ACTA SCIENTIFIC AGRICULTURE (ISSN: 2581-365X)

Volume 4 Issue 7 July 2020

Research Article

The Arsenal of Morphological and Physiological Mechanisms Adopted by Barley (*Hordeum vulgare*. L) to Face Salt Stress Damage

Zied Hammami[1]*, Nawel Ahmed[2], Nejia Ben Hmida[2], Soumaya Tounsi[2], Sawsen Ayadi[2] and Youssef Trifa[2]

[1]*Crop Diversification and Genetics Section, International Center for Biosaline Agriculture (ICBA), Dubai, UAE*

[2]*Laboratory of Genetics and Cereal Breeding, National Institute of Agronomy of Tunisia, Carthage University, Tunis, Tunisia*

***Corresponding Author:** Zied Hammami, Crop Diversification and Genetics Section, International Center for Biosaline Agriculture (ICBA), Dubai, UAE.

Received: April 16, 2020

Published: June 29, 2020

Abstract

The success of programmes improving barley performance under abiotic stress must go through an understanding of mechanisms developed by the plant to counteract this stress. Our study fits into this framework. It aims to evaluate six barley genotypes from the MENA region, treated with two salinity levels: 1.2 and 14 dS/m. Three genotypes are salt-tolerant, and three are sensitive. They were grown under a controlled environment and in 40L PVC tubes using sand and perlite as substrate. The evaluation was based on 15 morpho-physiological parameters related to water, ion content, temperature, and chlorophyll fluorescence.

The results confirm the existence of genetic variability for salt tolerance. Two Tunisian landraces 'Suihli' and 'Ardhaoui' and Omanis landraces 'Batini 100/1B' were not affected. Conversely, 'ICARDA20' and 'Barley Mednine' appeared to be sensitive to salt stress with a maximum reduction of 35% for improved genotype 'Konous'.

Results also show that salt tolerance in barley cannot be exclusively attributed to a single mechanism. All studied parameters significantly ($p < 0.001$) contributed to it. However, Stepwise regression revealed that plant water status expected by RWC is the key for salinity tolerance as well as a positive effect of K+ content, Fm/Fv, and leaf Temperature on proper water status.

The results highlight the effeteness of one visual trait, the salinity damage index (DI), to estimate barley tolerance. Indeed, a strong correlation was observed between DI and the biomass reduction ($P < 0.001$, $r^2 = 0.96$). In addition, correlation analyses showed that all parameters were inversely correlated with the DI.

Keywords: Salinity; Barley; Morpho-Physiological Parameters Traits; Tolerance; Damage Index

Abbreviations

SSI: Salinity Sensitivity Index; DI: Damage Index; TDW: Total Dry Weight; LA: Leaf Area; T: Leaf Temperature; RWC: Relative Water Content; SPAD: SPAD Value; RV: Root Volume; Fv/Fm: Chlorophyll Fluorescence; Chl_a: Leaf Content in Chlorophyll a; Chl_b: Leaf Content in Chlorophyll b; Car: Carotenoids; Na-L: Na^+ Content in Leaves; Na_R: Na^+ Content in Roots; K_L: K^+ Content in Leaves; K_R: K^+ Content in Roots; Ca_L: Ca^{2+} Content in Leaves; Ca_R: Ca^{2+} Content in Roots

Introduction

The Near East and North Africa (NENA) region is characterized by an arid and semi-arid climate that extends over the majority of the territory as well as by a significant intra- and inter-annual variability of rainfall and temperature, hence the obligation to make complementary and even total irrigations to ensure the survival and productivity of the culture. Water supplies for irrigation are scarce, and many contain high levels of salt. The weather forecasts for the years ahead are even harder.

Indeed, studies about the resilience of ecosystems to climate change indicate an increase in temperature and a decrease in precipitation by the year 2030. Thus, the lack of water and its poor quality are the most limiting factors facing NENA region agriculture, mainly in arid and semi-arid areas. The valorization of these areas is particularly challenging due to the degree of salt tolerance of the cultivated species, which are, for the most part, sensitive glycophytes [1]. The best approach is to use species that are tolerant of salt. It thus requires an adequate varietal choice. Barley is among the crops that value arid areas and salty environments [2], but its productivity remains insufficient, and the average grain yield in those areas never exceeds 0.5 t ha–1 [3]. Breeding efforts should be made to select productive varieties that are adapted to abiotic stresses such as salinity.

Indeed, the breeding program's success is based on the understanding of the mechanisms of salt stress on plants and their response to overcome it. Thus, studying the morpho-physiological behavior of barley genotypes in response to salinity is crucial for their discrimination and the selection of tolerant varieties. In this context, for marginal areas characterised by a multitude of concurrent stress (salinity, heat stress, low soil fertility, etc.), local barley could be a source of discovery of interesting characters for tolerance to these constraints [4-6]. These genotypes could be a source of genetic variation for crop improvement. Wu., *et al*. [7] reported that salinity tolerance mechanisms could be grouped into three main mechanisms, namely, Na^+ exclusion, osmotic tolerance, and tissue tolerance.

