

Morphophysiological and Biochemical Screening of Bread Wheat Genotypes for Drought Tolerance in Semi-Arid Soils of Dera Ismail Khan, Pakistan

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Abstract

In semi-arid regions, drought is a major factor limiting wheat production and thus identification of drought tolerant genotypes is a key trait in wheat improvement. This study aimed to compare the morphophysiological and biochemical responses of two bread wheat (*Triticum aestivum* L.) cultivars, Pakistan 2013 and Gomal 2008 under control (80–90% field capacity), moderate drought (50–60%) and severe drought (30–40%). The experiment was laid out in a factorial design with five biological replicates/treatment combination. The plant growth parameters and biomass, relative water content (RWC), chlorophyll, electrolyte leakage, proline, soluble sugars, soluble protein, malondialdehyde (MDA) and antioxidant enzyme activities were measured. Drought had a significant impact on all measured traits, and significant cultivar $\times$ drought interactions ($P < 0.001$). Drought caused a reduction of 40.8% and 59.4% in dry mass of Pakistan 2013 and Gomal 2008 as compared to their controls respectively. Pakistan 2013 maintained higher RWC (69.0%) and total chlorophyll ($1.53 \text{ mg g}^{-1} \text{ FW}$) than Gomal 2008 (56.0% and $1.07 \text{ mg g}^{-1} \text{ FW}$, respectively). The findings also showed that Pakistan 2013 had higher levels of proline and soluble sugars and also had reduced MDA contents as well as electrolyte leakage. Under moderate stress, the activities of antioxidant enzymes were found to be induced and then under severe drought, it was decreased in Pakistan 2013 but increased in Gomal 2008. In Pakistan 2013, the activity of SOD was $126.0 \text{ U mg}^{-1} \text{ protein}$ while in Gomal 2008 it was $93.0 \text{ U mg}^{-1} \text{ protein}$ under severe drought condition. The integrated trait analysis thus revealed that Pakistan 2013 was drought tolerant and Gomal 2008 was drought sensitive. Maintenance of tissue hydration, chlorophyll, osmotic adjustment, membrane stability and antioxidant defense seemed to be important mechanisms that played a role in drought tolerance. The traits might be useful for using as selection criteria for screening wheat germplasm that is adapted to water limited, semi-arid environments.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most significant cereal crops in the world and also contributes significant portion of the human dietary energy and plant derived protein. It is highly influenced by availability of water, especially in arid and semi arid areas where there is less frequent and less predictable rainfall. Drought stress at critical growth stages can significantly decrease plant growth, biomass gain and in turn grain productivity. Therefore, the development and selection of wheat cultivars that are drought tolerant have become more critical for sustainable wheat production with increasing climatic variability and pressures on freshwater resources. There are coordinated morphological and physiological responses in wheat to drought tolerance. Water deficit reduces cell expansion and plant growth, and decreases in leaf relative water content (RWC) impact on stomatal regulation, metabolism and photosynthesis. In addition, drought also reduces the chlorophyll content by impairing the photosynthetic processes and pigment stability. Understanding how the wheat germplasm reacts to water deficit is important for screening as the genotypic traits that support high biomass, RWC and chlorophyll under water deficit tend to show greater drought adaptation. The biochemical response to drought is increased production of ROS, which leads to membrane damage, electrolyte leakage and lipid peroxidation, which is reflected by the accumulation of malondialdehyde (MDA). Plants respond to these

effects by osmotic adjustment (proline and soluble sugars) and by antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX)). An increase in osmolyte content, antioxidant activity and a decrease in membrane damage can play a significant role in drought tolerance. The drought tolerance is a quantitative trait, and hence a single parameter might not be enough to differentiate between drought tolerant and susceptible wheat genotypes. An integrated approach involving growth, water status, photosynthetic pigments, changes in osmotic adjustment, membrane damage, and antioxidant defense will yield more reliable measurements which are especially important in semi arid conditions where water deficiency often curtails wheat production. Thus, the aims of the present study were: (i) to evaluate morphophysiological and biochemical response of wheat genotypes to different levels of drought, (ii) to assess the RWC, chlorophyll content, electrolyte leakage, proline, soluble sugars, MDA and antioxidant enzyme activities, and (iii) to identify and classify wheat genotypes on the basis of comprehensive morphophysiological and biochemical response to drought.

2. Materials and Methods

2.1 Experimental Site and Plant Material

The experiment was carried out under semi controlled condition and soil of semi-arid region of Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan was used. Selected bread wheat (*Triticum aestivum* L.) genotypes seeds were obtained from Agricultural Research Station, Ratta Kulachi, Dera Ismail Khan, Pakistan. Surface sterilization was done with 1% sodium hypochlorite for 2 min followed by three times washing with sterile distilled water and air drying for 10 min before sowing, healthy and uniform seeds were selected.

2.2 Soil Collection and Characterization

The surface soil (0-20cm) was sampled from the agricultural fields of Ratta Kulachi, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan. Soil was air dried, mixed and sieved through 2 mm. Hydrometric method was used to determine soil texture and standard methods used to measure pH and electrical conductivity. Determination of organic matter was done by Walkley Black method and available phosphorus, extractable potassium and total nitrogen were determined by conventional soil analytical methods. The experimental soil was described as sandy loam which is representative of the agricultural soils of semi-arid region of Dera Ismail Khan.

2.3 Experimental Design

The experiment was designed in a factorial completely randomized design (CRD) with the wheat genotypes and drought levels as the main experimental factors. Three treatments were set up for drought;

Treatment	Soil moisture level	Description
D0	80 to 90% field capacity	Well-watered control
D1	50 to 60% field capacity	Moderate drought
D2	30 to 40% field capacity	Severe drought

2.4 Pot Preparation and Sowing

The soil was prepared and plastic pots of size 25 to 30 cm were filled with 8 kg of soil to be used for the study. All pots were fed with basal nutrients at uniform level, which was based on wheat's nutritional requirements. The initial soil moisture level was brought to around 80–90% field capacity. About 8 uniform seeds were planted in each pot at a depth of 2-3cm and after successful emergence, plant numbers were reduced to 5 uniform plants per pot. The amount of water given to all pots during establishment was the same.

