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Physiochemical Mechanisms of Post Drought Recovery and Yield Stability in Wheat – Unfolding the Resilience Dynamics

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Abstract

Drought is one of the most important environmental factors limiting wheat productivity, particularly during critical growth stages, and is expected to intensify under changing climatic conditions. Developing drought-tolerant cultivars requires a comprehensive understanding of the physiological and biochemical processes involved in stress adaptation and recovery. Here, we evaluated the drought responses in four wheat (*Triticum aestivum* L.) cultivars, namely C-306, RSP-561, RSP-566, and HD-2888, under controlled pot conditions. The experimental plants were exposed to three irrigation treatments - well-watered control (100% irrigation), severe drought stress (0% irrigation), and drought stress followed by re-watering. A comprehensive evaluation of biochemical, physiological, antioxidant, and yield-related traits was carried out to identify mechanisms associated with drought tolerance. Drought-tolerant *cvs*. C-306 and RSP-561 showed higher accumulation of phenols, carotenoids, ascorbic acid, proline, and soluble sugars, indicating enhanced antioxidant protection and osmotic adjustment under water deficit. These cultivars also maintained greater antioxidant enzyme activity, reflecting an efficient defense system against oxidative damage. In contrast, HD-2888 and particularly RSP-566 showed higher lipid peroxidation and lower membrane stability, indicating greater cellular injury under stress. Superior growth, phenological performance, and yield-related traits were recorded in C-306 and RSP-561 compared with the more susceptible cultivars. Following re-watering, C-306, RSP-561, and HD-2888 exhibited substantial recovery in chlorophyll content, relative water content, and leaf area, whereas recovery was limited in RSP-566. Overall, C-306 and RSP-561 demonstrated greater drought resilience, with RSP-561 emerging as a promising genetic resource for breeding wheat cultivars with improved drought tolerance and stable yield.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide and constitutes a staple food for a substantial proportion of the world's population. However, its productivity is increasingly challenged by climate change, particularly because of the rising frequency and severity of drought events (Gupta et al., 2023). In arid and semi-arid regions, limited water availability has become a major limitation to sustainable wheat production, resulting in substantial yield losses and threatening food security (Abdullah et al., 2026).

Drought stress affects plant growth and productivity through a range of morphological, physiological, biochemical, and molecular alterations (Ali et al., 2025; Seleiman et al., 2021). Reduced soil moisture restricts water uptake, causing cellular dehydration, loss of turgor, and inhibition of plant growth. Photosynthesis is also adversely affected due to stomatal closure, limited carbon dioxide assimilation, and damage to chlorophyll pigments and thylakoid membranes, resulting in reduced chlorophyll content, leaf area, and biomass accumulation (Qiao et al., 2024). Nevertheless, many plants exhibit considerable recovery following rewatering, highlighting the importance of evaluating both stress responses and post-stress recovery mechanisms (Wang and Zu, 2026). One of the primary effects of drought is the over accumulation of reactive oxygen species (ROS), such as superoxide radicals, hydrogen peroxide, and

hydroxyl radicals, which cause oxidative damage to lipids, proteins, nucleic acids, and cellular membranes (Sachdev et al., 2021). Lipid peroxidation, commonly assessed through the measurement of malondialdehyde (MDA) levels, serves as a widely used marker of oxidative damage. To counteract ROS-induced oxidative stress, plants employ antioxidant defense systems comprising enzymatic antioxidant components, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and polyphenol oxidase (PPO), together with non-enzymatic antioxidants such as phenolic compounds, ascorbate, and carotenoids (Sachdev et al., 2021). The effectiveness of these defense mechanisms often determines the degree of drought tolerance among genotypes.

In addition to antioxidant protection, plants maintain cellular water balance through osmotic adjustment. Accumulation of osmoprotective compounds, including proline and soluble sugars, contributes to maintaining osmotic balance, membrane stability, and safeguarding cellular structures against dehydration stress (Iqbal, 2018). Similarly, relative water content (RWC) and cell membrane stability (CMS) serve as key physiological indicators of plant water status and drought tolerance (Nihunkar et al., 2024). Drought stress also alters crop phenology by accelerating developmental stages such as heading, anthesis, and maturity, often shortening the grain-filling period and reducing yield potential (Shavrukov et al., 2017; Sumitra et al., 2024; Seileman et al., 2021). Consequently, reductions in plant height, biomass production, grain number, and grain weight are commonly observed under water-deficit conditions (Ullah et al., 2024).

Although, the impact of drought on wheat has been widely reported, considerable variation exists among cultivars in their ability to maintain physiological function, recover after re-watering, and sustain productivity under stress. Identifying traits associated with drought adaptation can facilitate the development of wheat cultivars better adapted to water-limited environments. Therefore, the present investigation was designed to examine the responses of four wheat cultivars under control, drought, and re-watering conditions by assessing biochemical, physiological, antioxidant, and yield-related parameters. The study aimed to elucidate the mechanisms underlying drought tolerance and recovery through the evaluation of antioxidant defense, osmotic adjustment, membrane stability, water relations, and yield performance. It was hypothesized that drought-tolerant cultivars would exhibit enhanced antioxidant activity, greater osmolyte accumulation, improved maintenance of cellular water status and membrane integrity, and superior recovery following re-watering, thereby sustaining plant growth and productivity under drought conditions.

Materials and Methods

Plant Growth Conditions

A pot experiment was carried out under controlled environmental conditions to investigate drought responses in four bread wheat (*Triticum aestivum* L.) cultivars exhibiting contrasting levels of drought tolerance, namely C-306, HD-2888, RSP-561, and RSP-566. Uniform, healthy seeds were sown in pots filled with a well-homogenized mixture of soil, sand, and farmyard manure (2:1:1, v/v/v). Throughout the

crop establishment phase, plants were maintained under optimum growing conditions, where the temperature ranged between 20–25°C during the day and 8–12°C at night, with 60–75% relative humidity. And supplied with adequate irrigation to ensure uniform establishment. All recommended agronomic practices were implemented consistently across treatments until the initiation of the drought experiment.

Drought stress application and management

Water-deficit stress was imposed by withholding irrigation at the predetermined crop growth stage (45 days after sowing) to simulate severe drought conditions. Three irrigation treatments were given - (i) well-watered control (Soil moisture was maintained at 35 ± 5% using a gravimetric method), (ii) severe drought stress achieved through complete withdrawal of irrigation (Soil moisture 8 ± 2%), and (iii) rewatering, in which drought-stressed plants received irrigation after completion of the stress period to restore soil moisture. This experimental approach enabled assessment of both plant responses during drought and their capacity to recover following rehydration. Throughout the stress period, plants were regularly monitored for visible symptoms of moisture deficit, including leaf rolling, wilting, and growth suppression. Upon completion of the drought treatment, soil moisture in the rewatering treatment was restored to levels comparable with those maintained under the well-watered control.

Experimental design of the study

The experiment included four wheat cultivars (C-306, HD-2888, RSP-561, and RSP-566) subjected to three irrigation regimes comprising well-watered control (Soil moisture content 35 ± 5%), drought stress (withholding irrigation, reduced Soil moisture 8 ± 2%), and rewatering after drought. A broad range of biochemical, physiological, antioxidant, phenological, growth, and yield-related parameters was evaluated under each irrigation treatment to characterize drought tolerance and post-stress recovery. Observations were recorded immediately after completion of the drought period as well as following the rewatering phase. The study followed a completely randomized design (CRD) with three independent biological replicates (Fig. 1).

Plant sampling

Fully expanded leaves were collected at the end of the drought treatment and again after the rewatering phase. Sampling was carried out during the morning hours to minimize diurnal variation in physiological and biochemical measurements. Leaf samples intended for biochemical and antioxidant analyses were immediately immersed in liquid nitrogen and subsequently stored at − 80°C until further analysis.

Assessment of drought responsive traits

The influence of drought stress and subsequent rewatering on wheat cultivars was investigated through comprehensive measurements of biochemical, physiological, antioxidant, phenological, growth, and yield-related traits. All observations were recorded at two sampling stages, namely immediately after the drought treatment and following recovery by rewatering, to evaluate both stress-induced responses and the extent of physiological recovery.

Determination of non-enzymatic antioxidants and osmolytes

Total phenolic content

Total phenolic content (TPC) was quantified using the Folin-Ciocalteu colorimetric assay following the procedure of Singleton and Rossi (1965), with slight modifications. Approximately 0.2 g of fresh leaf tissue was homogenized in 5 mL of 80% methanol and centrifuged at 12,000 × g for 10 min at 4°C. A 0.5 mL aliquot of the resulting supernatant was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and allowed to react for 5 min at room temperature. Subsequently, 2.0 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added, and the reaction mixture was incubated in darkness for 30 min to facilitate colour development. Absorbance was recorded at 765 nm using a UV–Visible spectrophotometer.

The phenolic concentration was determined from a calibration curve prepared with gallic acid and expressed as mg gallic acid equivalents (GAE) per 100 g fresh weight (FW) (Dvojković et al., 2023). Total phenolic content was calculated as:

:

$$TPC\ (mg\ GAE\ 100\ g^{-1}\ FW) = (C \times V \times 100)/(W \times 1000)$$

where C represents the phenolic concentration obtained from the gallic acid standard curve (mg L^{-1}), V is the extract volume (mL), and W denotes the fresh weight of the sample (g).

Total Carotenoid Content

Carotenoid content was estimated according to the spectrophotometric procedure described by Lichtenthaler and Wellburn (1983). Fresh leaf tissue (0.2 g) was homogenized in 10 mL of 80% (v/v) acetone, followed by centrifugation at 12,000 × g for 10 min. The absorbance of the clear extract was measured at 470, 645, and 663 nm using a UV–Visible spectrophotometer. Chlorophyll *a*, chlorophyll *b*, and carotenoid concentrations were determined using the equations proposed by Lichtenthaler and Wellburn (1983).

Carotenoid concentration was calculated using

$$Carotenoids\ (mg\ L^{-1}) = [1000 \times A_{470} - 1.82 \times Chl\ a - 85.02 \times Chl\ b] / 198$$

The final carotenoid content was expressed as mg 100 g^{-1} fresh weight (FW) using the following equation:

Total carotenoids (mg 100 g^{-1} FW) = (C × V × 100)/(W × 1000)

where C is the carotenoid concentration (mg L^{-1}), V is the extraction volume (mL), and W represents the fresh weight of the tissue (g) (Lichtenthaler and Wellburn, 1983; Kumari et al., 2025)

Ascorbic Acid Content

The concentration of ascorbic acid was determined following the protocol of Mukherjee and Choudhuri (1983) with minor modifications. Fresh leaf tissue (0.2 g) was extracted in 5 mL of 6% (w/v) trichloroacetic acid (TCA) and centrifuged at 12,000 × g for 15 min at 4°C. A 0.5 mL portion of the supernatant was combined with 0.5 mL of 2% dinitrophenylhydrazine (DNPH) reagent containing thiourea and copper sulfate. The reaction mixture was incubated at 37°C for 3 h, after which 2.5 mL of chilled 85% sulfuric acid was added slowly with continuous stirring. The mixture was allowed to stand at room temperature for 30 min, and absorbance was measured at 530 nm.

Ascorbic acid concentration was estimated using a standard curve prepared with L-ascorbic acid and expressed as mg 100 g^{-1} fresh weight (FW) (Mukherjee and Choudhuri, 1983; Ashraf et al., 2022).

The concentration was calculated using:

Ascorbic acid (mg 100 g^{-1} FW) = (C × V × 100)/(W × 1000)

where C denotes the concentration obtained from the standard curve (mg L^{-1}), V is the extract volume (mL), and W is the fresh weight of the sample (g).

Proline content

Free proline was quantified using the acid ninhydrin method described by Bates et al. (1973). Fresh leaf tissue (0.1 g) was homogenized in 2 mL of 3% (w/v) sulfosalicylic acid and centrifuged at 13,000 rpm for 10 min. One millilitre of the supernatant was mixed with an equal volume of acid ninhydrin reagent and 1 mL of glacial acetic acid. The reaction mixture was incubated in a boiling water bath (100°C) for 1 h and then cooled to room temperature. Subsequently, 2 mL of toluene was added, and the contents were vortexed for 20 s. Following phase separation, the absorbance of the toluene layer was measured at 520 nm using a UV–Visible spectrophotometer.

Proline concentration was determined from an L-proline standard curve and expressed as µmol g^{-1} fresh weight (FW) (Bates et al., 1973; Raza and Khan, 2023).

The concentration was calculated using:

Proline (µmol g^{-1} FW) = (C × V)/(115.5 × W)

where C represents the proline concentration obtained from the standard curve (μg mL^{-1}), V is the volume of toluene (mL), 115.5 is the molecular weight of proline, and W is the fresh weight of the sample (g)

Total Soluble Sugar Content

Total soluble sugars were estimated by the anthrone colorimetric method of Yemm and Willis (1954). Fresh leaf tissue (0.1 g) was homogenized in 5 mL of 80% ethanol and incubated in a water bath at 80°C for 30 min. The homogenate was centrifuged at 10,000 × g for 10 min, and the supernatant was collected for analysis. A 1 mL aliquot of the extract was mixed with 5 mL of freshly prepared anthrone reagent (0.2% anthrone in concentrated sulfuric acid) and heated in a boiling water bath for 10 min. After cooling to room temperature, absorbance was measured at 620 nm using a UV–Visible spectrophotometer.