Plant physiology offers several parameters for investigating the effects of abiotic stresses on plant growth and yield. Salinity tolerance is the phenotypic expression of a complex set of biochemical and morpho-physiological properties attributed to multiple mechanisms, including Na^+ exclusion, Na^+ sequestration in vacuoles, K^+ retention in the cytosol, osmotic adjustment and xylem control [7]. The absorption of Na^+ and Cl^- must be limited, while maintaining the absorption of macronutrients such as K^+, NO^{3-} and Ca^{2+} [8,9]. Cytosolic K^+ is essential for the activation of several metabolic enzymes and for the reduction of the activity of endonucleolytic enzymes responsible for triggering programmed cell death in cells affected by salt [10]. Several regulatory mechanisms based on the presence of calcium and its role in Ca^{2+} signaling have been identified as indicators of salt tolerance [11]. Salinity tolerance was correlated with Na^+/Ca^{2+} selectivity [12] based on a simple exchange of ions at the plasma membrane surface [13]. Jones., *et al*. [14] mentioned that leaf temperature is an indicator of the stomatal conductance of barley grown under a range of salt treatments. Indeed, the effeteness of this parameter as selected criteria. Measurements of photosynthetic activity can, therefore, also transmit valuable information about the "state of health" of plants [15] and particularly facing salt stress [16].

Discoveries on salinity tolerance mechanisms must be applied to crops to improve their degree of tolerance [17]. Effective phenotyping, meant to differentiate between genotypes, is needed for yield improvement in saline environments [18]. However, cost and time can affect the efficiency of varietal selection and breeding programs. For that reason, we have to reduce the number of traits to consider only a few key ones and/or a quick efficacy visual test.

This study focuses on salinity tolerance in contrasting pairs of barley genotypes for a better understanding of the physiological origins of this tolerance and the easiest method to estimate it.

Materials and Methods

Plant material

Six barley varieties (*Hordeum vulgare*. L) of different origins were studied, three are salinity-sensitive, and three are tolerant to salt stress. Three varieties were obtained from the International Center for Biosaline Agriculture (ICBA), Dubai, United Arab Emirates (Table 1).

Genotypes	Genetic Class	Origin	Tolerance	Reference
Konouz	IV	Tunisia (North)	Sensitive	[45]
Ardhaoui	LA	Tunisia (Mareth)	Tolerant	[46,47]
Suihli	LA	Tunisia (Mahdia)	Tolerant	[45,46]
Barley Mednine	LA	Tunisia (ICARDA)	Sensitive	[46]
ICARDA 20	IV	Introduced (ICARDA)	Sensitive	[47]
100/1B	LA	Introduced (Oman)	Tolerant	[48,49]

Table 1: Characteristics of the tested varieties.
IV: Improved Variety; LA: Local Accession.

Environment, treatments and experimental design

Source of Barley salt tolerance was studied in glass greenhouse using a semi-hydroponic culture technique at the National Agronomic Institute of Tunisia (INAT). Six genotypes were grown in PVC tubes (1.2m length and 0.2m in diameter) containing a mixed substrate of 70% perlite and 30% sand. Twelve seeds were sown manually per tube. The plants were regularly irrigated with 120 ml of ½ Hoagland solution (pH: 5.5 to 6) (Arnon and Hoagland, 1940). At the three-leaf stage, ten plants were left to ensure the uniformity of the test. Then salinity treatment commenced and two water irrigation treatments differing by the level of salinity: 1.2 dS/m and 13 dS/m were applied, similar to standard salinity water in arid Tunisian area [6]. The essay was arranged in a completely randomized block device with five repetitions. Harvesting was done at the flowering stage, 121 days after sowing.

Measured parameters

Morphological parameters

At 120 days after sowing, plants were collected, roots, and shoots part were separated. The roots were cleaned and immersed in a known quantity of water to determine their volume in cm^3, which was equal to the difference between water level before and after immersion. The flag leaf area of each plant was determined in cm^2 using a LICOR benchtop planimeter. Both aerial and root parts were dried in the oven for 72 hours at 80°C and weighed to determine their total dry weight (TDW).

Physiological parameters

Ten flag leaf taken at random from each tube were arbitrary sampled to determine selected salinity tolerance parameters were measured according to Tester M and Davenport R [19].

Canopy temperature

The Canopy temperature measurement was performed using Sixth Sense LT300 Infrared Thermometer Technologies, USA).

