

Exogenous proline enhances redox homeostasis and growth performance in drought-stressed wheat

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Abstract

This study investigated the role of foliar-applied proline in mitigating drought-induced damage in two wheat (*Triticum aestivum* L.) varieties, Subhani-21 and Dilkash-20, grown under 100% and 40% field capacity. Proline was applied as a foliar spray at concentrations of 50 and 100 mM. Plants were assessed for growth attributes, photosynthetic pigments, osmolyte accumulation, antioxidant enzyme activities, and ion homeostasis. Drought stress markedly reduced shoot and root growth, biomass accumulation, and chlorophyll content. Exogenous proline significantly alleviated these adverse effects, with 100 mM proving most effective. Proline-treated plants under water deficit exhibited notable increases in chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids, indicating improved photosynthetic stability. Furthermore, proline application enhanced the accumulation of endogenous proline, soluble proteins, and phenolic compounds, along with increased activities of key antioxidant enzymes (SOD, POD, and CAT), reflecting improved oxidative stress tolerance. Enhanced accumulation of potassium (K⁺) and reduced accumulation of sodium (Na⁺) suggested better ionic balance under drought conditions. Correlation analysis revealed strong positive associations among growth parameters, pigment content, osmolytes, and antioxidant responses, supporting a coordinated proline-mediated stress tolerance mechanism. Overall, foliar application of proline, particularly at 100 mM, effectively enhanced drought resilience in wheat through integrated physiological and biochemical adjustments. These findings highlight the potential of proline as a practical strategy to improve wheat performance under water-limited conditions.

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Introduction

Drought stress lowers growth and development of plants by limiting several physiological processes, including photosynthetic activity (Qiao et al., 2024). Drought stress interferes with plant water relations, nutrient uptake, and metabolic homeostasis. A decline in soil moisture restricts the absorption of water, leading to stomatal closure and decreased CO₂ assimilation, which in turn reduces the chlorophyll content and photosynthetic capacity (Qiao et al., 2024; Khamis et al., 2025). This physiological imbalance is further extended with oxidative stress that is developed due to the

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excessive formation of reactive oxygen species (ROS), such as superoxide radicals and hydrogen peroxide, which may result in severe damage to cell membranes and macromolecules (Jomova et al., 2024; Keya et al., 2025; Rao et al., 2025). As a result, the growth, biomass, and reproductive development are significantly decreased, resulting in severe losses in the yield of drought-impacted wheat. Future climate change projects increased frequency and severity of droughts, likely escalating food security threats, especially in arid and semi-arid areas (Seleiman et al., 2021; Wang et al., 2024).

Plants have evolved a wide range of adaptive strategies to cope with drought stress, which are broadly categorized into morphological, physiological, and biochemical mechanisms. Morphologically, drought conditions often lead to reduced leaf area, leaf thickening, increased leaf rolling, and enhanced cuticle development, all of which contribute to minimizing transpirational water loss (Ali et al., 2022; Khan et al., 2026). In addition, root system architecture is frequently modified through deeper and more extensive rooting to improve water acquisition from deeper soil layers (Kaur et al., 2026). Physiologically, plants regulate stomatal conductance to reduce water loss, adjust photosynthetic activity, and maintain cellular turgor through osmotic adjustment, thereby sustaining essential metabolic functions under water-deficit conditions (Haghpanah et al., 2024). Biochemically, drought stress triggers the accumulation of compatible solutes such as proline, glycine betaine, and soluble sugars, which contribute to osmotic balance and stabilization of proteins and cellular membranes, while antioxidant defense systems, including superoxide dismutase, catalase, and peroxidases, are upregulated to detoxify reactive oxygen species generated under stress (Kapoor et al., 2020; Haghpanah et al., 2024). Together, these integrated responses enable plants to maintain growth, survival, and reproductive potential under limited water availability.

Proline is one of the osmoprotectants that has multiple effects on plant metabolism. It is an osmolyte that sustains cellular water homeostasis, protein and membrane stabilization, ROS scavenging, and redox homeostasis (Renzetti et al., 2025). Moreover, proline acts as a signaling molecule that regulates stress-related gene activity, including *P5CS* and *ProDH*, and interacts with hormonal pathways such as abscisic acid (ABA) to modulate plant responses to drought stress (Balooch et al., 2024). In this context, exogenous application of proline has shown promising results in several crops. For example, in maize, proline treatment improved shoot and root growth, maintained chlorophyll content, and reduced lipid peroxidation under drought stress (Khan et al., 2022; 2025). Similarly, in wheat, foliar application of proline enhanced water retention, antioxidant enzyme activities, and grain yield under water deficit (Li et al., 2024; Pecka et al., 2025).

Foliar application is particularly important as it facilitates rapid uptake through leaf tissues and directly influences key physiological processes, including osmotic adjustment and antioxidant defense, under water-deficit conditions (Jacomassi et al., 2024; Metwally et al., 2026). Despite its practical importance, the optimal concentrations and underlying mechanisms by which foliar-applied proline mitigates drought-induced stress in wheat remain insufficiently understood. Therefore, this study was conducted to evaluate the effects of exogenous proline applied as a foliar spray on the growth, physiological, and biochemical responses of wheat under drought stress (40% field capacity). It was hypothesized that foliar application of proline would alleviate drought-induced oxidative and osmotic stress by upregulating the antioxidant defense system, enhancing osmolyte accumulation, and regulating ion homeostasis, thereby improving plant growth performance. Overall, this study aimed to provide insights into the potential use of proline as a bio-stimulant for improving drought tolerance in wheat.

Material and Methods

Experimental site and environmental conditions

The current research aimed to explore the ameliorative role of exogenously applied proline in enhancing drought tolerance of wheat (*Triticum aestivum* L.) cultivars. The work was conducted in the Old Botanical Garden, University of Agriculture, Faisalabad (UAF), Pakistan, in the open air and in the daytime. The Environmental Biology and Plant Ecology Laboratory, Department of Botany, UAF, was used to carry out physiological and biochemical analyses. During the experiment, the overall climatic conditions were characterized by the average temperatures of 24.29 °C in daytime, 14.18 °C in nighttime, average relative humidity of 55.7 %, and the natural photosynthetic photon flux density (PPFD) of 850 to 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The mean photoperiod was 11 to 13 h.

Experimental design and treatments

The experiment involved a completely randomized design (CRD), which had three replications

per treatment. The treatments were two water regimes and two levels of proline. The water regimes constituted control (100% field capacity) and drought stress (40% field capacity). Two wheat varieties, namely, Subhani-21 and Dilkash-20, were procured from the Ayub Agricultural Research Institute (AARI), Faisalabad. In addition to the control treatment, proline was applied at two concentrations, 50 mM and 100 mM, representing the low and high effective doses without causing phytotoxic effects.

Soil preparation and pot details

Pots were filled each with 8 kg of air-dried loamy soil. Before sowing, the soil was analyzed for different physicochemical properties. The experiment used soil which contained a loamy texture having a pH of 7.6 ± 0.2 , and electrical conductivity (EC) 1.35 dS m^{-1} . The percentage of organic matter was 1.2, and the field capacity determined through the gravimetric method was 23%.

