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Original Research Article

Assessment of salt tolerance of some Tunisian barley accessions using gas exchange attributes and Na^+ content

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A B S T R A C T

Barley (*Hordeum vulgare* L.) is one of the most salt tolerant crop species and it is the fourth largest cereal crop in the world. The effect of salinity on gas exchange parameter, Na^+ content in shoots, selectivity K^+/Na^+, instantaneous water use efficiency and total dry matter were studied in fourteen barley accessions from two regions in the Southern Tunisia. Experiments were conducted in a greenhouse. The accessions were grown in soil and exposed to three salinity levels (5, 13 and 20.5 dS/m). Salinity decreased significantly the net photosynthesis assimilation, the transpiration rate, the stomatal conductance, the K^+/Na^+ and didn't induce significant variability on the intracellular CO_2 concentration. Sodium content in shoots increase with intensifying salinity levels. The total dry matter vary significantly with increasing salinity levels and the highest averages were recorded for the accessions 9, 1 and 2 with, respectively, 34.66, 33.75 and 31.46 g/pot. Measurement of stomatal conductance provided the best information to assess genetic differences in barley for their tolerance to salinity stress. A significant positive correlation was recorded between the TDM, the net photosynthesis assimilation, the transpiration rate and the stomatal conductance. The accessions Ettalah, Chneni Tatouine and Elhezma showed more salt tolerance as indicated by their relatively high Salinity susceptibility index for total dry matter and a relatively low impairment of stomatal conductance, photosynthesis assimilation and transpiration rate. Furthermore, these accessions are usually classified as tolerant or moderate in all parameters.

Introduction

Salinity is one of the important limiting factor for agriculture overall in the Mediterranean region. It affects about 7% of the world's total land area (Flowers *et al*., 1997). Approximately 930 million ha of cultivated land affected are currently threatened by salinity (Munns 2002; Kefu et al., 2002). Tunisia is concerned by the salinity problem. Based on FAO (2005), about 1.8 million hectares representing

11.6% of the total surface of the country, are affected by salinity. Barley (*Hordeum vulgare* L.) is one of the most salt tolerant crop species and it is the fourth largest cereal crop in the world (Jiang et al., 2006). However, salinity limits barley production and it is one of the major abiotic stresses, especially in arid and semi-arid regions where salt concentration can be close to that in the seawater (Shannon, 1998). Several accessions of barley exist in Tunisia and the most known are "Souihlis", "Ardhaoui", "Frigui", "Beldi", "Djebali", "Sfira" and "Djerbi" (El Faleh and Mdimagh 2005).

Salt stress affects plant growth and development at different levels of plant organization (Munns 2002). It has a threefold effect on plant health. On the osmotic level, the presence of high *concentrations* of *salt (NaCl*) in the *soil solution* decreases the osmotic potential of the plant by inducing the inhibition of the uptake of certain nutrients such as K^+, Ca_2^+ and NO_3^- and the accumulation of Na^+ and Cl^- to potentially toxic levels within cells (Mengel and Kirkby, 2001; Krouma, 2009). On the metabolic level, some authors have shown that salinity induces the production of reactive oxygen species (ROS) (Ashraf and Harris, 2004), reducing the activity of certain enzymes (Munns, 2002), and impaired nitrogen (Mansour 2000; Santos et al., 2002; Krouma, 2009) and carbon (Balibera et al., 2003) metabolism. