophyll-a value among the tested varieties under NoDS conditions. Akber-19 exhibited the highest mean chlorophyll a value (3.47 mg/g FW), followed by FSD-08 (3.33 mg/g FW), Ujala-16 (3.26 mg/g FW), Fakhar e Bhakkar (3.20 mg/g FW), Anaj-17 (3.17 mg/g FW), Urooj-22 (3.13 mg/g FW), Subhani-21 (3.06 mg/g FW), MH-21 (2.94 mg/g FW), Ghazi-19 (2.56 mg/g FW) and Dilkash-20 (2.36 mg/g FW). However, the chlorophyll-a content decreased significantly for all varieties under DS conditions compared to the NoDS condition. The percentage changes for chlorophyll-a (compared to NoDS) were as follows (from highest decrease to lowest): Akber-19 (-18.17%), Ghazi-19 (-9.78%), FSD-08 (-9.2%), Anaj-17 (-7.76%), Ujala-16 (-7.69%), Dilkash-20 (-6.14%), MH-21 (-5.5%), Subhani-21 (-4.49%), Urooj-22 (-4.46%) and Fakhar-e-Bhakkar (-3.46%). Based on these results, the variety Fakhar-e-Bhakkar exhibited the least decrease in chlorophyll-a values under DS conditions (-3.46%), indicating better tolerance to drought stress. On the other hand, the variety Akber-19 showed the highest decrease in chlorophyll-a (-18.17%), indicating poor tolerance to drought stress (Fig. 3-A).

Chlorophyll b: Under NoDS conditions, the varieties performed as follows in terms of chlorophyll-b (from highest to lowest mean value): Akber-19 (2.51 mg/g FW), Fakhar e Bhakkar (2.35 mg/g FW), Ujala-16 (2.14 mg/g FW), Subhani-21 (2.03 mg/g FW), Anaj-17 (1.91 mg/g FW), FSD-08 (1.8 mg/g FW). MH-21 (1.63 mg/g FW), Ghazi-19 (1.46 mg/g FW), Urooj-22 (1.39 mg/g FW) and Dilkash-20 (1.02 mg/g FW). Under DS conditions, the varieties displayed varied responses in chlorophyll-b contents compared to NoDS conditions. The percentage changes for chlorophyll-b (compared to NoDS) were as follows (from highest decrease to lowest): Subhani-21 (-30.06%), Ghazi-19 (-26.73%), MH-21 (-26.63%), Dilkash-20 (-22.74%), Akber-19 (-22.38%), Anaj-17 (-21.91%), Urooj-22 (-21.13%), FSD-08 (-16.4%), Ujala-16 (-14.39%) and Fakhar-e-Bhakkar (-6.31%) (Fig. 3-B). Based on these results, Fakhar-e-Bhakkar exhibited the least decrease in chlorophyll-b under DS conditions (-6.31%), indicating better tolerance to drought stress. On the other hand, the variety Subhani-21 showed the highest decrease in chlorophyll b (-30.06%) indicating poor tolerance to drought stress.

Total chlorophyll: In case of NoDS conditions, the varieties exhibited varying levels of total chlorophyll contents, with Akber-19 (5.97 mg/g FW) having the highest mean value, followed by Fakhar-e-Bhakkar (5.55 mg/g FW), Ujala-16 (5.4 mg/g FW), FSD-08 (5.13 mg/g FW), Subhani-21 (5.09 mg/g FW), Anaj-17 (5.08 mg/g FW), MH-21 (4.57 mg/g FW), Urooj-22 (4.52 mg/g FW), Ghazi-19 (4.03 mg/g FW) and Dilkash-20 (3.38 mg/g FW). However, when subjected to DS conditions, all varieties experienced a reduction in total chlorophyll contents as compared to the NoDS condition. Varieties exhibiting the highest reduction in total chlorophyll under DS conditions were Akber-19 (-19.93%), Ghazi-19 (-15.94%), Subhani-21 (-14.69%), Anaj-17 (-13.09%), MH-21 (-13.05%) FSD-08 (-11.73%), Dilkash-20 (-11.15%), Ujala-16 (-10.35%) Urooj-22 (-9.59%). and Fakhar-e-Bhakkar (-4.67%). The results indicate that drought stress negatively affected all varieties, as evidenced by decreased total chlorophyll values. Among the varieties, Akber-19 exhibited the most pronounced reduction (-19.93%), signifying its limited ability to tolerate drought stress. Conversely, Fakhar-e-Bhakkar demonstrated the least reduction (-4.67%) in total chlorophyll, suggesting greater resilience to drought stress (Fig. 3-C).

Chlorophyll a/b: The results revealed distinct variations in chlorophyll a/b contents among the tested varieties under NoDS conditions. Urooj-22 exhibited the highest mean chlorophyll a/b value (2.26), followed by Dilkash-20 (2.18), FSD-08 (1.85), MH-21 (1.81), Anaj-17 (1.66), Ghazi-19 (1.64), Ujala-16 (1.53), Subhani-21 (1.52), Akber-19 (1.38) and Fakhar-e-Bhakkar (1.36). Under DS conditions, the varieties displayed increase in chlorophyll a/b contents as compared to NoDS conditions with Ghazi-19 exhibiting the noteworthy increase of +24.28% followed by Subhani-21 (+23.27%), Urooj-22 (+17.81%), Dilkash-20 (+16.6%), Anaj-17 (+16.44%), MH-21 (+14.91%), FSD-08 (+7.83%), Ujala-16 (+7.13%), Akber-19 (+5.14%) and Fakhar-e-Bhakkar (+3%). Fakhar-e-Bhakkar displayed least increase in Chlorophyll a/b contents, indicating better tolerance to drought stress (Fig. 3-D).

Carotenoids: The results revealed distinct variations in carotenoid contents among the tested varieties under NoDS conditions. Akber-19 exhibited the highest mean value of carotenoid (1.57 mg/g FW), followed by FSD-08 (1.55 mg/g FW), Anaj-17 (1.49 mg/g FW), Ujala-16 (1.48 mg/g FW), Subhani-21 (1.47 mg/g FW), Ghazi-19 (1.37 mg/g FW), Urooj-22 (1.33 mg/g FW), MH-21 (1.28 mg/g FW), Fakhar-e-Bhakkar (1.26 mg/g FW) and Dilkash-20 (0.99 mg/g FW). Under DS conditions, the varieties displayed varied responses in carotenoid contents compared to NoDS conditions. Notably, some varieties showed an increase in carotenoids, with Fakhar-e-Bhakkar exhibiting the most significant increase of +14.23% followed by Dilkash-20 (+8.55%), Ujala-16 (+4.36%) and Urooj-22 (+3.09%). All other varieties showed considerable decrease in carotenoids with Anaj-17 exhibiting the highest decrease (-21.97%), further Ghazi-19 (-15.42%), FSD-08 (-14.97%), Akber-19 (-8.76%), Subhani-21 (-6.3%), and MH-21 (-1.23%) experienced notable decrease in carotenoids under DS conditions (Fig. 4-A). Based on these results, the variety Fakhar-e-Bhakkar exhibited the maximum increase in carotenoids under DS conditions, indicating better tolerance to drought stress.

