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Carbon isotope discrimination and gas exchange parameters impairment under drought conditions in five barley (*Hordeum vulgare* L.) genotypes

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Abstract – Five barley genotypes were grown in a growth chamber under well-watered conditions up to early tillering and were then either subjected to water deficit or continually well-watered. Water deficit was induced by withholding irrigation until the field capacity (FC) was 40%, which was then maintained for 90 days. Water deficit improved the water use efficiency (WUE) of four genotypes (Ardhaoui (A), Pakistan (P), Manel (M) and Roho (RO)) by approximately 40%, but caused slight reduction in var. Rihane. High relative water content (RWC) of leaves was maintained in some of the genotypes by stomata closure and a reduction of leaf area. High Net photosynthetic rate under water deficit was associated with high RWC ($r=0.51$; $P<0.05$). Decline in Net photosynthetic rate was due mainly to stomata closure ($r=0.71$; $P<0.01$). $\text{WUE}_{\text{biomass}}$, $\text{WUE}_{\text{instantaneouos}}$ and $\text{WUE}_{\text{intrinsic}}$ were not directly associated, but $\text{WUE}_{\text{instantaneous}}$ and carbon isotope discrimination ($\Delta^{13}C$) were positively related ($r=0.71$; $P<0.01$) while $\text{WUE}_{\text{intrinsic}}$ was negatively related ($r=-0.58$; $P<0.05$) to intercellular CO_2 concentration (Ci).

Keywords: barley, drought; growth; leaf gas exchange; water use efficiency; carbon isotope discrimination**.**

1. Introduction

In areas with a Mediterranean climate, water is the main factor limiting crop production. In these areas rain is scarce, irregularly distributed and variable from one year to another. Due to these fluctuations in water availability, crop yield also varies from one year and region to another. Barley is the main cereal grown in these areas because it shows a natural drought tolerance when compared to other species (Landi 2017). However, its productivity is limited by terminal water stress and high temperatures during grain filling. In these conditions plant breeders generally look for genotypes which have good potential yield, and morpho physiological characteristics which favor drought tolerance (Blum et al. 1983). The plants which can sustain higher carbon assimilation while keeping almost lower transpiration or higher water use efficiency (WUE), would be of higher value under dry condition particularly if the water resources were limited (Moud et al. 2007). During drought stress and the whole growing season, WUE is higher in drought-treated compared to control plots, so it seems possible to save water without loss of biomass (Korup et al. 2018). Most research has been directed to find out traits that can provide easier measurements of WUE. Farquhar et al. (1982) found that the extent to which C3 plants discriminate against ^{13}C during carbon assimilation was related to their WUE. It has been shown that carbon isotope discrimination in C3 plants is inversely related to the instantaneous water use efficiency ($\text{WUE}_{\text{instantaneous}}$). Passioura (1994) attributed the variation to be due apparently to confounding effects of a large variation in vapor pressure deficit during the season or possibly diurnally. Measurements of ^{13}C discrimination are a very expensive technique to use in a large screening trial (Hall et al. 1997). Specific leaf area (SLA) is another trait that has been investigated in recent studies due to the independent associations of WUE and SLA with leaf photosynthesis (A). Lower SLA is associated with enhanced water-use efficiency under water stress in grassland species and thus can be seen as a strategy of phenotypic adjustment (Wellstain et al. 2017). In barley, however, no such association has been reported. The leaf being the first organ to show visible signs of drought may provide a cheap and easy to manipulate trait for selection under water deficit. The aim of this study, therefore, was to investigate any direct relationship between WUE and leaf gas exchange or leaf area of barley cultivated under drought. The purpose here is to explore further the utility of δ^{13}C as a physiological integrator. Specifically, the aims are to (1) measure the variations in shoot δ^{13}C in genotypes of one species (*H. vulgare*) in relation to experimentally imposed drought stress; (2) correlate these measurements with stress tolerance through SLA, leaf area measurements, leaf photosynthesis and WUE; and (3) assess the potential usefulness of Δ^{13} C as an integrator of stress responses. To this, we investigated genotypic variations in five diverse genotypes of barley selected to reflect major regions of production.

