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ASSESSMENT OF ANTIOXIDANT POTENTIAL IN DIVERSE WHEAT CULTIVARS UNDER NATURAL GROWTH CONDITIONS IN PAKISTAN

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Abstract

Wheat (*Triticum aestivum* L.) is a globally important cereal crop and a natural source of phytochemicals with significant antioxidant properties. Despite the well-established therapeutic potential of wheat leaf extracts, comparative biochemical profiling of locally adapted Pakistani cultivars remains limited. Five Pakistani wheat cultivars, PERWAZ, MEXI-PAK, PAVON, LU-26, and IQBAL-2000, were grown under natural outdoor conditions for 60 days without fertilizer application. Growth parameters (plant height, shoot length, root length, fresh weight, dry weight) were recorded at harvest. Total phenolic content (TPC) was determined using the Folin-Ciocalteu micro-colorimetric method and total chlorophyll content (TCC) by spectrophotometry. One-way ANOVA, Tukey's HSD post-hoc test ($\alpha = 0.05$), and Pearson correlation analysis were performed using Statistics 8.1. Highly significant differences were observed in TPC [F(4,10) = 707,645.5, $p < 0.001$] and TCC [F(4,10) = 25,543.4, $p < 0.001$] across cultivars. LU-26 recorded the highest TPC (723.200 ± 0.300 mg/g F.W) and TCC (0.1895 ± 0.0005 mg/g), while IQBAL-2000 exhibited the lowest TPC (350.867 ± 0.666 mg/g F.W) and PERWAZ the lowest TCC (0.1057 ± 0.0003 mg/g). Shoot length and dry weight also differed significantly among cultivars ($p < 0.001$). A strong negative correlation was detected between dry weight and TCC ($r = -0.844$, $p < 0.001$), indicating a trade-off between structural biomass and chlorophyll accumulation. LU-26 demonstrated the highest antioxidant potential among the five cultivars tested, supporting its suitability for wheatgrass-based functional food applications and as a priority candidate in breeding programs targeting enhanced phytochemical profiles.

Key words: Antioxidants, Wheat, Total Chlorophyll Content (TCC), Total Phenolic Content (TPC), Oxidative stress, Reactive Oxygen Species (ROS)

INTRODUCTION

Wheat (*Triticum aestivum* L.), belonging to the family Poaceae, is one of the three most widely cultivated cereal crops globally, grown across diverse climatic and soil conditions (Neupane *et al.,* 2022; Rehman *et al.,* 2025). It constitutes a primary source of dietary energy, contributing approximately 60-70% carbohydrates, 10-15% protein, and 2-3% fat, and supplying around 340 kcal per 100 g; it is estimated that 35% of the world's population relies on wheat as a principal food source (Khalid *et al.,* 2023). Beyond its nutritional role, wheat is recognized as a rich natural source of phytochemicals bioactive secondary metabolites that contribute substantially to human health (Mani *et al.,* 2021). Among these, phenolic compounds and chlorophylls have attracted considerable scientific interest for their antioxidant properties and potential therapeutic applications.

Reactive oxygen species (ROS), including superoxide anions, hydrogen peroxide, and hydroxyl radicals, are continuously generated as by-products of aerobic cellular metabolism in organelles such as chloroplasts, mitochondria, and peroxisomes (Garcia-Caparros *et al.,* 2021; Hernansanz-Agustín and Enríquez, 2021; Lennicke and Cochemé, 2021; Mansoor *et al.,* 2022). At moderate concentrations, ROS serve important physiological roles in cell signaling, pathogen defense, and tissue repair; however, an excess accumulation of ROS relative to the antioxidant capacity of the cell leads to oxidative stress (Afzal *et al.,* 2023; Jomova *et al.,* 2023).

Oxidative stress induces oxidative modifications to proteins, lipids, and nucleic acids, and has been implicated in the pathogenesis of numerous chronic conditions, including cardiovascular disease, cancer, neurodegeneration, and diabetes (Dubois-Deruy *et al.,* 2020; Juan *et al.,* 2021). To counteract ROS-induced damage, plants have evolved both enzymatic antioxidant systems, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), and non-enzymatic antioxidants such as phenolic compounds, ascorbic acid, tocopherols, and chlorophylls (Rajput *et al.,* 2021; Rao *et al.,* 2025).

Phenolic compounds are among the most abundant and well-characterized non-enzymatic antioxidants in plants. They include a diverse array of structural classes, flavonoids, hydroxycinnamic acids, hydroxybenzoic acids, and tannins, all sharing the capacity to donate hydrogen atoms to neutralize free radicals and chelate pro-oxidant metal ions, thereby limiting oxidative chain reactions (Kumar and Goel, 2019; Sun and Shahrajabian, 2023; Khawula *et al.,* 2023). Phenolic biosynthesis in plants is regulated by both genetic factors and environmental stimuli, including light intensity, temperature, and oxidative stress signals (Jan *et al.,* 2021).

Total phenolic content (TPC), typically expressed as gallic acid equivalents (GAE) per unit of fresh or dry weight, is widely used as a standardized measure of the overall antioxidant capacity of plant material (Ainsworth and Gillespie, 2007). Significant inter-varietal variation in TPC has been documented across cereal crops, highlighting the role of genetic background in determining antioxidant profiles (Zhan *et al.,* 2022).

Chlorophyll, the primary photosynthetic pigment in plant leaves, serves a dual function as both the driver of carbon assimilation and a potent antioxidant molecule. Its porphyrin ring structure enables direct quenching of singlet oxygen and other reactive species generated during photosynthetic electron transport (Krieger-Liszkay and Shimakawa, 2022).

Total chlorophyll content (TCC) is therefore not only a key indicator of photosynthetic efficiency and plant nutritional status, but also a component of the leaf antioxidant network. Young wheat shoots, commonly, known as wheatgrass, are particularly rich in chlorophyll, alongside amino acids, vitamins, and minerals, leading to their recognition as a nutrient-dense functional food (Moustakas *et al.,* 2022; Nikolić *et al.,* 2025).