Sugar concentration was estimated using a glucose standard curve and expressed as mg g^{-1} dry weight (DW) (Yemm and Willis, 1954; Szablińska-Piernik and Lahuta, 2023).

Total soluble sugar content was calculated as:

Total soluble sugars (mg g^{-1} DW) = (C × V)/(W × 1000)

where C is the sugar concentration derived from the glucose standard curve (mg L^{-1}), V is the extraction volume (mL), and W is the dry weight of the sample (g).

Assessment of Physiological Traits

Chlorophyll Content

Total chlorophyll content was estimated in fully expanded wheat leaves following the method of Arnon (1949) with slight modifications. Fresh leaf tissue (0.2 g) was homogenized in 10 mL of 80% (v/v) acetone and centrifuged at 14,000 × g for 5 min. The absorbance of the resulting supernatant was recorded at 645 and 663 nm using a UV–Visible spectrophotometer (Eppendorf AG 22331 Hamburg, Biospectrometer). Chlorophyll concentration was calculated according to the equation proposed by Arnon (1949) and expressed as mg g^{-1} fresh weight (FW).

Total chlorophyll (mg g^{-1} FW) = $[(20.2 \times A_{645}) + (8.02 \times A_{663})] \times V / (1000 \times W)$

where A_{645} and A_{663} denote the absorbance values measured at 645 and 663 nm, respectively, V represents the final extract volume (mL), and W is the fresh weight of the tissue sample (g) (Arnon, 1949; Ashraf et al., 2022)

Relative water content

Leaf relative water content (RWC) was determined according to the procedure described by Barrs and Weatherley (1962). Fully expanded leaves were harvested and immediately weighed to obtain their fresh

weight (FW). The leaves were then floated on distilled water in Petri dishes for 24 h at room temperature to achieve full turgidity. After incubation, excess surface moisture was removed by gentle blotting, and the leaves were weighed to determine their turgid weight (TW). Subsequently, the samples were oven-dried at 70°C until a constant weight was attained, and the dry weight (DW) was recorded.

Relative water content was calculated using the following equation:

$$RWC\ (\%) = [(FW - DW)/(TW - DW)] \times 100$$

where FW is the fresh weight, TW is the fully turgid weight, and DW is the oven-dry weight of the leaf sample (Barrs and Weatherley, 1962; Nithinkumar et al., 2024).

Leaf Area

Leaf area was estimated following the method described by Kemp (1960). Fully expanded leaves were selected, and both the maximum leaf length and the widest portion of the leaf blade were measured using a graduated ruler. The measured dimensions were used to calculate leaf area by applying a correction factor of 0.75, which accounts for the natural shape of wheat leaves. Leaf area was expressed as cm^2 per leaf.

Leaf area was calculated as:

$$\text{Leaf area } (cm^2) = \text{Leaf length} \times \text{Maximum leaf width} \times 0.75$$

where both leaf length and maximum leaf width are expressed in centimeters (Kemp, 1960; Silva et al., 2022).

Assessment of Oxidative Stress and Membrane Integrity

Malondialdehyde Content

Malondialdehyde (MDA), an indicator of lipid peroxidation and membrane damage, was determined following the procedure of Stewart and Bewley (1980). Fresh leaf tissue (0.2 g) was homogenized in 2 mL of ethanol and centrifuged at 10,000 × g for 10 min. A 1 mL aliquot of the supernatant was mixed with 2 mL of reaction solution containing 0.5% (w/v) thiobarbituric acid (TBA) prepared in 20% (w/v) trichloroacetic acid (TCA). The reaction mixture was heated in a boiling water bath for 30 min and immediately transferred to an ice bath to terminate the reaction. After centrifugation at 10,000 rpm for 5 min, the absorbance of the supernatant was measured at 532 and 600 nm using a UV–Visible spectrophotometer.

The MDA concentration was calculated using the following equation:

$$\text{MDA } (nmol\ mL^{-1}) = [(A_{532} - A_{600})/155000] \times 10^6$$

where A_{532} and A_{600} represent absorbance values at 532 and 600 nm, respectively, while 155000 M^{-1} cm^{-1} is the extinction coefficient of the MDA–TBA complex (Stewart and Bewley, 1980).

Membrane Stability Index

Membrane stability index was estimated using the method proposed by Abid et al. (2018). Approximately 200 mg of fresh leaf tissue was thoroughly rinsed with double-distilled water and transferred into two separate test tubes containing distilled water. One set of samples was incubated at 40°C for 30 min, after which the electrical conductivity was recorded as T_1. The second set was subsequently heated at 100°C for 10 min, and the final electrical conductivity was measured as T_2.

The membrane stability index was calculated as follows:

$$MSI\ (\%) = [1 - (T_1/T_2)] \times 100$$

where T_1 and T_2 correspond to the electrical conductivity values obtained after incubation at 40°C and 100°C, respectively (Abid et al., 2018).

Membrane Injury

Membrane injury was estimated according to the procedure described by Dhanda et al. (2004) using membrane stability values obtained from drought-stressed and well-watered control plants. The percentage of membrane injury was calculated using the following relationship:

$$MI\ (\%) = [1 - \{MSI\ (drought)/MSI\ (control)\}] \times 100$$

where MSI (drought) and MSI (control) represent the membrane stability indices recorded under drought and control conditions, respectively. Higher membrane injury values indicate greater disruption of membrane integrity caused by drought-induced oxidative stress (Dhanda et al., 2004).

Determination of enzymatic antioxidant activities.

For antioxidant enzyme assays, 0.2 g of fresh leaf tissue was homogenized in 5 mL of ice-cold 50 mM potassium phosphate buffer (pH 7.8) supplemented with 0.5 mM EDTA and 2% (w/v) polyvinylpyrrolidone (PVP-40). The homogenate was centrifuged at 15,000 × g for 15 min at 4°C, and the resulting supernatant was collected for the estimation of antioxidant enzyme activities.

Superoxide Dismutase Activity

Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed according to the method described by Beyer and Fridovich (1987). The assay is based on the ability of SOD to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture contained potassium phosphate buffer, methionine, NBT, EDTA, riboflavin, and an appropriate volume of enzyme extract. Following illumination, absorbance was recorded at 560 nm using a UV–Visible spectrophotometer.

One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT photoreduction under the assay conditions and was expressed as U mg^{-1} protein (Beyer and Fridovich, 1987).

Catalase Activity

Catalase (CAT; EC 1.11.1.6) activity was determined according to the protocol of Hadwen et al. (2024) by monitoring the enzymatic decomposition of hydrogen peroxide (H_2O_2). The decrease in absorbance resulting from H_2O_2 consumption was measured spectrophotometrically at 240 nm. Enzyme activity was expressed as µmol H_2O_2 decomposed min^{-1} mg^{-1} protein (Hadwen et al., 2024).

Ascorbate Peroxidase Activity

The activity of ascorbate peroxidase (APX; EC 1.11.1.11) was measured following the method reported by Hong et al. (2018). The assay was based on the oxidation of ascorbate in the presence of hydrogen peroxide. Changes in absorbance were monitored at 290 nm for 2 min using a UV–Visible spectrophotometer. Enzyme activity was calculated using the extinction coefficient of ascorbate and expressed as µmol ascorbate oxidized min^{-1} mg^{-1} protein (Hong et al., 2018).

Polyphenol oxidase Activity

Polyphenol oxidase (PPO; EC 1.14.18.1) activity was determined spectrophotometrically using catechol as the substrate following the method described by Ndzi et al. (2021) with slight modifications. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 6.8), catechol solution, and the crude enzyme extract. The reaction was initiated by adding the enzyme extract, and the increase in absorbance resulting from catechol oxidation was monitored at 420 nm using a UV–Visible spectrophotometer. PPO activity was expressed as the change in absorbance per minute per milligram of protein (ΔA_{420} min^{-1} mg^{-1} protein)

Assessment of growth, phenological and yield traits

Growth Traits

Growth Traits

Growth-related observations were recorded at physiological maturity. Plant height was measured from the soil surface to the tip of the spike, excluding the awns, using a graduated measuring scale and expressed in centimetres (cm). To estimate dry matter accumulation, harvested plants were dried in a hot-air oven at 70°C until a constant weight was achieved. The oven-dried samples were then weighed using an analytical balance, and dry matter accumulation was expressed as g $plant^{-1}$ (Slafer et al., 2015).

Yield Traits

Yield attributes were recorded after the crop reached physiological maturity. Representative spikes from each treatment were harvested and used for yield assessment. The number of grains per spike was determined by manually counting the grains from each spike. After threshing, the grains obtained from individual spikes were weighed to determine grain weight per spike (g). Thousand-grain weight (TGW) was estimated by randomly selecting and weighing 1000 grains using an analytical balance, and the results were expressed in grams (FAO, 2023; Reynolds et al., 2022).

Phenological Traits

Phenological development was monitored throughout the crop growth period. Days to heading were recorded as the number of days from sowing until approximately 50% of the plants had emerged spikes. Days to anthesis were noted when nearly half of the spikes within a treatment reached the flowering stage. Days to physiological maturity were recorded when spikes exhibited complete senescence and the grains had attained full physiological maturity. The grain-filling duration was determined by calculating the interval between anthesis and physiological maturity using the following equation:

Grain filling duration (days) = Days to physiological maturity – Days to anthesis

Data analysis

All statistical analyses were carried out using IBM SPSS Statistics for Windows, Version 30.0 (IBM Corp., Armonk, NY, USA). Prior to analysis, the data were examined for compliance with the assumptions of normality and homogeneity of variance. A two-way analysis of variance (ANOVA) based on the General Linear Model (GLM) was performed to determine the effects of cultivar (V), irrigation treatment (T), and their interaction (V × T) on all biochemical, physiological, antioxidant, growth, phenological, and yield-related parameters. Mean comparisons were conducted using Duncan's Multiple Range Test (DMRT) at a significance level of $P \leq 0.05$. Experimental data are presented as the mean ± standard error (SE) of three biological replicates. Graphical visualization and figure preparation were performed using GraphPad Prism Version 10.4.1 (GraphPad Software, Boston, MA, USA).

Results

Biochemical Responses

Drought stress markedly altered the biochemical profile of all wheat cultivars, with significant effects of genotype, treatment, and genotype × treatment interaction observed for all measured traits ($P \leq 0.05$).

Non-enzymatic antioxidants

Total phenolic and ascorbic acid contents increased under drought stress in all cultivars (Fig. 2A-C). The highest accumulation of phenolics was recorded in C-306 (305.92 mg 100 g^{-1} FW), followed by RSP-561 (285.58 mg 100 g^{-1} FW), whereas RSP-566 exhibited the lowest concentration. A similar trend was observed for ascorbic acid, with C-306 and HD-2888 exhibiting the greatest increases under drought.

Following rewatering, both metabolites declined but remained above control levels. In contrast, carotenoid concentration declined under drought in all genotypes (Fig. 2B). The reduction was most pronounced in C-306 and RSP-566, and only partial recovery was observed after rewatering.

Figure 2. A) Total phenolics, B) carotenoid and C) ascorbic acid content in wheat cultivars subjected to drought stress. Bars are mean ± SE (n = 3). Different letters above bar within each cultivar indicates significant differences at $P \leq 0.05$ (DMRT).

Osmolyte accumulation

Drought stress promoted substantial accumulation of proline and soluble sugars in all cultivars (Fig. 3A-B). C-306 and RSP-561 accumulated the highest proline concentrations (0.966 and 0.950 mg g^{-1} FW, respectively), whereas RSP-566 showed markedly lower accumulation. Likewise, soluble sugar content increased across genotypes, reaching maximum values in C-306 (185.52 mg g^{-1} DW) and RSP-561 (180.46 mg g^{-1} DW). Although both metabolites declined after rewatering, their concentrations remained significantly higher than those of well-watered controls. Collectively, the greater accumulation of antioxidants and osmoprotectants in C-306 and RSP-561 suggests a more effective biochemical adjustment to water deficit than in HD-2888 and particularly RSP-566.

Physiological Responses

Drought significantly affected chlorophyll content, relative water content (RWC), and leaf area, with responses varying among cultivars ($P \leq 0.05$). Chlorophyll concentration declined under drought in all genotypes (Fig. 4A). The largest reduction occurred in RSP-566, whereas RSP-561 maintained comparatively higher chlorophyll levels throughout the stress period. Rewatering partially restored chlorophyll content but values remained below those of control plants. Similarly, drought reduced leaf water status, as reflected by lower RWC values (Fig. 5B). C-306 maintained the highest RWC under stress (63.46%), followed by HD-2888, whereas RSP-561 and RSP-566 exhibited greater dehydration. Rewatering improved leaf hydration in all cultivars, with C-306 showing the strongest recovery response. Leaf area was also adversely affected by water limitation (Fig. 4C). HD-2888 maintained the largest leaf area under stress, whereas RSP-566 consistently exhibited the smallest canopy size. Despite partial recovery following rewatering, leaf expansion did not return to pre-stress levels. These results indicate that drought-induced reductions in photosynthetic surface area and tissue hydration were less pronounced in C-306 and RSP-561 than in RSP-566.