Sowing and crop management

Surface-sterilization of the seeds was done by placing them in 1% sodium hypochlorite solution for 3 min. They were thoroughly rinsed with distilled water to eliminate all traces of the disinfectant. Each pot contained 10 seeds, and once they had completely emerged, the seedlings were thinned to five similar plants per pot. All pots were maintained under natural light and ambient temperature. Basal fertilizers of N, P, and K ($100:80:60 \text{ kg ha}^{-1}$) were applied using urea, single superphosphate, and muriate of potash, respectively, after converting the field-recommended doses to pot-equivalent quantities according to the soil mass per pot. Weeding was performed manually, and the pots were rotated periodically to minimize positional effects from sunlight variation.

Induction of drought stress and application of proline

Germination was complete after 10 days of sowing. Drought stress was imposed 20 days after sowing by maintaining soil moisture at 40% of field capacity (FC), while the control plants were maintained at 100% FC. Field capacity was monitored gravimetrically by weighing the pots every three days, and the water loss was replenished to maintain the desired moisture levels. Proline treatments were applied as a foliar spray seven days after the imposition of drought stress. Solutions of 0 mM, 50 mM, and 100 mM proline were prepared in distilled water and sprayed uniformly onto the leaves until runoff. The treatments were repeated at two-week intervals to maintain the effect of proline throughout the growth period.

Harvesting and sample preparation

Plants were harvested after four months of growth. Two plants per pot were carefully uprooted and separated into shoot and root. Fresh samples were used immediately for the estimation of physiological and biochemical parameters, while portions collected for morphological measurements were oven-dried at 70°C . Leaf tissues collected for enzyme assays were rapidly frozen in liquid nitrogen.

Morphological measurements

Shoot and root lengths were measured using a ruler, whereas shoot and root fresh and dry weights were determined using an analytical balance.

Determination of photosynthetic pigments

The photosynthetic pigments were isolated and determined by the procedure outlined by Arnon (1949), but with minor modifications. New leaf samples (each 0.5 g) were homogenized in 10 mL of 80% acetone and centrifuged at 10,000 rpm for 10 minutes. The supernatant was then gathered, and the absorbance was measured at 663 nm and 645 nm using a UV-V spectrophotometer (Shimadzu UV-1800). The concentrations of chlorophyll *a*, chlorophyll *b*, and total chlorophyll were then determined using the following standard equations:

$$\text{Chl } a \text{ (mg g}^{-1} \text{ FW)} = [12.7(A_{663}) - 2.69(A_{645})] \times V / (1000 \times W)$$

$$\text{Chl } b \text{ (mg g}^{-1} \text{ FW)} = [22.9(A_{645}) - 4.68(A_{663})] \times V / (1000 \times W)$$

$$\text{Total Chlorophyll} = \text{Chl } a + \text{Chl } b$$

V = volume of extract (mL), and W = fresh weight (g) of the sample.

Antioxidant enzyme assays

Homogenization of leaf tissues (0.25g) was done in 5 mL ice-cold 50 mM potassium phosphate buffer (pH 7.8) with 1 mM EDTA and 1% polyvinylpyrrolidone (PVP). The homogenate was

centrifuged at 12,000 rpm for 15 min at 4 °C, and the resulting supernatant was utilized in enzymatic activity assays.

Superoxide dismutase

The SOD activity was determined based on its capacity to inhibit the photochemical reduction of nitroblue tetrazolium (NBT), following the method of Beauchamp and Fridovich (1971). Absorbance was recorded at 560 nm, and one unit of SOD was defined as the amount of enzyme required to inhibit 50% of NBT reduction.

Peroxidase

The POD activity was assessed using the procedure described by Chance and Maehly (1955). The reaction mixture contained phosphate buffer (pH 7.0), guaiacol, hydrogen peroxide (H₂O₂), and the enzyme extract. The formation of tetraguaiacol was monitored spectrophotometrically at 470 nm at 30 sec intervals.

Catalase

The CAT activity was measured by tracking the decomposition of H₂O₂ at 240 nm according to Chance and Maehly (1955). The enzyme activity was expressed as $\mu\text{mol H}_2\text{O}_2$ decomposed per minute per milligram of protein.

Estimation of osmolytes and biochemical attributes

Proline content

Free proline levels in the leaf samples were quantified following Bates et al. (1973). Fresh leaf samples (each 0.5 g) were homogenized in 3% sulfosalicylic acid and filtered. The filtrate was reacted with acid ninhydrin and glacial acetic acid in a boiling water bath for 1 h. The chromophore was extracted with toluene, and the absorbance was taken at 520 nm after cooling.

Total soluble proteins (TSP)

According to the method of Bradford (1976), 0.25 g of fresh leaf tissue was homogenized in 5 mL of phosphate buffer. The homogenate was transferred to Eppendorf tubes and centrifuged at 12,000 rpm for 15 min. The supernatant was collected in fresh Eppendorf tubes after discarding the pellet. An aliquot of the supernatant was mixed with 5 mL of Bradford reagent in a test tube. The mixture was vortexed, and absorbance was measured at 595 nm using a spectrophotometer.

Total phenolics

Total phenolics were estimated by using the method of Julkenen-Titto (1985) using Folin-Ciocalteu reagent. Fresh plant material (0.5 g) was extracted in 80% acetone; the extract was centrifuged at 12,000 and the supernatant collected. A 100 μL of the extract was mixed with 0.5 mL of Folin-Ciocalteu's phenol reagent and 2.5 mL of 20% Na₂CO₃. The volume of the mixture was made up to 5 mL and vortexed. Absorbance of the reaction mixture was noted at 750 nm.

Estimation of mineral ions (Na⁺, K⁺, Ca²⁺)

Oven-dried shoot and root samples (each 0.1 g) were digested with a mixture of concentrated H₂SO₄ and H₂O₂ (2:1, v/v) on a hot plate until the solution became clear. The digested samples were filtered and diluted to 50 mL with distilled water. Sodium (Na⁺), potassium (K⁺), and calcium (Ca²⁺) concentrations were measured using a flame photometer (Sherwood Model 410) and expressed as mg g⁻¹ dry weight.

Statistical analysis

All experimental data were analyzed using Statistix 8.1 software. A two-way ANOVA was applied to examine the effects of proline concentration and water regime. The treatment means were compared using Tukey's HSD test at a 5% significance level ($p \leq 0.05$). The results are presented as mean $\pm$ standard error (SE), and graphical representations were prepared using OriginPro 2023 and Microsoft Excel.