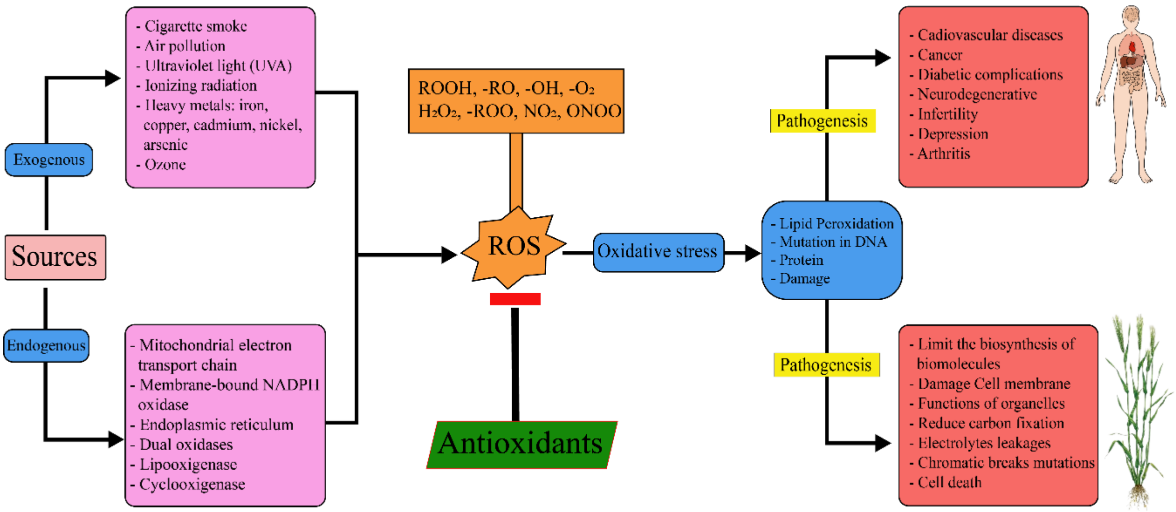


Fig 1. Schematic figure of the link between ROS, oxidative stress and their effects on the human body, and Plants.

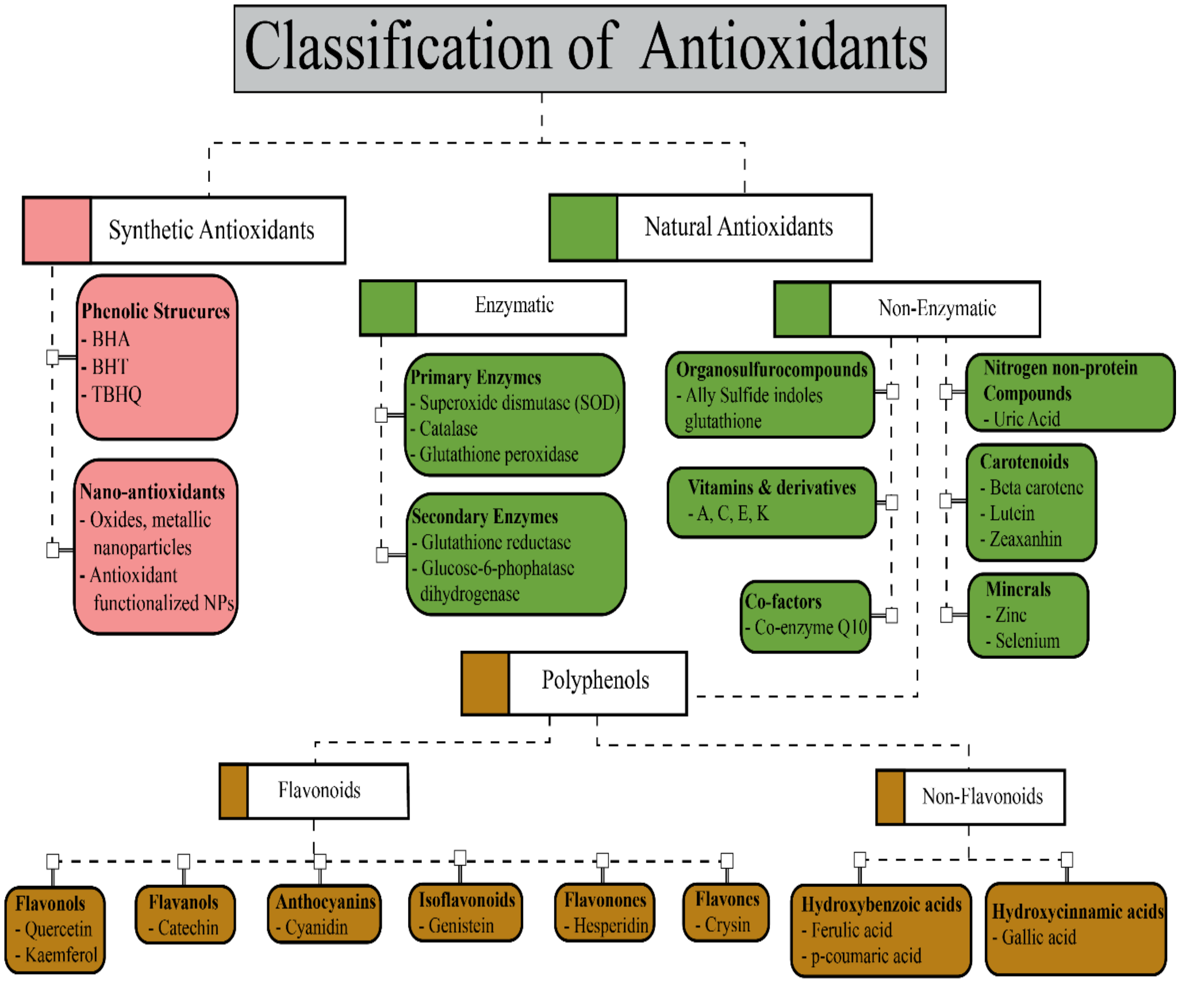


Fig 2. Schematic figure of Clasification of Antioxidants.

