



Morpho-physiological Responses of Durum Wheat (*Triticum durum* Desf.) Seedlings to Drought Stress and Their Correlation with Yield



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THIS STUDY aimed to investigate the response of durum wheat seedlings to water stress and to analyze the relationships between seedling-stage parameters and their behavior under field conditions. Sixteen (16) genotypes of durum wheat (*Triticum durum* Desf.) were subjected to two water regimes: control and stressed. Parameters measured included accumulated biomass, seedling height, specific leaf weight, relative water content, proline content, drying rate of the flag leaf, and cell integrity. Water stress significantly affected all measured parameters, though the degree of impact varied among genotypes. Some genotypes demonstrated lower sensitivity to stress than others. Proline accumulation at the seedling stage showed a correlation with genotype performance under field conditions. Additionally, genotypes with higher specific leaf weight under stress better retained relative water content. The findings suggest that the ability to accumulate proline and sugars contributes to minimizing yield loss under water stress. This highlights potential physiological traits for breeding durum wheat varieties with improved drought tolerance.

Keywords: *Triticum durum* Desf., water stress, seedling, proline, soluble sugar, leaf desiccation.

Introduction

The variation in grain yields of durum wheat (*Triticum durum* Desf.) in semi-arid regions largely originates from the effects of abiotic stresses, primarily water and temperature-related. Water stress, a major limiting factor for wheat production (Zhang et al., 2018), is typically intermittent and highly unpredictable, except towards the end of the growing cycle when it commonly interacts with high temperatures (Laala et al., 2021).

Different plant species respond variably to water stress, as observed by Ghobadia et al. (2013).

The efficiency of a breeding program can thus be enhanced by selecting traits associated with stress performance that are less likely to show genotype x environment interactions (Gonzalez et al., 2007). The nature and timing of these stresses allow limited flexibility for breeders, who must favor

genotypes with optimal earliness. To better adapt wheat to environmental variability, selection assisted by physiological approaches stands out as a valuable alternative (Fellahi et al., 2018; Fellahi et al., 2020).

Among the traits associated with environmental adaptation is the ability of a genotype to develop a dense and deep root system, which improves water stress tolerance (Vogt, 2023). The storage of carbon substrates in the spike peduncle and their potential transfer for grain filling is another mechanism that helps minimize yield loss under intense water and heat stress towards the end of the cycle (Medina et al., 2016).

The ability of the plant to accumulate organic and mineral substances at the cellular level helps maintain water balance, thereby better tolerating

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external stresses (Mensink *et al.*, 2017). This ability is linked to osmotic regulation mechanisms (McElrone *et al.*, 2013). Among the substances that accumulate at relatively high levels under stress are proline and soluble sugars (Sami *et al.*, 2016).

Above-ground biomass production and its distribution among various sinks are characteristics that enhance adaptation to a given environment (Bouzerzour *et al.*, 2000; Ashinie and Kindie, 2016). Stem height, which benefits from these effects during dry years due to stored reserves, is a trait that aids in better adaptation to variable environments (Tahir *et al.*, 2023). Leaf water status, associated with the capacity for adjustment, is one of many parameters that appear to play a role in stress tolerance (Osakabe *et al.*, 2014).

Studying these mechanisms involved in stress tolerance control in durum wheat (*Triticum durum* Desf.) is crucial for selecting parent plants and for choosing progeny during breeding. Such studies are more cost-effective if conducted on seedlings, allowing for faster screening and sorting of a larger number of subjects. This study aims to investigate proline content, relative water content, and cellular integrity as mechanisms related to water stress tolerance in durum wheat seedlings (*Triticum durum* Desf.) and to analyze their associations with field performance.

Materials and Methods

Experimental design

The study was conducted at the laboratory of the Institute of Natural and Life Sciences at Larbi Ben M'hidi University in Oum El Bouaghi. Sixteen durum wheat genotypes were provided by the Technical Institute of Field Crops (TIFC) in Sétif (Table 1).

Table 1. List of the studied durum wheat varieties.

Order	Pedigree	Origin
1	439/Ads/97	Italy
2	Belikh2	Syria
3	Massara1	Syria
4	Cyprus1	Cyprus
5	Mrb5	Syria
6	Sahel77	Algeria
7	Mrb16//Enté/Mario	Syria
8	Waha	Algeria
9	Bicre	Syria
10	Beliouni 3852	Algeria
11	Derraa	Syria
12	Semito	Italy
13	Daki	Syria
14	Heïder	Syria
15	Hd/Mt//Ho	Algeria
16	MBB	Algeria

These genotypes served as the experimental material in a trial set up in a greenhouse. The trial was conducted in vegetation pots, each containing ten seeds planted in 4500 g of soil sourced from the lands of the Technical Institute for Vegetable and Industrial Crops (TIVI) in Oum El Bouaghi (OEB). The soil physico-chemical analysis are presented in Table 2. Analyzing of these results shows that the soil has a clay-loam texture, an alkaline pH, and a moderate cation exchange capacity. Levels of K₂O, MgO, Ca₂O, and Na₂O are adequate for cereal cultivation. The soil is well-supplied with organic matter, as its C/N ratio is sufficient to support the development of microorganisms responsible for organic nitrogen mineralization. The soil, while exhibiting good electrical conductivity, contains a high level of active limestone, rendering it susceptible to chlorosis.

Table 2. Physicochemical characteristics of the soil. The results are reported as mean ± SD of 3 different measurements.