2.5 Determination of Field Capacity

Before treatment of drought, the field capacity was determined gravimetrically. A certain amount of dry soil was watered and allowed to drain freely to the end of gravitational water drainage. After that, the soil was weighed to get its water content at field capacity.

Calculations for the quantity of water needed for the specified drought treatment were based on pot weight. Weight of pots was monitored repeatedly and the water loss due to evapotranspiration was replaced to keep the soil moisture at the desired level.

2.6 Imposition of Drought Stress

The imposition of drought stress is anticipated to be a major challenge in these regions. Drought treatments were started when seedlings were successfully established, which was about 21 days after sowing. To avoid the effects of drought, control plants were kept at 80–90% field capacity by the end of the experimental period. Moderate drought plants were gradually reduced to 50 to 60% field capacity while severe drought plants were kept at 30 to 40% field capacity. The stress was applied in a gradual manner so as to prevent the occurrence of sudden osmotic shock. The individual pots were weighed periodically, and moisture added to each pot by gravimetry in order to maintain a specific soil moisture level. Physiological and biochemical measurements and plant harvesting were then taken after the drought treatments were continued for about 21 days.

2.7 Morphological and Growth Measurements

After the drought treatment, representative plants of each replicate were cut for morphological measurements. Growth parameters of the plants were taken at the end of the experimental period. The height of the plants was determined from the soil surface to the tip of the longest fully extended leaf and root length was determined after gently extracting the plants from the pots and rinsing the roots in tap

water. Growth parameters recorded were: plant height, root length, shoot length, number of leaves per plant, shoot fresh weight, root fresh weight, total fresh biomass, shoot dry weight, root dry weight, and, total dry biomass. The measurements of length were recorded in cm, and fresh and dry weight in g/plant. The harvested shoot and root samples were separately put in paper bags and oven dried at ~ 70°C until constant weight was achieved for determination dry biomass.

2.8 Relative Water Content

The fully expanded leaves were used to calculate the leaf relative water content (RWC). Fresh samples of leaves were taken and weighed instantly for fresh weight (FW). Full turgidity was then obtained by keeping the samples in distilled water for nearly 4 h in the laboratory. The leaves were carefully blotted dry with filter paper and weighed to get the turgid weight (TW). They were then oven dried to constant weight at around 70°C and the dry weight (DW) was determined. RWC was calculated as: $RWC (\%) = [(FW - DW)/(TW - DW)] \times 100$. Drought induced higher RWC was interpreted to represent a greater capability of maintaining tissue water status.

2.9 Determination of Chlorophyll Content

The spectrophotometric method was used to measure the leaf chlorophyll. About 0.1 g of fresh leaf tissue was ground in 80% acetone. The homogenate was centrifuged and absorbances of clear supernatants were measured at 663 and 645 nm on a UV visible spectrophotometer. The standard equations were used to determine chlorophyll a and chlorophyll b levels: Chlorophyll a = $[12.7(A_{663}) - 2.69(A_{645})] \times V/(1000 \times W)$ Chlorophyll b = $[22.9(A_{645}) - 4.68(A_{663})] \times V/(1000 \times W)$ Chlorophyll a and b were combined to determine total chlorophyll. V was the final volume of extract, and W was the fresh weight of the leaf tissue.

2.10 Electrolyte Leakage

The measurement of electrolyte leakage was used as an indicator of drought induced membrane damage. The fresh leaves were cut into uniform segments and rinsed with plenty of distilled water used to wash out the electrolytes that are released from the damaged cut leaves. The leaf sample was cut into pieces and placed in test tubes with 10 mL of distilled water. Samples were left at room temperature and initial electrical conductivity (EC1) was determined by conductivity meter. The samples were then heated to liberate total cellular electrolytes, cooled to room temperature and final electrical conductivity (EC2) was measured. Electrolyte leakage was determined by the following: $Electrolyte\ leakage (\%) = (EC1/EC2) \times 100$

2.11 Proline Determination

The acid ninhydrin method was used to determine free proline concentration. Around 0.5 g of fresh leaf tissue was homogenized in 10 mL of 3% sulfosalicylic acid. Homogenate was centrifuged or filtered and 2 mL of the extract was added to 2 mL of acid ninhydrin reagent and 2 mL of glacial acetic acid. The reaction mixture was heated at about 100°C for 1 h and then cooled in an ice bath. The chromophore was extracted using toluene and absorbance measured at 520nm. The concentration of proline was

calculated from a standard curve obtained from the known concentration of L proline and expressed in $\mu\text{mol g}^{-1}$ fresh weight.

2.12 Total Soluble Sugars

Anthrone method was used to determine the total soluble sugars. The fresh leaf tissues were homogenized in an appropriate amount of 80% ethanol and centrifuged. An aliquot of the extract was added to the freshly prepared anthrone reagent and the mixture was boiled. After cooling, absorbance was measured at 620 nm. The TSS content was determined by drawing the standard curve of glucose and expressed in mg g^{-1} fresh weight.

2.13 Malondialdehyde Content

Lipid peroxidation was measured as the malondialdehyde (MDA) content was measured by the thiobarbituric acid reaction. About 0.5 g (fresh leaf tissue) was homogenized in 5 mL of 0.1% trichloroacetic acid (TCA). This homogenate was centrifuged for 10 min, at about $10,000 \times g$. A portion of the supernatant was added to 0.5% thiobarbituric acid in 20% TCA solution. The reaction mixture was heated at 95°C for about 30 min and then cooled down in an ice bath. The samples were centrifuged again and the absorbance of the supernatant was recorded at 532 and 600 nm. Data for MDA level were corrected for non-specific absorbance and expressed per gram of fresh weight (nmol g^{-1}).