Oxidative Damage and Membrane Integrity

Drought stress significantly influenced oxidative damage and membrane stability parameters ($P \leq 0.05$). Malondialdehyde (MDA) content increased substantially under drought in all cultivars (Fig. 5A). The highest levels were observed in RSP-566 and HD-2888, whereas C-306 maintained the lowest MDA

concentration, reflecting reduced lipid peroxidation. RSP-561 also exhibited relatively low MDA accumulation.

Cell membrane stability (CMS) declined in response to drought across all cultivars (Fig. 5B). C-306 retained the highest membrane stability, followed by RSP-561 and HD-2888, whereas RSP-566 exhibited the greatest loss of membrane integrity. Consistent with CMS, membrane injury was highest in RSP-566 and lowest in C-306 (Fig. 5C). Rewatering improved membrane stability in all genotypes; however, complete restoration was not achieved. The lower oxidative damage and greater membrane integrity maintained by C-306 and RSP-561 suggest superior protection of cellular structures under water deficit conditions.

Antioxidant Enzyme Responses

Activities of APX, CAT, SOD, and PPO increased significantly under drought stress, although the magnitude of induction differed among cultivars ($P \leq 0.05$). CAT activity nearly doubled under drought, with the highest activities observed in RSP-561 and C-306 (Fig. 6A). Similarly, SOD activity increased markedly, reaching maximum values in C-306, followed by RSP-561 (Fig. 6B). APX activity exhibited a comparable response, with C-306 displaying the greatest induction and RSP-566 the lowest (Fig. 6C). PPO activity also increased under drought across all cultivars (Fig. 7D). The strongest response was observed in C-306 and RSP-561, whereas RSP-566 consistently recorded lower activity. Although enzyme activities declined after rewatering, they remained above control levels. The stronger antioxidant response observed in C-306 and RSP-561 likely contributed to their reduced oxidative damage and improved drought tolerance.

Figure 6: Heatmap showing cultivar-specific changes in (A) CAT, (B) SOD, (C) APX, and (D) PPO activities under control, drought, and rewatering conditions. Higher color intensity denotes greater enzyme activity. Different letters indicate significant differences among treatment means (DMRT, $P \leq 0.05$).).

3.5 Growth and Yield Attributes

Drought stress significantly ($P \leq 0.05$) affected all growth and yield-related traits, including plant height, grain number per spike, grain weight per spike, 1000-grain weight, and dry matter accumulation. Analysis of variance revealed significant effects of variety (V), treatment (T), and their interaction (V × T), indicating differential genotypic responses to water deficit.

Plant height declined significantly under drought across all cultivars (Fig. 7). C-306 exhibited a reduction from 124.35 cm under control conditions to 114.14 cm under drought, whereas RSP-566 showed a more pronounced decrease from 111.42 to 98.45 cm. HD-2888 and RSP-561 also experienced significant reductions, although the magnitude of decline was comparatively lower, suggesting greater maintenance of vegetative growth under stress.

Grain number per spike was adversely affected by drought; however, C-306 (47.72 grains spike^{-1}) and RSP-561 (47.18 grains spike^{-1}) remained statistically at par with their respective control values, indicating limited impairment of reproductive sink capacity. In contrast, HD-2888 and RSP-566 recorded significant reductions, reaching 40.28 and 43.51 grains spike^{-1}, respectively, reflecting a greater sensitivity of reproductive development to moisture stress (Fig. 7).

Significant varietal differences were also observed for grain weight per spike. RSP-561 recorded a slight increase under drought, reaching 2.19 g spike^{-1}, and remained statistically superior or at par with other cultivars, suggesting efficient assimilate partitioning under stress conditions. Conversely, RSP-566 exhibited a marked reduction to 1.43 g spike^{-1}, indicating impaired grain filling under water deficit (Fig. 7).

Figure 7: Cultivar-specific responses of plant height, grain number per spike, and grain weight per spike under control and drought stress conditions. Lines connect treatment means for each cultivar. Different letters indicate significant differences among treatment means (DMRT, $P \leq 0.05$).

The response of 1000-grain weight varied among cultivars (Fig. 8). HD-2888 recorded the highest value under control conditions (48.63 g), which declined to 43.15 g under drought. In contrast, C-306 and RSP-561 exhibited slight increases under stress, reaching 45.12 g and 46.27 g, respectively, suggesting their ability to sustain grain filling despite limited water availability. RSP-566 recorded the lowest 1000-grain weight under drought (32.47 g), indicating substantial impairment of grain development.

Dry matter accumulation was significantly reduced by drought in all cultivars (Fig. 8). HD-2888 produced the highest dry matter under control conditions (29.41 g plant^{-1}) but declined to 20.76 g plant^{-1} under drought. C-306 and RSP-561 showed comparatively moderate reductions, whereas RSP-566 recorded the lowest biomass accumulation under stress (19.75 g plant^{-1}), reflecting a substantial reduction in growth and assimilate production.

Overall, C-306 and RSP-561 exhibited greater yield stability under drought, characterized by minimal reductions in grain number, maintenance of grain weight, and comparatively higher biomass accumulation. In contrast, RSP-566 consistently recorded the largest reductions across growth and yield traits, confirming its susceptibility to water deficit. These findings indicate that drought resilience in wheat is closely associated with the ability to sustain reproductive performance and biomass production under moisture-limited conditions.

Phenological Traits

Drought stress markedly altered crop phenology, significantly affecting days to heading, anthesis, maturity, and grain filling duration ($p \leq 0.05$). Analysis of variance revealed highly significant effects of variety (V), treatment (T), and their interaction (V × T), indicating that wheat cultivars differed in the magnitude of their phenological responses to moisture deficit. Overall, drought accelerated crop

development, resulting in earlier heading, flowering, and maturity, accompanied by a shortened grain-filling period (Fig. 9).

Exposure to drought stress significantly reduced the time required for heading in all cultivars. The greatest reduction was observed in HD-2888, where days to heading declined from 91.27 days under control conditions to 81.55 days under drought stress. Similarly, C-306 exhibited a decrease from 90.72 to 82.80 days. Comparable reductions were recorded in RSP-561 and RSP-566, confirming that water deficit accelerated the transition from vegetative to reproductive growth. The observed differences were statistically significant, as reflected by the separation of means into distinct LSD groups (Fig. 9).

A similar pattern was evident for anthesis, with drought stress inducing earlier flowering across all cultivars. C-306 flowered at 89.75 days under drought compared with 101.25 days under control conditions, while HD-2888 showed a reduction from 98.67 to 87.14 days. Significant decreases were also recorded for RSP-561 and RSP-566. These results indicate that moisture stress hastened reproductive development, leading to a substantial shortening of the pre-anthesis growth period (Fig. 9).

Drought stress significantly shortened the crop life cycle by accelerating physiological maturity. In C-306, maturity was reached at 125.34 days under drought compared with 138.43 days under non-stress conditions. Similar reductions were observed in HD-2888 (132.39 to 119.51 days), RSP-561 (142.91 to 135.42 days), and RSP-566 (139.50 to 128.53 days). The consistent decline in maturity duration across cultivars highlights the tendency of wheat plants to complete their life cycle more rapidly under moisture-limited environments, thereby reducing exposure to prolonged stress (Fig. 9).

Grain filling duration was significantly curtailed under drought stress, although the extent of reduction varied among cultivars. RSP-566 recorded the shortest grain-filling period (36.37 days), indicating a pronounced adverse effect of moisture deficit on assimilate accumulation and grain development. In contrast, RSP-561 maintained the longest grain-filling duration (41.25 days) under stress, suggesting a greater capacity to sustain reproductive growth despite limited water availability. C-306 and HD-2888 exhibited intermediate durations of 38.28 and 37.57 days, respectively. The relatively longer grain-filling period maintained by RSP-561 may contribute to its superior adaptation under drought conditions by allowing continued assimilate deposition into developing grains (Fig. 9).

Discussion

Drought profoundly altered the biochemical, physiological, and agronomic performance of the evaluated wheat cultivars, although the magnitude of these responses differed considerably among genotypes. The significant cultivar × treatment interaction observed for nearly all measured traits demonstrates that drought tolerance is not governed by a single mechanism but results from the coordinated regulation of multiple physiological and biochemical processes. Cultivars C-306 and RSP-561 consistently exhibited greater resilience by maintaining stronger antioxidant protection, improved osmotic adjustment, enhanced membrane stability, and comparatively better growth and yield performance under water deficit. In contrast, RSP-566 displayed pronounced physiological impairment, indicating limited adaptive

capacity under prolonged moisture stress. These findings reinforce the concept that drought tolerance is a complex quantitative trait controlled by integrated defense mechanisms rather than by the expression of any single physiological attribute.

One of the clearest biochemical responses to water deficit was the increased accumulation of phenolic compounds, particularly in C-306 and RSP-561. The higher phenolic concentration observed in these cultivars suggests an enhanced capacity to counteract oxidative stress through non-enzymatic antioxidant defense. Besides directly scavenging reactive oxygen species (ROS), phenolic metabolites contribute to membrane protection and help preserve cellular homeostasis during dehydration. The comparatively lower phenolic content recorded in RSP-566 indicates a weaker antioxidative response, which is consistent with its greater susceptibility to oxidative damage. Similar genotype-dependent enhancement of phenolic metabolism under drought has been documented in wheat and other cereals, where increased phenolic accumulation has been associated with improved stress adaptation and maintenance of cellular function (Lv et al., 2022; Laddomada et al., 2021; Dvojković et al., 2023; Nardino et al., 2022; Chaouachi et al., 2024; Tanbakuyi et al., 2025; Yan et al., 2026).

Unlike phenolic compounds, carotenoid concentration declined in response to drought across all cultivars. Because carotenoids play a central role in protecting chloroplasts against photooxidative damage by dissipating excess excitation energy, their depletion indicates progressive disruption of the photosynthetic apparatus under moisture deficit. The greater reduction recorded in RSP-566 further supports its higher sensitivity to oxidative stress, whereas the relatively moderate decline observed in C-306 and RSP-561 suggests more effective preservation of the photosynthetic machinery. Similar reductions in carotenoid content under drought have been reported in wheat, where degradation of photosynthetic pigments has been linked with impaired photochemical efficiency and chloroplast instability (Hu et al., 2023; Kumari et al., 2025).

The accumulation of ascorbic acid further demonstrates the importance of non-enzymatic antioxidant defence during drought adaptation. Elevated ascorbate concentrations, particularly in C-306 and HD-2888, indicate activation of the ascorbate–glutathione pathway, which plays a fundamental role in detoxifying hydrogen peroxide and maintaining intracellular redox balance. The enhanced antioxidant status observed in these cultivars likely contributed to limiting oxidative injury during water deficit. Similar protective functions of ascorbic acid have been widely reported, with increased endogenous or externally supplied ascorbate improving chlorophyll retention, photosynthetic activity, nutrient uptake, and drought tolerance in wheat (Al-Kazzaz, 2023; Foyer and Kunert, 2024).

Compatible solutes also contributed substantially to drought adaptation. Both proline and soluble sugars accumulated to significantly higher levels under drought, with the greatest increases observed in C-306 and RSP-561. Rather than functioning solely as osmolytes, these metabolites stabilize proteins and membranes, protect enzyme activity, and contribute to ROS detoxification while supporting osmotic balance during cellular dehydration. Their greater accumulation in the tolerant cultivars reflects superior metabolic plasticity, enabling maintenance of cellular function under limited water availability.

Conversely, the relatively lower concentrations recorded in RSP-566 suggest a less efficient adaptive response. Comparable increases in osmoprotectant accumulation have consistently been reported in drought-stressed wheat and are considered a characteristic feature of stress acclimation through metabolic reprogramming (Ghosh et al., 2021; Szablińska-Piernik et al., 2023; Dvojković et al., 2023).

Collectively, these biochemical responses indicate that drought tolerance in the present study was associated with a coordinated enhancement of antioxidant metabolism and osmotic regulation rather than the alteration of individual metabolites. The consistently stronger biochemical adjustment exhibited by C-306 and RSP-561 provides a physiological basis for their improved performance under drought and supports their potential use as valuable genetic resources in breeding programmes targeting enhanced tolerance to water-limited environments.

Maintenance of photosynthetic function emerged as one of the principal characteristics distinguishing drought-tolerant and drought-sensitive cultivars. Although chlorophyll concentration declined in all cultivars under water deficit, the reduction was considerably smaller in C-306 and RSP-561 than in RSP-566. This indicates that the tolerant cultivars were more effective in preserving chloroplast integrity during dehydration. Chlorophyll degradation under drought is primarily associated with oxidative disruption of thylakoid membranes, inhibition of chlorophyll biosynthesis, and accelerated pigment catabolism, all of which ultimately restrict light harvesting and carbon assimilation. Therefore, the greater retention of chlorophyll observed in C-306 and RSP-561 likely contributed to sustaining photosynthetic activity during stress, thereby supporting continued growth and grain development. Similar relationships between chlorophyll preservation and enhanced drought tolerance have been demonstrated in wheat and several other crop species, where maintenance of photosynthetic pigments was positively associated with improved stress adaptation (Hu et al., 2023; Wang et al., 2025; Yan et al., 2024).

