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Original Paper

Identification of barley landrace genotypes with contrasting salinity tolerance at vegetative growth stage

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Abstract In the present study, we evaluated the behavior of 21 Tunisian barley landraces under salt stress. The evaluation was performed using 14 morphological and physiological traits at vegetative growth stage under severe salt stress (200 and 250 mM). A multivariate analysis was used in order to select the genotypes with contrasting behavior towards salinity and to identify the major traits conferring salinity tolerance. According to the PCA analyses the genotypes exhibited diverse behavior with the salt stress concentration, indeed 3 different clustering profiles were obtained. Eleven quantitative characters were considered the most pertinent for the ranking of genotypes for salt tolerance. Among them the total fresh weight and the net CO_2 assimilation rate were the most discriminating descriptors at 250 mM NaCl. These parameters allowed as the identification of the contrasting pair genotypes toward salinity. "Testour" was classified as the most sensitive and "Enfidha" the most tolerant toward salinity stress. These findings would be of great relevance in breeding programs.

Key words: barley landrace genotypes, multivariate analysis, salt stress tolerance, vegetative growth stage.

Salinity is one of the principal abiotic stresses in agriculture worldwide, especially in the Mediterranean area. It's considered as the principal factor depressing plant growth and productivity in the arid and semi-arid regions (Sayar et al. 2010). Saline soils are formed by an excess of soluble salts and usually results from natural processes or from irrigation with saline water. Salt stress induces various morphological, physiological, biochemical and molecular changes in plants (Kafi 2009). It affects almost all plant functions, such as osmotic adjustment, drop of stomata, root hydraulic conductance, reduced growth rate, changes of the root to shoot ratio, nutritional disorders, metabolic imbalance and change of the photosynthetic active pigments concentration (James et al. 2011). Nevertheless, plants have different degree of tolerance to salt stress conditions (Munns and Tester, 2008). Indeed, Salt stress tolerance of plants is varying according to species, genotypes, level of salinity and growth stage (Shafi et al. 2011). In recent decades, studies on salinity tolerance are increasingly made in crop species through conventional selection and breeding techniques (Azizpour et al. 2010; Bazrafshan and Ehsanzadeh 2014). However, efforts to breed for salt tolerance has become limited due to lack of understanding of the complexity of the tolerance mechanism and the lack of reliable, quick, and convenient screening techniques (Munns and James 2003). Indeed, Munns et al. (2000) suggested that physiological traits are able to supply more objective information than agronomic parameters or visual assessment and are more important to appraise the degree of salt tolerance of whole plant species.

In view of many researchers, special interest was attributed to barley since it has a long history of cultivation and adaptation in North Africa, West Asia and East Asia; especially, in drought and saline areas (Badr et al. 2000). Barley's salt tolerance and growth maintain were related to its capacity to accumulate high concentrations of Na^+ in its leaves (Munns et al. 2006).

In the unfavorable areas of Tunisia, barley is mostly grown as landraces by subsistence farmers without application of fertilizers, pesticides and herbicides. These landraces are well adapted to harsh environmental conditions and are considered as a large reservoir of genetic diversity and of great importance to varieties improvement (Ben Naceur et al. 2012). In this study, we aim to evaluate salt tolerance in barely landraces populations from Tunisia at vegetative growth stage using multivariate analysis and based on morphological and physiological parameters in order to select genotypes with contrasting behavior toward salinity (tolerant/sensitive) that could be useful in breeding programs and "omics" investigations.

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Materials and methods

Plant material

Twenty-one Tunisian barley landraces (*Hordeum vulgare* L.) well representative of genetic variation among local barley landraces in Tunisia (Zoghlami et al. 2011) were chosen to evaluate their tolerance to salt stress (0, 200, 250 mM; Table 1).

Growth conditions

Whole experiments were carried out under semi-controlled greenhouse from December 2010 to June 2011. Barley seeds were surface-sterilized for 5 min in 10% sodium hypochlorite, and then thoroughly rinsed with distilled water. Ten seeds were sowed in PVC pots, filled with sand, and thoroughly washed. A randomized complete block design was used, with five replications (pots) per each treatment. All pots were irrigated for 15 days with distillated water (0 mM NaCl). After 15 days planting, the Hoagland solution was provided to each pot (Hoagland and Arnon 1950). Plants were daily watered at a rate of 100 ml per pot. When the third leaf was completely expanded, the number of plants was reduced to two seedlings per pot and a gradual salt stress was applied. In the present experiment two salt stress gradients were analyzed: 200 and 250 mM of NaCl as well as control. Salt stress was applied gradually by increments 50 mM per day (except for the control treatment which is 0 mM NaCl). In order to avoid an increase in soil salt concentration, all plants were watered with tap water for two weeks after the salt stress application. Plants were harvested 45 days after sowing and used for morphological and physiological measurements.

