Indian Journal of Biochemistry & Biophysics
Vol. 61, September 2024, pp. 563-573
DOI: 10.56042/ijbb.v61i9.10326

Deciphering the physio-biochemical dynamics of wheat (*T. aestivum* L.) genotypes during water deficit stress

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Received 10 April 2024; *revised 02 June 2024*

Water deficit stress poses a significant challenge to wheat cultivation worldwide, necessitating the identification of indicator traits for plant productivity under such conditions. This study investigated the physiological and biochemical responses of four wheat varieties (C-306, DBW 110, DBW 136, and GW 451)under varying levels of water deficit stress (100%, 75%, and 35% FC). The results indicated that increasing water deficit stress resulted in notable decreases in physiological parameters such as the relative water content (RWC), chlorophyll content, chlorophyll stability index (CSI) and membrane stability index (MSI) at both the booting and grain filling stages. In response to water deficit stress, the plants increased their levels of proline, total soluble sugars (TSS), total phenol and free amino acids (FAA). Furthermore. oxidative damage, indicating that parameters such as proline concentration and malondialdehyde (MDA) content increased. Water deficit conditions markedly promoted the activities of key antioxidant enzymes, including catalase and peroxidase. Among the varieties, C-306 and DBW 110 were less affected by water deficit stress, as shown by significant changes in their physiological and biochemical parameters. These results highlight the importance of assessing physio-biochemical traits related to phenotypic water deficit stress tolerance in wheat varieties, providing valuable insights for breeding programs aimed at developing and selecting drought-tolerant cultivars.

Keywords: Catalase, Field capacity, Membrane stability index, Peroxidase, Relative water content

Wheat is an important crop in India that faces challenges from high temperatures and water scarcity, which often impede its growth and yield. Drought, in particular, significantly reduces wheat productivity, leading to potential yield losses ranging from 50% to 90%[1]. Among various stressors, water scarcity is a primary limitation to crop productivity[2]. It disrupts the plant's life cycle and grain-filling period, reducing photosynthesis and accelerating senescence[3]. Water scarcity affects wheat plants in diverse ways, causing changes in protein composition, stimulating antioxidant production, facilitating osmotic adjustment, modulating hormone levels, influencing root morphology and depth, regulating stomatal conductance, and affecting chlorophyll content and transpiration rates[4]. The impact of water deficiency varies across different growth stages, altering yield components such as plant density, spike and grain numbers per plant or unit area, and individual grain weight, each assessed at various growth phases[5]. Notably, water stress during the spike-growing period, particularly around anthesis, significantly diminishes wheat yield potential[6]. Improving water deficit stress tolerance and productivity remains a challenging task for breeders. Understanding how plants respond to water deficit stress is essential for creating stress-tolerant crops that can meet the demand for food grains. Maintaining a greater yield throughout various stress and non-stress conditions is crucial for cultivars[7,8]. Under stressful conditions, plant survival depends on the ability of plants to perceive, transmit, and respond to stimuli, initiating various physiological and biochemical adaptations[9]. In this context, the current study aimed to evaluate and characterize the physio-biochemical responses of wheat to water deficit stress, contributing insights into the mechanisms essential for developing stress-tolerant cultivars.

Materials and Methods

Plant material and water stress induction

This study utilized four wheat genotypes, C-306, DBW 110, DBW 136, and GW 451, sourced from the Wheat Research Station at JAU, Junagadh. The experiment was performed in a completely randomized

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design (CRD) with four replications in the net house of the Department of Genetics and Plant Breeding, JAU, Junagadh, during the *Rabi* season of 2019-20. Plastic bags (8×5 inch) filled with a soil mixture consisting of soil, sand, and vermicompost at a 1:1:1 ratio and NPK applied at a ratio of 4:2:1 were used to sow the seeds. All pots were initially kept wet until 30 days following seeding. After that, irrigation was adjusted to maintain three different levels of field capacity (FC): T1 Control (100% FC), T2 (75% FC), and T3 (35% FC). The field capacity of the soil was determined using the gravimetric method. Stress conditions were induced at the booting stage by withholding water to reach 75% and 35% of field capacity (FC). After one week of stress imposition, watering was resumed to facilitate recovery. Plant samples were randomly selected per treatment in each replicate to record observations of various traits, and their averages were utilized for statistical analysis. Physiological parameters, biochemical parameters, and antioxidant enzyme activities were measured in flag leaves at the booting stage, five days after stress induction.

Physiological parameters

Leaf relative water content (RWC)

The leaf relative water content (RWC) was determined according to the method of Barrs[10]. Fully developed leaves from each pot were cut at the base of the leaf blade and then weighed immediately to obtain the fresh weight (FW). They were then placed in a Petri dish filled with distilled water. After 24 h, the leaves were removed from the Petri dish, placed on blotting paper to water from the surface, and then weighed to obtain the turgid weight (TW). The samples were finally placed in a hot air oven at 80°C for 48h and weighed to obtain the dry weight (DW). The leaf relative water content was calculated by the following formula according to Clark and Mac-Caig[11]:

RWC (%) = [(FW-DW)/(TW-DW)] x 100.

Membrane Stability Index (MSI)

For MSI determination, 0.5 g of leaf sample was weighed and placed in a test tube containing 50 mL of distilled water. One set of samples was placed in a water bath at 40°C for 30 min, and the conductivity (C1) for electrolytic leakage was measured using a conductivity meter. Another set was exposed to boiling water (100°C) for 10 min, followed by recording the conductivity (C2) after cooling to room temperature[12]. The MSI was calculated using the following formula: MSI = 1-(C1/C2) × 100.

