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Morpho-Physiological Behaviour of Four Genotypes of Durum Wheat (*Triticum durum* Desf.) Grown under Different Levels of Water Stress

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Abstract: As part of a struggle against drought and its adverse effects on agricultural production particularly cereals and in order to understand the effect of water stress and to identify or discriminate the most resistant variety to the water deficit. The adaptation of durum wheat varieties drought is expressed by its ability to maintain a satisfactory level of main factors: physiological, morphological and biochemical. The study is focused on four varieties of durum wheat (*Triticum durum* Desf.) under different levels of water stress (35 %, 25%, 15% and 10% of the field capacity) and 100% of the field capacity (Terms of normal irrigation). The assays are performed by measuring sheet states five morpho-physiological parameters (leaf area, relative water content and pigments of chlorophyll content) and biochemical parameters of the assay (soluble sugar and proline). The three varieties manifest an almost similar behaviour via- α-via to the stress applied; varieties marked high levels of most osmoticums studied a net increase of proline and total of sugars soluble are distinguished of: Sémito, Bidi17 and Citra respectively, against a low content of this osmoticums in Wahbi. Therefore are tolerant varieties compared to Wahbi Durum wheat actually manifest traits of morpho-physiological and biochemical adaptation to deficit conditions.

Key words: Durum wheat · Water stress · RWC · Proline · Soluble sugars · Chlorophyll pigment · Adaptation

INTRODUCTION

Historically, grains are an important part of resources and economic exchanges among this grain durum wheat is the staple crop, which accounts for 8 % of the wheat area in the world [1]. Over 70 % of this area is Mediterranean conditions. In this region, drought is a major cause yield losses that vary from 10-80% depending on the years [2]. At the annual level, the consequences of drought depend on its period and its duration of action [3].

Cereal production in Algeria is still very inadequate to meet the demand of this product in wide use. He faces several biotic and a biotic constraint of soil orders and / or climate among these stresses, drought is considered the most important factor limiting grain production is one of the leading factors leading to differences not only between average yields and potential but also between different cereal seasons [4].

Cereal crops with an annual production ranges from independence 1between 10 and 45 million quintals, seems to be the vulnerable area as it is practiced in rainfed areas [5]. Most authors agree to consider that the Algerian cereal is long, largely dominated by durum wheat [6], a million hectares production is characterized by a wide variation and yields remain low and irregular, it is closely related to total precipitation [7]. Most often this rainfall is insufficient and irregular in time varies from one year to another.

This irregularity results in drought conditions can affect cereals during one or more phases of their development cycle. The selection process of local

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cultivars had a regression from 1965 largely due to the lack of human and material resources at various levels of existing structures having load the collection and storage of existing genotypes'. To this end, it became imperative to consider a better strategy for conservation and development of genetic resources.

The objectives of this study is to: testing durum wheat varieties (local and introduced) in relation to their resilience to water shortages and capacity in production by morphological approaches (Identification responses these varieties of durum wheat used by the biometric characters), physiological (changes in water status parameters: the relative water content RWC and chlorophyll content) and biochemical (assessing the technical capacity on the accumulation of compatible osmolytes, proline and soluble sugars).

MATERIAL AND METHODS

Plant Material: Four varieties of durum wheat (*Triticum durum* Desf.), local and introduced origins seeds were provided by the Technical Institute Crop El-khroub Constantine (ITGC) are the subject of this study. These cultivars are characterized by contrasting agricultural productivity (Table 1).

Growing Conditions: Experiment was conducted under greenhouse (in semi-controlled conditions. The grains of these varieties were germinated in Petri dishes on filter paper Wattman no soaked with water. After germination, seedlings were transplanted into plastic pots containing the same amount of the mixture of soil, sand and compost in the proportions 3:1: 1. At 8 pars pot seedlings for each genotype 6 treatments and 3 pots per treatment are defined which were 24 pots per treatment and genotype pots are watered three times a week and kept at maximum hydration up at stage four well developed leaves. At this stage , five different water regimes were imposed.