Results

Proline-mediated responses of wheat growth traits under drought stress

All growth parameters, including shoot length (SL), root length (RL), shoot fresh weight (SFW),

root fresh weight (RFW), shoot dry weight (SDW), and root dry weight (RDW), were significantly influenced by drought stress, as confirmed by highly significant main effects ($p < 0.001$; **Table 1**, **Figure 1**). In the absence of proline at 40% field capacity, shoot length declined by 3.85% in Subhani-21 and 11.53% in Dilkash-20. In contrast, root length increased by 30% and 60%, respectively, under drought conditions. A similar trend was observed in biomass traits, where shoot fresh weight decreased by 18.18% in Subhani-21 and 17.47% in Dilkash-20, while shoot dry weight declined by 18.36% and 19.4%, respectively. Conversely, root biomass exhibited an increasing trend, with root fresh weight rising by 16.66% in Subhani-21 and 50% in Dilkash-20, and root dry weight by 25% and 50%, respectively. Application of 100 mM proline markedly improved plant performance under drought stress. In Subhani-21, SL and RL increased by 3.8% and 41.66%, respectively, while RFW and RDW rose by 100% and 150%. The decline in SFW and SDW was considerably reduced to -4.54% and -3.06% , respectively. In Dilkash-20, proline treatment resulted in partial restoration of shoot growth (SL: -3.8%) and substantial enhancement of RL (120%), RFW (100%), and RDW (200%). Additionally, SFW showed a slight increase, and SDW reduction was limited to -3.2% . Significant differences between cultivars were observed for most traits ($p < 0.001$), with Subhani-21 demonstrating relatively greater stability in shoot-related parameters under drought stress. However, the absence of significant interaction effects ($C \times Dr$, $C \times Pro$, $Dr \times Pro$) indicates that both cultivars responded similarly to proline application across varying moisture conditions (**Table 1**).

Proline-mediated changes in photosynthetic pigments under drought stress

Drought stress significantly affected photosynthetic pigments in both cultivars, as indicated by significant drought main effects ($p < 0.01$; **Table 2**). Under 40% field capacity without proline application, chlorophyll *a* decreased by 2.96% in Subhani-21 and 56.74% in Dilkash-20, while chlorophyll *b* declined by 11.69% and 45.03%, respectively. Total chlorophyll was reduced by 22.52% in Subhani-21 and 12.81% in Dilkash-20, whereas carotenoids decreased by 19.08% and 34.60%, respectively (**Figure 2**). Application of 100 mM proline under drought improved pigment levels, with greater recovery observed in Subhani-21. Chlorophyll *a* increased by 8.26%, chlorophyll *b* by 36.37%, total chlorophyll by 31.36%, and carotenoids by 12.06% in proline-treated drought-stressed plants compared to drought-stressed plants without proline. In Dilkash-20, proline treatment resulted in partial recovery, as chlorophyll *a* and *b* remained below the control levels (-20.50% and -13.05% , respectively), while total chlorophyll showed a slight increase ($+8.51\%$) and carotenoids remained reduced (-12.32%). Cultivar differences were significant ($p < 0.05$ – 0.001), with Subhani-21 maintaining relatively better pigment stability under drought stress. However, interaction effects were non-significant, indicating a consistent response to proline across the cultivars.

Antioxidant enzyme responses under drought and proline application

Antioxidant enzyme activities (SOD, POD, and CAT) were significantly affected by drought stress in both cultivars ($p < 0.01$ – 0.001 ; **Table 2**). Under 40% field capacity without proline, Subhani-21 showed a slight increase in SOD ($+2\%$), while the activities of POD and CAT decreased by 17.91% and 27.08%, respectively. In contrast, Dilkash-20 exhibited a slight decrease in SOD activity (-1.37%), an increase in POD activity ($+10.83\%$), and a pronounced reduction in CAT activity (-45.60%) (**Figure 3**). Application of 100 mM proline under drought markedly enhanced antioxidant enzyme activities.

Table 1: Mean squares from analysis of variance (ANOVA) of the data for growth parameters of wheat subjected to different levels of proline under drought stress conditions.

SoV	df	SL	RL	SFW	RFW	SDW	RDW
Cultivar (C)	1	132.25***	20.25 ***	0.13*	0.30*	0.17**	0.30**
Drought (Dr)	1	42.25***	42.25***	0.28**	0.30*	0.34***	0.30**
Proline (Pro)	2	42.5 ***	18.75***	0.21**	0.54***	0.26***	14.4***
C $\times$ Dr	1	0.25ns	0.25 ns	0.004ns	0.002 ns	0.01 ns	0.06ns
C $\times$ Pro	2	0.25ns	0.75ns	0.004ns	0.002 ns	0.01ns	0.06 ns
Dr $\times$ Pro	2	0.25ns	0.25ns	0.004ns	0.002 ns	2.77e-4 ns	0.06 ns
C $\times$ Dr $\times$ Pro	2	0.25ns	0.25ns	0.004ns	0.002 ns	0.01 ns	0.06 ns
Error	24	1	1	0.02	0.04	0.02	0.03

*, **, *** = significant at 0.05, 0.01, and 0.001 levels, respectively. ns = non-significant.

Abbreviations: Exponent (e), Shoot Length (SL), Root Length (RL), Shoot Fresh Weight (SFW), Root Fresh Weight (RFW), Shoot Dry Weight (SDW), Root Dry Weight (RDW)

