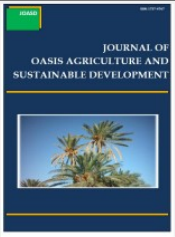

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Assessment of the physio-biochemical performance of Tunisian barley landraces under deficit saline-irrigation during grain filling stage

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Abstract

Salinity is one of the main and important abiotic stresses that adversely affects crop growth, development and production. In this study, two barley (*Hordeum vulgare* L.) landraces were subjected to three treatments of deficit saline-irrigation (12 dS/cm) (T0 = 100%ETc, T1 = 75%ETc, and T2 = 50%ETc) during grain filling stage. Carbon isotope discrimination ($\Delta^{13}C$) was associated with some physio-biochemical parameters to evaluate barley response to saline conditions. Results of this study showed that deficit saline-irrigation significantly ($p < 0.05$) decreases $\Delta^{13}C$ in both barley landraces. Moreover, photosynthetic rate (A), transpiration (E), stomatal conductance (*gs*), and instantaneous water use efficiency (iWUE) were significantly affected by treatments. Relative water content (RWC), chlorophyll *a*, and chlorophyll (SPAD) value were significantly ($p < 0.01$ and $p < 0.001$) were affected by deficit saline-irrigation. In addition, phenolic compounds were affected by treatments and landraces (except syringic and p-coumaric acids), and their interactions (except syringic acid). Moreover, high correlations were noticed between $\Delta^{13}C$ and physio-biochemical parameters. Results suggested that both barley landraces make a higher iWUE, and a weak variation in phenolic compounds. Moreover, $\Delta^{13}C$ associated with physio-biochemical traits can also be good criteria for screening of salt-tolerance of barley during grain filling stage. Taken together, our study suggests that the response to deficit saline-irrigation in barley landraces involves an interplay between various physiological and biochemical mechanisms mainly related to $\Delta^{13}C$.

1. INTRODUCTION

In the arid and semi-arid regions of the world, soil salinization is one of the major abiotic stresses, which leads to serious environmental problems (Hamid et al., 2008) and limit plant growth, development, and productivity (Shahbaz et al., 2012; Mbarki et al., 2018). Recent investigations have estimated that the increase in salinization will affect more than 50% of all cultivated land (Jamil et al., 2011). Barley (*Hordeum vulgare* L.) is an important cereal crop worldwide. However, barley fields are exposed to severe salt and drought stresses which seriously affects their productivity (Ceccarelli et al., 2007; El-Wahed et al., 2015). Indeed, it has been widely demonstrated that salinity affects almost every aspect of plant physiology and biochemistry by three ways: (1) osmotic stress caused by the low water potential in the root surface (2) toxic effects due to the specific Cl^- and Na^+ stresses, and (3) nutrient imbalance caused by excess of theses ions (Munns and Tester, 2008). In addition, salinity affects plant growth and development through the perturbation of gas exchange and photosynthesis process. In fact, the photosynthetic rate (A), stomatal conductance (*gs*), transpiration (E) and water use efficiency (WUE) are susceptible to salt stress (Eisa et al., 2012; Goussi et al., 2018). All these processes are very complex and related to many factors

such as stress duration, plant development stage, genotype tolerance and the genotype x environment interaction (Negrão et al., 2017; Manaa et al., 2019).

Previous studies have demonstrated that the biosynthesis of efficient antioxidants such as phenolic compounds are considered one of the main defense mechanisms in stressed plants (Hafsi et al., 2016, 2017). Indeed, polyphenols (including phenolic acids, flavonoids and proanthocyanidins) are described to be important and effective agents in scavenging free radicals (Georgiev et al., 2014; Skrovankova et al., 2015). The antioxidative properties of phenolic compounds come from (i) high reactivity as hydrogen or electron donors, (ii) the ability of the polyphenol derived radical to stabilize and dissociate unpaired electrons and (iii) they chelate transition metal ions ability (Bharti et al., 2014). Importantly, in recent years, the carbon isotope discrimination ($\Delta^{13}C$) has been successfully employed to evaluate salt-tolerance by the evaluation of WUE (Keller et al., 2017), photosynthetic activity and yield (Monneveux et al., 2006) as well as diverse biochemical features (Mansour et al., 2020) in C3 plants. Furthermore, previous studies have investigated the stable isotope carbon composition in diverse plants in order to understand the photosynthetic responses under the major environmental stresses such as salt, drought, cold stresses, (Tsialtas and Maslaris, 2006; Khazaei et al., 2008). The physical and chemical processes that lead to the ultimate fractionation in plant tissues are divided into components of carbon isotope fractionations, which are mostly concerned with photosynthesis (Rouhi et al., 2007). The main advantage of using carbon isotope discrimination to characterize plant stress over instantaneous measurements of gas exchange or water capacity is that it offers an integrated index of stress history rather than a snapshot in time (Koyro, 2006). In this context, our research aims to (1) evaluate the effect of deficit saline-irrigation on some physio-biochemical parameters of two barley landraces and (2) determination of the relationships between $\Delta^{13}C$ and these parameters in order to evaluate barley growth and development under salt condition during grain filling stage.

