
Research Article

Morphological, physiological and anatomical traits in durum wheat (*Triticum durum* Desf.) as affected by semi-arid conditions

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Article history:

Submitted 16 March 2024

Accepted 04 May 2024

Published 25 June 2024

Keywords:

Flag leaf

Morpho-physiological

Semi-arid

Water deficit

Wheat

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Abstract

Durum wheat (*Triticum durum* Desf.) is one of Algeria's primary cereal crops. Although the areas reserved for this species are estimated at approximately 40%, the production remains low to meet national grain requirements. This shortfall is primarily due to the impact of biotic and abiotic stresses, particularly drought, which significantly limit wheat yields. For that, this study assessed the behavior of five genotypes of durum wheat (Waha, ACSAD 1351, Vitron, Oued Zenati and Langlois) under water stress conditions. The experiment was carried out in Tiaret (Western Algeria). The studied parameters are morpho-physiological and anatomical of the flag leaf. The obtained results showed that the water deficit greatly affected the dimensions of the plant and triggered a decrease in the water content. Structural modifications due to cellular resizing of the structural constituents of the last leaf, reflected by variations in the rate of the outer epidermal wall thickness and reductions of leaf mesophiles. These modifications remain effective in maintaining the hydration of the aerial part by limiting water loss and increasing the hydraulic resistance of the leaves. According to this study, it seems that an inter-varietal difference has been highlighted. However, a genetic cross is recommended to combine the maximum number of resistant genes into a single variety. This strategy is considered as an effective solution for mitigating the effects of abiotic stresses, especially in semi-arid regions.

How to cite:

Arous, A., Zemour, K., Mehdeb, D., Labdelli, A., Zemour, H., Chouhim, K. M. A., Adda, A., & Belkhouja, M. (2024). Morphological, physiological and anatomical traits in durum wheat (*Triticum durum* Desf.) as affected by semi-arid conditions. *Journal of Agriculture and Applied Biology*, 5(1): 97 - 109. doi: 10.11594/jaab.05.01.08

1. Introduction

Globally, water deficit is the major limiting factor in agricultural production (Zemour et al., 2021; Zeng et al., 2023). This abiotic stress has led to major losses in production in many regions (Godoy et al., 2021). Thirty-five per cent of the world's farmland is classified as arid or semi-arid. This situation characterises the Mediterranean area, which is defined by spatial and temporal irregularities in the declaration of this constraint. Among the crops cultivated in the world, durum wheat is considered the most important plant in the diet and is particularly affected by drought in Algeria (Boudiar et al., 2019), Tunisia (Chaouachi et al., 2023) and Egypt (Mansour et al., 2020).

The durum wheat sensitivity to water deficit is particularly important during the grain filling period, as this reproductive phase is highly vulnerable to the water state of the plant (Mäkinen et al., 2018). Consequently, the water deficit limits the grain filling phase and eventually the final yield (Fuentelba-Sandoval et al., 2022). This phase usually occurs under high temperatures associated with drought effect (Qi et al., 2022; Zemour et al., 2019). The weight and quality of the grain are the main reference characteristics for the assessment of yield (Oustani et al., 2023) and therefore productivity in the different cereal species. The grain filling process in durum wheat depends on the supply of carbonaceous products from two main sources, the translocation of reserves stored in the different parts of the plant and the current elaboration of photoassimilates by the different organs (Shewry et al., 2012; Joshi et al., 2022). The involvement of these organs is conditioned by their position in relation to the developing grain as well as their longevity. The translocation of the assimilates, mainly fructose, during the post-anthesis period, takes place from the accumulated reserves in the stem (Arous et al., 2020). The contribution of the flag leaf is essential in determining of grain yield during grain filling (Lou et al., 2021; Ma et al., 2021; Mickky et al., 2020). The physiological and micro-morphological activity of the leaf, in particular photosynthesis and transpiration, is affected by severe water deficit (Wang et al., 2018; Urban et al., 2017). The reduction of photosynthesis, linked to the reduction of leaf water potential, is expressed by the closure of the stomata and the reduction of the supply of CO₂ (Sakoda et al., 2021; Canavar, 2015; Martin-StPaul, et al., 2017; Seleiman et al., 2021).