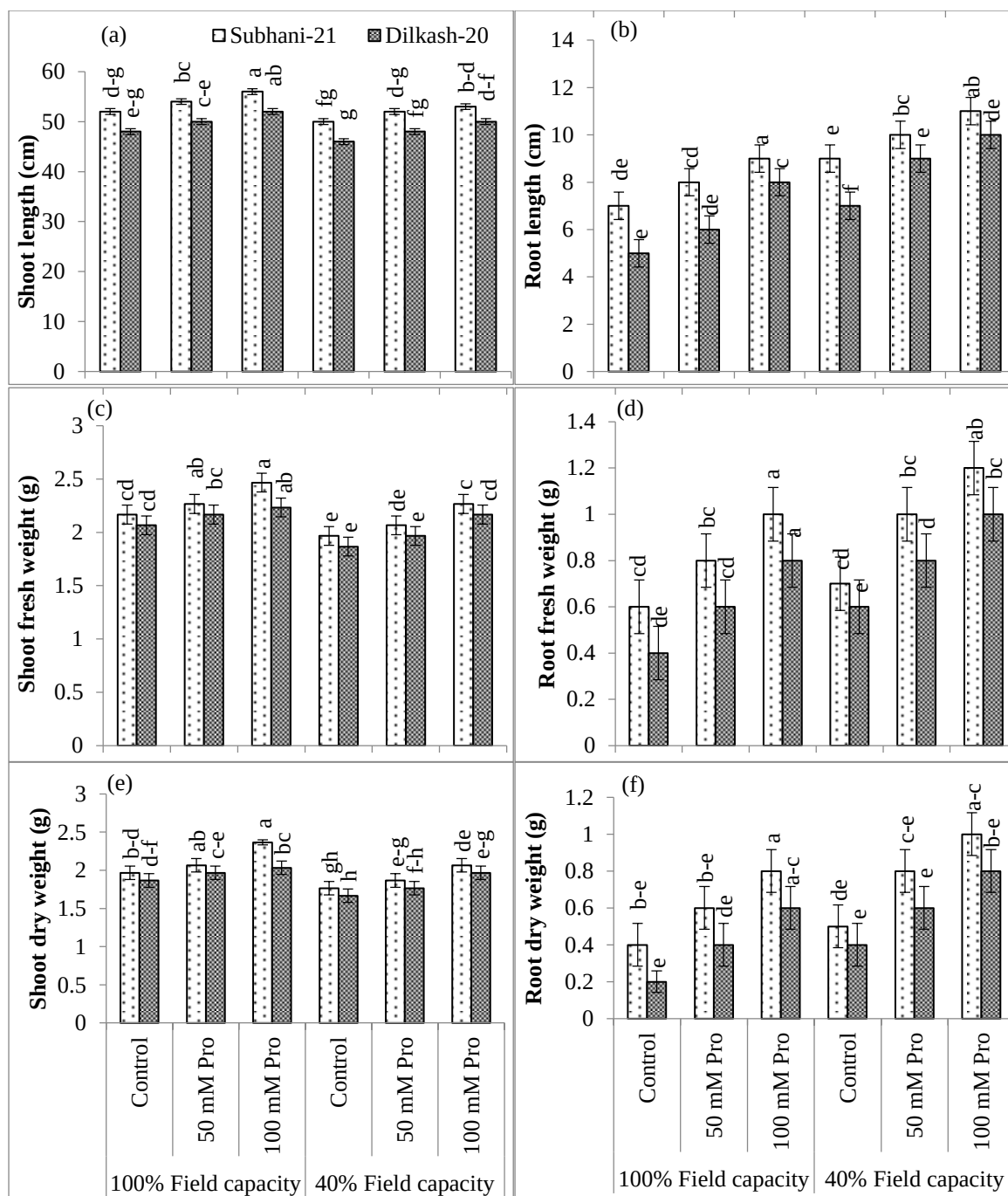


Figure 1: Effect of proline (Pro) on growth attributes of wheat (*Triticum aestivum* L.) cultivars under drought stress (a) shoot length (cm), (b) root length (cm), (c) shoot fresh weight (g), (d) root fresh weight (g), (e) shoot dry weight (g), and (f) root dry weight (g). Data represent mean $\pm$ SE ($n = 3$). Different letters on bars indicate significant differences at $p = 0.05$ according to Tukey's HSD test.

Table 2: Analysis of variance (ANOVA) of the data for plant pigments, antioxidants, and organic osmolytes parameters of drought-stressed wheat plants fed with varying levels of proline.

SoV	df	Chl a	Chl b	Total Chl	Carotenoids	SOD	POD	CAT	TSP	Proline	Phenolics
Cultivar (C)	1	3.08***	0.15***	0.24**	0.07*	0.17***	0.07**	0.2*	0.007*	0.02***	4.6e ⁻⁴ ns
Drought (Dr)	1	0.04**	0.16***	0.32***	0.08**	0.04*	0.11***	1.6***	0.01**	0.15***	0.003***
Proline (Pro)	2	0.21***	0.31***	0.75***	0.09***	0.22***	0.37***	2.13***	0.015***	0.15***	0.004***
C $\times$ Dr	1	7.75e ⁻⁵ ns	0.006ns	0.01ns	0.01ns	0.001ns	0.05**	0.03ns	0.003ns	0.004ns	2.3ns
C $\times$ Pro	2	0.05***	0.01ns	0.07*	0.001ns	0.01ns	0.003ns	0.02ns	2.94e ⁻⁴ ns	0.001ns	1.67ns
Dr $\times$ Pro	2	6.45e ⁻⁴ ns	0.003ns	0.002ns	0.003ns	0.001ns	0.007ns	0.24*	3.69e ⁻⁵ ns	0.004ns	2.40ns
C $\times$ Dr $\times$ Pro	2	0.001ns	6.69e ⁻⁴ ns	0.006ns	0.001ns	0.001ns	0.003ns	0.03ns	3.76e ⁻⁴ ns	3.008e ⁻⁴ ns	8.55ns
Error	24	0.005	0.004	0.019	0.009	0.009	0.006	0.04	0.001	0.001	2.3e ⁻⁴

* ** *** = significant at 0.05, 0.01, and 0.001 levels, respectively. ns = non-significant.

Abbreviations: Exponent (e), Chlorophyll (Chl), Superoxide Dismutase (SOD), Peroxidase (POD), Catalase (CAT), Total Soluble Proteins (TSP)

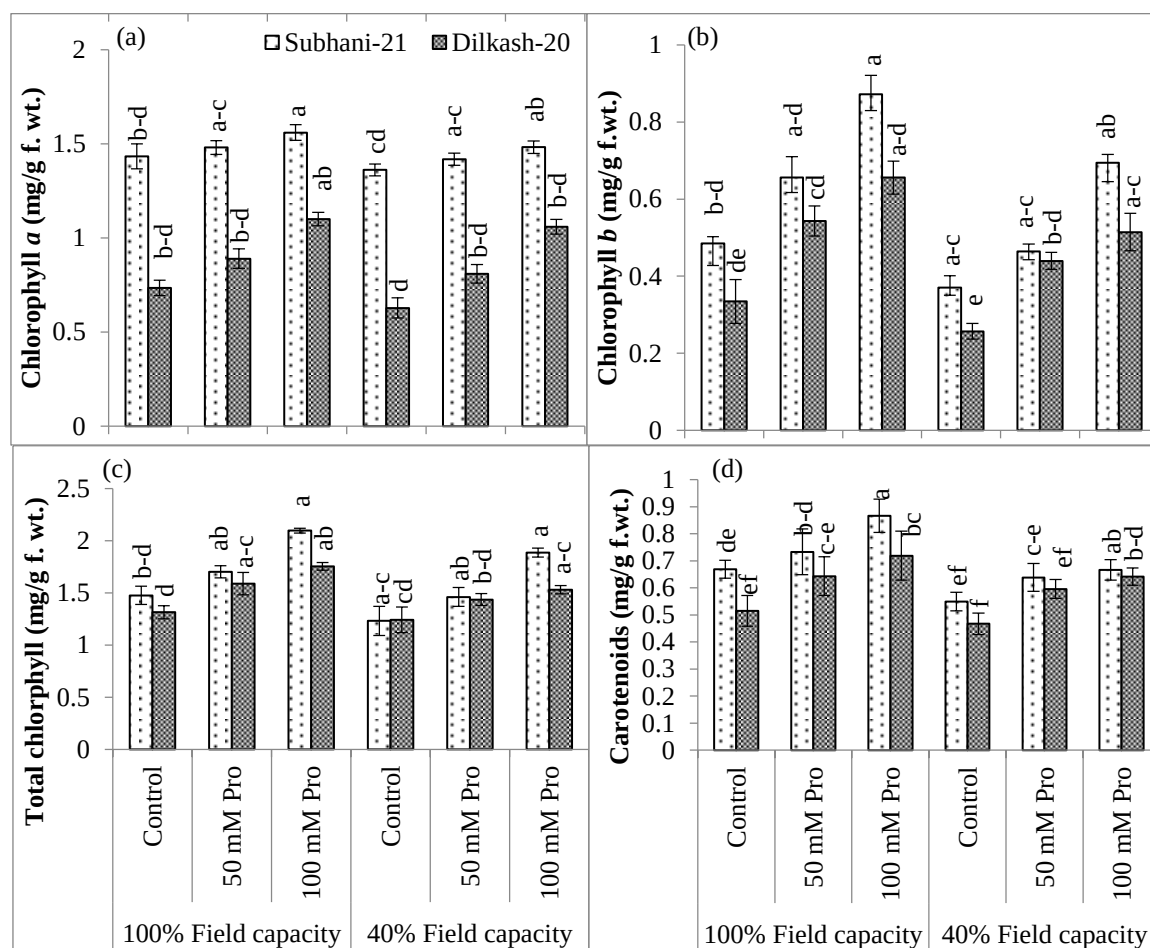


Figure 2: Effect of proline (Pro) on photosynthetic pigment contents of wheat (*Triticum aestivum* L.) cultivars under drought stress: (a) chlorophyll a (mg g⁻¹ f. wt.), (b) chlorophyll b (mg g⁻¹ f. wt.), (c) total chlorophyll (mg g⁻¹ f. wt.), and (d) carotenoids (mg g⁻¹ f. wt.). Numbers are a mean and SE ($n = 3$). The letters on bars indicate significant differences at $p = 0.05$ according to Tukey's HSD test.