Wheatgrass extracts have been reported to exhibit antioxidative, anti-inflammatory, antimicrobial, and anticancer properties, and have been linked to beneficial effects on red blood cell formation and tissue regeneration (Rana *et al.,* 2011; Sundaresan *et al.,* 2015).

Despite the well-established nutritional and therapeutic value of wheat and wheatgrass, the majority of biochemical characterization studies in wheat have focused on grain fractions rather than leaf tissue and have been conducted predominantly on internationally developed genotypes (Adom *et al.,* 2003; Şavşatli, 2020). While a limited number of investigations have examined antioxidant properties in wheatgrass at varying growth stages or under different cultivation conditions (Agrawal *et al.,* 2015; Virdi *et al.,* 2021), comparatively few studies have profiled the antioxidant potential of locally adapted Pakistani wheat cultivars at a defined vegetative stage under natural, fertilizer-free conditions. Given that Pakistan is a major wheat-producing nation and that regionally adapted cultivars may exhibit substantially different phytochemical profiles from internationally studied varieties, such investigations are of both agricultural and public health significance.

The present study addresses this gap by providing a comparative biochemical profile of five Pakistani wheat cultivars, PERWAZ, MEXI-PAK, PAVON, LU-26, and IQBAL-2000, released by the Pakistan Agricultural Research Council (PARC) and representing a range of genetic backgrounds and regional adaptations. Unlike previous studies that have examined grain fractions or employed artificial fertilizers and controlled lighting, this study assessed leaf tissue collected at 60 days post-sowing under entirely natural outdoor growth conditions, providing data that more closely reflects the phytochemical composition achievable under typical field or low-input wheatgrass cultivation scenarios. Furthermore, in addition to TPC and TCC, this study measured a comprehensive set of growth parameters, plant height, shoot length, root length, fresh weight, and dry weight, enabling correlation analyses between vegetative growth performance and antioxidant capacity.

In light of the foregoing, the present study was designed with the following objectives: (i) to determine and compare the total phenolic content (TPC) and total chlorophyll content (TCC) of leaf extracts from five Pakistani wheat cultivars harvested at 60 days post-sowing; (ii) to record and compare key vegetative growth parameters, plant height, shoot length, root length, fresh weight, and dry weight across the five cultivars under natural growth conditions; (iii) to identify cultivar(s) exhibiting the highest antioxidant potential for potential application in functional food development and wheatgrass-based therapeutic products; and (iv) to examine correlations between growth parameters and phytochemical characteristics to better understand the relationship between vegetative development and antioxidant capacity in wheat leaves.

MATERIALS AND METHODS

Biochemical Characteristics Analysis Based Profiling of Diverse Wheat Cultivars (Resource from Pakistan)

Five wheat varieties (PERWAZ, MEXI-PAK, PAVON LU-26 and IQBAL-2000), released by the Pakistan Agricultural Research Council (PARC) and widely cultivated across Punjab, Pakistan, were selected for this study on the basis of their agronomic diversity and regional significance. These cultivars represent a range of genetic backgrounds, providing a suitable basis for comparative biochemical profiling.

Wheat seeds were washed thoroughly and soaked in tap water for 24 hours at room temperature prior to sowing. Seeds were germinated in pots containing locally collected soil and grown under natural outdoor conditions. The experiment was conducted under ambient temperature (20-30°C) and natural humidity, without the application of artificial light or fertilizers. After 60 days of sowing, wheat leaves were harvested for biochemical analyses.

Soil Collection and Analysis

Soil was collected from the vicinity of the University of Management and Technology (UMT), Lahore. The collected soil was air-dried, crushed, and passed through a 2 mm sieve to obtain a uniform texture. Soil pH and electrical conductivity (ECe) were determined using a saturated soil-water paste prepared with deionized water. The extract from the saturated paste was used to measure ECe using a conductivity meter.

Experimental Design

The experiment was conducted using a completely randomized design (CRD) with five varieties and three replicates per variety, yielding a total of 15 experimental units. Each pot received 7000 g of sieved soil and was sown with 5-7 seeds per pot. All pots were labelled and arranged systematically. The experimental layout is presented in Table 1.

Table 1. Experimental Layout showing varieties (V) and replicates (R).

	Varieties (V)				
	V1	**V2**	**V3**	**V4**	**V5**
Replicates(R)	R_1 V_1	R_1 V_2	R_1 V_3	R_1 V_4	R_1 V_5
	R_2 V_1	R_2 V_2	R_2 V_3	R_2 V_4	R_2 V_5
	R_3 V_1	R_3 V_2	R_3 V_3	R_3 V_4	R_3 V_5

Growth Parameters

Plant height, shoot length, and root length in centimeter were measured using a measuring scale after 60 days of seed sowing. Fresh biomass (g) was recorded using an electronic balance immediately after harvest. Dry weight was determined after drying the plant material at room temperature in the dark for 24 hours and subsequently re-weighing.

Total Chlorophyll Content (TCC) Analysis

Dried wheat leaves were ground to a fine powder using a mortar and pestle. A 0.5 g of aliquot of the homogenized material was mixed with 85% (v/v) aqueous acetone to extract leaf pigments, following the method described by (Metzner *et al.,* 1965). The extract was centrifuged at 4000 rpm for 7-10 minutes at 4°C. The resulting supernatant was carefully separated and diluted with 85% acetone to a concentration suitable for spectrophotometric analysis. Absorbance was recorded at 453, 645, and 663 nm using a UV-Vis spectrophotometer, and blank readings of pure acetone were subtracted from each sample reading. Total chlorophyll content was expressed as mg/g F.W.

Total Phenolic Content (TPC) Analysis

Dried leaf samples were ground using a mortar and pestle, and 0.5 g of the homogenized powder was used for TPC determination. Total phenolic content was estimated using the micro-colorimetric Folin-Ciocalteu method described by

(Ainsworth and Gillespie 2007). A standard curve was constructed using gallic acid at varying concentrations, and a linear regression equation derived from the standard curve was applied to calculate phenolic content in samples. Results were expressed as gallic acid equivalents (GAE) in mg/g F.W.