Chlorophyll and Carotenoid Contents

Fresh leaf samples (0.2 g) were ground in a mortar and pestle using 80% acetone. The mixture was then filtered, and the resulting solution was centrifuged to remove any insoluble particles. The supernatant was carefully transferred to a fresh test tube. Subsequently, the concentration of chlorophyll in the extract was measured using a spectrophotometer at wavelengths of 645 nm, 663 nm and 470 nm. The concentrations of chlorophyll and carotenoids were calculated following the method described by Lichtenthaler (1987) and are expressed as mg g^{-1} FW.

Chlorophyll Stability Index (CSI)

The CSI was determined by subjecting leaf samples to a water bath at 56°C ± 1°C for 30 min, after which the samples were ground in 100 mL of 80% acetone. The absorbance of the filtrate was measured using a spectrophotometer at wavelengths of 645 nm and 663 nm. The CSI was calculated by using the formula provided by Sairam[14].

Biochemical parameters

Total Phenol

Total phenol was measured by using the method outlined by Bray and Thorpe[15]. Leaf samples (0.2 g) were ground in a mortar and pestle with 5 mL of 80% methanol. From the methanol extract prepared for analysis, 0.1 mL of sample extract was removed and evaporated to dryness in a water bath. Subsequently, 1 mL of distilled water and 0.5 mL of folin-Ciocaltu (F-C) assay (diluted 1:1 with water) were added to each test tube. The reaction mixture was left to stand for 3 min before adding 2 mL of 20% sodium carbonate and thorough mixing. The tubes were then placed in boiling water for exactly 1min and cooled in ice water. The absorbance of the filtrate was measured at 650 nm using a spectrophotometer against a reagent blank, and the amount was calculated in mg g^{-1} FW using a standard curve prepared with different concentrations of catechol.

Free amino acids

Leaf samples (0.25 g) were homogenized with 5 mL of phosphate buffer (pH 7) and centrifuged for 10 min at 14000 rpm. One milliliter of extract was mixed with 2% ninhydrin and 10% pyridine, and the final volume was adjusted to 15 mL. The mixture was heated in a water bath at 90°C for 30 min and then cooled. The absorbance of the solution was measured at 570 nm following the method described by Crampton *et al.*[16].

Total soluble sugar content

The soluble sugar content was determined spectrophotometrically from the soluble and residual fractions of ethanol-water extracts described by Dubois *et al.*[17]. First, 0.5 mL of soluble sugar extract and 4.5 mL of 80% ethanol were combined in test tubes. The test tubes were placed in an ice bath, and 10 mL of anthrone reagent was slowly added. The test tubes were placed in a boiling water bath for 10 min and immediately cooled in an ice bath after the absorbance of the extract was measured at 630 nm after 1 hr.

Malondialdehyde (MDA) Content

The MDA content was determined by using the method described by Hodges *et al.*[18] with some modifications. Leaf samples (0.5 g) were homogenized in 4 mL of 1% (*w/v*) trichloroacetic acid (TCA) and then centrifuged at 10,000 × g for 10 min. Then, 1.5 mL of the supernatant was mixed with 1.5 mL of 0.5% (*w/v*) TBA in 20% (*w/v*) TCA. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10000 × g for 5 min, the absorbance of the supernatant was recorded at 440, 532 and 600 nm. The concentration of TBARS (µmole g^{-1} FW) was calculated by using an extinction coefficient of 155 mM^{-1} cm^{-1}, and the results are expressed as µmole MDA equivalents per gram.

Proline Content

The proline content was estimated by using the method described by Bates *et al*[19]. Free proline was extracted by using 100 mg of leaf samples at 3% sulfosalicylic acid and estimated by ninhydrin reagent. The absorbance of the extract with toluene aspirated from the liquid phase was read at 520 nm. The proline concentration was determined using a standard curve and was expressed as µmole g^{-1} FW.

Enzyme Assay

Five hundred milligrams of fresh leaf sample was crushed in 2 mL of extraction buffer containing 50 mM sodium phosphate buffer (pH 7.0) and 0.1 mM Na-EDTA through a mortar and pestle. The extract was centrifuged at 15000 × g for 15 min, and the collected supernatants were utilized for assessing various antioxidant enzymes. All steps involved in the preparation of the enzyme were carried out at 4°C.

Peroxidase (EC 1.11.17) activity was estimated by using the method of Perur[20] (1962). The reaction mixture for the estimation of peroxidase activity contained 2.99 mL of 0.03% H_2O_2 in 0.1 M phosphate buffer (pH 6.0) containing 0.01% Ortho dianisidine dye (freshly prepared and dissolved in methanol). The reaction was initiated by the addition of 10 µL of enzyme extract. The absorbance was measured at 460 nm for up to 1 min at intervals of 15 sec when there was a change in the color of the oxidized dye. The blank was run without the addition of enzymes. The specific activity was expressed as the Δ OD. g^{-1} protein. min^{-1}.

Catalase (EC 1.11.1.6) activity was estimated by using the method of Aebi[21]. The 3 mL reaction mixture for the catalase estimation contained 50 mM sodium phosphate buffer (pH 7.0), 30 mM H_2O_2 and 50 µL of enzyme extract. The catalase activity was estimated by measuring the decrease in absorbance at 240 nm. The specific activity was measured as Δ O.D. g^{-1} protein. min^{-1}.

