



MORPHOLOGICAL CHARACTERISTICS AND WATER STATUS OF SOME TUNISIAN BARLEY GENOTYPES SUBMITTED TO WATER STRESS

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Abstract:

Tunisia has been qualified as a country vulnerable to climate change that will be unregistered a great drop of annual rainfall and an increase of evaporation. Response strategies of agriculture to drought will be critical because drought is one of the major abiotic stresses which adversely affect crop growth and yield. Among strategies to be developed to cope with the effect of climate change, recourse of genetic diversity and new varietal creation can be a solution among other methods.

In this study, four barley genotypes were cultivated in semi-controlled conditions and submitted to three levels of water stress. Data were recorded on number of grain per plant (NGP), one thousand grains weight (PMG), total leaf surface (TLS), plant height (HAT), stomata density (DS), leaf water content (RWC) and leaf water potential (LWP).

Results showed that morphological characteristics (HAT, TLS, DS), yield components (NGP, PMG) and water status (LWP, RWC) of barley genotypes were decreased significantly. However, moderate water deficit didn't affect significantly the most of parameters studied. Study had demonstrated also that barley genotypes developed different strategies and mechanisms to cope with water deficit, based essentially on their osmotic adjustment capacity.

Keywords:

barley, water stress, leaf water potential, relative water content, yield, height, leaf surface, stomata.

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1. INTRODUCTION

Climate change is one of the determinants that will influence the development of agriculture in the twenty-first century, particularly in Tunisia which has been qualified as a country vulnerable to climate change [1] and among the « hot spot for climate change» countries [2].



Model projections available for Tunisia [3] indicate a clear increase in annual average temperature (1.1°C by 2030). Heat waves would then be more numerous, longer and more intense, with frequent days of scorching heat. Model simulations also suggest a drying trend in the region. Tunisia would be particularly affected by droughts that would be more frequent, more intense and longer-lasting [4]. In fact, [5] predicted a drop of 4 to 28% in annual rainfall.

The water deficit, that represents a major stake for the countries concerned, will be worsened by increased evaporation [6], the fact that resources will become scarcer [7] and will be over-exploited, and that coastal aquifers will become more salty [8]. The availability of water is the major limiting (due to global warming) factor.

Response strategies of agriculture to drought will be critical because drought is one of the major abiotic stresses which adversely affect crop growth and yield and thus a constraint for plant productivity worldwide [9]. Indeed, drought stress reduces agricultural products but adaptation with climate changes beside of other methods could improve agriculture [10]. Among strategies to be developed to cope with the effect of climate change, recourse of genetic diversity and new varietal creation can be a solution among other methods. In fact, large germplasm collection could help to development of new crops germplasms that have high water use efficiency and tolerant to drought, which can withstand the effects of climate change [11] because old species creation will be less well adapted to this new climate. Genetic variability accumulated in various plant species and involved with increase of production and adaptation to less favorable environments is being used by humankind since beginning of agriculture by selection of seeds collected from the best genotypes.

Barley (*Hordeum vulgare* L.) is among cereals relatively tolerant to drought [12] making it a primary candidate for cultivation in arid and semi-arid lands that are highly vulnerable to climate change [13]. Barley is currently gaining popularity in the world and in Tunisia [14] due to its adaptability to different climates, failure of other crops, and its new industrial uses in specific food applications [15],[16]. In Tunisia, barley is cultivated in all regions of the country and occupied between 34% and 38% of the cereal cultivated area [17]. It is used both as feed and food [18], [19]. It is noted that most of barley improved varieties that are being introduced into the country from various sources are not sufficiently adapted to abiotic stress. Therefore, development of tolerant varieties to water stress with high potential of production under climate change and identifying landraces growing under local agricultural conditions with specific adaptations remain the main goals of the sector.

The present research aims to study Tunisian barley varieties and accessions on the physiological and anatomical level that will be helpful for understanding their genetic diversity, for managing their effective utilization in breeding programs and in adaptation to climate change.

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL



Four barley genotypes (*Hordeum vulgare* L.) were used. Three improved varieties (Manel, Rihane and Kounouz) were obtained from Crops Laboratory of the National Institute of Agronomic Research of Tunisia. One local landrace (Ardhaoui) was collected from Medenine in the South of Tunisia. All of them were six-rowed spring barley (Table 1).