The contrasting responses among cultivars were further reflected in their ability to maintain plant water status. Relative water content (RWC) declined significantly under drought in all genotypes; however, C-306 and HD-2888 retained comparatively higher tissue hydration than RSP-566. Maintenance of RWC under moisture deficit reflects efficient regulation of water uptake, reduced transpirational losses, and improved osmotic adjustment, thereby allowing essential physiological processes to continue despite limited water availability. In the present study, the higher RWC recorded in the tolerant cultivars corresponded well with their greater accumulation of proline and soluble sugars, indicating that osmotic adjustment played an important role in sustaining cellular hydration. Similar associations between improved water status and drought tolerance have been widely reported in wheat, where cultivars capable of maintaining higher RWC generally exhibit superior physiological performance and improved productivity under stress (Nithinkumar et al., 2024).

Leaf area also responded markedly to drought, reflecting the strong influence of water limitation on vegetative growth. All cultivars exhibited reduced leaf expansion under stress; however, the decline was substantially greater in RSP-566 than in the tolerant cultivars. Restriction of leaf development represents

an adaptive response that reduces transpirational water loss, but it also limits the photosynthetically active surface available for carbon fixation. Consequently, prolonged reductions in leaf area are frequently accompanied by decreased biomass production and lower assimilate availability for reproductive growth. The comparatively larger leaf area maintained by C-306 and RSP-561 therefore represents an important adaptive advantage, allowing these cultivars to sustain greater photosynthetic capacity while simultaneously minimizing excessive water loss. Comparable reductions in leaf expansion under drought have been attributed to inhibition of cell division and cell enlargement resulting from reduced turgor pressure and carbon assimilation (Nyaupane et al., 2024).

Measurements of malondialdehyde (MDA) and membrane stability further confirmed that oxidative injury differed substantially among cultivars. The pronounced increase in MDA observed under drought indicates enhanced lipid peroxidation resulting from excessive accumulation of reactive oxygen species. Nevertheless, the tolerant cultivars accumulated considerably lower amounts of MDA than RSP-566, demonstrating a greater capacity to restrict oxidative damage. Lower lipid peroxidation in C-306 and RSP-561 is consistent with their stronger antioxidant defence and higher concentrations of protective metabolites observed in the biochemical analyses. Similar inverse relationships between antioxidant capacity and MDA accumulation have been consistently reported in drought-stressed wheat, where reduced lipid peroxidation is considered a reliable indicator of stress tolerance (Hameed et al., 2012; Ishfaq et al., 2024).

Changes in membrane stability closely paralleled the patterns of MDA accumulation. Drought substantially reduced membrane stability in all cultivars, although the magnitude of decline varied according to genotype. Higher membrane stability recorded in C-306 and RSP-561 indicates improved preservation of membrane structure despite prolonged water deficit, whereas the pronounced loss of membrane integrity in RSP-566 reflects severe oxidative disruption of cellular membranes. Membrane stability is widely recognized as an integrative indicator of drought tolerance because it reflects the combined effects of oxidative stress, osmotic regulation, and antioxidant protection. The partial restoration of membrane stability following rewatering demonstrates that some physiological recovery occurred after stress relief; however, recovery remained incomplete in the susceptible cultivar, suggesting that prolonged drought caused irreversible cellular injury that could not be fully repaired after irrigation was restored. Similar observations have been reported in wheat, where membrane damage persisted even after rehydration, particularly in drought-sensitive genotypes.

Collectively, these physiological responses indicate that successful drought adaptation depends not only on minimizing water loss but also on preserving photosynthetic efficiency and cellular integrity throughout the stress period. The superior performance of C-306 and RSP-561 demonstrates that effective maintenance of chlorophyll content, tissue hydration, leaf expansion, and membrane stability enables plants to sustain metabolic activity under drought and enhances their capacity to recover following rewatering. These coordinated physiological adjustments provide a mechanistic explanation for the improved growth and productivity exhibited by these cultivars under water-limited conditions.

The enhanced activities of antioxidant enzymes observed under drought stress demonstrate that enzymatic ROS detoxification constitutes a major defense mechanism against oxidative stress in wheat. Although, drought increased the activities of SOD, CAT, APX, and PPO in all the cvs., the magnitude of induction differed markedly among genotypes. Wheat cv. C-306 and RSP-561 consistently exhibited higher enzyme activities than HD-2888 and particularly RSP-566, indicating a more efficient antioxidant system capable of maintaining cellular redox homeostasis during water deficit stress. This enhanced enzymatic response corresponded closely with the lower MDA accumulation and improved membrane stability recorded in these cvs., suggesting that efficient ROS scavenging effectively minimized the oxidative injury.

Superoxide dismutase represents the first enzymatic barrier against oxidative stress by rapidly converting superoxide radicals into hydrogen peroxide. However, because hydrogen peroxide itself is cytotoxic when accumulated in excess, its rapid removal by catalase and ascorbate peroxidase is equally essential for maintaining cellular homeostasis. The coordinated increase in SOD, CAT, and APX activities observed in the tolerant cultivars therefore indicates that these enzymes functioned as an integrated antioxidant network rather than as independent defence components. Such coordinated regulation has been recognized as a characteristic feature of drought-tolerant genotypes, enabling efficient detoxification of ROS while protecting cellular membranes, proteins, and photosynthetic structures from oxidative damage. Similar relationships between enhanced antioxidant enzyme activity and improved drought tolerance have been reported in wheat and other cereal crops (Lv et al., 2022; Chaouachi et al., 2024; Tanbakuyi et al., 2025; Yan et al., 2026).

In addition to the primary antioxidant enzymes, polyphenol oxidase also responded positively to drought stress. Although, PPO is commonly associated with phenolic metabolism and plant defence, increasing evidence indicates that it also contributes indirectly to antioxidant protection by regulating phenolic oxidation and reinforcing cellular defence mechanisms during environmental stress. The greater PPO activity recorded in C-306 and RSP-561 therefore complements the enhanced accumulation of phenolic compounds observed in these cultivars, suggesting coordinated regulation between enzymatic and non-enzymatic antioxidant systems. Such integration strengthens the overall antioxidant capacity of the plant and enhances its ability to withstand prolonged dehydration.

An important outcome of the present study was the persistence of relatively high antioxidant enzyme activities even after re-watering. Although, restoration of irrigation alleviated drought stress, enzyme activities did not immediately return to control levels, particularly in the tolerant cultivars. This sustained activation indicates that physiological recovery extends beyond the simple restoration of water availability and reflects the retention of protective metabolic adjustments established during the stress period. Such responses are consistent with the concept of stress memory, whereby previous exposure to drought primes plants for a faster and more efficient response to subsequent stress events. Rather than representing delayed recovery, the prolonged maintenance of antioxidant activity may provide an adaptive advantage by protecting recovering tissues from secondary oxidative bursts that frequently occur during the transition from drought to rehydration. Comparable post-stress persistence of

antioxidant metabolism has been documented in wheat and other crops and has been proposed as an important component of drought resilience (Lukić et al., 2020).

The contrasting antioxidant responses among cultivars further emphasize that drought tolerance depends not only on the induction of defence enzymes but also on the efficiency with which these enzymes function collectively. Despite exhibiting some increase in antioxidant activity, RSP-566 accumulated substantially higher levels of lipid peroxidation and experienced greater membrane disruption than the tolerant cultivars. This indicates that activation of antioxidant enzymes alone is insufficient unless the response is rapid and adequately coordinated with other protective mechanisms, including osmotic adjustment and non-enzymatic antioxidant metabolism. Conversely, the balanced enhancement of enzymatic and biochemical defence systems observed in C-306 and RSP-561 effectively restricted oxidative damage and contributed to superior physiological performance under drought.

Overall, the present findings demonstrate that antioxidant enzymes constitute a central component of the drought adaptation strategy in wheat. Their coordinated induction, together with sustained activity during the recovery phase, enabled tolerant cultivars to maintain cellular homeostasis, preserve membrane integrity, and reduce oxidative damage under prolonged water deficit. These responses provide a mechanistic explanation for the improved physiological performance and drought resilience exhibited by C-306 and RSP-561, highlighting the importance of integrated antioxidant regulation in breeding wheat cultivars adapted to increasingly water-limited environments.

The biochemical and physiological adjustments observed under drought were ultimately reflected in differences in plant growth and yield performance. Water deficit significantly reduced plant height, biomass accumulation, grain number, grain weight, and thousand-grain weight; however, the magnitude of these reductions varied considerably among cultivars. The comparatively smaller decline recorded in C-306 and RSP-561 indicates that these cultivars were able to sustain vegetative growth and reproductive development despite limited water availability. Their superior agronomic performance can be attributed to the combined maintenance of photosynthetic activity, improved cellular hydration, stronger antioxidant defence, and enhanced osmotic regulation, all of which collectively supported assimilate production and its subsequent allocation to developing grains.

Plant height is widely regarded as a sensitive indicator of drought-induced growth inhibition because stem elongation depends largely on cell expansion and turgor-driven growth. The reduction in plant height observed across all cultivars therefore reflects the direct influence of moisture limitation on cell enlargement and vegetative development. Nevertheless, the tolerant cultivars maintained greater plant stature than RSP-566, suggesting that preservation of water status and metabolic activity enabled continued growth even under prolonged drought. Similar reductions in vegetative growth have been widely reported in wheat, where decreased turgor pressure and restricted photosynthesis suppress stem elongation and biomass production under water deficit (Slafer et al., 2015; Ullah et al., 2024).

Reproductive traits exhibited clear genotypic differences in drought sensitivity. Although grain number per spike declined under stress, C-306 and RSP-561 maintained values comparable to their respective controls, indicating that reproductive development was less affected than in the susceptible cultivars. The ability to preserve grain set under drought is particularly important because grain number is established during sensitive developmental stages and contributes substantially to final yield. In contrast, the pronounced reduction observed in RSP-566 indicates greater disruption of floral development and sink establishment during moisture stress.

A similar trend was evident for grain weight and thousand-grain weight. Interestingly, RSP-561 maintained, and in some cases slightly increased, grain weight under drought despite reduced water availability. This response suggests efficient assimilate remobilization and preferential allocation of available photoassimilates towards grain filling. Such compensatory allocation has been reported as an adaptive mechanism that partially offsets yield reductions when the number of developing grains is restricted under stress. Conversely, the substantial decline in grain weight and thousand-grain weight observed in RSP-566 indicates inadequate assimilate supply during grain filling, most likely resulting from reduced photosynthetic capacity and accelerated leaf senescence. Similar relationships between assimilate partitioning, grain filling efficiency, and drought tolerance have been described in wheat under terminal drought conditions (Maiti and Satya, 2014; Reynolds et al., 2022).

Dry matter accumulation also reflected the contrasting adaptive capacities of the evaluated cultivars. Although biomass production declined under drought in all genotypes, C-306 and RSP-561 exhibited comparatively smaller reductions than RSP-566. Biomass accumulation integrates the cumulative effects of photosynthesis, water relations, respiration, and carbon partitioning throughout plant development. Therefore, maintenance of higher dry matter production in the tolerant cultivars provides additional evidence that these genotypes were able to sustain metabolic activity despite prolonged moisture limitation. The lower biomass recorded in RSP-566 further confirms its reduced capacity to maintain growth under stress.

Drought also modified crop phenology by accelerating developmental progression, leading to earlier heading, anthesis, and physiological maturity. Advancement of reproductive development under water deficit is generally regarded as a drought escape strategy that enables plants to complete their life cycle before environmental conditions deteriorate further. While this strategy may improve survival, it also reduces the duration available for assimilate production and grain filling, thereby limiting final yield. In the present study, this trade-off was particularly evident in RSP-566, where the marked reduction in grain-filling duration was accompanied by substantial losses in grain weight and biomass accumulation. By contrast, RSP-561 maintained a relatively longer grain-filling period, allowing continued assimilate deposition into developing grains and contributing to its superior yield stability under drought. Similar relationships between phenological plasticity, grain-filling duration, and drought adaptation have been reported in recent wheat studies (Haghpanah et al., 2024; Gulino and Lopes, 2024).

Collectively, these findings demonstrate that maintenance of yield under drought depends on the coordinated integration of biochemical protection, physiological stability, and efficient assimilate partitioning rather than on any individual trait. The superior performance of C-306 and RSP-561 illustrates that effective drought adaptation results from the combined ability to preserve photosynthetic function, limit oxidative damage, maintain water status, sustain grain filling, and protect reproductive development. This integrated response enabled these cultivars to maintain greater productivity under water-limited conditions and identifies them as promising genetic resources for breeding programmes aimed at improving drought resilience in wheat.