Table 1: Origins of studied genotypes

Varieties	Origin
Cirta	*ITGC* Elkhroub, Algeria
Bidi 17	*ITGC* Guelma, Algeria
Wahbi	*ITGC* Elkhroub, Algeria
Sémito	*ITALY*

They consisted of 100% cc (control), in the case of plants stressed lot, irrigation is suspended until the obtaining different levels of deficit hydrique,10, 15 and35% of field capacity.

Measurements

Relative Water Content (RWC): Relative water content (RWC) was measured according to method described by Barrs [8]. The calculation is done using the formula [1]: [*RWC (%) = [(FW -DW) / (TW- DW)* 100*] [9], where FW is the fresh weight (the blade of the leaves cut at its core is immediately weighed), TW: fresh weight at full turgidity (the same sheet placed in a test tube containing distilled water for 24 h at 4°C), DW is the dry weight determined after the passage of sample in an oven at 80°C for 48 hours (dry weight of leaf).

Proline: was assayed on the middle third of the last two sheets by the method developed by Troll and Lindsly [10] the values obtained are converted to proline levels by means of an equation:

$$Y = 0.62 * OD (528nm) / MS.$$

These equations determine the levels of proline in the leaves of plants. The spectrometer is set to zero through 40% methanol.

Total Soluble Sugars: The total soluble sugars (sucrose, fructose and their derivatives methyl and polysaccharides) are assayed by the phenol method as described by Dubois *et al* [11].

Leaf Area: Is determined by a measuring device scanner leaf area.

Chlorophyll Pigments: Are assayed by the method as described by Mackiney *et al*. [12] and are determined by the concentration of chlorophyll a and b are expressed and given by the equations:

$$Chl(a)\ (\mu g/100mg\ MF)=12,3DO(663)-0,86(645)\ /10$$
$$Chl(b)\ (\mu g/100mg\ MF)=22,9DO(645)-4,86(663)/10$$

Statistical Analysis: The statistical analysis of the results obtained in different experiments was tested by analysis of variance performed using the (Xl- STAT-PRO 2006) software program. The Newman-Keuls test has given the average and ranks with a significance levels (α=5%).

RESULTS AND DISCUSSION

Morpho-Physiological Parameter: The analysis of variance of the results obtained revealed the existence of very highly significant difference between the levels of stress, between varieties insignificant and significant for the interaction between the two factors (Table 2).

Relative Water Content: A comparison between the evolutions of the relative water content of four varieties studied showed that water stress causes a drop in the percentage of water. This fall is becoming clearer as and when the stress level is increasing (Fig. 1), in both varieties Bidi 17 and Wahbi, the values of RWC fluctuate between a 84, 71 % to 27, 57 % consequently scoring percentage decrease of 5, 88 % to 67, 45 % among the four levels of stress (35 %, 25%, 15% and 10% of the field capacity), respectively compared to control (100% of field capacity). About Sémito and Cirta, the RWC is varies 78, 65 % to 25, 26% and decreases in the order of 4, 10% to 44, 59 %. This means that the greater the intensity of water stress s' increases, the higher the relative water content (RWC) is lowered, while maintaining relatively high compared to control values (100% FC).

Content of Chlorophy ll Pigments: The chlorophyll (a + b) was decreased correspondingly over the level of stress among the four genotypes (Fig.2). The Bidi 17, Wahbi and Cirta varieties are mark grades ranging between 0, 99 to 0, 57 µg/100mg MF. Decline rates are from 10,10 to 25,25% in Bidi 17, 19,14% to 39,36% in Wahbi and 21,50% to 33, 33% in Cirta four levels of stress (35%, 25%, 15% and 10% FC) compared with baseline values at 100% FC, the variety Sémito records levels fluctuating between 1,08 and 0,54 µg/100mg MF, marking rates decrease 20,37% to 50%.

This allowed us to conclude that Bidi 17 decreased its total chlorophyll content unless Sémito, Cirta and Wahbi.

Leaf Area: The analysis of variance of the results reveals the existence of very highly significant difference between the levels of stress, highly significant between varieties and not significant for the interaction between the two factors (irrigation levels and variety) (Table 1 and Figure 3). In our experiment is the Bidi 17 variety which records high values of leaf area at 35% and 25 % compared to the control, followed by Wahbi, Sémito and Citra.