Chlorophyll fluorescence and chlorophyll content measurements

Chlorophyll characteristics were directly recorded using a chlorophyll meter SPAD (Soil Plant Analysis Development, Minolta SPAD 502 Meter, Osaka, Japan) and Chlorophyll fluorescence characteristic (Fv/Fm) was recorded using a portable multimode chlorophyll fluorometer (Model, OS5P Optisciences, Inc. Winn Avenue Hudson, USA). Chlorophyll pigments a, b, and carotenoids (m/gFM) were extracted and determined from the same leaves, according to Torrecillas A., *et al*. [20], Arnon., DI [21] respectively.

Ion concentration Na+, K+ and Ca2+

Ion measurements were obtained as described by Pauwels JM., *et al* [22]. The Na^+, K^+, and Ca^{2+} were measured using standard flame photometer procedure (MODEL PFP7 Flame photometer, JENWAY, Bibby Scientific Ltd, UK) and reported as mg g^{-1} dry weight.

Relative water content

The relative water content (RWC) of each fresh leaf sampled is determined and calculated according to Gonzalez L and Gonzalez-Vilar M [23].

Damage index (DI)

Saline stress damage index was scored averaging the damage index from zero to ten (0-no visual symptoms of the damage, 10-symptoms are very visual) using criteria such as the extent of leaf chlorosis, the number of dead leaves and the survival rate of the tillers [24,25]. The final value of DI for each genotype was the average of five replicates (each replicate was the average of 8 plants per pot).

Statistical analysis

Salinity sensitivity index (SSI)

Tolerance to salinity was assessed for each genotype using the Salinity Sensitivity Index (SSI). The SSI values were calculated for each genotype and each parameter as the ratio between the parameter value (X) measured under salt stress (Xs) and the measured value without salt stress (control) (Xc) [26]:

$$SSI = (Y_S/Y_C) * 100$$

The data were subjected to statistical analysis using the statistical software R version 3.1.2. All variables were analyzed using a linear variance analysis model (ANOVA $p < 0.05$) to study the significance of genotype effects, salt treatments, and interaction treatment*genotype. Multiple comparisons were processed using Duncan´s multiple range test ($\alpha = 0.05$). Descriptive statistics were performed on the original scores of the variables. A correlation

matrix with significance levels (p-value). Correlation analysis using Pearson parametric correlation test was performed to determine the correlation coefficients and the p-value of the correlation for all possible pairs of columns in the data table. Stepwise regression was carried out to group the different parameters and to schematize the relationships between the parameters and their contributions to salinity tolerance.

Results

Effect of salinity on the studied parameters

The analysis of variance performed on the different morphological and physiological parameters measured in the six barley genotypes is summarized in table 2. The salinity of irrigation water appears to induce significant growth and development disturbances for all the tested varieties. The results showed a significant difference ($p < 0.001$) between control (EC: 1.2 dS/m) and saline (EC: 13 dS/m) conditions according to the Fisher variance comparison test for most of the measured parameters, except for chlorophyll b and carotenoids. Besides, a significant difference was observed ($p < 0.001$) between varieties for all parameters. The interaction Treatment*Genotype was also significant for all parameters except the SPAD value indicating a difference in varietal behavior to the salinity treatment.

The comparison between biomass produced by the six barley genotypes irrigated with the two contrasted salty water treatments (1.2 dS/m and 14 dS/m) confirms that the two local accessions, Ardhaoui and Suihli, and the Oman local one Batini (100/1B), are the most tolerant to salt stress. Ardhaoui and Suihli are especially not affected by stress, unlike the three genotypes BarleyMednine, ICARDA20, and Konouz that recorded the most significant reduction in biomass (35%) (Table 3).

Parameters	Treatment	Genotypes	Interaction
SDW	<0.0001	<0.0001	0.00612
LA	<0.0001	<0.0001	<0.0001
RV	<0.0001	<0.0001	0.00499
T	<0.0001	0.00958	<0.0001
RWC	<0.0001	<0.0001	0.0157
SPAD	0.2887	<0.00109	0.61532
Fv/Fm	<0.0001	<0.0001	<0.0001
Na_L	0.00916	<0.0001	0.00925
Na_R	<0.0001	<0.0001	<0.0001
K_L	0.01190	<0.0001	0.00561
K_R	<0.0001	<0.0001	<0.0001
Ca_L	0.0444	<0.0001	0.0176
Ca_R	<0.0001	<0.0001	<0.0001
Chl_a	0.0200	0.0136	<0.0001
Chl_b	0.306	<0.0001	<0.0001
Car	0.42	<0.0001	<0.0001

Table 2: Analysis of variance and significance levels observed (p-values) for the effects of Saline Treatment, Barley Genotype and their Interaction for Total Dry Weight (TDW), Leaf Area (LA), Leaf Temperature (T), Relative Water Content (RWC), SPAD value (SPAD), root volume (RV), chlorophyll fluorescence (Fv/Fm), leaf content in chlorophyll a (Chl_a), b (Chl_b) and carotenoids (Car) Na^+ content in leaves (Na-L) and roots (Na_R), K^+ content in leaves (K_L) and roots (K_R) and Ca^{2+} content in leaves (Ca_L) and roots (Ca_R).