Table 1. Tunisian barley genotypes analyzed: their origin of sampling and bioclimatic layer.

	Genotypes	Origin of sampling	Bioclimatic layer
1	Abbessa	Jendouba	humid
2	Barrage Malleg	Elkef	humid
3	Souidia	Bizerte	sub-humid
4	Utique	Bizerte	semi-arid
5	Boulifa	Elkef	semi-arid
6	Testour	Beja	semi-arid
7	Mograne	Zaghouan	semi-arid
8	Saoef	Zaghouan	semi-arid
9	Sabkhet Solimene	Nabeul	semi-arid
10	Sidi Mtir	Sousse	semi-arid
11	Enfidha	Sousse	semi-arid
12	Ouled Salah	Mahdia	Semi-arid
13	Skhira	Sfax	semi-arid
14	Sabkhet Wadrane	Sfax	semi-arid
15	Mezouna	Sidi Bouzid	arid
16	Bredaa	Mahdia	arid
17	Kerkennah	Kerkennah	arid
18	Manzel Habib	Gabes	arid
19	Kettana	Gabes	arid
20	Hessi Jalleb	Medenine	arid
21	Ardhaoui	Medenine	sahara

Morphological measurements

Four morphological traits (Table 2) were scored on the 21 barley genotypes. Six individual plants from the control and each salt treatment were used.

The distances from crown to leaf tip and root tip were measured as shoot length (SL) and root length (RL), respectively. The number of leaves (NL) and tiller (TN) per plant was counted and the mean values of each replication were used for statistical analyses.

Physiological measurements

Biomass

Biomass was determined from control and salt stressed plants. At harvest times, the roots and shoots of plants from each replication were separated. The fresh weight was measured for shoot (SFW), root (RFW) and whole plant (TFW). After being

Table 2. Morphological and physiological traits used with their contribution to the definition of the axis F1 and F2 of the PCA under 0, 200 and 250 mM salt concentration.

		NaCl (mM)					
		0		200		250	
No.	Traits	F1	F2	F1	F2	F1	F2
1	Shoot fresh weight (SFW)	12.472	13.827	11.524	1.319	12.802	2.303
2	Root fresh weight (RFW	2.522	14.976	9.829	2.906	11.048	0.080
3	Total fresh weight (TFW)	12.068	19.543	12.125	1.762	13.995	1.623
4	Shoot dry weight (SDW)	16.910	1.710	2.633	18.292	4.142	31.398
5	Root dry weight (RDW)	0.670	0.310	9.131	0.001	11.645	0.037
6	Total dry weight (TDW)	15.113	1.250	5.174	15.047	2.952	27.721
7	Tiller number (TN)	4.182	0.362	2.008	7.053	0.066	0.701
8	Leaf number (LN)	9.776	0.261	0.140	22.086	0.040	11.851
9	Shoo lengtht (SL)	6.659	24.230	0.191	0.017	0.487	0.326
10	Root length (RL)	6.134	12.214	3.384	11.478	1.060	7.496
11	Relative water content (RWC)	0.098	9.625	9.779	7.514	8.320	5.509
12	Net CO_2 assimilation (A)	1.948	0.869	12.781	3.739	13.029	2.302
13	Stomatal conductance (gs)	4.853	0.298	10.706	3.464	11.509	3.752
14	Transpiration (E)	6.596	0.525	10.597	5.322	8.907	4.902

F1 and F2 correspond to the first axis and the second axis of PCA blot for each salt concentration applied.

dried at 70°C in an oven until the samples reached a constant weight, the dry weight of roots (RDW) and shoots (SDW) per plant, were measured (Table 2).

Relative water content (RWC)

To determine the relative water content (RWC), the harvested flag leaves were immediately weighed to obtain the fresh weights (FW), and then floated on distilled water to reach saturation. After 24 h of incubation the turgid weights (TW) were measured. Leaf samples were oven dried at 70°C for 72 h and dry weights (DW) were assessed.

RWC was calculated according to Barrs and Weatherley formula (1968).

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

Photosynthetic parameters

Leaf gas exchange parameters, such as net CO_2 assimilation (A), stomatal conductance (gs), and transpiration rate (E) were measured using a portable photosynthesis system (LC pro+). Measurements were taken from the mid-lamina portion of the abaxial surface of the youngest fully expanded leaf at the harvesting day.