At 15% and 10%, the Sémito variety keeps the same value, while varieties Cirta and Bidi 17 mark low rate of decline compared to the Wahbi variety that experiences high decrease in these levels.

Biochemical Parameters: The analysis of variance of the results obtained revealed the existence of very highly significant difference between the levels of stress, between varieties insignificant and significant for the interaction between the variables of biochemical factors, proline and sugar content (Table 3)

Proline Content: Analysis of variance of the results reveals the existence of very highly significant difference for stress levels but not significant between the varieties studied. Proline is known to be widely present in plants and normally accumulates in large quantities in response to environmental stress as well due to an increase in production by reducing degradation (Fig. 4).

In a normal irrigation condition, the proline content was estimated from the degree of stress 35 % of field's capacity, content ranges from 9,16 ± 0,97 to 5, 34 ± 2,1 µg /100 mg DM among genotypes, the minimum value is recorded in the genotype Sémito.

Table 2: Analysis of variance of Morph physiological parameter. (The repetition in the table, this is correction in the page 1215 of manuscript)

	Genotype effect			Treatment effect			Genotype × Treatment effect		
Genotype variables	MS	F	Pr > F	MS	F	Pr > F	MS	F	Pr > F
RWC	104,90	1,897	0,15 Ns	3436,212	62,12	0,0001***	115,15	2,082	0,049**
Chl (a+b)	0,072	3,767	0,020**	0,124	6,519	0,001***	0,006	0,315	0,981 Ns
La (cm^2)	106,12	5,6	0,0001***	62,589	9,21	0,0001***	4,296	0,632	0,977 Ns

MS: Mean square, F: calculated, Pr > F: Probability RWC : the relative water content, Chl (a+b) : Content of chlorophyll a+b, La (cm^2): leaf area, *p = 0,1, **p = 0,05, ***p = 0,001 : its respectively the existence of significant, highly significant and the existence of very highly significant difference; Ns : not significant.

Table 3: Analysis of variance of Biochemical parameter

	Genotype effect			Treatment effect			Genotype × Treatment effect		
Genotype Variables	MS	F	Pr > F	MS	F	Pr > F	MS	F	Pr > F
Prol	30,315	0,807	0,49Ns	3018,135	80,32	0,0001***	48,568	1,293	0,270 Ns
Sugar	131,733	3,209	0,036*	398,749	9,7 15	0,0001***	57,087	1,391	0,221 Ns

MS: Mean square, Pr> F: Probability, Prol: Proline content, Sugar: soluble sugar content *p = 0, 1, **p = 0, 05, ***p = 0,001: its respectively the existence of significant, highly significant et the existence of very highly significant difference; Ns: not significant.

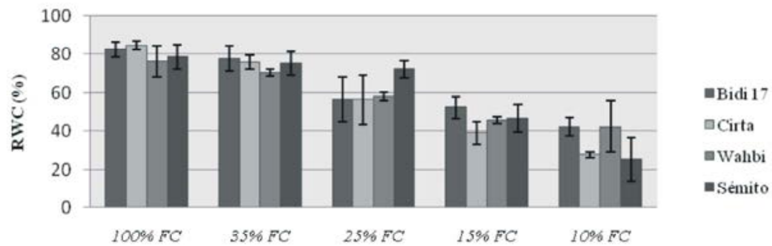

Fig. 1: Variation of relative water content in leaves of four genotypes of durum wheat depending on levels of water stress

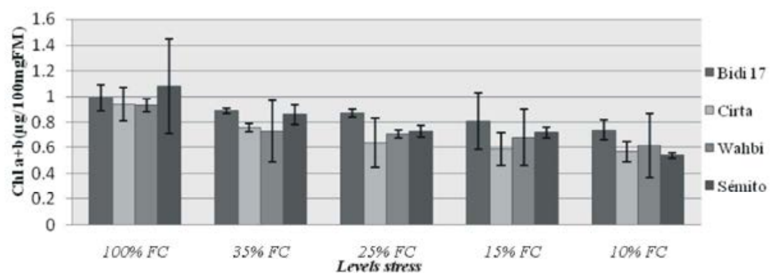

Fig. 2: Variation of chlorophyll (a + b) in leaves of four genotypes of durum wheat depending on levels of water stress