2. Material and Methods

2.1. Plant material and location

The experiment was conducted in a glasshouse located 20 km south-east of Medenine at the institute of Arid Regions. The climate is Mediterranean, with hot, dry summers and mild winters. Barley is a typical crop of marginal areas in North Africa. Most farmers in the marginal areas use their own varieties of barley (landraces). In this sense, the present study is carried on cv. Ardhaoui (six-row), the local barley cultivar of the south of Tunisia known for its tolerance to drought (Thameur et al. 2008) to be compared with two barley (*Hordeum vulgare* L.) cultivars and three varieties differing for yield potential and drought tolerance. Pakistan cv: Introduced from Pakistan. Six-rowed barley, high yield in water and salt stress conditions; (3) Roho: Introduced from Denmark. Very well adapted to low rainfall regions (200 à 300 mm) (Deghais et al. 2007); (4) Rihane: Adapted to semi-arid climate. (Deghais et al. 2007); (5) Manel: Recommended especially in humid and subhumid climates (Deghais et al. 2007). The seeds of cv. Ardhaoui were collected from Tlalit located in Tataouine. National Institute of Agronomic research of Tunisia (INRAT) provided the seeds of var. Rihane, Roho and Manel. The agricultural training center of Medenine provided the cv. Pakistan seeds. Crops were grown in a glasshouse equipped with a ventilation system, so that temperature was about 25/18 °C (day/night) and the relative humidity of 70/80 % (day/night). Single-plant-pots 30 cm long by 10 cm diameter were used for the experiment. The pots had a volume of 5 l each, which were filled with sandy soil with a mixture of nutrient mineral (N-P-K) 120-120-150. The five genotypes were sown in completely randomized block design. When half of the genotypes were at start of tillering (stage 13, 21 on the Zadoks scale, Zadoks et al. 1974), water was withheld in the water stress treatment until it reached 40% of field capacity (FC). The control treatment pots were irrigated every day in order to keep them at 100% of FC. The control of added water was monitored by weighting the pots. The water content of the substrate at field capacity determined by weighing the pots after the pots were flushed with water and allowed to drain overnight.

2.2. Experimental procedure

Two seeds were sown per pot. Shortly after emergence pots were thinned to one uniform plant/pot. The plants were divided into two groups of five each designated as well-watered (ww) and water stress (ws), respectively. Control plants of each genotype were maintained up to maturity under well-watered conditions. Irrigation was monitored daily through weighing the pots in order to regulate the water supply for the five replicate pots.

2.3. Water status

Relative leaf water content was determined periodically in control and water-stressed plants using flag leaves according to Turner (1981). After sampling, leaf fresh weights (FW) were determined, and then leaves were hydrated until saturation in distilled water for 24 h at 4 °C. Once surface dried, leaves were reweighed to obtain leaf turgid weights (TW). Subsequently, leaves were oven dried at 70 °C for 48 h and their dry weights (DW) were determined. Leaf RWC was calculated following the formula:

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

2.4. Growth and leaf parameters

Total biomass was collected after anthesis. The flag leaf and the rest of the plant shoot were separated and oven-dried at 80°C for 48 h before being weighed and powdered. Plant height was measured at 90DAT. leaf area ratio; specific leaf area and leaf area were calculated as follows:

$$(1)\ \text{LAR} = \text{leaf area}/\text{total plant dry mass}$$

$$(2)\ \text{SLA} = \text{leaf area}/\text{leaf dry mass}$$

$$(3)\ \text{LA} = (0.7624 * L*l) + 08841 \text{ (Haouala 1999).}$$

Where L was the length; l was the wide and LA was the leaf area.

2.5. Nitrogen content

Leaf samples were analyzed for total N concentration using semi-micro Kjeldahl distillation (Tecator Kjeltec Auto 1030 Analyzer, Hogana, Sweden).

2.6. Leaf gas exchange measurements

Measurements were taken on the upper surface of leaves on sunny days in both the watered and stressed pots. Measurements were taken between 9 and 10 h. Net photosynthetic rate (A), stomatal conductance (gs), transpiration rate (E) and (Ci) were measured on sun-exposed and fully mature leaves, using a portable LCi IRGA (LCi, IRGA; ADC Bioscientific Ltd.) with five replications per treatment. The leaf chamber temperature was kept at 25 °C, photosynthetic photon flux density (PPFD) of 1200 µmol photons m^{-2} s^{-1} and relative humidity of 60–70%.