The water stress results in a series of changes that influence growth, morphological and physiological characters of crops (Tuan et al., 2019; Culman et al., 2019). For that, the objective of this study was to evaluate these characteristics in durum wheat grown in the semi-arid area in Algeria.

2. Materials and methods

2.1 Experimental conditions and plant material

The experiment is carried out in the open-field at the faculty of nature and life sciences of IBN Khaldoun University of Tiaret in Northwestern Algeria (E°1°33'53", N°34°04' 11"). The climatic conditions are presented in Figure 1).

The cumulative rainfall for the 2014-2015 campaign is around 445 mm and is characterized by a heterogeneous distribution between months, with peaks on December (95 mm) and February (121 mm). The annual average temperature is 14.7°C, with the highest monthly mean temperature of 4.91°C occurring in February and the lowest monthly mean temperature of 21.07°C recorded in June (Figure 1). This climatic situation pushes the crops to complete rapidly their development cycle.

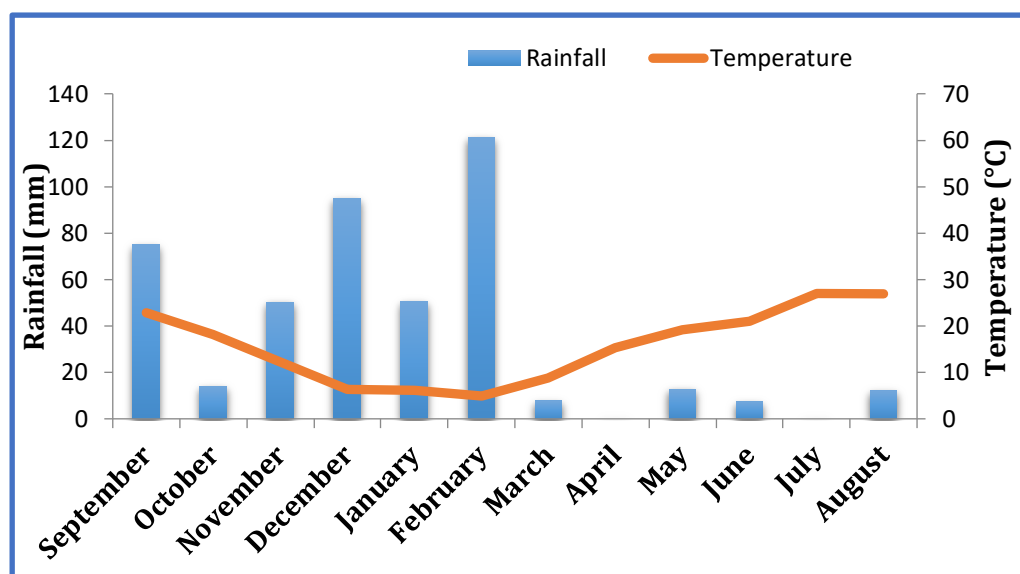


Figure 1. Climatic conditions prevailing in the study area in Tiaret (2014-2015)

The used plant material consists of five genotypes of durum (Table 1), provided by the Technical Institute of Field Crops of Tiaret (ITGC). The adopted device includes two treatments, one irrigated and the other under rainy conditions. From stem elongation stage, irrigation has been applied until the soil moisture reaches a sufficient capacity in the field. The basic plots are 1.2 m wide (6 rows with an inter-row space of 20 cm) and 5 m long, i.e. a plot area of 6 m². The genotypes are set up in a complete randomized block experimental design with three replicates.

