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RESEARCH ARTICLE

Morpho-physiological markers associated with Water Use Efficiency in Algerian durum wheat at different water regimes

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Abstract

Water use efficiency is controlled by several physiological mechanisms. It is considered as an important trait that has been associated with drought tolerance of crop plants. In this work, we investigated WUE and its components on ten Algerian durum wheat cultivars of different origins under controlled conditions. Plants were sown at different water regimes (95% considered as well watered treatment, 60%, 20% considered as dry treatments). A set of parameters (relative water content RWC, chlorophyll content (SPAD index), leaf temperature LT°, stomatal conductance (gs),Leaf area (LA) and Specific leaf weight (SLW)) describing plant response to water deficit were measured. WUE was estimated as the ratio of total dry matter on the total water consumption. Our objective was to evidence differences among cultivars in response to drought stress. ANOVA analysis reveals a significant difference between varieties in response to the applied water treatments, Genotypes that have shown high values of WUE, TDM, RWC, Chlorophyll content and LA under dry treatments could be suggested as Algerian drought tolerant genotypes. Based on the correlations between WUE and the studied parameters our study may suggest some morpho-physiological traits associated with water use efficiency and drought tolerance.

INTRODUCTION

Durum wheat is the most widely grown cereal species in the Mediterranean basin. It holds about 45% of durum wheat cultures in the word. In Algeria cereals holds 60% of cultivated lands. Unfortunately the production is still very low because of the adverse climatic variations and water stress conditions resulting from the dry climate of the region **(Bousba et al., 2009).**Drought is the most devastating abiotic stress affecting crop productivity (**Reyazul el al., 2012)**. It occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation **(Xiao-li, el al., 2012;Sankar et al., 2008)**.Plant water use efficiency (WUE) is becoming a key issue in semiarid areas, where crop production relies on the use of large volumes of water**(Medrano et al 2015).**It is considered as an important trait that has been associated with drought tolerance of crop plants.WUE can be measured at different scales, ranging from instantaneous measurements on the leaf to more integrative ones at the plant and crop levels**(Medrano et al 2015),** at the plant scale and ignoring evaporation from the soil WUE is defined as the ratio of biomass production and the amount of water used over a certain period (**Boogaard, 1997)**. It was observed that plants can improve their WUE in response to drought stress condition .The Water Use Efficiency (WUE) characteristic as an important screening technique can help in discovering significant variation among the genotypes **(Condon et al., 2002).**

In response to water stress, plants develop different mechanisms (morphological, physiological and biochemical) which inhibit or remove the harmful effects of stresses. **(Izabelaet al., 2013)**.Generally, reduction in stomatal conductance (gs) and evapotransipiration (E) under drought result in higher WUE due to the plant rapidly adjusting water loss through transpiration and absorption of CO2 through stomata regulation **(Xiao-li, el al.,2012)**. It has been reported by Cowan (1988) that regulating stomata apertures in leaves can regulate leaf WUE under limited water condition. Moreover, change in leaf temperature may be an important factor in controlling leaf water status under drought stress **(Shakeel et al., 2011)**. Some researchers reported that higher leaf temperature may have important consequences on the longevity and photosynthetic capacity of individual leaves and have further affects on leaf WUE **((Xiao-li, el al.,2012;McNaughton and Jarvis, 1991)**. Leaf water status may accurately define the demand and supply of water. Relative Water Content (RWC) is an important determinant of metabolic activity and the survival of leaves **(Öner el al., 2014**) and is an attribute for discriminating drought tolerant and sensitive genotypes **(Rauf, 2008)**.Chlorophyll concentration is known to be one of the major factors affecting photosynthetic capacity; **(Öner el al.,2014)** .The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation. The permanent or temporary water deficit severely hampers the plant growth and development more than any other environmental factor **(Shakeel et al., 2011).** Water deficit reduces the number of leaves per plant and individual leaf size, leaf longevity by decreasing the soil's water potential. Leaf area expansion depends on leaf turgor, temperature, and assimilating supply for growth **(Shakeel et al., 2011)**. Drought-induced reduction in leaf area is ascribed to suppression of leaf expansion through reduction in photosynthesis **(Rucker et al., 1995).** A common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production **(Zhao et al., 2006).** Specific leaf weight SLW indicates leaf dry mass per area. It has been widely exploited as a reliable morpho-physiological marker contributing to drought tolerance for various crop plants **(Mohammadreza et al., 2014).**The present study aims to study ten durum wheat genotypes responses to different water regimes and to investigate the relationship between WUE and the morpho-physiological studied traits.

Materials and Methods

Plant material and growing conditions

Ten varieties of durum wheat (Triticum Durum Desf L.) local and introduced listed in (Table 1) and provided by the ITGC, Institut Technique des Grandes Cultures (station El Khroub Algeria) were used in this study. Seeds were germinated on petri dishes, after germination seedlings were transferred to plastic pots with mixture of (clay soil / sand 3:1) in greenhouse with an average temperature 25±10 C° and relative humidity 45± 15%.The pots were arranged completely in a randomized block design with three replications for each treatment.

Drought stress treatment

To adjust the amount of pots watering in terms of the irrigation regimes of field capacity (95% considered as control treatment, 60% and 20% considered as dry treatments), the soil water content was continuously monitored and maintained by watering at 95%, 60% and 20% levels of field capacity during the experiment. Changes in the soil water of each pot were measured and checked daily by weighing each pot at the beginning and end of the removed plant. The total amount of water used was calculated as the difference between final and initial pot weight and the amount of water supplied to each pot. Plants were harvested at the flag leave stage.