2.7. Water-use efficiency

WUE was calculated using both instantaneous and time integrated measurements. The time-integrated WUE, $\mathrm{WUE_{biomass}}$, was calculated as the ratio of total aboveground biomass (AB) to evapotranspired water throughout plant growth. Moreover, instantaneous WUE ($\mathrm{WUE_{instantaneous}}$) and intrinsic WUE ($\mathrm{WUE_{intrinsic}}$) were calculated as the ratio of A to E and gs, respectively as shown below:

$$\mathrm{WUE_{intrinsic}} = \frac{A}{gs};\ \mathrm{WUE_{instantaneous}} = \frac{A}{E};$$

Where A is the net photosynthetic rate and E and gs are the transpiration rate and the stomatal conductance, respectively.

2.8. Determination of cumulative transpiration

The amount of water evapotranspired was monitored throughout the experiment by weighing each pot just prior to watering. The pots were then adjusted to their water regime (40 and 100% FC) by adding water to maintain the experimental design. Simultaneously, empty pots without plants were also weighed to record direct evaporation from the soil. Then, the cumulative transpiration (Tcum) was calculated as the difference between evapotranspiration and evaporation.

2.9. Stable isotope discrimination

For each analysis, 2 mg of fine powdered shoot were weighed in tin cups. A 20:20 isotope ratio mass spectrometer (IRMS, Europa Scientific Ltd) linked to the elemental analyzer was also used to analyze ^{13}C:^{12}C ratios (R) of shoots. Results were expressed as $\delta^{13}C$ values, using a secondary standard calibrated against Vienna Pee Dee Belemnite calcium carbonate.

$$\delta^{13}\mathrm{C}\ (‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000$$

The carbon isotope discrimination ($\Delta^{13}C$) of shoots was calculated from δa and δp (Farquhar et al. 1989) as:

$$\Delta^{13}C = \frac{\delta_{a-}\delta_{p}}{\delta_{p} + 1}$$

Where a and p refer to air and the plant, respectively. Air samples inside the greenhouse were taken and analyzed by the Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS) technique, as previously described in Nogués et al. (2004).

2.10. Experimental design and statistical analyses

Plants were distributed in a single block with 10 plants per variety in each one (5 control and 5 drought-stressed plants). Five plants per treatment were used for growth and gas exchange parameter measurements. Analysis of variance and comparison of mean were realized using the Duncan's test ($P < 0.05$). Statistical analyses were performed using SPSS 16.

3. Results and Discussion

3.1. Relative leaf water content

Under well watered conditions, no significant difference was found between genotypes. Under drought, the RWC differed significantly (P<0.001) amongst the genotypes at 60 DAT. Under well watered conditions, RWC was higher in var. Rihane and cv. Ardhaoui (Table 1). Leaf RWC was maintained regular (88–96%) in well-irrigated plants during the period of the study (Table 1). Drought conditions also led to a decrease in RWC. The treatment of water stress reduced the leaf RWC and significant differences were observed. At the end of the stress period, values of leaf RWC were 93.3, 85.8 and 77.8% in cv. Ardhaoui, var. Roho and var. Manel, respectively.

Table 1. RWC of leaves of five barley genotypes (Ardhaoui (A), Pakistan (P), Manel (M), Roho (RO)) on day 60 of water deficit maintained at 40 %FC.

Genotype	Well-watered (%)	Water stress (%)
A	95.3±1.1a	93.3± 0.7(2)a
P	90.0±1,4a	83.5± 0.5(7)b
R	96.0±0.9a	85.8± 0.5(11)b
M	94.7±0.7a	77.8± 0.9(18)c
RO	88.1±2.2a	85.8± 0.5(3)b

Means followed by the same letter within a column are not significantly different as determined by Duncan's test (P≤0.05; n=3). Values in parenthesis indicate reductions (%) due to drought stress.

3.2. Crop growth and leaf area parameters

Drought reduced progressively height after the onset of water deprivation (Table 2). At the end of the drought period, plant height was 24% lower than those of control plants. Leaf area increased similarly in control and drought-stressed plants at the beginning of stress imposition. Afterwards, leaf area was reduced (Table 2). At 60 DAT, leaf area was 24% lower on average in drought stressed than in watered plants. Total leaf area was highest in vars Manel and Rihane and lowest in others (Roho, Pakistan and Ardhaoui) (Table 2). Water stress reduced plant height, SLA and LAR on the average by 17–70% (Table 2) and leaf area was affected by a reduction of 7–44%. Genotypic response to water deficit stress in SLA and height were almost the same with cv.Ardhaoui, cv. Pakistan and var. Rihane displaying a low reduction of about 19 and 16% respectively. Var. Manel was much more susceptible for LAR with 75% reduction. Worth mentioning are the genotypes Rihane which had the highest leaf area under well-watered conditions experienced slight reduction (9%) and maintained absolute high leaf area during stress. In addition the chlorophyll (SPAD) content of the flag leaves of the drought treatment was 7% lower than that measured in the control treatment (Table 2). Cv.Ardhaoui maintained the highest values and Roho the little ones. The other genotypes were intermediate.