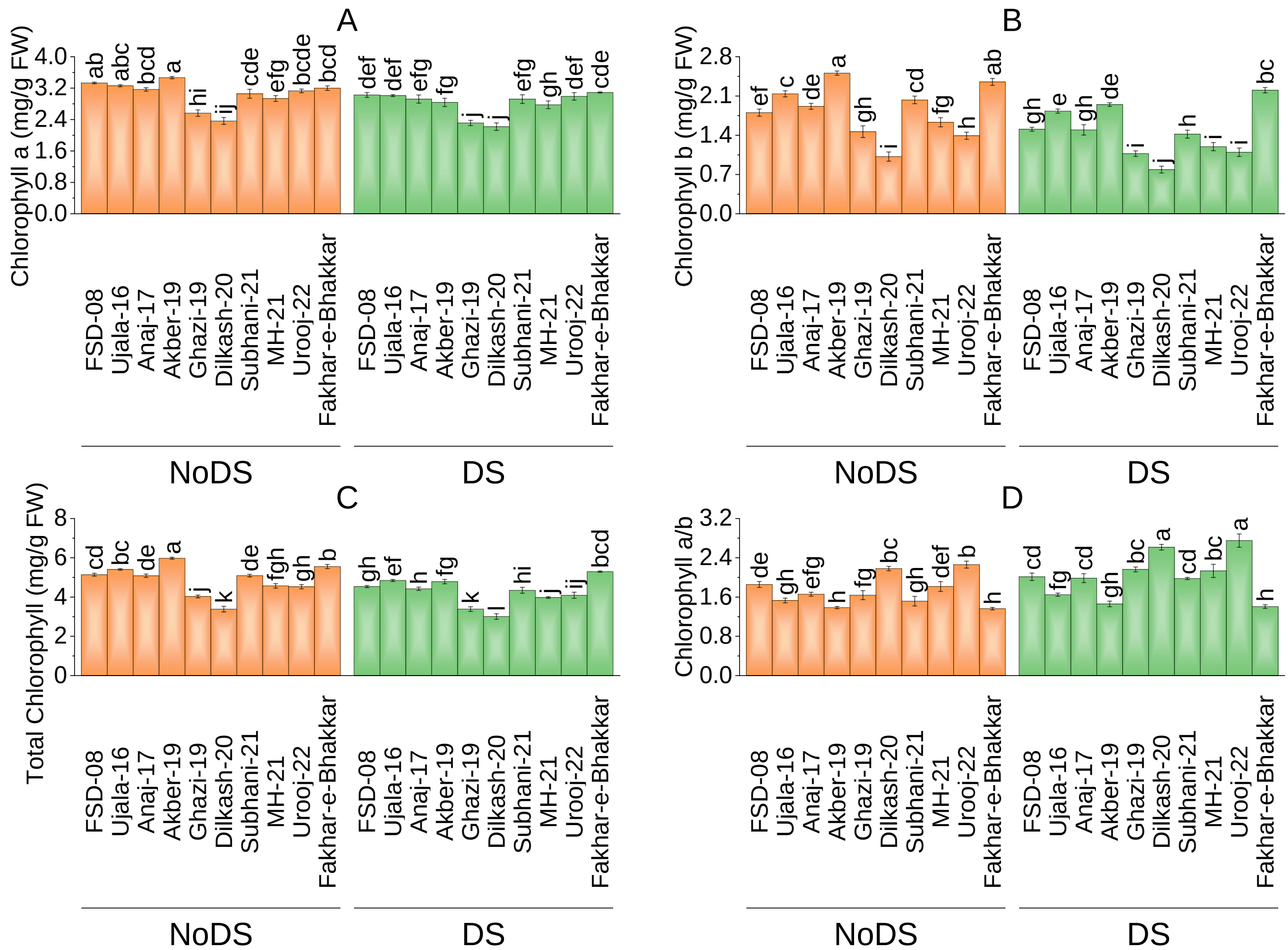

Fig. 3. Influence of control (NoDS) and drought stress (DS) on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), chlorophyll a/b (D) of 10 wheat cultivars. Bars indicate mean ± SE (n=4); compared using Fisher's LSD at significance $p \leq 0.05$.

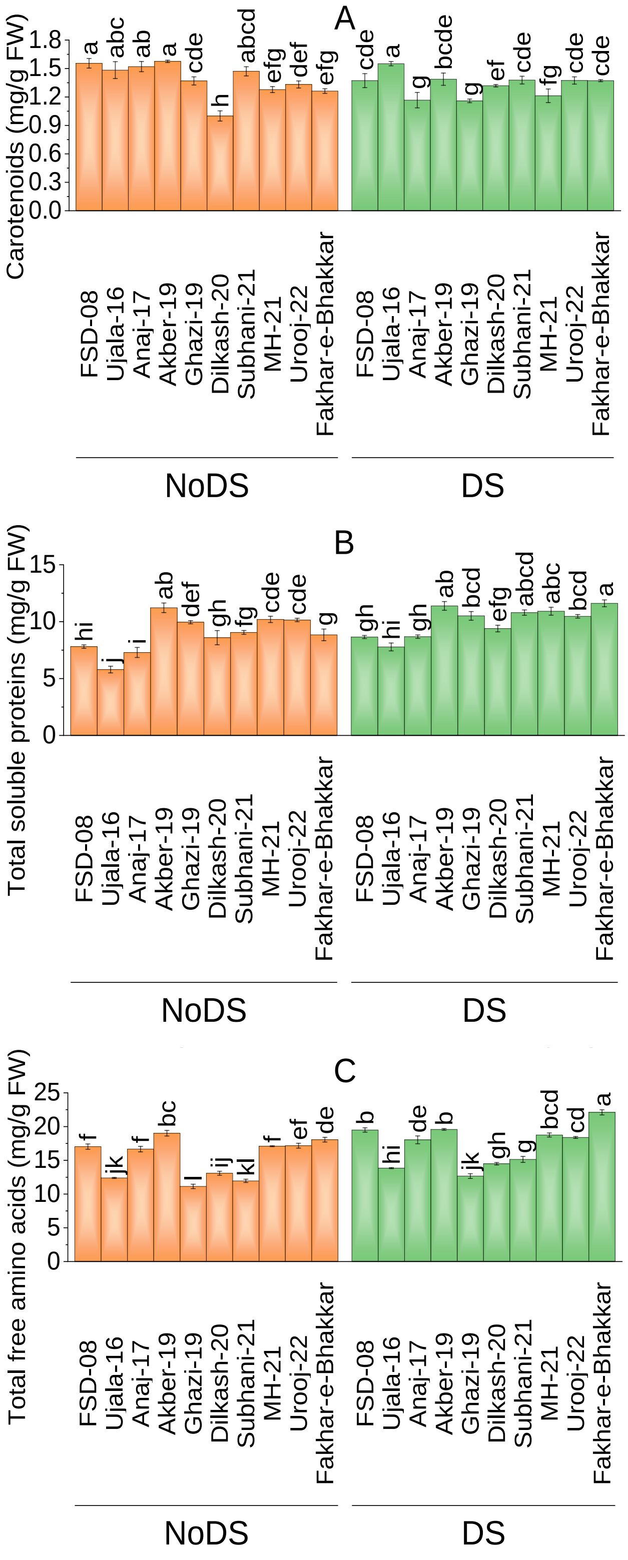

Fig. 4. Influence of control (NoDS) and drought stress (DS) on carotenoids (A), total soluble proteins (B), total free amino acids (C) of 10 wheat cultivars. Bars indicate mean ± SE (n=4); compared using Fisher's LSD at significance $p \leq 0.05$.