The Morpho-physiological analysis

For determination of Relative Water Content (RW, fresh leaves were taken from each genotype and each replication at flag leaf stage and weighted immediately to record fresh weight (FW). Than they were placed in distilled water for 4 h and then weighted again to record turgid weight (TW), and subjected to oven drying at 70°C for 24 h to record dry weight (DW). (RWC) was determined according to the method adopted by Turner (1986): RWC % = [(FW– DW) / (TW-DW)] × 100. Chlorophyll content was assessed using a SPAD chlorophyll meter (Minolta crop, USA) before any measure, the device must be set (number of signal tower) and size (N = 0). In this protocol the rate of chlorophyll is estimated per unit SPAD. The leaf temperature (LT) was measured using a hand held infrared thermometer (IRT), the stomatal conductance was measured at mid-morning on the adaxial leaf surface with a hand-held porometer (AP4) which measured the stomatal resistance (rs), the stomatal conductance values (gs) were deduced from the ratio 1/ (rs).

in addition to the previous parameters, total leaf area (cm^2) was measured according to Paul et al., (1979) and the specific leaf weight (SLW) was calculated by dividing the total leaf area by leaf dry weight (LDW/LA) ($g\ m^{-2}$) according to **(Lambrides et al., 2004).**

WUE was calculated as total dry weight divided by total amount of transpiration according to **(Qiao et al., 2010).**The amount of water loss from the pots, weighed on daily basis, represented the transpiration**.**

Statistical analysis

To evaluate differences among the wheat genotypes in response to different water regimes, Data were subjected to an ANOVA analysis at the $P<0.05$ significance level. Pearson's correlations among the studied parameters were tested using SPSS 22.0 software package.

Results

Water use efficiency and its components (WUE, TWC, TDM)

We observed a noticeable variation in the response of the wheat genotypes to the different water regimes imposed. Results in (Fig.1a) show that WUE increased under the two dry treatments (60%, 20%) compared to the control 95%.The variety Bous displayed the highest value of WUE for the treatment 20% field capacity (FC) with 14.66 mg/g.water followed by the variety GGR 11.48mg/g.water whereas the lowest values were registered by the varieties Cir, Rah and Bidi (3.05 3.08 and 3.09 mg/g.water) respectively .Data in Figure.1b showed a slight decrease in the total dry matter (TDM) accumulated for all the genotypes under both treatments 60% and 20%. Despite of the severity of the water regime imposed, some genotypes maintained high values of TDM, such as the variety Bous under the treatment 20% with (619.66mg), the same as Sim under 60% (690mg). Moreover, our results revealed a significant drop in the total water consumption (TWC) under the two regimes 60%, 20% compared to the control treatment, all the studied varieties decreased the amount of TWC under the dry treatments except the varieties Bous, GGR and Sim. The ANOVA Results **(Table 2)** show that for WUE and its compounds, the different genotypes displayed a significant difference in response to the water treatments ($P\leq0.0001$).

The Morpho-physiological traits

Our study showed that RWC (relative water content) decreased under drought stress conditions, for the water regime 20 % FC, the highest reduction in RWC was obtained from the genotypes Cir and Bidi. Also, the highest RWC value was obtained from Bous cultivar under the same water regime (Fig.2.a).otherwise our results indicated that the studied genotypes were not very affected by the moderate dry treatment 60% FC. According to the data in **(Table 2)** RWC presents a highly significant difference between genotypes and the water treatment $P\leq0.0001$.

The results of our study indicated that stomatal aperture was significantly affected by the dry treatments imposed. Data illustrated in (Fig 2.b) show that the cultivars Dk and Bidi presented the lowest values of (gs) for the water regime 20%, (0.06 and 0.07mmol m-2s-1) respectively. The same was registered for the regime 60% FC, genotypes Dk,Bidi and Cirta exhibited the lowest values of (gs) (Fig 2.b). The ANOVA analysis presented a highly significant difference between genotypes $P\leq0.0001$, water regimes and the interaction GXWR. **(Table2).**

Leaf temperature was positively influenced by the severity of the water regime imposed; we registered increased values for all the studied genotypes under the two regimes 60% and 20% comparing to the control (Fig 2.c). The difference among genotypes and the water treatments was highly significant$P\leq 0.0001$ as shown in **(Table2).** In addition, our results revealed that there was a significant effect of drought conditions on the chlorophyll content (SPAD Index) in all genotypes. The chlorophyll content decreased under the two water regimes 60% and 20% as shown in (Figure 2.d) and it presents highly significant differences among genotypes, water regimes and the interaction with $P\leq0.0001$ **(Table2).**

Moreover, the water regimes imposed had a significant effect on the morphological studied traits, Leaf area (LA) decreased for all the genotypes under the dry treatments compared to the Control **(Fig.3a)** except for the genotype Bous which maintained the highest leaf area for both dry treatments (18.01 cm^2). Leaf area results showed highly significant differences among genotypes and the water treatments $P\leq0.0001$ **(Table 2)** .The ANOVA analysis revealed that the effect of the applied water regimes on specific leaf weight (SLW) varied among genotypes, the varieties Cir, Rah and Bidi registered a significant increase in the SLW for the treatment 20% FC (Fig.3.b), in contrast to the varieties W, MBB, Dk, Bouss, GGR, Sim which presented almost the same values of SLW for both dry treatments. The results showed that there was a highly significant difference between genotypes and the water regimes $P\leq0.0001$ (Table2)