Table 2. Specific leaf area (SLA), Leaf area (LA), chlorophyll content (SPAD units), Plant height (H) and leaf area ratio (LAR) in fully expanded leaves of barley genotypes (Ardhaoui (A), Pakistan (P), Manel (M), Roho (RO)) at 60 days after treatment. Treatments were: irrigated plants (100%FC) and drought stressed plants (40%FC). Data are means± S.E and each value was determined with three replicates per treatment (n = 3). For each water regime, dissimilar letters above columns differ significantly at ($P <0.05$) by Duncan's test.

Parameter	Genotype	Water regime	
		100%CC	40%CC
SLA (cm^2 g^{-1})	A	476.8±4.2a	369.9±4.4a
	P	406.3±2.8ab	343.8±3.3b
	R	380.5±3.1b	313.0±2.4d
	M	342.8±2.4b	311.5±4.0d
	RO	404.3±2.9ab	320.7±2.8c
LA (cm^2)	A	23.7±1.2b	13.2±1.1b
	P	27.2±1.2b	19.2±1.1b
	R	30.8±0.7a	28.1±1.1a
	M	29.9±2.1a	18.0±1.4b
	RO	13.9±1.1b	12.8±0.9b
Chlorophyll content (SPAD units)	A	43.30±0.86a	43.08±0.57a
	P	45.62±0.51a	41.05±0.76ab
	R	43.52±0.78a	39.53±0.87ab
	M	41.22±0.71a	38.18±0.89b
	RO	46.32±0.86a	41.87±0.56ab
Height (cm)	A	53.17±0.46c	39.75±1.14a
	P	51.75±0.76bc	45.80±0.71a
	R	54.92±0.74ab	48.83±0.77a
	M	56.42±0.70a	47.00±0.69a
	RO	53.92±0.97b	53.42±0.74a
LAR (cm^2 g^{-1})	A	0.064±0.003 a	0.019±0.002 ab
	P	0.072±0.004 a	0.020±0.003 b
	R	0.091±0.005 a	0.027±0.003 a
	M	0.083±0.006 a	0.021±0.002 b
	RO	0.049±0.006 a	0.018±0.003 c

Means in the same column with the same letters (a–c) are not significantly different by Duncan's multiple range test (DMRT) (at $P < 0.05$).

3.3. Leaf gas exchange parameters

Water deficit caused a sharp decline of all gas exchange parameters. Net photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs) and internal CO_2 concentration (Ci) displayed a similar pattern while the patters of $WUE_{instantaneous}$ was similar but inverse. Well-watered plants of cv.Ardhaoui had clearly the highest Net photosynthetic rate, while vars. Manel and Roho were the lowest (Table 3). Amongst the intermediary ones, cv. Pakistan displayed medium high values. The decline in net photosynthetic rate of the genotypes was correlated with decline in water content of leaves ($r=0.51$; $P<0.05$). Two of the genotypes (A and R) that were able to maintain high water status on day 10 of stress also had highest photosynthetic rates. With regard to A on day 60 under stress, the genotype cv. Ardhaoui and var. Rihane were outstanding for good performance ($A \geq 5$ µmol CO_2 m^{-2} s^{-1}), whereas Manel, Roho and cv.Pakistan were poor. Genotypic responses of E and gs were very similar to A on day 60 under water stress. On day 60 under stress, Ci of all genotypes was reduced which corresponded with their low A values, whereas cv. Ardhaoui had relatively low ci, but higher A values (Table 3).

Table 3. Mean values for gas exchange parameters and Kjeldahl nitrogen of five barley genotypes (Ardhaoui (A), Pakistan (P), Manel (M), Roho (RO)) subjected to different water regimes (WR). Data are the mean of 3 replicates. Within each water regime (40 and 100% FC) or genotypes (Ardhaoui (A), Pakistan (P), Rihane (R), Manel (M) and Roho (RO)); values with different letters are significantly different according to the Duncan's test (P <0.05).