Total soluble proteins: Under NoDS conditions, the varieties exhibit varying levels of total soluble proteins as follows (from highest to lowest mean value): Akber-19 (11.22 mg/g FW), MH-21 (10.19 mg/g FW), Urooj-22 (10.14 mg/g FW), Ghazi-19 (9.95 mg/g FW), Subhani-21 (9.05 mg/g FW), Fakhar-e-Bhakkar (8.84 mg/g FW), Dilkash-20 (8.59 mg/g FW). FSD-08 (7.81 mg/g FW), Anaj-17 (7.29 mg/g FW) and Ujala-16 (5.79 mg/g FW). However, total soluble proteins increased significantly for all varieties under DS conditions compared to the NoDS condition. The percentage changes for total soluble proteins (compared to NoDS) were as follows (from highest increase to lowest): Fakhar-e-Bhakkar (+25.55%), Ujala-16 (+23.87%), Subhani-21 (+16.14%), Anaj-17 (+15.99%), FSD-08 (+9.72%), Dilkash-20 (+8.56%), MH-21 (+6.57%), Ghazi-19 (+5.32%), Urooj-22 (+3.21%), and Akber-19 (+1.46%). Based on these results, the variety Fakhar-e-Bhakkar exhibited the maximum increase in total soluble proteins under DS conditions (+25.55%), indicating better tolerance to drought stress. On the other hand, the variety Akber-19 showed least increase in total soluble protein (+1.46%), indicating poor tolerance to drought stress (Fig. 4-B).

Total free amino acids: Results under optimal NoDS conditions showed that all the varieties exhibited differences in total free amino acids, with Akber-19 (19.00 mg/g FW) displaying the highest mean value, followed by Fakhar-e-Bhakkar (18.05 mg/g FW), Urooj-22 (17.16 mg/g FW), MH-21 (17.08 mg/g FW), FSD-08 (17.01 mg/g FW), Anaj-17 (16.66 mg/g FW), Dilkash-20 (13.08 mg/g FW), Ujala-16 (12.38 mg/g FW), Subhani-21 (11.94 mg/g FW) and Ghazi-19 (11.13 mg/g FW). However, when subjected to DS conditions, all varieties experienced enhanced total free amino acids compared to the NoDS condition. Varieties exhibiting the highest increase in total free amino acid under DS conditions were Fakhar-e-Bhakkar (+21.04%), Subhani-21 (+18.31%), FSD-08 (+12.67%), Ghazi-19 (+12.07%), Ujala-16 (+10.52%), Dilkash-20 (+9.71%), MH-21 (+8.86%). Anaj-17 (+7.61%), Urooj-22 (+6.64%), and Akber-19 (+2.91%). The results indicate that all varieties were affected by drought stress, as evidenced by the increase in total free amino acids. Fakhar-e-Bhakkar exhibited the most pronounced increase among the varieties, signifying its high ability to tolerate drought stress. Conversely, Akber-19 demonstrated the least increase in free amino acids, suggesting lesser resilience to drought stress (Fig. 4-C).

Hydrogen peroxide (H_2O_2): Under NoDS conditions, the results revealed distinct variations in H_2O_2 contents among the tested varieties. Ujala-16 exhibited the highest mean value of H_2O_2 (18.45 µmol/g FW) followed by Anaj-17 (17.85 µmol/g FW), Ghazi-19 (16.62 µmol/g FW), FSD-08 (16.41 µmol/g FW), MH-21 (16.19 µmol/g FW), Dilkash-20 (16.11 µmol/g FW), Fakhar-e-Bhakkar (14.24 µmol/g FW), Urooj-22 (13.14 µmol/g FW) and Subhani-21 (12.5 µmol/g FW). Notably, the varieties exhibit significantly higher H2O2 species under DS conditions than NoDS conditions. Varieties exhibiting the highest increase in H_2O_2 under DS conditions were Akber-19 (+52.28%). Urooj-22 (+33.75%), Subhani-21 (+32.51%), MH-21 (+31.76%), Dilkash-20 (+28.62%), FSD-08 (+26.38%), Ujala-16 (+25.39%), Ghazi-19 (+24.69%), Anaj-17 (+23.66%) and Fakhar-e-Bhakkar (+23.54%). Based on these results, Fakhar-e-Bhakkar exhibited the least increase in H_2O_2 under DS conditions, indicating better tolerance to drought stress (Fig. 5-A).

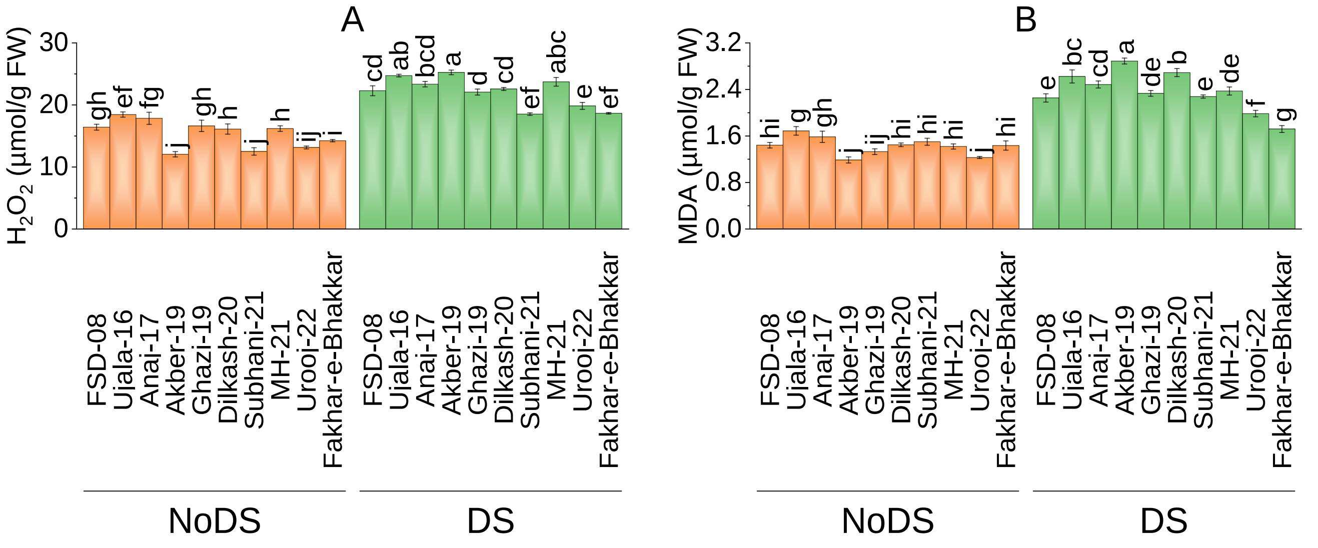

Fig. 5. Influence of control (NoDS) and drought stress (DS) on H_2O_2 (A) and MDA (B) contents of 10 wheat cultivars. Bars indicate mean ± SE (n=4); compared using Fisher's LSD at significance *p*≤0.05.