Parameter	Value
Clay (%)	40.8 ± 1.2
Fine silt (%)	24.6 ± 1.1
Coarse silt (%)	24.2 ± 3.1
Fine sand (%)	5.6 ± 0.75
Coarse sand (%)	1.8 ± 0.01
Total limestone (%)	20.9 ± 2.3
Active limestone (%)	19.0 ± 2.0
pH	8.7 ± 0.5
EC (mmhos cm ⁻¹)	0.35 ± 0.01
Ca (meq 100g ⁻¹)	23.5 ± 4.1
Mg (meq 100g ⁻¹)	3.1 ± 0.8
Na (meq 100g ⁻¹)	1.7 ± 0.03
K (%)	1.0 ± 0.05
C (%)	1.0 ± 0.02
Organic matter (%)	1.7 ± 0.4
N (%)	0.24 ± 0.05
C/N	4.12 ± 1.6
CR (%)	27.7 ± 3.6
CEC (meq 100g ⁻¹)	27.2 ± 3.0

To irrigate the pots, the water retention capacity of the soil was determined and they were irrigated every four days following emergence, using the evaporation method. Each irrigation replenished the water lost through evapotranspiration, which was determined by weighing the pots on an electronic Bosch scale. Irrigation was stopped at the four-leaf stage for half of the pots to apply the stressed treatment, while it was continued for the other half, serving as the control. Water stress was maintained

for a period of 10 days at an average temperature of 25°C and a day length of 14 hours. After this period, measurements and observations described below were initiated.

Measurements

Leaf Area (LA)

Leaf area was estimated by measuring and summing half of the product of length (L) and maximum width (I) for ten leaves.

$$LA \text{ (cm}^2\text{)} = \sum [0,5(L \times I)]$$

Leaf Drying Rate (DR) and Turgor (RWC)

Six leaves were weighed to obtain the fresh weight (FW). They were then placed in test tubes filled to ¾ with distilled water and left in darkness for two hours. Afterward, the turgid weight (TW) was measured. The leaves were then allowed to lose water content at ambient temperature (25°C), and fresh weight at time $t_1=120$ minutes (FW120) was recorded. The drying rate (DR) was calculated using:

$$TS1 \text{ (mg water/Ment)} = (TW-FW120)/120$$

The leaves were then dried in an oven at 85°C for 12 hours to obtain the dry weight (DW). The relative water content (RWC) was calculated as:

$$RWC \text{ (\%)} = 100[(FW-DW)/(TW-DW)]$$

Leaf Specific Weight (LSW)

Leaf specific weight was assessed by the ratio of fresh or dry weight of a leaf to its surface area:

$$LSW \text{ (mg.cm}^2\text{)} = FW/LA$$

Above-Ground Biomass (Bio)

Biomass was estimated by weighing the harvested sample after drying at 70°C for 12 hours. Results were expressed as g of dry matter/m².

Seedling Height (SH)

Seedling height was measured from the soil surface to the base of the last leaf.

Cellular Integrity (CI)

Cellular integrity was measured on three leaf samples per genotype for both the control and stressed treatments. Cellular integrity is measured using the method described by Saadallah and Shanahan (1990). Fifteen leaf discs were divided into two groups and placed in test tubes. The treated tubes were placed in a 50°C water bath for 60 minutes, while the control tubes remained at

ambient conditions. The conductivity of the treated tubes was measured before being placed in a 100°C water bath to destroy all cells. The percentage of cells damaged by heat stress was calculated

Proline Assay (Pro)

Proline was quantified following the method described by Monneveux and Nemmar (1986). A sample of leaf blade material was heated in methanol, then mixed with a solution of distilled water, acetic acid, orthophosphoric acid, acetic acid, and ninhydrin. The solution separated into two layers, with the clear upper layer containing proline. The optical density was measured at 528 nm using a spectrophotometer.

Soluble Sugars Assay (Sug)

Water-soluble sugars were determined from 100 mg of plant material extracted in an 80% ethanol solution. The extract was incubated at 60°C for 6 hours. Anthrone reagent was prepared by dissolving 150 mg of anthrone in a 72% H₂SO₄ solution. The plant extract was pipetted into glass tubes, and 6 ml of anthrone reagent was added to each sample, heated in a water bath at 100°C for 10 minutes, then cooled on ice for 10 minutes. Optical density was measured at 625 nm using a Sontays Techtron 635 spectrophotometer. Soluble sugar concentrations were estimated by referring to a standard sugar calibration curve and expressed on a fresh weight basis.

Statistical analysis

A factorial design with two factors was used with three repetitions. The first factor was the variety effect with 16 levels, and the second was the stress effect with 2 levels. The experiment was conducted following the method of Troll and Lindsley (1955), improved by Lahrer and Magne as cited by Lepout (1992). Data collected for various measured parameters were analyzed using a two-way analysis of variance. Relationships among variables measured on seedlings were examined using principal component analysis. All analyses were performed using Statistica 08 software.

Results and discussion

Impact of genotype and water stress on seedling traits

The analysis of variance for the measured traits indicates a significant genotype effect for seedling height, leaf specific weight, and cellular integrity. The genotype effect was not significant for above-ground biomass, leaf area, and relative water content. Water stress had a significant to highly significant effect across all measured variables, while the genotype x stress interaction was not significant for leaf drying rate (Table 3).

It must be highlighted that the genotype and water stress effects are tested relative to the interaction variance when the interaction effect is statistically significant. A significant genotype effect indicates

varietal differences in behavior, while a significant water stress effect points to differences caused by the applied constraint.

Table 3. Mean squares from the analysis of variance for traits measured on durum wheat seedlings.

Source of variation	DF	SH	BIO	LSW	LA	RWC	CI	Pro	Sug	DR
Genotype (G)	15	25.4**	292.9 ^{ns}	177.2**	16187 ^{ns}	166.5 ^{ns}	750.3**	268340.7***	8364610.1***	0.09***
Stress (S)	1	151.7**	6972.6**	2381.1**	234689**	2593.8**	--	20480.7***	4404722.6***	0.33***
G x S	15	3.7**	206.4**	70.8**	9425.3**	161.2**	--	7747.2***	642395.6***	0.005 ^{ns}
Error	62	1.23	10.8	4.5	720.3	6.8	8.4	6.6	21.3	0.004

Ns, Not significant. ** Highly significant $p < 0.01$ *** Very Highly significant $p < 0.001$. The G x S variance is used to test the genotype effect when it is significant.

SH, Seedling Height; BIO, Above Ground Biomass; LSW, Leaf Specific Weight; LA, Leaf Area; RWC, Relative Water Content; CI, Cellular Integrity; Pro, Proline Content; Sug, Soluble Sugars; DR, Drying Rate of the Flag Leaf.

Genotypic means of measured seedling traits

The means for the traits expressing the specific genotype effect are given in Table 4. Seedling height varies from 7.5 cm for 439/Ads-97 to 13.8 cm for Mrb16/Ente/Mario. Seedling height at this stage reflects the influence of plant form rather than internode elongation. A prostrate growth habit is beneficial as it covers the soil early in the cycle, reducing evaporation and favoring soil water use by the plant. In contrast, an erect form indicates early heading and does not facilitate soil moisture retention early in the cycle (Othmani *et al.*, 2015; Ltaief and Krouma, 2023). Water stress is one of the environmental factors that significantly affect plant growth and development, with notable impacts on plant life (Iqbal *et al.*, 2023a).

In the presence of water stress, a positive relationship between grain yield and plant height suggests that yield depends on stronger vegetative growth and greater mobilization of stem reserves (Khan *et al.*, 2010).

Above-ground biomass produced shows means ranging from 12.2 g/pot for MBB to 39.0 g/pot for Belikh2 (Table 4). Although the difference between these two means is large, the genotype effect is not significant due to a wide interaction variance (Table 4). Biomass at the seedling stage is influenced by leaf area, which is related to the tillering capacity of the plant (Li *et al.*, 2023). High biomass at the beginning of the cycle indicates better water use and lower sensitivity to low temperatures (Pour-Aboughadareh *et al.*, 2019). Dry matter accumulation at early growth stages sometimes

contributes to variations in dry matter accumulated at heading and maturity stages, and ultimately to grain yield (Bouzerzour *et al.*, 2000).

Leaf specific weight varies from 17.9 mg/cm² in Beliouni to 36.5 mg/cm² in Sahell77, with significant differences among genotypes. Varieties 439/Ads-97, Belikh2, and Mrb16, known for their relative earliness, have heavier foliage than the later varieties Beliouni, Derraa, Heider, and MBB. Early varieties Waha and Cyprus have low specific leaf weights of 18.80 and 21.1 mg/cm², respectively (Table 4). A high specific leaf weight indicates better photosynthetic capacity, reduced sensitivity to photo-inhibition, improved water-use efficiency, and the consistency of the photosynthetic apparatus in a given genotype (Fang *et al.*, 2024).

Genotype effect on leaf area is not significant despite differences among genotypes, which range from 134.7 cm² for Derraa to 361.7 cm² for Semito. This lack of significance is due to high genotype x water stress interaction variance. Semito, MBB, Daki, Waha, Mrb5, and Sahell77 exhibit larger leaf areas compared to Derraa, Cyprus, and Beliouni (Table 4). A variety with a small leaf area can achieve good yield due to greater light-use efficiency per unit leaf area (Li *et al.*, 2023). Plant response to drought varies significantly according to species, growth stage, stress level, and duration (Jaleel *et al.*, 2008).

Comparing genotypic means shows no significant differences in relative water content. **Leaf turgor** varies from 68.2% in Daki to 88.7% in Mrb16, with Sahell77, Mrb5, and Semito showing the highest RWC values (Table 4). A reduction in relative water content affects nutrient uptake and leads to decreased photosynthetic activity due to stomatal closure. Bouzerzour *et al.*, (2000) noted that the ability to maintain high leaf turgor is influenced by earliness in durum wheat. Physiologically, stress causes a reduction in relative water content in leaves and a progressive decline in CO₂ uptake due to reduced stomatal conductance, which in turn

reduces chlorophyll content (Habash et al., 2014; Outoukarte et al., 2019).

Cellular integrity values range from 18.67% in Heider to 82% in Waha, indicating that Waha is particularly sensitive to heat stress. Comparisons of varietal means show that, in addition to Heider, 439/Ads-97, Derraa, MBB, and Heider/Martes/Huevos de Oro are less sensitive to heat shock. Highly sensitive varieties, in addition to Waha, include Massara and Bicre (Table 4).

Drought tolerance may also be indicated by biochemical traits. Compared to non-stressed

plants, stressed plants produce higher levels of alcohols, sugars, proline, glycine, betaine, and putrescine, and accumulate more solutes. Studies show that drought-resistant wheat cultivars exhibit higher proline content under water stress than sensitive cultivars (Slama et al., 2018). Furthermore, tolerance mechanisms and osmotic control are influenced by the accumulation of soluble carbohydrates (Hisyam et al., 2017; Bhutto et al., 2023).

Table 4. Means of the traits measured on seedlings of different durum wheat genotypes. The results are reported as mean \pm SD of 3 different measurements.

Genotype	HT	BIO	LSW	LA	RWC	CI	Pro	Sug	DR
1	7.5 \pm 0.9	19.1 \pm 1.7	30.6 \pm 5.0	234.3 \pm 5.0	78.5 \pm 0.7	25.7 \pm 0.1	119.3 \pm 8.3	330.5 \pm 7.7	1.4 \pm 0.2
2	13.3 \pm 0.2	39.8 \pm 2.4	33.5 \pm 0.6	210.0 \pm 3.2	76.7 \pm 1.2	31.3 \pm 0.1	241.4 \pm 3.2	23.0 \pm 4.5	1.3 \pm 0.02
3	12.0 \pm 0.3	27.8 \pm 1.9	26.7 \pm 3.7	215.3 \pm 3.6	74.0 \pm 1.3	53.3 \pm 1.5	125.0 \pm 6.4	602.4 \pm 6.6	1.1 \pm 0.04
4	9.9 \pm 0.5	22.2 \pm 4.7	18.8 \pm 2.8	173.5 \pm 1.2	71.5 \pm 2.8	24.3 \pm 0.6	40.0 \pm 1.2	546.6 \pm 3.5	0.9 \pm 0.05
5	10.3 \pm 0.1	22.4 \pm 2.5	24.2 \pm 5.8	264.7 \pm 4.5	81.5 \pm 3.4	34.0 \pm 3.3	81.6 \pm 3.4	131.6 \pm 4.7	0.7 \pm 0.07
6	13.8 \pm 1.5	36.2 \pm 4.0	36.2 \pm 1.2	260.3 \pm 7.1	83.0 \pm 1.4	33.7 \pm 1.1	78.6 \pm 2.6	962.1 \pm 2.8	1.9 \pm 0.12
7	13.9 \pm 0.9	26.4 \pm 1.5	31.7 \pm 6.1	224.8 \pm 1.0	88.7 \pm 3.5	42.0 \pm 3.4	65.2 \pm 0.8	60.8 \pm 0.5	0.8 \pm 0.06
8	12.4 \pm 2.3	20.6 \pm 0.4	21.1 \pm 3.4	275.5 \pm 2.9	81.3 \pm 0.7	82.0 \pm 2.0	71.3 \pm 3.4	2518.0 \pm 11.4	0.5 \pm 0.01
9	10.4 \pm 0.8	21.1 \pm 3.3	25.6 \pm 2.9	237.3 \pm 3.8	78.5 \pm 1.1	52.3 \pm 3.7	12.3 \pm 0.4	173.1 \pm 1.9	0.7 \pm 0.09
10	10.0 \pm 1.3	15.0 \pm 2.4	17.9 \pm 1.0	194.0 \pm 0.8	71.0 \pm 1.9	46.3 \pm 2.9	58.2 \pm 1.0	168.4 \pm 3.8	1.0 \pm 0.09
11	8.8 \pm 0.6	28.0 \pm 1.2	18.3 \pm 0.05	134.7 \pm 6.5	82.0 \pm 2.7	26.0 \pm 0.1	118.2 \pm 7.5	195.4 \pm 3.6	1.3 \pm 0.02
12	10.3 \pm 0.7	22.0 \pm 0.8	28.2 \pm 1.3	361.7 \pm 1.9	82.8 \pm 4.4	32.3 \pm 1.4	198.0 \pm 9.1	2715.0 \pm 10.4	1.2 \pm 0.11
13	9.4 \pm 1.8	19.4 \pm 4.3	27.3 \pm 0.04	269.3 \pm 2.5	68.2 \pm 0.3	47.0 \pm 1.8	190.0 \pm 9.7	980.0 \pm 8.7	1.0 \pm 0.06
14	7.5 \pm 0.5	26.6 \pm 1.3	22.6 \pm 1.7	251.7 \pm 3.3	80.8 \pm 3.8	18.7 \pm 0.4	49.5 \pm 2.9	545.2 \pm 4.5	0.9 \pm 0.08
15	13.1 \pm 2.7	22.9 \pm 3.7	24.1 \pm 1.3	244.7 \pm 9.1	77.3 \pm 2.9	27.3 \pm 0.2	88.5 \pm 4.3	1626.0 \pm 12.4	1.3 \pm 0.21
16	9.6 \pm 1.2	12.4 \pm 1.9	23.9 \pm 1.9	294.3 \pm 4.3	80.0 \pm 0.6	26.7 \pm 1.1	47.8 \pm 1.1	195.4 \pm 8.6	0.9 \pm 0.13
LSD 5%	2.83	5.6	21.5	151.0	4.1	4.7	2.96	144.3	0.001

1=Ads, 2=Belikh2, 3=Massara1, 4=Cyprus1, 5=Mrb5, 6=Sahell77, 7=Mrb16, 8=Waha, 9=Bicre, 10=Beliouni, 11=Derraa, 12=Semito, 13=Daki, 14=Heider, 15=Hd/Mt/HO, 16=MBB

LSD 5% Least Significant difference.

HT: Seedling height (cm); BIO: Above-ground biomass (g pot⁻¹); LSW: Leaf specific weight (mg cm⁻²); LA: Leaf area (cm²); RWC: Relative water content (%); CI: Cellular integrity (%); Pro: Proline content (μ g g⁻¹ of fresh weight); Sug: Soluble sugars (μ g g⁻¹ of fresh weight) DR: Drying rate of the flag leaf (mg H₂O min⁻¹)

Proline content ranges from 12.3 μ g g⁻¹ of fresh matter in Bicre to 214.3 μ g/g in Belikh2, indicating varying proline accumulation capacities among genotypes. Similarly, flag leaf drying rate varies from 0.7 mg H₂O min⁻¹ in Mrb5 to 1.90 mg H₂O min⁻¹ in Sahell77 (Table 4). Understanding the relationship between water and biochemical

changes in response to drought can contribute to wheat tolerance, which is essential for the stability of future yields (Akter et al., 2023). These findings align with those of Saghour El Idrissi et al., (2023), who reported that water stress significantly impacts stomatal conductance, relative water content, leaf

area, temperature, SPAD values, proline, soluble sugars, glycine betaine, and yield traits.

Soluble sugar content varies by variety, ranging from 23.0 $\mu\text{g g}^{-1}$ of fresh matter in Belikh2 to 2715 $\mu\text{g g}^{-1}$ in Semito. Numerous researchers have shown that varieties capable of maintaining high water content accumulate more soluble sugars, enabling them to resist stress for longer (Slama *et al.*, 2018). According to Chaib *et al.*, (2015), increased soluble sugar content protects membranes from drying. Certain sugars, such as trehalose, are known to bind to membrane lipids and stabilize membrane structure (Mensink *et al.*, 2017).