Statistical analysis

The collected data were subjected to analysis of variance (ANOVA), and the mean differences were compared using the Duncan multiple range test ($P \leq 0.05$). The results are expressed as the means ± standard errors (SEs). Correlation analysis was performed to identify significant correlations among the studied attributes. Figures were plotted using Prism GraphPad 9.2 software (GraphPad Software Inc., CA, USA).

Results

Physiological parameters

This study revealed significant impacts on physiological traits, including the relative water content (RWC), chlorophyll content, chlorophyll stability index, and membrane stability index (MSI), due to different water deficit stress levels at both the booting and grain filling stages (Table 1). Notably, the various water regimes significantly influenced the physiological traits of the wheat plants, especially compared to those of the control. Changes in RWC in wheat leaves as a result of increasing water deficit stress. Under irrigated conditions (100% FC), all varieties maintained high RWC levels at both the booting and grain filling stages. However, when exposed to progressively decreasing moisture levels, all varieties exhibited a reduction in RWC. The greatest percentage reduction in RWC was noted in GW 451 and DBW 136, which also exhibited the lowest RWC values relative to those of the other varieties under low moisture conditions (35% FC) at

Table 1 — Effect of different levels of drought stress on the relative water content (RWC), chlorophyll content, chlorophyll stability index (CSI) and membrane stability index (MSI) of different bread wheat cultivars at the booting and grain filling stages

		RWC (%)*		Chlorophyll content (mg g^{-1} FW)*		CSI (%)*		MSI (%)*	
Genotypes	Treatments	Booting stage	Grain filling stage	Booting stage	Grain filling stage	Booting stage	Grain filling stage	Booting stage	Grain filling stage
C-306	T_1: (100% FC)	89.19±4.48a	82.66±3.03a	3.00±0.12a	2.28±0.07a	28.03±1.53a	23.91±0.76a	33.92±1.21a	38.76±2.67a
	T_2: (75% FC)	74.21±3.01b	71.57±3.17b	2.00±0.09c	1.45±0.07b	19.91±1.24c	17.03±0.96b	25.73±3.31c	31.66±4.03c
	T_3: (35% FC)	56.06±3.24d	49.21±2.69c	1.04±0.12e	0.89±0.11de	10.50±0.98e	10.81±1.62c	17.44±0.74e	21.97±0.72e
DBW 110	T_1: (100% FC)	89.77±2.52a	83.02±3.03a	2.99±0.17a	2.16±0.08a	29.20±2.22a	24.14±1.32a	35.02±2.49a	39.25±2.19a
	T_2: (75% FC)	73.14±2.59bc	70.55±3.84b	1.87±0.11c	1.38±0.09b	19.15±2.14c	16.44±0.69b	24.17±1.76cd	27.97±1.64d
	T_3: (35% FC)	55.47±2.48d	50.59±4.12c	0.89±0.10f	0.94±0.11d	11.66±1.49e	10.01±1.10c	15.95±0.86ef	19.96±1.52ef
DBW 136	T_1: (100% FC)	86.55±2.67a	81.28±1.90a	2.85±0.06b	2.19±0.09a	25.34±0.74b	23.43±1.08a	32.44±2.51a	38.19±2.51a
	T_2: (75% FC)	67.77±3.90c	66.30±4.26b	1.64±0.05d	1.33±0.09bc	15.940.90d	16.37±0.49b	28.37±0.79b	33.11±0.94bc
	T_3: (35% FC)	35.97±7.47e	39.78±5.19d	0.55±0.05g	0.83±0.09de	8.42±1.03f	10.38±2.05c	14.02±1.25f	18.30±0.69f
GW 451	T_1: (100% FC)	84.15±3.63a	82.15±3.63a	2.84±0.05b	2.18±0.08a	24.70±0.77b	22.44±0.80a	29.81±1.86b	34.92±2.55b
	T_2: (75% FC)	70.29±1.07bc	70.11±3.59b	1.53±0.05d	1.23±0.04c	16.93±1.69d	16.53±0.63b	21.89±1.27d	27.51±1.31d
	T_3: (35% FC)	35.21±3.81e	41.09±2.90d	0.54±0.05g	0.81±0.07e	8.35±1.21f	10.27±0.70c	16.92±1.32e	20.87±1.50ef
	G	513.0**	464.5**	2134.2**	1027.1**	586.0**	563.0**	351.0**	285.3**
F value	T	27.0**	6.6**	45.5**	5.5**	19.9**	1.2^{NS}	5.6**	4.6**
	G x T	8.4**	2.6*	3.7**	1.4**	0.8^{NS}	0.7^{NS}	6.4**	4.1**