In Subhani-21, SOD activity increased by 24.29%, POD by 52.5%, and CAT showed a substantial rise (+254.94%) compared to drought-stressed plants fed with no proline. Similarly, in Dilkash-20, proline treatment increased SOD activity by 28.62%, POD by 54.30%, and CAT by 152.31% (Figure 3). Cultivar effects were significant, with Subhani-21 generally showing stronger recovery of enzyme activities. However, interaction effects were non-significant, indicating a consistent response to proline across cultivars (Table 2).

Osmolytes, protein content, and phenolic contents in drought-stressed wheat plants fed with proline

Biochemical parameters, including total soluble proteins (TSP), endogenous proline, and phenolic content, were significantly affected by drought stress in both cultivars ($p < 0.01$ – 0.001 ; Table 2). Under 40% field capacity without proline application, Subhani-21 showed increases in TSP (+18.8%), proline (+6.32%), and phenolics (+14.44%). Similarly, Dilkash-20 exhibited increases in TSP (+28.16%), proline (+7.29%), and phenolics (+17.77%) under drought conditions (Figure 4). Application of 100 mM proline under drought further enhanced these biochemical traits. In Subhani-21, TSP increased to +22.2%, proline to +32.19%, and phenolics to +32.77% compared to drought-stressed plants receiving no proline. In Dilkash-20, proline treatment resulted in increases of +25.30% in TSP, +27.29% in proline, and a substantial rise in phenolics (+42.77%) (Figure 4). Cultivar effects were significant, with Dilkash-20 accumulating relatively higher phenolic content, while Subhani-21 maintained comparatively stable protein levels. However, interaction effects were non-significant, indicating a consistent response to proline across cultivars (Table 2).

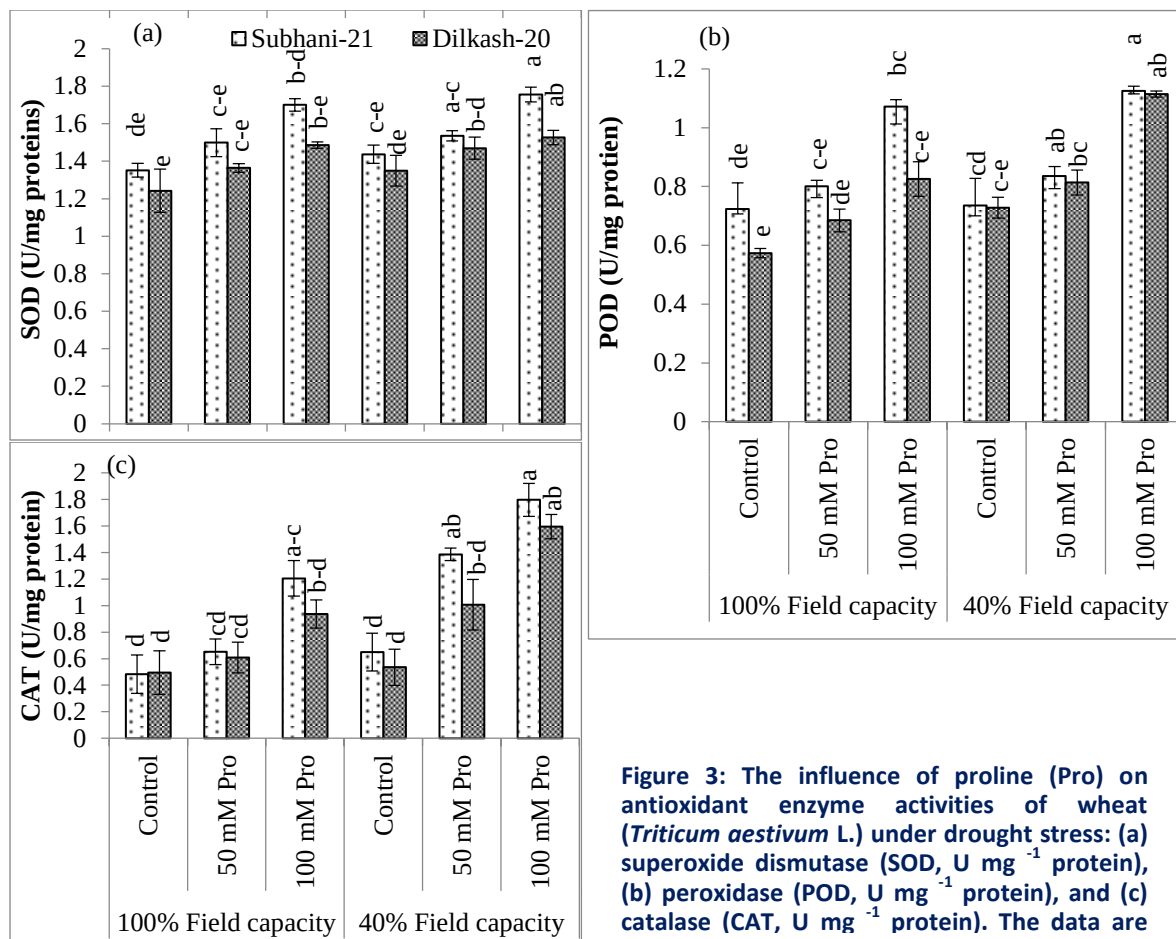


Figure 3: The influence of proline (Pro) on antioxidant enzyme activities of wheat (*Triticum aestivum* L.) under drought stress: (a) superoxide dismutase (SOD, U mg⁻¹ protein), (b) peroxidase (POD, U mg⁻¹ protein), and (c) catalase (CAT, U mg⁻¹ protein). The data are

