ASSESSMENT OF WATER STRESS TOLERANCE IN DURUM WHEAT (*Triticum durum* Desf.): EFFECTS ON GROWTH AND BIOCHEMICAL PARAMETERS

Meriem RICHA*, Mohamed LAZALI*

*Research laboratory ERP, Faculty of Natural and Life Sciences & Earth Sciences. Djilali Bounaama University of Khemis Miliana, Algeria 44015

Corresponding author: Meriem Richa, Research laboratory ERP, Faculty of Natural and Life Sciences & Earth Sciences. Djilali Bounaama University of Khemis Miliana, Algeria 44015, telephone: +213 793948761, E-mail: m.richa@univ-dbkm.dz

Abstract. Water stress represents a major limitation to crop growth and yield, particularly in semi-arid ecosystems. The objective of this research is to assess the impact of water stress (30% of field capacity) on the growth and biochemical traits of four varieties of durum wheat (Oued El Bared, Simeto, Prospero, and Vitron). The parameters examined include shoot length (SL), root length (RL), fresh shoot weight (FSW), dry shoot weight (DSW), fresh root weight (FRW), dry root weight (DRW), and the concentrations of chlorophyll, proline, and soluble sugars. The results show that the different types of plants reacted to water stress in quite different ways. Vitron and Simeto varieties exhibited enhanced tolerance, which was evidenced by increased root development and a heightened accumulation of proline and soluble sugars. Overall, this research highlights considerable inter-varietal variability and gives useful information that can help breeding programs that want to make durum wheat varieties that can grow in dry conditions, thereby contributing to more sustainable agriculture in arid and semi-arid regions.

Keywords: durum wheat; drought effects; proline; *Triticum durum* Desf.; water stress; chlorophyll.

INTRODUCTION

Water stress is one of the most significant problems that is harming wheat production around the world right now. This has serious consequences for food security as climate change continues. The implications of drought stress on durum wheat represent a pivotal subject within contemporary agronomy. Durum wheat (*Triticum durum* Desf.), extensively cultivated across semi-arid and Mediterranean territories, exhibits pronounced sensitivity to fluctuations in soil moisture availability [29, 48]. Drought stress can profoundly influence plant development, yield potential, and modifications in the chemical and nutritional profiles of the grains. The implementation of effective water management strategies and the development of drought-tolerant durum wheat cultivars are instrumental in mitigating these adverse effects, thus ensuring consistent and good yields under different environmental conditions. In Algeria, drought stress exerts a considerable influence on durum wheat production, particularly in a nation marked by arid and semi-arid climatic zones [11]. Given that durum wheat is significantly dependent on sufficient water availability during critical developmental phases, it encounters considerable obstacles under conditions of water stress. The repercussions of drought stress are reflected in stunted plant growth, reduced photosynthetic efficiency, and compromised quality metrics of the grains, such as protein concentration and gluten strength [13, 17]. A comprehensive understanding of these dynamics is essential for the formulation of resilient agricultural methodologies, encompassing the selection and breeding of drought-resistant durum wheat cultivars, the optimization of irrigation practices, and the exploration of physiological adaptations to water stress, thereby ensuring sustainable wheat production under Algeria's challenging climatic conditions.

Historically, much of the research conducted on durum wheat in the context of improving genetic tolerance to drought stress has primarily aimed at enhancing productivity, an approach grounded in agronomic performance metrics. Presently, wheat enhancement initiatives are increasingly oriented towards the genetic augmentation of tolerance to drought stress. This requirement emphasizes how crucial it is to look into, recognize, and validate the phenological, morpho-physiological, and biochemical characteristics linked to yield performance in water stress situations [40].

Drought stress adversely affects key physiological processes in plants, including photosynthesis, nutrient uptake, stomatal conductance, and water relations, ultimately impairing plant growth and productivity [1]. One central adaptive mechanism to counteract osmotic stress under drought is the accumulation of compatible solutes, such as proline and soluble sugars. Proline acts as an osmoprotectant, stabilizing subcellular structures, scavenging reactive oxygen species, and maintaining cellular turgor [2]. Similarly, soluble sugars such as sucrose, glucose, and fructose contribute to osmotic adjustment and serve as energy reserves during stress recovery [9]. Chlorophyll content, particularly chlorophyll a and b, is also a key indicator of plant health and photosynthetic efficiency. Under drought conditions, chlorophyll degradation often occurs due to oxidative stress, leading to reduced photosynthesis and impaired energy production [10]. Thus, monitoring proline, sugars, and chlorophyll levels provides crucial insights into the physiological strategies employed by plants to withstand water deficit.

Under such circumstances, both the production and quality of wheat are detrimentally impacted, particularly during critical growth periods, such as the heading and grain development phases [2, 20]. For example, extreme drought conditions experienced by wheat plants during the grain-filling phase have been shown to diminish yields by 44% [2, 42]. Additionally, a separate investigation indicated that prolonged mild drought stress at the heading stage resulted in a 58% reduction in grain yield [2, 15]. A variety of yield-related characteristics, including spike length, grains per spike, overall biomass, and grain yield, are anticipated to be impacted under drought conditions [2,32, 38]. Consequently, there exists an imperative to develop novel wheat varieties endowed with climate-resilient traits capable of withstanding an array of abiotic stressors, including drought [2, 3].

To this end, we investigated the effects of water stress on four durum wheat varieties, assessing their resistance and sensitivity through growth, physiological, and biochemical perspectives.

Specifically, this study aims to:

1. Evaluate the growth responses, including SL and RL as well as fresh and dry biomass.
2. Assess biochemical parameters such as chlorophyll content, proline, and soluble sugars.
3. Identify varietal differences in drought tolerance to distinguish between sensitive and tolerant genotypes.

This comprehensive approach provides valuable insights for future breeding programs targeting drought resilience in durum wheat under semi-arid conditions. Based on the hypothesis that durum wheat genotypes exhibit differential responses to water deficit, we hypothesized that the selected varieties would display distinct morphological and physiological adaptations. To capture key adaptive responses, drought stress was imposed from the 3-leaf stage (Zadoks 13) and maintained until flowering (Zadoks 65), covering the transition from vegetative to early reproductive growth. This period is critical for determining plant performance under drought, as it includes major physiological and biochemical adjustments that influence yield potential. The study, therefore, addresses the following questions: Which variety demonstrates the highest drought tolerance based on agronomic and biochemical indicators? How does water deficit influence chlorophyll content, proline accumulation, and soluble sugar levels in the different genotypes?