Conclusion

The present investigation demonstrated substantial genotypic variation in drought tolerance and post-stress recovery among the four wheat cultivars evaluated under controlled irrigation regimes. Drought-induced reductions in growth, physiological performance, and yield were closely associated with oxidative damage and impaired cellular water status. However, cultivars C-306 and RSP-561 effectively mitigated these adverse effects through coordinated enhancement of antioxidant defence, greater accumulation of compatible osmolytes, improved membrane stability, and better maintenance of photosynthetic capacity. These integrated responses enabled both cultivars to sustain superior growth and yield performance under water-deficit conditions while exhibiting a greater capacity to recover following rewatering. Among the evaluated genotypes, RSP-561 consistently combined efficient biochemical and physiological adaptation with improved yield stability, indicating its strong potential as a drought-resilient breeding resource. The persistence of enhanced antioxidant activity after rewatering further suggests that sustained protective mechanisms contribute to recovery and may represent an important component of drought adaptation in wheat. Overall, the findings emphasize that effective drought tolerance results from the coordinated interaction of antioxidant regulation, osmotic adjustment, maintenance of cellular integrity, and preservation of physiological function rather than the influence of individual traits alone. The identified drought-responsive characteristics provide valuable physiological markers for screening germplasm and support the utilization of RSP-561 and C-306 as promising parental material in breeding programmes aimed at developing climate-resilient wheat cultivars capable of maintaining stable productivity under increasingly water-limited environments.

Declarations

Author contributions statement

G.K.R. conceived and designed the study, supervised the research, guided the experimental work, interpreted the results, and critically revised the manuscript. **D.M.K.** curated the data, performed the statistical analyses, prepared the figures, interpreted the findings, and wrote the original draft of the manuscript. **G.J.** contributed to the experimental work, methodology, data validation, and manuscript revision. **R.R.K.** assisted with data analysis, interpretation of results, visualization, and critical editing of the manuscript. **P.K.R.** supervised the manuscript, reviewed the manuscript, and contributed to its

improvement. **S.B.** editing of the manuscript. All authors read and approved the final version of the manuscript.

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References

1. Abdullah, H., Al-Haddad, A. & Abusam, A. Climate-driven changes and impact on arid lands water resources for agriculture production and food security. In: (eds Suleiman, M. K. & Shahid, S. A.) Fostering Arid Lands Agriculture in the Face of Climate Change. Advances in Global Change Research, Vol. 81. Cham: Springer. https://doi.org/10.1007/978-3-031-98490-7_3 (2026).
2. Abid, M. et al. Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L). *Sci. Rep.* **8** (1), 4615. https://doi.org/10.1038/s41598-018-21441-7 (2018).
3. Ali, S. et al. Exploring physiological and molecular dynamics of drought stress responses in plants: challenges and future directions. *Front. Plant Sci.* **16**, 1565635. https://doi.org/10.3389/fpls.2025.1565635 (2025).
4. AL-Kazzaz, A. G. M. Role of ascorbic acid in drought stress tolerance of wheat plant *Triticum aestivum* L. *Ibn AL-Haitham J. Pure Appl. Sci.* **36** (3), 21–27. https://doi.org/10.30526/36.3.3101 (2023).
5. Arnon, D. I. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* **24** (1), 1–15. https://doi.org/10.1104/pp.24.1.1 (1949).
6. Ashraf, M., Shahzad, S. M., Imtiaz, M. & Rizwan, M. Salinity and drought stress tolerance in plants through antioxidant regulation and osmolyte accumulation. *Environ. Exp. Bot.* **201**, 104942. https://doi.org/10.1016/j.envexpbot.2022.104942 (2022).
7. Barrs, H. D. & Weatherley, P. E. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian J. Biol. Sci.* **15** (3), 413–428. https://doi.org/10.1071/BI9620413 (1962).
8. Bates, L. S., Waldren, R. P. & Teare, I. D. Rapid determination of free proline for water-stress studies. *Plant. Soil.* **39** (1), 205–207. https://doi.org/10.1007/BF00018060 (1973).
9. Beyer, W. F. & Fridovich, I. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Anal. Biochem.* **161** (2), 559–566. https://doi.org/10.1016/0003-

2697(87)90489-1 (1987).

10. Beyer, W. F. Jr. & Fridovich, I. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Anal. Biochem.* **161** (2), 559–566. https://doi.org/10.1016/0003-2697(87)90489-1 (1987).
11. Chaouachi, L. et al. The opportunity of using durum wheat landraces to tolerate drought stress: screening morpho-physiological components. *AoB Plants*. **15** (3), plad022. https://doi.org/10.1093/aobpla/plad022 (2023).
12. Dhanda, S. S., Sethi, G. S. & Behl, R. K. Indices of drought tolerance in wheat genotypes at early stages of plant growth. *J. Agron. Crop. Sci.* **190** (1), 6–12. https://doi.org/10.1046/j.1439-037X.2003.00092.x (2004).
13. Dhanda, S. S., Sethi, G. S. & Behl, R. K. Indices of drought tolerance in wheat genotypes at early stages of plant growth. *J. Agron. Crop. Sci.* **190** (1), 6–12. https://doi.org/10.1046/j.1439-037X.2003.00092.x (2004).
14. Dvojković, K. et al. Early antioxidative response to desiccant-stimulated drought stress in field-grown traditional wheat varieties. *Plants* **12** (2), 249. https://doi.org/10.3390/plants12020249 (2023).
15. Dvojković, K. et al. Early antioxidative response to desiccant-stimulated drought stress in field-grown traditional wheat varieties. *Plants* **12**, 249. https://doi.org/10.3390/plants12020249 (2023).
16. Foyer, C. H. & Kunert, K. The ascorbate–glutathione cycle coming of age. *J. Exp. Bot.* **75** (9), 2682–2699. https://doi.org/10.1093/jxb/erae023 (2024).
17. Ghosh, U. K., Islam, M. N., Siddiqui, M. N. & Khan, M. A. R. Understanding the roles of osmolytes for acclimatizing plants to changing environment: a review of potential mechanism. *Plant Signal. Behav.* **16** (8), 1913306. https://doi.org/10.1080/15592324.2021.1913306 (2021).
18. Gulino, D. & Lopes, M. S. Phenological adaptation of wheat varieties to rising temperatures: implications for yield components and grain quality. *Plants* **13** (20), 2929. https://doi.org/10.3390/plants13202929 (2024).
19. Gupta, A. K. et al. The effect of climate change on wheat production: present patterns and upcoming difficulties. *Int. J. Environ. Clim. Change*. **13** (11), 4408–4417. https://doi.org/10.9734/ijecc/2023/v13i113621 (2023).
20. Hadwan, M. H. et al. An improved method for measuring catalase activity in biological samples. *Biology Methods Protocols*. **9**, bpae015. https://doi.org/10.1093/biomethods/bpae015 (2024).
21. Haghpanah, M., Hashemipetroudi, S., Arzani, A. & Araniti, F. Drought tolerance in plants: physiological and molecular responses. *Plants* **13** (21), 2962. https://doi.org/10.3390/plants13212962 (2024).
22. Hameed, A., Goher, M. & Iqbal, N. Drought induced programmed cell death and associated changes in antioxidants, proteases and lipid peroxidation in wheat leaves. *Biol. Plant.* **57**, 370–374. https://doi.org/10.1007/s10535-012-0286-9 (2013).
23. Hong, S. H. et al. Functional switching of ascorbate peroxidase 2 of rice between peroxidase and molecular chaperone. *Sci. Rep.* **8**, 9171. https://doi.org/10.1038/s41598-018-27459-1 (2018).

24. Hong, Y. et al. Comprehensive profiling of ascorbate peroxidase gene family and enzymatic responses under abiotic stress in wheat. *Front. Plant Sci.* **9**, 1254. https://doi.org/10.3389/fpls.2018.01254 (2018).
25. Hu, C., Elias, E., Nawrocki, W. J. & Croce, R. Drought affects both photosystems in *Arabidopsis thaliana*. *New Phytol.* **240** (2), 663–675 (2023).
26. Hu, F., Zhang, Y. & Guo, J. Effects of drought stress on photosynthetic physiological characteristics, leaf microstructure and related gene expression of yellow horn. *Plant Signal. Behav.* **18** (1), 2215025. https://doi.org/10.1080/15592324.2023.2215025 (2023).
27. Iqbal, M. J. Role of osmolytes and antioxidant enzymes for drought tolerance in wheat. In: Global Wheat Production. London: IntechOpen. https://doi.org/10.5772/intechopen.75926 (2018).
28. Ishfaq, N. et al. Mitigating drought-induced oxidative stress in wheat (*Triticum aestivum* L.) through foliar application of sulfhydryl thiourea. *Sci. Rep.* **14**, 15985. https://doi.org/10.1038/s41598-024-66506-y (2024).
29. Kemp, C. D. Methods of estimating the leaf area of grasses from linear measurements. *Ann. Botany*. **24** (95), 491–499. https://doi.org/10.1093/oxfordjournals.aob.a083734 (1960).
30. Kumari, M. et al. Effect of drought stress on physiological, biochemical and antioxidant activity of wheat genotypes at booting stage. *Plant. Sci. Today*. **12** (sp4), 1–8. https://doi.org/10.14719/pst.8842 (2025).
31. Kumari, M. et al. Effect of drought stress on physiological, biochemical and antioxidant activity of wheat genotypes at booting stage. *Plant. Sci. Today*. **12** (sp4), 1–8. https://doi.org/10.14719/pst.8842 (2025).
32. Laddomada, B. et al. Drought and heat stress impacts on phenolic acids accumulation in durum wheat cultivars. *Foods* **10** (9), 2142. https://doi.org/10.3390/foods10092142 (2021).
33. Lichtenthaler, H. K. & Wellburn, A. R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **11** (5), 591–592. https://doi.org/10.1042/bst0110591 (1983).
34. Lichtenthaler, H. K. & Wellburn, A. R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **11** (5), 591–592. https://doi.org/10.1042/bst0110591 (1983).
35. Lukić, N., Kukavica, B., Davidović-Plavšić, B., Hasanagić, D. & Walter, J. Plant stress memory is linked to high levels of anti-oxidative enzymes over several weeks. *Environ. Exp. Bot.* **178**, 104166. https://doi.org/10.1016/j.envexpbot.2020.104166 (2020).
36. Lv, L. et al. Different adaptive patterns of wheat with different drought tolerance under drought stresses and rehydration revealed by integrated metabolomic and transcriptomic analysis. *Front. Plant Sci.* **13**, 1008624. https://doi.org/10.3389/fpls.2022.1008624 (2022).
37. Maiti, R. K. & Satya, P. Research advances in major cereal crops for adaptation to abiotic stresses. *GM Crops Food*. **5** (4), 259–279. https://doi.org/10.4161/21645698.2014.947861 (2014).

38. Nardino, M. et al. Understanding drought response mechanisms in wheat and multi-trait selection. *PLoS ONE*. **17** (4), e0266368. https://doi.org/10.1371/journal.pone.0266368 (2022).
39. Ndzi, D. L., Okoniewska, M., Nzikou, J. M. & Low, N. H. Role of ascorbic acid in the extraction and quantification of potato polyphenol oxidase activity. *Foods* **10** (10), 2486. https://doi.org/10.3390/foods10102486 (2021).
40. Nithinkumar, K. et al. *Relative Water Content: Concept and Practical Implications*. IntechOpen. (2024). https://doi.org/10.5772/intechopen.100551
41. Nithinkumar, K. et al. Venkata Sai Chakradhar Reddy, A. and Relative water content: concept and practical implications. (2024).
42. Nyaupane, S. et al. Drought stress effect, tolerance and management in wheat – a review. *Cogent Food Agric.* **10** (1), 2296094. https://doi.org/10.1080/23311932.2023.2296094 (2024).
43. Qiao, M., Hong, C., Jiao, Y., Hou, S. & Gao, H. Impacts of drought on photosynthesis in major food crops and the related mechanisms of plant responses to drought. *Plants* **13** (13), 1808. https://doi.org/10.3390/plants13131808 (2024).
44. Raza, A. & Khan, N. Assessment of proline function in higher plants under abiotic stress. *Plant Biol.* **25** (1), 73–98. https://doi.org/10.1111/plb.13510 (2023).
45. Raza, A. & Khan, N. Assessment of proline function in higher plants under abiotic stress. *Plant Biol.* **25** (1), 73–98. https://doi.org/10.1111/plb.13510 (2023).
46. Sachdev, S., Ansari, S. A., Ansari, M. I., Fujita, M. & Hasanuzzaman, M. Abiotic stress and reactive oxygen species: generation, signaling and defense mechanisms. *Antioxidants* **10** (2), 277. https://doi.org/10.3390/antiox10020277 (2021).
47. Seleiman, M. F. et al. Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants* **10** (2), 259. https://doi.org/10.3390/plants10020259 (2021).
48. Shavrukov, Y. et al. Early flowering as a drought escape mechanism in plants: how can it aid wheat production? *Frontiers in Plant Science*, 8, 1950. (2017). https://doi.org/10.3389/fpls.2017.01950
49. Silva, S. H. M. G., Vendruscolo, E. P., Seleguini, A., Benett, K. S. S. & Nascimento, A. R. Non-destructive models for estimating leaf area in wheat. *Pesquisa Agropecuária Trop.* **52**, e70609. https://doi.org/10.1590/1983-40632022v5270609 (2022).
50. Singh, M., Vaishali & Kumar, L. Phenolic acid analysis in Indian wheat cultivar exposed to drought stress. *Journal Pharmacognosy Phytochemistry Special Issue*. **9** (5), 287–294 (2020).
51. Stewart, R. R. C. & Bewley, J. D. Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiol.* **65** (2), 245–248. https://doi.org/10.1104/pp.65.2.245 (1980).
52. Stewart, R. R. C. & Bewley, J. D. Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiol.* **65** (2), 245–248. https://doi.org/10.1104/pp.65.2.245 (1980).
53. Sumitra, P., Kilian, B., Özkan, H., Zeibig, F. & Frei, M. Physiological and biochemical changes induced by drought stress during the stem elongation and anthesis stages in the *Triticum* genus. *Environ. Exp. Bot.* **228** (B), 106047. https://doi.org/10.1016/j.envexpbot.2024.106047 (2024).