Genotypes		Salinity Sensitivity Index (SSI)															
		TDW	AS	RV	T	RWC	SPAD	Fv/Fm	Na_L	Na_R	K-L	K-R	Ca-F	Ca-R	Chl_a	Chl_b	Car
P-value		0.00176	0.00108	0.00405	0.00131	0.0023	0.00092	0.00074	0.0399	<0.0001	<0.0001	<0.0001	0.00157	0.0011	<0.0001	<0.0001	0.002
Ardhaoui	11	99.99a	99.65a	101.2a	99.34c	99.93a	91.68b	97.18a	113.7a	85.39ab	94.12a	100a	106.7a	100a	89.61de	166.9a	81.03cd
100/1B	10	89.44abc	88.81a	68.12bc	99.42c	99.99a	104.8a	97.16a	87.04ab	76.79b	94.74a	95.56a	95.83a	100a	117.9b	106.2b	141.8ab
Barley Mednine	2	81.21bcd	53.8b	57.91c	116.8ab	92.48ab	91.01b	83.81b	69.52b	53.07d	69.49b	66.71c	58.93b	58.61cd	96.48cd	102.2b	85.11cd
ICARDA 20	4	74.85cd	65.57b	80.16ab	120.4a	92.72ab	91.89b	87.75b	87.88ab	91a	60.58b	59.69d	67.62b	43.45d	80.14^e	90.29b	169.2a
Konouz	5	66.92d	62.55b	89.63ab	106bc	89.79b	102.8a	75.86c	92.35ab	63.32c	83.68b	86.63b	95.24a	73.21bc	104.5c	86.22b	54.12d
Suihli	12	97.19ab	88.03a	84.38ab	98.75c	100.8a	106.3a	96.36a	99.53a	86.09a	98.15a	95.69a	95.24a	88.89ab	151.9a	81.56b	108.5bc

Table 3: Significance levels (p-values) for differences and pairwise comparison of 6 barley genotypes for Salinity Sensitivity Index (SSI) for Total Dried Weight (Total and Root) (TDW)), Leaf area (LA), Leaf temperature (T), Relative water content (RWC), SPAD value (SPAD), Root volume (RV), Chlorophyll fluorescence (Fv/Fm), leaf chlorophyll content (Chl_a), b (Chl_b) and carotenoid (Car), Na^+ ion content in leaves (Na-L) and roots (Na_R), K^+ content in leaves (K_L) and roots (K_R) and Ca^{2+} content in leaves (Ca_L) and roots (Ca_R).

A strong correlation was observed between the salinity damage index and the reduction in biomass (SSI-TDW) ($P < 0.001$, $r^2 = 0.96$). Thus, the salt effect can be estimated visually by visual index (Figure 1).

Figure 1: Correlation between the salinity sensitivity index calculated for the total dry weight (SSI-TDW) and the salinity-induced damage index (DI). Data are means ± standard error of eight plants.

The average comparison test of the SSI with the studied parameters in table 3 showed that the three tolerant genotypes had the best values for water content (RWC), SPAD, photosynthesis activity (chlorophyll pigments and chlorophyll fluorescence), retention capacity of K^+ and Ca^{2+} leaf and root and a low concentration of Na^+, thus, an arsenal of mechanisms of tolerance to salinity.

Involvement of measured parameters in the salinity tolerance process

The number of morphological and physiological features per genotype implicated in the tolerance among the 15 traits studied was determined by counting the parameters that significantly have the best tolerance index values according to the Duncan mean comparison test. A strong negative correlation was observed in figure 2 between the damage index and the number of traits involved in the tolerance ($R^2 = -0.82$, $p < 0.001$).

Damage index: Correlation between the studied parameters and the impact of the salinity

In order to identify the physiological processes that are responsible for a genotype tolerance, a correlation between the ISSs relative to all measured parameters and the damage index was performed.

Figure 2: Correlation between the numbers of morphological and physiological traits per genotype, involved in salt tolerance among the 15 traits studied, and the salinity-induced damage index (DI).

DI is inversely correlated with all the studied parameters. DI proves how effective they are in estimating salinity-induced damage and, therefore, tolerance to salinity.

The results show a perfect relationship ($r \sim 1$, $p < 0.001$) between the SSI-RWC and the ID (Figure 3). Besides, a strong correlation was observed ($R^2 = 0.8$) between SSI-T and salinity-induced damage (ID) (Figure 3). Barley leaf temperature increases at 14dS/m.

Other vital traits in avoiding the salt effect are K^+ capacity retention in roots and in shoots, with a correlation ($R^2 = -0.8$ and -0.9, respectively) for both (Figure 4). The three salt-tolerant barley genotypes had higher K^+ content at the root than the three salt-sensitive genotypes (Table 3).