Table 1. Characteristics of the studied genotypes

Genotype	Type	Origin	Beard Color
Waha (CHAMI)	ICARDA	ICARDA (Syria)	Pale Brown
ACSAD1351	ACSAD	ACSAD (Syria)	Whitish
Vitron (T)	CIMMYT	IAO (Italy)	Whitish
Oued Zenati	Variety (local population)	Algeria	Black
Langlois	Variety (local population)	Algeria	Brown

2.2 Measured parameters

Relative water content (RWC)

The relative water content expressed by the RWC (Relative Water Content) is determined according to the Ladiges method (Ladiges, 1975; Clarke et Mc, 1982; Raissac, 1992). This content is deduced by the following formula:

$$\text{RWC \%} = (\text{Pi} - \text{Ps}) / (\text{PPt} - \text{Ps}) \times 100$$

Pi: initial fresh weight

PPt: weight at full turgor

Ps: dry weight

Rate Water loss from excised leaves (RWL)

The rate water loss (RWL) from excised leaves is estimated using the method of Horne et Kahn (2000). This parameter is determined after 20 and 60mn by the following formula:

$$\text{RWL t (mg.cm-2.mn-1)} = (\text{Pi} - \text{Pt} / \text{Ps}) (1 / \text{LA} * \text{t})$$

Pi: Initial weight

Pt: weighing time (20 and 60 min)

Ps: dry weight

LA: leaf area

Biometric measurements

Using a tape, the following measurements are taken:

- ❖ The limb area, obtained by using MESERUM Pro software. version 3.4
- ❖ The length of the flag leaf sheath.
- ❖ The diameter of last leaf sheath
- ❖ The diameter estimation is carried out using a caliper.

2.3 Histological sections

Firstly, a sample of 1cm long is taken from the middle part of the last leaf for each genotype, and immediately soaked in fixative solution (ethanol 17%, formalin 3% and acetic acid 1%) for 12 h. Next, the samples are washed in running water for the same time as the fixation and dehydrated by passage through ethanol solutions of increasing concentration (50%, 70%, 90%, 100% and 100%). Then, the samples are impregnated and embedded in molten paraffin (at 56°C) and cut using a microtome (LEIKA RM2145) at a thickness of 7µm. After removing the paraffin with toluene, the sections are washed and dehydrated with ethanol and colored with alum carmine and methyl green. Finally, the measurements were performed using a ZEISS brand microscope equipped with an ocular micrometer. The observations focused on the thickness of leaf mesophiles and of outer epidermal walls.

2.4. Statistical analysis

Datas were subjected to statistical analysis of variance (ANOVA) at 5 % threshold using Statistica 8.0. Two-way ANOVA (Factorial Experiment Based on RCBD) were used in order to determine the effect of genotype, water regime, and their interaction.

3. Result and discussion

3.1. Limb area of the flag leaf

The obtained results (Table 2) showed that the variations of the limb area vary significantly through the used genotypes and the applied water regime ($p < 0.001$). There is a clear reduction in the leaf area by the water regime ($p < 0.001$).

Table 2. Effect of genotype and water treatment on the Leaf limb area, Sheath diameter and Sheath length in durum wheat under irrigated and rainfed conditions in Tiaret (2014-2015)

Trait	Leaf limb area (cm ²)	Sheath diameter (cm)	Sheath length (cm)
Genotypes	86.1***	4.17**	96.98***
Water regime	172.7***	31.9***	3.99 ns
Genotypes*Water regime	40.95***	2,3ns	0,06 ns

** significant at $p < 0.01$; *** significant at $p < 0.001$. ns: not significant at $p > 0.05$

In irrigated regime, the average value of the leaf area is estimated at 91.53 cm² (Figure 2). Indeed, Oued Zenati genotype is distinguished by the highest value (128.49 cm²) compared to the lowest area recorded by ACSAD1351 genotype (57.40 cm²). Also, the results indicated that the water deficit decreased the leaf area with average rate of 43%.