54. Szablińska-Piernik, J. & Lahuta, L. B. Polar metabolites profiling of wheat shoots (*Triticum aestivum* L.) under repeated short-term soil drought and rewatering. *Int. J. Mol. Sci.* **24** (9), 8429. https://doi.org/10.3390/ijms24098429 (2023).
55. Szablińska-Piernik, J. & Lahuta, L. B. Polar metabolites profiling of wheat shoots (*Triticum aestivum* L.) under repeated short-term soil drought and rewatering. *Int. J. Mol. Sci.* **24** (9), 8429. https://doi.org/10.3390/ijms24098429 (2023).
56. Tanbakuyi, F., Chaichi, M., Razavi, K. & Sanjarian, F. Tissue-specific antioxidant responses and PAL gene expression in bread wheat under drought stress. *Iran. J. Biotechnol.* **23** (1), e4005. https://doi.org/10.30498/ijb.2025.483262.4005 (2025).
57. Ullah, N., Nawaz, M. A. & Alsafran, M. Physiological mechanisms regulating source–sink interactions and grain yield formation in heat-stressed wheat. *Plant. Stress*. **14**, 100654 (2024).
58. Wang, J. & Xu, Z. Plant response to and recovery from drought. *Curr. Biol.* **36** (8), R343–R362 (2026).
59. Wang, S., Ma, Q., Li, C., Zhang, S. & Liu, X. Chloroplast responses to drought: integrative mechanisms and mitigation strategies. *Int. J. Mol. Sci.* **26** (24), 11872. https://doi.org/10.3390/ijms262411872 (2025).
60. Yan, H. et al. Metabolic response strategies of spring wheat under osmotic stress. *Front. Plant Sci.* **17**, 1766233. https://doi.org/10.3389/fpls.2026.1766233 (2026).
61. Yan, W. et al. Effects of drought stress on photosynthesis and chlorophyll fluorescence in blue honeysuckle. *Plants* **13** (15), 2115. https://doi.org/10.3390/plants13152115 (2024).
62. Yemm, E. W. & Willis, A. J. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.* **57** (3), 508–514. https://doi.org/10.1042/bj0570508 (1954).

Figures

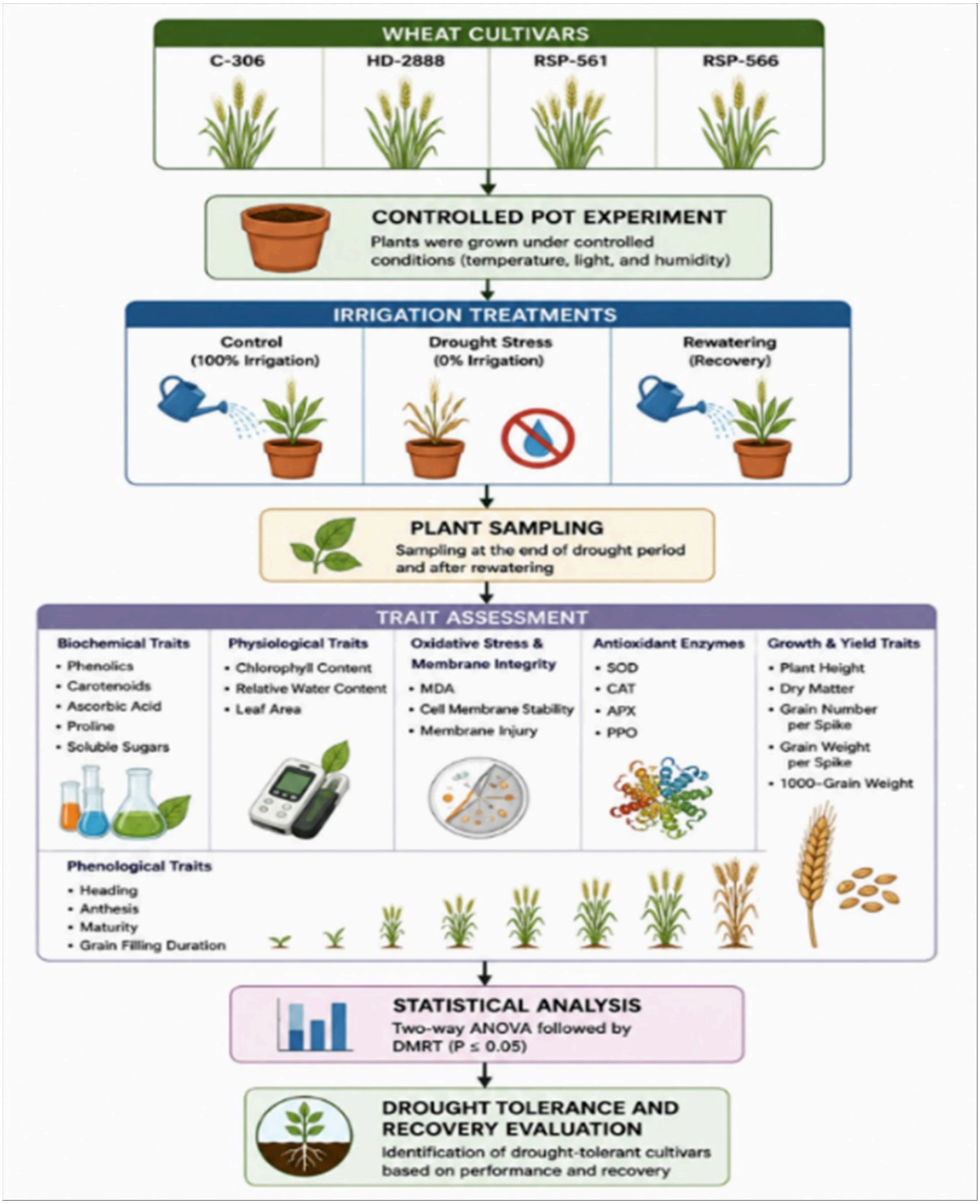


Figure 1

Experimental workflow illustrating drought stress, rewatering treatment, trait assessment, and drought tolerance evaluation in four wheat cultivars.

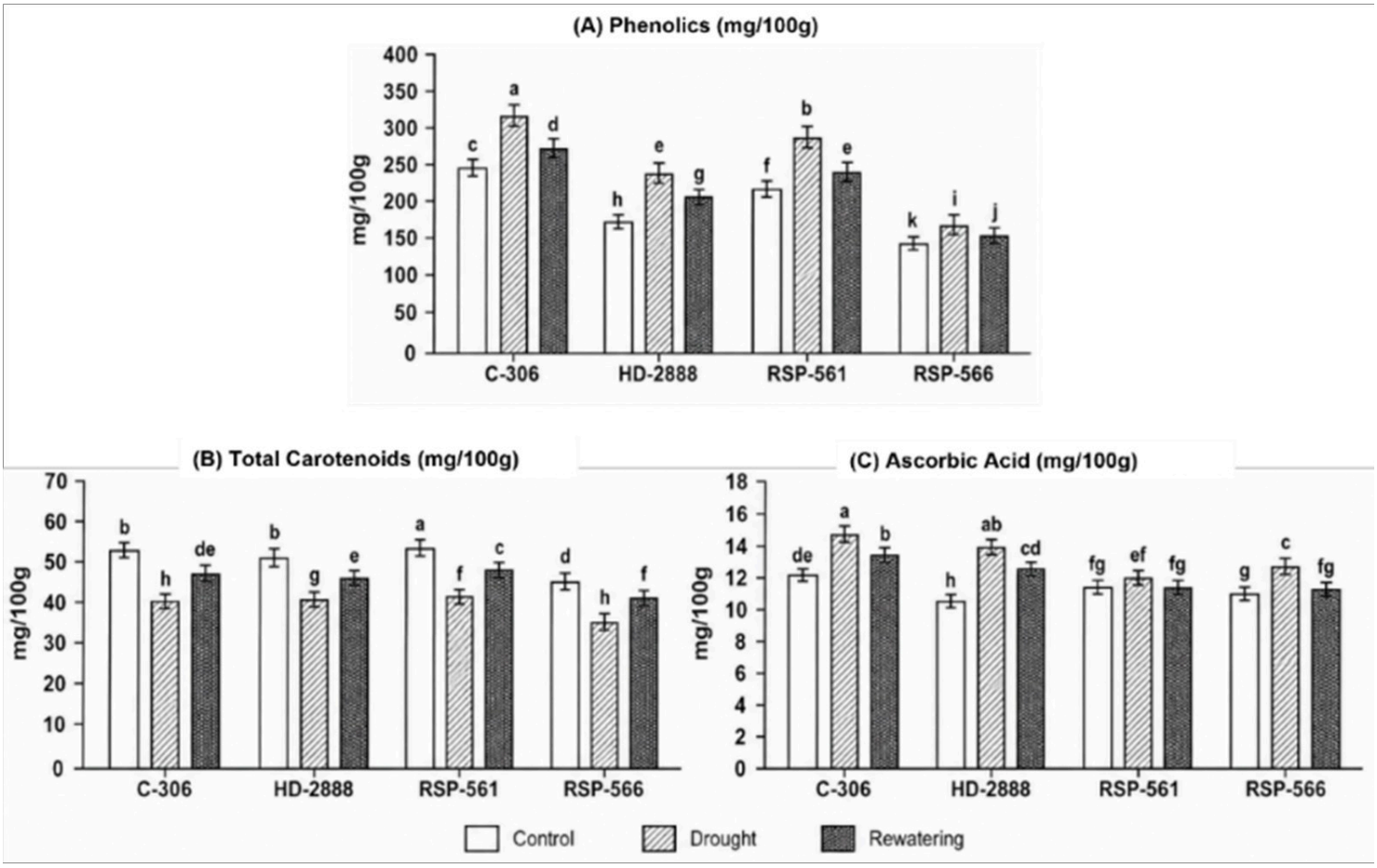


Figure 2

A) Total phenolics, B) carotenoid and C) ascorbic acid content in wheat cultivars subjected to drought stress. Bars are mean ± SE (n=3). Different letters above bar within each cultivar indicates significant differences at $P \leq 0.05$ (DMRT).

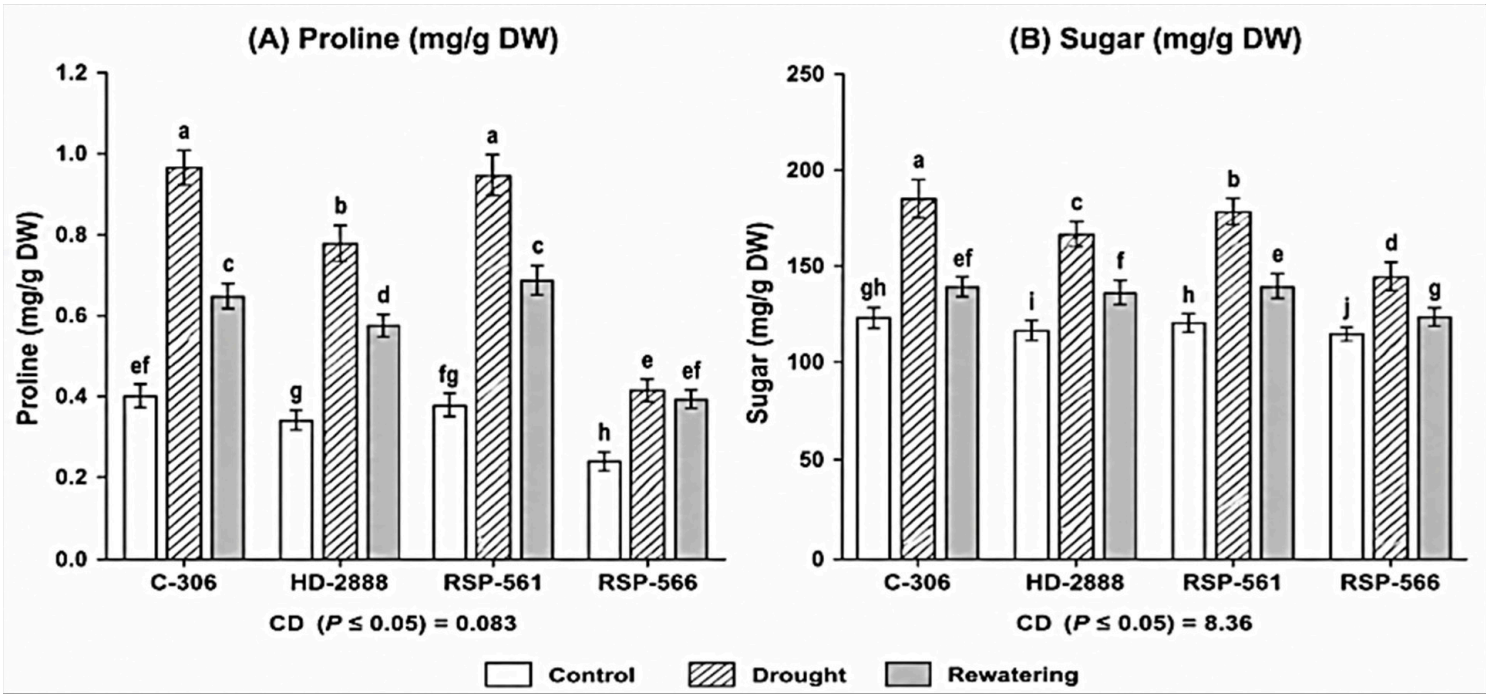


Figure 3

A) Proline content and B) Total sugars in wheat cultivars subjected to drought stress. Bars are mean ± SE (n=3). Different letters above bar within each cultivar indicates significant differences at P ≤ 0.05 (DMRT).