Correlations among the studied parameters

The correlation analysis revealed parameters strongly linked to WUE under both dry treatments. WUE was negatively correlated with total water consumption TWC (r=-0.88** for 60% FC and r=-0.69** for 20%FC), stomata conductance gs(r=-0.42* for 60%FC and r= -0.68** for 20% FC), and leaf temperature (r= -0.52** for 60%FC and r= - 0.53** for 20% FC). A marked positive correlation was found between WUE and TDM under both dry treatment (r=0.63 for 60% FC and r =0.90** for 20% FC). Under the dry treatment 20% FC we registered a significant positive correlation between WUE and LA and between WUE and RWC (r= 0.72**, r=0.47** respectively). These results are shown in Table 3. Data illustrated in Fig 4(A and B) reveals that TDM, TWC and LA are strongly linked to WUE under the dry treatment 20% FC.

Table1. Origin of the studied genotypes

Genotype	Code	Origin
Waha	W	CIMMYT-Mexico cross, released by ICARDA Syria and Algeria
Cirta	Cir	ITGC Constantine ,Algeria
Beliouni	BEL	Algeria
Bidi 17	Bidi	Algeria
Guemgoum Rkhem	GGR	Algeria
Mohamed Ben Bachir	MBB	Hauts plateaux Est, Algeria.
Djennah Khetifa	Dk	Alegria/Tunisia
Simeto	Sim	Italy
Bousselam	Bous	ITGC Setif, Algeria
Rahouia	RAH	TIARET ,Algeria

Table 2. Variance analysis for all measured parameters of ten wheat genotypes subjected to three water regimes 95 %, 60% and 20%

Variance Source	df	TDM		WUE		TWC	
Genotypes G	9	113998,989	***	62,632	***	116019,2	***
Water Regimes WR	2	611040,811	***	169,023	***	1516176	***
Interaction (GxWR)	18	8005,774	Ns	5,179	***	58335,044	***

Variance Source	df	RWC		g(s)		LT	
Genotypes G	9	505,454	***	0,052	***	70,164	***
Water Regimes WR	2	1595,472	***	0,398	***	243,709	***
Interaction (GxWR)	18	48,753	**	0,008	***	2,126	ns

Variance Source	df	SPAD		LA		SLW	
Genotypes G	9	79,441	***	52,236	***	54,123	***
Water Regimes WR	2	253,654	***	213,434	***	98,121	***
Interaction (GxWR)	18	4,021	*	2,828	ns	13,17	***

Values are mean square. *(P<0.05), ** (P<0.01), *** (P<0.0001).

(RWC)Relative Water Content %, g(s) Stomatal Conductance, (LT) Leaf Temperature,(LA) Leaf Area, (SLW) Specific Leaf Weight, (TDM) Total Dry Matter, (WUE) Water Use Efficiency, (TWC) Total Water Consumption.

Table 3. Pearson's correlation among the studied parameters of ten durum wheat genotypes under two different dry treatments

Correlation coefficients at 60% field capacity

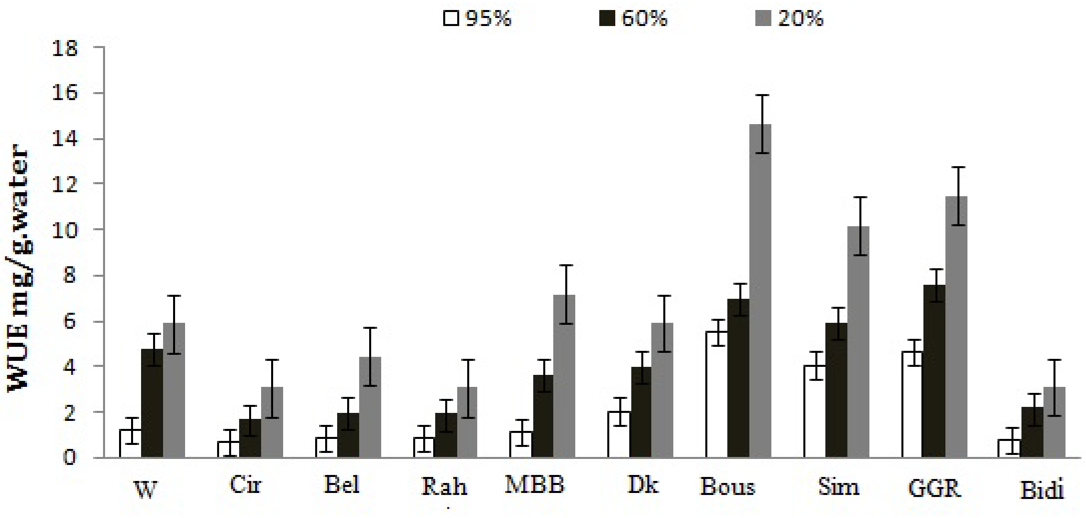
	WUE	TWC	gs	TDM	LT°	SPAD Index	LA	SLW	RWC
WUE	**1**	-,885**	-0,42*	,636**	-,526**	-0,027	0,338	-,488**	0,046
TWC		**1**	-,372*	-,452*	,531**	0,032	-0,35	,370*	0,018
g(s)			**1**	,607**	-0,204	-0,255	,468**	-0,216	,494**
TDM				**1**	-,580**	-0,106	,442*	-,639**	,567**
LT°					**1**	0,316	-0,235	,393*	-,408*
SPAD Index						**1**	-0,147	-0,091	-,587**
LA							**1**	-,638**	,440*
SLW								**1**	-0,258
RWC									**1**