2. MATERIALS AND METHODS

2.1. Plant material and growth conditions

The plant material used in our study consists of two Tunisian barley landraces (*Hordeum vulgare* L.), "Karkeni" and "Bengardeni". For each landrace, healthy seeds were cultivated for a period of six months between November 2015 and May 2016 during season of 2015-2016 in a common garden characterized by a sandy soil with electrical conductivity of 3.75 dS/m. Once sown, the seeds were irrigated using a salty well-water (salinity averaged on 10 dS/m) via a drip-irrigation system and subjected to three different irrigation regimes based on the cultural evapotranspiration ETc; T0 = 100%ETc, T1 = 75%ETc, and T2 = 50%ETc. The ETc was calculated using FAO-56 model. The experiment were conducted at the Institute of Arid Regions, located 22.5 km southeast of Medenine (10°38'30.34''E, 33°29'53.23''N, 106m a.s.l.). The climate is Mediterranean, with hot and dry summers and mild winters, with an average annual rainfall of 125 mm. During the experiment, Minimum temperatures ranged from were between 3.5 and 15.7 °C, while maximum temperatures were between 16 and 39.8 °C for the same period (Bagues et al., 2020). At the grain filling stage, three replicates were randomly chosen from each landrace and irrigation regimes for the physio-biochemical analyses.

2.2. Analyses of carbon isotope discrimination ($\Delta^{13}C$)

The carbon isotope composition was determined on leaf dry matter. After freeze-drying of leaf samples (three replicates per treatment and landrace), they were finely ground with a Retsch MM200 mill ball (Bioblock Scientific, Illkirch, France) to ensure homogeneity. Then 600–800 µg per sample weighed in stain capsules (Courtage Analyse Service, Mont Saint-Aignan, France) and used for ^{13}C analysis with an isotope ratio mass spectrometer, IRMS (VG Isotech, Manchester, Royaume-Uni) coupled to an elemental analyzer (Flash A, Thermo-Finnigan, Villebonsur-Yvette, France) at the Institute of Plant Sciences Paris-Saclay (IPS2, Orsay, France). Carbon isotope composition ($\delta^{13}C$) was calculated as the deviation of the carbon isotope ratio ($^{13}C/^{12}C$, called R) from the international standard (Vienna Pee Dee Belemnite):

$$\delta^{13}C\ (‰) = \left[\frac{R\ sample - R\ standard}{R\ standard}\right] x\ 100$$

The carbon isotope discrimination ($\Delta^{13}C$) was calculated using the formula:

$$\Delta^{13}C\ (‰) = \left[\frac{(\delta a - \delta p)}{(1 + \delta p)}\right]$$

where, δp is the $\delta^{13}C$ of the leaves and δa is the $\delta^{13}C$ of the atmospheric CO_2 (assumed to be −8‰). Two standards (glutamic acid and sucrose) from IAEA, and one laboratory standard (glutamic acid), were also measured in order to correct for the drift of the IRMS (1 capsule of laboratory glutamic acid every 6 samples, and 2 capsules of each AIEA standard every 24 samples).

2.3. Gas exchange, photosynthetic pigments, and SPAD analysis

Gas exchange parameters such as photosynthetic rate (A), transpiration rate (E), stomatal conductance (*gs*), and instantaneous water use efficiency (iWUE) were measured using a portable gas-exchange system (ADC BioScientific LC ProSystem Serial No. 3302) on the flag leaves. Leaf temperature was maintained at 25 °C, light intensity was set at 800 µmol photons m/s with a red/blue light source and the CO_2 concentration was set at 400 µmol/mol. Leaf to air VPD was maintained at 1 KPa.

Chlorophyll a and Chlorophyll b were extracted and quantified using the modified method of Arnon (1949). After the extraction and analysis, the relative amount of Chlorophyll a and Chlorophyll b were calculated using the following formulae:

Chlorophyll a (mg/g) = [(12.7xA_{663} - 2.69xA_{647}) V/W]
Chlorophyll b (mg/g) = [(22.9xA_{647} - 4.68xA_{663}) V/W].

Chlorophyll content (SPAD) was evaluated using a chlorophyll meter Type (Minolta 1500) at a rate of 6 repetition per treatment.

2.4. Relative water content

The relative water content (RWC) was determined according to the approach proposed by Sharp et al. (1990). Briefly, 10 leaves from each treatment were weighed immediately (FW) after harvesting. Leaves were then placed in distilled water for 4 h and then turgid weight (TW) was measured. Then the leaves were dried in oven at 80 °C for 24 h to obtain their dry weight (DW).. The RWC was finally calculated as follow:

RWC (%) = [(FW–DW) / (TW–DW)] x 100

2.5. Analysis of phenolic compounds by analytical LC-ESI-MS

Dry leaf methanolic extract was analyzed using an LCMS-2020 mass spectrometer (Shimadzu, Kyoto, Japan). LC system was equipped with an electrospray ionization source (ESI). Spectra were recorded in negative ion mode, monitored and processed using Shimadzu Lab Solutions LC–MS software. The LC-20AD XR binary pump system, the SIL-20AC XR auto sampler, the CTO-20AC column oven and the DGU-20AS degasser (Shimadzu, Kyoto, Japan) were the main elements of the LC system. For analysis, Thermo Electron (Dreieich, Germany) was provided as with an Aquasil C18 column thermostatted at 40 °C (150 mm x 3 mm, 3 µm) preceded by an Aquasil C18 guard column (10 mm x 3 mm, 3 µm). The solvents were: A (0.1% formic acid in H_2O, v/v) and B (0.1% formic acid in methanol, v/v). The elution gradient established was 10–100% B for 0–45 min, 100% B over 45–55 min and re-equilibration of the column lasted 5 min between individual runs. The flow rate of the mobile phase was 0.4 mL/min, and the injection volume was 5 mL. High-purity nitrogen served as the nebulizer and auxiliary gas. The ion spray voltage was set at -3.5 V in the negative mode. The following settings were applied: nebulizing gas flow of 1.5 L/min, a dry gas flow rate of 12 L/min, a DL (dissolving line) temperature of 250 °C, a block source temperature of 400 °C and a voltage detector of 1.2 V.

2.6. Data analysis

First, we conducted a two ways ANOVA using SPSS 25.0 for Windows (SPSS Inc., United States) in order to explore the effects of landraces, the deficit saline-irrigation and their interaction on the measured parameters. Statistical differences were checked by Duncan test with significance threshold of $P < 0.05$. Furthermore, in order to investigate the associations between the studied physio-biochemical features in each of the three saline-irrigation doses were analyzed using Pearson coefficient (*r*). In order to determine the patterns of differentiation among the measured

traits and to show their contribution to the landraces studied under tested treatments, the obtained data were analyzed using Principal Components Analysis (PCA) and hierarchical clustering analysis (HCA) through XLSTAT software version 2014.5.03 (Addinsoft) and ClustVis online tool, respectively. PCA results were visualized using a scatter plot formed by the two first PCs, whereas those obtained by HCA illustrated in an interactive heat map combining the studied landraces, treatments and the physio-biochemical data.

3. RESULTS

3.1. Effect of deficit saline-irrigation on $\Delta^{13}C$

As shown in Fig. 1, the two studied barley landraces "Bengardeni" and "Karkeni" differed significantly in their $\Delta^{13}C$, with "Bengardeni" landrace displaying higher levels in all saline-irrigation treatments. Moreover, $\Delta^{13}C$ seems to be significantly influenced by the deficit saline-irrigation ($P < 0.05$) in both barley landraces. Although significant for both landraces, the reduction of $\Delta^{13}C$ from T0 to T1 was relatively similar, reaching 1.19% in "Karkeni" and 1.21% in "Bengardeni". In the severe saline-irrigation treatment (T2), the reduction was found more pronounced in "Bengardeni" (2.12%) compared to "Karkeni" (1.69%). However, ANOVA results revealed that $\Delta^{13}C$ was non-significantly affected by the combined effect of treatment and landrace (Table 1).

3.2. Effect of deficit saline-irrigation on gas exchange parameters

The effects of deficit saline-irrigation on photosynthetic rate (A), transpiration rate (E), stomatal conductance (*gs*), and instantaneous water use efficiency (iWUE) in both barley landraces are illustrated in Fig. 2A-D, respectively. Based in ANOVA result (Table 1),

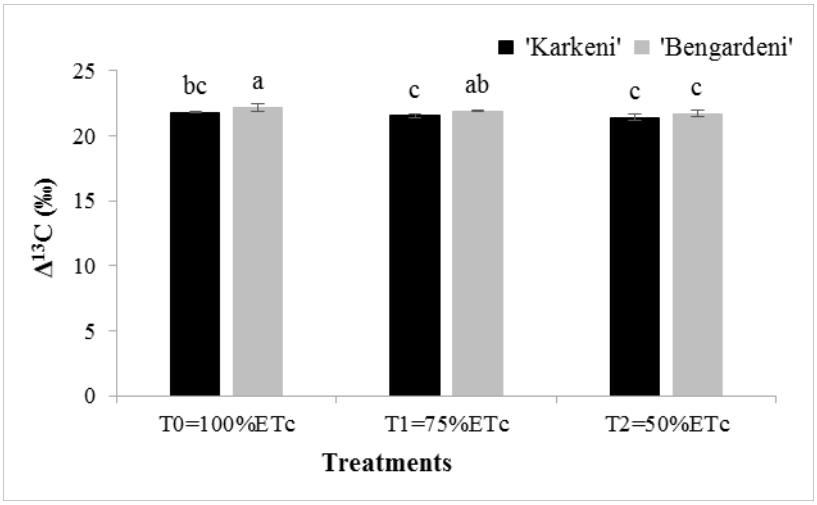

Fig. 1. Effects of deficit saline-irrigation on $\Delta^{13}C$ of "Karkeni" and "Bengardeni" barley landraces. Data are means ± standard errors (SE), n = 3. Column values with different letters are significantly different at $P < 0.05$, according to Duncan's multiple range test.

Table 1. Analysis of variance of carbon isotope discrimination ($\Delta^{13}C$), photosynthetic rate (A), transpiration rate (E), stomatal conductance (*gs*), and instantaneous water use efficiency (iWUE)

ANOVA	$\Delta^{13}C$	A	E	*gs*	iWUE
Treatment (T)	**	***	***	***	**
Landrace (L)	*	**	***	*	***
T x L	ns	*	ns	ns	***
Error	0.038	0.850	0.288	0.001	0.219

ns: non-significant; *, **, *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

A, E, *gs*, and iWUE were significantly affected by treatment, landraces and their interactions (for A and iWUE). As shown in Fig. 2A-C, A, E and *gs* decreased under deficit saline-irrigation, from T0 to T2, in ''Karkeni'' (47.66%, 67.63% and 65%, respectively) and in ''Bengardeni'' (48.03%, 45.04% and 36.36%, respectively). Moreover, from T0 to T2, iWUE increased just in ''Karkeni'' by 60.23%. The higher values of iWUE were observed in ''Karkeni'' (4.2, 4.58 and 6.73 µmol CO_2/mmol H_2O) compared to (3.64, 4.39 and 3.41 µmol CO_2/mmol H_2O) in ''Bengardeni'', for T0, T1 and T2, respectively (Fig. 2D).

