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The effect of water stress on some physiological and biochemical traits in five durum wheat (*Triticum durum* Desf.) genotypes

Hizia Kelaleche[1], Ali Guendouz*[2], Miloud Hafsi[3]

[1]*Department of Agronomy, University of Mohamed El bachir El Ibrahim, BBA, Algeria*

[2]*National Institute of Agronomic Research of Algeria, Research Unit of Setif (INRAA), Algeria*

[3]*Department of Agronomy, Ferhat ABBAS University of Setif, Algeria*

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Abstract

Water stress is the major environmental stresses that affect agricultural production worldwide, especially in arid and semi-arid regions. This research investigated the effect of water stress in leaf, root and leaf growing zone on five durum wheat genotypes grown in the greenhouse until 3rd leaf. We use morphological (leaf and root length) and biochemical parameters (Proline, Sugar and relative water content) to quantify the effect of water stress. The rustles showed a significant effect of water stress for all parameters just an exception for the root length. The results indicated that the effect of water deficit on biochemical parameters depended on the combination of water stress and wheat cultivars and organs. The analyses carried show that under water deficit stress leaf, root and leaf growing zone a RWC was sharply reduced due a combination of leaf growth reduction. Water deficits impose leaf, root and leaf growing zone proline content increase. Based on the biochemical parameters the genotypes Bousselem, Mexicali75 and Waha are the most tolerant genotypes. The use of the morphological traits showed that the genotypes Mexicali75 and Altar84 are the most tolerant for the leaf length and Waha and Bousselem are the most tolerant when we based in our evaluation on the root length. Over all, the use of the Proline, Sugar and relative water content to evaluate the tolerance of the genotypes to water stress are very suitable under these conditions.

* **Corresponding Author:** Ali Guendouz ✉ guendouz.ali@gmail.com

Introduction

Water stress (drought) is the most important factor that affecting the productivity of wheat. Across plant species, drought imposes various physiological and biochemical limitations and adverse effects (Mukherjee and Choudhuri, 1983; Chaves and Oliveira, 2004). Exposing plants to water stress adversely affect plant growth and productivity (Namich, 2007). The decrease in soil water potential causes alteration in minerals uptake by plant roots and reduction in leaf expansion under drought or salinity stress conditions (Pospíšilová *et al.*, 2000) Durum wheat production is severely affected by water stress in many parts of the world. A considerable area comprises on semi-arid environments with low water posing a major constraint on wheat production (Shafeeq *et al.*, 2006).

Water stress causes the establishment of a state of water regulation of the plant that is manifested by the stomatal closure and by a regulation of the osmotic potential (Anjum *et al.*, 2011). This regulation is achieved by the accumulation of compounds osmoregulators leading to a reduction of the osmotic potential, allowing the maintenance of the potential of turgidity. The accumulation of these organic compounds has been highlighted in several plant species subject to the constraint of water stress such as rice, wheat and potato (Farhad *et al.*, 2011; Xiong *et al.*, 2012). The connection between the ability of accumulation of these solutes and the tolerance of plants to water stress has been the subject of many discussions (Tahri *et al.*, 1998; Qayyum *et al.*, 2011). Proline accumulation is one of the most common and direct biochemical responses to water deficit (Hanson and Hitz 1982). The accumulation of low molecular compatible solutes including proline leads to a decrease in cell osmotic potential and permits osmotic adjustment, which results in water retention and prevention of dehydration (Heuer, 1994; Yoshiba *et al.*, 1997). Accumulation of sugars in different parts of plants is enhanced in response to the variety of environmental stresses (Prado *et al.*, 2000). Various authors point to the role of soluble sugars in the protection against stresses. Metabolisation of storage reserves in the endosperm of cereal seeds is tightly regulated and has a primary pivotal role in the interactions among sugars, ABA and gibberellins pathways responsible for the response to drought (Finkelstein and Gibson, 2001). A central role of sugars depend not only on direct involvement in the synthesis of other compounds, production of energy but also on stabilization of membranes (Hoekstra *et al.*, 2001), action as regulators of gene expression (Koch, 1996) and signal molecules (Sheen *et al.*, 1999; Smeekens, 2000). Soluble sugar content has proved to be a better criterion than proline content in screening durum wheat (*Triticum durum* Desf.) for drought tolerance (Al Hakimi *et al.*, 1995). In this experiment, only the total sugar content was determined without the identification of specific sugar components. Therefore osmotic regulation will help to cell development and plant growth in water stress (Pessarkli, 1999). It is defined that decrease of relative water content (RWC) close stomata and also after blocking of stomata will reduce photosynthesis rate (Cornic, 2000). It is reported that high relative water content is a resistant mechanism to drought, and that high relative water content (RWC) is the result of more osmotic regulation or less elasticity of tissue cell wall (Ritchie *et al.,* 1990). The aim of this study is to evaluate the performance of five durum wheat genotypes based on some physiological and biochemical traits under stressed and irrigated conditions.