Data were taken earlier from 10.30 to 12.00 a.m. according to the following conditions: leaf surface area 5.8 cm^2, ambient CO_2 concentration (Cref) 377.5 $mmol.mol^{-1}$, temperature of the leaf chamber varied from 28.8 to 33.3°C, ambient pressure (P) 1022 mbar, PPFD (Qleaf) at the leaf surface was maximum up to 650 $\mu mol/m^2$. Relative intercellular CO_2 concentration (Ci/Ca) was calculated using the formula: Ci/Ca = intercellular CO_2 concentration/ambient CO_2 concentration.

Data analysis

Data were analyzed by multivariate analysis, clustering and ANOVA analysis using XLSTAT software (Addinsoft, www.xlstat.com). The principal component analysis (PCA) was applied on two data sets from control and stressed plants. Each matrix contains 21 genotypes in rows and the measured parameters in columns. PCA was performed to identify accession groups, to determine the axes and the characters significantly contributing to the variation and to identify accessions with contrasting behavior towards salinity (tolerant/sensitive). In this procedure, the similarity matrix was used to generate Eigen values and scores for the genotypes. The first two principal components, which accounted for the highest variation, were then used to plot two-dimensional scatter plots.

The ranking of genotypes for salt tolerance using the most discriminating physiological descriptors under 250 mM of salt concentration was performed. The data were converted to salt tolerance indices using the method of (Zeng et al. 2002). The salt tolerance indices were defined as the observation under salt stress divided by the average of the controls. Both TFT and (A) percentage decrease in comparison to control were calculated as bellow:

$$\text{Percentage decrease}\ (\%) = [(\text{control} - \text{stress}) / (\text{control})] \times 100$$

Data variance between treatments was assessed by STATISTICA software (ANOVA/MANOVA) and comparison of means by higher significant difference (HSD) Duncan's test ($p \leq 0.05$). Six replicates were used for each parameter.

Results

In this study, fourteen morphological and physiological descriptors (Table 2) were used in order to analyze the response of a set of 21 barley landraces genotypes under two severe salt stress concentrations (200 and 250 mM) as well as under control condition. The Principal Component Analysis (PCA) based on all the variables was used for discrimination between genotypes.

Morpho-physiological response under control conditions

Fourteen morphological and physiological descriptors were used for the characterization of the genotypic response under control condition.

The PCA plot setup for control plants (Figure 1a) shows 43.92% of cumulative variance. The first axis (25.41%) was highly correlated to the shoot dry weight and to the total dry weight ($r = 0.655$, $r = 0.733$, respectively) (Table 2). The second axis (18.51%) was correlated to the total fresh weight and the shoot length.

The distribution of the 21 control genotypes on the first two PCA axis is shown in Figure 1a. On the positive side of axis1, we found the genotypes which are characterized by the highest shoot dry weight and the total dry weight. Whereas, the genotypes located on the negative side of the axis are characterized by the lowest shoot length.

Morpho-physiological characterization at 200 mM NaCl

Under 200 mM of salt concentration, we obtained different clustering schema of the genotypes with different response against salt stress. In fact, the PCA plot revealed high level of variation (65% of cumulative variance between axis 1 and 2) (Figure 1b). The first axis (F1 = 44.66%) was defined by the total fresh weight parameter and the net CO_2 assimilation (Table 2) ($r = 0.871$, $r = 0.894$, respectively). The second axis (F2 = 20.33%) was defined by both leaf number and shoot dry weight. The distribution of the 21 stressed genotypes on the two PCA axis (Figure 1b) shows that the genotypes characterized by the highest total fresh weight and the net CO_2 assimilation are located on the positive side. The genotypes on the negative side are characterized by the lowest values. These data indicate that several