All analyses were performed in triplicate (n = 3). Data were analyzed using Statistics version 8.1. One-way analysis of variance (ANOVA) was applied to assess differences among wheat varieties for each parameter. Means were compared using Tukey's Honestly Significant Difference (HSD) post-hoc test. A significance threshold of $p < 0.05$ was used for all statistical comparisons.

RESULTS AND DISCUSSION

Growth parameters and biochemical characteristics of five Pakistani wheat cultivars (PERWAZ, MEXI-PAK, PAVON, LU-26, and IQBAL-2000) were assessed after 60 days of sowing under natural outdoor conditions without fertilizer application. Seven variables were measured: plant height, shoot length, root length, fresh weight, dry weight, total phenomenon (TPC), and total chlorophyll content (TCC). One-way ANOVA, Tukey's HSD post-hoc test, and Pearson correlation analysis were performed using Statistics 8.1 at a significance level of $\alpha = 0.05$.

Plant height varied across the five cultivars, ranging from 103.040 ± 11.176 cm in MEXI-PAK to 121.920 ± 2.540 cm in LU-26, with intermediate values in PERWAZ (114.810 ± 11.940 cm), PAVON (112.777 ± 1.665 cm), and IQBAL-2000 (108.373 ± 1.940 cm). The high standard deviations observed in PERWAZ and MEXI-PAK indicate considerable replicate-level variation, reflecting sensitivity of these cultivars to micro-environmental differences within the experimental pots. Plant height is a complex quantitative trait governed by multiple genetic loci and modulated by environmental factors including temperature, photoperiod, and nutrient availability (Wang *et al.,* 2017). The absence of significant variation under natural, fertilizer-free conditions is consistent with reports that genotypic differences in height are most pronounced under contrasting agronomic inputs (Khalid *et al.,* 2023).

Despite the non-significant ANOVA outcome, the numerically higher mean height of LU-26 is consistent with its generally superior agronomic performance observed across other parameters in this study.

Pearson correlation analysis revealed a significant positive relationship between plant height and root length ($r = 0.711$, $p = 0.003$) and between plant height and shoot length ($r = 0.556$, $p = 0.031$), confirming coordinated above- and below-ground vegetative development across varieties. Shoot length differed highly significantly among the five wheat cultivars [$F(4,10) = 58.141$, $p < 0.001$]. PAVON recorded the greatest mean shoot length (68.920 ± 0.815 cm), closely followed by LU-26 (66.803 ± 1.833 cm), while MEXI-PAK exhibited the shortest shoots (51.057 ± 1.833 cm). PERWAZ and IQBAL-2000 recorded identical means of 57.743 cm.

Tukey's HSD post-hoc analysis (HSD = 4.475) confirmed that MEXI-PAK was significantly shorter than all other varieties, and that PAVON and LU-26 did not differ significantly from each other (mean difference = 2.117 cm, $p > 0.05$), nor did PERWAZ and IQBAL-2000 (mean difference = 0.000 cm).

Shoot elongation in wheat is primarily regulated by gibberellin biosynthesis and sensitivity pathways, as well as by genetic determinants of internode elongation (Wang *et al.,* 2017).

Table 2: Descriptive statistics for all study variables across wheat cultivars (N = 15)

Variable	Cultivar	Mean	SD	Min	Max	Overall Mean
Plant height (cm)	PERWAZ	114.810 ±11.940	11.940	102.87	126.75	**112.184 ± 9.108**
	MEXI-PAK	103.040±11.176	11.176	91.95	114.30	
	PAVON	112.777±1.665	1.665	111.00	114.30	
	LU-26	121.920±2.540	2.540	119.38	124.46	
	IQBAL-2000	108.373±1.940	1.940	106.68	110.49	
Shoot Length (cm)	PERWAZ	57.743 ± 2.464	2.464	55.63	60.45	**60.453 ± 6.932**
	MEXI-PAK	51.057 ± 1.833	1.833	49.53	53.09	
	PAVON	68.920 ± 0.815	0.815	68.33	69.85	
	LU-26	66.803 ± 1.833	1.833	64.77	68.33	
	IQBAL-2000	57.743 ± 0.639	0.639	57.15	58.42	
Root Length (cm)	PERWAZ	56.387 ± 10.306	10.306	45.72	66.29	**53.999 ± 8.114**
	MEXI-PAK	51.983 ± 12.958	12.958	38.86	64.77	
	PAVON	51.393 ± 8.932	8.932	44.45	61.47	
	LU-26	58.333 ± 2.443	2.443	56.13	60.96	
	IQBAL-2000	51.900 ± 6.431	6.431	46.99	59.18	
Fresh Weight (g)	PERWAZ	19.233 ± 1.021	1.021	18.50	20.40	**21.508 ± 2.638**
	MEXI-PAK	22.267 ± 1.662	1.662	20.50	23.80	
	PAVON	20.633 ± 0.961	0.961	19.60	21.50	
	LU-26	19.640 ± 1.296	1.296	18.42	21.00	
	IQBAL-2000	25.767 ± 0.322	0.322	25.40	26.00	
Dry Weight (g)	PERWAZ	5.273 ± 0.155	0.155	5.10	5.40	**4.261 ± 0.650**
	MEXI-PAK	4.227 ± 0.241	0.241	4.00	4.48	
	PAVON	4.483 ± 0.226	0.226	4.25	4.70	
	LU-26	3.623 ± 0.256	0.256	3.33	3.80	
	IQBAL-2000	3.697 ± 0.248	0.248	3.42	3.90	
Total Phenolic Content (mg/g F.W)	PERWAZ	443.000 ± 0.173	0.173	442.9	443.2	**583.460 ± 160.872**
	MEXI-PAK	690.167 ± 0.231	0.231	689.9	690.3	
	PAVON	710.067 ± 0.153	0.153	709.9	710.2	
	LU-26	723.200 ± 0.300	0.300	722.9	723.5	
	IQBAL-2000	350.867 ± 0.666	0.666	350.1	351.3	
Total Chlorophyll Content (mg/g)	PERWAZ	0.1057 ± 0.0003	0.0003	0.1055	0.1060	**0.1517 ± 0.0317**
	MEXI-PAK	0.1287 ± 0.0006	0.0006	0.1280	0.1290	
	PAVON	0.1598 ± 0.0003	0.0003	0.1595	0.1600	
	LU-26	0.1895 ± 0.0005	0.0005	0.1890	0.1899	
	IQBAL-2000	0.1750 ± 0.0001	0.0001	0.1749	0.1750	

Values presented as Mean ± SD. SD = Standard Deviation.