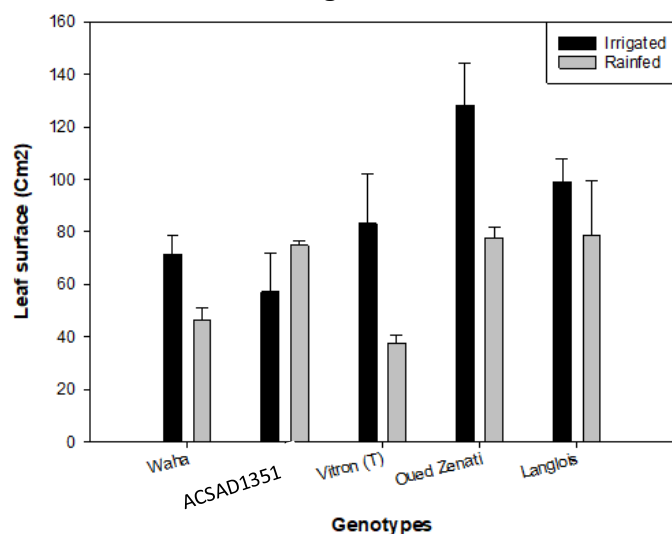


Figure 2. The limb area of the flag leaf (cm²) in the five durum wheat genotypes grown under irrigated and rainfed conditions. Data are the means of three replicates harvested at the mature stage of the plants.

Among the tested genotype, VITRON proves to be the most sensitive to water deficit (-55%). The involvement of flag leaf characteristics in the development of grain in durum wheat has been proven by previous studies (Sanchez-Bragado et al., 2014). Thus, during grain filling, the photosynthetic activity of the last leaf ensures the availability of photoassimilates directly to this organ. The retained parameters of flag leaf relate to the limb area, the diameter and the length of the sheath. The decrease in leaf dimensions follows a decrease in light energy amount leading to a reduction in photosynthetic activity amount and therefore the diminution of the final grain yield. Leaf limb area determines the drought resistance and is involved in determining final grain weight, as high leaf area will lose more water than low leaf area (Belkharchouche et al., 2020).

In this study, the leaf area of Oued Zenati genotype is characterized by the highest leaf limb area. Then, it presents the genotype which loses more water consequently. The decrease of leaf elongation leads to opening reduction or even closing of the stomata (Prasad et al., 2008; Abdelhakim et al., 2022). Indeed, under water stress the reduction of leaf area is a strategy developed by plants to reduce their water losses (Seleiman et al., 2021). The flag leaf limb area showed a positive correlation with the rate of water content ($r^2=0.6$). This relation suggests that large-sized leaves lose less water per unit leaf area by transpiration. A larger flag leaf area captures more light and fixes more carbohydrates which are favorable for high yield. Reducing water loss by closing stomata is one way plants adapt to drought (Chen et al., 2022).

Length and diameter of the flag leaf sheath

The results (Table 2) showed that the sheath length of the last leaf is variable according to the tested genotypes ($p<0.001$), while the practiced irrigation had no effect on this parameter ($p>0.05$). The obtained results (Figure 3) indicated that the application of the water deficit affected all the sheath length of genotypes. Under an irrigated regime, the average sheath length is 19.49cm. Thus, the values of this length oscillated between 15.52cm (ACSAD1351) and 25.02cm (Oued Zenati). However, the application of water deficit caused a slight reduction in all the tested

genotypes. Therefore, the values of sheath lengths are between 24.36cm (Oued Zenati) and 14.95cm (ACSAD1351).