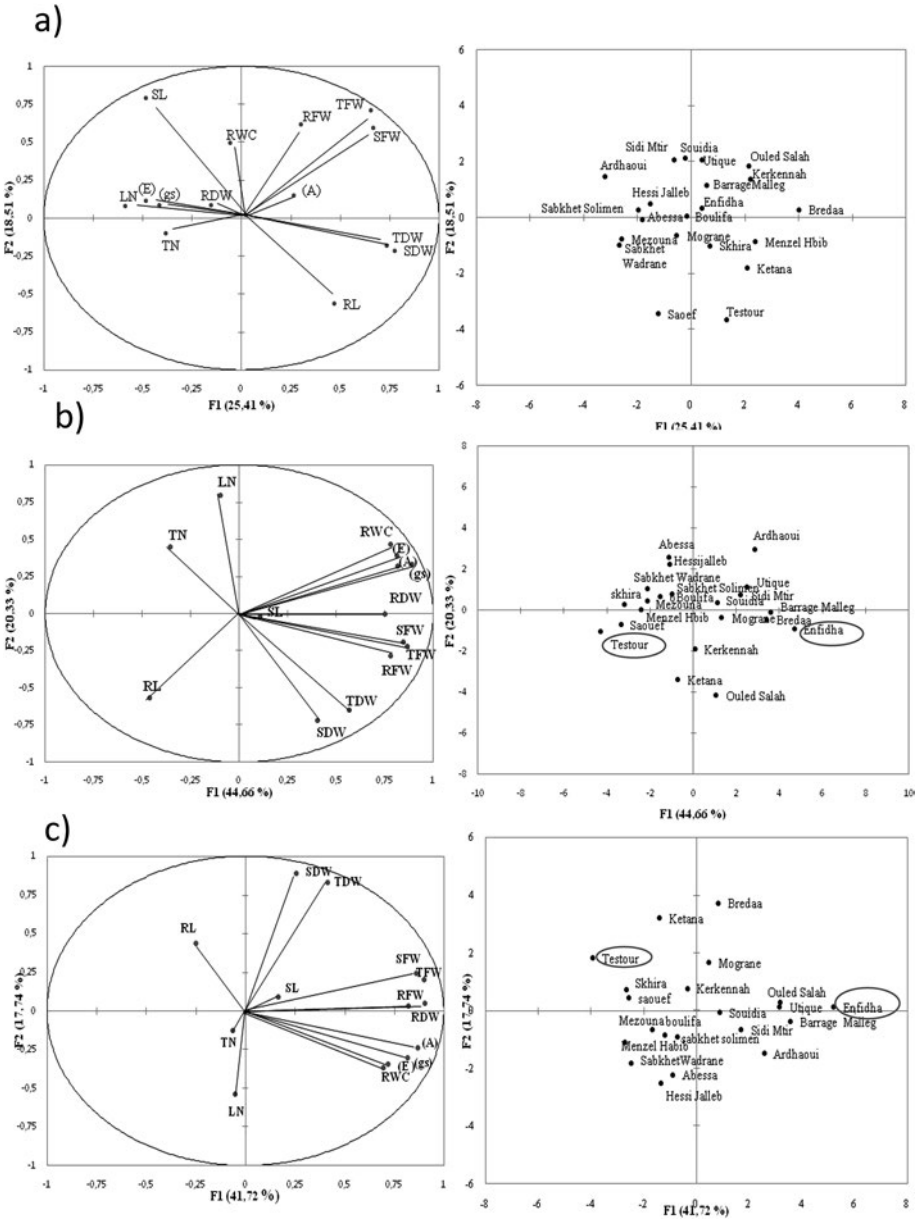

Figure 1. Principal component analyses PCA plots showing the contribution of the 14 morphological and physiological parameters to the variation under control and salt stress conditions (on the left) and grouping of the 21 Tunisian barley landraces according to F1 and F2 axes (on the right). (a) Control condition, (b) 200 mM NaCl and (c) 250 mM NaCl.

genotypes were able to maintain their productivity and overcome salt stress. These genotypes are considered as the most tolerant genotypes. According to the dendrogram (Figure 2) and based on all descriptors analyzed on 200 mM salt stress, the 21 studied genotypes exhibited different responses toward salt treatment. Indeed, genotypes were grouped into three major groups: C1 (13 genotypes), C2 (3 genotypes) and C3 (5 genotypes), as illustrated in the dendrogram. Per variation class, the genotypes "Manzel Habib", "Mograne" and "Barrage Malleg" were identified as the barycenters of the groups C1, C2 and C3, respectively (Figure 2).

Morpho-physiological characterization at 250 mM NaCl

The PCA plot at 250 mM salt treatment, revealed 59.46% of cumulative variance between axis1 and 2 (Figure 1c). The first axis (F1 = 30.52%) is defined by the total fresh weight and the net CO_2 assimilation parameters. The second axis (F2 = 41.72%) is characterized by the total dry weight and the shoot dry weight. Whereas, tiller number, leaf number and root length were the least effective traits (Table 2). Thus, eleven descriptors out of fourteen were identified as the most useful descriptors for the classification of the genotypes.