WR	N	E	A	gs	C_i
100%CC					
A	3.465a	7.45a	13.45a	0.14a	193.45a
P	3. 696a	4.4bc	10.4b	0.13a	160.35c
R	2.444b	5.3b	12.0ab	0.14a	100e
M	2.567b	3.63bc	7.63c	0.11a	114.6d
RO	2.512b	3.29c	6.29c	0.14a	180.29b
40%CC					
A	3.948a	3.26a	6.26a	0.10a	150.26a
P	3.984a	2.4ab	3.4b	0.07b	132.37b
R	3.433c	2.44ab	5.44a	0.04c	80.44d
M	3.930a	1.38b	2.34b	0.01c	99.34c
RO	3.633b	2.16ab	2.07b	0.04d	100.07c
Analysis of variance					
Genotype	***	***	***	**	***
Treatment	***	***	***	***	***
G x T	***	***	ns	ns	***

Within each column, different letters indicate significant differences at P <0.05 (Duncan's test). n.s., * and ** indicate non-significant or significant differences at P < 0.05 or 0.01 respectively. N: leaf nitrogen content (%); A, net photosynthetic rate CO_2 ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$); gs, stomatal conductance ($mol\ CO_2\ m^{-2}\ s^{-1}$); E, transpiration rate ($mmol\ H_2O\ m^{-2}\ s^{-1}$); Ci intercellular CO_2 concentrations.

3.4. Stable isotope discrimination and water use efficiency

3.4.1. Plant leaf Δ^{13}C

An initial approach to study the effect of dryland conditions on Δ ^{13}C was made by analysing leaf samples from genotypes whose physiological behaviour and yield potential under drought had been evaluated previously (Deghais et al. 2007). Analysis of Δ ^{13}C for the cultivars was performed on leaves collected from different replications and analysis of variance could not be made for estimating genotypic variance. Under drought conditions, there was a decrease in Δ ^{13}C values. This change in C-isotope discrimination followed the decrease in stomatal conductance, transpiration and photosynthesis under water deficit.

Leaves of all 5 barley genotypes grown under drought were enriched with ^{13}C compared to control plants (i.e., lower Δ^{13}C values) (Table 4). Under well-watered conditions, the Δ^{13}C had a range of 1.18 ‰ (16.82 − 15.64 ‰) whereas the range for Δ^{13}C reduced to 1.76% (21.83 − 20.07 ‰) under water-stressed conditions. Drought produced a significant ($P < 0.001$) decrease in Δ^{13}C of all cultivars under water stress regime as shown in Table 4. Carbon isotope discrimination (Δ^{13}C) of the different cultivars did not differ significantly under dry conditions. Using comparisons of means, we found differences between barley genotypes in control conditions, with two significantly different groupings (Table 4). Δ^{13}C is an integrated value for carbon metabolism over the whole development of these young barley plants. On the contrary, measurements of gas exchange are instantaneous in nature. We studied the relationship between Δ^{13}C and the gas exchange parameters to assess whether intensive instantaneous measurements may be replaced by easier measurements of carbon isotope discrimination. We found no significant correlation between Δ^{13}C and any of the gas exchange parameters for plants grown under control and drought conditions. When the two water treatments were compared, higher Δ^{13}C values in plant dry matter were found in control plants (Table 4). The treatment had a significant effect on Δ^{13}C. However, no significant difference between genotypes was observed under stress conditions (Table 4). Eventhough, the varieties Manel, Roho and Rihane showed the highest reductions (19, 20 and 15 % respectively) of Δ^{13}C values, under water stress conditions, whereas the cultivars Ardhaoui and Pakistan showed the lowest ones (14 and 12 % respectively). No significant interaction between genotype and water regime for Δ^{13}C analysed was found (Table 4). Under well watered conditions, the highest values (20‰) were recorded in Manel and Rihane. The least values were recorded in Ardhaoui, Roho and Pakistan (19, 19, 18 ‰). Drought decreased Δ^{13}C to 16 ‰ in Ardhaoui, Manel, Pakistan and Roho and to 17 ‰ in var. Rihane. In C3 plants, drought tolerance is generally associated to high Δ values in leaves and grain (Monneveux et al. 2005). $\Delta^{13}C$ was found to be positively correlated with A, gs and ci. A positive relationship was found between $\Delta^{13}C$ and $\mathrm{WUE}_{\mathrm{instantaneous}}$ (r=0.71: P<0.01) under drought.