Melondialdehyde (MDA): In the case of NoDS conditions, the varieties exhibited varying levels of MDA contents, with Ujala-16 (1.69 µmol/g FW), having the highest mean value, followed by Anaj-17 (1.58 µmol/g FW), Subhani-21 (1.5 µmol/g FW), Dilkash-20 (1.45 µmol/g FW). FSD-08 (1.44 µmol/g FW), Fakhar-e-Bhakkar (1.43 µmol/g FW), MH-21 (1.42 µmol/g FW), Ghazi-19 (1.33 µmol/g FW), Urooj-22 (1.23 µmol/g FW), and Akber-19 (1.19 µmol/g FW). However, when subjected to DS conditions, all varieties experienced an increase in MDA contents as compared to the NoDS condition. Varieties exhibiting the highest increase in MDA under DS conditions were Akber-19 (+58.89%), Dilkash-20 (+46.19%), Ghazi-19 (+42.97%), MH-21 (+40.19%) Urooj-22 (+38.04%). Anaj-17 (+36.21%), FSD-08 (+36.07%), Ujala-16 (+35.71%), Subhani-21 (+34.12%) and Fakhar-e-Bhakkar (+16.49%). The results indicate that drought stress negatively affected all varieties, as evidenced by increased MDA values. Among the varieties, Akber-19 exhibited the most pronounced increase (+58.89%), signifying its limited ability to tolerate drought stress. Conversely, Fakhar-e-Bhakkar demonstrated the least increase (+16.49%) in MDA content, suggesting greater resilience to drought stress (Fig. 5-B).

Peroxidase (POD): Results under optimal (NoDS) conditions, showed that all the varieties exhibited differences in peroxidase activity, with Fakhar-e-Bhakkar (2.91 U/mg Protein), displaying the highest mean value, followed by FSD-08 (2.68 U/mg Protein), MH-21 (2.56 U/mg Protein), Ujala-16 (2.51 U/mg Protein), Dilkash-20 (2.26 U/mg Protein), Urooj-22 (2.18 U/mg Protein), Anaj-17 (2.06 U/mg Protein), Subhani-21 (1.99 U/mg Protein), Ghazi-19 (1.91 U/mg Protein) and Akber-19 (1.68 U/mg Protein). However, when subjected to DS conditions, all varieties experienced enhanced peroxidase activity compared to the NoDS condition. Varieties exhibiting the highest increase in peroxidase activity under DS conditions were Fakhar-e-Bhakkar (+27.4%), Ujala-16 (+23.53%), Anaj-17 (+21.47%), Dilkash-20 (+21.03%), MH-21 (+20.23%), Ghazi-19 (+16.92%), Subhani-21 (+16%), FSD-08 (+15.43%), Urooj-22 (+11.91%), and Akber-19 (+7.81%). The results indicate that all varieties were affected by drought stress, as evidenced by the increase in peroxidase activity. Fakhar-e-Bhakkar exhibited the most pronounced increase among the varieties, signifying its high ability to tolerate drought stress. Conversely, Akber-19 demonstrated the least increase in peroxidase, suggesting lesser resilience to drought stress (Fig. 6-A).

Catalase (CAT): Under optimal (NoDS) conditions, the varieties performed as follows in terms of catalase activity (from highest to lowest mean value): Fakhar e Bhakkar (0.97 U/mg Protein), Ujala-16 (0.96 U/mg Protein), Subhani-21 (0.89 U/mg Protein), Dilkash-20 (0.81 U/mg Protein), Urooj-22 (0.79 U/mg Protein), FSD-08 (0.75 U/mg Protein), Ghazi-19 (0.63 U/mg Protein), Anaj-17 (0.6 U/mg Protein), MH-21 (0.57 U/mg Protein) and Akber-19 (0.53 U/mg Protein). The percentage changes for catalase activity under DS conditions (compared to NoDS) were as follows (from highest increase to lowest): Fakhar-e-Bhakkar (+41.13%), MH-21 (+38.73%), Anaj-17 (+37.83%), FSD-08 (+32.55%), Ghazi-19 (+31.24%), Subhani-21 (+28.74%), Dilkash-20 (+26.85%), Urooj-22 (+25.55%), Ujala-16 (+22.54%) and Akber-19 (+10.53%) (Fig. 6-B). Based on these results, the variety Fakhar-e-Bhakkar exhibited the maximum increase in catalase activity under DS conditions (+41.13%), indicating better tolerance to drought stress. On the other hand, the variety Akber-19 showed the lowest increase in catalase activity (+10.53%), indicating poor tolerance to drought stress.

Superoxide dismutase (SOD): Results under optimal NoDS conditions, showed that all the varieties exhibited differences in SOD activity, with Fakhar-e-Bhakkar (5.25 U/mg Protein), displaying the highest mean value, followed by Ujala-16 (5.21 U/mg Protein), Dilkash-20 (4.72 U/mg Protein), Anaj-17 (4.17 U/mg Protein), Ghazi-19 (4.15 U/mg Protein), FSD-08 (3.88 U/mg Protein), MH-21 (3.84 U/mg Protein), Urooj-22 (3.01 U/mg Protein), Subhani-21 (3.00 U/mg Protein) and Akber-19 (2.29 U/mg Protein). However, when subjected to

DS conditions, all varieties experienced significantly enhanced SOD activity as compared to the NoDS condition. Varieties exhibiting the highest increase in SOD activity under DS conditions were Fakhar-e-Bhakkar (+39.44%), Urooj-22 (+39.30%), Subhani-21 (+35.26%), FSD-08 (+33.19%), MH-21 (+32.42%), Ghazi-19 (+30.43%), Anaj-17 (+30.14%), Dilkash-20 (+27.43%), Ujala-16 (+24.72%) and Akber-19 (+20.34%). The results indicate that all varieties were affected by drought stress, as evidenced by the increase in SOD activity. Fakhar-e-Bhakkar exhibited the most pronounced increase among the varieties, signifying its high ability to tolerate drought stress. Conversely, Akber-19 demonstrated the least increase in SOD activity, suggesting lesser resilience to drought stress (Fig. 6-C). These findings underscore the importance of selecting appropriate cultivated varieties under DS conditions. Varieties such as Fakhar-e-Bhakkar, which can maintain H_2O_2 and MDA contents and enhanced antioxidant activities, may prove valuable in mitigating the adverse effects of drought stress on crop productivity (Fig. 6-C).