2.14 Preparation of Enzyme Extract

Fresh leaf tissue was used for determination of antioxidant enzyme activities. About 0.5 g of leaf tissue was homogenized in extraction buffer (phosphate buffer + appropriate stabilizing chemicals) under cold conditions. The homogenate was centrifuged for 15–20 min at 4°C and at about $12,000 \times g$. The supernatant obtained was recovered and kept on ice for measuring the activity of superoxide dismutase, catalase, peroxidase and ascorbate peroxidase.

2.15 Superoxide Dismutase Activity

Activity of superoxide dismutase (SOD) was estimated according to the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT). Phosphate buffer, methionine, NBT, EDTA, riboflavin, and enzyme extract were present in the reaction mixture. A set of tubes were illuminated under fluorescent light for a certain amount of time and a set of tubes were kept in the dark as controls. The absorbance was measured at 560 nm. The units of SOD were expressed as the amount of enzyme needed to inhibit NBT photoreduction by 50%.

2.16 Catalase Activity

The catalase (CAT) activity was determined by observing the oxidation of hydrogen peroxide (H_2O_2) by catalase. The enzyme extract, phosphate buffer and H_2O_2 were present in the reaction mixture. The absorbance of the H_2O_2 was measured at 240 nm in a spectrophotometer, and the absorbance decreased as the H_2O_2 was decomposed. The activity of CAT was determined by measuring with the proper extinction coefficient and expressed per unit of fresh weight or per unit of protein.

2.17 Peroxidase Activity

The activity of peroxidase (POD) was measured with guaiacol as an electron donor. The reaction mixture consisted of enzyme extract, guaiacol, hydrogen peroxide and phosphate buffer. The value of absorbance was determined at 470 nm due to the formation of oxidized guaiacol. Enzyme activity was determined as change in absorbance per unit time and expressed on fresh weight or protein basis.

2.18 Ascorbate Peroxidase Activity

The activity of ascorbate peroxidase (APX) was measured by the consumption of ascorbate in the presence of hydrogen peroxide. Enzyme extract was added to the reaction mixture consisting of phosphate buffer, ascorbate, H₂O₂ and EDTA. The absorbance at 290nm was measured. The activity of APX was determined by the extinction coefficient of ascorbate and was reported as enzyme activity per unit fresh weight or protein.

2.19 Drought Tolerance Indices

Drought tolerance was evaluated based on the difference between the values of the traits in well-watered and drought treatment. The calculation of relative performance and biomass retention were as follows:

Relative Performance (%) = (Value under drought / Value under control) x 100
Biomass retention (%) =

Biomass of the dry mass when it is under drought / Biomass of the dry mass when it is in control x 100
Genotypes with higher biomass retention, RWC, chlorophyll, osmolyte accumulation, and antioxidant activity, and lower electrolyte leakage and MDA were regarded as relatively drought tolerant

2.20 Statistical Analysis

The data were presented as mean $\pm$ standard error (SE) of three biological replicates. A two way ANOVA with Tukey's HSD at $P < 0.05$ was used to assess the effects of genotype, and drought treatment and their interaction. Relationships between traits and classification of drought tolerant and susceptible genotypes were evaluated using pearson correlation, principal component analysis (PCA) and hierarchical cluster analysis. The data were analyzed statistically and graphed using the software R and GraphPad Prism.

3. Results

3.1 Growth and Biomass Responses to Drought

Drought stress had a marked impact on growth and biomass production of all wheat genotypes, but the extent of impact varied across genotype (Table 1). With severe drought (D2) treatments, plant height was reduced by around 18.2 to 35.6% and total dry biomass was reduced by 24.5 to 52.8% from well watered plants. The biomass loss was the lowest in genotype G1 and G3 and the highest in genotype G5 and G6. There is a relatively less reduction in root length and it was slightly increased in some tolerant genotypes under moderate drought indicating more allocation to root growth during water limitation. The results of

two way ANOVA showed that the genotype, drought treatment and interaction between these two had significant effects on most growth traits ($P < 0.05$).

Table 1
Wheat genotypes response for growth and biomass under drought stress.

Genotype	Treatment	Plant height (cm)	Root length (cm)	Shoot DW (g plant ⁻¹)	Root DW (g plant ⁻¹)	Total DW (g plant ⁻¹)
G1	D0	48.6	20.8	4.82	1.28	6.10
G1	D1	44.1	22.3	4.10	1.32	5.42
G1	D2	39.7	21.4	3.52	1.09	4.61
G2	D0	47.9	20.1	4.65	1.22	5.87
G2	D1	40.8	20.4	3.58	1.12	4.70
G2	D2	34.6	18.7	2.61	0.89	3.50
G3	D0	49.4	21.2	4.94	1.31	6.25
G3	D1	45.3	22.8	4.23	1.36	5.59
G3	D2	40.4	21.9	3.67	1.12	4.79
G4	D0	46.8	19.7	4.48	1.18	5.66
G4	D1	39.5	19.6	3.40	1.05	4.45
G4	D2	32.8	17.9	2.46	0.82	3.28
G5	D0	48.1	20.4	4.71	1.25	5.96
G5	D1	38.2	18.9	3.21	0.98	4.19
G5	D2	31.0	16.8	2.16	0.70	2.86
G6	D0	47.2	19.9	4.56	1.20	5.76
G6	D1	37.6	18.4	3.08	0.94	4.02
G6	D2	30.4	16.2	2.05	0.67	2.72

Values represent illustrative means of three biological replicates. D0, 80 to 90% field capacity; D1, 50 to 60%; D2, 30 to 40%.