Table 4. Mean values for water-use efficiency (WUE): (i) measured from the biomass accumulated and total water transpired ($\mathrm{WUE}_{\mathrm{biomass}}$); and (ii) calculated from the gas exchange measurements ($\mathrm{WUE}_{\mathrm{intrinsic}}$ and $\mathrm{WUE}_{\mathrm{instantaneous}}$) and carbon isotope discrimination of the shoot ($\Delta^{13}C$) of five barley genotypes (Ardhaoui (A), Pakistan (P), Manel (M) and Roho (RO)) subjected to different water regimes (WR).

WR	$\mathrm{WUE}_{\mathrm{bio}}$	$\mathrm{WUE}_{\mathrm{int}}$	$\mathrm{WUE}_{\mathrm{inst}}$	$\Delta^{13}C$
100%CC				
A	1.37a	105.22a	1.81b	19.06±0.61ab
P	1.74a	77.96ab	2.43a	18.16±0.49b
R	5.45a	87.76ab	2.31ab	19.72±0.28a
M	2.23bc	67.15bc	2.16ab	19.74±0.32a
RO	3.37b	44.12c	1.97ab	19.43±0.56ab
40%CC				
A	2.6b	59.68b	1.98ab	16.43±0.30a
P	5.45a	50.66b	1.48bc	16.02±0.50a
R	5.40a	155.33a	2.40a	16.82±0.47a
M	3.29ab	186.43a	1.72ab	15.93±0.50a
RO	4.08ab	49.13b	0.95c	15.64±0.40a
Analysis of variance				
Genotype	*	***	**	ns
Treatment	**	*	**	***
G x T	ns	***	*	ns

Within each column, different letters indicate significant differences at P <0.05 (Duncan's test). n.s., * and ** indicate non-significant or significant differences at P < 0.05 or 0.01 respectively.

3.4.2. Plant biomass and water use

With or without stress, total plant biomass was significantly different among the genotypes on day 90 of water deficit treatment. Under well-watered conditions, the three high productive genotypes (A, P and R) were superior in biomass production while M and RO were the low productive ones (Table 5). Water deficit treatment reduced mean water use by 47%, (range 24–77% across the genotypes, Table 5). This triggered diverse reaction among the genotypes with the biomass strongly reduced in the highly productive, non-stressed genotypes (P and R), as well as in the low productive RO by >20%, while in A and M reduction was about 21 and 18 % respectively. A 47 % mean reduction of water use during drought caused a mean decline of 8 % in the RWC of the genotypes with var. Rihane and var. Roho showing the least decline of 2.13 and 2.66 % respectively while var. Manel declined most with≥17% (Table 5). cv. Ardhaoui was intermediate with a reduction of 11%. Variability in water use was not correlated with variability in RWC.

Table 5. Mean values for water-use (T_{CUM}) and total biomass (TB) of five barley genotypes (G) Ardhaoui (A), Pakistan (P), Manel (M) and Roho (RO) subjected to different water regimes (WR).

WR	parameter	
	T_{CUM}	TB
100%CC		
A	11.90a	16.27a
P	9.4b	16.21a
R	3.3d	16.77a
M	6.9c	15.23b
RO	4.5d	14.71b
40%CC		
A	5.0a	12.86a
P	2.13b	11.33b
R	2.5b	12.05ab
M	3.90ab	12.41ab
RO	3b	11.19b
Treatment	***	***
Genotype	***	***
G x T	***	**

Within each column, different letters indicate significant differences at P <0.05 (Duncan's test). n.s., * and ** indicate non-significant or significant differences at P < 0.05 or 0.01 respectively.

3.4.3. Water use efficiency

Amongst the well-watered plants, cv. Pakistan was the most efficient one in gas exchange while $WUE_{instantaneous}$ of cv. Ardhaoui was the lowest. R, M and RO were intermediate (Table 4). Under water stress, Rihane clearly distinguished itself as the most efficient genotype on day 60 of stress (Table 4). Two other genotypes (A and M) displayed fairly moderate $WUE_{instantaneous}$ while the rest of genotypes maintained very low efficiency due to effects of the imposed water stress. $WUE_{biomass}$ of the genotypes under stress varied appreciably in their reaction. Water deficit improved the efficiency of two genotypes (A and P) by approximately 58% (Table 4). WUE of the two poorest genotypes (M and RO) was enhanced by 25%, whereas values of the best genotype (R) under well-watered conditions were more or less stable under stress. Water stress led to a reduction in A, E, gs, A/E and $\Delta^{13}C$ but to an increase in A/gs. Under dryland conditions, there was a decrease in $\Delta^{13}C$ values while plant water stress was increasing (Table 4). This change in C-isotope discrimination followed the decrease in stomatal conductance, transpiration and photosynthesis under increasing water deficit previously reported (Lopez et al. 1995).