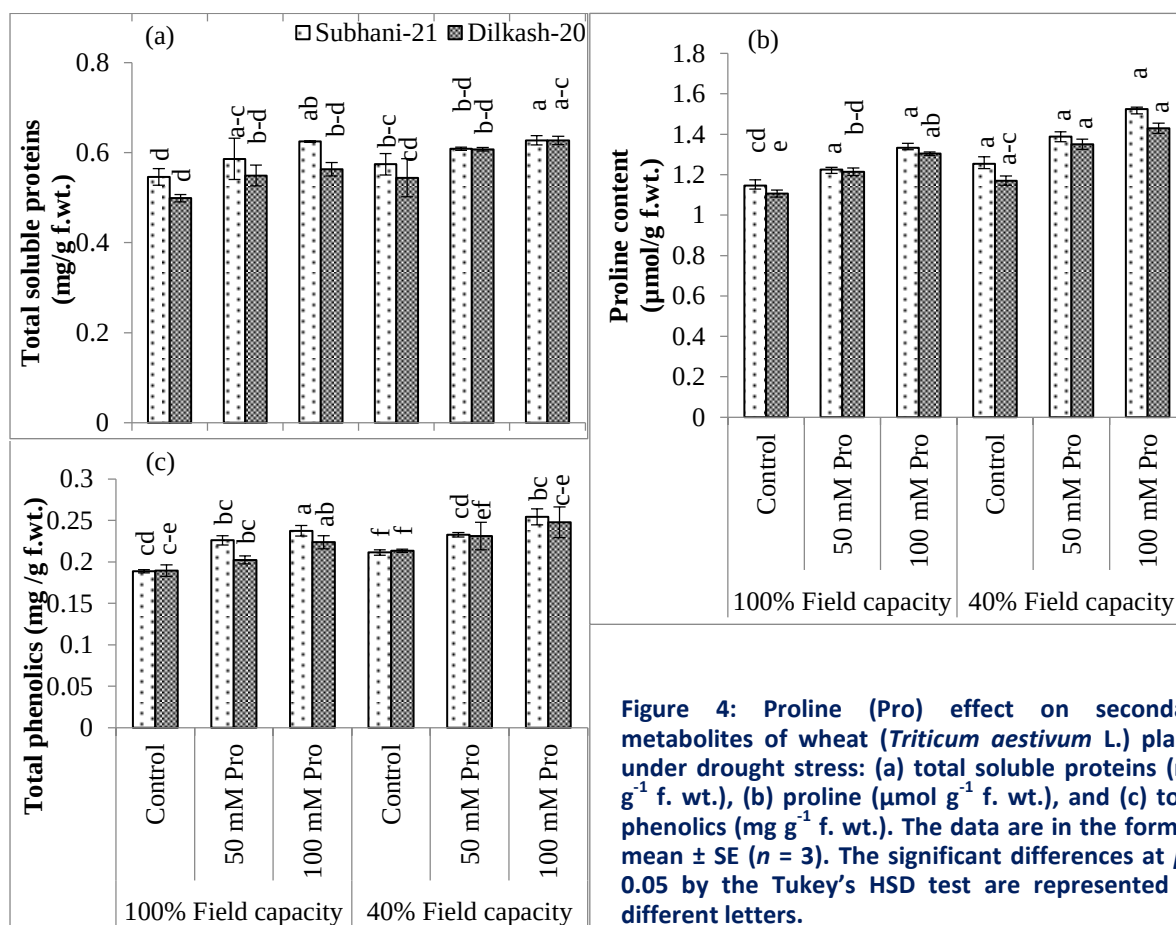


Figure 4: Proline (Pro) effect on secondary metabolites of wheat (*Triticum aestivum* L.) plants under drought stress: (a) total soluble proteins (mg g⁻¹ f. wt.), (b) proline (μmol g⁻¹ f. wt.), and (c) total phenolics (mg g⁻¹ f. wt.). The data are in the form of mean ± SE (*n* = 3). The significant differences at *p* = 0.05 by the Tukey's HSD test are represented by different letters.

Ionic regulation under drought and proline application

Drought stress significantly influenced the ionic composition (Na^+ , K^+ , and Ca^{2+}) of both shoot and root tissues ($p < 0.001$; **Table 3**). Under 40% field capacity without proline application, Subhani-21 exhibited increased Na^+ levels in both shoots (+10.11%) and roots (+10.75%), along with elevated Ca^{2+} in shoots (+12.12%) and roots (+11.42%). In contrast, K^+ content decreased in shoots (−14.28%) and roots (−13.51%). Similarly, in Dilkash-20, Na^+ increased in shoots (+6.25%) and roots (+6.66%), while Ca^{2+} also increased slightly in shoots (+6.25%) and roots (+5.88%). However, K^+ content declined in both shoots (−3.44%) and roots (−3.22%) (**Figure 5**). Application of 100 mM proline under drought further modified the ionic balance in both cultivars. In Subhani-21, Na^+ levels increased in shoots (+13.09%) and roots (+13.92%), and Ca^{2+} also increased in shoots (+15.15%) and roots (+14.28%). In contrast, K^+ content was maintained at control levels (0% change) in both shoots and roots. In Dilkash-20, proline treatment enhanced the accumulation of Na^+ in shoots (+18.75%) and roots (+20%), K^+ in shoots (+3.44%) and roots (+3.22%), and Ca^{2+} in shoots (+18.75%) and roots (+17.64%) (**Figure 5**). Cultivar effects were significant, whereas interaction effects were non-significant, indicating a consistent pattern of both cultivars to proline across treatments (**Table 3**).