Regarding the genotypes clustering, one more different scheme was obtained. Indeed, the genotypes are divided

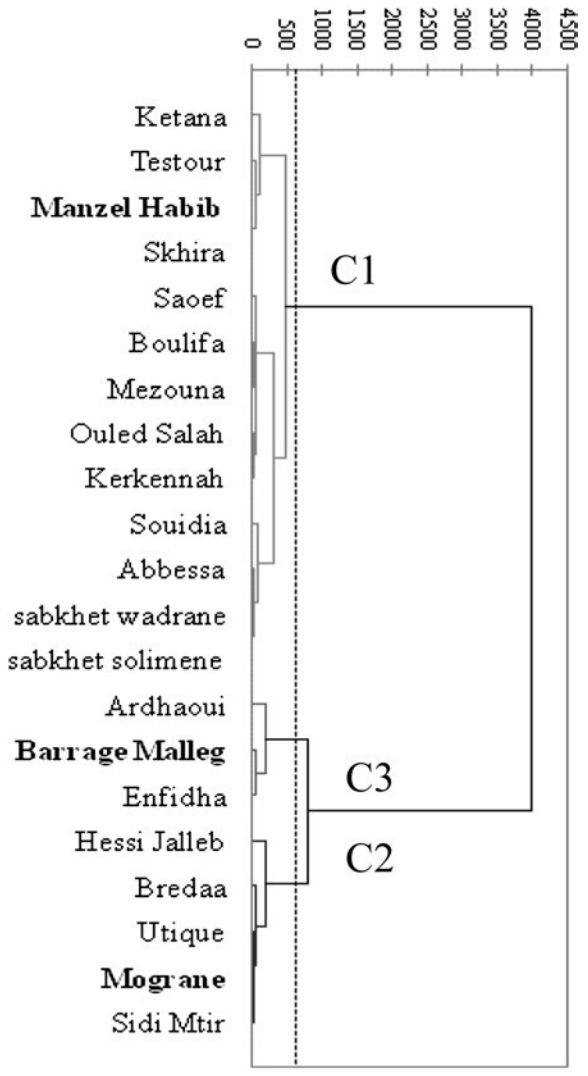

Figure 2. Cluster analyses of Tunisian barley landraces based on Euclidian distances calculated with 14 morphological and physiological traits under 200 mM NaCl. C1, C2 and C3 represent the groups individualized and the accessions in bold represent the barycenters of the groups.

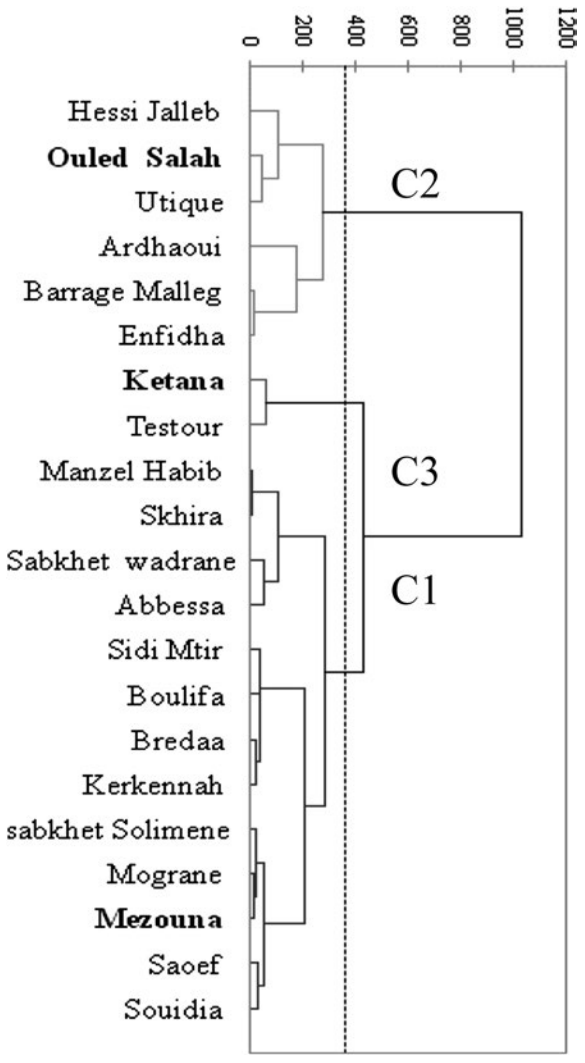

Figure 3. Cluster analyses of Tunisian barley landraces based on Euclidian distances calculated with 14 morphological and physiological traits at 250 mM NaCl. C1, C2 and C3 represent the groups individualized and the accessions in bold represent the barycentres of the groups.

in two groups and each groups characterized by a specific behavior towards salt stress (Figure 3). The first group contains genotypes with high fresh weight and net CO_2 assimilation which means that these genotypes are able to maintain high photosynthesis and productivity level. The second group is characterized by low fresh weight and net CO_2 assimilation representing salt-sensitive genotypes.