A strong negative relationship between salinity damage was found between photosynthetic apparatus reactions estimated by the efficiency of light-harvesting of photosystem II (SSI-Fm/Fv) and DI in the presence of stress ($R^2 = -0.9$, $p < 0.01$) (Figure 3).

Stepwise regression to schematize the physiological sources of tolerance

The relationships between the studied parameters and their contributions to salinity tolerance expected by DI were analyzed using multiple linear regressions (stepwise) and schematized in figure 4 using stepwise regression. It shows the direct impact of the RWC ($r^2 = 0.95$, $p = 0.04$) on the tolerance variation expressed by the DI as well as the impact of the retention capacity of K^+ ($r^2 =$

Figure 3: Correlation matrix with significance levels (p-value) between salinity-induced damage index (DI) and salinity sensitivity index calculated for all stated traits. Positive correlations are displayed in green and negative correlations in red color. The color intensity and the size of the circle are proportional to the correlation coefficients. On the right side of the correlogram, the legend color shows the correlation coefficients and the corresponding colors.

Figure 4: Schematization of the relationship found after stepwise analysis with salinity tolerance expected by DI for the whole set of six barley genotypes as a dependent variable and the fifteen tested parameters as independent variables.

0.8, p = 0.02) and the Fm/Fv (r^2 = 0.28, p = 0.02) on the RWC. The direct involvement of RWC in salinity tolerance and involvement of K^+ and Fm/Fv in RWC was also noticed. It is correlated with the drop in yield due to salt stress.

Discussion

The comparison between biomass produced by the six barley genotypes irrigated with no saline (EC: 1.2 dS/m) and saline water (EC: 13 dS/m) and the other 14 parameters confirms that the response to salinity generally manifests in barley by a negative effect on all major processes such as photosynthesis, energy metabolism, and the water status [7,17].

This study further confirms that the two local accessions Ardhaoui and Suihli and the Oman local one Batini (100/1B) are more tolerant to salt stress. Ardhaoui and Suihli are particularly not affected by stress, unlike the three genotypes BarleyMednine, ICARDA20, and Konouz that recorded the most significant reduction in biomass (33%) (Table 3). Thus, local barley genotypes could be an essential source for finding new sources for salinity tolerance [5,6].

Salt stress effects can be observed visually from morphological symptoms (Table 3). Thus, a correlation was observed between these symptoms (damage index) and the reduction in biomass (SSI-TDW) (P < 0.001, r^2 = -0.96). This study highlights the importance and effectiveness of this visual index for estimating the stress and thus deducing the level of tolerance of the barley genotype [25-27].

Involvement of measured parameters in the salinity tolerance process

The number of traits involved in the tolerance per genotype among the 15 traits studied was determined by counting the parameters that have the best tolerance index values, according to the Duncan test (p < 0.05). We found a robust negative relationship between the damage index and the number of traits involved in the tolerance (R^2 = -0.82, p < 0.001) (Figure 2). On the one hand, more barley genotypes use defense mechanisms. The higher is its level of tolerance to salinity. On the other hand, under stress conditions related to salinity, tolerance is the phenotypic expression of a set of complex physiological, biochemical, and morphological properties that may interact with one another [28].

The tolerant local genotype has developed many mechanisms to tolerate salt stress [7,17].

Correlation analysis using Pearson parametric correlation test presented in the matrix in figure 3 shows that DI is inversely correlated with all the studied parameters. This proves how effective they are to estimate salinity-induced damage and, therefore, tolerance to salinity, and these traits can be summarised in one visual trait.

A perfect correlation ($r \sim 1$, $p < 0.001$) between the SSI-RWC and the ID (Figure 3) has shown that relative water content (RWC) is associated with stress tolerance. RWC is widely used to determine the water status of plants compared to their turgescent state [29]. Irrigation with salt-water reduces the ability of the roots to draw water from the soil [30].

Studying variations of temperature allowed the classification of the six genotypes on tolerant or sensitive (Table 3) and confirmed the work of Munns R., *et al* [31]. Also, a correlation was observed ($R^2 = 0.8$) between leaf temperature (T) and salinity-induced damage (ID) (Figure 3). Sirault XRR., *et al*. [32] found that the difference between barley leaf temperature grown at 200 mM and 0 mM NaCl is about 1.6°C.

A strong correlation between leaf and root capacity to retain K^+ and barley salinity tolerance was found ($R^2 = -0.8$) for both (Figure 3). This shows that the retention of K^+ in roots is another crucial trait for salinity tolerance [33]. This correlation has been reported for several species, including barley [34-36]. The retention of potassium in the mesophyll is essential for salt tolerance [33]. Cytosolic K^+ is essential for the activation of several metabolisms [10].