Materials and methods

Plant material and stress conditions

Five durum wheat genotypes (*Triticum durum* Desf.) (Table 1) were used in our study.

The experiment was conducted at the university of Mohamed El bachir El ibrahimi bordj bou arréridj, Algeria. Durum wheat seeds were surface sterilized by dipping the seeds in 1% mercuric chloride solution for 2 min and rinsed thoroughly with sterilized distilled water. Seeds were pre-germinated in Petri dishes. After the emergence of the first leaf, the seedlings were grown in PVC cylinders of 50 cm height and 10 cm diameter filled with a mixture of sand, soil and organic dry matter (8:1:1). Seedlings were irrigated by sufficient water each two days.

Diurnal and nocturnal temperatures were 24-27 °C and 16-19 °C respectively with 14 hours/day photoperiod. At the 3rd leaf stage, treated plants are subjected to water stress by stop irrigation for 9 days and control plants are regularly irrigated.

Extraction and measurements

The growing leaf three was disclosed, the location of the elongation zone of the growing leaf and the exact distance of growth zone was found to be 3 cm long from leaf base (Hu *et al.*, 2000) it was verified by measuring displacement rates along the leaf axis by the pricking method (Schnyder *et al.*, 1987). Leaf tissue of the elongation zone was quickly cut into small segments for Measurements.

The soil was separated from the roots by a jet moderate of tap water. The roots were then washed in a tray before proceeding to the measures.

Biochemical analysis

Soluble sugar estimation: Sugars were extracted from the three organs (root, 3rd leaf and leaf elongation zone). Total soluble sugars content was measured by the method described by Dubois *et al.* (1956).

Proline content: Proline was extracted from a sample of 100 mg of fresh organs materials (3rd leaf, leaf elongation zone and roots) by 2 ml méthanol and estimated the proline content according to the method of Troll and Lindsley (1955).

Morphological parameters

The 3rd leaf and root length was measured in centimeter with ruler at the end of the experiment.

Physiological parameters

The relative water content (RWC)

Determined according to the method of Ritchie *et al.* (1990). Large broadleaves Organs (leaf, root, leaf elongation zone) discs were cut from the organs, to obtain about 5-10 cm²/sample. In the Lab, vials were weighed to obtain leaf sample weight (W), after which the samples were immediately hydrated to full turgidity for 4 hrs under normal room light and temperature. Organs samples were then rehydrated by floating on distilled water in close Petri dishes. After 4 hrs the samples were then taken out of water and blotted dry for any surface moisture quickly and lightly with filter paper and immediately weighed to obtain fully turgid weight (TW).

Samples were then oven dried at 800C for 24 hrs and reweighed to determine the dry weight (DW). All weighing were done to the nearest mg.

Calculation

RWC (%) = [(DW-FW) /(TW-FW)] × 100,

Where FW = fresh weight and TW = turgid weight.

Statistical analysis

All collected data were subjected to the statistical analysis (ANOVA) by STATISTICA software.

Results and discussion

The study of physiological responses of durum wheat genotypes to water stress is a useful tool to understanding the mechanisms of drought resistance. Drought induces significant alterations in plant physiology. Some plants have a set of physiological adaptations that allow them to tolerant water stress conditions.

Table 1. Origin of the five genotypes used in the study.

Genotypes	Name	Origin
1	Waha	ICARDA/CIMMYT
2	Bousselem	ICARDA/CIMMYT
3	Mexicali75	CIMMYT
4	Hoggar	Espagne
5	Altar 84	CIMMYT

Effect of water stress on proline content

Proline is an amino acid known for its sensitivity to drought and it is produced under drought. In plants proline accumulation had been well correlated with tolerance to salinity and drought.

In this study water stress caused a significant increase in proline content (Table 2). As shown in Table 3, and in the Leaf organ the proline content ranged from 9.88 µg/g for Bousselem to 0.62 µg/g for Altar 84 with an average of 4.57 µg/g. In addition, and in the root organ the values varied between 11.39 µg/g for Waha to 1.96 µg/g for Hoggar, but in leaf growing zone organ the proline content ranged from 15.07 µg/g for Hoggar to 6.21 µg/g for Altar84.

Table 2. Mean of physiological and biochemical traits studied in leaves, leaf growing zone and roots under irrigated and stressed conditions.

Organ	Leaf			Leaf growing zone			Root			Morphological traits	
Conditions	PRO (ug)	SUG (ug)	RWC (%)	PRO (ug)	SUG (ug)	RWC (%)	PRO (ug)	SUG (ug)	RWC (%)	Leaf length	Root length
Irrigated	3,14 (b)	1,84(b)	87,56(a)	6,12(b)	2,98 (b)	76,92(a)	3,88(b)	2,83(b)	87,89(a)	17,30(a)	8,06(a)
Stressed	7,70 (a)	4,90(a)	65,18(b)	11,35(a)	5,02(a)	54,39(b)	7,17(a)	4,07(a)	62,36(b)	13,31(b)	7,86(a)
LSD 5%	0,4	0,2	3,32	0,19	0,35	3,55	1,03	0,33	2,81	0,43	0,35

PRO: Proline content; SUG: Sugar content; RWC: Relative water content. Means followed by the same latter are not significantly different at $p<0.05$.

According to the rustles of Chorfi1 and Taïb (2011) the proline content increased proportionally in response to water deficit both in leaves and roots. High accumulation of proline content has been advocated as a parameter of selection for stress tolerance (Jaleel *et al.*, 2007). Based on the mean of proline content over all organs the highest content registered in Bousselem genotype.

Effect of water stress on soluble sugar

Accumulation of soluble carbohydrates increased resistance to the drought on the plant (Table 4), showed significant difference between sugar content under stressed and irrigated conditions. The highest values registered under stressed condition. Various authors point to the role of soluble sugars in the protection against stresses.

Table 3. Effect of water stress on proline in leaves, leaf growing zone and root of five durum wheat genotypes.

Genotypes	Proline content (ug)		
	Leaf	leaf growing zone	Root
Waha	8,13 (a)	8,94(b)	11,39(a)
Bousselem	2,19 (d)	7,04 (c)	8,33(b)
Méxicali75	7,69 (a)	6,21 (d)	3,32(c)
Hoggar	2,85 (c)	15,07(a)	1,96(c)
Altar84	6,23 (b)	6,41(d)	2,64(c)
Mean	5,42	8,73	5,53
Min	2,19	6,21	1,96
Max	8,13	15,07	11,39
LSD 5%	0,64	0,31	1,63

In the leaf organ sugar content varied between 5.00 ug/g for Waha to 1.95 ug/g for $Altar_{84}$. In addition, and in the roots organ the soluble sugar ranged from 8.51 ug/g in $Mexicali_{75}$ to 1.14 ug/g for $Altar_{84}$, but in leaf growing zone the values varied between 10.75 ug/g for $Mexicali_{75}$ ug/g to 1.09 ug/g for Hoggar. There are several reports on carbohydrate accumulation during various abiotic stresses in the temperate grasses and cereals from the Gramineae family where long term carbohydrate storage occurs during reproductive development (Meier and Reid, 1982) Accumulation of sugars in different parts of plants is enhanced in response to the variety of environmental stresses (Prado *et al.*, 2000).

Table 4. Effect of water stress on sugar content of leaves , leaf growing zone and root of five durum wheat genotypes.