According to the dendrogram and based on all descriptors, the genotypes were clustered into three main groups (C1, C2 and C3) (Figure 3). The group C1 was the largest one, it contains 13 genotypes, the group C2 enclosed 6 genotypes, and the group C3 included 2 genotypes.

Per variation class, the genotypes "Mezouna", "Ouled Salah" and "Kettana" were identified as the barycenters of the identified groups C1, C2 and C3, respectively.

Ranking of Genotypes for Salt Tolerance

According to the multivariate analysis method, only the most discriminating physiological descriptors were maintained for the evaluation of the physiological response within the accessions studied at 250 mM salt stress. Therefore, only total fresh weight and net CO_2 assimilation (parameters defining axis1 of the PCA plot at 250 mM) were used for the ranking of all genotypes for salt stress tolerance using salt tolerance indices and percentage decrease values (Table 3, Figures 4, 5).

Results have shown that salinity had negative effect on vegetative development. Indeed, salt tolerance indices decreased with the increase of salt stress. Besides, salt tolerance indices varied among genotypes (Table 3). For instance, The salt tolerance indices of total fresh weight were ranged from 0.27 "Souidia" and "Testour" genotypes to 0.65 "Enfidha" genotype. In the same way,

Table 3. Salt tolerance indices calculated from the most discriminating parameters in Tunisian local barely genotypes under 250 mM salinity concentration.

Genotypes	TFW	(A)
Testour	0.267	0.125
Souidia	0.267	0.228
Ouled Salah	0.302	0.295
Barrage Malleg	0.315	0.264
Skhira	0.319	0.128
Boulifa	0.338	0.241
Saouef	0.395	0.138
Sabkhet Wadrane	0.413	0.155
Abbessa	0.413	0.220
Sabkhet Solimene	0.421	0.196
Mezouna	0.424	0.146
Mograne	0.452	0.228
Manzel Habib	0.461	0.138
Utique	0.464	0.257
Kerkennah	0.489	0.217
Sidi Mtir	0.493	0.244
Hessi Jalleb	0.514	0.221
Kettana	0.528	0.174
Bredaa	0.540	0.146
Ardhaoui	0.554	0.260
Enfidha	0.646	0.313

TFW: Total fresh weight, (A): Net CO_2 assimilation

the salt tolerance indices of net CO_2 assimilation were ranged from 0.13 "Testour" to 0.31 "Enfidha" genotypes. The genotypes showing the highest salt tolerance indices are considered as the most tolerant to salt stress.

Figures 4 and 5 illustrate the percentage decrease of total fresh weight and net CO_2 assimilation relative to control under 250 mM of salt concentration. These data showed a decrease from 76.55 to 35.35% for the total fresh weight and from 77.47 to 58.69% for the net CO_2 assimilation. The genotypes showing the highest percentage decrease are considered as the most sensitive to salt stress. According to Duncan test, significant differences were detected between the analyzed genotypes. The total fresh weight percentage decrease allowed the classification of the 21 analyzed genotypes into 16 different groups. The genotypes marked 'a' were the most sensitive genotypes to salt stress. Whereas, those marked 'l' were the most tolerant. The others genotypes are considered intermediate (Figure 4).

The classification based on the net CO_2 assimilation percentage decrease classified the genotypes into 15 groups. The genotypes marked with the letter 'a' were the most affected by salt stress, whereas the one marked with 'm' was the most tolerant (Figure 5).