The correlation between SFW and RFW was -0.22854, indicating a weak negative correlation. Similarly, SDW showed a moderate positive correlation with SFW (0.76322) and a weak negative correlation with RFW (-0.11908). RDW exhibited a weak positive correlation with RFW (0.52702) and a weak negative correlation with SDW (0.07914). The variables SL and RL showed a strong positive correlation with each other (0.74619), indicating a close relationship. Additionally, both SL and RL demonstrated moderate positive correlations with LA (0.70396 and 0.74489, respectively). LA, in turn, exhibited moderate positive correlations with SDW (0.78228) and SL (0.7875). Regarding the pigments, Chla and Chlb showed moderate positive correlations with each other (0.71569), indicating their co-occurrence. Both Chla and Chlb demonstrated moderate positive correlations with SL (0.37316 and 0.44886, respectively) and RL (0.45117 and 0.55725, respectively). However, Chla exhibited a weak positive correlation with RFW (-0.03556), while Chlb showed a weak negative correlation with RFW (0.08073). The relationship between Car and the other variables was relatively weaker. Car exhibited a weak positive correlation with RFW (0.11954) and a weak negative correlation with RDW (-0.0148). Similarly, Car showed weak positive correlations with SL (0.27477) and RL (0.22518). The Chla/b ratio showed a strong negative correlation with Chlb (-0.89411), indicating an inverse relationship. It also exhibited moderate negative correlations with SDW (-0.4952) and SL (-0.37618). However, Chla/b showed a weak positive correlation with Car (-0.33282). Furthermore, TChl demonstrated moderate positive correlations with SDW (0.54263), RDW (0.22165), SL (0.44902), and RL (0.5519). It also showed a strong positive correlation with Chla (0.90171) and Chlb (0.9473), indicating a close association with these pigments. The correlations with TSP were relatively weak, with slight positive correlations observed with RFW (0.11458) and RDW (0.3804), and a slight negative correlation with SL (-0.01715). Similarly, TFAA showed weak positive correlations with RFW (0.25071), RDW (0.55123), and SL (-0.08073) (Fig. 7).

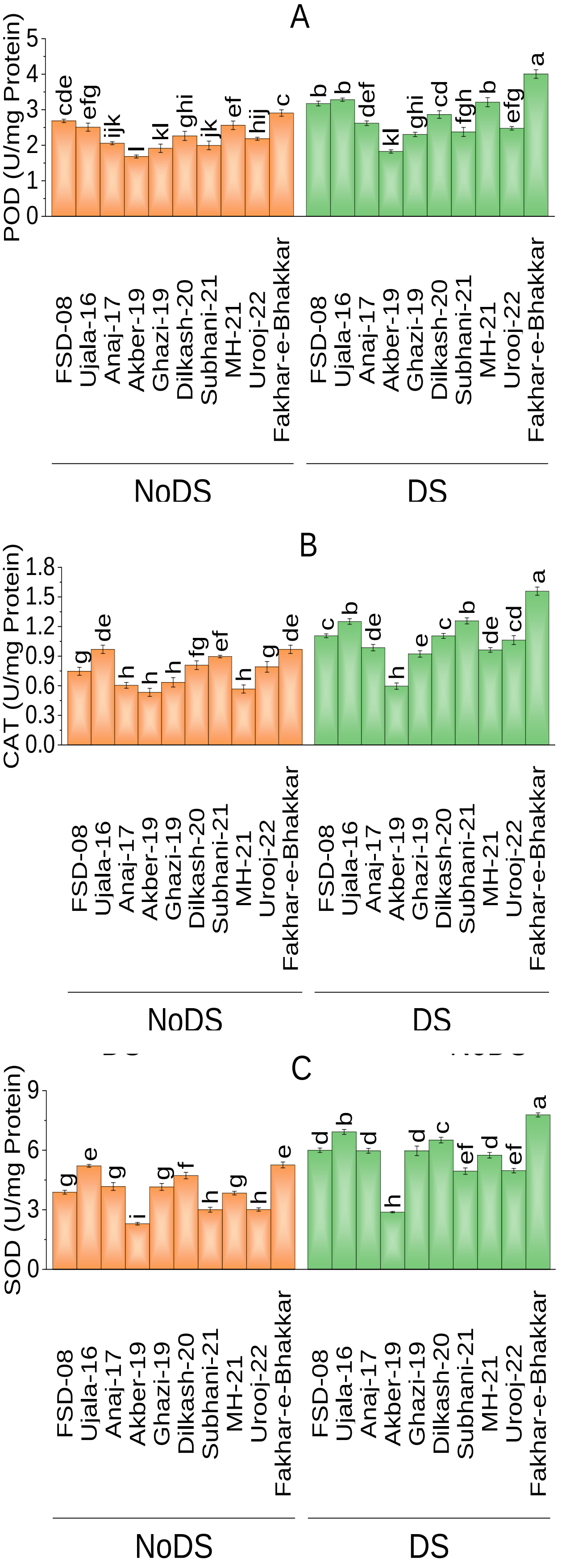

Fig. 6. Influence of control (NoDS) and drought stress (DS) on peroxidase (POD) (A), catalase (CAT) (B), superoxide dismutase (SOD) (C) contents of 10 wheat cultivars. Bars indicate mean ± SE (n=4); compared using Fisher's LSD at significance $p \leq 0.05$.