Correlation coefficients at 20% field capacity

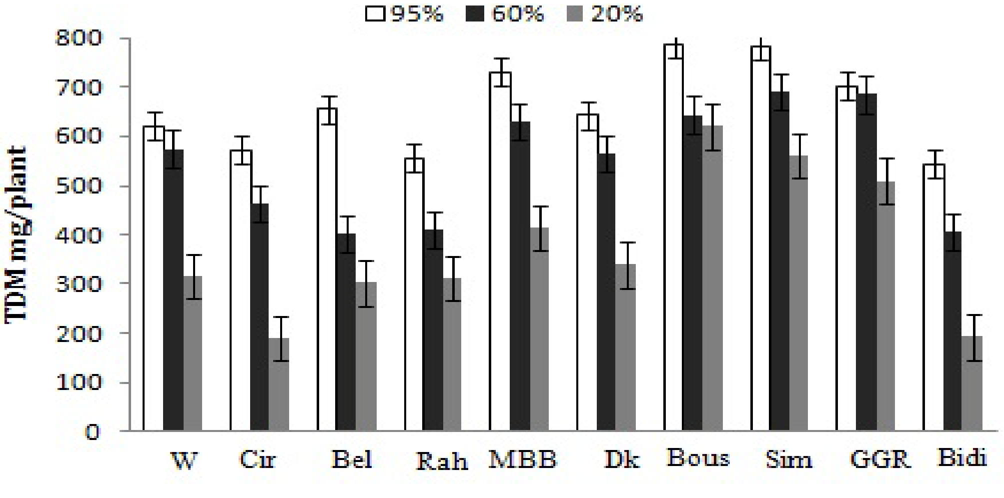
	WUE	TWC	gs	TDM	LT°	SPAD Index	LA	SLW	RWC
WUE	**1**	-,690**	-,682**	,901**	-,532**	,363*	,729**	-,599**	,479**
TWC		**1**	-,483**	-,391*	,533**	-,650**	-,494**	,632**	-0,29
Gs			**1**	,693**	-,410*	0,351	0,305	-,506**	,475**
TDM				**1**	-,457*	0,136	,622**	-,552**	,465**
LT°					**1**	-0,099	-,426*	,831**	-0,249
SPAD index						**1**	0,204	-0,305	0,136
LA							**1**	-,463**	,367*
SLW								**1**	-,378*
RWC									**1**

**. The correlation is significant at the 0.01 level (bilateral).

*.The correlation is significant at the 0.05 level (bilateral).


☐ 95%
■ 60%
■ 20%
WUE mg/g.water
0
2
4
6
8
10
12
14
16
18
W
Cir
Bel
Rah
MBB
Dk
Bous
Sim
GGR
Bidi

a)


☐ 95%
■ 60%
■ 20%
TDM mg/plant
0
100
200
300
400
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600
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800
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Cir
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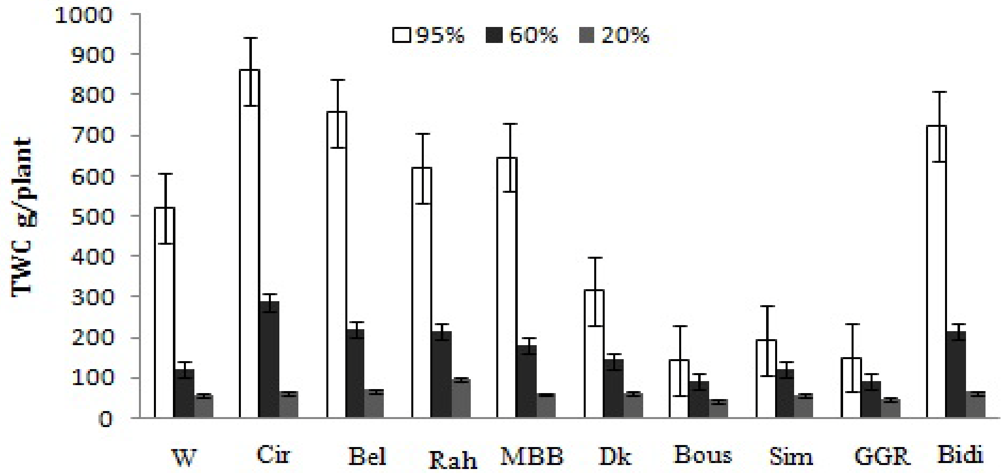
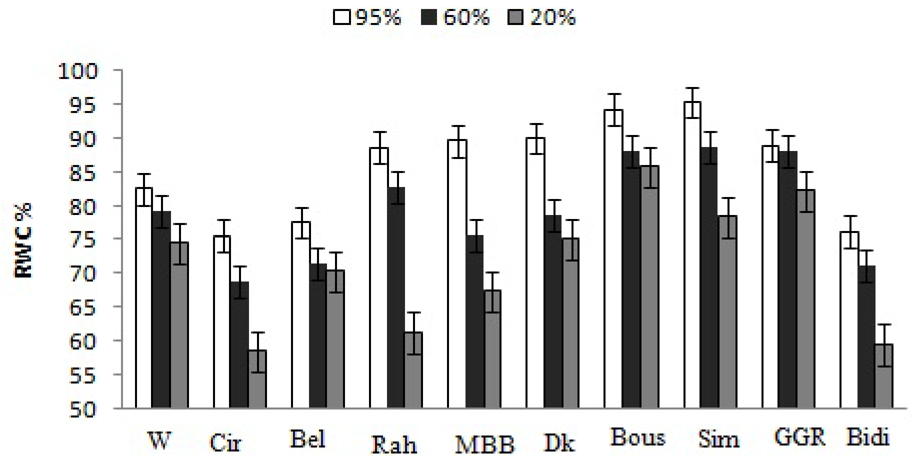
Rah
MBB
Dk
Bous
Sim
GGR
Bidi