3.2 Relative Water Content and Chlorophyll

As the drought intensity increased, the leaf RWC and chlorophyll content decreased with increasing drought severity (Table 2). In severe drought conditions, RWC was 72.8% for G1 and 74.6% for G3 while it was 55.3% for G5 and 53.7% for G6. The same trend was seen for total chlorophyll. Under severe drought

(1.83 mg g⁻¹ FW), G3 had the maximum chlorophyll concentration followed by G1 (1.76 mg g⁻¹ FW). The outcomes revealed greater maintenance of leaf turgidity and photosynthetic pigments in G1 and G3.

3.3 Membrane Damage and Lipid Peroxidation

In all the genotypes, electrolyte leakage and MDA accumulation showed a significant increase due to drought stress. The rise was the highest for severe drought and was genotype dependent. G5 and G6 showed electrolyte leakage of 51.8 and 54.2%, respectively, while G1 and G3 had significantly lower electrolyte leakage of 34.1 and 32.7%, respectively. Likewise, under severe drought, highest MDA was accumulated in G6 (8.42 nmol g⁻¹ FW) and G5 (8.05 nmol g⁻¹ FW) while lowest MDA concentration was observed in G3 (5.12 nmol g⁻¹ FW).

Figure 4. Drought stress effect on membrane damage and lipid peroxidation of wheat genotypes. Contents of electrolyte leakage (EL) and malondialdehyde (MDA) for six wheat genotypes (G1–G6) under well-watered control (D0), moderate drought (D1) and severe drought (D2). Values are given as mean ± SEM.

Table 2
Physiological and biochemical responses of wheat genotypes under severe drought

Genotype	RWC (%)	Total chlorophyll (mg g ⁻¹ FW)	Electrolyte leakage (%)	MDA (nmol g ⁻¹ FW)	Proline (μmol g ⁻¹ FW)	Soluble sugars (mg g ⁻¹ FW)
G1	72.8	1.76	34.1	5.36	8.42	31.6
G2	63.5	1.43	43.6	6.71	6.84	27.3
G3	74.6	1.83	32.7	5.12	8.91	33.2
G4	61.8	1.36	46.2	7.14	6.31	25.8
G5	55.3	1.18	51.8	8.05	5.47	22.6
G6	53.7	1.12	54.2	8.42	5.18	21.9

3.4 Osmolyte Accumulation

Under drought stress, the concentration of proline and soluble sugar increased and significantly higher levels were observed with resistant varieties. G3 had the highest concentration of proline (8.91 μmol g⁻¹ FW) while G1 had the highest concentration of proline (8.42 μmol g⁻¹ FW) during severe drought. The highest level of soluble sugar was obtained by G3 (33.2mg g⁻¹ FW).

3.5 Antioxidant Enzyme Activities

The effect of drought on the activities of SOD, CAT, POD, and APX is shown in Table 3. Drought stress caused a significant increase in the activity of these enzymes, especially in G1 and G3. G3 exhibited the

maximum SOD and CAT activity while G1 was closely followed in case of severe drought. The increase in antioxidant activity was relatively small in G5 and G6, however. An increase in the antioxidant activity was correlated with a decrease in electrolyte leakage and MDA accumulation.

Table 3
Antioxidant enzyme activities under severe drought

Genotype	SOD (U mg ⁻¹ protein)	CAT (U mg ⁻¹ protein)	POD (U mg ⁻¹ protein)	APX (U mg ⁻¹ protein)
G1	92.4	31.8	48.6	19.7
G2	78.6	26.2	40.5	16.3
G3	96.8	34.1	51.3	21.2
G4	72.5	23.8	37.2	14.8
G5	64.3	20.1	31.6	12.4
G6	61.8	18.9	29.7	11.8

3.6 Trait Relationships and Genotype Classification

Correlation analysis revealed positive relationship between total dry biomass and other variables as RWC ($r = 0.86$), total chlorophyll ($r = 0.81$), proline ($r = 0.74$), soluble sugars ($r = 0.77$) and antioxidant enzyme activities ($r = 0.68-0.82$). On the other hand, biomass was negatively correlated with electrolyte leakage ($r = -0.84$) and MDA ($r = -0.87$). These relationships were confirmed by principal component analysis. The first two principal components accounted for about 76.4% of the overall variance. The increases in biomass retention (RWC), chlorophyll, accumulation of osmolyte and antioxidant activities were grouped with G1 and G3 while the increase in electrolyte leakage and increase in MDA accumulation were grouped with G5 and G6. According to the integrated morphophysiological and biochemical responses, the plants were classified as drought tolerant (G3 and G1) drought moderately tolerant (G2 and G4) and drought sensitive (G5 and G6). In general, the traits related to tissue water status maintenance, enhanced osmotic adjustment and oxidative stress protection, along with reduced oxidative damage in membranes were the most prominent features of drought tolerance in the wheat varieties that were tested.

4. Discussion

The growth, physiological performance, osmotic adjustment, membrane stability, and antioxidant defense of the wheat genotypes evaluated were significantly affected by drought stress. These responses were more intense with increasing drought severity and significant differences between genotypes identified, suggesting that there was useful variation for drought tolerance. G1 and G3 overall performance was better than the other genotypes while G5 and G6 were more sensitive towards water deficit. The decrease in shoot height and plant biomass in drought compared with well-watered indicates