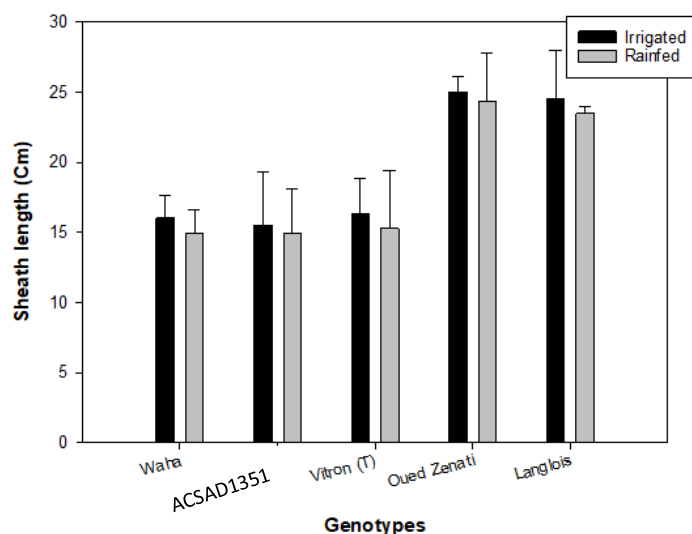


Figure 3. Sheath length (cm) in the five durum wheat genotypes grown under irrigated and rainfed conditions. Data are the means of three replicates harvested at the mature stage of the plants

The analysis of the results show that the sheath length is positively correlated with the limb surface, thus indicating that the leaves with the largest surfaces also record the longest sheaths ($r^2 = 0.711^*$). Whereas, the sheath diameter is negatively correlated with limb surface of the last leaf ($r = -0.437$).

The diameter of the flag leaf sheath (Table 2) is conditioned by the applied water regime and the nature of the tested genotypes ($p < 0.001$). The obtained average results (Figure 4) in rainfed regime illustrated that Oued Zenati genotype has the largest diameter (2.12 cm), while the smallest diameter is presented by ACSAD1351 genotype (1.78 cm).

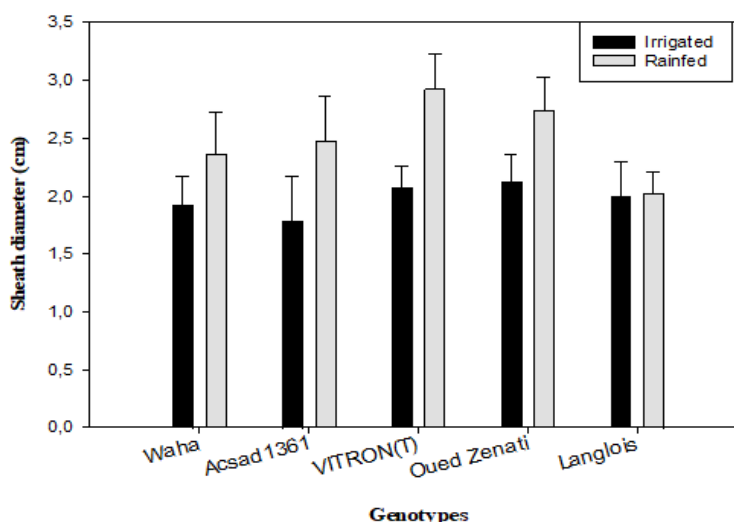


Figure 4. Sheath diameter of the Flag leaf (cm) in the five durum wheat genotypes grown under irrigated and rainfed conditions. Data are the means of three replicates harvested at the mature stage of the plants

Medyouni et al. (2021) and Prasad et al. (2008) demonstrated that leaf growth is inhibited as soon as the plant is subjected to water deficit. It results in a decrease of the amount of absorbed light energy leading to a reduction in the amount of accumulated aerial biomass. These results confirm the role played by this organ in ensuring the availability of photoassimilates under severe conditions. Indeed, it has been reported that water stress causes a multitude effects on the functioning of the plant, of which the reduction in cell elongation and division are the first symptoms, leading to the decrease of photosynthetic leaf area (Wu et al. 2022). Water deficit accelerates leaf senescence, which promotes the translocation of carbon and nitrogen reserves from the leaves to the grain, to the detriment of photosynthesis activity (Amigues et al., 2006).

Relative water content (RWC %)

The analysis of variance showed that the water deficit reduces significantly the levels of RWC (Table 3).