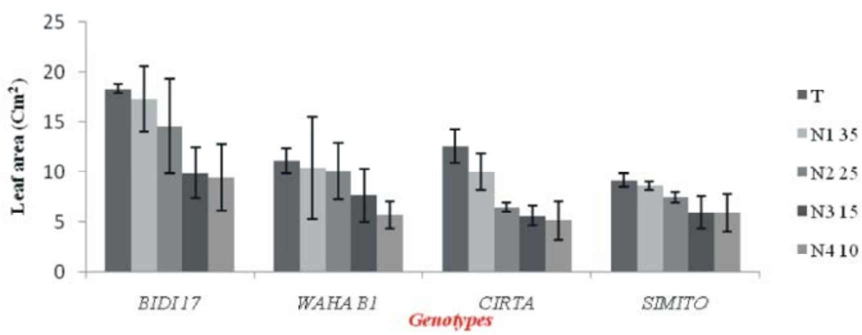

Fig. 3: Variation of the leaf area in leaves of four genotypes of durum wheat depending on level of water stress

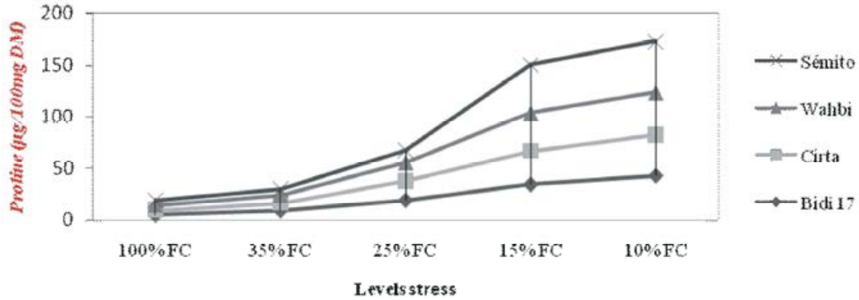

Fig. 4: Variation of proline content in leaves of four durum wheat genotypes based on level of water stress

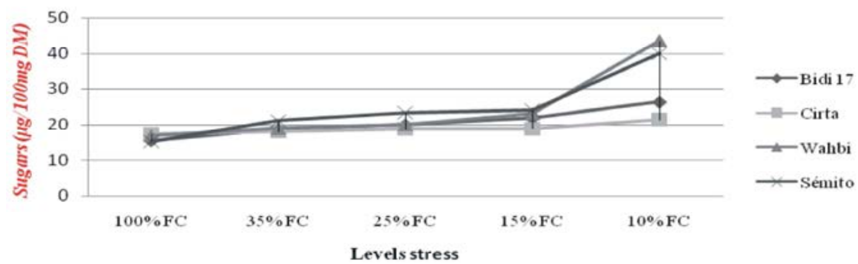

Fig. 5: Variation of total soluble sugar content in leaves of four durum wheat genotypes on level of water stress.

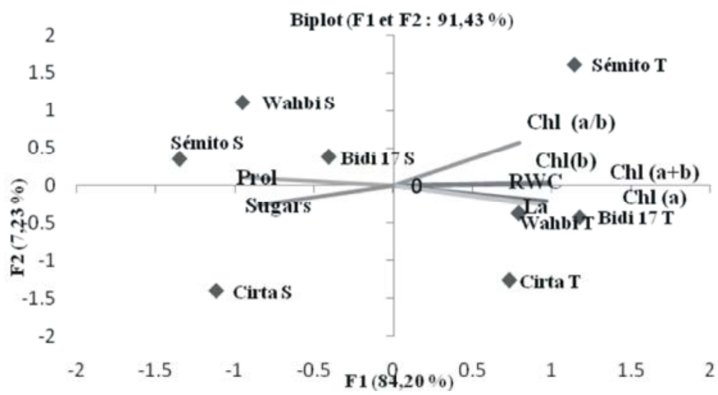

Fig. 6: Diagrams of the distribution of variables and individuals to moisture 10% of field capacity.