b)



c)

Figure1. (a) Water use efficiency (mg/MS/plant), (b) Total dry Matter, (c) Total water consumption of ten wheat genotypes under different water regimes.


□95% ■60% ■20%
RWC %
100
95
90
85
80
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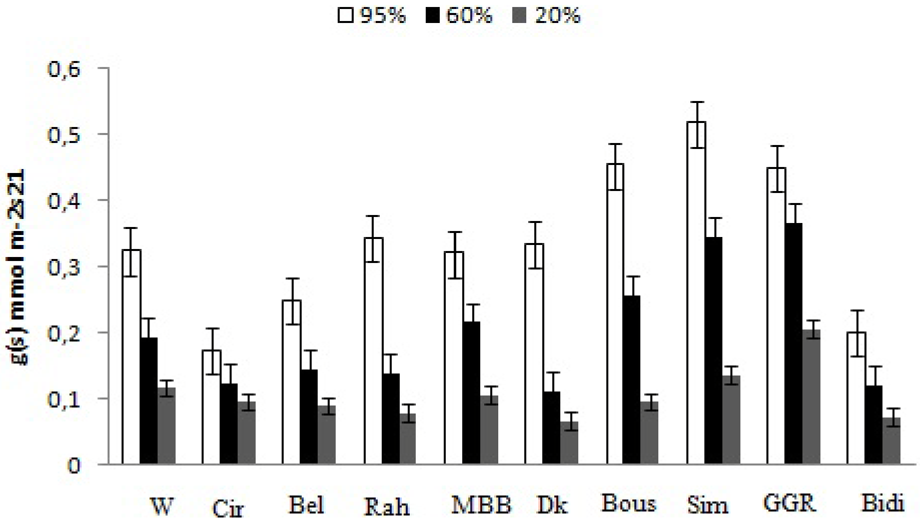
Dk
Bous
Sim
GGR
Bidi

a)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g(s) mmol m-2s21
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0,4
0,3
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0,1
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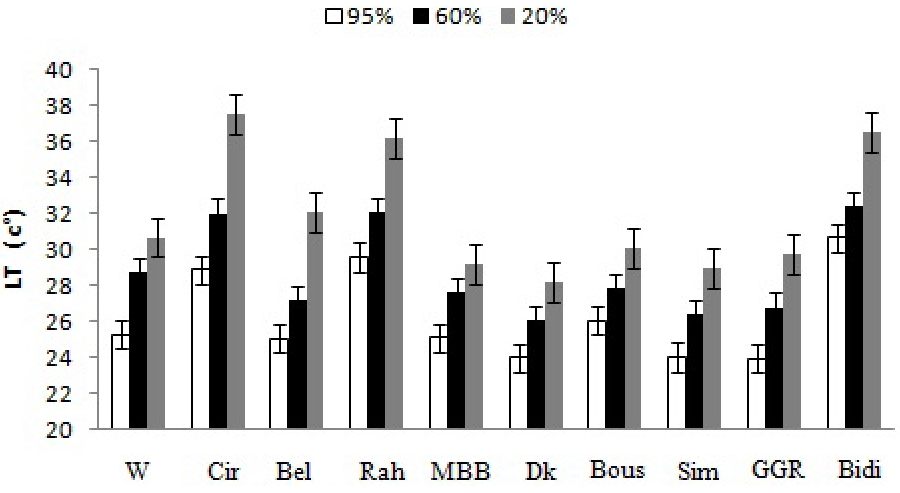
Cir
Bel
Rah
MBB
Dk
Bous
Sim
GGR
Bidi

b)