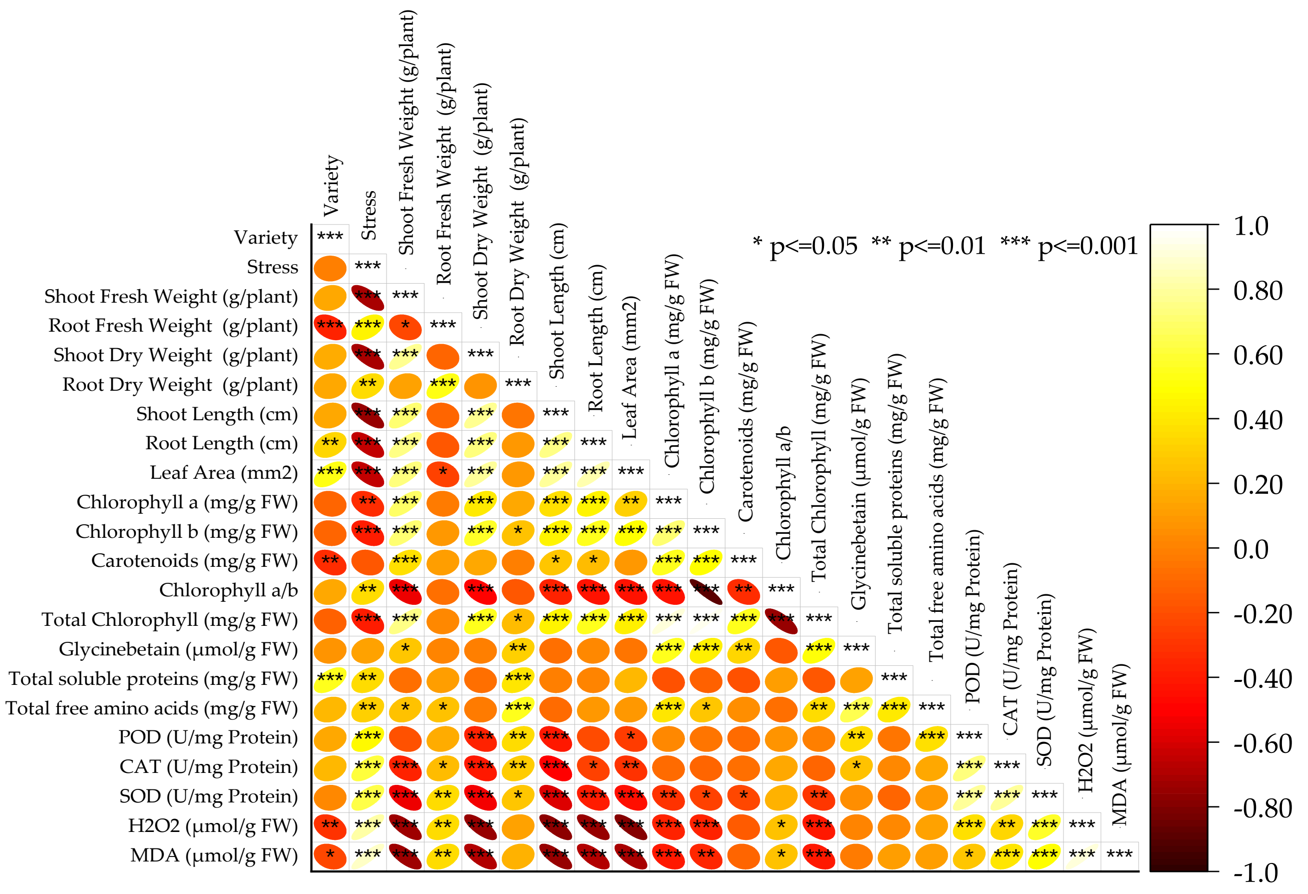

Fig. 7. Pearson correlation for studied attributes.

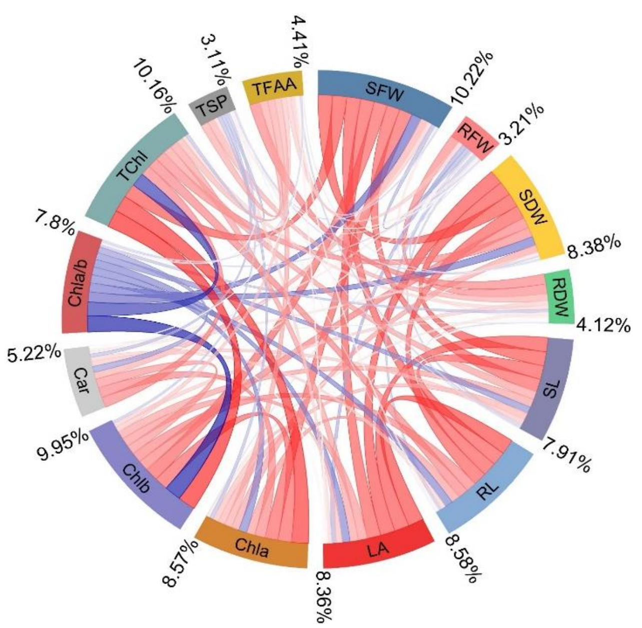

Fig. 8. Correlation chord plot for studied attributes.

The chord correlation plot shows the correlation coefficients between plant growth and physiological parameters. The correlation coefficients range from -1 to 1, where a value of 1 indicates a perfect positive correlation, -1 indicates a perfect negative correlation, and 0 indicates no correlation. The analysis reveals several significant correlations. Shoot fresh weight (g/plant) shows a positive correlation with shoot dry weight (0.76322), shoot length (0.70396), root length (0.74489), leaf area (0.72337), chlorophyll a (0.69702), chlorophyll b (0.7106), carotenoids (0.37472), and total chlorophyll (0.75959). It also exhibits a negative correlation with chlorophyll a/b (-0.54937). Root fresh weight (g/plant) demonstrates a positive correlation with shoot fresh weight (0.22854), root dry weight (0.52702), shoot length (0.11757), root length (0.17193), leaf area (0.24091), chlorophyll b (0.08073), carotenoids (0.11954), and total soluble proteins (0.11458). However, it negatively correlates with chlorophyll a (-0.03556) and chlorophyll a/b (-0.06917). Shoot dry weight (g/plant) is positively correlated with shoot fresh weight (0.76322), shoot length (0.76625), root length (0.72273), leaf area (0.78228), chlorophyll a (0.41458), chlorophyll b (0.56939), and total chlorophyll (0.54263). It negatively correlates with chlorophyll a/b (-0.4952) and total soluble proteins (-0.0667). Root dry weight (g/plant) shows positive correlations with root fresh weight (0.52702), shoot dry weight (0.07914), root length (0.08929), leaf area (0.08172), chlorophyll a (0.15372), chlorophyll b (0.24416), carotenoids (-0.0148), total chlorophyll (0.22165), total soluble proteins (0.3804), and total free amino acids (0.55123). However, it negatively correlates with chlorophyll a/b (-0.16999). Other parameters such as shoot length, root length, leaf area, chlorophyll a, chlorophyll b, carotenoids, chlorophyll a/b, total chlorophyll, total soluble proteins, and total free amino acids also show significant correlations with various growth and physiological parameters (Fig. 8).