*Means (±SE) (n=4) followed by different letters in each column are significantly different ($P \leq 0.05$) according to DMRT

both the booting and grain filling stages. On the other hand, at both the booting and grain filling stages, DBW 110 and C-306 maintained relatively high RWC values and exhibited a lower percentage reduction than did the other varieties under the same moisture level (35% FC). In the present study, there was an inverse relationship between the moisture stress level and total chlorophyll content for all the varieties at both stages. Differences in chlorophyll content were also observed among the varieties. Under irrigated conditions (100% FC), all varieties maintained a high total chlorophyll content at both the booting and grain filling stages. However, when exposed to progressively decreasing moisture levels, all varieties exhibited a reduction in total chlorophyll content. The greatest percentage reduction in total chlorophyll was noted in GW 451 and DBW 136, which also exhibited the lowest total chlorophyll content values relative to those of the other varieties under low moisture conditions (35% FC) at both the booting and grain filling stages. On the other hand, at both the booting and grain filling stages, DBW 110 and C-306 maintained relatively high total chlorophyll contents and exhibited a lower percentage reduction than did the other varieties under the same moisture level (35% FC). The chlorophyll stability index was significantly influenced by different genotypes and moisture stress levels. The C-306 and DBW 110 genotypes exhibited greater chlorophyll stability under low moisture (35% FC) conditions, while all the other genotypes experienced a decrease in chlorophyll stability under the same moisture levels. Among all the varieties examined, C-306 and DBW 110 retained relatively high chlorophyll contents when exposed to low moisture stress at both the booting and grain filling stages. The sensitive varieties (DBW 136 and GW 451) had lower chlorophyll contents under low moisture stress. The low moisture stress-induced decrease in the total chlorophyll content signifies that moisture stress induced a significant decrease in the number of photosynthetic reaction centres. The membrane stability index (MSI) decreased with increasing water deficit stress compared to that of the control (100% FC) at both stages. However, when exposed to progressively decreasing moisture levels, all varieties exhibited a reduction in the MSI. The greatest percentage reduction in MSI was noted in GW 451 and DBW 136, which also exhibited the lowest MSI values relative to those of the other varieties under

Table 2 — Effect of different levels of drought stress on the free amino acid (FAA), total soluble sugar (TSS), total phenol, and carotenoid contents of different bread wheat cultivars at the booting and grain filling stages

		FAA (mg g^{-1} FW)*		TSS (mg g^{-1} FW)*		Total phenol (mg g^{-1} FW)*		Carotenoid (mg g^{-1} FW)*	
Genotypes	Treatments	Booting stage	Grain filling stage	Booting stage	Grain filling stage	Booting stage	Grain filling stage	Booting stage	Grain filling stage
C-306	T_1: (100% FC)	0.75±0.05e	0.82±0.02ef	8.14±0.56ef	7.98±0.67d	1.35±0.10d	2.45±0.10d	0.64±0.07a	0.65±0.03a
	T_2: (75% FC)	0.99±0.01c	1.08±0.07cd	8.99±0.74abc	9.03±0.30ab	1.68±0.09bc	2.90±0.04b	0.50±0.06c	0.53±0.05de
	T_3: (35% FC)	1.26±0.18a	1.35±0.09a	9.67±0.23a	9.57±0.43a	2.17±0.08a	3.36±0.17a	0.46±0.03cd	0.45±0.03gh
DBW 110	T_1: (100% FC)	0.73±0.03e	0.88±0.02e	8.26±0.45def	8.29±0.24cd	1.27±0.24d	2.52±0.16cd	0.58±0.06b	0.60±0.04b
	T_2: (75% FC)	0.91±0.02cd	1.10±0.08bc	9.36±0.60ab	9.16±0.49ab	1.63±0.20c	2.91±0.09b	0.47±0.02cd	0.50±0.02ef
	T_3: (35% FC)	1.14±0.14b	1.34±0.07a	9.67±0.21a	9.60±0.13a	2.19±0.10a	3.40±0.11a	0.43±0.03de	0.45±0.03g
DBW 136	T_1: (100% FC)	0.67±0.00e	0.79±0.01ef	7.65±0.53f	7.91±0.94d	1.27±0.11d	2.33±0.26d	0.51±0.01c	0.58±0.03bc
	T_2: (75% FC)	0.87±0.00d	1.04±0.09cd	8.58±0.39cde	8.55±0.39bcd	1.79±0.12bc	2.89±0.11b	0.42±0.02def	0.47±0.01fg
	T_3: (35% FC)	1.01±0.02c	1.19±0.08b	9.49±0.35ab	9.52±0.41a	2.20±0.10a	3.16±0.40ab	0.37±0.01f	0.41±0.01hi
GW 451	T_1: (100% FC)	0.67±0.01e	0.75±0.05f	7.76±0.07f	7.92±12d	1.32±0.13d	2.44±26d	0.50±0.02c	0.56±0.02cd
	T_2: (75% FC)	0.87±0.00d	0.98±0.05d	8.91±0.09bcd	8.99±0.04ab	1.86±0.09b	2.92±0.08b	0.46±0.02cd	0.44±0.01gh
	T_3: (35% FC)	0.86±0.00d	1.13±0.09bc	9.01±0.52abc	8.79±0.29bc	2.23±0.17a	2.82±0.51bc	0.39±0.02ef	0.39±0.02i
F value	G	112.0**	175.5**	48.0**	38.6**	175.5**	41.7**	67.2**	166.4**
	T	19.0**	13.3**	4.4**	2.5^{NS}	1.3^{NS}	2.1^{NS}	18.1**	19.2^{NS}
	G x T	4.9**	1.5^{NS}	0.7^{NS}	1.4^{NS}	0.8^{NS}	1.7^{NS}	1.4^{NS}	0.7^{NS}

*Means (±SE) (n=4) followed by different letters in each column are significantly different ($P \leq 0.05$) according to DMRT

low moisture conditions (35% FC) at both the booting and grain filling stages. On the other hand, DBW 110 and C-306 sustained relatively high MSI values and showed a lower percentage reduction than did the other varieties under the same moisture level (35% FC) at both the booting and grain filling stages.