The longer shoots in PAVON and LU-26 suggest greater apical dominance and internode elongation capacity, which may contribute to their higher photosynthetic output and antioxidant accumulation observed in subsequent analyses. These findings are consistent with (Virdi *et al.,* 2021), who reported significant variation in shoot growth among Indian wheat cultivars under uniform growth conditions. The significant positive correlation between shoot length and TCC (r = 0.572, p = 0.026) further indicates that cultivars with greater shoot development tend to have higher chlorophyll accumulation, likely due to greater leaf area and light interception capacity.

Root length ranged from 51.393 ± 8.932 cm in PAVON to 58.333 ± 2.443 cm in LU-26, with high within-group variability particularly notable in MEXI-PAK (SD = 12.958 cm) and PERWAZ (SD = 10.306 cm). The lack of significant inter-varietal differences in root length under natural, unamended soil conditions is consistent with findings that root architecture in wheat is strongly influenced by soil nutrient status, moisture availability, and fertilizer inputs rather than genotype alone (Mehrabi *et al.,* 2021; Wang *et al.,* 2024).

Without the addition of differential nutrient treatments, genotypic differences in root growth potential may be masked by uniform resource constraints. The high within-group variability in MEXI-PAK and PERWAZ further suggests that these cultivars may be more responsive to micro-scale spatial heterogeneity in soil conditions. Nevertheless, the strong positive correlation between plant height and root length (r = 0.711, p = 0.003) confirms that overall vegetative growth remained coordinated across varieties, with taller plants tending to develop more extensive root systems regardless of variety.

Fresh weight differed significantly among the five wheat cultivars [F(4,10) = 16.205, p < 0.001]. IQBAL-2000 recorded the highest fresh biomass (25.767 ± 0.322 g), substantially greater than all other cultivars. MEXI-PAK followed with 22.267 ± 1.662 g, while PAVON (20.633 ± 0.961 g), LU-26 (19.640 ± 1.296 g), and PERWAZ (19.233 ± 1.021 g) recorded comparatively lower values. Tukey's HSD post-hoc analysis (HSD = 3.067): IQBAL-2000(a) > PERWAZ(b) = MEXI-PAK(b) = PAVON(b) = LU-26(b).

Fresh biomass is a composite measure of water content and structural plant material, and is influenced by cell expansion, turgor pressure, and overall metabolic activity (Pour-Aboughadareh *et al.,* 2019). The notably high fresh weight of IQBAL-2000, despite its moderate shoot and root dimensions, suggests a higher water retention capacity or greater leaf succulence in this cultivar. This pattern may be associated with osmotic adjustment mechanisms that enhance cellular water uptake under ambient conditions. Environmental factors including temperature, relative humidity, and soil water availability at the time of harvest are also known to affect fresh biomass considerably (Ferrante and Mariani, 2018).

Dry weight differed highly significantly among cultivars [F(4,10) = 25.952, p < 0.001]. PERWAZ recorded the highest dry weight (5.273 ± 0.155 g), significantly greater than all other varieties. PAVON (4.483 ± 0.226 g) and MEXI-PAK (4.227 ± 0.241 g) formed an intermediate group and did not differ significantly from each other. LU-26 (3.623 ± 0.256 g) and IQBAL-2000 (3.697 ± 0.248 g) recorded the lowest dry weights and were not significantly different from each other.

Dry weight reflects the structural and storage compounds within plant tissue, including cellulose, hemicellulose, stored carbohydrates, proteins, and secondary metabolites (Pisano *et al.,* 2021). The high dry weight of PERWAZ suggests greater accumulation of structural biomass components, potentially associated with thicker cell walls or higher starch content in its tissues. Notably, the strong negative correlation between dry weight and TCC ($r = -0.844$, $p < 0.001$), the most significant correlation detected in this study indicates an inverse relationship between structural biomass accumulation and chlorophyll content.

This trade-off may reflect a metabolic allocation strategy, whereby cultivars with high structural biomass (e.g., PERWAZ) invest proportionally less in photosynthetic pigment synthesis compared to cultivars such as LU-26 that prioritize photosynthetic efficiency. Similar biomass-photosynthesis trade-offs have been reported in other cereals under resource-limited conditions (Gill and Tuteja, 2010).

Total phenolic content differed highly significantly among the five cultivars [$F(4,10) = 707{,}645.500$, $p < 0.001$], with each variety exhibiting a distinctly different level of phenolic accumulation. LU-26 recorded the highest TPC (723.200 ± 0.300 mg/g F.W), followed sequentially by PAVON (710.067 ± 0.153 mg/g F.W), MEXI-PAK (690.167 ± 0.231 mg/g F.W), PERWAZ (443.000 ± 0.173 mg/g F.W), and IQBAL-2000 (350.867 ± 0.666 mg/g F.W).

Tukey's HSD post-hoc analysis confirmed that all ten pair-wise comparisons were statistically significant (HSD = 0.961), indicating complete differentiation in phenolic content across all cultivars. The extraordinarily high F-value reflects extremely low within-group variance (MSE = 0.128), confirming the high measurement reproducibility: LU-26(a) > PAVON(b) > MEXI-PAK(c) > PERWAZ(d) > IQBAL-2000(e).