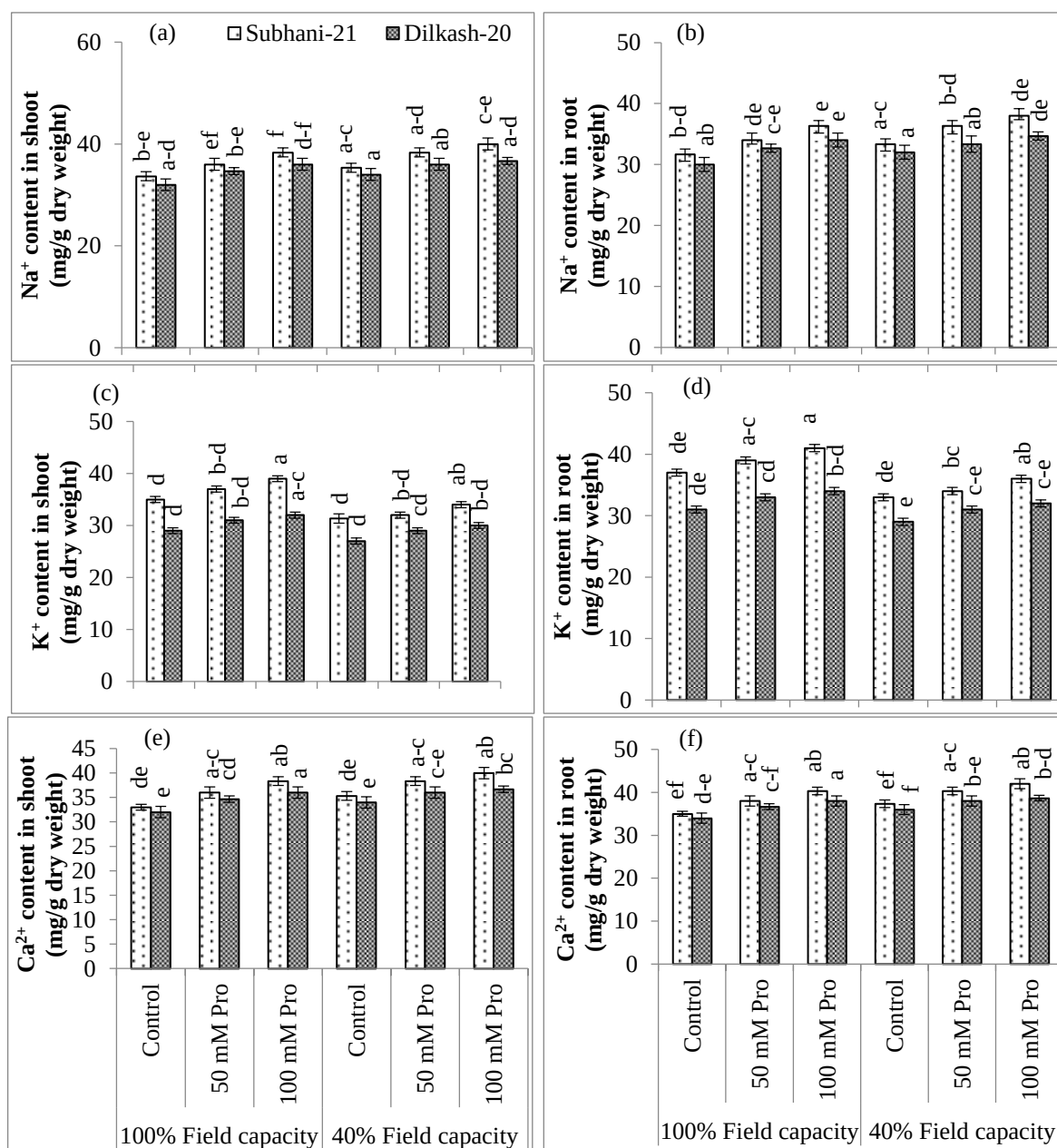


Figure 5: Effect of proline (Pro) on ionic composition of wheat (*Triticum aestivum* L.) cultivars under drought stress: (a) sodium (Na^+) content in shoot, (b) sodium (Na^+) content in root, (c) potassium (K^+) content in shoot, (d) potassium (K^+) content in root, (e) calcium (Ca^{2+}) content in shoot, and (f) calcium (Ca^{2+}) content in root (mg g^{-1} DW). Data represent mean $\pm$ SE ($n = 3$). Different letters on bars indicate significant differences at $p = 0.05$ according to Tukey's HSD test.

Table 3: Mean squares from analysis of variance (ANOVA) of the data for inorganic elements of drought-stressed wheat plants fed with varying levels of proline.

SoV	df	Na (S)	Na (R)	K (S)	K(R)	Ca (S)	Ca (R)
Cultivar (C)	1	38.02**	13.58**	230.02***	225***	34.02**	34.02**
Drought (Dr)	1	23.36*	6.51*	69.69***	100***	26.69**	26.69**
Proline (Pro)	2	49***	48.44***	30.11***	31.75***	53.44***	53.44***
C × Dr	1	0.69ns	1.36ns	14.69**	16***	1.36ns	1.36ns
C × Pro	2	1.44ns	1.33ns	0.77ns	0.75ns	2.11ns	2.11ns
Dr × Pro	2	0.44ns	0.33ns	0.44ns	0.25ns	0.77ns	0.77ns
C × Dr × Pro	2	0.44ns	0.77ns	0.44ns	0.25ns	0.11ns	0.11ns
Error	24	3	3.11	1.11	1	2.88	2.88

*, **, *** = significant at 0.05, 0.01, and 0.001 levels, respectively. ns = non-significant.

Abbreviations: Exponent (e), Sodium (Na), Potassium (K), Calcium (Ca), S (Shoot), R (Root)

Correlation analysis

The correlation matrix (**Figure 6**) revealed significant relationships among morphological, physiological, and biochemical traits under drought and proline treatments. Strong positive correlations were observed between shoot length, root length, and biomass components (SFW, RFW, SDW, RDW). Growth traits also showed positive associations with photosynthetic pigments (Chl *a*, Chl *b*, and total chlorophyll). Endogenous proline and phenolic content were positively correlated with antioxidant enzyme activities (SOD, POD, and CAT). In contrast, Na⁺ exhibited negative correlations with growth traits and chlorophyll content, whereas K⁺ showed positive relationships with physiological and growth parameters.

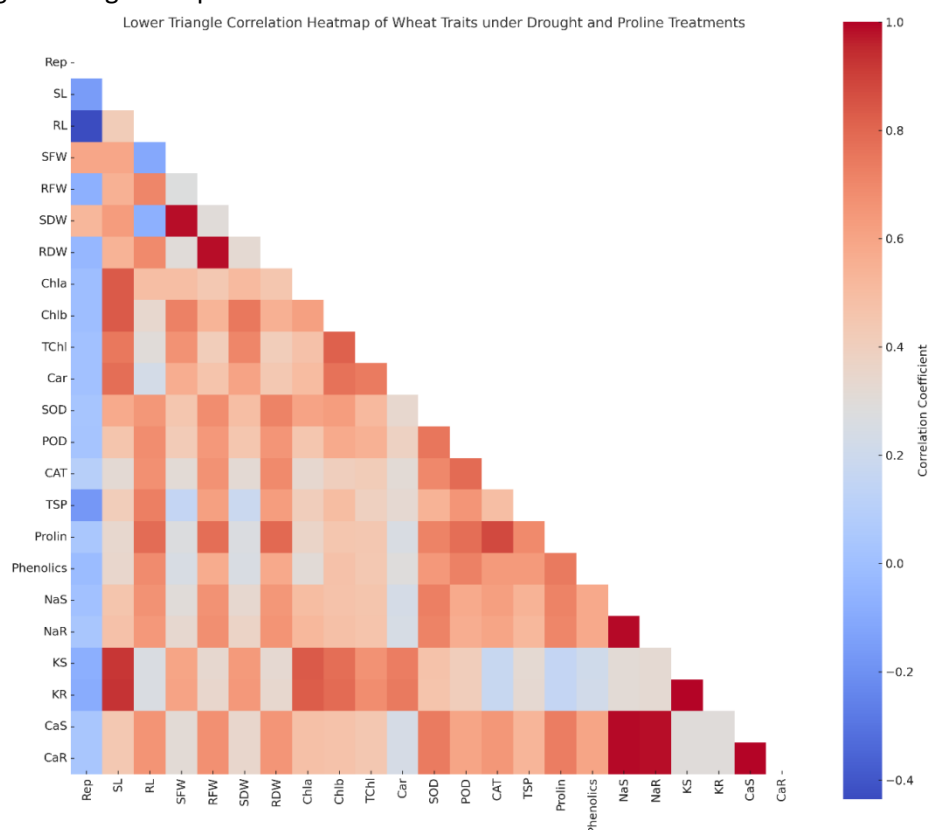


Figure 6: A correlogram shows the associations between growth parameters, physiological attributes, and biochemical parameters of two different wheat cultivars. Red colors indicate positive correlations, whereas blue colors indicate negative correlations.

Abbreviations: SL: shoot length; RL: root length; NOL: number of leaves; LA: leaf area; SFW: shoot fresh weight; RFW: root fresh weight; SDW: shoot dry weight; RDW: root dry weight; Chl *a*: chlorophyll *a*; Chl *b*: chlorophyll *b*; T Chl: total chlorophyll; Carot: carotenoids; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; TSP: total soluble protein; NaS: sodium content in shoot; NaR: sodium content in root; KS: potassium content in shoot; KR: potassium content in root; CaS: calcium content in shoot; CaR: calcium content in root.