3.3. Effect of deficit saline-irrigation on RWC, photosynthetic pigments, and SPAD value

As shown in Table 2, RWC, *Chl a*, and SPAD value were significantly ($P < 0.01$ and $P < 0.001$)

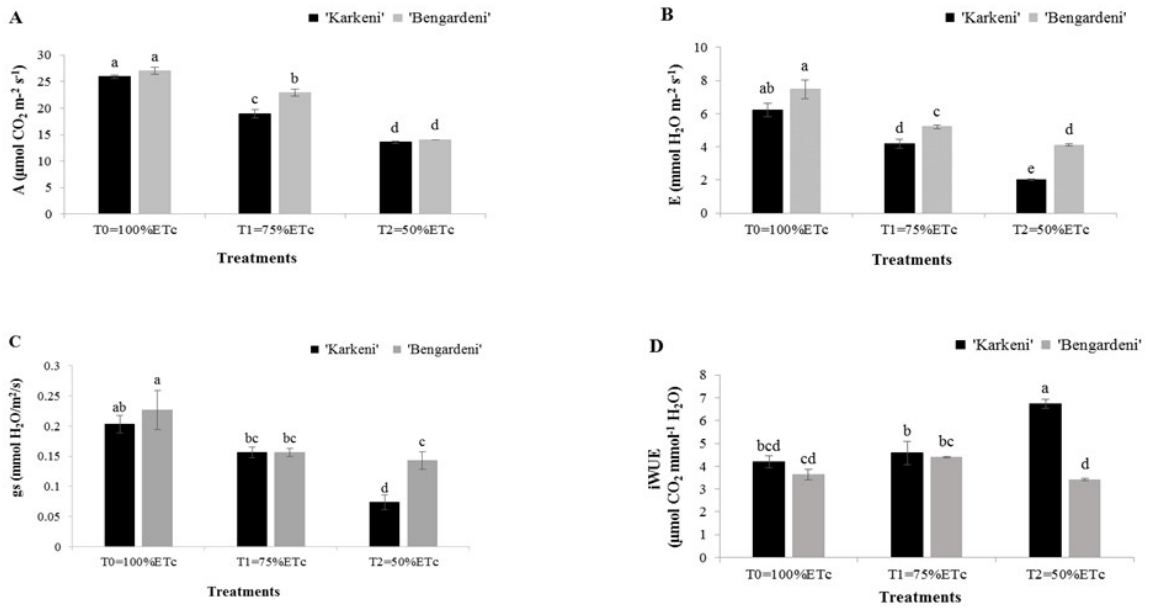

Fig. 2. Effects of deficit saline-irrigation on the (A) photosynthetic rate (A), (B) transpiration rate (E), (C) stomatal conductance (*gs*) and (D) instantaneous water use efficiency (iWUE) of "Karkeni" and "Bengardeni" barley landraces. Data are means ± standard errors (SE), n = 3. Column values with different letters are significantly different at $P < 0.05$, according to Duncan's multiple range test.

Table 2. Effects of deficit saline-irrigation on relative water content (RWC), Chlorophyll a, (*Chl a*), Chlorophyll b (*Chl b*), and Chlorophyll content (SPAD) in "Karkeni" and "Bengardeni"landraces.

Treatment	Landrace (L)	RWC (%)	*Chl a* (mg/g FW)	*Chl b* (mg/g FW)	SPAD
T0=100%ETc	'Karkeni'	70.80 ± 0.34a	12.81 ± 0.47a	7.67 ± 0.31b	39.13 ± 0.4a
T0=100%ETc	'Bengardeni'	68.06 ± 0.96ab	12.41 ± 0.4a	7.23 ± 0.95b	37.45 ± 0.39b
T1=75%ETc	'Karkeni'	64.76 ± 1.00c	11.39 ± 1.46b	8.75 ± 0.11a	36.03 ± 0.42c
T1=75%ETc	'Bengardeni'	64.35 ± 2.44c	11.21 ± 1.1bc	7.63 ± 1.13b	36.55 ± 0.18c
T2=50%ETc	'Karkeni'	59.23 ± 0.49dc	11.37 ± 0.42b	7.56 ± 0.49b	35.28 ± 0.51c
T2=50%ETc	'Bengardeni'	56.89 ± 4.51d	11.08 ± 0.18c	7.59 ± 0.32b	35.80 ± 0.54c
ANOVA	T	***	**	ns	***
	L	ns	ns	ns	ns
	T x L	ns	ns	ns	***

Data are means ± standard errors (SE), n = 3. Values with different letters are significantly different at $P < 0.05$, according to Duncan's multiple range test.
ns, non-significant, *, **, *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

affected by deficit saline-irrigation. Indeed, from T0 to T2, reductions in RWC, *Chl a* and SPAD value were observed in ''Karkeni'' (16.34%, 11.24% and 9.83%, respectively) and in ''Bengardeni'' (16.41%, 10.71% and 4.40%, respectively). However, no significant effect was detected for *Chl b*.

3.4. Effect of deficit saline-irrigation on phenolic profile

According to ANOVA results, except for syringic and p-coumaric acids, all the phenolic compounds contents were significantly influenced by treatment and landraces. However, it should be noted that the syringic acid content seems to be significantly affected by the combined effect T x L ($P < 0.05$) (Table 3). As shown in Table 3, phenolic compounds varies differently between treatments and landraces. Indeed, in full irrigation T0, ''Karkeni'' shows higher values compared to ''Bengardeni'', for example, 4-O-caffeoylquinic acid (0.94 µg/g DW compared to 0.70 µg/g DW), syringic acid (4.97 µg/g DW compared to 4.46 µg/g DW), trans-ferulic acid (27.80 µg/g DW compared to 18.31 µg/g DW). On the other hand, in deficit irrigation T2, these values decreases in ''Karkeni'' compared to ''Bengardeni'' (0.56 µg/g DW compared to 1.51 µg/g DW, 3.20 µg/g DW compared to 3.44 µg/g DW, 15.97 µg/g DW compared to 17.12 µg/g DW, respectively).