Average leaf drying rates range from 0.5 mg water min^{-1} in Waha to 1.4 mg water min^{-1} in 439/Ads-97 (Table 4). According to Seleiman *et al.*, (2021), varieties that are more resistant to water stress are characterized by a lower drying rate per unit area compared to relatively sensitive varieties. Water-stress-resistant genotypes retained 43.36% more water in their leaves under well-watered conditions than under limited water conditions, while drought-sensitive genotypes retained only 15.69% more water (Roy *et al.*, 2024).

Table 5. Average effect of water stress on measured traits in durum wheat seedlings.

Treatment	HT	BIO	LSW	LA	RWC	CI	Pro	Sug	DR
NS	12.0	32.4	30.7	289.9	83.7	37.7	54.4	411.5	1.4
S	9.5	15.3	20.7	190.9	73.3	37.7	143.8	1002.0	0.5
S – NS	-2.5	-17.1	-10.0	-99.0	-10.4	--	+89.4	+590.5	-0.9
(S-NS) /NS (%)	-20.8	-52.8	-32.6	-34.2	-12.4	--	+164.3	+143.0	-64.3
LSD _{5%}	0.86	10.2	4.91	236.0	8.54	--	1.05	1.88	0.03

LSD _{5%} Least Significant difference.

NS: Non-stressed treatment

S: Stressed treatment

HT: Seedling height (cm); BIO: Above-ground biomass (g pot^{-1}); LSW: Leaf specific weight (mg cm^{-2}); LA: Leaf area (cm^2); RWC: Relative water content (%); CI: Cellular integrity (%); Pro: Proline content ($\mu\text{g g}^{-1}$ of fresh weight); Sug: Soluble sugars ($\mu\text{g g}^{-1}$ of fresh weight) DR: Drying rate of the flag leaf ($\text{mg H}_2\text{O min}^{-1}$).

Genotype x Water Stress Interaction

A significant genotype x stress interaction indicates differential genotype responses to water stress. The means of the non-stressed and stressed treatments are provided in Table 6.

Varieties such as Mrb16, Waha, Daki, and Sahell77 were able to minimize the reduction in their relative

Effect of water stress on measured traits

The imposed water stress had varying effects on the different measured traits. Significant differences were observed between the means of non-stressed and stressed genotypes (Table 5). These variations, expressed relative to the means of the non-stressed treatment, ranged from -12.4% for relative water content to -52.7% for above-ground biomass (Table 5).

These results indicate that dry matter accumulation, followed by leaf area and specific leaf weight, are the traits most sensitive to the applied water stress. Relative water content is the least sensitive trait, at least for the level of stress applied in this experiment. Proline and sugars increased by 164.3% and 150.9%, respectively, relative to the control, while the drying rate of the flag leaf decreased by 64.5%.

The current findings are in accordance with those of Nezhadahmadi *et al.* (2013), who observed that the adverse effects of water stress on wheat plants led to significant reductions in their morphological traits and productivity.

water content under water stress compared to the non-stressed condition. Among these, Mrb16/Ente/Mario displayed high relative water content under non-stressful conditions, while Waha and Sahell77 had high relative water content under water stress. In contrast, genotypes 439/Ads-97, Derraa, Mrb5, and Massara1 appeared most affected by water stress, with a considerable reduction in relative water content. Among these,

Mrb5 and Derraa exhibited high relative water content under non-stress conditions (Table 6). Boutraa et al. (2010) reported that the continued growth of durum wheat despite reduced soil moisture is explained by the osmoregulation ability of the plant, which helps maintain the leaf turgor necessary for cell elongation. Variations in RWC are considered a useful indicator of plant water balance and sensitivity to stress (Ru et al., 2020; Khorsandi et al., 2018).

Genotypes with high leaf area under non-stressed conditions include Semito, Waha, and Mrb5. Under water deficit, Semito, MBB, and Daki exhibit the best leaf area. MBB, Heider/Martes/Huevos de Oro, Daki, and Semito minimize leaf area reduction due to water stress, while varieties such as Beliouni, Bicre, Mrb16/Ente//Mario, and Mrb5 show large reductions in leaf area (Table 6). Leaf area reduction can be beneficial as it decreases evaporative surface and sun exposure.

The quantity of carbon fixed through photosynthesis and the amount of water used in transpiration are both determined by leaf area (Dwivedi et al., 2017). Saghour El Idrissi et al., (2023) reported that water stress led to a significant reduction in leaf area (LA) in several examined lines, with the extent of reduction correlated with stress level.

The accumulation of above-ground dry matter reflects the productive capacity of a variety. Varieties with better performance under non-stress conditions are Belikh2 and Derraa, while Sahell77 and Heider/Martes//Huevos de Oro showed the highest performance under water stress (Table 6). Varieties that minimize dry matter reduction under water stress include Sahell77, Beliouni, and Heider/Martes//Huevos de Oro, whereas Cyprus1, Belikh2, 439/Ads-97, Derraa, and Mrb5 show considerable reductions. According to Ding et al., (2018), stomatal conductance was 28% lower in water deficit conditions compared to the control, while transpiration rate decreased by 34%.

Heider/Martes//Huevos de Oro is the only variety that minimizes the reduction in all three traits under water stress. Daki, Semito, and MBB possess the

ability to minimize the reduction in both relative water content and leaf area under water stress, whereas Sahell77 and Beliouni minimize reductions in relative water content and above-ground biomass simultaneously. According to Habash et al. (2014), greater accumulated dry matter in foliage can be advantageous for capturing light energy. However, excess foliage may lead to water waste due to increased leaf area during periods of high-water demand.

Leaf specific weight is an indicator of photosynthetic apparatus consistency. Varieties that produce denser foliage under non-stressed conditions include Belikh, Sahell77, and 439/Ads-97. Under water stress, varieties with high leaf specific weight include Sahell77, Bicre, and Daki (Table 6). Daki, Bicre, Hd/Mt//Ho, and Mrb5 demonstrate the ability to minimize reductions in specific leaf weight under water stress. In contrast, Cyprus1, Belikh2, Beliouni 3852, and 439/Ads-97 significantly reduce their specific leaf weight under water stress. According to Ali et al. (2023), reduced water loss through morphological changes in foliage, such as reduced leaf elongation, early leaf senescence, and leaf rolling, contributes to water stress avoidance. These adaptations affect specific leaf weight.

Tall, erect varieties include Sahelle77 and Hd/Mt//Ho under non-stressed conditions, while under water stress, Sahell77 and Mrb16/Ente//Mario are the tallest (Table 6). Mrb16/Ente//Mario, Cyprus, Bicre, and MBB minimize height reduction under water stress.

The highest proline increases under water stress were recorded in Massara1, Cyprus1, Mrb5, Waha, Derraa, and Daki, with increases over four times that under non-stress conditions. Increased proline inhibits protein synthesis or enhances protein degradation due to reduced transport to other plant organs or increased synthesis of glutamate proteins (Wu et al., 2022).

Table 6. Mean values of traits measured on non-stressed (NS) durum wheat seedlings and relative changes under stress (S).

G	HT		BIO		LSW		LA		RWC		Pro		Sug		DR									
	NS	S	R %	NS	S	R %	NS	S	R %	NS	S	R %	NS	S	R %	S								
1	8.6±0.4	6.2±0.0	-28	28.7±1.3	9.5±0.0	-67	41.6±0.7	19.6±0.7	-53	299±6.2	170±1.1	-43	85±3.0	72±1.5	-15	92.9±1.7	146±6.1	57	234±8.3	661±9.3	183	2.1±0.4	0.75±0.1	-64
2	14.7±0.1	11.8±0.5	-20	60.5±0.4	19.1±0.3	-68	44.7±0.1	22.3±1.6	-50	265±6.3	155±6.5	-42	79±1.3	74.3±1.4	-6	172±3.3	311±3.3	81	9.4±0.2	36.6±7.3	288	2.3±0.5	0.16±0.1	-93
3	13.3±0.1	10.6±0.2	-20	38.8±0.1	16.7±0.5	-57	32.7±0.3	20.6±2.7	-37	247±8.8	184±8.9	-26	84.3±4.0	63.7±0.1	-25	46.9±1.2	203±0.2	333	238±5.4	967±5.2	307	2±0.1	0.16±0.0	-92
4	10.2±0.2	9.6±0.1	-6	38.2±0.2	6.1±0.1	-84	24.8±2.3	12.8±0.6	-49	200±5.9	147±3.1	-26	73.7±2.9	69.3±0.8	-6	12.1±0.7	67.7±0.6	458	81.2±1.5	130±4.1	60	1±0.1	0.16±0.0	-84
5	13.3±0.3	7.3±0.1	-45	38.3±1.0	6.4±0.7	-83	25.5±1.3	22.9±0.1	-10	351±9.4	177±5.2	-49	91.3±1.8	71.7±1.4	-26	27.8±0.5	136±6.6	387	67.8±1.7	195±5.8	188	1.1±0.2	0.25±0.3	-77
6	15.5±0.4	12.1±0.4	-22	38.3±1.2	34.1±0.2	-11	44.3±1.3	28.1±1.8	-37	330±3.7	190±8.8	-42	85±1.6	81±0.3	-5	42.4±0.7	115±0.7	170	95.5±1.7	1342±8.3	1305	2±0.4	0.16±0.1	-92
7	13.8±0.3	13.3±0.5	-4	35.9±2.3	16.9±0.1	-53	38.7±2.3	24.8±0.4	-36	311±8.1	138±5.7	-55	89.2±1.7	88±2.7	-1	38.2±0.3	92.1±0.3	141	22.8±0.1	98.8±1.6	333	1.3±0.3	0.33±0.1	-74
8	13.8±0.1	11±0.2	-20	23.9±1.0	17.2±0.3	-28	23.9±0.1	18.3±1.6	-23	359±3.9	192±2.8	-47	82.7±1.5	80±1.2	-3	26.1±0.9	116±8.5	346	1806±9.8	3232±9.0	79	0.8±0.1	0.25±0.1	-69
9	10.7±0.2	10.2±0.1	-5	25.5±0.1	16.7±1.3	-35	26.1±0.4	25.1±2.3	-4	333±8.2	141±8.3	-58	83.3±1.4	73.7±4.1	-12	6.9±0.3	17.4±0.5	152	128±2.8	219±7.4	71	0.9±0.0	0.5±0.1	-45
10	11.3±0.0	8.8±0.3	-22	16±0.1	13.9±0.1	-13	26.1±0.5	9.7±0.2	-63	314±7.8	74±4.4	-76	92±1.1	80±2.0	-13	40±1.6	76.4±0.1	91	90.1±1.3	247±2.4	174	1.8±0.1	0.25±0.0	-86
11	10.5±0.1	7±0.3	-33	43.2±4.3	12.7±0.2	-71	22.3±1.3	14.3±1.3	-36	158±2.9	111±3.6	-30	89.3±0.8	74.7±1.2	-16	41.7±0.1	195±3.0	367	104±2.1	287±5.0	176	2.1±0.5	0.41±0.3	-80
12	12.6±0.1	8.1±0.1	-36	26.8±1.3	17.2±0.0	-36	31.5±0.8	24.9±0.5	-21	386±9.4	338±4.3	-12	85.3±2.0	80.3±1.4	-6	106±0.2	290±9.3	174	1623±8.6	3814±5.1	135	1.9±0.3	0.41±0.0	-79
13	10.3±0.2	8.5±0.2	-17	25.5±1.3	13.3±0.8	-48	28.7±0.9	25.8±0.2	-10	304±8.2	286±9.1	-6	69.3±0.8	67±0.7	-3	67.2±1.9	313±3.7	365	324±2.7	1637±6.6	406	1.6±0.1	1.08±0.3	-33
14	8.8±0.1	6.2±0.2	-30	34±2.3	19.1±1.3	-44	26.5±2.1	18.7±0.1	-30	295±1.9	208±5.2	-30	83.3±1.4	78.3±0.9	-6	41.8±0.4	57.3±0.5	37	524±4.2	765±7.2	46	1.6±0.0	0.16±0.1	-90
15	14.8±0.4	11.3±0.1	-24	25.4±2.3	20.4±1.1	-20	24.7±0.6	23.4±1.8	-5	250±2.6	240±1.8	-4	83±1.4	71.7±3.1	-14	79.8±1.5	97.3±0.1	22	1134±2.2	2121±2.9	87	2.2±0.5	0.33±0.2	-85
16	9.8±0.2	9.5±0.2	-3	18.9±1.3	5.9±0.2	-69	28.1±1.3	19.7±2.7	-30	30±0.6	289±5.5	-4	83.3±1.3	77±1.4	-7	27.6±0.2	67.3±0.2	144	113±2.1	278±2.4	146	1.5±0.2	0.41±0.1	-73
LSD _{5%}	1.81		5.36		3.5		43.8		4.25		4.18		7.5		0.01									