A cluster plot with convex hulls was generated to visualize the relationships between the variables PC 1, PC 2, and Variety. The plot displayed the percentage of variance explained by PC 1 (58.31%) and PC 2 (21.72%). The scores for each data point were plotted by their coordinates on PC 1 and PC 2, along with the corresponding Variety labels. The data points within each variety formed distinct clusters enclosed by convex hulls to illustrate the grouping pattern. The variety "FSD-08" was represented by multiple data points with scores ranging from 0.15447 to -3.31178 on PC 1 and -0.60104 to 1.09457 on PC 2. Similarly, the variety "Ujala-16" exhibited a cluster of data points with scores varying from 0.06702 to -2.95665 on PC 1 and -0.04411 to 0.66894 on PC 2. The variety "Anaj-17" was characterized by data points ranging from 1.36813 to 2.27963 on PC 1 and -0.96806 to 1.17891 on PC 2. The variety "Akber-19" displayed a distinct cluster of data points with scores ranging from 3.70862 to 4.43715 on PC 1 and 2.6008 to 3.14861 on PC 2. Similarly, the variety "Ghazi-19" exhibited a cluster with scores varying from 0.11566 to -3.21928 on PC 1 and -1.65726 to 1.21012 on PC 2. The variety "Dilkash-20" showed data points ranging from -1.03236 to 0.94443 on PC 1 and -2.11761 to 1.21012 on PC 2. The variety "Subhani-21" formed a distinct cluster with data points varying from 1.60441 to 2.87809 on PC 1 and -1.36707 to -0.22993 on PC 2. The variety "MH-21" exhibited a cluster with scores ranging from 1.32988 to 2.03592 on PC 1 and -1.06444 to 1.00575 on PC 2. The variety "Urooj-22" displayed data points varying from 1.47014 to 2.53336 on PC 1 and -1.98292 to -0.3991 on PC 2. Lastly, the variety "Fakhar-e-Bhakkar" showed a distinct cluster of data points with scores ranging from 2.9386 to 3.62114 on PC 1 and -0.81391 to 2.34206 on PC 2. The scores on PC 1 and PC 2 for the data points labeled "NoDS" range from 0.15447 to 4.32477 and from -2.11761 to 3.14861 respectively. Conversely, for the "DS" labeled data points, the PC 1 and PC 2 scores range from -3.31178 to -1.02779 and from -1.65726 to 1.21012 respectively (Figure 8). Hierarchical cluster plot for studied attributes showed that shoot dry weight was most representable variable while CAT was least representable variable (Fig. 9).

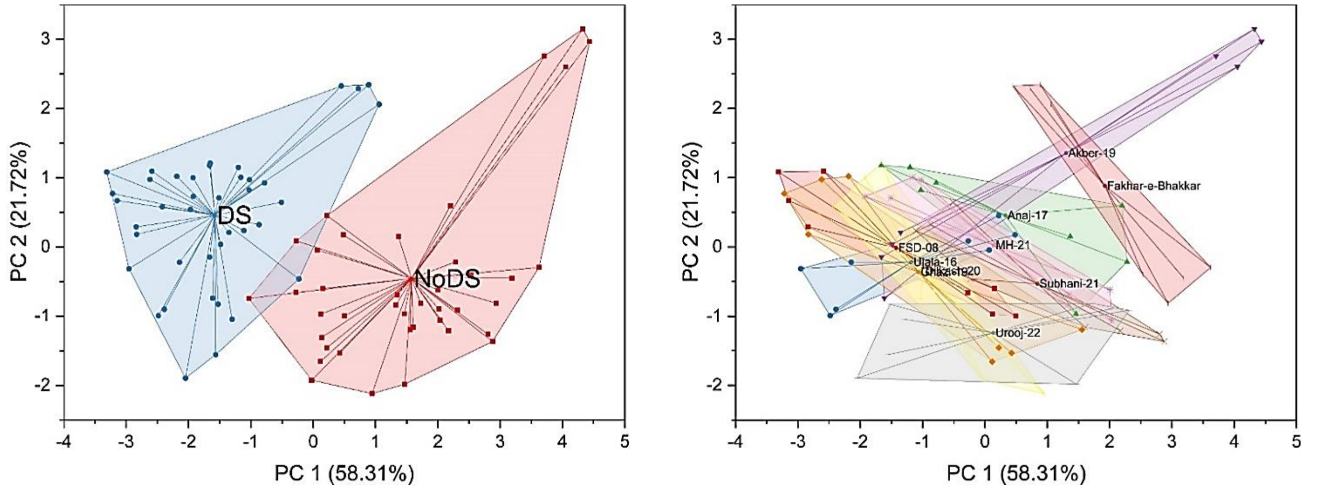

Fig. 9. Cluster plot convex hull for drought levels (No DS and DS) and cultivated varieties.

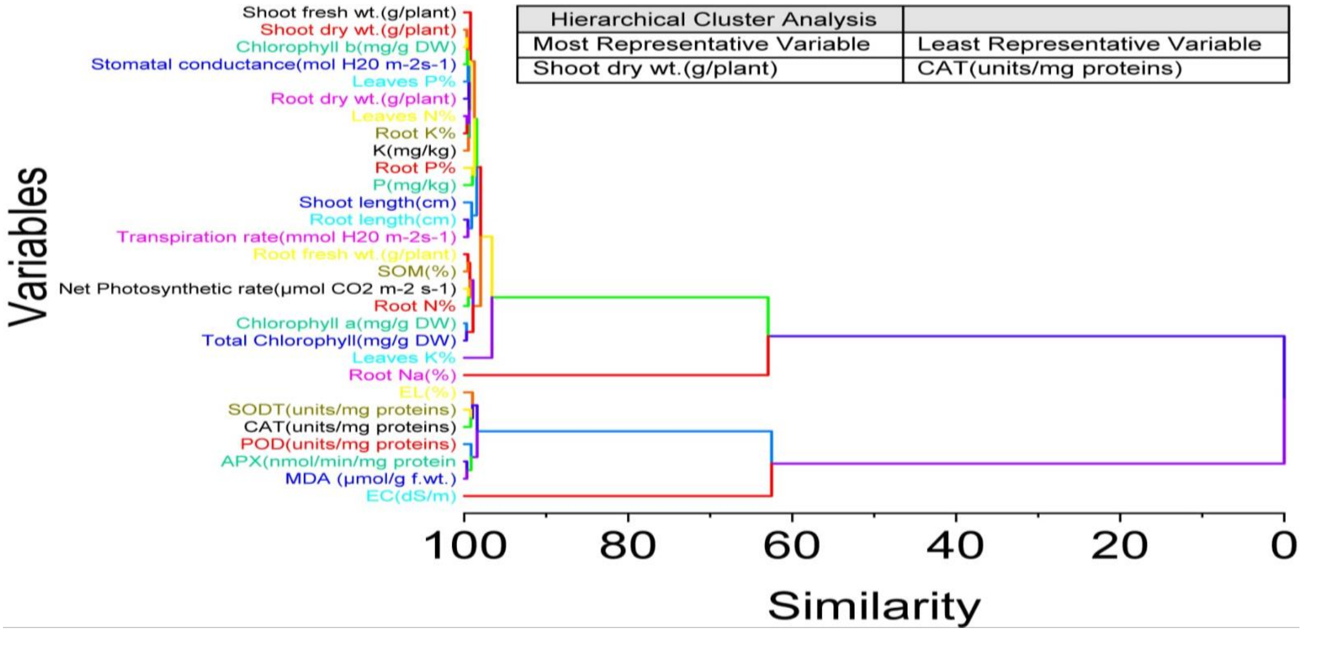

Fig. 10. Hierarchical cluster plot for studied attributes.