3.5. Relationships between $\Delta^{13}C$ and physio-biochemical parameters

A highly considerable correlation was noted between $\Delta^{13}C$ and physio-biochemical parameters under deficit saline-irrigation (Tables 4 and 5). A highly significant positive correlations (r = 0.903, r = 0.811, and r = 0.906; $p < 0.01$) were observed between $\Delta^{13}C$ and E, *gs*, A, respectively. No significant relationship (r = -0.485) was observed between $\Delta^{13}C$ and instantaneous water use efficiency (iWUE). A significant positive correlations (r = 0.709, r = 0.616; $p < 0.05$) also were founded between $\Delta^{13}C$ and RWC, SPAD, respectively.

In the other hand, a very weak positive correlations (r = 0.465, r = 0.246, r = 0.117 and r = 0.115) were founded between $\Delta^{13}C$ and quinic

Table 3. Effects of deficit saline-irrigation on the levels of phenolic compounds (µg/g DW) in flag leaves of two barley landraces ("Karkeni" and "Bengardeni").

Treatment (T)	Landrace (L)	Quinic acid	Proto-catechuic acid	4-O-caffeoyl quinic acid	Syringic acid	p-coumaric acid	Trans-ferulic acid	Kaemp-ferol	Cirsiliol
T0=100%ETc	**'Karkeni'**	176.51 ± 5.66a	6.84 ± 0.10e	0.94 ± 0.01b	4.97 ± 0.80a	9.32 ± 0.04abc	27.80 ± 1.01a	7.05 ± 0.01a	55.20 ± 0.62c
T0=100%ETc	**'Bengardeni'**	169.91 ± 4.48a	13.72 ± 0.10b	0.70 ± 0.00bc	4.46 ± 0.36abc	10.29 ± 0.66ab	18.31 ± 0.48c	5.17 ± 0.00c	55.93 ± 0.50c
T1=75%ETc	**'Karkeni'**	132.40 ± 4.68b	7.99 ± 0.06d	0.78 ± 0.09bc	4.20 ± 0.38abc	7.35 ± 0.17c	22.09 ± 0.69b	7.02 ± 0.18a	61.64 ± 0.68bc
T1=75%ETc	**'Bengardeni'**	161.28 ± 0.12a	8.60 ± 0.01d	0.58 ± 0.04c	4.82 ± 0.39ab	8.36 ± 0.13bc	17.04 ± 0.07c	6.63 ± 0.17a	77.03 ± 1.20a
T2=50%ETc	**'Karkeni'**	176.58 ± 7.10a	9.92 ± 0.57c	0.56 ± 0.08c	3.20 ± 0.07c	11.03 ± 1.57a	15.97 ± 0.89c	6.08 ± 0.14b	54.08 ± 4.12c
T2=50%ETc	**'Bengardeni'**	82.67 ± 5.55c	19.35 ± 0.10a	1.51 ± 0.16a	3.44 ± 0.10bc	7.64 ± 0.68c	17.12 ± 1.24c	5.85 ± 0.19b	64.11 ± 4.34b
ANOVA	**T**	***	***	**	ns	ns	***	***	***
	L	***	***	*	ns	ns	***	***	**
	T x L	***	***	***	ns	*	***	***	*
	Error	77.747	0.185	0.023	0.550	1.725	2.059	0.062	19.196

acid, p-coumaric acid, trans ferulic acid, cirsiliol, respectively, and a very weak negative correlations (r = -0.102, r = -0.331 and r = 0.327) were observed between $\Delta^{13}C$ and protocatechuic acid, 4-O-caffeoylquinic acid, kaempferol, respectively. A significant positive correlation (r = 0.728; $p < 0.05$) was observed between $\Delta^{13}C$ and syringic acid.

SPAD, and $\Delta^{13}C$, $| r | \geq 0.291$. The second PC accounted for 25.10% of the total variance and displayed strong positive correlations with iWUE (r = 0.409) and quinic acid content (r = 0.399) and significant negative associations with protocatechuic acid (r = -0.325) 4-O-caffeoylquinic acid (r = -0.392). The two remaining axes explained 43.80% and 25.09%

Table 4. Pearson's correlation among $\Delta^{13}C$ and different physiological traits of barley landraces under irrigation treatments.

Variables	$\Delta^{13}C$	E	*gs*	A	iWUE	RWC	*Chl a*	*Chl b*	SPAD
$\Delta^{13}C$	1								
E	0.903	1							
gs	0.811	0.980	1						
A	0.906	0.922	0.887	1					
WUE	-0.199	-0.539	-0.640	-0.233					
iWUE	-0.485	-0.739	-0.780	-0.466	1				
RWC	0.709	0.776	0.786	0.932	-0.273	1			
Chl a	0.593	0.711	0.722	0.780	-0.234	0.854	1		
Chl b	-0.470	-0.312	-0.162	-0.222	0.105	0.004	-0.296	1	
SPAD	0.616	0.784	0.796	0.846	-0.434	0.881	0.906	-0.230	1

Significant correlation at $P < 0.05$ (*) and $P < 0.01$ (**)

Table 5. Pearson's correlation among $\Delta^{13}C$ and different biochemical traits of barley landraces under irrigation treatments.