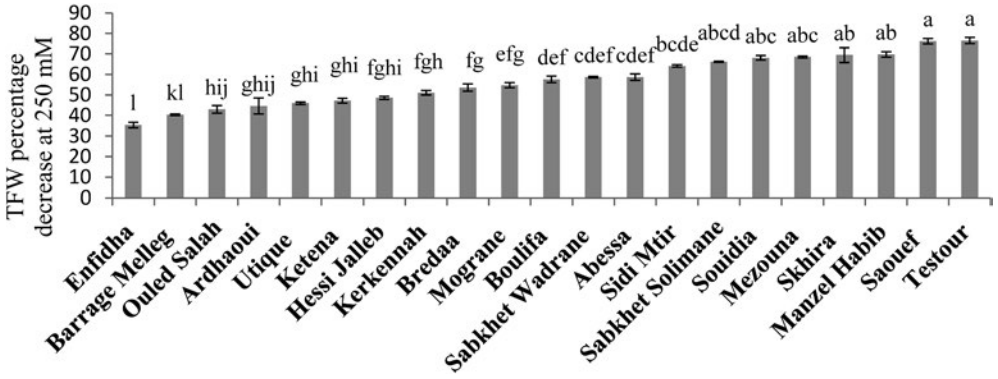

Figure 4. Total fresh weight (TFW) percentage decrease at 250 mM NaCl. Means of 6 replicates ± standard error. For each genotype, means with the same letter are not significantly different at $p \leq 0.05$, according to Duncan's test.

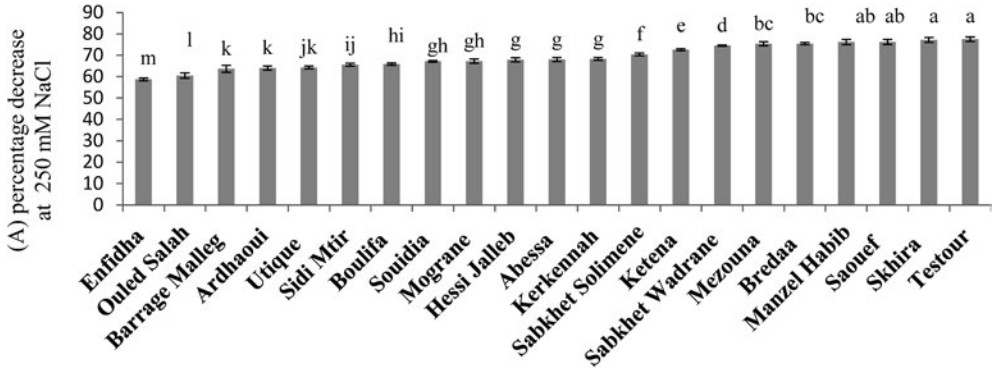

Figure 5. Net CO_2 assimilation (A) percentage decreases at 250 mM NaCl. Means of 6 replicates ± standard error. For each genotype, means with the same letter are not significantly different at $p \leq 0.05$, according to Duncan's test.

Identification of the contrasting pair genotypes towards salinity

It was released from the salt tolerance indices (Table 3) and the percentage decreases (Figures 4, 5) that "Testour" and "saoef" genotypes were the most sensitive and "Enfidha" the most tolerant one. However, the integration of all parameters using PCA analysis allowed to distinguish "Testour" genotype as the most sensitive one. Indeed, this pair accession, with contrasting behavior toward salinity delimited the variation area on the PCA plot on which they were diametrically opposite (Figure 1c). Thus, the PCA analysis seems to be more efficient to discriminate between contrasting genotypes.

Discussion

The selection of the most tolerant genotypes toward salinity can be considered as one of the main targets in plant breeding. In the present study, salt tolerance was evaluated at vegetative stage utilizing twenty-one local barley landraces, well representative of the barley genetic diversity in Tunisia and based on fourteen morphological and physiological traits. The collection has shown good candidates according to our objectives: a rich gene-pool with a large geographical distribution and diversity in adaptation to abiotic stresses (Zoghlami et al. 2011). Besides, we were able to select for the first time genotypes with contrasting behavior toward salinity using multivariate analyses. Multivariate analyses based on morphological and physiological parameters proved to be valuable tools for the description and the classification of the genotypes but also for the identification of salt tolerant genotypes. Previously, these statistical tools were also identified as powerful for the identification of salt tolerant accession in crop collection species such as rice (Cha-um et al. 2009), green gram (Ahmad et al. 2005), wheat (El-Hendawy et al. 2005), tomato (Juan et al. 2005), sugarcane (Cha-um et al. 2012), pea-nut (Liu et al. 2012) and cauliflower (Zhu et al. 2012).The advantages of using this method in the evaluation of salt tolerance are: (i) PCA allows a simultaneous analysis of multiple parameters to increase the accuracy of the genotype ranking at different salt levels (Jianjie et al. 2013) (ii) it allows the visualization of differences among individuals as well as (iii) the identification of possible groups and the establishment of relationships among individuals and variables (Martinez-Calvo et al. 2008).