Discussion

Drought, being a limiting factor, is well documented and changes various phases of plant's development and growth (Alghbari & Ihsan, 2018). Severe drought stress alters plant bio-chemical and physiological activities, ultimately affecting growth (Kapoor *et al*., 2020). However, Plants hardly recover from drought stress at early developmental stages mainly seedling. As regards the screening of economical wheat varieties under drought stress, growth-related factors such as seedling fresh/dry biomass and shoot & and root length were generally thought of as important selection standards to select drought tolerance levels in wheat (Munns & James, 2003; Foito *et al*., 2009). Plant responses to drought are different and involve the participation of defence systems and modifications in morphology, physiology, biochemistry, anatomy, and short and prolonged growth-linked adaptation developments (Abobatta, 2019). In the current research, wheat plants under drought stress exhibited lower growth at the seedling stage. These outcomes are in accordance with previous investigations on quinoa (Elewa *et al*., 2017), flax (Sadaq & Bakry, 2020) and moringa (Elhamid *et al*., 2021). During early seedling growth, severe drought stress negatively affects all growth parameters and limits photosynthesis, resulting in growth inhibition, plant height, and dry biomass reduction (Ghotbi-Ravandi *et al*., 2014). These reductions could result from limited cell expansion and division under stress that reduced its apical growth. Furthermore, leaf development is considered more susceptible to drought in wheat; a decrease in leaf area occurs to lessen the transpiration rate (Kapoor *et al*., 2020). Shrinkage of leaf area in wheat (Dhakal, 2021), increase in leaf thickness, and root system enlargement all is linked with adaptive responses (Anjum, 2011; Kapoor *et al*., 2020). Under a drought environment, plants try to find and absorb water from lower soil layers by improving their root's structural design and preserving more water (Abobatta, 2019). Hence, root to shoot ratio increases under drought stress to assist water absorption (Smirnoff, 1998). The most frequent and harmful effect of drought on plants is a decline in fresh plus dry biomass (Farooq *et al*., 2009) that is caused by stomatal closure during drought and restricting the ability of the Calvin cycle to fix CO_2 effectively (Ozturk *et al*., 2021).

Drought declines plant development due to physiological alterations and growth limitations (Adl *et al*., 2020). Dehydration of plant cells causes disintegration of thylakoid membranes, resulting in considerable reduction of chlorophyll contents and increasing drought stress (Liu *et al*., 2016). A decline in chlorophyll contents under drought has been highlighted in previous studies, including wheat (Lalarukh *et al*., 2014; Chen *et al*., 2016; EpeeMisse, 2018; Dhakal, 2021); moringa plant (Ezzo *et al*., 2018) and chickpea (Bakhoum *et al*., 2020) and maintenance of these contents under drought is the adaptation of tolerant genotypes (Seher *et al*., 2015). Drought stress effect on photosynthetic pigments is cultivar dependent, tolerant cultivars displayed higher chlorophyll contents under drought due to their genetic makeup (Alaei, 2011). A decline in chlorophyll contents due to thylakoid membrane damage and photosynthetic pigment decay was observed under drought conditions (Taibi *et al*., 2016). Chloroplast lipid oxidation, pigments and protein structural modifications and degradation in chlorophyll by proteolytic enzymes resulted in overall chloroplast molecule deterioration and, ultimately, closure of stomata (Jomo *et al*., 2016). Also, higher chlorophyll contents and plant height were observed in the control treatment than in drought (Yavas & Unay, 2016).

The surge in soluble-protein contents was also examined under drought stress (Mohammadkhani & Heidari, 2008), and this may be credited to the increased formation of amino-acids that were formed due to drought stress (Jin *et al*., 2016; Ozturk *et al*., 2021). There are numerous findings about the buildup of free amino acids (FAA) under stress in different crops for osmotic adjustment (Lalarukh & Shahbaz, 2020b). Among drought-stress plant responses related to proteins, dehydrins (Kosova *et al*., 2014) and heat-shock proteins (Di Donato & Geisler, 2019) are thought to be involved. Heat-shock proteins are engaged in numerous stresses, either abiotic or biotic (Kumar *et al*., 2014; Guo *et al*., 2021) apart from heat stress, whereas dehydrins are proteins that are specifically generated under drought stress and play a crucial part in plant's adaptation and response to abiotic stress (Thomas, 2015). Several studies on plant responses, especially in wheat and barley, against stress have shown a positive correlation between dehydrin protein levels and plants' stress tolerance (Vitamvas *et al*., 2019).

The implication of severe drought stress limits photosynthesis, which induces the action and accumulation of signalling molecules in the form of reactive oxygen species (ROS) due to abiotic stress. Carotenoids also maintain ROS homeostasis in plants (Hasanuzzaman *et al*., 2020). We noticed a significant buildup of stress markers (MDA and H_2O_2) in drought-stressed cultivars. The fact that drought-induced ROS caused damage to cellular membranes and, ultimately, cells is in accordance with previous investigations on drought-stressed wheat (Abid *et al*., 2018; Kirova *et al*., 2021) and tobacco (Cvikrova *et al*., 2013). Excessive formation of ROS like O^{2-} and H_2O_2 provokes injury and peroxidation of cellular membranes and results in MDA accumulation (Kohli *et al*., 2019; Hasanuzzaman *et al*., 2020). In turn, this initiated the cell's internal detoxification machinery to protect it from oxidative harm (Mittler, 2017). The rise in H_2O_2 concentration following stress exposure has been described in many experiments, plus the intensity of stress and time affects its production (Caverzan *et al*., 2016; Jiang *et al*., 2019). The documented rise and decline in enzymatic activity and oxidative damage respectively are directly associated. A larger representation of antioxidant activity is surely connected to greater tolerance under abiotic stress (Lalarukh & Shahbaz, 2020a). Antioxidant enzymes boosted considerably under stress conditions (Dhakal, 2021) and were described to give drought tolerance to plants, involving scavenger enzymes like catalase (CAT), peroxidases (POD), and superoxide dismutase (SOD) (Laus *et al*., 2021). SOD performs as a front-line defence converting O^{2-} to H_2O_2 (Gill *et al*., 2015) then CAT and POD detoxify H_2O_2 forming H_2O. The stress markers, a substantial induction of antioxidant enzyme activities, and accumulation of compatible solutes were also reported in wheat (Abid *et al*., 2018; Sallam *et al*., 2019). Additionally, several articles explain that the abiotic stress effect in the wheat plant is specific to its genotype, i.e., various genotypes exhibit separate responses under similar stress conditions and drought-tolerant genotypes normally kept an elevated antioxidant enzyme levels that lessen the oxidative injury (Caverzan *et al*., 2016).

Conclusion

Wheat crops have capacity to sustain its development and growth during drought stress by adjusting biochemical and physiological attributes. Tolerated wheat cultivar must be identified under severe drought to improve wheat production. In view of above findings, we suggested that cultivar Fakhar-e-Bhakkar could be used for cultivation in arid and semi-arid areas of Pakistan as it performed well during drought conditions by retaining morphological, physiological and biochemical attributes. In well-irrigated areas, farmers could grow Akber-19 for better and improved yield.

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