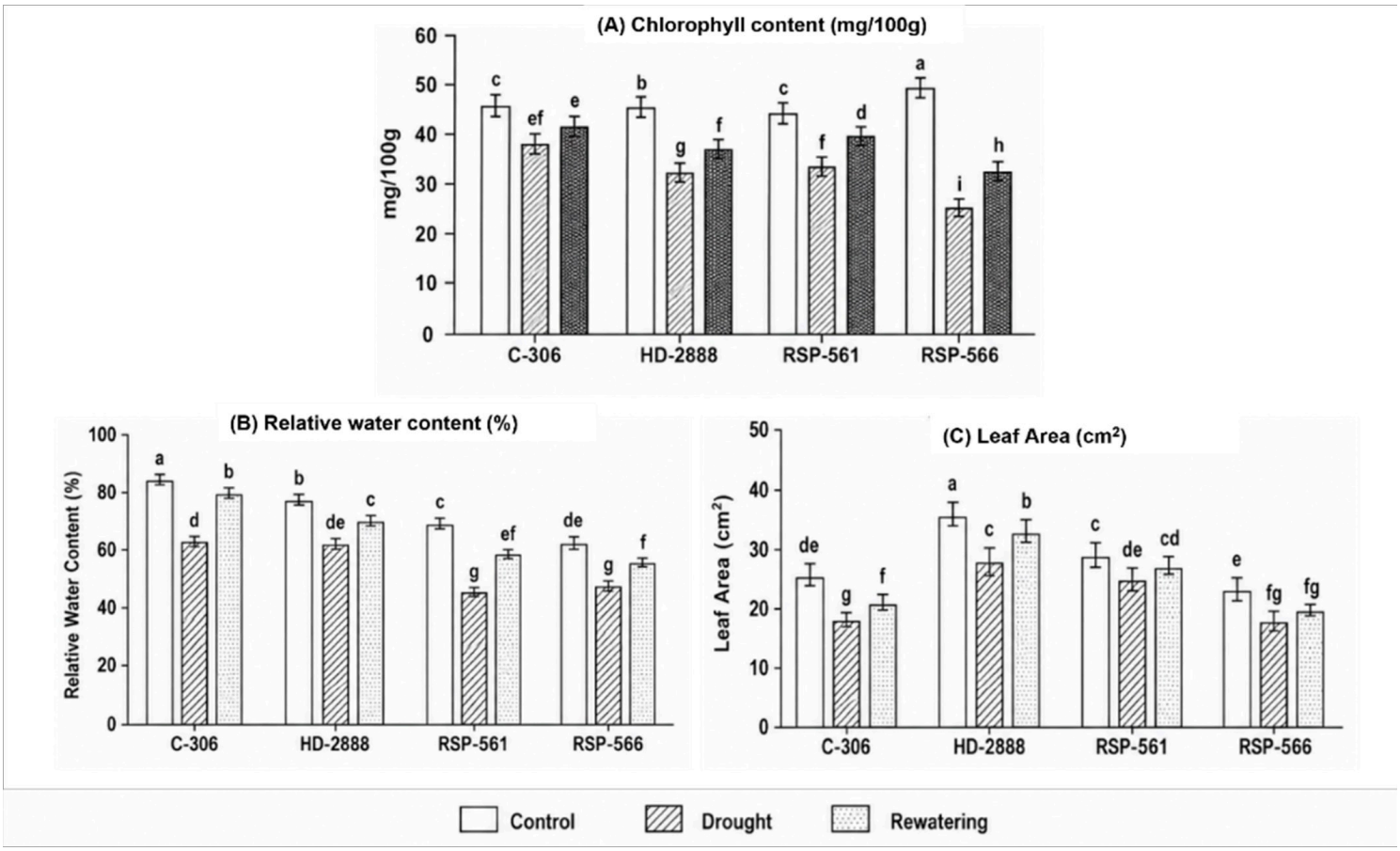
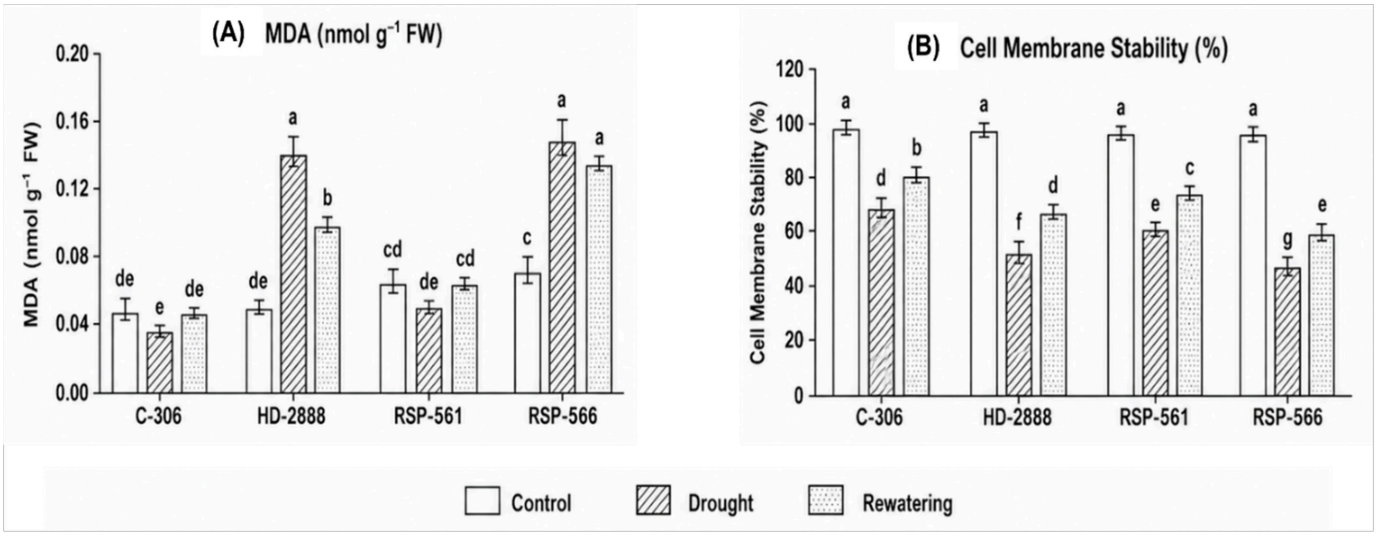
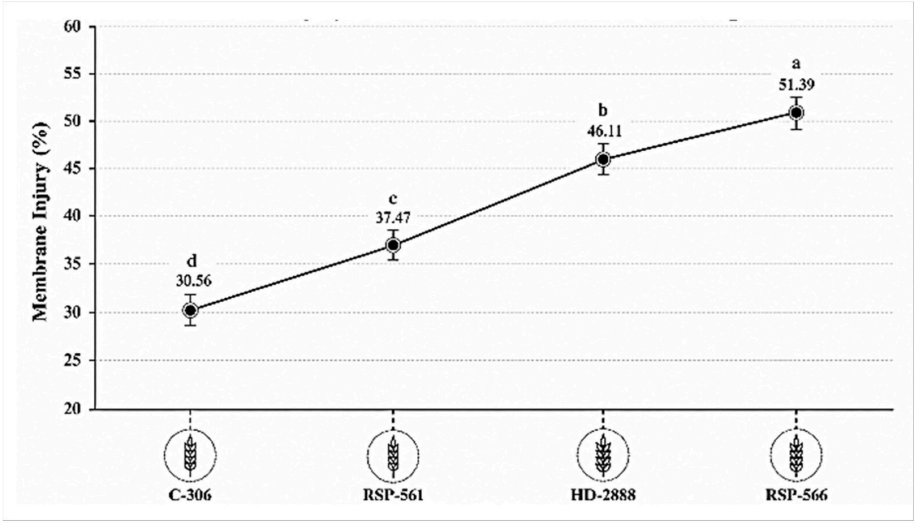


Figure 4

Effect of drought stress on physiological traits of wheat cultivars. A) Chlorophyll content, B) Relative water content, and C) Leaf area. Bars are mean ± SE (n=3). Different letters above bar within each cultivar indicates significant differences at P ≤ 0.05 (DMRT).





C

Figure 5

Effect of drought stress on A) MDA content and B) cell membrane stability of wheat cultivars. Different letters above bar within each cultivar indicates significant differences at P ≤ 0.05 (DMRT).

C: Membrane injury (%) in four wheat cultivars exposed to drought stress. Different letters indicate significant differences among cultivars according to Duncan's Multiple Range Test (DMRT) at *P* ≤ 0.05.

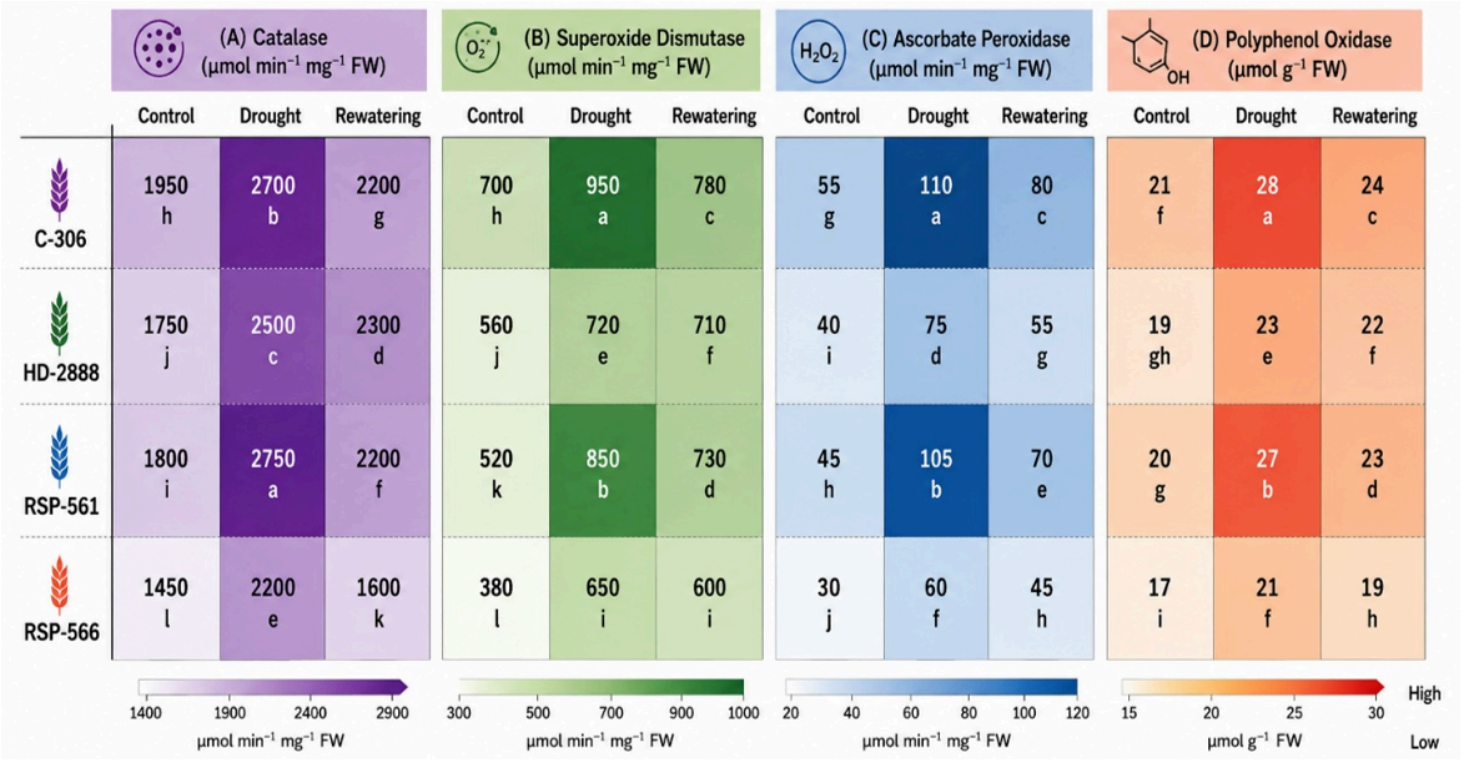


Figure 6

Heatmap showing cultivar-specific changes in (A) CAT, (B) SOD, (C) APX, and (D) PPO activities under control, drought, and rewatering conditions. Higher color intensity denotes greater enzyme activity. Different letters indicate significant differences among treatment means (DMRT, $P \leq 0.05$).).