Parameters	$\Delta^{13}C$	quinic acid	Proto-catechuic acid	4-O-caffeoylquinic acid	syringic acid	p-coumaric acid	Trans-ferulic acid	Kaemp-ferol	Cirsiliol
$\Delta^{13}C$	1								
quinic acid	0.465	1							
protocatchuic acid	-0.102	-0.718	1						
4-O-caffeoylquinic acid	-0.331	-0.834	0.711	1					
syringic acid	0.728	0.426	-0.544	-0.267	1				
p-coumaric acid	0.246	0.741	-0.138	-0.517	-0.155	1			
trans ferulic acid	0.117	0.229	-0.528	0.109	0.611	-0.167	1		
Kamepferol	-0.327	0.094	-0.731	-0.109	0.395	-0.453	0.630	1	
Cirsiliol	0.115	-0.300	0.015	-0.006	0.280	-0.596	-0.326	0.229	1

Significant correlation at $P < 0.05$ (*) and $P < 0.01$ (**)

3.6. PCA and Heatmap Analyses

PCA results (Fig. 3) revealed that the first four PCs, which were the most important in explaining the variation between the two barley landraces on the basis of the analyzed parameters (Eigenvalues > 1), summarized 68.90% of the total variance (Table 6). With 43.80% of the explained variance, PC1 was mainly associated with E, *gs*, A, RWC, *Chl a*, of the variance, respectively. PC3 was mainly associated with *Chl b*, p-coumaric acid and Kaempferol ($| r | > 0.5$), whereas PC4 was found strongly associated with *Chl a* (r = -0.320), 4-O-caffeoylquinic acid (r = -0.325), trans ferulic acid (r = -0.375) and cirsiliol (r = 0.629) (Table 6). The scatter plot formed by PC1 and PC2 revealed that in the full irrigation treatment (T0 = 100%ETc) for both landraces and the moderate irrigation treatment (T1 = 75%ETc)

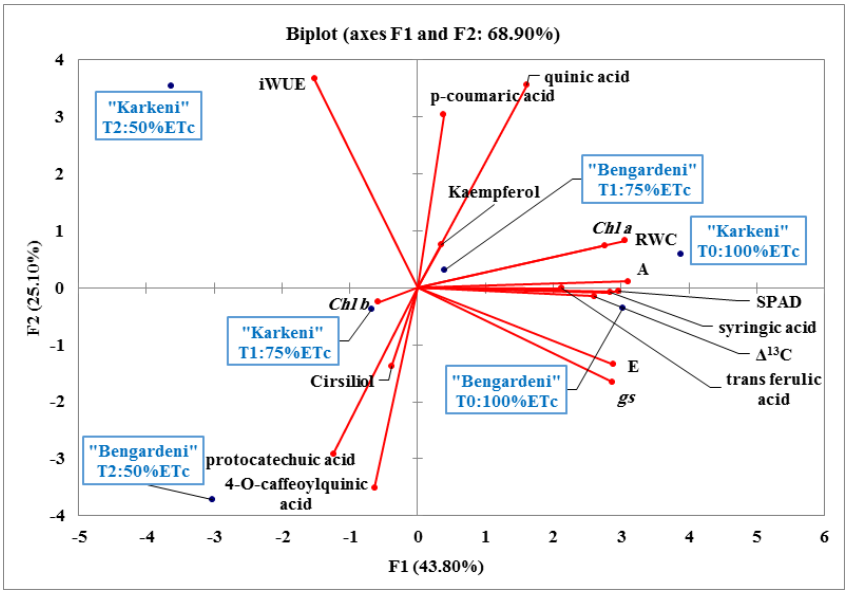

Fig. 3. The biplot for F1 and F2 showing the relationships between the studied barley landraces and all the measured traits under deficit saline-irrigation during the grain filling stage.

Table 6. Eigenvalue, percentage variation and cumulative variance for the 4 factors derived from principal component analysis (PCA).

Parameters	PC1	PC2	PC3	PC4
E	0.3233	-0.1502	-0.1256	0.0960
Gs	0.3210	-0.1845	-0.0579	0.0082
A	0.3483	0.0122	-0.0352	0.1417
iWUE	-0.1703	0.4098	0.0401	-0.0652
RWC	0.3421	0.0923	0.0923	-0.0255
Chl a	0.3092	0.0832	-0.0926	-0.3206
Chl b	-0.0652	-0.0275	0.4838	-0.0431
SPAD	0.3315	-0.0077	0.0306	-0.1808
quinic acid	0.1806	0.3991	-0.0706	0.0602
protocatechuic acid	-0.1391	-0.3258	-0.3340	-0.0775
4-O-caffeoylquinic acid	-0.0708	-0.3922	-0.0075	-0.3256
syringic acid	0.3185	-0.0097	0.1666	0.2300
p-coumaric acid	0.0434	0.3399	-0.3629	-0.1473
trans ferulic acid	0.2369	-0.0006	0.3029	-0.3738
Kaempferol	0.0401	0.0857	0.5309	-0.0287
Cirsiliol	-0.0434	-0.1544	0.1618	0.6295
Δ^{13}C	0.2915	-0.0167	-0.2210	0.2997
Eigenvalue	7.8847	4.5179	3.2007	1.8201
Variability (%)	43.8040	25.0994	17.7818	10.1116
Cumulative %	43.8040	68.9034	86.6852	96.7968

for ‘‘Bengardeni’’ landrace, a strong positive association between Δ^{13}C and most of analyzed parameters. However, when subjected to severe stress (T2 = 50% ETc), both landraces seem to be characterized by a relatively low of *Chl b*. Such association patterns seem to be a regulation mechanism developed by both landraces to cope with the deficit saline-irrigation.