MATERIALS AND METHODS

The study used four durum wheat (*Triticum durum* Desf.) varieties: Vitron, Simeto, Prospero, and Oued El Bared, selected based on their contrasting responses to drought stress. Vitron and Simeto were widely studied for their drought tolerance across the Mediterranean region[4], Oued El Bared was a local Algerian cultivar adapted to semi-arid zones[28]. Prospero was included based on recommendations from the experimental station of the Field Crops Technical Institute ITGC of Khemis Miliana, following breeder observations suggesting potential stress resilience. Seeds were provided by the ITGC station of Khemis Miliana (Algeria), harvested during the 2022 campaign, and sown in sterile sand-compost substrate (2:1).

The experiment was conducted in a greenhouse at Djilali Bounaama University (Khemis Miliana, Algeria), within the Water–Rock–Plant research laboratory. The greenhouse operated under natural environmental conditions, without artificial control of temperature or light. Temperature was monitored daily using a thermometer placed inside the greenhouse. Between December and April, average daytime temperatures ranged from 15 °C to 21 °C, while nighttime temperatures dropped to between 4 °C and 9 °C.

Substrate and pot preparation: The pots used had dimensions of 15 cm in diameter and 20 cm in height, corresponding to a soil volume of approximately 4L. Each container was filled with a substrate formulated from two-thirds river sand and one-third compost, with a pH of 6,5-7,2 and a moderate EC estimated at 0,3-0,8 $dS \cdot m^{-1}$, with a thin layer of gravel positioned at the base to enhance drainage efficacy.

Field capacity (FC) of the substrate was determined gravimetrically following the classical method of (Richard, L.A., 1949):

$$FC(\%) = \frac{(M_{after} - M_{before})}{M_{before}} \times 100$$

Where *Mafter* denoted the substrate mass following drainage and *Mbefore* signified the substrate mass prior to saturation. This determined field capacity subsequently served as a baseline reference for the implementation of drought stress treatment. The pots designated for the stressed treatments were irrigated to maintain moisture levels at 30% of this established field capacity; in contrast, the control pots were routinely watered to sustain optimal hydration levels consistent with field capacity.

Growing conditions and drought stress protocols: For each durum wheat variety, three seeds were sown per pot, with five pots per condition (control and drought). No thinning was applied; all seedlings were measured, and their average was applied as one replicate. Drought stress was imposed at the 3-leaf stage (Zadoks 13) and maintained until flowering (Zadoks 65). Soil moisture was gravimetrically monitored and maintained at approximately 30 % of field capacity in drought-stressed pots, and at 100 % in controls, following the classical gravimetric method. Water was added at regular intervals to maintain target field capacity, ensuring consistent soil moisture despite plant growth and evapotranspiration. This threshold was commonly used to simulate moderate to severe drought conditions in wheat [20]. Pots were arranged by variety in rows, with regular intra-row rotation and periodic inter-row repositioning to minimize positional effects.

Growing monitoring: The monitoring of plant growth was performed by evaluating both morphological and biomass-related parameters. The assessed traits included shoot length (SL in cm), root length (RL in cm), fresh shoot weight (FSW in mg), dry shoot weight (DSW in mg), fresh root weight (FRW in mg), and dry root weight (DRW in mg). Fresh biomasses were measured immediately after harvest, while dry weights were obtained after oven-drying the shoot and root samples at 80 °C for 48 hours, followed by weighing using a precision balance. All measurements were conducted at the flowering stage to assess the impact of water deficit on plant development.

Biochemical analyses: To investigate the physiological responses of the four durum wheat varieties under drought stress, biochemical traits were evaluated as follows:

Relative chlorophyll content was measured non-destructively using a BS_TAB SPAD chlorophyll meter. SPAD values were recorded directly on intact leaves and expressed in SPAD units.

Soluble sugars were extracted from fresh leaf material using ethanol. After evaporation of the solvent, the extract was reconstituted in distilled water. The anthrone method was used to measure the amount [35]. We used a glucose calibration curve to measure absorbance at 585 nm and show the results in µg/g of fresh weight.

We used 40% methanol to extract free proline from 100 mg of fresh leaf tissue and then used the method in [6] to measure the colorimetric reaction with acid ninhydrin. We measured the absorbance at 528 nm and used a standard curve of L-proline to figure out how much proline was in the sample. The results were given in µg/g of fresh weight.

Statistical analyses: All data were tested for normality and homogeneity of variances using the Shapiro–Wilk and Levene's tests, respectively. Two-way ANOVA was performed to assess the effects of water stress, variety, and their interaction on the morpho-physiological and biochemical parameters. One-way ANOVA was followed by Tukey's HSD test, whereas two-way ANOVA analyses were followed by pairwise t-tests with Bonferroni adjustment. Significance was set at $p < 0.05$. Results were expressed as means ± standard deviation (SD). All statistical analyses were conducted using R software (version 4.2.3; RStudio). Additionally, a Principal Component Analysis (PCA) was carried out using the FactoMineR package (version 2.10) [30], to explore multivariate relationships among traits and to discriminate among the wheat varieties based on their responses to water stress. Graphical visualizations were produced with the factoextra package.

The PCA and hierarchical clustering were performed on the full dataset comprising 40 biological replicates (4 varieties × 2 water regimes × 5 replicates). Each individual in the factorial map represented a single plant, allowing the analysis to capture both inter- and intra-group variability. This replicate-level approach was chosen to preserve the natural variation among replicates and to reveal patterns that could be obscured when using treatment group means.

RESULTS

Impact of water deficit on the growth parameters: The imposition of water deficit (30% of field capacity) significantly affected the growth performance of four durum wheat varieties: Oued El Bared (O), Simeto (S), Prospero (P), and Vitron (V). The control groups are denoted as OC, SC, PC, and VC. In contrast, the drought-stressed groups are labelled OS, SS, PS, and VS. Figure 1 summarizes the changes in growth traits under water deficit and presents a comparative analysis among the stressed varieties. Statistical significance levels are indicated as follows: ns (not significant), * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

In addition, a two-way ANOVA was conducted to assess the effects of variety, treatment, and their interaction on the measured traits. The complete ANOVA results, including degrees of freedom, F-values, and P-values, are provided in Table 1.

Table 1. Results of two-way ANOVA showing the effects of variety, treatment, and their interaction on growth parameters of durum wheat under water deficit.

Traits	Anova results				
	Source of variation	Df	F-value	P-value	Significance
SL(cm)	Variety	3	11.52	2.78e-05	***
	Variety x Treatment	4	201.48	<2e-16	***
	Residual	32			
RL(cm)	Variety	3	5.794	0.00279	**
	Variety x Treatment	4	14.032	1.02e-06	***
	Residual	32			
FSW(mg)	Variety	3	4.485	0.009748	**
	Variety x Treatment	4	6.283	0.000754	***
	Residual	32			
DSW(mg)	Variety	3	12.73	1.21e-05	***
	Variety x Treatment	4	10.86	1.12e-05	***
	Residual	32			
FRW(mg)	Variety	3	23.60	3.01e- 08	***
	Variety x Treatment	4	18.41	3.42e-10	***
	Residual	32			
DRW(mg)	Variety	3	9.396	0.000133	***
	Variety x Treatment	4	15.12	1.54e-08	***
	Residual	32			

Note: Significance levels: ns (not significant); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Based on the ANOVA results, the main differences among varieties and treatments are described below for each growth parameter.

SL significantly decreased in all varieties under water deficit except for Prospero, with the most substantial reductions observed in Oued El Bared and Vitron (Fig. 1a). Among stressed plants (Fig. 1b), Vitron maintained a significantly longer SL compared to Oued El Bared and Simeto.

RL was significantly reduced in Oued El Bared and Simeto. At the same time, no significant effect was observed in Prospero and Vitron (Fig. 1a). Among stressed plants (Fig. 1b), Vitron maintained relatively greater RL compared to Simeto and Prospero, though differences were not statistically significant.

FSW and DSW were significantly reduced for all varieties (Fig. 1a). Nevertheless, Prospero and Simeto maintained relatively higher fresh weights under stress conditions. Among the stressed plants (Fig. 1b), Prospero exhibited a significantly higher SFW than Oued El Bared. Regarding DSW, Vitron, Prospero and Simeto showed higher values compared to Oued El Bared.

Across all varieties, water stress had an impact on root biomass as measured by FRW and DRW (Fig. 1a). Simeto showed a notable decline. Prospero, Vitron, and Oued El Bared, on the other hand, did not exhibit any notable changes. Vitron preserved comparatively more root biomass during drought, indicating a possible adaptive advantage. Among the stressed plants (Fig. 1b), Prospero exhibited a significantly higher FRW compared to Oued El Bared. For DRW, both Prospero and Vitron showed elevated values, although the differences among stressed varieties were not statistically significant.

Effect of water stress on the biochemical parameters: This study examined the effect of drought stress on key biochemical parameters in four durum wheat varieties. The measured traits included chlorophyll content (SPAD), soluble sugars, and proline (µg/g FW). A two-way ANOVA revealed significant effects of variety, treatment, and their interaction on these traits, as summarized in Table 2.

Chlorophyll content, measured using a SPAD meter, significantly decreased under drought in all varieties. The most pronounced decreases were observed in Prospero and Simeto ($p < 0.001$), while Vitron exhibited a slight but significant reduction ($p < 0.05$), and Oued El Bared showed a moderate decline ($p < 0.01$). Vitron and Oued El Bared maintained higher chlorophyll levels under water-limited conditions compared to the other varieties (Fig. 2a,b).

Soluble sugars significantly increased in Vitron and Oued El Bared ($p < 0.001$) under drought, indicating osmotic adjustment. In contrast, Simeto and Prospero showed no significant changes ($p > 0.05$). As illustrated in Fig. 2a,b, significant differences were observed among the stressed varieties, with Oued El Bared and Prospero displaying higher soluble sugar concentrations compared to Vitron and Simeto ($p < 0.05$).

Proline accumulation increased significantly in all varieties under water stress. Vitron and Simeto exhibited the highest levels ($p < 0.001$), suggesting stronger osmoprotection. Prospero and Oued El Bared had moderate increases, with the lowest response recorded in Oued El Bared ($p < 0.05$) (Fig. 2b). When comparing the stressed genotypes, Simeto and Vitron accumulated significantly more proline than Prospero and Oued El Bared ($p < 0.05$). At the same time no significant difference (ns) was observed between the latter two (Fig. 2a).

Evaluation of the relationships between the growth parameters of durum wheat varieties using PCA and hierarchical clustering:

PCA variables: A Principal Component Analysis (PCA) and hierarchical clustering were conducted on the 40 biological replicates (4 varieties × 2 treatments × 5 replicates). This replicate-level analysis allowed capturing both inter-varietal and intra-varietal variability in response to water stress, thereby providing a higher resolution of phenotypic differences.

The PCA biplot illustrates the contribution of the growth parameters to the first two principal components. Dim1 explains 51.59% of the total variance. It is mainly driven by SL, RL, FRW, and DRW, identifying these traits as primary discriminators among varieties and treatments. FSW and DSW also contribute to Dim1 and Dim2, but their vector orientations suggest distinct correlation structures.

Table 1. Results of two-way ANOVA showing the effects of variety, treatment, and their interaction on biochemical parameters of durum wheat under water deficit.

Traits	Anova results				
	Source of variation	**Df**	**F-value**	**P-value**	**Significance**
Chlorophyll	Variety	3	2.274	0.989	***
(SPAD)	Variety x Treatment	4	45.853	8.23e-13	
	Residual	32			
Soluble sugars	Variety	3	3.838	0.0187	*
(µg/g FW)	Variety x Treatment	4	9.170	4.71e-05	***
	Residual	32			
Proline	Variety	3	3.102	0.0403	*
(µg/g FW)	Variety x Treatment	4	16.000	2.71e-07	***
	Residual	32			

Note: Significance levels: ns (not significant); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

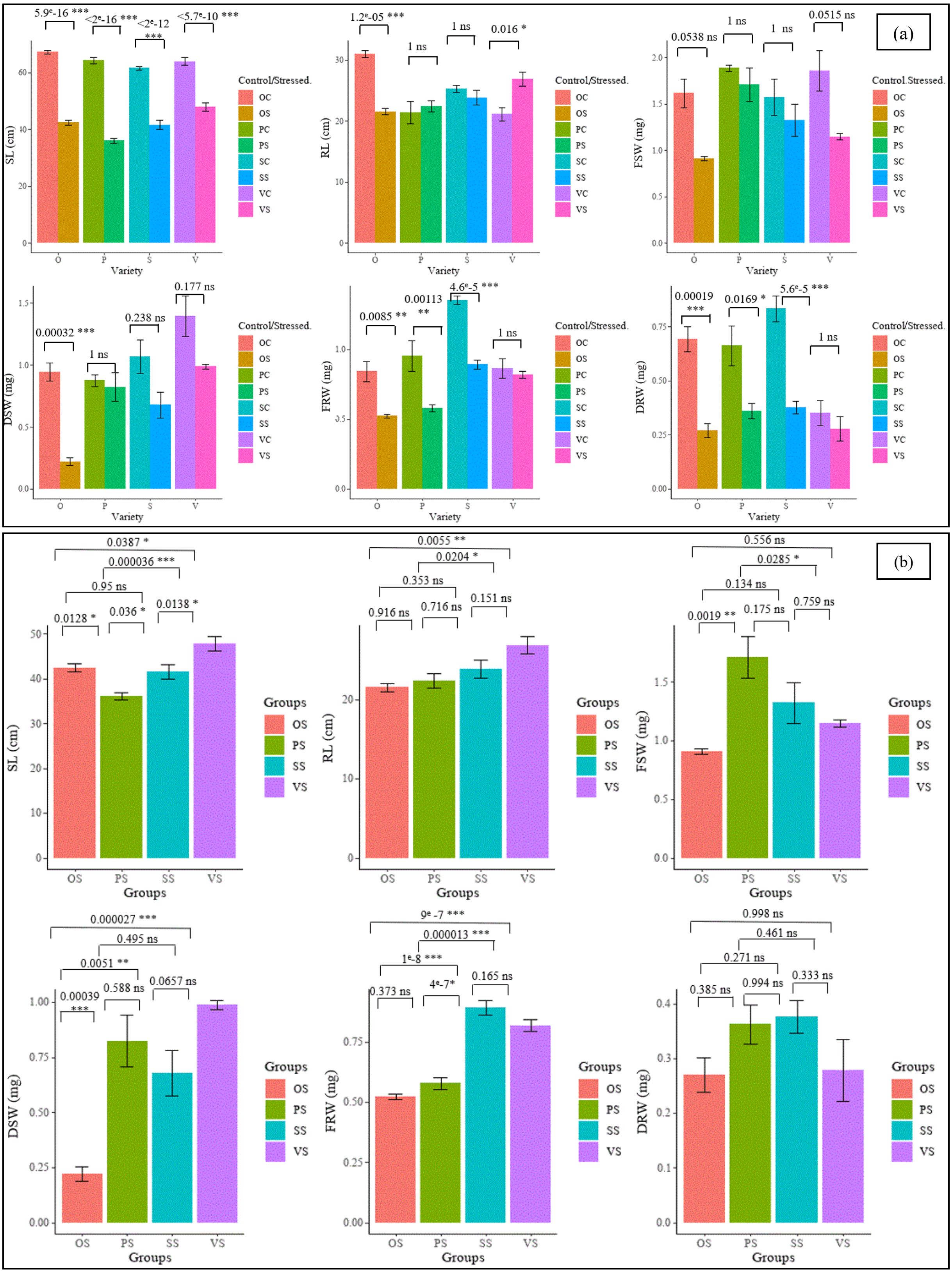

Figure 1. Effect of drought stress on growth parameters in four durum wheat varieties. (a) Control vs. stress groups. (b) Comparison among stressed varieties.Traits: AL, RL (cm); FAW, DAW, FRW, DRW (mg). Values are mean ± SD. Significance levels: ns (not significant); * p < 0.05; ** p < 0.01; *** p < 0.001.

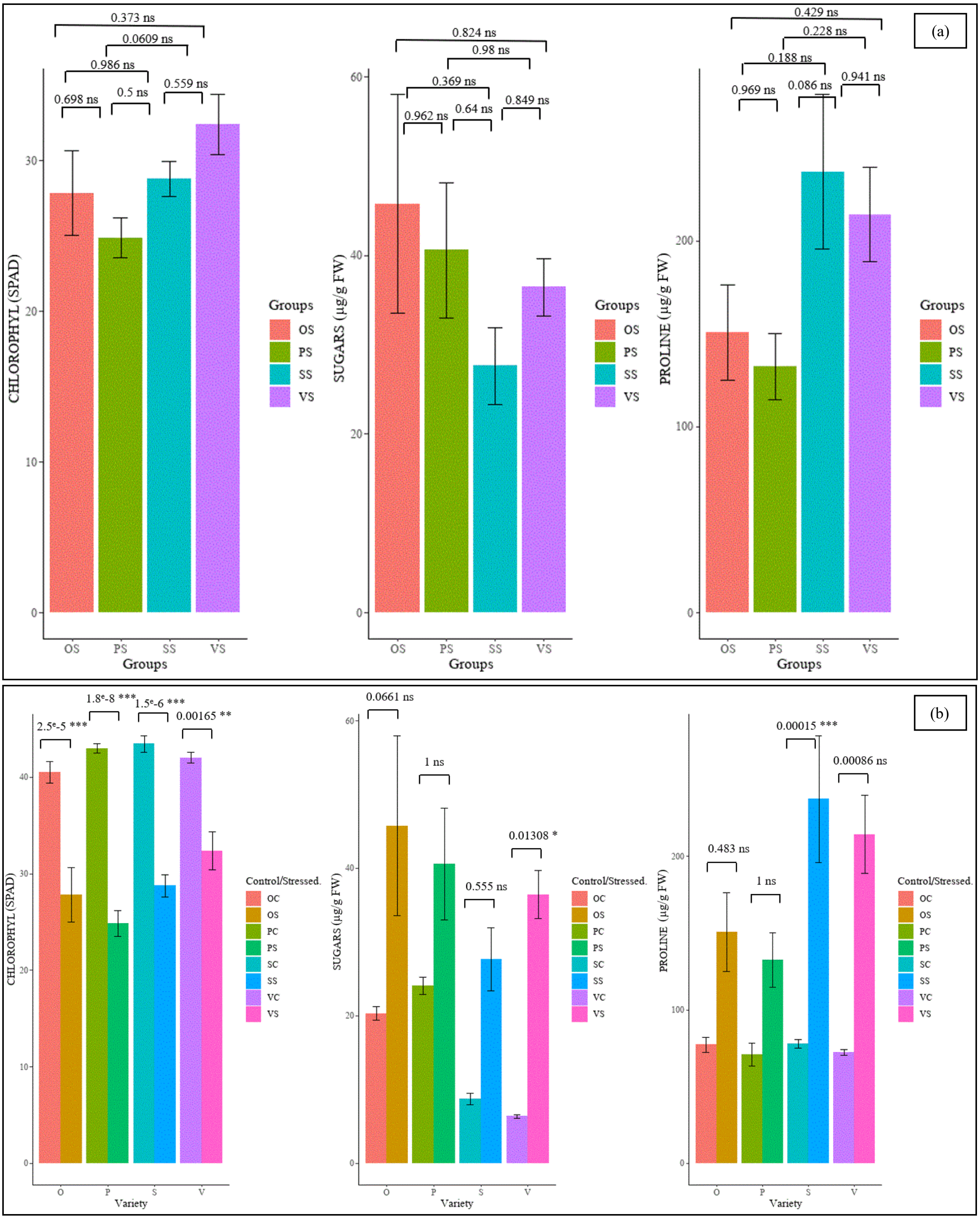

Figure 2. Effect of water stress on biochemical parameters in four durum wheat varieties. (a) Comparison among stressed varieties. (b) Control vs. stress groups. Traits: chlorophyll content (SPAD), soluble sugars, and proline (µg/g FW). Values are mean ± SD. Significance levels: ns (not significant); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Closely aligned vectors denote strong positive correlations, while divergent vectors indicate weaker or negative associations.

Factor Map: The factor map projects the 40 individual observations onto the first two principal components (Dim1 and Dim2), which together account for 69.39% of the total variance. This plot reveals patterns of distribution based on treatment and varietal effects. Clusters of closely positioned individuals indicate similar growth responses, while those located at the periphery of the plot reflect atypical or extreme responses to drought stress, likely due to marked differences in shoot and/or root development. The third component (Dim3), which explained 15.01% of the variance, brought the cumulative variance to 84.36%. Although not shown, inspection of Dim3 confirmed and reinforced the clustering observed in the PC1–PC2 plane.

To complement PCA, Hierarchical Clustering on Principal Components (HCPC) was applied to group individuals with similar response profiles. This analysis, represented in three-dimensional space, revealed two separate clusters:

Cluster 1 includes individuals who have not experienced drought stress. The growth parameters SL, RL, FRW, and DRW are more effectively sustained within this group, signifying optimal growth conditions under controlled treatments.

Cluster 2 includes individuals who have been exposed to drought stress. When compared to Cluster 1, this shows a notable decrease in growth traits, demonstrating the detrimental effects of water deficit on the development of both shoots and roots. These clustering results highlight varietal differences in stress adaptation strategies and reinforce the biological relevance of the multivariate approach, despite the limited number of genotypes. Furthermore, the use of a three-dimensional PCA projection enhanced the separation among varieties, particularly under stress, compensating for the moderate variance (69.39%) captured by the first two dimensions alone.