□95% ■60% ■20%
LT (c°)
40
38
36
34
32
30
28
26
24
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20
W
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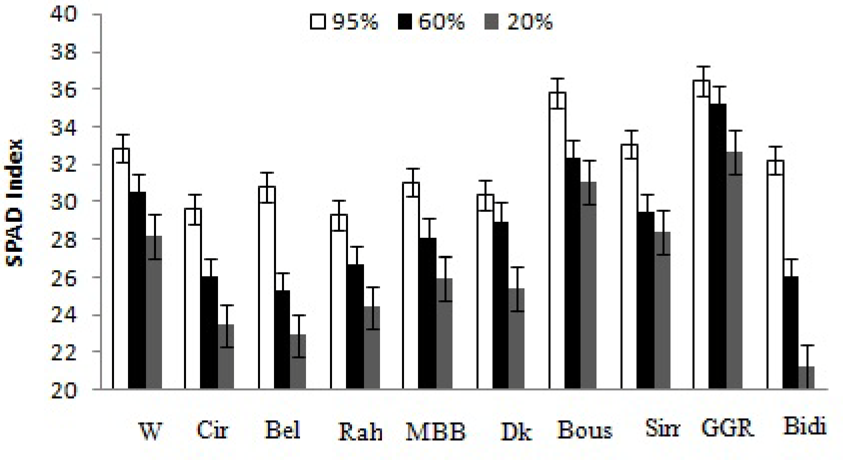
Rah
MBB
Dk
Bous
Sim
GGR
Bidi

c)



d)