that drought stress reduced cell expansion, photosynthesis, nutrient uptake and dry matter production, consistent with other reports of inhibitory effects of drought. The highest biomass reductions were due to severe drought in G5 and G6. G1 and G3 conversely had relatively high amounts of shoot and root biomass. Maintenance of biomass under water deficit is regarded as an important indicator of drought adaptation, as it is closely related to water conservation, photosynthetic performance and metabolic stability. The relative greater root development found in tolerant genotypes treated with moderate drought condition may also have helped to acquire water from soil. As the drought severity increased, RWC decreased gradually. G1 and G3 however, exhibited much higher RWC than susceptible genotypes under severe drought. Water deficit avoidance and water uptake efficiency, stomatal regulation, osmotic adjustment and reduced water loss are correlated with maintenance of leaf hydration during water deficit. These genotypes also had a higher RWC, which was correlated with better biomass retention and therefore directly accounted for a better drought performance. Drought stress also caused a loss of chlorophyll and the highest loss was seen in the G5 and G6 treatments. Under drought, the loss of chlorophyll can occur due to its degradation, inhibition of its biosynthesis, oxidative damage and disruption of chloroplast membranes. The higher chlorophyll concentration that was generally maintained by G1 and G3 plants, however, suggested more stability in the photosynthetic apparatus of the plants. The higher retention of chlorophyll under drought-conditions might explain in part the higher biomass production of these genotypes, and could help maintain carbon assimilation. The amount of electrolyte leakage and MDA significantly rose with increasing drought severity, thus demonstrating that drought stress caused injury to membranes and lipid peroxidation. The two most susceptible genotypes, G5 and G6 had the highest electrolyte leakage and MDA levels. G1 and G3 showed a lower level of both parameters, on the contrary. The present results indicate that tolerant genotypes had higher membrane stability and were more efficient in the prevention of oxidative damage. The strong negative relationships also observed between electrolyte leakage and MDA with biomass further indicates their usefulness as drought sensitivity indicators. Another key difference between the genotypes was the build-up of compatible osmolytes. The levels of proline and soluble sugars were higher during drought, but much higher in G1 and G3. These compounds are involved in osmotic adjustment, protein and membrane stabilization, maintenance of cellular hydration and protection against oxidative stress. This increased accumulation of osmolyte could thus have played a role in the increased RWC and reduced injury to membranes observed in tolerant genotypes. However, concentrations of each osmolyte must be read in conjunction with growth and physiological responses as an accumulation of these does not indicate drought tolerance. Growth in activities of SOD, CAT, POD and APX were stimulated by drought stress, specifically in G1 and G3. Antioxidant enzymes activity levels are increased and protect from excessive production of ROS in water deficits. The SOD activity is a crucial first-line defense against the production of superoxide radicals, while the activities of CAT, POD and APX are involved in the following steps of hydrogen peroxide detoxification. The higher antioxidant activities along with the decreased MDA and electrolyte leakage of the genotypes G1 and G3 indicate that these genotypes had more efficient ROS scavenger system. Correlation analysis also revealed that drought tolerance was the result of coordinated response and not a single trait. Biomass was positively correlated with RWC, chlorophyll, proline, soluble sugars and antioxidant enzyme activities whereas negatively related with MDA and

electrolyte leakage. The same was true for PCA, which distinguished the relatively tolerant from the susceptible genotypes. These results justify the choice of integrated screening for drought tolerance (morphophysiological and biochemical) over screening on one parameter alone.

Overall, G3 had the most positive drought adaptive response, closely followed by G1. They exhibited increased biomass retention, reduction in leaf water status loss, maintenance of chlorophyll, increased accumulation of osmolytes, improved antioxidant defense, and decreased oxidative membrane damage. G5 and G6 were drought susceptible (OPPOSITE) and G2 and G4 intermediate. These contrasting genotypes are good sources for the molecular characterization of the drought responsive genes and drought tolerance mechanism in wheat.

5. Conclusion

The present study showed significant differences in drought tolerance in wheat genotypes, under the semi arid soil conditions. Droughts caused a significant decrease in growth, biomass, relative water content, and chlorophyll but an increase in the accumulation of osmolytes, oxidative damage, and antioxidant enzyme activities. These reactions varied greatly from genotype to genotype. The genotypes G3 and G1 were found as the most drought tolerant, as they retained higher biomass, RWC and chlorophyll content with high proline, soluble sugars and antioxidant enzyme activities. They also showed less electrolyte leakage and MDA accumulation, which suggested that they had better membrane stability and protection against oxidative stress. However, G5 and G6 were determined to be drought sensitive and G2 and G4 moderate drought tolerant. The findings show that the physiological responses such as RWC, chlorophyll retention, biomass maintenance, proline, soluble sugars, MDA, electrolyte leakage and antioxidant enzyme activities could be utilized together as effective screening traits for drought tolerance in wheat. These tolerant and susceptible genotypes identified represent contrasting plant material for more in-depth molecular characterization of genes associated with stress and are also a resource for wheat breeding programs seeking to develop improved wheat cultivars for water limited and semi arid conditions.

Declarations

Author Contribution

S.B. conducted the experiments, collected the data, performed laboratory analyses, and contributed to preparation of the original manuscript. K.B. contributed to experimental methodology, data analysis, and interpretation of the results. S.A. assisted with biochemical analyses, data interpretation, and manuscript preparation. S.Z.U.A. contributed to experimental work, data collection, and statistical analysis. N.U. conceived and designed the study, supervised the research, interpreted the results, and critically revised the manuscript. A.Z. contributed to data interpretation, manuscript review, and scientific editing. All authors read and approved the final manuscript.

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Figures



Figure 1

Wheat plants' response to various intensities of drought stress is presented visually. Well watered control (D0, 80 to 90% field capacity), moderate drought (D1, 50 to 60% field capacity), and severe drought (D2, 30 to 40% field capacity) were represented by plants of a single cultivar of wheat. As water deficit increased, plant growth and leaf greenness decreased and significant leaf senescence and growth inhibition occurred under severe drought.