1=Ads, 2=Belikh2, 3=Massara1, 4=Cyprus1, 5=Mrb5, 6=Sahell77, 7=Mrb16, 8=Waha, 9=Biere, 10=Belioumi, 11=Derraa, 12=Semito, 13=Daki, 14=Heider, 15=Hd/Mi/HO, 16=Mbb

R%=[(ST-NS)/NS].100

LSD_{5%} Least Significant difference.

NS: Non-stressed treatment,

S: Stressed treatment

Soluble sugar accumulation varies among varieties. Genotypes Daki, Sahell77, Massara1, and Mrb16/Ente/Mario show significant accumulation under water stress, nearly four times higher than that observed in control plants (Table 6). The increase in soluble sugars is due to enhanced catabolism of insoluble sugars (mainly starch) through amylase activity in stressed plants, or reduced sugar transport from leaves to other organs, or growth reduction due to stress (Rosa et al., 2009). Sucrose and polyfructose play an important role in drought adaptation, as sucrose preserves membrane phospholipids and protects soluble protein structure (Kerpesi and Galiba, 2000). Polyfructose contributes to water stress tolerance by reducing cellular osmotic potential, thus maintaining turgor (Yang et al., 2021).

For the flag **leaf drying rate**, reductions ranged from -33% in Daki to -93% in Belikh2. Varieties adapted to arid regions are characterized by lower drying rates compared to those adapted to more favorable environments (Manga et al., 2015). Anatomical changes in leaves, such as reduced

elongation, wilting, premature death, and rolling, contribute to the plant's ability to avoid water stress (Araus et al., 1998).

Varietal Characterization

Relationships were studied using principal component analysis (PCA), separately for the control and stressed treatments, with average field yield included for each variety.

In the control PCA, Axis 1 integrates information from physiological parameters such as proline, flag leaf drying rate, and leaf specific weight, as well as yield, sugars, and cellular integrity. These two sets of variables are opposed along Axis 1. Axis 2 integrates information on seedling above-ground biomass and relative water content (Figure 1a). The distribution of genotypes on the plane formed by Axes 1 and 2 indicates that, along Axis 1, genotypes Waha and Bicre (negatively correlated) are opposed to genotypes Belikh and Semito (positively correlated) (Figure 1b).

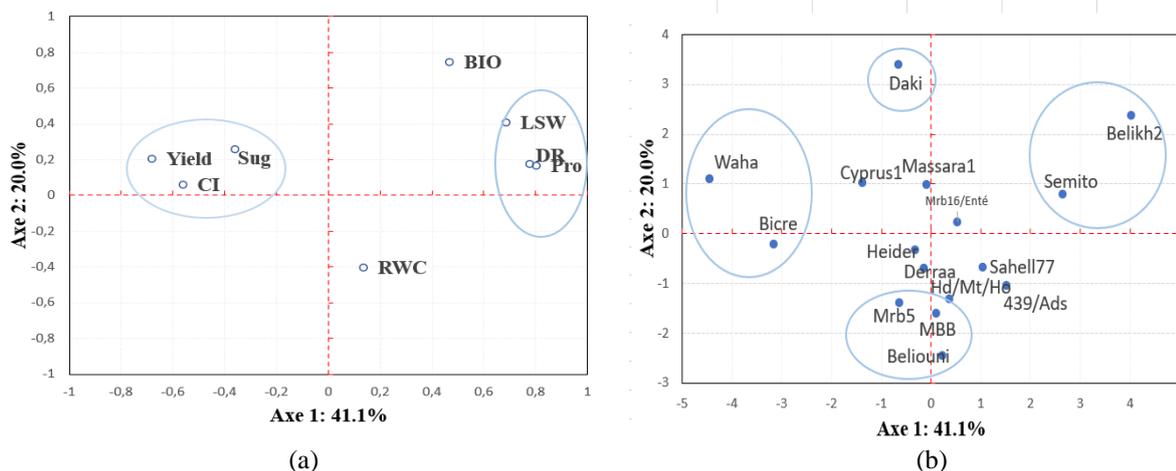


Fig. 1. Principal component analysis of the control treatment.

(a): Correlation circle of variables measured for the control treatment. (BIO). leaf specific weight (LSW). leaf area (LA). relative water content (RWC). proline content (Pro). soluble sugars (Sug). and the drying rate of the flag leaf (DR). CI: Cellular integrity (%).