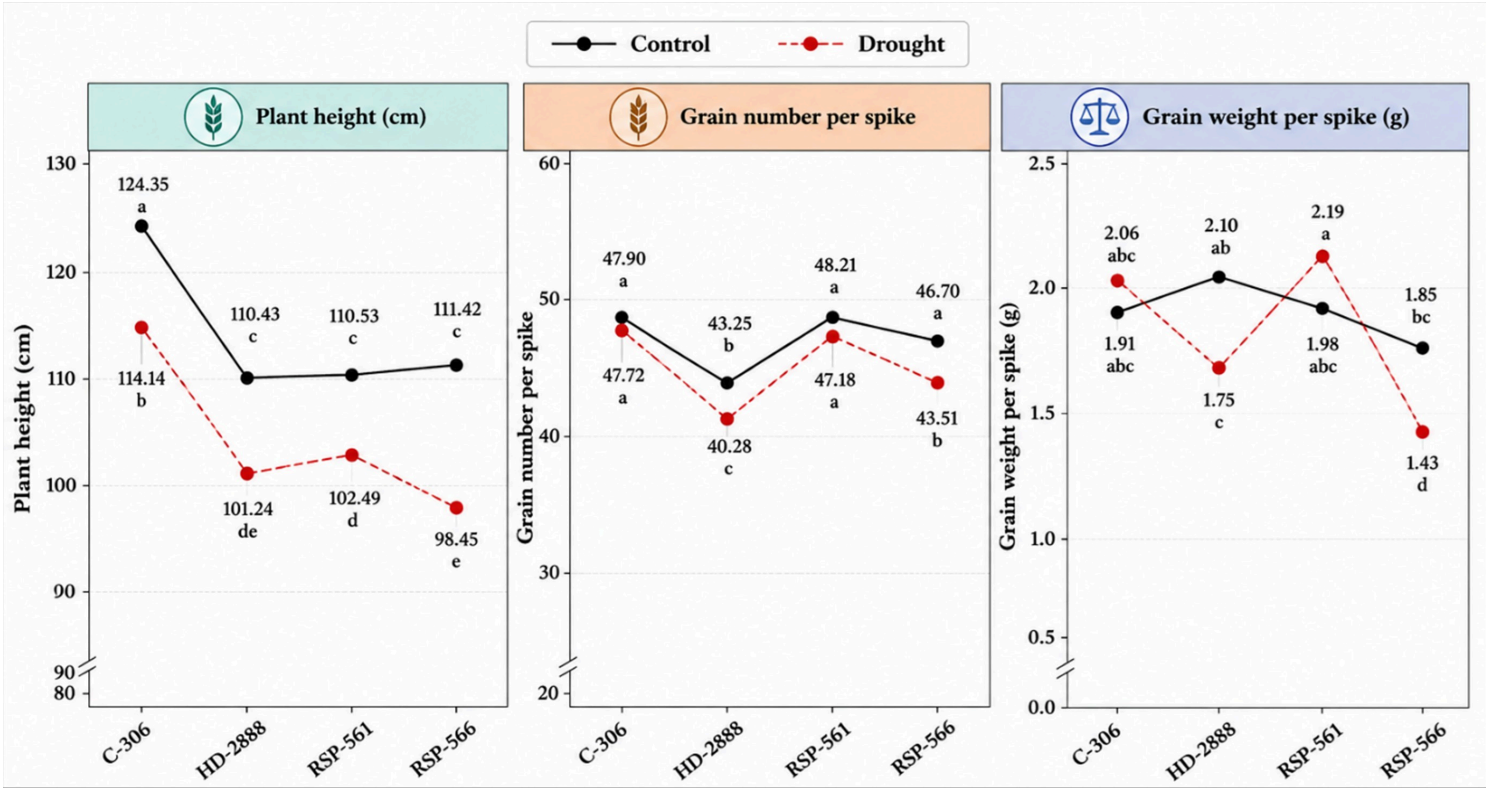


Figure 7

Cultivar-specific responses of plant height, grain number per spike, and grain weight per spike under control and drought stress conditions. Lines connect treatment means for each cultivar. Different letters indicate significant differences among treatment means (DMRT, $P \leq 0.05$).

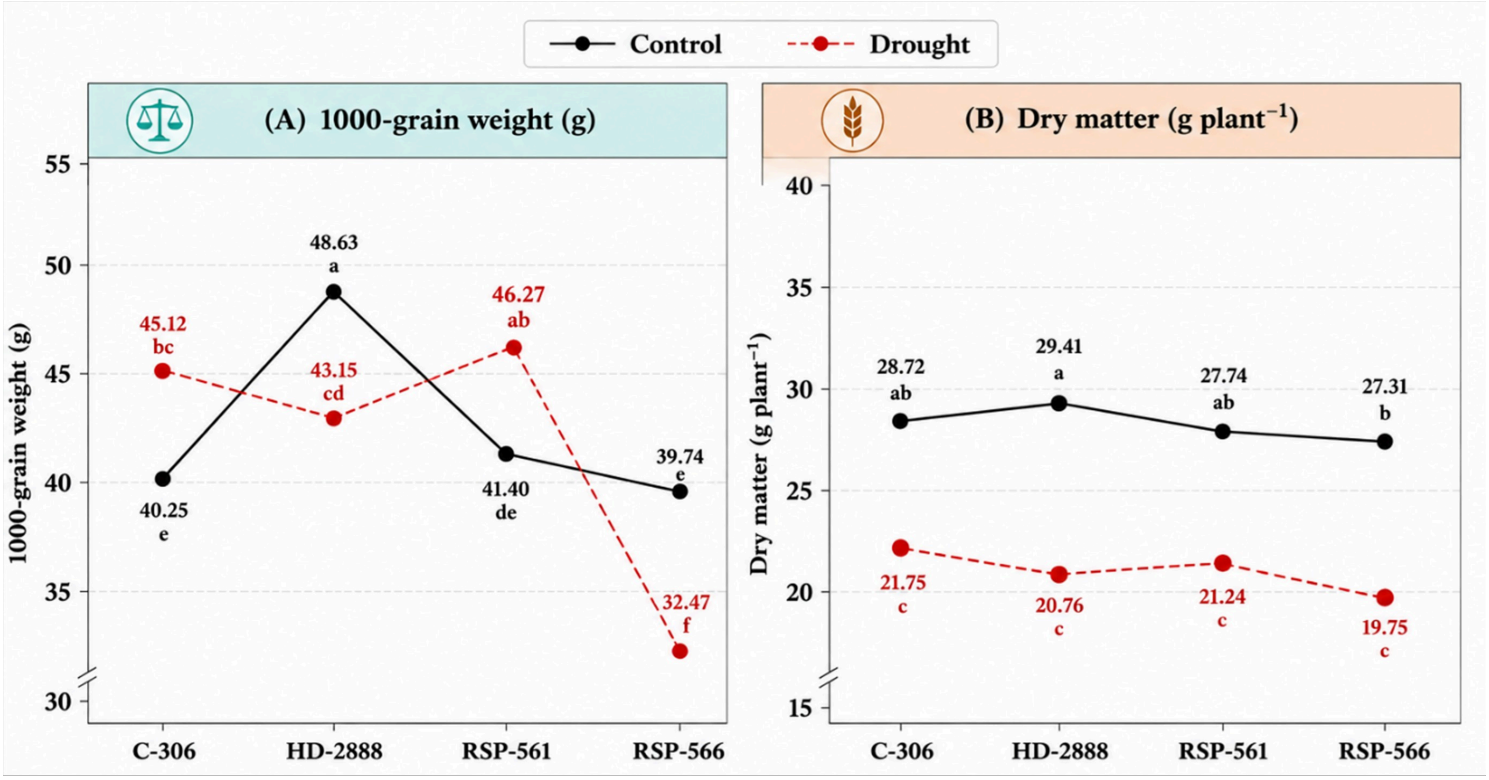


Figure 8

Cultivar-specific variation in 1000-grain weight and dry matter accumulation under control and drought stress conditions. Lines connect treatment means for each cultivar. Different letters denote significant differences among treatment means (DMRT, $P \leq 0.05$).

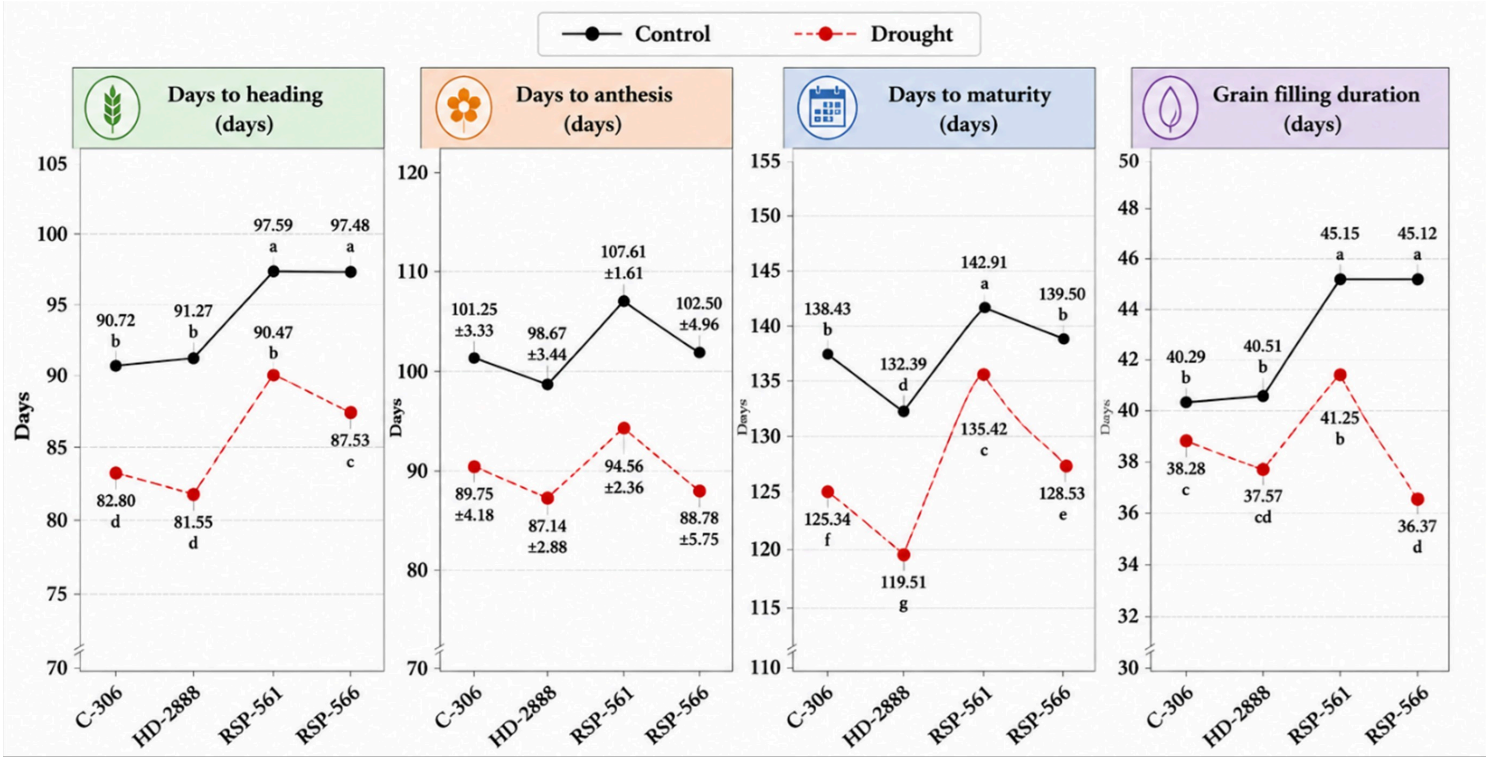


Figure 9

Effects of drought stress on days to heading, anthesis, maturity, and grain filling duration in four wheat cultivars. Drought accelerated phenological development and shortened the grain filling period across cultivars. Different letters denote significant differences among treatment means (DMRT, $P \leq 0.05$).