Figure 2. a).Relative water content (RWC), b).Stomatal conductance (gs), c).Leaf Temperature (LT) and d).SPAD index of ten wheat genotypes under different water regimes.

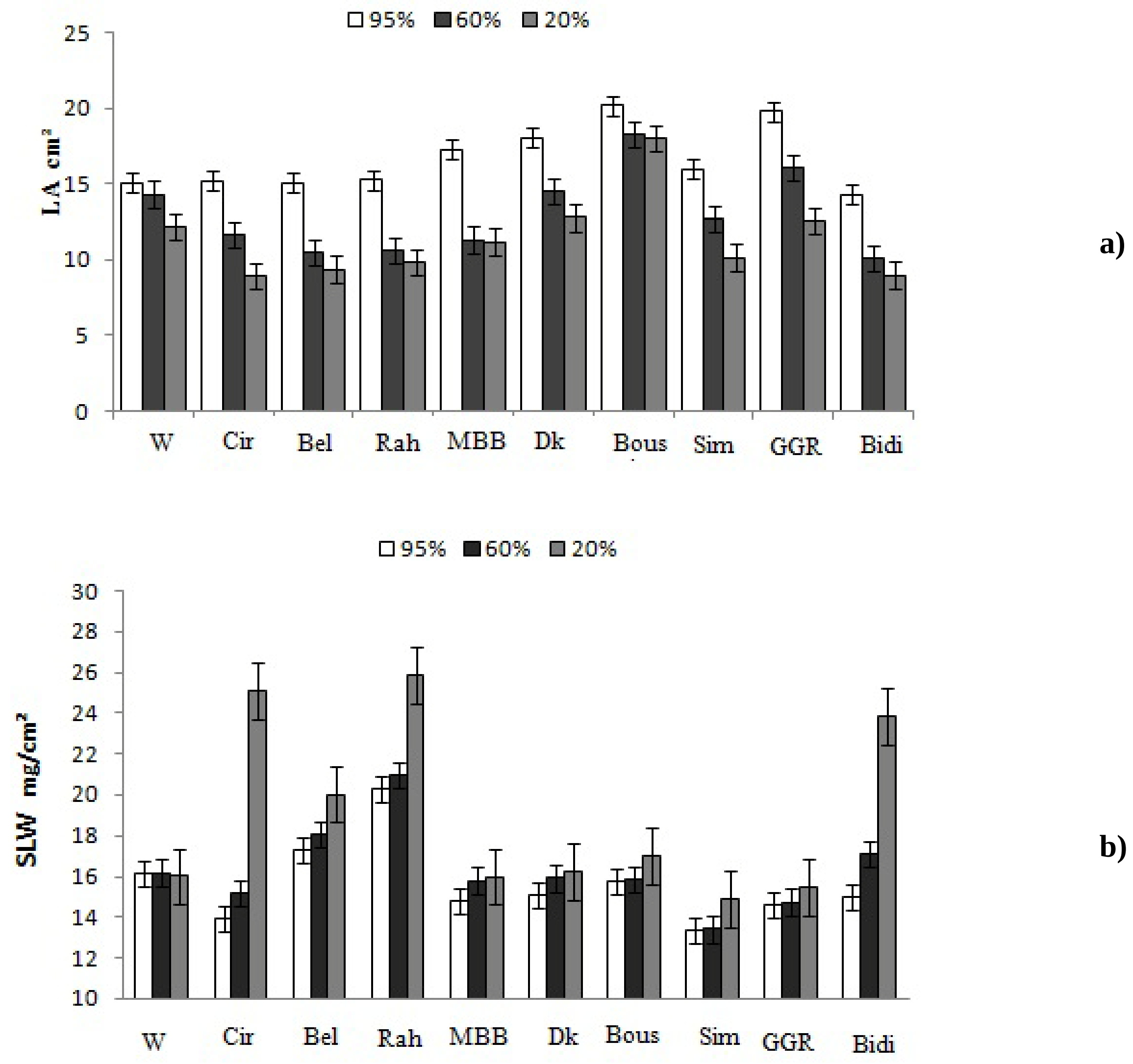
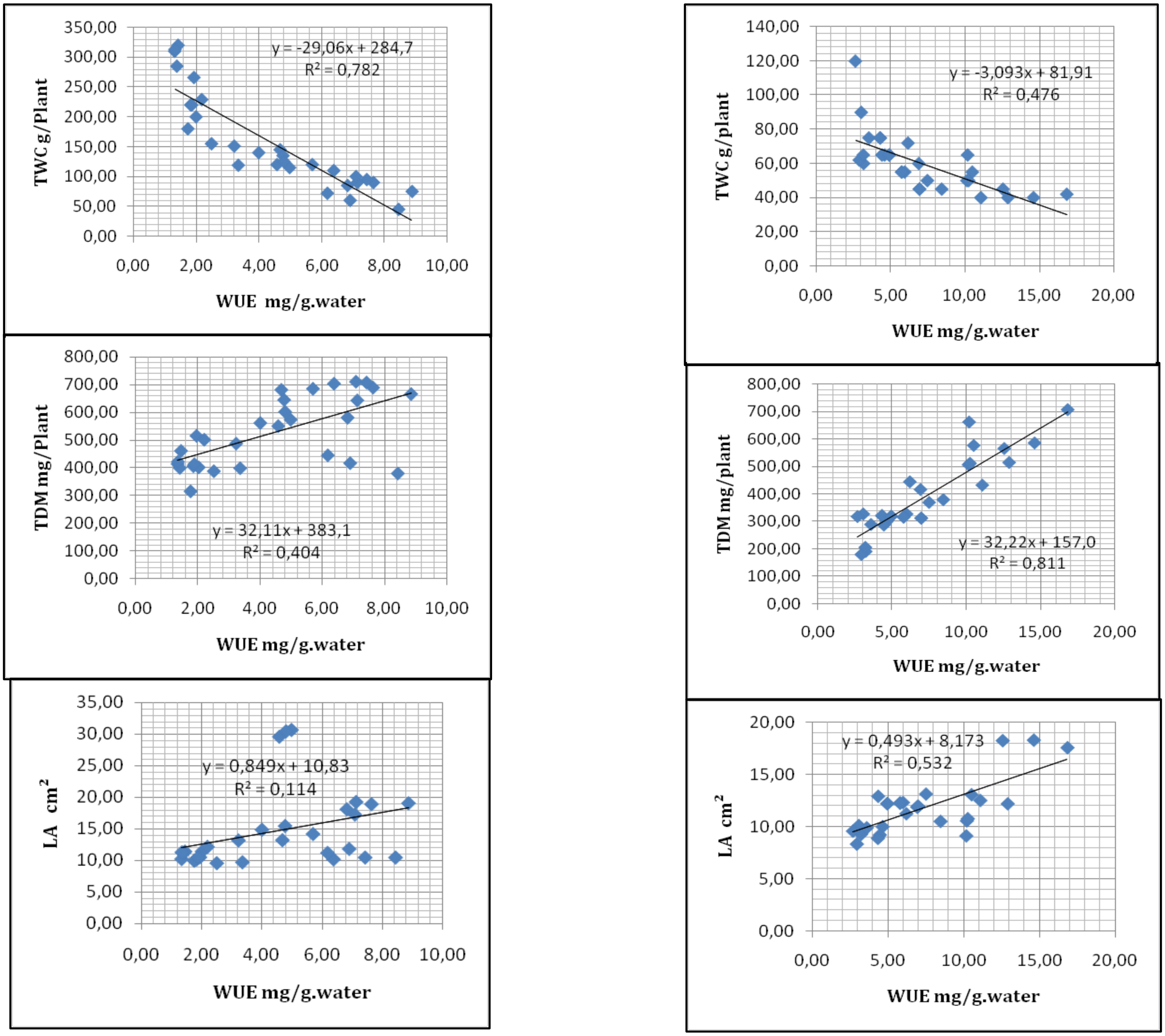