Our results show that calcium retention is an indicator of salt tolerance. Maathuis FJM., *et al*. [11] have identified its role in signaling and several regulatory mechanisms.

However, we did not find a clear distinction in Na^+ content in leaves between tolerant and sensitive genotypes. The two sensitive genotypes, ICARDA20 and Konouz, behave as tolerant (Table 3). The tolerant genotype Ardhaoui contained significantly more Na^+ than the other varieties. Some research has mentioned the absence of a clear correlation between leaf Na^+ content and plant salt tolerance [37]. This is probably due to the sequestration of Na^+ in the vacuole in order to protect the cytoplasm. Indeed, the total amount of Na^+ absorbed by the other genotypes is located in the cytoplasm, where all the biochemical mechanisms sensitive to Na^+ exist. Wu H., *et al*. [38] defended this hypothesis by visualizing the intracellular distribution of Na^+ in leaf tissues using fluorescent sodium in two different barley varieties ('Numar' tolerant, 'Gairdner' sensitive).

A correlation was observed ($R^2 = 0.86$) between the efficiency of light-harvesting of photosystem II (Fm/Fv) and DI (Figure 3). The chlorophyll fluorescence enlightens us on how the photosynthetic apparatus reacts in the presence of stress.

Kalaji HM., *et al*. [39] confirmed this hypothesis following comparison between the salt-tolerant barley genotype 'Arabi Aswad' and the sensitive genotype 'Arabi Abyad'. As a result, early reactions of the photosynthetic apparatus in barley may play a key role in salt stress tolerance. Jiang Q., *et al*. [26] indicate that this selection criterion has proven to be reliable about the photosynthetic efficiency of 14 barley genotypes subjected to salt stress.

Chlorophyll contents a and b and SPAD values were inversely correlated with DI (Figure 3). According to Shah SH., *et al*. [40] the decrease in chlorophyll content follows the application of salt treatment. These results are consistent with those found by Cheikh MH., *et al*. [41] in barley. The recorded reduction of chlorophyll content a or b is responsible for the decrease of photosynthesis, plant growth and productivity [40]. However, this reduction is variable depending on the level of tolerance of the genotypes. For instance, chl_a content in Ardhaoui and Batini (100/1B) is almost unaffected by salinity (Table 3).

The correlation between leaf area (LA) and DI ($r = -0.9$) confirmed that LA was significantly reduced after irrigation with salt water. Alem C., *et al*. [42] prove that the decrease in grain yield is positively correlated with the decrease in leaf area, which confirms our results (Table 3). It is also correlated with a decrease in photosynthesis and transpiration.

Stepwise regression to schematize the physiological sources of tolerance

Stepwise regression was used to show the impact of the RWC ($r^2 = 0.95$, $p = 0.04$) on the tolerance expressed by the DI. As well as the impact of the retention capacity of K^+ ($r^2 = 0.8$, $p = 0.02$) and the Fm/Fv ($r^2 = 0.28$, $p = 0.02$) on the RWC.

The involvement of RWC, K^+ and Fm/Fv in salinity tolerance was noted, which is correlated with the drop in yield due to salt stress. Indeed, our results show that in salt conditions, water status is the key to tolerance. Thus, the tolerant genotype adjusts its osmotic potential to ensure, on the one hand, the absorption of soil water and, on the other hand, the retention of intracellular water and the

maintenance of turgor [43]. Barley can ensure this by maintaining K^+ cytosolic homeostasis via releasing the vacuolar K^+ in the cytosol and thus replacing it by Na^+ to maintain the osmotic pressure in the vacuole. Therefore, the retention of potassium in the mesophyll is essential for salinity tolerance [33].

The role of light-harvesting of photosystem II on the water's status can be explained by the sensitivity of some components in the photosynthetic electron transport chain, notably the efficiency of the water-splitting complex on the donor side of PSII (Fv/Fo).

Leaf temperature is also an indicator of the water's status. Indeed, there was an active correlation between direct measurements of leaf temperature and stomatal conductance of barley grown under different salt treatments [14]. Thus, it makes it possible to estimate water status for each genotype.

Tolerant genotypes can maintain physiological activity while maintaining a functional water status, which translates into better vegetative development. K^+ and stoked water in the vacuole does not cost energy to the detriment of production when they are liberated to the cytoplasm [44].

Conclusion

A study of specific parameters made it possible to differentiate the levels of salinity tolerance of different barley genotypes and to show the arsenal of the physiological mechanisms adopted by the two genotypes coming from Tunisian local population Suihli and Ardhaoui and the Omani local accession introduced Batini 100/1B against salt stress. In recent years, several physiological variables, such as photosynthetic efficiency and plant temperature, have begun to be widely used in plant breeding programs to evaluate crop performance during growth under various adverse environmental conditions such as salinity. The results obtained in this study confirmed the efficiency of these variables for selection. Therefore, more weight should be assigned to the RWC and K^+.