Table 3. Effect of genotype and water treatment on the physiological and anatomical parameters of the flag leaf in durum wheat under irrigated and rainfed conditions in Tiaret (2014-2015)

Trait	Genotypes	Water regime	Genotypes *Water regime
RWC (%)	0.67ns	104.9***	5.15ns
RWL (mg.cm-2.mn-1) (20mn)	1.8ns	1,03ns	2.9*
RWL (mg.cm-2.mn-1) (60mn)	7.4***	1.2ns	0.6ns
Leaf mesophylic thickness (μm)	19.9***	58.9***	2.4ns
Outer epidermal walls thickness (μm)	124.7***	317.2***	24.56***

*significant at $p < 0.05$, *** significant at $p < 0.001$. ns: Not significant at $p > 0.05$

The all genotypes grown under rainfed conditions are the most sensitive (Figure 5). Indeed, ACSAD1351 revealed the highest rate (68.44%). While, Oued Zenati genotype recorded the lowest value of RWC (55.70%). However, under irrigation supply, the highest RWC is noted by Oued Zenati genotype (90.04%). In the same conditions, the lowest RWC value (75.58%) is recorded by Waha genotype.

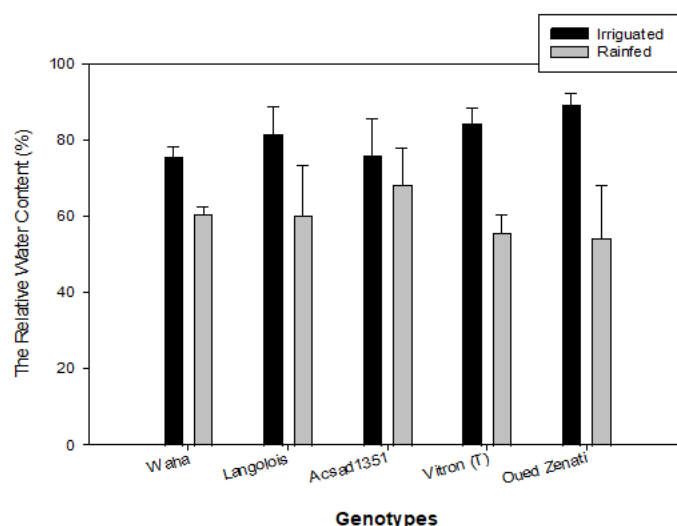


Figure 5. Relative water content (%) in the five durum wheat genotypes grown under irrigated and rainfed conditions. Data are the means of three replicates harvested at the mature stage of the plants

The obtained results showed that the water deficit leads to different impacts on the studied parameters. Relative water content (RWC) is an important trait that relates to drought stress. RWC has been proposed as a more important indicator of water status under drought conditions (Ogbaga et al., 2020). Several researchers have shown that RWC is the most commonly used physiological method to assess the susceptibility of plant tissues and the tolerance of cells to dehydration due to drought (Boyer et al., 2008; Shivakrishna et al., 2018). Our data revealed that water stress significantly reduced RWC. Results confirmed by Mehere et al. (2018) and Siddique et al. (2000). Several researchers have also documented that drought-resistant genotypes retain more water in their leaves than sensitive genotypes (Rampino et al., 2006; Ahmed et al., 2019, 2020; Pouraboughadareh et al., 2017).

Rate water Loss (RWL)

The results (Tab. 3) revealed that the RWL (60mn) is variable according to the tested genotypes ($p < 0.001$), while the applied irrigation had no effect on this parameter ($p > 0.05$). The results have pointed up that the highest value of RWL is registered after 20mn of measurements. In the same time and under rainfed conditions Vitron has loosed greatly than the other genotypes (Table 4). So, the recorded values are 4.87, 4.03, 2.57, 2.07 and 2.08 ($\text{mg.cm}^{-2}.\text{mn}^{-1}$) for Vitron, Oued Zenati, Langlois, ACSAD1351 and Waha respectively (Table 4). While, under irrigation this parameter revealed slight increasing in all the genotype with values of 1.2($\text{mg.cm}^{-2}.\text{mn}^{-1}$) (Vitron), 6.3($\text{mg.cm}^{-2}.\text{mn}^{-1}$) (Oued Zenati), 3.4($\text{mg.cm}^{-2}.\text{mn}^{-1}$) (Langlois), 3.75($\text{mg.cm}^{-2}.\text{mn}^{-1}$) (ACSAD1351) and 4.17($\text{mg.cm}^{-2}.\text{mn}^{-1}$) (Waha). The analysis of the links between the measured variables indicates that the relative water content is positively related to the water loss rate of the excised leaves RWL ($r^2 = 0.69$).