Phenolic compounds are a major class of plant secondary metabolites with well-established antioxidant activity, functioning through hydrogen atom donation to neutralize free radicals and reactive oxygen species (ROS), chelation of pro-oxidant metal ions, and inhibition of lipid peroxidation (Zeb, 2020; Rajashekar, 2023). The high TPC in LU-26 (723.200 mg/g F.W) indicates this cultivar possesses superior constitutive antioxidant defense capacity.

Phenolic biosynthesis in plants is primarily governed by the shikimate pathway and is influenced by both genetic determinants and abiotic stress signals (Zagoskina *et al.,* 2023; Kumar *et al.,* 2023). The considerable range in TPC across cultivars from 350.867 mg/g in IQBAL-2000 to 723.200 mg/g in LU-26 underscores the strong genotypic control over phenolic metabolism. High phenolic accumulation at the 60-day vegetative stage has been linked to antioxidative requirements during active growth and reproduction.

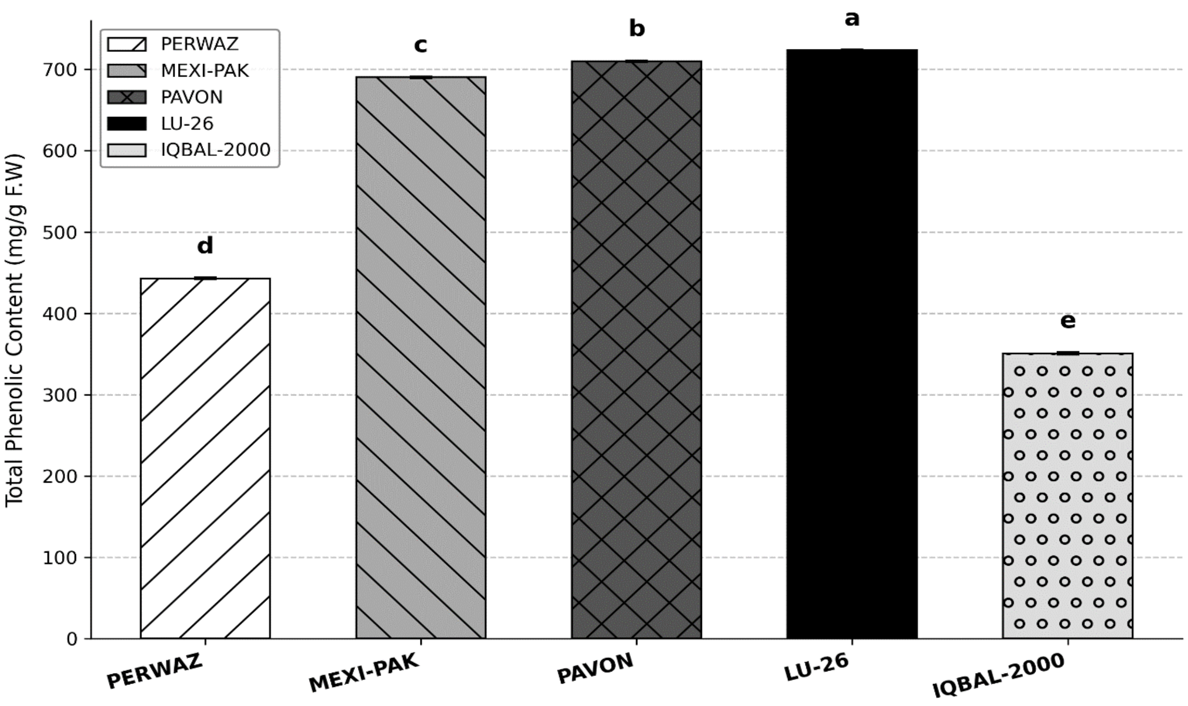
These findings align with Şavşatli (2020) and Durairaj *et al.* (2014), who reported variety-dependent phenolic content in wheat species. The elevated TPC in LU-26 supports its potential application in functional food formulations and wheatgrass-based therapeutic products targeting oxidative stress-related conditions.

Total chlorophyll content differed highly significantly among the five wheat cultivars [$F(4,10) = 25{,}543.362$, $p < 0.001$]. LU-26 recorded the highest TCC (0.1895 ± 0.0005 mg/g), followed by IQBAL-2000 (0.1750 ± 0.0001 mg/g), PAVON (0.1598 ± 0.0003 mg/g), MEXI-PAK (0.1287 ± 0.0006 mg/g), and PERWAZ (0.1057 ± 0.0003 mg/g). All ten pair-wise comparisons were statistically significant (HSD = 0.0010), confirming complete differentiation in chlorophyll content across all cultivars. The extremely low within-group variance (MSE ≈ 0.00000) confirms high measurement precision: LU-26(a) > IQBAL-2000(b) > PAVON(c) > MEXI-PAK(d) > PERWAZ(e).

Photosystems I and II, and its content directly determines photosynthetic efficiency, carbon assimilation capacity, and ultimately, plant productivity (Zhang *et al.,* 2016). The substantially higher TCC in LU-26 (0.1895 mg/g) compared to PERWAZ (0.1057 mg/g) a nearly 79% difference indicates markedly superior photosynthetic potential in LU-26 under the natural growth conditions employed in this study.

Chlorophyll content in wheat is strongly linked to leaf nitrogen status, as chlorophyll molecules contain a nitrogen-rich porphyrin ring structure; its accumulation is typically progressive through the vegetative stage until anthesis (Croft *et al.,* 2020; Wu *et al.,* 2023). The moderate positive correlation between shoot length and TCC (r = 0.572, p = 0.026) observed here suggests that cultivars with more developed shoots had proportionally greater chlorophyll synthesis, consistent with the higher leaf surface area available for photosynthetic apparatus assembly in taller plants.

Furthermore, the strong negative correlation between dry weight and TCC (r = −0.844, p < 0.001) reveals a significant metabolic trade-off: cultivars that accumulated more structural dry biomass (e.g., PERWAZ, dry weight = 5.273 g) invested proportionally less in chlorophyll synthesis, while high-TCC cultivars such as LU-26 (dry weight = 3.623 g) appeared to prioritize photosynthetic efficiency over structural biomass accumulation.