Figure 3.Effect of different water regimes 95%, 60% and 20% on a).Leaf Area cm², b).Specific Leaf Weight SLW g/cm² of ten wheat genotypes.



A) B)

Figure 4 .Relationship between WUE and Total water Consumption (TWC), Total Dry Matter (TDM) and Leaf Area (LA) of ten wheat genotypes A) under 60% Filed capacity regime, B) under 20% Filed capacity regime .

Discussions

Our goal was to impose drought conditions similar to those found in agricultural settings that cause reductions in seed yield **(Christine et al., 2012and Jones, 2007).** We found that drought stress affected the water use efficiency components; we registered a significant decrease in Total water consumption (TWC) and total dry matter (TDM) for the water regime 20%. In our study, we found that WUE increase across water treatments, the obtained results showed a genotypic variation for the wheat genotypes in response to the water regimes imposed for WUE and its components **(Fig.1).** The studied genotypes registered a slight decrease in TDM across the water regimes, this decrease in TDM for wheat cultivars has been reported by many authors **(Ykhlef et al., 1998) and (Changhai et al., 2010).**Likewise, the correlation analysis revealed a positive significant correlation between WUE and TDM under drought conditions **(Table.3, Fig.4A and 4B)**, the same results were obtained by **(Alirezan and Farshad, 2013**).
Ykhlef et al (1998) reported that water use efficiency increase with the intensity of the water stress and it affects the most water consumption more than dry mass accumulated which was in agreement with our study. For the water regime 20%, all wheat genotypes showed a considerable decrease in TWC under water stress conditions and maintained high values of TDM and WUE **(Fig.1.a, b,c).**Moreover the applied water regimes had a significant effect on water consumption and stomata conductance which decrease dramatically under drought **(Fig1.c and Fig2.b)** This decrease of g(s) has been mentioned by many authors **(Ykhlef and Djekoun., 2000, Baloch et al., 2012.,Hamla et al 2014).** According to the simple correlation analysis, total water consumption TWC and stomata conductance were negatively correlated with WUE under drought stress treatments **(Table.3, fig.4 A and B).** Our results are in good agreement with the results of (**Xiao-li1 et al., 2012**) and support the findings of the pervious results done by (**Bhagsari 1986; Johnson 1993; Cowan 1997; Zhang*et al*. 2005; Zhao *et al*. 2006;Yu *et al*. 2008 and Xiao-li1 et al., 2012),** who found that increasing of leaf WUE was due to the reduction of transpiration and an increase in photosynthesis. Furthermore, it has been reported by **(Baker *et al*., 2007; Sharkey *et al*., 2007; Tambussi*et al*., 2007 and Xiao-li1 et al., 2012)** that reductions in g(s) and transpiration were parallelly accompanied only by reductions in (Ci) intercellular CO2 concentration resulted in increasing water use efficiency, this was mainly due to plant rapidly adjusting water loss through transpiration and absorption of CO2 through stomatal regulation thereby resulting in increased water use efficiency.
Our results revealed a decrease in the relative water content (RWC) in response to drought stress which has been reported by Nayyar and Gupta (2006) that when leaves are subjected to drought, leaves exhibit large reductions in RWC. It has been also reported in previous investigations done by **(El Tayeb, 2006)** and **(Geravandia, 2011**) that drought tolerant genotypes showed the higher RWC rather than drought sensitive genotypes. Moreover, our data analysis indicated a positive correlation between WUE and RWC under water stress conditions (Table.3), the same results were reported by **(Ykhlef et al., 1998).**Likewise, drought affects wheat leaves by increasing leaf temperature as has been reported by **(Hamla, 2014)** which was in accordance with our results, all wheat genotypes showed increased values of LT under drought condition. Moreover, change in leaf temperature may be an important factor in controlling leaf water status under drought stress, drought-tolerant species maintain water-use efficiency by reducing the water loss **(Shakeel, 2011)** which was in good agreement with our results; genotypes exhibiting low values of LT maintained high WUE under drought condition **(fig 1.a and 2.c).**The negative correlation between WUE and LT was not significant **(Table.3),** similar results were found by**(Zaho el al.,2006) and (Xiao-li1, et al., 2012).**
The variation of chlorophyll content in leaves under different levels of water regimes provides information about the behavior of genotypes towards water efficiency **(Bousbaet al., 2009).** In the present study, chlorophyll content of durum wheat leaves dropped across the water regimes **(Fig.2d).**Our results supported the findings of other authors **(Bousba, 2012;Izabelaet al., 2013;Hamla, 2014) and (Gao et al., 2004**) who showed that a lack of water leads to a drop of chlorophyll in the leaves. Studies have shown that, more tolerant wheat varieties tend to keep higher relative water content and higher chlorophyll content **(Parwata et al., 2012).**In addition to the previous parameters**,** leaf area of wheat genotypes was also induced by drought stress, LA decreased across the water regimes. Boutraa(2010) reported that according to the study of Lu et al.(1998) 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).**The mechanism, by which plant leaf area is reduced under water stress, is through the reduction of cell elongation, which leads to the reduction of cell size and therefore the reduction of leaf area **(Schuppler,1998).** In the present study, results revealed a significant positive correlation between WUE and leaf area under the dry treatments :(r= 0.49** for 60%FC; r= 729** for 20%FC)**(Fig 4 A and B).**The association of high WUE with high LA is in accordance with results of Boogaard (1997) who reported that a greater investment in leaf area was associated with higher water-use efficiency at the plant level as well as at the leaf level which was the case of our study. In fact, Boogaard (1997)

suggested that the correlation of high water-use efficiency with a high leaf area is promising and should be further tested under field conditions.

Our results revealed that specific leaf weight (SLW) increase across the water regimes applied and presented variation among the studied genotypes (Fig.3.b), Furthermore, SLW had a positive correlation with WUE under the water stress treatments as shown in (Table.3).These results support the findings of Ykhlef (1998). Drought stress was found to have caused an increase in SLW in almost all studies, increases in SLW under drought conditions have also been reported in durum wheat **(Ykhlef,1998)** and in some fruit trees such as peaches **(Martinez, 2010),** wild almond **(Mohammadreza,2014).**

Conclusions

The aim of our work was to study the response of ten wheat genotypes to different water regimes and to investigate the relationship between WUE and the morpho-physiological studied traits. The obtained results indicated a genotypic variation between varieties in response to the applied water treatments. This variation is due to the different morpho-physiological mechanisms developed by the cultivars to overcome the water stress conditions. In addition, the correlation analysis revealed a significant relationship between WUE and the studied parameters such as TWC and (gs) which are considered as the most important traits to regard while studying WUE, Furthermore, the significant positive correlation between leaf area and WUE could be an important result to consider in further studies and should be confirmed in field conditions. Thus, these traits may be considered as morpho-physiological markers of WUE under drought conditions. Moreover, our findings may suggest tolerant genotypes such as Bous, GGR and Sim which presented high values of Water use efficiency (WUE), Total Dry Matter (TDM) and chlorophyll content, relative water content (RWC), as well as high values of leaf area and specific leaf weight under drought stress conditions. Likewise, these genotypes presented less values of TWC and could be suggested as less water consumer genotypes under water limited conditions.

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