Biochemical parameters

Biochemical traits, including free amino acid (FAA), total soluble sugar (TSS), total phenol, and carotenoid content, were significantly affected by both genotype and water deficit stress level at both the booting and grain-filling stages (Table 2). The minimum free amino acid content in leaves was recorded at field capacity (100% FC, Control), and it increased with decreasing moisture level in all the genotypes. The greatest FAA content was noted in DBW 110 and C-306 under low moisture conditions (35% FC) at both the booting and grain filling stages. On the other hand, DBW 136 and GW451 sustained relatively low FAA values and showed greater reductions than did the other varieties under the same moisture levels (35% FC) at both the booting and grain filling stages. The total sugar content increased significantly under the water stress treatments at both stages, irrespective of the genotype. The minimum TSS in leaves was recorded at field capacity (100% FC, Control), and it increased with decreasing moisture level in all the genotypes. Sugar content was highest at the booting stage. The increase was considerably greater in the tolerant genotype C 306, followed by DBW 110, under low moisture conditions (35% FC) at both the booting and grain filling stages. The water deficit stress-induced accumulation of soluble sugars decreased in DBW 136 and GW 451. The total phenol content increased under water deficit stress compared to that under control conditions, with a greater increase observed under severe and moderate stress. Compared with the tolerant genotypes, the sensitive genotypes exhibited a lower increase in total phenol content. Under irrigated conditions (100% FC), all varieties had lower phenol contents at both the booting and grain filling stages. However, when exposed to progressively decreasing moisture levels, all varieties exhibited an increase in phenol content. The greatest increase in phenol content was noted in C-306 and DBW 110 relative to the other varieties under low moisture conditions (35% FC) at both the booting and grain filling stages. On the other hand, DBW 136 and GW 451 sustained relatively lower phenol contents under the same moisture levels (35% FC) at both the booting and

grain filling stages. The carotenoid content decreased significantly in all the genotypes at both the booting and grain filling stages. The carotenoid content was greater at the booting stage than at the grain filling stage. The water stress-induced decrease in carotenoids was less pronounced in tolerant genotypes at both the booting and grain filling stages. The decreases in carotenoid content were greater in susceptible genotypes at the booting and grain filling stages. The maximum reduction in carotenoid content under water stress occurred in DBW 136 and GW 451, and the minimum occurred in C-306 and DBW 110 at both stages. These findings demonstrate the significant influence of different water deficit stress levels on physiological and biochemical traits in wheat genotypes, highlighting the importance of selecting tolerant varieties for improved stress resilience.

The free proline content generally increased with increasing severity of stress compared to that of the control at both the booting and grain-filling stages (Fig. 1A and B). In response to water deficit stress, all varieties exhibited a steep increase in leaf proline content, and the rate of increase increased with increasing severity of water stress. The greatest proline accumulation was noted for genotypes C-306 and DBW 110 relative to the other varieties under low moisture conditions (35% FC) at both the booting and grain filling stages. The lowest proline concentration was recorded for DBW 136 and GW 451 under the same moisture level (35% FC) at both the booting and grain filling stages.

The level of lipid peroxidation, indicated by MDA accumulation, was assessed to determine the degree of oxidative damage caused by stress. Our findings indicated that MDA levels were significantly influenced by stress severity and variety (Fig. 1C and D). The MDA content was lower in the control (100% FC) treatment than in the water-stressed plants. Moreover, there was a progressive increase in lipid peroxidation levels with increasing stress severity. Under severe water deficit stress conditions (T3, 35% FC), GW 451 and DBW 136 exhibited the maximum MDA content, while C-306 and DBW 110 showed the least MDA accumulation under similar conditions during both the booting and grain-filling stages.