2.6 Imposition of Drought Stress

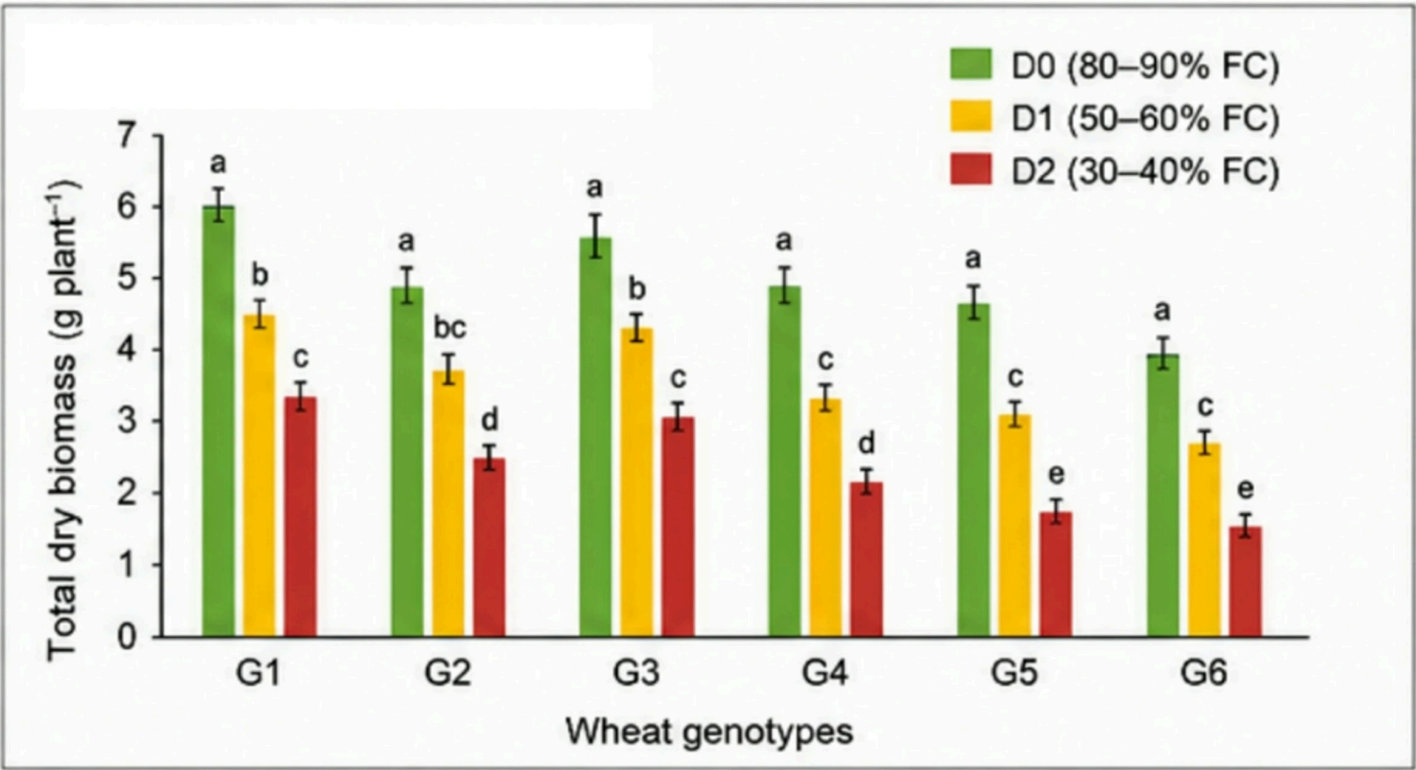


Figure 2

The effect of drought stress on total dry biomass of wheat genotypes. Dry biomass of six wheat genotypes (G1 to G6) in well watered (D0: 80 to 90% field capacity), moderate drought (D1: 50 to 60% field capacity) and severe drought (D2: 30 to 40% field capacity). Values are presented as means $\pm$ SE.

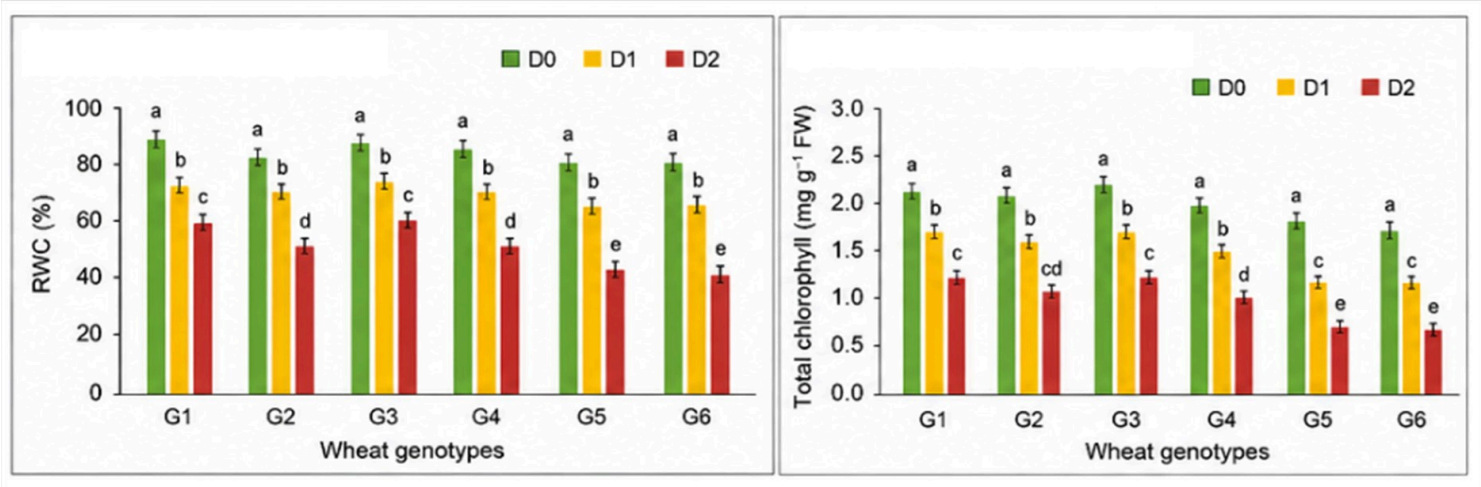


Figure 3

Drought stress effects on RWC and TChl of wheat genotypes. Relative water content (left) and total chlorophyll content (right) of six wheat genotypes (G1-G6) under well-watered control (D0), moderate drought (D1) and severe drought (D2). All values are given as mean $\pm$ SE.