Table 4. Principal component analysis of different variables of four varieties of durum wheat

Variables'	RWC	Chl (a+b)	Chl (a)	Chl(b)	Chl (a/b)	La (cm2)	Prol
Chl (a+b)	0,957***						
Chl(a)	0,952***	0,999***					
Chl (b)	0,975***	0,955***	0,948***				
Chl (a/b)	0,653	0,809***	0,821***	0,603			
La	0,777***	0,743**	0,746**	0,767**	0,539		
Prol	-0,971***	-0,940***	-0,938***	-0,941***	-0,680	-0,738**	
Sugar	-0,737**	-0,795***	-0,797***	-0,732**	-0,757**	-0,688	0,817***

RWC : relative water content; Chl (a+b); Chl(a); Chl (b); Chl (a/b): is respectively Chlorophyll(a+b), Chlorophyll(a), Chlorophyll(b) and rapport of Chlorophyll(a/b); La (cm^2): leaf area; Prol: Proline content; Sugar: soluble sugar content *p = 0,1, **p = 0,05, ***p = 0,001 : its respectively the existence of significant, highly significant et the existence of very highly significant difference; Ns : not significant.

While the maximum value is recorded in the Bidi17. But those are confused by 100% FC (control), this may be due to the presence of a thermal stress. The order of increase is 1, 81 times; 1, 66 times; 1, 50 times and 1, 33 times in Bidi 17 Wahbi, Cirta and Sémito respectively compared to control. At 25% FC, the four varieties accumulate more proline. A net increase is distinguished it is about 3.83 times, 4.09 times, 3.51 times and 2.97 times in Bidi 17, Wahbi, Cirta and Sémito respectively compared to control.

Total of Soluble Sugars: In stressed lot 35% of field capacity, the four varieties marked values vary from 18 ± 1, 89 to 21,09 ± 2,18 µg / 100 mg DM. The maximum value is recorded in the genotype Sémito with a rate of 21, 09 ± 2, 18 µg /100 mg DM, while the minimum value is recorded in the Wahbi variety that marks a rate of 18 ± 1, 89 µg /100mg DM. Stressed at the two lots 25 % and 15 % FC, the four varieties show a slight increase (Fig. 5). The levels range from 18, 64 ± 3, 24 to 23, 32 ± 8, 28 µg /100 mg DM at Wahbi and Sémito. The rate of increase is 1,03 to 1,72 times in Bibi17; from 1,07 to 1.23 times in Wahbi; of 1,19 to 2,60 times in Cirta and 1,53 to 2,62 times in Sémito compared to the control lot and 35% of field capacity.

Relationship Between Physiological and Biochemical Variables: The principal component analysis reveals for both 100% and 10% of field capacity levels shows that the percentage of information given by the F1 axis is 84, 20% and that given by the axis F2 is 7, 23% a total of 91, 43%. Interpretation may be limited to these two axes large discriminating power (Table 4 and Figure 6).

Highly significant positive correlation can be found between the Relative water content and the pigment of Chlorophylls Chl (a+b), Chl (a), Chl (b) and between the Relative water content and the leaf area (Table 4 and Figure 6). A negative correlation very highly significant between the Relative water content and sugars (r=-0,737**) and between the Relative water content and Proline (r=-0,971***)

The parameters Chlorophylls (a+b), (a), (b), leaf area and 1Chlorophylls (a/ b) are negatively correlated with the Proline so r =-0,940***; r =-0,938***; r =-0,941***; r =-0,738** and r = -0,680* respectively. Leaf area is negatively correlated with sugars and proline. Chl (a) has a very highly significant positive correlation with Chl (a + b) so r = 0,996 *** and with the ratio Chl (a/ b) so r = 0,764 *** (fig. 6).

This latter, have a significant positive correlation with Sugars. The correlations obtained between sugar accumulation and proline on the one hand and between the RWC, chlorophyliens pigment and other sugars shows that more and more increases the proline and the RWC diminuend pigment, lowering the RWC and chlorophyll content and has a strong assessing accumulation of osmolytes (Fig.6).

DISCUSSION

The analysis of the relative water content and the content of chlorophyll pigments is used to describe a comprehensive way, the water status in response to water stress and evaluate the ability to achieve a good osmoregulation and maintain turgid cell [13]. Comparison of relative water content in four varieties based treatments applied, shows that there is a negative correlation between the level of stress and Relative water content. Newman Keuls test (α = 5%) ranks variable stress levels into four groups and the class variable in one group at: Bidi 17, Sémito, Cirta and Wahbi (59, 15 %, 56, 73 %, 55, 69 % and 52,304 %).