Results also show that tolerance to salinity and these fifteen traits can be summarised in one visual trait, salinity damage index, to estimate salinity-induced damage and then the tolerance or the sensitivity. This index can be adequately applied to accelerate and reduce the cost of breeding programmes to improve yield under salt stress.

Bibliography

1. Maas EV. "Salt tolerance of plants". *Applied Agricultural Research* 1 (1986): 12-26.

2. El Felah M and Medimagh S. "Food Barley in Tunisia". In Food Barley: Importance, Uses and Local Knowledge Grando S and Gomez MH. (Editions). "International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo (2005): 29-35.

3. GDAP. "General Direction of Agriculture Production". Ministry of Agriculture and Hydraulics Resources, Tunisia (2015).

4. Chalak L., *et al.* "Performance of 50 Lebanese barley landraces (*Hordeum vulgare* L. subsp. vulgare) in two locations under rainfed conditions". *Annals of Agricultural Sciences* 60 (2015): 325-334.

5. Bodner G., *et al.* "Management of crop water under drought: a review". *Agronomy for Sustainable Development* 35 (2016): 401-442.

6. Z Hammami., *et al.* "Predicting yield reduction in improved barley (*Hordeum vulgare* L.) varieties and landraces under salinity using selected tolerance traits". *Field Crops Research* 211 (2017): 10-18.

7. Wu H., *et al.* "Linking salinity tolerance with tissue-specific Na^+ sequestration in wheat roots". *Frontiers in Plant Science* 6 (2015a): 71.

8. Munns R and Tester M. "Mechanism of salinity tolerance". *Annual Review of Plant Biology* 59 (2008): 651-681.

9. Shabala S and Pottosin I. "Regulation of potassium transport in plants under hostile conditions: implications for abiotic and biotic stress tolerance". *Physiologia Plantarum* 151 (2014): 257-279.

10. Demidchik V., *et al.* "Stress-induced electrolyte leakage: the role of K^+ permeable channels and involvement in programmed cell death and metabolic adjustment". *Journal of Experimental Botany* 65 (2014): 1259-1270.

11. Maathuis FFJM., *et al.* "Regulation of Na^+ fluxes in plants". *Frontiers in Plant Science* 5 (2014): 467.

12. Deinlein U., *et al.* "Plant salt-tolerance mechanisms". *Trends in Plant Science* 19.6 (2014): 371-379.

13. Cramer GR and Läuchli A. "Ion activities in solution in relation to Na^+-$Ca2^+$ interactions at the plasmalemma". *Journal of Experimental Botany* 37 (1986): 321-330.

14. Jones HG., *et al.* "Thermal infrared imaging of crop canopies for the remote diagnosis and quantification of plant responses to water stress in the field". *Functional Plant Biology* 36.10-11 (2009): 978-989.

15. Papageorgiou G and Govindjee. "Chlorophyll a fluorescence: a signature of photosynthesis". Springer, Ordrecht (2010): 1-42.

16. Wang G., *et al.* "Effects of salinity stress on the photosynthesis of *Wolffia arrhiza* as probed by the ojip test". *Fresenius Environmental Bulletin* (2011): 20.

17. Roy SJ., *et al*. "Salt resistant crop plants". *Current Opinion in Biotechnology* 26 (2014): 115-124.

18. Hu YO., *et al.* "Diversity of pico-to mesoplankton along the 2000 km salinity gradient of the Baltic Sea". *Frontiers in Microbiology* 7 (2016): 679.

19. Tester M and Davenport R. "Na^+ tolerance and Na^+ transport in higher plants". *Annals of Botany* 91 (2003): 503-507.

20. Torrecillas A., *et al*. "Rapid determination of leaf Clorofila in discos limonero". *Fruits* 39 (1984): 617-622.

21. Arnon DI. "Copper enzymes in isolated chloroplasts, polyphenoxidase in beta vulgaris". *Plant Physiology* 24 (1949): 1-15.

22. Pauwels JM., *et al*. "Manuel de laboratoire de pédologie: Méthodes d'analyses des sols et des plantes". *Publications Agricole, France* (1992): 28.

23. Gonzalez L and Gonzalez-Vilar M. "Determination of relative water content". In: Reigosa, M.J. (Ed.), Handbook of Plant Ecophysiology Techniques. Kluwer Academic Publishers, Dordrecht (2001): 207-212.

24. Zhou Y., *et al.* "MICAL-1 is a negative regulator of MST-NDR kinase signaling and apoptosis". *Molecular and Cellular Biology* 31 (2011): 3603-3615.

25. Wu H., *et al.* "Durum and bread wheat differ in their ability to retain potassium in leaf mesophyll: implications for salinity stress tolerance". *Plant and Cell Physiology* 55 (2014): 1749-1762.