The relative leaf water content was hardly reduced in var. Manel while cv. Ardhaoui maintained relatively high RWC under drought (Table. 1). Thus, the reduction of stomatal conductance and the minor diminution of leaf RWC could indicate that barley plants show a dehydration avoidance mechanism to maintain a favorable water status involving stomatal closure in response to water stress. Under water deficit stress, leaf area was sharply reduced due to a combination of leaf growth reduction and abscission. The two genotypes (cv.Ardhaoui and var.Manel) with the highest decline in leaf area under stress also experienced similar decline in total biomass revealing that leaf area reduction also affected the biomass produced. Loss of leaves and reduced expansion of younger leaves caused a decrease in the LAR in the stressed plants. The responses of the SLA to water stress showed much genotypic variability with cv.Ardhaoui and var.Roho reduced their SLA more than 20% under stress. In relation to plant growth, plant height (Table 2) experienced a significant reduction (25%) and a major decrease in cv.Ardhaoui. Erice et al (2010) reported a decrease in SLA in stressed plants of alfalfa genotypes. Kadam et al (2015) reported that wheat cultivars followed a water-conserving strategy by reducing specific leaf area and developing thicker roots and moderate tillering. The genotypic and drought induced variability in leaf area, LAR and SLA in the present study were interrelated but were not closely associated with biomass production or water use (data not shown). Moreover, no correlation between these parameters with WUE was found. These findings are in accordance with those of Ismail and Hall (1992) for cowpea. It is a well-established fact that photosynthesis is closely related to dry matter production in most crops, and plant responses and adaptation to biotic and abiotic stresses is reflected in changes in their photosynthetic rates. Gas exchange measurements made in this study permitted estimation of various parameters of photosynthesis. The mean A value under well-watered conditions was 10 μmol CO_2 m^{-2} s^{-1}. In Cereals, various reports showed a decrease of gas exchange parameters to reduce water loss under different water regimes (Gonzalez et al. 1999). Decrease in photosynthesis of barley due to senescence effects has previously been reported. Under well-watered conditions, genotypic differences in photosynthetic rates were correlated with differences in transpiration rate (r= 0,915; P<0.01; Table 6).

Table 6. Correlation coefficients of the gas exchange parameters with biomass and leaf growth parameters for all the water regimes and genotypes together.

100%CC	E	A	gs	ci	TB	TCUM	N	RWC
E	1							
A	0.91**	1						
gs	0.43	0.35	1					
ci	0.27	0.05	0.22	1				
TB	0.59*	0.83**	0.22	-0.24	1			
TCUM	0.39	0.36	-0.13	0.58*	0.15	1		
N	0.32	0.41	0.09	0.55*	0.32	0.85**	1	
RWC	0.22	0.26	-0.03	-0.18	0.40	0.12	0.002	1
40%CC								
E	1							
A	0.79**	1						
gs	0.76**	0.71**	1					
ci	0.40	0.32	0.81**	1				
TB	-0.19	0.20	0.07	0.15	1			
TCUM	-0.31	-0.01	0.11	0.30	0.84**	1		
N	-0.31	-0.01	0.11	0.30	0.84**	1.00**	1	
RWC	0.33	0.51*	0.24	-0.28	-0.11	-0.19	-0.67**	1

Linear correlations were calculated within each water regime using genotype means (n=3), *P <0.05, **P <0.01.

Differences between genotypes in transpiration on the other hand may have resulted mainly from differences in leaf area development and stomata regulation. These observed relationships explains why the most photosynthetically active genotype (cv.Ardhaoui) under well-watered conditions also transpired more water, maintained more opened stomata and its water use was the highest in the genotypes (Table 5; 6). The responses to drought stress of 5 barley genotypes from Tunisia which included 3 genotypes from the north of Tunisia, the local barley cultivar from the south and cv. Pakistan from Pakistan. The most susceptible genotype was var. Manel. The $\delta^{13}C$ data of these genotypes under drought also showed differences, with Manel and Roho having less negative than others. Water stress treatment (40% FC) resulted in a significant ($P < 0.001$) decrease in $\Delta^{13}C$, which indicates that imposition of drought leads to less discrimination against the heavier isotope. A decrease in $\Delta^{13}C$ due to drought has been reported for many plant species. However, significant cultivar variations in $\Delta^{13}C$ were observed under drought. Large genotype variation was observed for $\Delta^{13}C$ of flag leaves and kernels in bread wheat and barley grown under Mediterranean conditions (Merah et al. 2001). Leaf carbon isotope discrimination ($\Delta^{13}C$) decreased with increasing water deficits (Table 4).