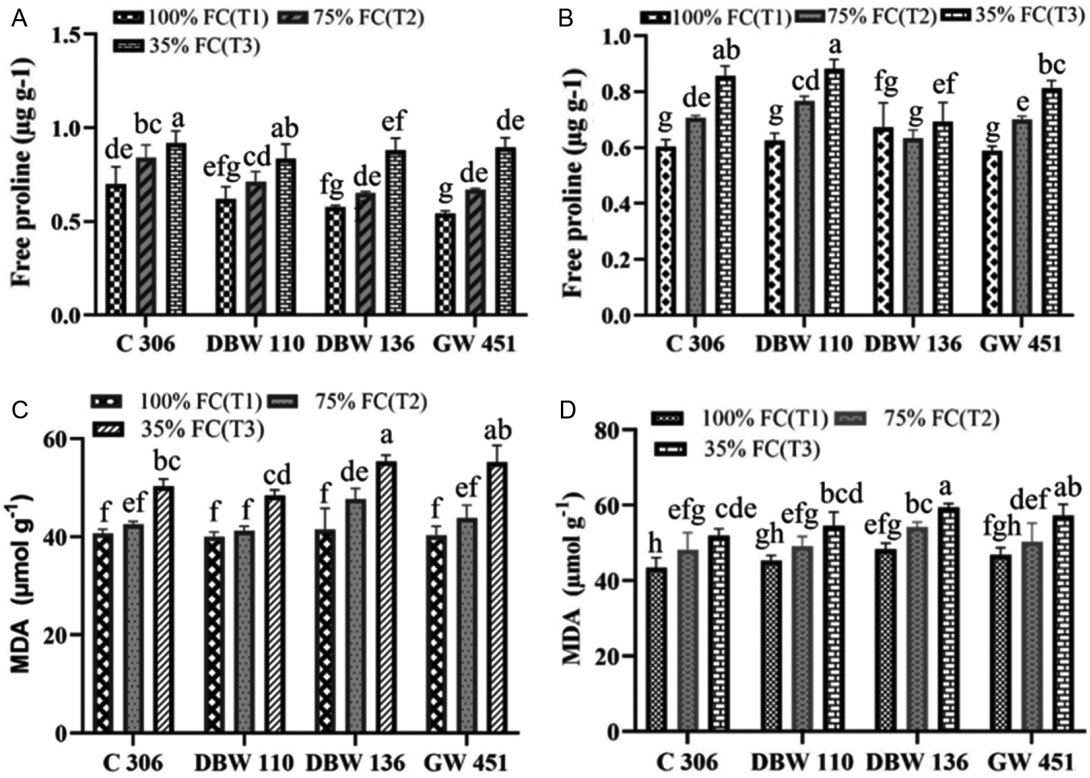

Fig. 1 — Effect of different levels of drought stress on the free proline content (µg^{-1} FW) at the (A) booting stage; (B) grain filling stage; the MDA content (µmol g^{-1} FW) at the; (C) booting stage; and (D) grain filling stage of different bread wheat cultivars. The data represent the means ± SEs of four separate measurements. The different letters on the top of the error bars indicate significantly different means between treatments at $P \leq 0.05$

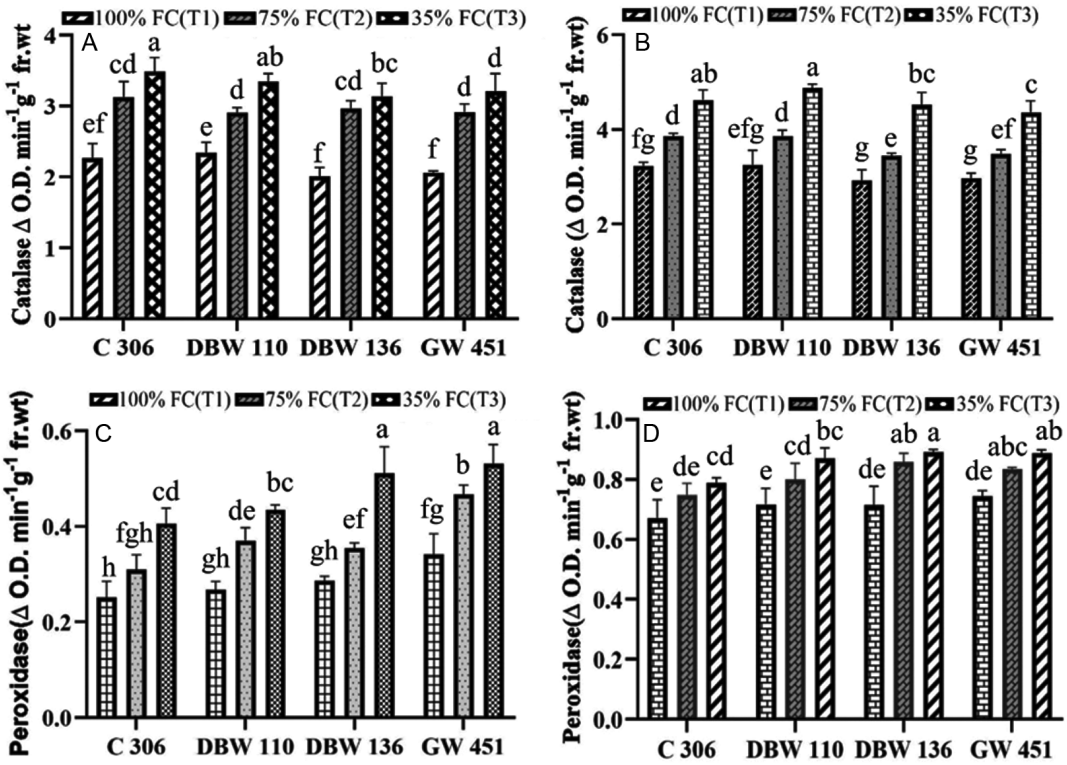

Fig. 2 — Effect of different levels of drought stress on the enzymatic activity of catalase (Δ O.D. min^{-1} g^{-1} FW) at the (A) booting stage; (B) grain filling stage; peroxidase (Δ O.D. min^{-1} g^{-1} FW) activity at the; (C) booting stage; and (D) grain filling stage of different bread wheat cultivars. The data represent the means ± SEs of four separate measurements. The different letters on the top of the error bars indicate significantly different means between treatments at $P \leq 0.05$

Antioxidant enzyme assays

During periods of water deficit stress, the activities of the enzymatic antioxidants catalase (CAT) and peroxidase (POD) tended to increase (Fig. 2A-D). This increase was particularly notable, peaking at 35% FC. Overall, the activities of antioxidant enzymes were significantly greater under severe stress conditions (35% FC) than under the control conditions, with a marked distinction between growth stages. Notably, enzyme activity was greater during the grain-filling stage than during the booting stage under water deficit stress. The C-306 cultivar displayed the highest catalase activity, which was statistically comparable to that of the DBW 110 genotype, while GW 451 exhibited the lowest catalase activity at both the booting and grain-filling stages. Furthermore, C-306 and DBW 110 demonstrated significantly greater catalase activity than DBW 136 and GW 451 across both growth stages ($P \leq 0.05$). In terms of peroxidase activity, genotype GW 451 exhibited the highest peroxidase activity, which was statistically comparable to that of DBW 136, while C-306 exhibited the lowest peroxidase activity at both the booting and grain-filling stages.