26. Jiang Q., *et al.* "Gas exchange, chlorophyll fluorescence parameters and carbon isotope discrimination of 14 barley genetic lines in response to salinity". *Field Crops Research* 96 (2006): 269-278.

27. Chunthaburee S., *et al.* "Physiological and biochemical parameters for evaluation and clustering of rice cultivars differing in salt tolerance at seedling stage". *Saudi Journal of Biological Sciences* (2015).

28. Negrao S., *et al.* "Evaluating physiological responses of plants to salinity stress". *Annals of Botany* 119 (2017): 1-11.

29. Boyer JS., *et al.* "Osmotic adjustment leads to anomalously low estimates of relative water content in wheat and barley". *Functional Plant Biology* 35 (2008): 1172-1182.

30. Song J., *et al.* "Strategies for Adaptation of Suaeda physophora, Haloxylon ammodendron and Haloxylon persicum to a Saline Environment During Seed-Germination Stage". *Annals of Botany* 96 (2005): 399-405.

31. Munns R., *et al.* "New phenotyping methods for screening wheat and barley for beneficial responses to water deficit". *Journal of Experimental Botany* 61 (2010): 3499-3507.

32. Sirault XRR., *et al.* "A new screening method for osmotic component of salinity tolerance in cereals using infrared thermography". *Functional Plant Biology* 36 (2009): 970-977.

33. Wu H., *et al.* "Ability of leaf mesophyll to retain potassium correlates with salinity tolerance in wheat and barley". *Physiologia Plantarum* 149 (2013): 515-527.

34. Bose J., *et al.* "Kinetics of xylem loading, membrane potential maintenance, and sensitivity of K^+ -permeable channels to reactive oxygen species: physiological traits that differentiate salinity tolerance between pea and barley". *Plant, Cell and Environment* 37 (2014): 589-600.

35. Chen Z., *et al.* "Screening plants for salt tolerance by measuring K^+ flux: a case study for barley". *Plant, Cell and Environment* 28 (2005): 1230-1246.

36. Chen Z., *et al.* "Potassium and sodium relations in salinised barley tissues as a basis of differential salt tolerance". *Functional Plant Biology* 34 (2007): 150-162.

37. Wu Honghong. "Plant salt tolerance and Na^+ sensing and transport". *The Crop Journal* 6 (2018): 215-225.

38. Wu H., *et al.* "K^+ retention in leaf mesophyll, an overlooked component of salinity tolerance mechanism: a case study for barley". *Journal of Integrative Plant Biology* 57.2 (2015): 171-185.

39. Kalaji HM., *et al.* "Effects of salt stress on photosystem II efficiency and CO2 assimilation of two Syrian barley landraces". *Environmental and Experimental Botany* 73 (2011): 64-72.

40. Shah SH., *et al.* "Response of Chlorophyll, Carotenoid and SPAD-502 Measurement to Salinity and Nutrient Stress in Wheat (Triticum aestivum L.)". *Agronomy* 7 (2017): 61.

41. Cheikh MH., *et al.* "Evaluation de la tolérance au stress salin de quelques accessions d'orge (Hordum Vulgare L.) cultivées en Tunisie approche physiologique". *Science and Technology* 28 (2008): 30-37.

42. Alem C., *et al.* "Adaptations hydrique et photosynthétique du blé dur et du blé tendre au stress salin. C. R". *Biologies* 325 (2002): 1097-1109.

43. Ottow E., *et al.* Populus euphratica Displays Apoplastic Sodium Accumulation, Osmotic Adjustment by Decreases in Calcium and Soluble Carbohydrates, and Develops (2005).

44. Kamboj A., *et al.* "Identification of salt-tolerant barley varieties by a consolidated physiological and molecular approach". *Acta Physiologiae Plantarum* 37 (2015): 1716.

45. Kadri karim., *et al.* "Etude du comportement morpho- physiologique et agronomique vis-à-vis la tolérance à la salinité chez des accessions d'orges (*Hordeum vulgare* L.) cultivées dans un écosystème oasien". *Revue des Régions Arides - Numéro Spécial* 35 (2014)

46. Sbei H., *et al.* "Phenotypic diversity analysis for salt tolerance of Tunisian barley populations (*Hordeum vulgare* L.)". *Journal of Arid Land Studies* 21 (2012): 54-58.

47. Hammami Z., *et al.* "Evaluation of performance of different barley genotypes irrigated with saline water in South Tunisian Saharan conditions". *Environmental and Experimental Biology ISSN* (2016): 2255-9582.

48. Jaradat AA., *et al.* "Genetic diversity in the Batini barley landrace from Oman: Spike and seed quantitative and qualitative traits". *Crop Science* 44 (2004): 304-315.

49. Al-Dakheel., *et al.* "Evaluation of Batini barley landraces from Oman and breeding lines under various irrigation salinity levels". *Agricultural Science Research Journal* 2.1 (2012): 42-50.