One-way ANOVA: F(4,10)=707645.5, p<0.001 (**)
Bars sharing the same letter are not significantly different (Tukey's HSD, α=0.05). Error bars = ±1 SD.

Fig 3. Mean Total Phenolic Content (± SD) across five wheat cultivars

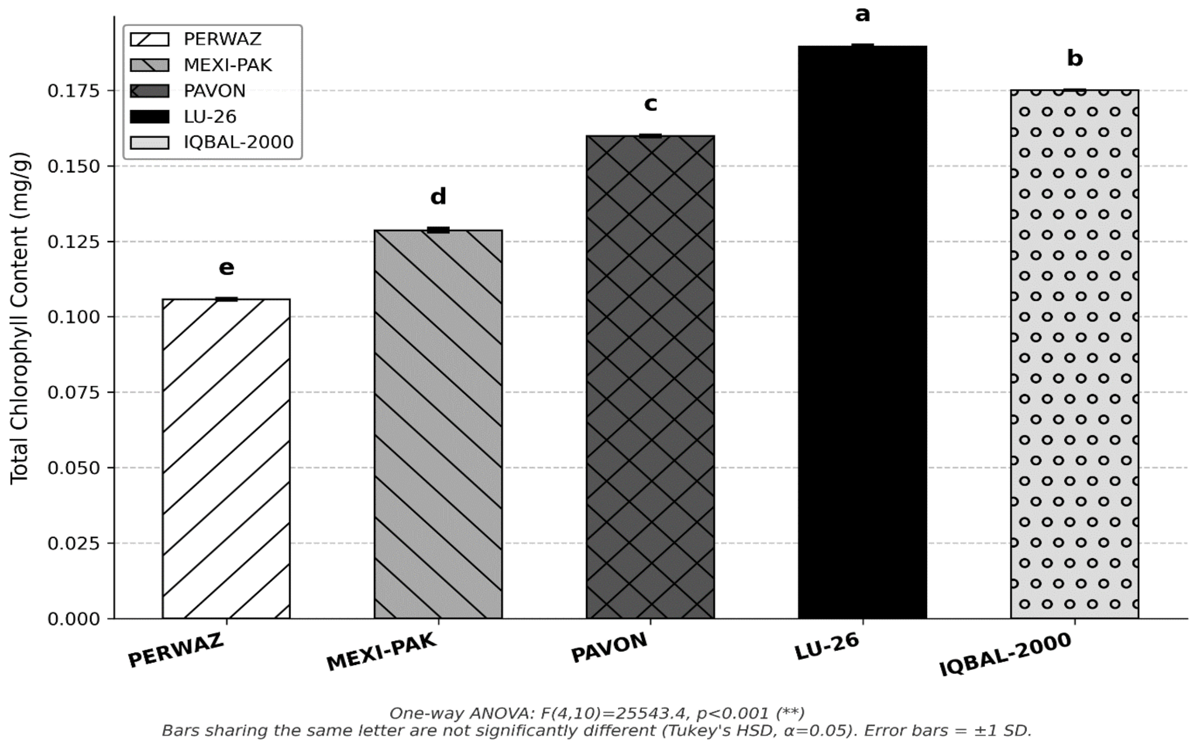


Fig 4. Mean Total Chlorophyll Content (± SD) across five wheat cultivars

Table 3: Summary of ANOVA and post-hoc results for all variables

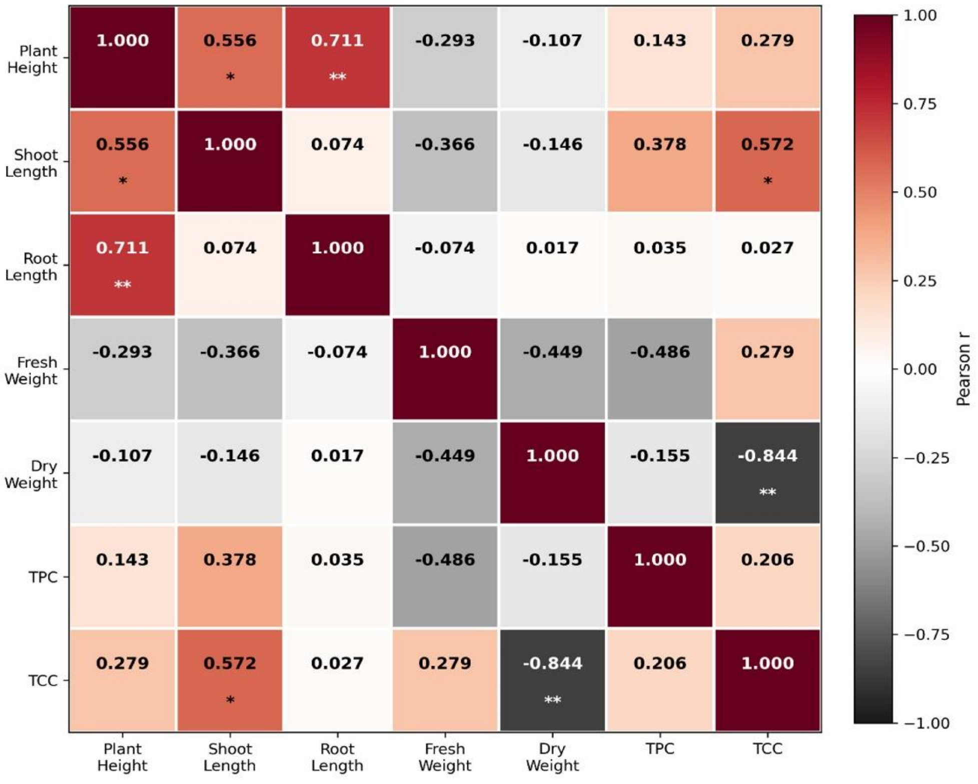
Variable	**ANOVA Result**	**Post-Hoc**	**Key Finding**
Plant Height (cm)	F(4,10)=2.677, p=0.094	ns - no post-hoc	No significant difference among varieties
Shoot Length (cm)	F(4,10)=58.141, p<0.001	**** - Tukey applied**	PAVON and LU-26 highest; MEXI-PAK lowest
Root Length (cm)	F(4,10)=0.372, p=0.824	ns - no post-hoc	No significant difference among varieties
Fresh Weight (g)	F(4,10)=16.205, p<0.001	**** - Tukey applied**	IQBAL-2000 highest; PERWAZ and LU-26 lowest
Dry Weight (g)	F(4,10)=25.952, p<0.001	**** -Tukey applied**	PERWAZ highest; LU-26 and IQBAL-2000 lowest
TPC (mg/g F.W)	F(4,10)=707645.5, p<0.001	**** -Tukey applied**	LU-26 highest (723.2); IQBAL-2000 lowest (350.9)
TCC (mg/g)	F(4,10)=25543.4, p<0.001	**** - Tukey applied**	LU-26 highest (0.1895); PERWAZ lowest (0.1057)