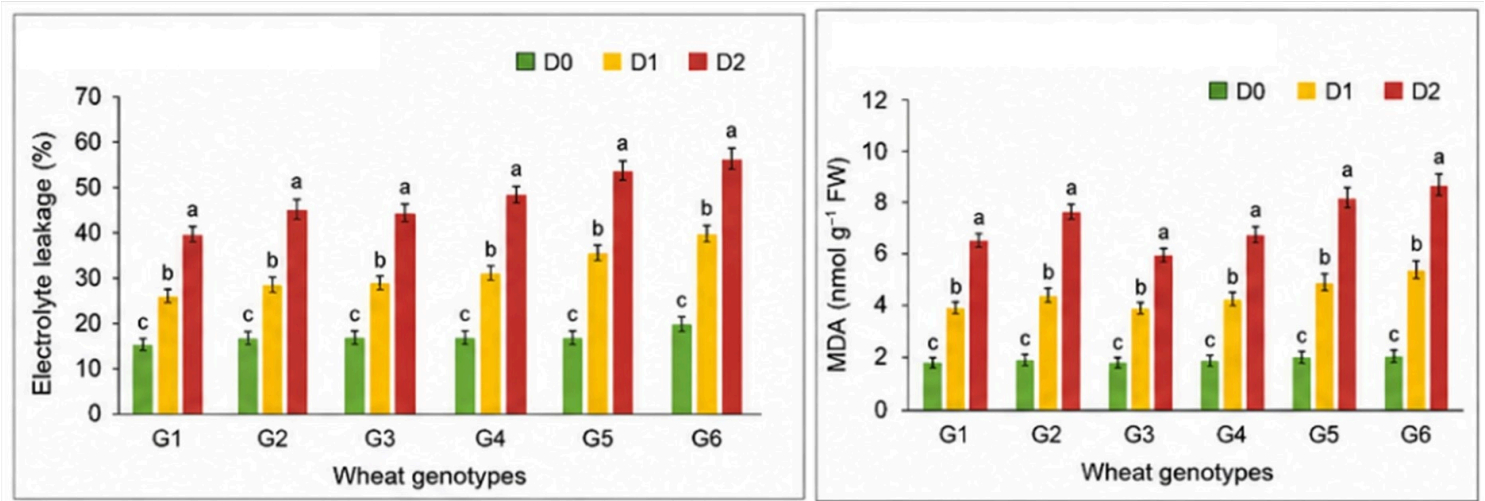


Figure 4

Drought stress effect on membrane damage and lipid peroxidation of wheat genotypes. Contents of electrolyte leakage (EL) and malondialdehyde (MDA) for six wheat genotypes (G1–G6) under well-watered control (D0), moderate drought (D1) and severe drought (D2). Values are given as mean $\pm$ SEM