(b): Projection of experimental points according to representation of genotypes for the control treatment. Ads. Belikh2. Massara1. Cyprus1. Mrb5. Sahell77. Mrb16. Waha. Bicre. Beliouni. Derraa. Semito. Daki. Heider. Hd/Mt/HO. MBB.

Waha and Bicre are distinguished by high mean values for yield, soluble sugars, and cellular integrity, and low mean values for proline, flag leaf drying rate, and specific leaf weight. Conversely, Semito and Belikh2 show high mean values for proline, flag leaf drying rate, and specific leaf weight. Along Axis 2, genotype Daki stands out for

high biomass, while varieties Mrb5, MBB, and Beliouni retain more water in their leaves.

Genotypes with low yield performance also accumulate more proline, dry out faster, have higher specific weight, and show greater thermal stress tolerance as measured by cellular integrity.

For the stressed treatment, Axis 1 captures information related to proline accumulation, yield performance, and cellular integrity, while Axis 2 incorporates data on leaf turgor, above-ground biomass, soluble sugars, flag leaf drying rate, and leaf specific weight (Figure 2a).

In the PCA, genotypes Waha and Bicre are positively associated with Axis 1, while Semito and Belikh2 are negatively associated. Along Axis 2, genotypes Sahell77 and Mb16/Ente/Mario contrast with Beliouni, Cyprus1, and MBB (Figure 2b)

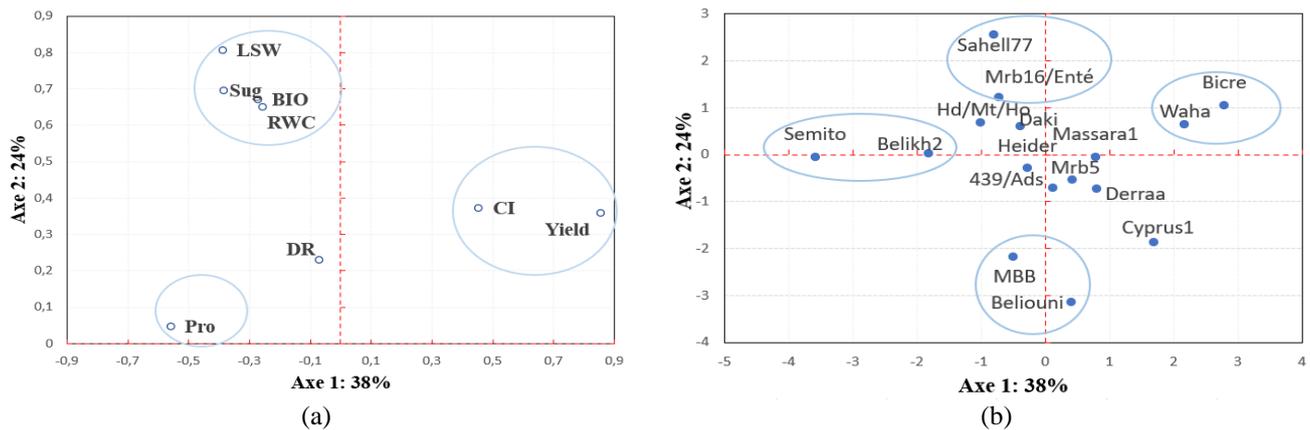


Fig. 2. Principal component analysis of the stressed treatment.

(a): Correlation circle of variables measured for the stressed treatment. (BIO). leaf specific weight (LSW). leaf area (LA). relative water content (RWC). proline content (Pro). soluble sugars (Sug). and the drying rate of the flag leaf (DR). CI: Cellular integrity (%).

(b): Projection of experimental points according to representation of genotypes for the stressed treatment. Ads. Belikh2. Massara1. Cyprus1. Mrb5. Sahell77. Mrb16. Waha. Bicre. Beliouni. Derraa. Semito. Daki. Heider. Hd/Mt/HO. MBB.

Under water stress, proline remains the only discriminating parameter between genotypes that are tolerant and those that lack this ability. The proline test does not provide any indication of grain yield performance by environment. The results of this study differ from those of Saghour el Idrissi et al. (2023), who concluded that most physiological and biochemical measurements are significantly correlated with yield parameters, based on the average correlation coefficients between physiological, biochemical, and yield parameters studied under both water-stressed and non-stressed conditions.

Conclusion

The results indicate significant differences among genotypes for height, specific leaf weight, and cellular integrity, and no significant differences for above-ground biomass, leaf area, and relative water content, as measured at the seedling stage. The effect of water stress was significant to highly

Consent for publication:

All authors declare their consent for publication.

Author contribution:

The manuscript was edited and revised by all authors.

Conflicts of Interest:

The author declares no conflict of interest.

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significant for all measured variables, while the genotype x stress interaction was not significant for the flag leaf drying rate. The findings suggest that dry matter accumulation, leaf area, and specific leaf weight are the traits most sensitive to the applied water stress. Proline content increased by 164.3% relative to the control, while the drying rate of the flag leaf decreased by 64.3%. Varieties responded differently to water stress: Sahell77, Beliouni, and Heider/Martes//Huevos de Oro exhibited the smallest reductions in dry matter production under stress, whereas Cyprus1, Belikh2, 439/Ads-97, Derraa, and Mrb5 experienced substantial reductions. Results also show that in the control treatment, biomass was higher as plant height and specific leaf weight increased, and higher biomass led to a greater drying rate. Genotypes with high specific leaf weight accumulated more proline and sugar and dried out more quickly. Average grain yield was negatively correlated with proline and the drying rate of the flag leaf. Under water stress, genotypes with high specific leaf weight better retained their relative water content.

From a breeding perspective, effectively using this early test requires first enhancing grain yield performance. Following this, genotypes with comparable yield capacities should be selected. Finally, the proline test can be applied to identify those genotypes capable of minimizing yield performance variability across years or environmental conditions.

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