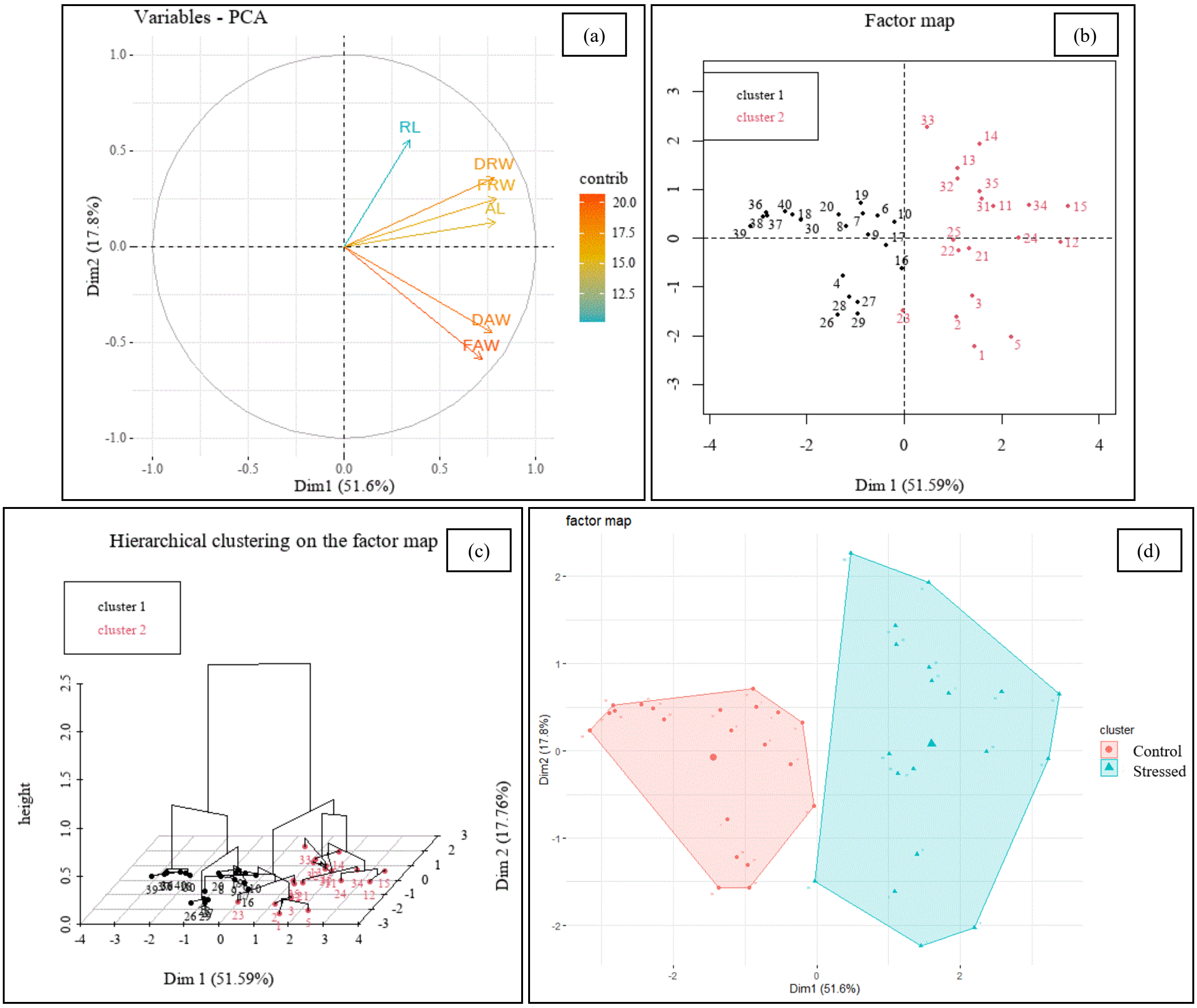

Figure 3. Principal component Analysis (PCA) and Hierarchical Clustering of growth Parameters in Four Durum Wheat Varieties under water stress (a) Projection of growth variables into the first two dimensions of the PCA (b) Factor Map of observations (individuals) showing the distribution of varieties based on the first two dimensions (c) Hierarchical clustering of individuals on the factor map to identify clusters (d) Factor map with visualization of groups (convex hulls) derived from hierarchical clustering.

Factor Map with Convex Hull: This graph illustrates the projection of individuals alongside a visual representation of groups utilizing convex hulls (polygons encircling the observations of each cluster).

The two polygons, depicted in distinct colors, symbolize the clusters of individuals derived from hierarchical clustering. The initial cluster (red) and the subsequent cluster (blue) display disparate growth responses to drought stress. The significant spatial separation between the clusters indicates that the wheat cultivars under study respond to water stress in morphologically different ways. Evaluation of the relationships between the biochemical parameters of durum wheat varieties using PCA and hierarchical clustering:

Variable correlation circle (PCA results): This graph demonstrates the correlations between biochemical parameters and the first two principal components (Dim 1 and Dim 2): Dim 1 (68.3%) accounts for the predominant portion of the variation, primarily associated with proline (exhibiting a positive correlation with Dim 1, coefficient > 0.8). This suggests that proline serves as a pivotal marker for drought stress. Dim 2 (19.8%) pertains to moderate variations in soluble sugars and chlorophyll (fig. 4a).

Chlorophyll exhibits a negative correlation with Dim 1 (coefficient < (-0.7)), indicating a decrease in its concentration under drought stress conditions.

Soluble sugars contribute moderately to both Dim 1 and Dim 2 (coefficients ranging between 0.4 and 0.6).

These findings imply that proline and chlorophyll represent the most discriminative parameters for differentiating water-stressed samples from control samples (fig. 4a).

Factorial representation of individuals: Individuals are categorized into two primary dimensions: The control group is situated within the negative quadrant of Dimension 1, which is correlated with elevated levels of chlorophyll and diminished concentrations of proline and soluble sugars. Conversely, samples subjected to water stress are positioned in the positive quadrant of Dimension 1, characterized by an augmentation in proline and soluble sugars.

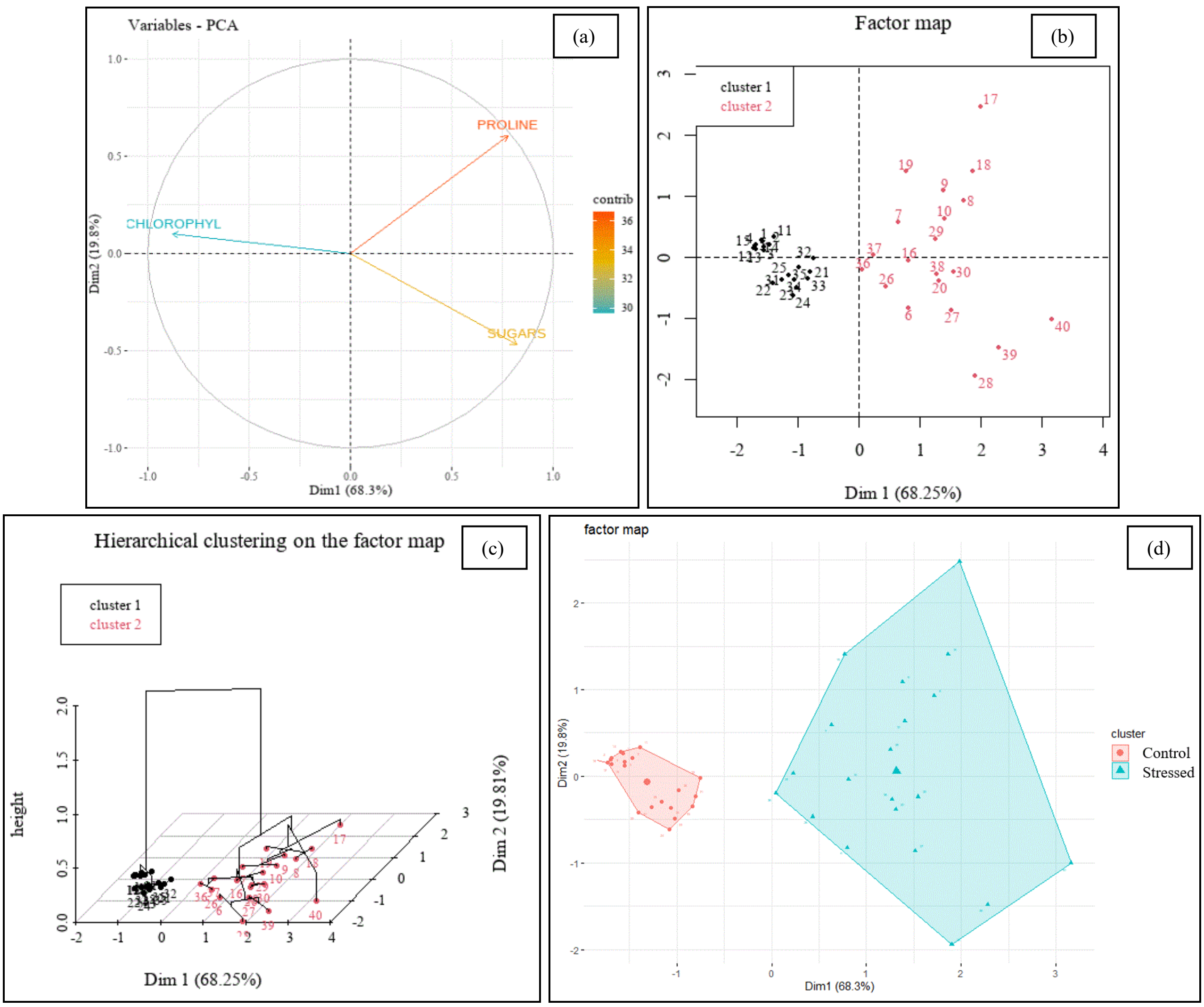

Figure 4. Principal component Analysis (PCA) and Hierarchical Clustering of Biochemical Parameters in Four Durum Wheat Varieties under water stress (a)Projection of biochemical variables into the first two dimensions of the PCA (b)Factor Map of observations (individuals) showing the distribution of varieties based on the first two dimensions (c) Hierarchical clustering of individuals on the factor map to identify clusters (d) Factor map with visualization of groups (convex hulls) derived from hierarchical clustering.