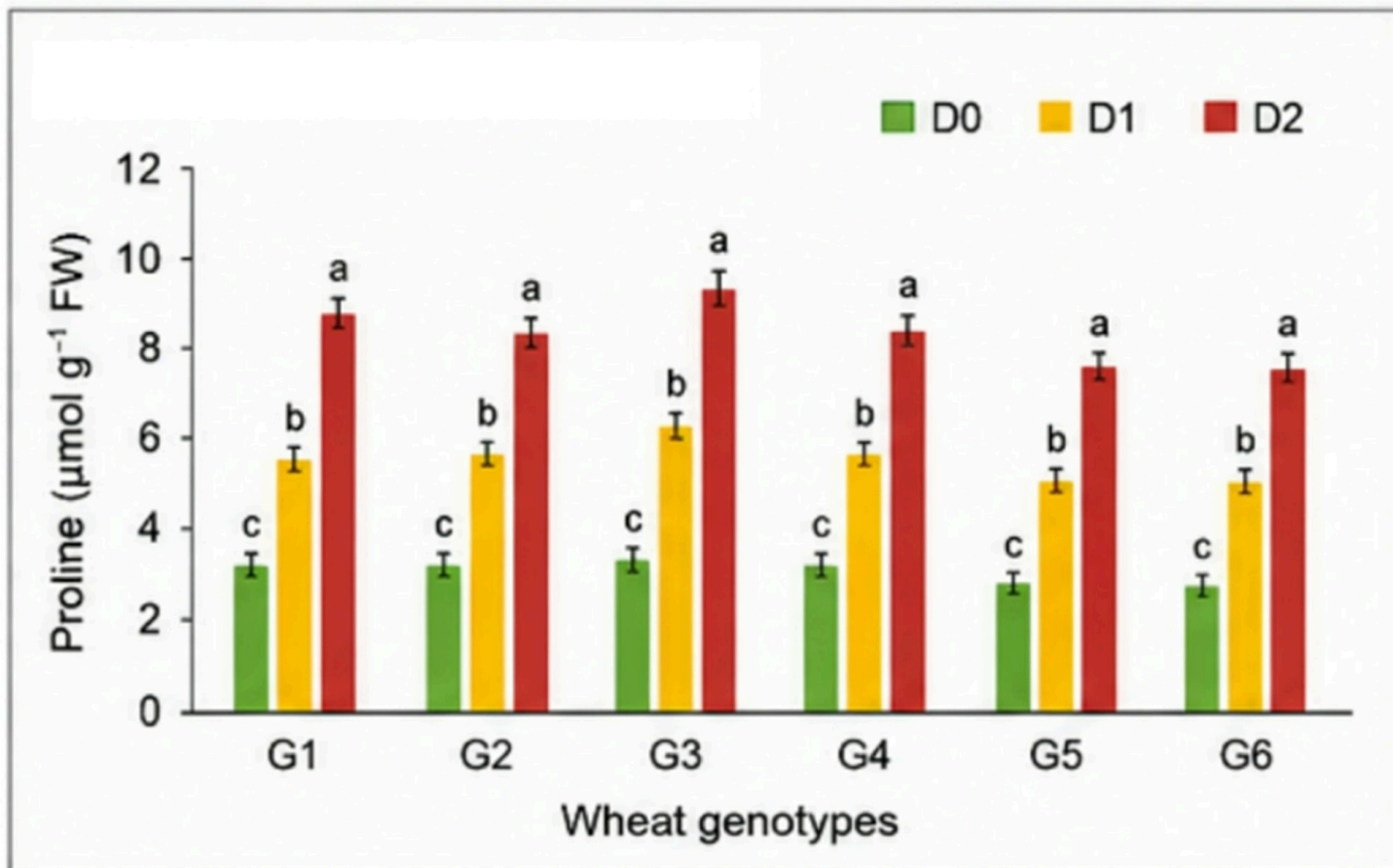


Figure 5

Response of wheat genotypes to drought stress by change in proline content. The content of proline in six wheat genotypes (G1 to G6) cultivated in well-watered (D0), moderate drought (D1), and severe drought (D2). Values are means $\pm$ SE.

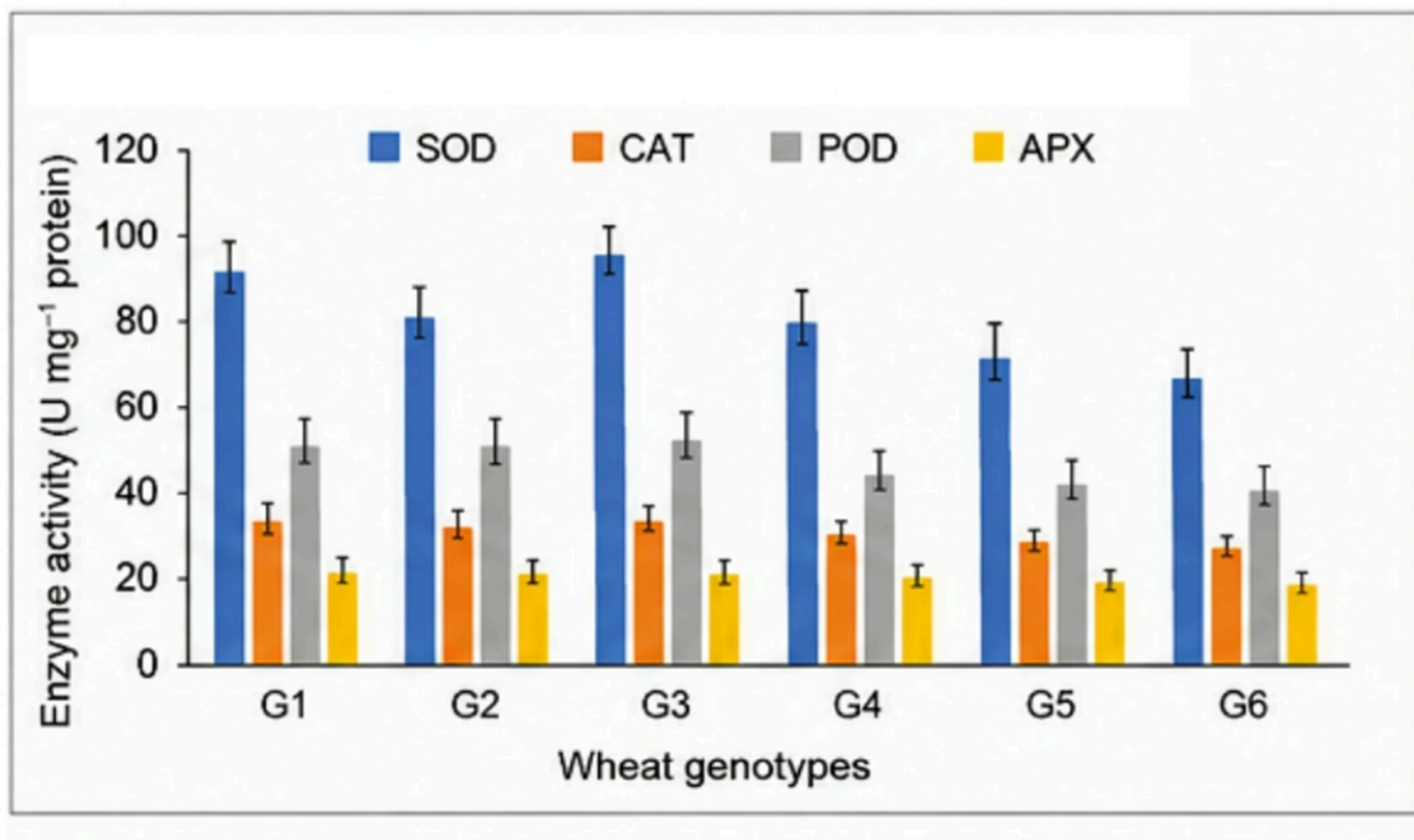


Figure 6

Wheat genotypes' activities of antioxidant enzymes under severe drought stress. Superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) activity of the six wheat genotypes (G1 to G6) subjected to severe drought (D2, 30 to 40% field capacity). Values are means $\pm$ SE.