We can conclude that all genotypes have almost the same behaviour via- à-via the levels of stress. The lack of water is a key element for the growth of plants, especially in arid and semi-arid region. It induces a decrease in the stressed plants of the relative water content and a significant reduction of the total biomass production.

The chlorophyll assimilation is the physiological process by which autotrophic plants are able to use solar energy for their nutrition exclusively with mineral feed; the amount of leaf chlorophyll can be influenced by many factors such as the age of the leaves, leaf position and environmental factors such as temperature and water availability [16; 17; 18]

The results of our experiments show that water stress induced a remarkable decrease in chlorophyll levels in almost all genotypes; this is confirmed by Organ [19] and Tambussi *et al* [20]. Proline is known to be widely present in plants and normally accumulates in large quantities in response to environmental stress as well due to an increase in production by reducing its degradation [21]. Given previous experience, the accumulation does not begin until level 40 % of field capacity when the plant feels the lack of water in non- limiting conditions of irrigation [22].

The four varieties show a slight increase. The levels range from 18,64 ± 3,24 to 23,32 ± 8,28 µg / mg DM at Wahbi and Sémito, The effect of water stress can result at the whole plant level and especially sheets, the net increase in the concentration of a number of constituent primary metabolites which may be carbohydrates. According to our results, the accumulation of soluble sugars is much lower than that of proline. Our results are in agreement with those obtained by Dib *et al* [23]. Long, it is known that the rate of sugar increases significantly in plants subjected to different types of stress among adult trees of eucalyptus under different stress [24] in wheat response to water deficit [25]

Stressed plants have responded by increasing amounts of sugars in their cells [25]. This increase is actually a parameter adaptation to water stress conditions. This is confirmation with the findings of researchers who have stated that the water deficit caused a significant accumulation of soluble sugars in the leaves. This accumulation is positively correlated with the degree of stress [14].

CONCLUSION

The value of this work is to evaluate the impact of water deficit on four varieties of durum wheat. The effect of drought may result, depending on the adaptive strategy of each variety to reduce sweating; these changes affect the air or underground part: reduction of leaf area and the number of sizes, leaf curling and /or better development root system. The aim is to characterize the effect of this stress on the morphological, physiological and biochemical behaviour of plants in deficit conditions.

The result seems to vary between the different treatments (35 %, 25 %, 15% and 10% field capacity) but does not reveal large variability between different varieties. In our study we have found that the rate of proline significantly higher compared to the control indicating that some metabolic perturbation follows the water stress intensity. The behaviour of four varieties is similar, but the maximum value is marked in Sémito and Bidi 17.

A slight increase in soluble sugar content was observed in four varieties in relation to the proline, despite the accumulation of soluble sugars is in positive correlation with the stress as to proline. Chlorophyll (a+b) shows a decrease depending on the degree of water stress. The RWC and leaf area marked decreases over the degree of stress. Significant positive between the first group of variables (proline, soluble sugars) and between the second group of variables (chlorophyll pigment surface correlations are found. The plants react appropriately to different levels of stress applied and control its metabolic functions following conditions governing culture medium to tolerate water deficit stress. Applying its analysis in several varieties and in other settings and at different levels of stress.

LIST OF ABBREVIATIONS

ITGC: The Grande's Cultures Technical Institute (Elkhroub).
CIMMYT: The International Center for Improvement of Maize and Wheat, Mexique.
RWC (%): The relative water content.
WF: the fresh weight.
WR: the weight of rehydration.
WD: the dry weight.
DO: The density obtique
Chl(A +B): Chlorophyll content.
Mol: moll
FM: fresh mass
DM: dry mass
XlSTAT-PRO 2006: a logician of Statistical treatment.
MS: Mean square.
Pr: Probability.
La (cm^2): Leaf area.
Prol: Proline content.
Sugar: soluble sugar content.
FC: field capacity.
35% FC, 25% FC, 15% FC and 10% FC: The lots of different levels stress of field capacity.
100% FC: control of field capacity

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