Table 4. Rate water loss (RWL $\text{mg.cm}^{-2}.\text{mn}^{-1}$) of the five durum wheat genotypes grown under irrigated and rainfed conditions in Tiaret (RWL1: after 20mn, RWL2: after 60mn)

Genotype	Water regime	RWL1	RWL2
Oued Zenati	Irrigated	6.34±0.4c	2.28±0.2ab
	Rainfed	4.03±0.3abc	2.19±0.04ab
Langlois	Irrigated	3.44±0.8abc	2.65±0.3ab
	Rainfed	2.57±0.6ab	2.12±0.25a
ACSAD1351	Irrigated	3.75±2.5abc	1.80±0.9a
	Rainfed	2.07±0.6ab	2.17±0.3a
Vitron	Irrigated	1.20±0.4a	4.62±1bc
	Rainfed	4.87±0.5bc	3.58±0.1c
Waha	Irrigated	4.17±1.4abc	1.98±0.3a
	Rainfed	2.08±0.3ab	1.63±0.03a

In the same column, means with the same letter were not significantly different at $p < 0.05$.

3.2. Leaf mesophilic thickness (μm)

Upon analyzing the data, the obtained results showed that the leaf mesophilic thickness is strongly influenced by the genotype and the adopted water regime (Table 3). The leaf mesophilic thickness under irrigated conditions varies between 100.9 μm (Oued Zenati, Tab. 5, fig. 6) and 81.67 μm (Langlois). Whereas, under rainfall conditions, Waha exteriorized the highest value (85.57 μm) and Langlois revealed the minimum value (60.33 μm). The reduction of the dimensions of the leaf mesophilic is imposed by a loss of turgidity. Seleiman et al. (2021) and Kefu et al. (2003) showed that the water deficit translated by a loss of turgidity thus minimizes the force of turgidity, reduces cell growth, thus explaining the inhibition of growth organs in plants. However,

this abiotic stress has a variable effect on the outer epidermal walls thickness of the leaf, depending on their location.

Table 5. Leaf mesophylic thickness (μm) and Outer epidermal walls thickness (μm) in durum wheat under two irrigation regimes in Tiaret (2014-2015)

Genotype	Water regime	Leaf mesophylic thickness (μm)	Outer epidermal walls thickness (μm)
Oued Zenati	Irrigated	100.9 $\pm$ 0.7a	2.82 $\pm$ 0.02a
	Rainfed	84.84 $\pm$ 0.8a	3.54 $\pm$ 0.06c
Langlois	Irrigated	81.67 $\pm$ 4.1ac	1.78 $\pm$ 0.03b
	Rainfed	60.33 $\pm$ 7.5b	2.73 $\pm$ 0.04a
ACSAD1351	Irrigated	81.79 $\pm$ 1.2ac	3.20 $\pm$ 0.12d
	Rainfed	70.44 $\pm$ 6.1bc	3.57 $\pm$ 0.1c
Vitron	Irrigated	84.39 $\pm$ 2.3a	1.85 $\pm$ 0.04b
	Rainfed	64.9 $\pm$ 7.5b	2.5 $\pm$ 0.1a
Waha	Irrigated	90.54 $\pm$ 1.2ad	2.6 $\pm$ 0.1a
	Rainfed	85.57 $\pm$ 1.6a	4.42 $\pm$ 0.3e

In the same column, means with the same letter were not significantly different at $p < 0.05$.