*** = Highly significant (p < 0.01); ns = not significant.*

Table 4. Pearson correlation matrix (r-values with significance) among study variables N = 15

Variable	Plant Height	Shoot Length	Root Length	Fresh Weight	Dry Weight	TPC	TCC
Plant Height	**1.000**	0.556*	0.711**	-0.293ns	-0.107ns	0.143ns	0.279ns
Shoot Length	0.556*	**1.000**	0.074ns	-0.366ns	-0.146ns	0.378ns	0.572*
Root Length	0.711**	0.074ns	**1.000**	-0.074ns	0.017ns	0.035ns	0.027ns
Fresh Weight	-0.293ns	-0.366ns	-0.074ns	**1.000**	-0.449ns	0.143ns	0.279ns
Dry Weight	-0.107ns	-0.146ns	0.017ns	0.017ns	**1.000**	-0.155ns	-0.844**
TPC	0.143ns	0.378ns	0.035ns	0.035ns	-0.449ns	**1.000**	0.206ns
TCC	0.279ns	0.572*	0.027ns	0.027ns	-0.844**	0.206ns	**1.000**

*** p < 0.01; * p < 0.05; ns = not significant. TPC = Total Phenolic Content; TCC = Total Chlorophyll Content. Diagonal = self-correlation (1.000).*



	Plant Height	Shoot Length	Root Length	Fresh Weight	Dry Weight	TPC	TCC
Plant Height	1.000	0.556 *	0.711 **	-0.293	-0.107	0.143	0.279
Shoot Length	0.556 *	1.000	0.074	-0.366	-0.146	0.378	0.572 *
Root Length	0.711 **	0.074	1.000	-0.074	0.017	0.035	0.027
Fresh Weight	-0.293	-0.366	-0.074	1.000	-0.449	-0.486	0.279
Dry Weight	-0.107	-0.146	0.017	-0.449	1.000	-0.155	-0.844 **
TPC	0.143	0.378	0.035	-0.486	-0.155	1.000	0.206
TCC	0.279	0.572 *	0.027	0.279	-0.844 **	0.206	1.000

** p < 0.01; * p < 0.05. TPC = Total Phenolic Content; TCC = Total Chlorophyll Content.

Fig 5. Pearson Correlation Heatmap among study variables (N=15)

This inverse relationship between biomass and photosynthetic pigment investment has been reported in other cereal species and may reflect differing source-sink dynamics among cultivars (Gill and Tuteja, 2010). The high TCC of LU-26 is particularly noteworthy for wheatgrass applications, as chlorophyll-rich extracts are valued for promoting red blood cell formation, tissue regeneration, and anti-inflammatory activity (Choudhary *et al.,* 2021; Minocha *et al.,* 2022; Anand and Singh, 2023).

Pearson correlation analysis identified four statistically significant associations among the seven study variables. Plant height was strongly and positively correlated with root length (r = 0.711, p = 0.003), indicating coordinated above- and below-ground growth, and moderately correlated with shoot length (r = 0.556, p = 0.031), reflecting an integrated vegetative growth pattern across cultivars.

Shoot length was moderately and positively correlated with TCC (r = 0.572, p = 0.026), suggesting that cultivars with more developed shoot architecture support greater chlorophyll accumulation, consistent with the larger photosynthetic leaf area available in taller plants. The most notable correlation was a strong negative relationship between dry weight and TCC (r = −0.844, p < 0.001), indicating a metabolic trade-off between structural biomass investment and photosynthetic pigment accumulation.

No significant correlations were detected between TPC and any of the growth or biomass parameters, confirming that phenolic biosynthesis in wheat leaves at the 60-day stage is largely independent of vegetative growth performance and is primarily determined by the genetic constitution of each cultivar (Ma *et al.,* 2022; Marcotuli *et al.,* 2025).

CONCLUSION

The present study demonstrated significant inter-varietal differences in antioxidant potential among five Pakistani wheat cultivars grown under natural conditions for 60 days. LU-26 recorded the highest total phenolic content (723.200 ± 0.300 mg/g F.W) and total chlorophyll content (0.1895 ± 0.0005 mg/g), with all pair-wise differences confirmed significant by Tukey's HSD (p < 0.001). A strong negative correlation between dry weight and TCC (r = −0.844, p < 0.001) indicated a metabolic trade-off between structural biomass and photosynthetic pigment accumulation. These results identify LU-26 as the most antioxidant-rich cultivar, making it a strong candidate for wheatgrass-based functional food applications and breeding programmes targeting enhanced biochemical profiles. Future studies should investigate antioxidant potential across multiple growth stages and under varying fertilizer regimes and validate the therapeutic efficacy of LU-26 leaf extracts through *in vitro and in vivo* models.

CONFLICTS OF INTEREST

There is no conflict of interest related to this article.

AUTHOR’S CONTRIBUTION

All authors have contributed equally.

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