Discussion

Drought stress markedly reduced shoot growth in both cultivars, reflecting constrained cell expansion and limited biomass accumulation under water deficit conditions (Khamis et al., 2025). The greater reduction observed in Dilkash-20 indicates its relatively higher drought sensitivity. In contrast, root traits in both cultivars increased under stress, suggesting adaptive biomass allocation toward belowground structures to enhance water acquisition, a well-established drought avoidance strategy in cereals (Dao et al., 2025). Foliar application of proline partially mitigated drought-induced growth inhibition in both cultivars. While improvements in shoot traits were modest, root growth was substantially enhanced, indicating preferential allocation toward water uptake under stress. The relatively larger increases in root traits may also reflect lower baseline values under drought, amplifying proportional responses. These effects are consistent with the known roles of proline in osmotic adjustment, membrane stabilization, and mitigation of oxidative damage (Candar et al., 2023; Avendaño et al., 2025; Khan et al., 2025). The broadly similar response patterns across cultivars suggest that foliar-applied proline is an effective strategy for enhancing drought tolerance in wheat.

Drought-induced reductions in chlorophyll and carotenoids indicate impairment of the photosynthetic apparatus, driven by inhibited pigment synthesis and enhanced degradation under oxidative stress (Song et al., 2025). The greater pigment loss in Dilkash-20, particularly chlorophyll *a*, reflects a higher susceptibility of its photosynthetic system. Foliar proline application alleviated pigment degradation, with more pronounced recovery in Subhani-21, suggesting genotype-dependent responsiveness. Improved chlorophyll *b* and total chlorophyll contents indicate enhanced stability of light-harvesting complexes. The protective effects of proline likely involve stabilization of thylakoid membranes, preservation of chlorophyll–protein complexes, and reduction of oxidative damage (Altuntaş et al., 2020; Masoumi et al., 2024). Recovery of carotenoids further supports improved photoprotection and reactive oxygen species (ROS) scavenging, contributing to redox homeostasis (Renzetti et al., 2024; Shehata, 2024).

The modulation of antioxidant enzymes underscores the central role of oxidative stress in drought responses. The limited change in superoxide dismutase (SOD), coupled with reductions in catalase (CAT) and peroxidase (POD) under drought, suggests impaired ROS detoxification and potential accumulation of hydrogen peroxide (Sachdev et al., 2021; Rao et al., 2025). Foliar proline application markedly enhanced antioxidant enzyme activities, particularly CAT and POD, indicating improved detoxification capacity. The coordinated upregulation of SOD, CAT, and POD reflects restoration of an efficient ROS-scavenging system. These effects are consistent with the role of proline in stabilizing enzyme structures, maintaining redox balance, and supporting antioxidant regeneration (Vettore et al., 2021; Gao et al., 2023). The stronger enzymatic response in Subhani-21 suggests greater intrinsic oxidative stress tolerance, although overall trends were consistent across the cultivars.

Drought-induced increases in endogenous proline and phenolic compounds indicate activation of osmotic and antioxidant defense mechanisms. Proline contributes to osmotic adjustment, while phenolics function as non-enzymatic antioxidants that scavenge ROS and stabilize cellular structures (Sana et al., 2025; Haider et al., 2026). The increase in total soluble proteins suggests maintenance of metabolic activity and induction of stress-responsive proteins. Foliar proline application further elevated endogenous proline and phenolic levels, indicating synergistic enhancement of stress defense pathways. The pronounced increase in phenolics in Dilkash-20 suggests greater reliance on non-enzymatic antioxidant systems, whereas Subhani-21 maintained more stable protein levels, reflecting genotype-specific adaptation strategies. Proline may also contribute to protein stability and reduced proteolysis under drought (Kesawat et al., 2023).

Alterations in ionic balance under drought reflect disruption of membrane selectivity and ion transport processes. Increased Na^+ and decreased K^+ levels indicate ionic imbalance and potential impairment of metabolic functions, given the essential role of K^+ in enzyme activation and osmotic regulation (Kumar et al., 2024; Cheema et al., 2025). Foliar application of proline improved ionic homeostasis by enhancing K^+ retention and increasing Ca^{2+} accumulation. Restoration of K^+ levels suggests improved membrane integrity and ion selectivity, likely through stabilization of transport systems and maintenance of electrochemical gradients (Riffat and Ahmad, 2020; Sobahan et al., 2024). Increased Ca^{2+} further indicates enhanced signaling and membrane stability (Kour et al., 2023). However, Na^+ levels were not reduced and in some cases increased, suggesting that proline-mediated tolerance may not involve Na^+ exclusion but rather intracellular sequestration or mitigation of Na^+ toxicity.

Correlation analysis revealed strong positive associations among growth traits, indicating coordinated regulation of plant performance under drought and proline treatments. Positive relationships between photosynthetic pigments and biomass highlight the importance of maintaining chlorophyll for carbon assimilation under stress (Karami et al., 2025). Significant correlations among antioxidant enzymes, proline, and phenolics demonstrate integrated activation of enzymatic and non-enzymatic defense systems. In contrast, the negative association of Na^+ with growth and chlorophyll confirms its detrimental effects, whereas positive correlations of K^+ with physiological traits underscore its central role in osmotic balance and metabolic stability (Karim et al., 2025). Collectively, these findings demonstrate that foliar-applied proline enhances drought tolerance through coordinated regulation of growth, photosynthesis, antioxidant defense, and ionic homeostasis.

Conclusion

Foliar application of proline effectively alleviated drought-induced growth and physiological constraints in wheat. Drought stress reduced biomass accumulation, photosynthetic pigments, and antioxidant enzyme activities, while disrupting ionic balance. In contrast, foliar-applied proline, particularly at 100 mM, improved shoot and root growth, enhanced chlorophyll content, stimulated antioxidant defense, and increased osmolyte and phenolic accumulation. Proline application improved ionic homeostasis mainly through enhanced K^+ retention and Ca^{2+} accumulation, although Na^+ levels remained largely unaffected. Both cultivars showed a consistent positive response to proline application. Overall, foliar-applied proline acts as an effective osmoprotectant and metabolic regulator, improving wheat performance under drought stress in controlled conditions. Subhani-21 exhibited superior performance compared to Dilkash-20, primarily due to its greater stability in growth attributes, better preservation of photosynthetic pigments, and a more efficient antioxidant defense system under drought stress conditions.

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Contribution of authors

Conceptualized and designed the study: SA, MSAA. Conducted field experiments and wrote up the first draft of the manuscript: IS, AJ, RA, SA, WR. Reviewed and edited the manuscript: AR, ANMR, EA, FA. All authors have read, reviewed, and agreed to publish the current version of the manuscript in IJAaEB.

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This study does not involve human/animal subjects, and thus no ethical approval is required.

Handling of bio-hazardous materials

The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Supplementary material

No supplementary material is included with this manuscript.

Conflict of interest

The authors declare no conflict of interest.

Availability of primary data and materials

As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with other author(s) as declared by the corresponding author (s) of this manuscript.

Authors' consent

All authors have critically read this manuscript and agreed to publish in IJAaEB.

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