It has been well documented that plant growth and development can be affected differently by several salt stress concentrations at various growth stages. In rice and wheat, the seedling stage is the most sensitive to salinity (Munns and Tester 2008). However, within barley, the most sensitive stages towards salinity seem to be the earlier growth stages (Adjel et al. 2013). Indeed, the vegetative stage which is characterized by its high tillering capacity appears to be a very important stage to evaluate genotypes response towards salinity (Ben Khaled et al. 2012). Thus salt tolerance at vegetative stage is considered as an important indicator of barley salt tolerance at later growth stages.

The present data shows that at the vegetative stage, the studied genotypes exhibited significant different responses to salt stress. Indeed, our results show a variation between genotypes of 65% and 59.46% at 200 mM and 250 mM, respectively. However, at control condition the variability is only 43.82% (Figure 1). This is consistent with previous findings that have indicated significant differences in the salt tolerance of barley genotypes (Bchini et al. 2011). The genotypic response to salinity also depended on the intensity of salt stress. The use of severe saline stress in our study was designed as a method to more rapidly, and visibly, help identifying salt-tolerant versus sensitive genotypes. Therefore, severe salt stress (200 and 250 mM NaCl) was used to elicit visible and measurable phenotypic differences among salt-tolerant and sensitive analyzed barley genotypes.

All the parameters here assessed were differently affected by salinity. Furthermore, the highest salt stress concentration used (250 mM) was the most damaging to all barely genotypes analyzed. Under this most severe salt stress concentration, the two physiological parameters: total fresh weight and net CO_2 assimilation (photosynthesis) were identified as the major traits conferring salinity tolerance as they exhibited the highest contribution value (13.99 and 13.03, respectively; Table 2). This goes in pair with Shafi et al. (2009) data, which demonstrate that biomass and photosynthesis were less affected in tolerant cultivars as compared with medium tolerant and sensitive one. Moreover, several studies explained the ultimate relation between these two parameters: the toxic effect of sodium at severe salt stress and physical damage to roots decreased their ability to absorb water and nutrient which caused marked reduction in photosynthesis enzymatic process and protein synthesis (Dadkhah 2011; Dulai et al. 2011). The decrease in the rate of photosynthesis due to leaf area might be responsible to decrease the shoot fresh and in turn the dry weight. It is evident from our results, that salt tolerant genotypes were able to maintain their biomass and photosynthesis and consequently able to overcome salt stress.

Based on all these reasons, these two characters were used as the most discriminating traits to evaluate genotypes for salt tolerance. The total fresh weight and net CO_2 assimilation were substantially reduced in all barely genotypes. Nevertheless, several genotypes had the highest biomass production and photosynthesis under control conditions could not maintain this level under salt stress conditions and seem to be the most highly affected genotypes by salt stress. For these reasons

it is very important to use the percentage decrease or tolerance indices in relation with the control average for genotype classification. Salt sensitive genotypes showed the maximum percentage reduction of total fresh weight and net CO_2 assimilation. However, the most tolerant ones showed minimal percentages decrease. These results corroborate those obtained by Ben Khaled et al. (2012).

To deeply analyze the different salt tolerance response of the studied genotypes, we further used the salt tolerance indices to distinguish between contrasting genotypes. Hence, it was released that two genotypes would be considered as the most sensitive "Testour" and "Saoef" and one as the most tolerant "Enfidha" (Table 3). The PCA plot was the most efficient to identify one pair genotypes with contrasting behavior towards salinity which are "Enfidha" (tolerant) and "Testour" (sensitive) (Figure 1c).

Obviously, knowledge of underlying physiological adaptation to salinity is very efficient for screening methods (Zhu 2000). Some researchers have suggested that screening for salt tolerance can be carried out using physiological markers (Bchini et al. 2011; Farissi et al. 2014). Our findings go in pair with them and showed that morpho-physiological traits could be effective for classification and screening of salt tolerant barley landraces. Our results indicate the existence of genetic potential for salt tolerance among this barely landraces collection and they are tolerant up to 250 mM salinity level during the vegetative growth stage. Hence, important consideration should be given to Tunisian barley landrace genotypes for breeding programs.

Conclusion

The multivariate data methodologies proved to be powerful tools for the selection of barley genotypes with contrasting behavior towards salinity and can be applied in salt tolerance breeding programs. Besides, due to salinity total fresh weight and net CO_2 assimilation can be used as suitable descriptors to evaluate salt tolerance in barely. The two contrasting barley genotypes in salt tolerance: "Enfidha" (tolerant) and "Testour" (sensitive) could be of great interest in future breeding programs for modern cultivar improvement.

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