To identify the key parameters for assessing the tolerance of barley landraces to the deficit saline-irrigation, both physiological and biochemical measurements were used to plot a heatmap. As shown in Fig. 4, the analyzed parameters were divided into two major groups. The parameters kaempferol, cirsiliol, *Chl b*, iWUE, p-coumaric acid, protocatechuic acid, 4-O-caffeoylquinic acid were associated together forming the cluster I, whereas all the remaining parameters formed together the second group (cluster II). Meanwhile, the generated heatmap showed a clear separation

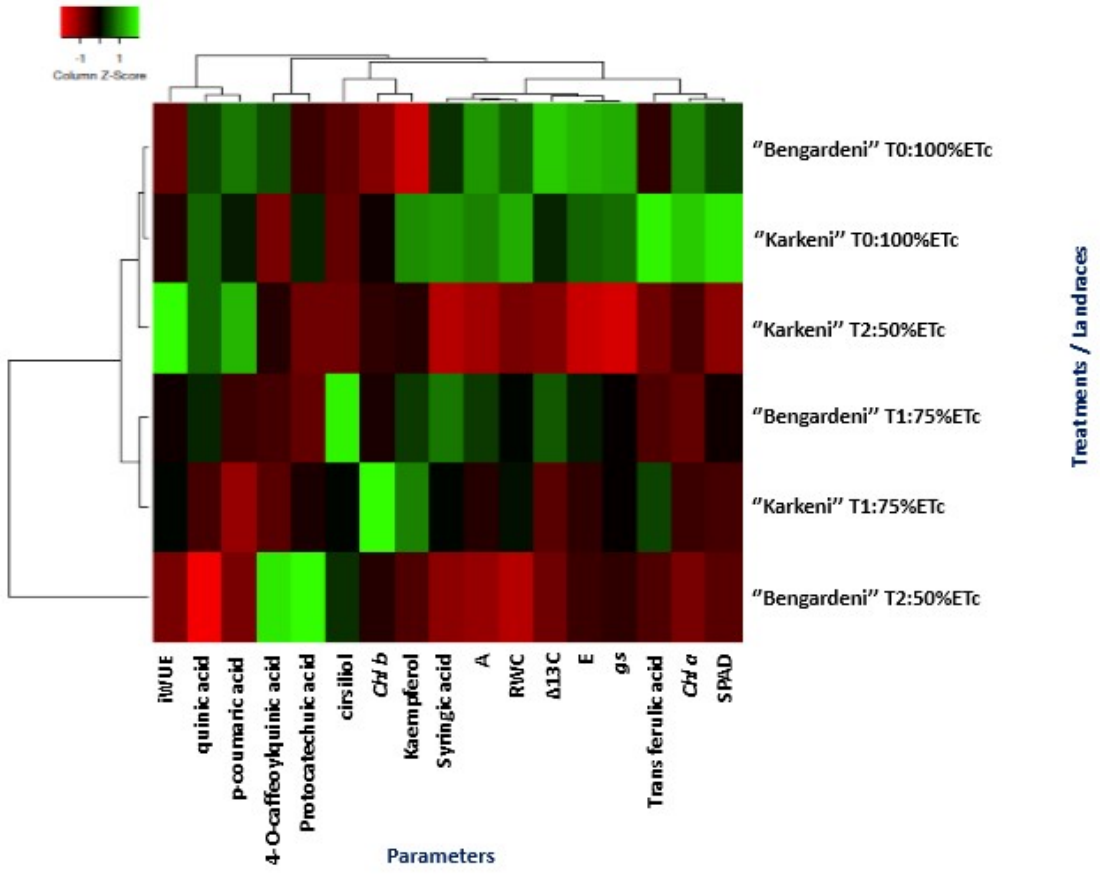

Fig. 4. Heatmap analysis of the physiological and biochemical parameters changes under deficit saline-irrigation across two barley landraces during the grain filling stage.

between ''Bengardeni'' and ''Karkeni'' landraces, without any separation among the deficit saline treatments. Overall, ''Bengardeni'' and landrace ''Karkeni'' showed similar patterns for all analyzed parameters under to severe stress (T2 = 50% ETc) and the full irrigation treatment (T0 = 100%ETc). However, the two landraces differed significantly at the moderate irrigation treatment (T1 = 75%ETc). Noticeably, compared to ''Karkeni'' landrace, a small decrease in the values of most physiological and biochemical parameters was observed in ''Bengardeni'' landrace suggesting that this latter maintains the main physiological and biochemical mechanisms that confers tolerance to deficit saline-irrigation.

4. DISCUSSION

Salinity is one of the main abiotic stresses. It adversely affects crop production by inducing water stress, specific ion toxicity and ionic imbalance (Anwar et al., 2011). In the present study, deficit saline-irrigation negatively affects $\Delta^{13}C$ in both barley landraces. A similar decrease in $\Delta^{13}C$ in response to salt stress has been reported in diverse crops including wheat (Shaheen and Hood-Nowotny, 2005), melon (Sarabi et al., 2017) and barley (Jiang et al., 2006). Interestingly, our results revealed a higher reduction in $\Delta^{13}C$ in ''Karkeni'', which is a salt-sensitive landrace compared to the salt-tolerant one ''Bengardeni'' (1.69% vs 2.12%). Similar patterns of reduction were observed by Shirazi et al. (2015) in wheat genotypes and Mustafa et al. (2019) in barley genotypes. Moreover, in this study, deficit saline-irrigation reduced photosynthetic rate (A), transpiration rate (E), stomatal conductance (*gs*) and increased instantaneous water use efficiency (iWUE) in both barley landraces. Similar results were found by Bagues et al. (2019, 2020) in barley under deficit irrigation with saline water at tillering and heading stages, respectively, and by Mansour et al. (2020) in bread wheat. The reduction in photosynthetic parameters could be explained by the Na^+ and Cl^- toxicity (Ashrafi

et al., 2014; Pirasteh-Anosheh et al., 2016). Salinity effect caused by deficit saline-irrigation on photosynthesis is directly attributed to the stomatal limitations for gases diffusion, which ultimately changes photosynthesis and mesophyll metabolism (Chaves et al., 2009). After the stomata closing, the internal reduction of CO_2 reduces the activity of several enzymes including Rubisco, thereby limiting carboxylation and reducing the photosynthetic rate (Negrao et al., 2017; Hosseinzadeh et al., 2018; Sayyad-Amin et al., 2018).