Correlation analysis

The correlations between different physio-biochemical traits, such as the carotenoid content (CAR), membrane stability index (MSI), relative water content (RWC), chlorophyll content (CC), chlorophyll stability index (CSI), peroxidase activity (POX), malondialdehyde content (MDA), total phenol (PHE), proline content (PRO), free amino acid (FAA), total soluble sugar (TSS), and catalase activity (CAT), of wheat genotypes grown under water deficit stress conditions at the booting and grain-filling stages were assessed using Pearson correlation analysis (Fig. 3A and B).

At the booting stage, oxidative stress factors, represented by MDA, exhibited a positive correlation ($P < 0.01$) and were negatively correlated with RWC, CC, carotenoid content, CSI, and MSI. The chlorophyll content was negatively correlated with the FAA content, TSS content, PRO content, and MDA content but positively correlated with the CSI and MSI. The total phenol content was positively correlated with the FAA content, TSS content, and proline content, while the carotenoid content was

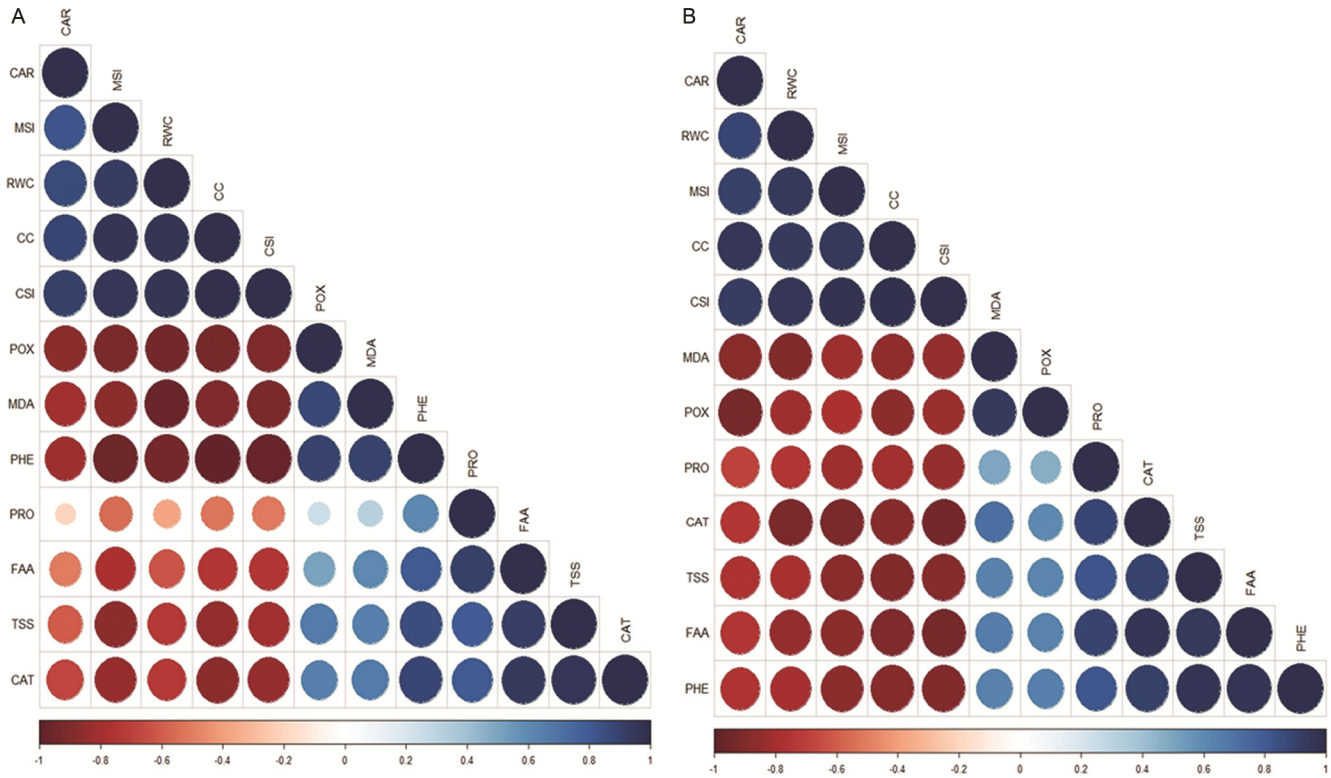

Fig. 3 —Correlation plot for measuring associations between measured physio-biochemical traits of bread wheat (*T. aestivum*) cultivars grown under different levels of drought stress (A) at the booting stage; and (B) at the grain filling stage. CAR, Carotenoid; MSI, Membrane stability index; RWC, Relative water content; CC, Chlorophyll content; CSI, Chlorophyll stability index; POX, Peroxidase activity; MDA, Malondialdehyde content; PHE, Total phenol; PRO, Proline content; FAA, Free amino acid; TSS, Total soluble sugar; CAT, Catalase activity

negatively correlated with the proline content. The proline concentration exhibited positive correlations (P< 0.01 and < 0.05) with the FAA content and TSS. Additionally, CAT and POX were positively correlated with FAA and TSS but negatively correlated with RWC, chlorophyll content, and CSI. Carotenoids were positively correlated with CC, CSI, and MSI but negatively correlated with TSS, MDA, PRO, CAT, PHE, and POX. The CSI exhibited positive correlations (P< 0.01 and <0.05) with the RWC, CC, and MSI.