2.2. PLANT GROWTH AND TREATMENTS

The experiment was conducted at the farm of National Agronomic Research Institute of Tunisia (INRAT) during the cropping season of 2014. The site is located at 36°51' latitude and 10° 11' longitude. This experimental station has a Mediterranean climate. The soil texture of the experimental site is clay loam (35.5% clay).

Seeds of each genotype were sown in 10 l plastic pots in five replications (5 plants per pot) and cultivated in a semi-controlled conditions (protect of rain) with natural day length. Irrigation was applied once a week during the early stage of growth. A basal dose of 50 kg/ha N in the form of ammonium nitrate (33% N) fertilizer was applied at sowing.

The experiment was carried out under three levels of water deficit stress (irrigation at 2/3 (T₁), 1/3 (T₂) and 1/6 (T₃), field capacity). The control plants were normally irrigated during all the experimental period and had received 100% of field capacity (T₀). Treatments were initiated at the emergence of fourth leaf. Soil moisture levels were maintained with manual irrigation by weighing individual pot at 9.00 H daily. The different treatment pots were randomized at each irrigation to avoid effects from other environmental factors, such as light conditions or temperature.

2.3. DATA

Data were recorded on number of grain per plant (NGP), one thousand grains weight (PMG), total leaf surface (TLS), plant height (HAT), stomatal density (DS), leaf water content (RWC), leaf water potential (LWP),

2.3.1. PLANT HEIGHT (HAT)

Plant height (cm) was determined using a graduated ruler (from the neck to the insertion of the ear).

2.3.2. TOTAL LEAF SURFACE (TLS)

All area leaves were estimated using Scion image software. The leaves were scanned using HP Scanner; the preliminary images were converted to from color to greyscale (selected from the output type menu). The final version was saved as a TIFF file. The leaf area (cm²) were calculated from the TIFF file using a public domain software (Scion image) as suggested by [20].



Table 1: Description of plant material used in this study.

Accession/Variety	Abbreviation	Description of agronomic characters
Ardhaoui	A	Six rows, local Tunisian barley landrace, grown in Southern Tunisia. It is characterized by its resistance to drought.
Kounouz	K	Six rows, Tunisian barley improved variety, registered in 2011, moderately precocious, productive and tolerant to fungi diseases
Manel	M	Six rows, Tunisian barley improved variety, registered in 1996, moderately precocious, productive and tolerant to fungi diseases
Rihane	R	Six rows, Tunisian barley improved variety, registered in 1987, moderately precocious and tolerant to drought and fungi diseases. It's now a widely grown variety (more than 40% of total barley cultivated areas in Tunisia).

2.3.3. STOMATAL DENSITY (DS)

A flag leaf was selected from each plant. The method to count stomata densities (stomata/mm²) began with the application of a thick layer of clear nail polish to the lower epidermis of each leaf. The nail polish was allowed to dry. A section of clear tape was firmly stuck to the section of nail polish then carefully peeled away from the leaf, leaving a leaf impression. The impression was then placed on a slide and viewed under 400X magnification of a light microscope. A representative section of stomata density was chosen and the stomata densities were counted under a photomicroscope system with a computer attachment (MPS 60, Leica, Wetzlar, Germany). Density stomatal was analyzed in the microphotographs with Image J 1.0 image processing software (National Institutes of Health, Bethesda, MD, USA).

2.3.4. YIELD PER PLANT (YGP)

The weight of gain per plant was measured by shelling mature ears and calculated individually (g).



2.3.5. THOUSAND GRAIN WEIGHT (PMG)

One thousand grain was counted for each treatment and weighted (g).

2.3.6. RELATIVE WATER CONTENT (RWC)

Percent of relative water content (RWC) was determined on flag leaf tissues excised in the morning (around 8:00 am). Excised leaves were measured for fresh weight (FW) and then rehydrated in a water- filled Petri dish at room temperature. Turgor weight (TW) was measured by allowing full rehydration (16 h), removing all water on the leaf surface, weighing, and then drying of leaves at 70°C for 48 h to determine DW [21].

The relative water content was calculated from the following equation:

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

2.3.7. LEAF WATER POTENTIAL (LWP)

The leaf water potential (ψ_f) of flag leaf (MPa) was measured at the abaxial surface of intact flag leaf with pressure chamber [22].

2.4. STATISTICAL ANALYSIS

Mean values were taken from measurements of 5 replicates and standard error of the means was calculated. Differences between means were determined by one-way ANOVA and Turkey's multiple range tests ($p < 0.05$). Analyses were done using the SPSS for windows (version 20.0).

The relationships between relative growth and the different variables examined were analysed by determining Pearson's correlation coefficients. Significance was determined using the Student t test.

3. RESULTATS

Drought stress induces many changes in barley morphology and physiology.

3.1. TOTAL LEAF SURFACE (TLS)

Leaf surface showed important variability among varieties and for the different water status of barley (fig.1). Leaf area was more important for genotypes which received a higher quantity of water. This result was verified for all varieties.

The variety, the irrigation treatment and their interaction showed significant effect ($P < 0.001$). In fact, the most adversely affected varieties are Rihane and Kounouz. These genotypes were lost about 50% of their leaf area when water stress was severe (T_3). In opposition, total leaf surface of local barley genotype (Ardhaoui) was only reduced by 27% for the same treatment.



3.2. STOMATA DENSITY (SD)

The stomata density of the lower leaf epidermis oscillated between 79 stomata/ mm² (Kounouz) and 125 stomata/mm² (Ardhaoui) for leaves issued from barley which received T₀ treatment (table 2). The effects of the variety and the irrigation treatment were significant on this parameter (P<0.001).

Water stress increased stomatal density for barley germplasm, but this increase is not uniform for all varieties. Indeed, Kounouz was the most affected: stomatal density was increased by 80% under severe treatment. Rihane variety seemed less impacted by water decrease.

3.3. PLANT HEIGHT (HAT)

Significant differences were observed for plant height among the genotypes (fig. 2). Kounouz variety (52 cm) was the tallest followed by Rihane and Manel varieties. Ardhaoui landrace attained minimum plant height (38 cm). Water stress treatments had significant effect on plant height of barley germplasm. The stem length decreased under water deficit conditions with significant differences among genotypes. Plant height for Rihane severely stressed was reduced by 40% significantly (p=0.01) than control plants while it decreased by 19% in Manel and Kounouz varieties. Ardhaoui landrace was the less affected (only 13% decrease).

A moderate water stress (T₁) did not affect or increased significantly plant height for all barley genotypes.

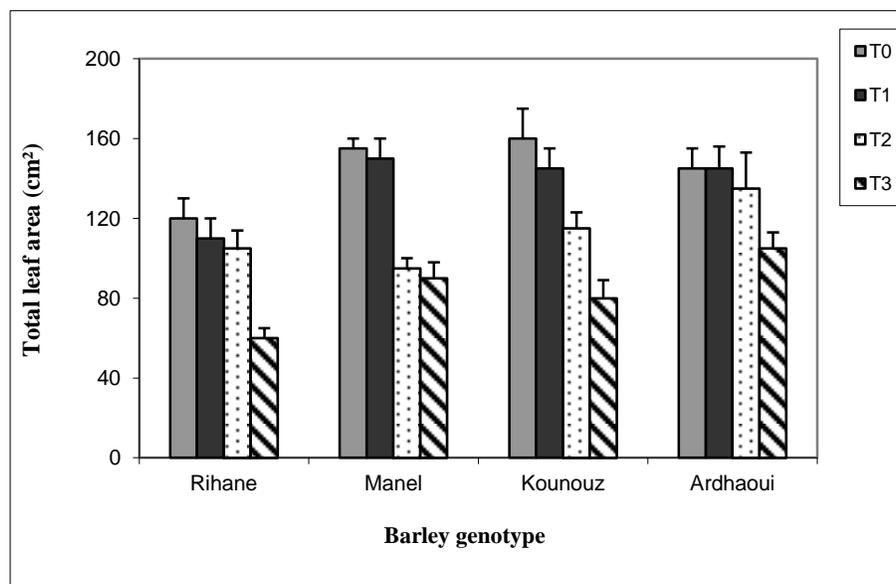


Fig.1: Total leaf area of barley genotypes in relation to drought treatments

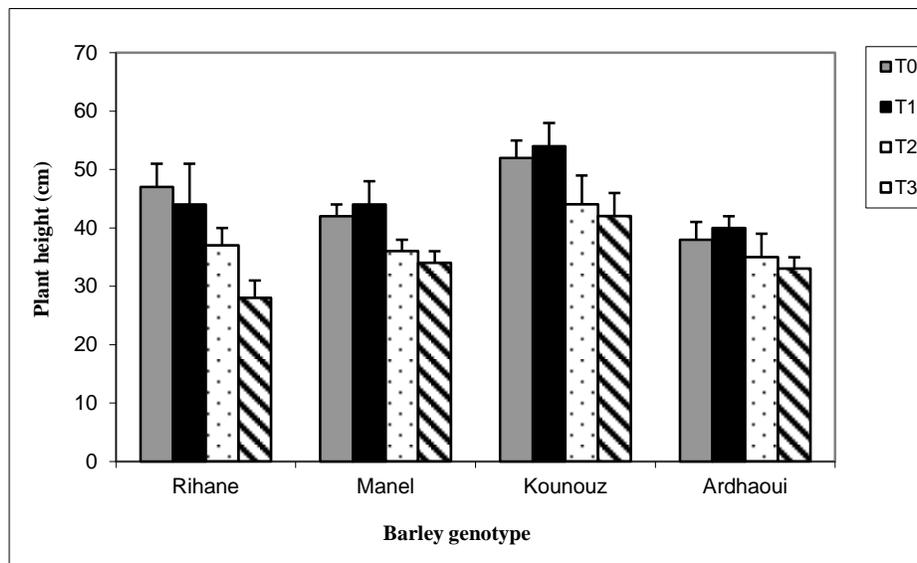


Fig.2. Plant height of barley genotypes in relation to drought treatments

3.4. YIELD PER PLANT (YGP)

Effects of water deficit on yield per plant of test barley cultivars indicated that this component was significantly decreased by water deficit treatments and the reduction increased with the increased water deficit (table 2). At higher water stress level, NGP of Rihane, Manel, Kounouz and Ardhaoui were reduced by 68, 48, 73 and 83% of control, respectively. But under moderate water stress (T_1), NGP for all genotypes were increased. The highest NGP (4.9 g) was recorded by Ardhaoui landrace.

3.5. THOUSAND GRAIN WEIGHT (PMG)

All barley genotypes exhibited a higher PMG under mild water stress (tab.2). Ardhaoui barley scored the superior weight of 1000 grains in T_1 treatment, followed by Manel variety at the same treatment, while Kounouz scored the least value in this trait in all water stress levels including control. When water deficit became severe, PMG of all barley genotypes decreased. The most affected was Kounouz.

Table 2: Stomatal density, yield per plant and thousand grain weight of the four barley genotypes under different water treatments

Parameters	Treatment	Rihane	Manel	Kounouz	Ardhaoui
Stomatal density (stomata/mm ²)	T ₀	96 a	112 a	79 a	125 a
	T ₁	104 a	129 a	121 b	146 b
	T ₂	121 b	162 c	125 b	158 b
	T ₃	121 b	175 c	142 b	171 c



Yield/Plant (g)	T ₀	2.2 a	2.1 a	1.1 a	2.9 b
	T ₁	2.5 a	2.4 a	1.4 a	4.9 a
	T ₂	1.4 b	1.2 b	0.4 b	0.9 c
	T ₃	0.7 c	1.1 b	0.3 b	0.5 c
Thousand grain weight (g)	T ₀	39.7 a	36.4 b	23.7 b	37.0 b
	T ₁	35.8 a	40.9 a	28.2 a	43.8 a
	T ₂	29.2 b	33.7 c	15.2 c	29.4 c
	T ₃	28.3 b	29.5 d	16.0 c	25.7 c

T₀ = 100% FC (control); T₁= 2/3 T₀; T₂= 1/3 T₀; T₃= 1/6 T₀; FC= field capacity

3.6. LEAF WATER POTENTIAL (LWP)

The leaf water potential of all barley genotypes was higher in control plants as compared to the three different treatments (fig.3). Water stress has reduced leaf water potential of 12, 48 and 74%, respectively, for T₁, T₂ and T₃ but under mild stress (T₁), LWP decrease wasn't significant. Effect of water deficit on leaf water potential of barley genotypes was significant (p=0.01). In fact, LWP in drought-treated varieties decreased significantly by 36, 57, 100 and 125% in the Manel, Kounouz, Rihane and Ardhaoui genotypes, respectively. Differences in the rate of LWP decrease among the genotypes allowed 2 groups to be defined. The first one was constituted by Manel and Kounouz which LWP decline was minor and the second group contained Rihane and Ardhaoui with LWP decrease was up to 100%.

3.7. RELATIVE WATER CONTENT (RWC)

RWC in flag leaves of control plants grown under well watered conditions (T₀) wasn't similar in all genotypes (varied from 80 to 91%), with statistical difference between them (Fig.4).

As water stress decreased to T₁, there was only a slight decline in RWC levels (average decline of 8%). Decline became important when stress was severe (21 and 27% respectively for T₂ and T₃) in comparison to control.

Like LWP, the differences in rate of water loss defined 2 homogeneous groups among the genotypes. Manel and Kounouz varieties had the lowest drop in RWC (average RWC decrease of 22%). While, Rihane and Ardhaoui possessed the highest water loss leaf tissues (up to 30%).

4. DISCUSSION

The barley genotypes showed differential response towards water stress and its impact on their morphological and physiological behaviour and performance. Differential response of barley varieties was also observed by [23], [24], [25], [26] and [27].

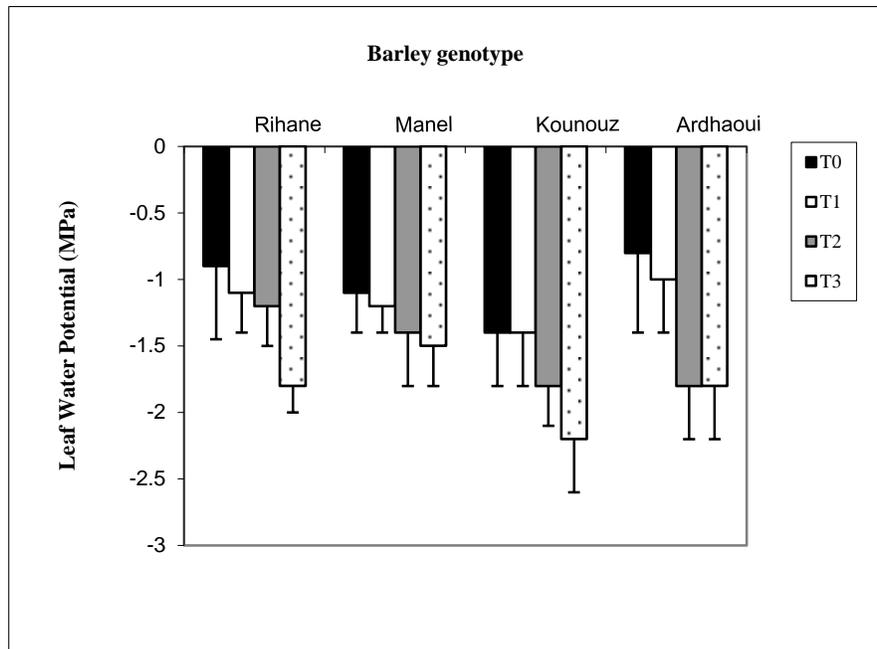


Fig.3: Leaf Water Potential of barley genotypes in relation to drought treatments

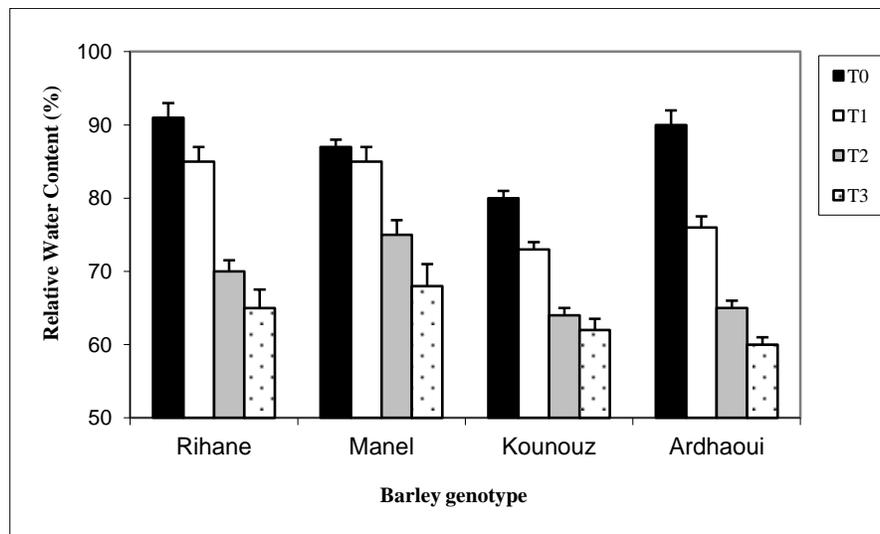


Fig.4: Relative Water Content of barley genotypes in relation to drought treatments

4.1. EFFECT OF WATER STRESS ON STEM HEIGHT

Drought stress reduces plant growth by affecting various physiological and biochemical processes such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters [28].



Plant height is a good indicator for determining the water stress [29]. Sammis et al. [30] reported that plant height can change depending on different level water deficiency. Under severe water stress, all barley height was reduced. This phenomenon was cited by several researchers on many crops [31], [32] and [33]. Decline in shoot length in response to drought might be due to either decrease in cell elongation resulting from the inhibiting effect of water shortage on growth promoting hormones which, in turn, led to a decrease in each of cell turgor, cell volume and eventually cell growth [34].

Moderate water stress had increased plant height. Similar result was found on sugar cane [35], on pearl millet [36] and on Foxtail Millet [37]. This may be difficult to explain due to the fact that control received excess water which caused oxygen deficiency that inhibited plant growth [38] and [39]. Excess water may also have some soil nutrients causing nutrient deficiency which reduced plant growth under such irrigation treatments.

4.2. EFFECT OF WATER STRESS ON TOTAL LEAF SURFACE

The effects of drought on leaf surface development are among the most cited in literature [40] and [41]. In fact, water deficit caused decrease in total leaf area. Reductions in leaf area during drought did not lead only to a reduced water loss [42] but also to a reduction in whole-plant carbon assimilation, and consequently reduced growth [34], [43] and [44].

4.3. EFFECT OF WATER STRESS ON STOMATA DENSITY

Stomatal density varied greatly among barley genotypes. Differences in stomatal characteristics among species, varieties and cultivars were reported in many studies [45] and [46]. It is clear that there is a strong relationship between environmental conditions and stomatal density, and the responses are variable between species [47].

The density of stomata is strongly influenced by water regime. It's found that increased water deficit causes an increase in stomata density at leaf surface of barley germplasm. For severe treatment, stomatal density of Kounouz variety has almost doubled. Similar result was cited by several authors [48] and [49].

Many reports showed an increase in stomatal density and a decrease in cell size under water deficit, indicating that an adaptation to drought could occur [50] and [48]. The compromise between stomatal size and density is related to the limitation of the leaf area allocated to stomata [43]. [51]and [52]suggested that smaller stomata are better at improving WUE, due to their more rapid response to changes in environmental conditions such as humidity [46].

[53]and [54] noted that great density with small size have been found to be a typical trait of species of xeric environments. We have obtained similar result for Ardhaoui landrace which is originated from arid region in south of Tunisia.



4.4. EFFECT OF WATER STRESS ON YIELD AND ON THOUSAND GRAIN WEIGHT

Drought can decrease both grain yield and quality of cereals [55] and [56]. Indeed, effects of water deficit on yield per plant and thousand grain weight of test barley cultivars were significantly decreased by water deficit treatments. The most affected was Kounouz. Reduction in the 1000 grain weight under drastic water stress can be attributed to low level of available water [57] causing low transition of photosynthesis matter and assimilates to kernels [29]. Other authors had shown that invertase is the enzyme responsible for the inhibition of grain filling during water stress [58].

Under moderate water stress (T_1), NGP and PMG for all genotypes were increased and Ardhaoui barley scored the superior YGP and PMG for this treatment. Similar results on effect of mild water stress on PMG and YGW were obtained on wheat [59] and [60].

4.5. EFFECT OF WATER STRESS ON WATER STATUS

Relative water content is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance.

Severe water stress has reduced leaf water potential and relative water content. Similar results were found by [31] and [61] on barley genotypes under water deficit. Water potential reduction is the result of a rapid osmotic adjustment and an increase of the concentrations osmotically [62] and [63].

Under moderate stress (T_1), LWP wasn't affected and RWC was declined slightly. Maintaining a high water content in the growing leaves and in leaves expansion in the presence of stress indicates osmotic adjustment effectiveness [64]. The osmotic adjustment (if any) results in a slower decrease of RWC when the leaf water potential continues to decline as observed by some authors on *T. durum* and *T. polonicum* [65].

Water status (LWP and RWC) had permitted to divide barley genotypes into two groups. The first one was characterised by minor decline in LWP and the lowest drop in RWC (Manel and Kounouz) and the second was constituted by Rihane and Ardhaoui that had the highest water loss leaf tissues and with LWP decrease was up to 100%. This group contained genotypes that had LWP of control below unity (ranging from -0.8 and -0.9 MPa).

When leaf water potential decrease was high (> 100%), varieties are characterized by adaptive osmotic adjustment type [66]. Both varieties (Ardhaoui and Rihane) are able to adjust their osmotic potential and, therefore, water potential depending on water availability in the soil. Important decline in LWP could indicate a drought tolerance [67] or sensitivity to dehydration [68]. Contrary, Manel and Kounouz varieties could have constitutive osmotic adjustment type permitting low decrease of LWP. Maintaining a high LWP under stress could indicate an avoidance strategy [69] that seems to be linked to morphological root characteristics (depth,



mass, ramification, volume) which optimize water absorption [70]. However, it is necessary to measure the osmotic potential of these accessions to confirm these results.

Previous studies had demonstrated that response of barley genotypes to drought stress were different [71],[72] and barley varieties capacity to cope with water deficit could show different strategies and mechanisms [73], [74] and [75].

To study four barley genotypes behaviour under drought, we have adopted one approach that consists to compare some morphological traits in relation to water status (LWP and RWC) of plant. Results showed that most of the genotypes tested exhibited tolerance to water deficit even under the most severe drought conditions imposed (T_3). However, some changes in different levels of adaptation to water deficit between the genotypes were noted. Indeed, Ardhaoui landrace that was the shortest genotype had the lowest height decline and was less affected in its total leaf surface. Moreover, Ardhaoui possessed the highest stomatal density in well watered treatment. Under severe water stress, it showed with Rihane the highest water loss leaf tissues and the highest leaf water potential decrease. This result was corroborated by de [61] but was in contradiction with the report of Thameur et al.[76] for two strains of cv. Adhaoui from the Switir and Tlalit regions of southern Tunisia.

5. CONCLUSION

Any drought tolerant mechanism would be welcome in the ongoing efforts to meet the challenge of global water deficits in crop production. In this research, it was demonstrated that barley germplasm studied under four water regimes had revealed a varietal effect of water stress and had permitted to divide barley genotypes in to two groups.

The first one contained Manel and Kounouz varieties which responded by a small reduction of leaf water potential, while maintaining a substantially higher RWC than other genotypes: decline LWP may in this case be the result of a significant reduction of the osmotic potential due to an accumulation of active solutes.

The second constituted by Rihane and Ardhaoui genotypes, was characterised by a significant reduction in LWP (> 100%) in parallel with important reduction in RWC. This might be due to a decrease of cell turgor, an absence of active osmotic adjustment and the presence of an adaptive osmotic adjustment.

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