Chlorophyll levels, conversely, exhibit a pronounced decline under stress conditions, with an average decrease of 45% observed in varieties under stress (Fig. 4b).

Hierarchical classification on the factorial map: Two principal clusters have been delineated: Cluster 1 (controls): This cluster encompasses non-stressed individuals, distinguished by elevated levels of chlorophyll and minimal concentrations of proline and soluble sugars.

Cluster 2 (water stress): This cluster comprises individuals experiencing stress, characterized by elevated concentrations of proline and soluble sugars.

Notably, chlorophyll levels demonstrate a significant reduction within this group (Fig. 4c).

Convex polygons of clusters: The graphical representation shows a clear separation between stressed and control individuals, as well as variability among the different varieties:

Cluster 1: The blue polygon encapsulates non-stressed individuals, which are marked by high levels of chlorophyll and low concentrations of proline and soluble sugars. These individuals occupy a confined region within the factorial plane, signifying a uniform biochemical response among varieties under control conditions.

Cluster 2: The red polygon includes individuals subjected to water stress. This group displays a wider dispersion, indicative of increased variability in biochemical responses among varieties experiencing water stress (Fig. 4d).

DISCUSSION

Plants exhibit responses to environmental stressors through a range of growth, physiological [47], and biochemical adaptations [46]. In the present study, we assessed the effects of water stress on the growth and biochemical characteristics of four varieties of Durum wheat by inducing water stress conditions at 30% field capacity. The findings revealed significant variability in the responses of the various wheat varieties, indicating a genetic diversity in their capacity to withstand water stress [5, 25].

One of the main consequences of water stress is the inhibition of shoot and root extension, reflecting impaired vegetative growth. This observation is consistent with previous reports showing that drought conditions restrict tissue expansion and thereby limit plant growth [31, 37, 39, 47].

This decline may be attributed to impairment in photosynthetic activity and a postponement in the allocation of resources, particularly sugars and nutrients, to various plant structures [29].

Stressed Varieties Groups: The results indicate a differential response among the four studied varieties of durum wheat. The superior performance of Vitron and Simeto under drought stress suggests that these varieties possess adaptive traits that confer greater tolerance to water deficit. This observation supports the hypothesis that genetic variation exists in the capacity of plants to manage water deficits, with more resilient varieties potentially evolving physiological and growth strategies to minimize water loss and sustain growth [23, 36].

Conversely, Prospero and Oued El Bared demonstrated a more substantial decline in growth parameters, which may be ascribed to an inadequate adaptive capacity or suboptimal response mechanisms to water stress.

Shoot and Root Biomass: The examination of FSW, DSW, FRW, and DRW exhibited trends consistent with those observed for SL and RL, revealing an overall decrease under water stress. However, the decrease in dry root biomass was more pronounced than that of the aerial parts, suggesting that root development was more negatively affected by the imposed water deficit. This finding aligns with previous studies [10, 16, 26, 27] reporting that water stress inhibits root growth by limiting cell division and elongation, reducing turgor pressure, and consequently restricting root expansion and biomass accumulation. Despite the commonly observed adaptive strategy of increasing root biomass under mild or moderate drought to enhance water uptake [18], our results clearly show that under severe stress conditions (30% field capacity), root growth was significantly constrained. Thus, both shoot and root biomasses were negatively impacted, with roots being more affected.

Aerial biomass plays a central role in photosynthesis and dry matter production. In our study, despite a significant reduction under water stress, aerial biomass remained higher than root biomass, reflecting the plant's natural tendency to prioritize light capture. Nevertheless, the observed reduction can be attributed to stomatal closure, decreased photosynthesis, and limited cell expansion, ultimately altering leaf growth and biomass production [34].

Interpretation of Inter-Varietal Differences: The analysis of inter-varietal differences elucidates that Vitron and simeto exhibit a greater capacity to withstand water stress in comparison to others. The varieties demonstrating enhanced tolerance to water stress may possess deeper root systems or exhibit more effective mechanisms for water regulation. The capacity to sustain root development, even in conditions of stress, may be associated with the production of finer or more prolific roots, thereby enabling the plant to more efficiently forage the soil for water and essential nutrients [18, 47].

Implications and Practical Perspectives: The findings derived from this study carry significant implications for the selection of durum wheat varieties that are adapted to conditions of water stress. In light of the challenges posed by climate change and fluctuations in precipitation, the development and selection of varieties that tolerate water stress is imperative to ensure the long-term sustainability of durum wheat cultivation. Future research endeavours should prioritize the identification of the physiological mechanisms that underpin water stress tolerance and assess the interactions between environmental variables and the growth traits that affect such tolerance.

Changes in Biochemical Parameters Under Water Stress: The principal component analysis (PCA) revealed that the three biochemical parameters investigated – proline, soluble sugars, and chlorophyll – serve as significant differentiators between individuals exposed to water stress and those in control conditions. These findings substantiate the notion that these biochemical parameters represent pertinent criteria for evaluating stress tolerance, corroborating the conclusions of numerous researchers [4, 9, 10, 13].

Proline and soluble sugars, both well-known osmoprotectants, accumulated significantly under drought conditions. The findings indicate a substantial elevation in proline accumulation in plants subjected to water stress. The substantial increase in proline observed in water-stressed plants aligns with its widely documented role as an osmoprotectant and stabilizer of cellular structures under drought stress [8, 22, 25]. In addition, this metabolite plays a central role in mitigating oxidative damage by functioning as an antioxidant [1, 7, 44]. The osmotic regulation facilitated by proline enables plants to maintain turgor pressure even at reduced water potential, which is critical for sustaining metabolic processes [9, 24]. Some genotypes exhibited an enhanced capacity for proline accumulation, a trait frequently associated with better stress resilience [12, 43, 45].

By increasing the synthesis of such osmoprotective compounds, plants can effectively reduce the generation of reactive oxygen species (ROS) and delay leaf senescence [21].

Under drought conditions, photosynthetic activity is markedly reduced, leading to a decrease in the synthesis of carbon compounds, particularly sugars. However, carbon allocation shifts toward the synthesis of soluble sugars, which play an essential role in plant adaptation to water stress [13, 14, 22, 37]. These sugars contribute to the maintenance of osmotic potential and also protect cellular membranes and proteins [10, 41]. Their accumulation thus constitutes a fundamental adaptive strategy enabling plants to tolerate dehydration and enhance drought resistance [22].

Chlorophyll content was also significantly reduced under water stress across the four durum wheat varieties studied. This decline can be attributed to the degradation of photosynthetic pigments, a typical response under stress conditions. It adversely impacts photosynthetic efficiency, thereby limiting plant growth. These findings are consistent with previous studies that identified chlorophyll content as a sensitive biomarker of drought-induced damage [3, 5, 19, 42, 44]. While a reduction in chlorophyll levels is typically associated with decreased photosynthetic capacity under stress, it may not always directly reflect impaired photosynthetic efficiency under optimal light conditions. Nevertheless, in our study, the significant correlation observed between chlorophyll content and growth parameters across all varieties reinforces the key role of chlorophyll in maintaining biomass production and overall plant growth under water stress.

Despite the limited number of genotypes and traits, PCA was justified due to the complexity of plant responses. While ANOVA revealed individual trait variations, PCA captured correlations among traits and distinguished stressed from control plants based on combined biochemical profiles. It thus provided an integrated view that complemented the univariate analysis.

Varietal differentiation: The multivariate analyses (the Principal Component Analysis (PCA) and hierarchical clustering) reveal significant inter-varietal variability in responses to water stress. Varieties such as Vitron and Simeto clustered closely with control individuals, reflecting better drought tolerance. This resilience could be linked to their capacity to accumulate higher levels of proline and soluble sugars, coupled with the maintenance of chlorophyll content traits often associated with physiological robustness under drought. In contrast, other varieties, located farther from the control cluster, showed more pronounced stress effects. These differences emphasize the importance of varietal selection in breeding programs aiming to enhance drought resilience in durum wheat.

Cluster analysis further distinguished two main groups: a homogeneous control group with high chlorophyll content and low levels of proline and sugars, and a more heterogeneous stressed group exhibiting the opposite pattern. The increased dispersion within the stressed group suggests a broader spectrum of physiological responses among genotypes. This highlights the relevance of biochemical markers, notably proline and soluble sugars, as valuable criteria for differentiating drought tolerance levels.

Relationships between growth and biochemical traits: The examination of the correlations between growth and biochemical characteristics under water stress conditions demonstrated distinct patterns that underscore the strategies employed by the analyzed durum wheat types. The PCA results demonstrated a positive correlation between shoot and root growth indices (SL, RL, FSW, DSW, FRW, DRW) and chlorophyll content, indicating that the preservation of chlorophyll during water stress aids in sustaining vegetative growth. Conversely, proline and soluble sugars exhibited negative associations with biomass-related features, indicating that varieties demonstrating greater growth decreases were also those accumulating elevated amounts of osmolytes.

These findings indicate two complementary yet divergent adaptation strategies. Varieties that sustain growth and biomass production during water stress seem to depend on the maintenance of photosynthetic activity, shown by elevated chlorophyll levels [12]. Conversely, more sensitive cultivars activate osmotic adjustment mechanisms by accumulating proline and soluble sugars, which protect cellular structures and sustain turgor, but are associated with a reduction in growth [21].

Correlations among growth maintenance, chlorophyll stability, and osmolyte accumulation align with prior findings in wheat and other cereals, indicating that drought-tolerant genotypes maintain photosynthetic capacity, while susceptible genotypes depend more on osmoprotective compounds [12, 20]. This duality emphasizes the necessity of examining both morphological and biochemical responses to enhance comprehension of the intricacies of drought tolerance in durum wheat.

Agronomic implications and prospects: The findings of this investigation bear significant implications for the enhancement of durum wheat varieties.

Selection criteria: Proline and soluble sugars are promising biochemical markers for drought response, but their reliability as selection criteria in breeding programs must be interpreted with caution because they are not always indicative of field performance.

Varietal differentiation: The study elucidates pronounced disparities among the tested genotypes, which may inform breeding initiatives targeting drought resilience.

Future perspectives: It is advisable to extend this investigation by incorporating plant growth-promoting rhizobacteria (PGPR), as these beneficial microbes can enhance drought tolerance through improved root architecture, hormonal modulation, and induction of stress-related metabolites. This study provides new insights into the morpho-physiological responses on four durum wheat genotypes(Simeto, Vitron, Prospero, and Oued El Bared) subjected to severe water stress (30% field capacity), a condition simulating arid field environments. in agreement with previous studies on wheat, maize, and legumes [14, 38, 39, 43], drought stress reduced aerial and root growth and biomass, while stimulating the accumulation of osmoprotectants such as proline and soluble sugars, and decreasing chlorophyll content. However, unlike most previous works that often focused on a single genotype, limited stress levels, or partial physiological traits, the originality of our research lies in its integrative analysis of both growth and biochemical parameters across multiple genotypes with contrasting geographic origins.. Notably, certain varieties such as Vitron and Simeto demonstrated superior drought resilience, maintaining higher biomass and pigment levels. To our knowledge, this is the first comparative study of these four genotypes under a standardized and well-controlled drought stress regime in Algeria, offering a novel perspective on intra-specific variability in durum wheat. These findings contribute to a better understanding of the complex mechanisms of drought adaptation and offer practical implications for the selection of drought-tolerant cultivars, supporting future breeding strategies for sustainable wheat production under climate change.

Limitations and outlook**:** This study has limitations despite its relevance. Limited number of genotypes examined, lack of field validation or performance-related data, the restriction to the vegetative stage, and the lack of physiological markers such as relative water content, stomatal conductance, or antioxidant activity should be acknowledged. Future studies should integrate these aspects to reinforce the robustness of selection criteria under field conditions.

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