During the early grain-filling stage, similar correlations were observed, with MDA positively correlated (P< 0.01) and MDA negatively correlated with RWC, CC, CSI, and MSI. CAT and POX were positively correlated with FAA and TSS but negatively correlated with RWC, chlorophyll content, and CSI. The total phenol content exhibited positive correlations with the FAA content, TSS content, and proline content, while the carotenoid content showed negative correlations. The proline content was positively correlated (P< 0.01 and < 0.05) with the FAA content and TSS. The CSI was positively correlated (P< 0.01 and < 0.05) with the RWC, CC, and MSI. Additionally, MSI exhibited positive correlations with RWC, CC, and CSI, which were negatively correlated with FAA, TSS, PRO, and MDA. The RWC was positively correlated with the CC, CSI, and MSI and negatively correlated with the TSS, CAT, POX, PHE, PRO, MDA, and FAA.

Discussion

Water deficit stress is a significant environmental factor that adversely affects crop growth and yield[22]. Plants of different species and cultivars respond differently to water deficit stress due to variations in their physiology, genetics, and metabolism[23]. Plant responses to water deficit stress depend on various factors, such as the intensity of stress, different stress interactions, species and developmental stages of plants[24]. This study aimed to estimate the physio-biochemical responses of wheat cultivars under different levels of water deficit stress. The results indicated a significant change in several physio-

biochemical parameters, including the relative water content (RWC), chlorophyll content, chlorophyll stability index, membrane stability index, lipid peroxidation, and proline content, during both the booting and early grain-filling stages under water deficit stress.

The decrease in crop growth under water stress can be attributed to water shortage and its impact on metabolic processes[25]. In our experiment, we observed a decrease in the leaf relative water content (RWC) under water deficit stress, particularly at the most severe level of stress (35% FC), indicating a disrupted plant water balance[26-28]. Variations in RWC among cultivars may be linked to differences in their ability to absorb water from the soil, with sensitive varieties being more affected by reduced RWC than are tolerant varieties[29]. Moreover, reductions in the chlorophyll content and chlorophyll stability index (CSI) under water deficit stress were observed, indicating photoinhibition and photodegradation of pigments induced by drought[30,31]. High CSI and chlorophyll content are indicative of drought tolerance and can serve as selection criteria for screening tolerant cultivars[32].

The membrane stability index (MSI) is a commonly used criterion for evaluating agricultural drought tolerance because water deficit results in water loss from plant tissue, which adversely affects membrane structure and function[33]. Our study revealed a decrease in MSI under water deficit stress, with susceptible genotypes exhibiting more pronounced reductions than tolerant ones[34-36]. Plants commonly respond to water deficit stress by accumulating suitable solutes, including proline, carbohydrates, and free amino acids, which enable osmotic adjustment[37]. These solutes help maintain turgor pressure and cell volume under low water potential conditions, thereby preserving metabolic functions[38]. In particular, proline plays a crucial role in mitigating oxidative damage and stabilizing cell membranes[39].

Increased lipid peroxidation, as indicated by elevated malondialdehyde (MDA) levels, reflects oxidative damage to cellular membranes under stress conditions[40,41]. Our study revealed that the C-306 and DBW 110 cultivars had the lowest MDA levels, suggesting that these cultivars are more tolerant to desiccation stress. To reduce the damage caused by oxidative stress, antioxidant enzymes such as peroxidase and catalase are essential for scavenging reactive oxygen species (ROS)[25]. Under drought stress, an increase in enzymatic antioxidant activities was observed, particularly in tolerant cultivars, indicating their importance in drought and desiccation tolerance mechanisms[38]. In summary, water deficit stress detrimentally impacts a range of physiological and biochemical functions in wheat, albeit with discrepancies across cultivars. Compared with their vulnerable counterparts, tolerant varieties exhibit superior preservation of cellular function and antioxidant defense systems when faced with water stress. This finding underscores the significance of selecting appropriate cultivars to enhance drought resilience in wheat crops.

Conclusion

In conclusion, our study provides a broad analysis of the physiological and biochemical features of wheat plants under water deficit stress. In the present study, there were two tolerance mechanisms employed in response to drought stress: the first involved the upregulation of antioxidant enzyme (CAT and POX) activity, whereas the second involved the accumulation of soluble sugars, free amino acids, and proline to facilitate osmotic adjustment. The cultivars C-306 and DBW 110 could tolerate water deficit better than the other varieties, as indicated by significant decreases in relative water content, leaf total chlorophyll content, proline accumulation and lipid peroxidation. Moreover, drought stress during grain filling is crucial for wheat yield; hence, drought stress may be avoided during this growth stage.

Acknowledgement

We sincerely acknowledge the Professor and Head, Department of Genetics and Plant Breeding, Junagadh Agricultural University, Junagadh, Gujarat for providing necessary experimental place and laboratory facilities for conduction and execution of this study.

Conflict of interest

All authors declare no conflict of interest.

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