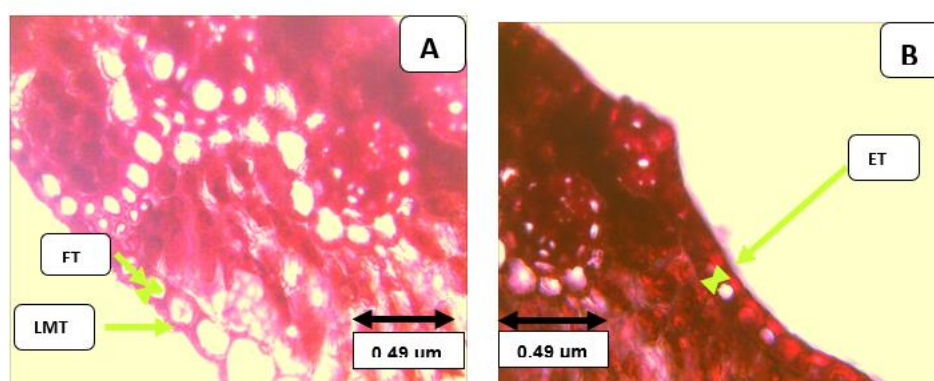


Figure 6. Transverse section of flag leaf showing the evolution of endoderm thickness in Oued Zenati genotype (A: Drought conditions, B: Irrigated conditions, ET: epidermal walls thickness, LMT: Leaf mesophylic thickness)

Outer epidermal walls thickness (μm)

The results (Table 3) showed that the outer epidermal walls thickness is strongly influenced by the adopted water regime ($p < 0.001$). According to the results (Table 5), it has been illustrated that Waha genotype exteriorized the highest value (4.42 μm), while the Vitron genotype recorded the lowest value of outer epidermal walls thickness (2.5 μm) under rainfed conditions. The control of transpiration by the flag leaf in wheat is an important characteristic of adaptation to very water-limiting situations (Wu et Bao, 2012). Also, the study of the structural transformations of the last leaf allows us to elucidate the relationships that exist between the morpho-physiological and structural modification. The obtained results indicated that the drought greatly affects the anatomical parameters of the flag leaf. Dry conditions tend to reduce mesophylic thickness ($r^2 = -0.366$). Thus, the water deficit causes a clear increase in this peripheral part ($r^2 = 0.350$). The thickening is of cellulosic nature or by synthesis of cutin deposition. These characteristics of epidermis cells (covering tissue) acts as a barrier between the external environment and the internal environment of the leaf. They are directly involved in the control of water loss and gas

exchange (Bacelar et al., 2004). This increase in the outer epidermal walls thickness would make it more resistant to an excessive reduction in turgor pressure and to collapse (tearing of the membrane and rupture of the walls).

4. Conclusion

Algeria wheat production fluctuated substantially in recent years. This species is cultivated under essentially rainfed conditions. Therefore, it is subject to highly fluctuate, often low, rainfall. This results in water deficits that can occur at any time during the vegetative cycle, leading to significant yield reductions. The obtained results in this study showed that water deficit is a limiting factor for durum wheat, affecting its morpho-physiological parameters. Also, the study revealed that there are significant correlations between the study parameters. Previous studies have proved these results (Chahbar and Belkhodja, 2016). These authors indicated that the role of these parameters in the adaptation and tolerance of durum wheat depends on the intensity of the water deficit. Selection on the basis of a low rate of water loss by excised leaves leads to variations in the other measured variables; reduction of relative water content, increase of flag leaf limb area and decrease of membrane stability. Identifying resistance mechanisms remain crucial in any manipulation aimed at creating a plant material tolerant to this abiotic stress. Moreover, this study highlighted that among tested genotype, it seems that Oued Zenati has an adaptation strategy corresponding to the hydric constraint of which it is native (Algerian semi-arid zone). This region presents high risks of late frosts and intermittent drought, especially at the end of the cycle.

Acknowledgement

We thanks all persons especially the engineers of the nature and life sciences department of Ibn Khaldoun University of Algeria

Author's declaration and contribution

The authors declare no conflict of interest. AA, ZK, CKMA, AA, carried out laboratory work and analysed data. AA, ZK, MD, LA, ZH, AA and BM advised about the laboratory technique and conducted manuscript proofreading before sub-mission. All authors read and approved the final version of the manuscript.

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