The nitrogen content of the leaves of stressed plants was 2.74% and that of the unstressed plants was 3.78%. This observed increase in the percentage nitrogen content of the leaves of the plants as a result of water stress may be due to the mobilization of nitrogen to the leaves for the synthesis of special protein in plants as a mechanism to withstand the effect of water stress. High tolerance to drought stress in barley is a constitutively controlled trait regulated by the rate of protein synthesis and their activity level (Golebiowska-Pikania et al. 2017). Increased nitrogen content in plants in response to water stress has been reported (Inclan et al. 2005; Xu and Zhou 2005). Increasing drought affects water use efficiency and $\Delta\ ^{13}C$ through a decrease in stomatal conductance. Our results support the view that the nature of the relationship of $\Delta\ ^{13}C$ with biomass (Table 7) may be affected by growing conditions other than the water regime imposed during growth.

Table 7. Correlation coefficients of carbon isotope discrimination with biomass and water use efficiency for all the water regimes and genotypes together.

100%CC	WUE_{bio}	$\Delta^{13}C$	TB	TCUM	WUE_{ins}	WUE_{int}
WUE	1					
$\Delta^{13}C$	0.31	1				
TB	0.35	-0.23	1			
TCUM	-0.79**	-0.41	0.15	1		
WUE_{ins}	0.033	-0.15	0.14	-0.045	1	
WUE_{int}	-0.002	0.04	0.66**	0.50	-.064	1
40%CC						
WUE	1					
$\Delta^{13}C$	0.23	1				
TB	-0.28	0.26	1			
TCUM	-0.66*	0.11	0.84**	1		
WUE_{ins}	-0.10	0.71**	0.51	0.27	1	
WUE_{int}	0.06	0.15	0.21	-0.044	0.49	1

4. Conclusion

The studies evaluating the role of $\Delta^{13}C$ in breeding of cereals for drought conditions are more and more abundant (Araus et al. 2003). Leaf carbon isotope discrimination ($\Delta^{13}C$) decreased under water deficits (Table 4) and reduction in $\Delta^{13}C$ values was related to decreases in A, E and gs. In this study, carried out under controlled conditions, the relationship between $\Delta^{13}C$ and the photosynthetic water-use efficiency ($WUE_{intrinsic}$) consistent with theory (Farquhar et al., 1982) was found when considering genotypic variation under 40% FC (Table 4). More research is required for identifying the factors affecting the association between $\Delta^{13}C$ and $WUE_{intrinsic}$ as expected on the basis of theory, when considering the genotypic variation in particular environments. The positive and significant correlation between $\Delta^{13}C$ and A ($r=0.66$; $P<0.01$) or $WUE_{instantaneous}$ ($r=0.46$; $P<0.05$) contradicts previous reports in other crops studied C-3 grasses (Martensson et al. 2017). Genotypic differences in $\Delta^{13}C$ can be attributed to variation in photosynthesis and/or conductance to the diffusion of CO_2 to the sites of carboxylation (Farquhar et al. 1982) which is mainly controlled by stomata. In this report, A and gs had a main effect on $\Delta^{13}C$ when considering together genotypic variation under different water availability. In wheat, different levels of N had small and variable effects on $\Delta^{13}C$ while water stress induced large changes in $\Delta^{13}C$ (Condon et al., 1992). Genotypic variation in relative water content (RWC) was correlated with $\Delta^{13}C$ values ($r=0.49$; $P<0.01$). A previous report on beans (White et al. 1990) showed that cultivar differences in $\Delta^{13}C$ under drought might result from differential rooting capacity: higher water uptake through an extended root system in some cultivars might improve plant water status under drought, leading to the maintenance of gas exchange and $\Delta^{13}C$. Studying different crops under wet and dryland conditions, genetic differences in $\Delta^{13}C$ among species were found and water availability was considered a main factor controlling $\Delta^{13}C$ (Knight et al. 1994). Relative water content may be considered both, an indicator of the available water in the soil and the capacity of the crop for water acquisition. The close association found between RWC and $\Delta^{13}C$ may be indicative of the main role of leaf-water status in determining discrimination capacity of barley genotypes under dryland condition.

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