Genotypes	Sugar content (ug)		
	Leaf	leaf growing zone	Root
Waha	5,29(a)	3,11(b)	3,15(b)
Bousselem	2,51(b)	3,23(b)	2,15 (c)
Méxicali75	5,001(a)	10,77(a)	8,51(a)
Hoggar	2,45(b)	1,09(d)	2,33(c)
Altar84	1,59(c)	1,82 (c)	1,14(d)
Mean	3,37	4,007	3,45
Min	1,59	1,09	1,14
Max	5,29	10,77	8,51
LSD 5%	0,32	0,55	0,52

The mean of sugar content over all organs showed that the genotype Mexicali$_{75}$ is the tolerant genotype than the other genotypes.

Effects of water stress on RWC

Relative water content is important character which related to drought stress. Relative water content (RWC) has been proposed as more important indicator of water status than other water potential parameters under drought conditions (Lugojan and Ciulca, 2011).

Table 5. Effect of water stress on RWC of leaves, Leaf growing zone and Root of five durum wheat genotypes.

Genotypes	Relative water content (%)		
	Leaf	leaf growing zone	Root
Waha	86,30(a)	72,34(ab)	70,64(c)
Bousselem	82,47(a)	58,53(c)	78,88(a)
Méxicali75	74,15(b)	76,71(a)	72,31(bc)
Hoggar	76,09(b)	69,01(b)	77,92(a)
Altar84	62,84(c)	51,67(d)	75,89(ab)
Mean	76,37	65,65	75,13
Min	62,84	51,67	70,64
Max	86,3	76,71	78,88
LSD 5%	5,26	5,61	4,44

As shown in Table 5, the RWC in leaf organ ranged from 86.30 % for Waha to 62.84 % for Altar84. In addition, and in the root organ the values of RWC varied between 78.88 % for bousselem to 70.64 % for Waha, but in the leaf growing zone the highest relative water content registered for Mexicali$_{75}$ (76.71 %) and the lowest values registered by Altar84 (51.67 %). Schonfeld *et al.* (1988) expressed with increase of drought stress of wheat, RWC decrease and usually but not always, in drought stress conditions, the cultivars that are resistant to drought have more RWC. In studies that performed on 4 cultivars of bread wheat, RWC reduced to 43 percent (from 88% to 45%) by moisture stress (Siddique *et al.*, 2000).

Based on the mean of the relative water content over all organ the genotype Waha registered the highest values, and we can noted that this genotype is the tolerant genotype than the other genotypes.

Effects of water stress on Morphological traits

Water stress had a significant effect on leaf and root length. Leaf length in response to water stress was decreased to 23% compared to well-watered conditions (Table 1). A significant variation in leaf length between genotypes registered in Table 6.

The highest values registered by Bousselem and Hoggar, 17.8 and 17.48 respectively.

Water stress affects negatively the root length (2.4%), the values ranged from 11.1 cm for Waha to 6.26 cm for Altar84. The impact of water stress on leaf growth can be explained as a method of adaptation to the conditions of water shortage to limit the rate of transpiration in order to maintain the water supply in the soil around plant roots to increases the chance of survival of the plant (Passioura, 2002).

Table 6. Effect of water stress on leaf length and root length of five durum wheat genotypes.

Genotypes	Leaf length (cm)	Root length
Waha	13,38(c)	11,1(a)
Bousselem	17,8(a)	7,78(b)
Méxicali75	14,5(b)	7,15(c)
Hoggar	17,48 (a)	7,51(bc)
Altar84	13,38(c)	6,26(d)
Mean	15,31	7,96
Min	13,38	6,26
Max	17,48	11,1
LSD 5%	0,68	0,55

Conclusion

The water stress decreases the morphological traits but increase the biochemical parameters, the genotypes showed a significant difference under this condition. Based on the biochemical parameters the genotypes Bousselem, Mexicali$_{75}$ and Waha are the most tolerant genotypes. In addition, the use of the morphological traits as an indicator of tolerance showed that the genotypes Mexicali$_{75}$ and Altar84 are the most tolerance genotypes based on the leaf length, but when we based on the root length the most tolerant genotypes are Waha and Bousselem.

Over all the combination between the morphological and biochemical parameters showed that the genotypes Bousselem, Mexicali$_{75}$ and Waha are the most tolerant genotypes.

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