Relative water content (RWC) decreased in both barley landraces due to deficit saline-irrigation. Similar results were found by Ghoulam and Fares (2001) in sugar beet and by Subramanyam et al. (2019) in rice. Reduction in RWC due to salinity indicates that salinity directly effects plant-water relations (Rasool et al., 2020). In the same context, photosynthetic pigments (*Chl a* and *Chl b*) were also affected by deficit saline-irrigation. Our findings are in agreement with those obtained by Rasool et al. (2020) in millet varieties under salt stress and by Mansour et al. (2020) in bread wheat. In fact, inhibition of chlorophyll activity may also possibly be due to superoxide radicals and H_2O_2 generated through salt stress, which disrupts thylakoid membranes and chloroplast (Subramanyam et al., 2019). Salinity has been reported to reduce chlorophyll content and escalate ROS (Jiang et al., 2017; Subramanyam et al., 2019). In addition, deficit saline-irrigation reduce significantly the relative chlorophyll content (SPAD). This result is similar to those observed by Sun et al. (2014) in which the SPAD reading of a wild *M. sinensis* population decreased with increasing salinity and their duration has been detected. Similar results were also observed by Wang et al. (2019) in ornamental grasses species irrigated with saline water. Indeed, diverse previous investigations have demonstrated that, salinity may inhibit chlorophyll synthesis and / or accelerate its degradation (Netondo et al., 2004; Zhao et al., 2007; Bonales-Alatorre et al., 2013).

In addition, deficit saline-irrigation affects significantly phenolic compounds in barley landraces. Similar results were detected in our previous studies in the two barley landraces at tillering and heading stage, respectively (Bagues et al., 2019, 2020). Also, by Stagnari et al. (2017) in durum wheat and by Jamalian et al. (2013) in strawberry cultivars under salt stress. In fact, the high salinity induces the formation of ROS in plant cells and its over-accumulation leads to oxidative damage to membrane lipids, proteins and nucleic acids (Gill and Tuteja, 2010). To recover high ROS levels, an efficient system of enzymatic and non-enzymatic antioxidants involved (Gill and Tuteja, 2010; Karuppanapandian et al., 2011). The general mechanism of free radical trapping by which these antioxidants work involves the donation of a p-hydroxyl hydrogen atom to ROS and the generation of a resonance stabilized carbon-based radical (Moussa et al., 2019).

To better understand the role of $\Delta^{13}C$ in the evaluation of barley under deficit saline-irrigation, a set of correlations was made between $\Delta^{13}C$ and some physio-biochemical parameters. High significant positive correlations were found between $\Delta^{13}C$ and (E, *gs*, and A). Our results are in agreement with the findings of Gao et al. (2018) in rice under drought stress. Nevertheless, Monneveux et al. (2006) showed that the positive relationship between $\Delta^{13}C$ and grain yield was explained by their close relationship with *gs*. The weak and negative correlation observed between $\Delta^{13}C$ and iWUE was in concordance with the results obtained in melon plants grown under saline condition (Sarabi et al., 2019) and in kiwifruit under deficit drip irrigation (Zheng et al. (2020). However, no significant correlation between $\Delta^{13}C$ and iWUE was obsreved, which is mainly caused by the rupture of the relationship between leaf efficiency and crop WUE (Condon et al., 1993). Changes in water use efficiency can arise from either changes in stomatal aperture or assimilation capacity (Dadkhah, 2013). If nevertheless, non-stomatal factors had the greatest influence on Ci, then the expectation would be a decrease in WUE and an increase in Ci and $\Delta^{13}C$ from the normal condition to highest stress (Dadkhah, 2013). The significant positive correlation between $\Delta^{13}C$ and RWC suggests that the effect of salinity on $\Delta^{13}C$ values may in part is related to the water status of leaves (Sarabi et al., 2019). In addition, the positive relationship between $\Delta^{13}C$ and syringic, p-coumaric acids is explicated by the no-significant effect of the treatment and landraces on this compounds. It can be suggested that, in addition to physiological (gas exchange, iWUE…) and agronomic (grain yield) traits, $\Delta^{13}C$ can be associated with biochemical traits like phenolic acids (syringic and p-coumaric acids…)

in order to evaluate salt-tolerance of barley during grain filling stage.

5. CONCLUSION

Photosynthesis, quantum efficiency of photosystem II, total chlorophyll content, leaf-to-air temperature difference, lipid peroxidation and carbon isotope discrimination are good physiological indicators that may be used in breeding programs to discriminate more yielding sugarcane genotypes under drought condition. In this study, various physiological and biochemical parameters were used to investigate their role in dealing with deficit-saline irrigation conditions in two barley landraces. Overall, our results showed that most traits were significantly correlated with $\Delta^{13}C$, either negatively or positively, indicating that the response to deficit-saline irrigation conditions involves an interplay between diverse physiological and biochemical mechanisms. We speculate that photosynthesis, instantaneous water use efficiency, chlorophyll content, minerals, phenolic acids and carbon isotope discrimination are key physio-biochemical indicators that may be used in breeding programs to evaluate barley under saline conditions.

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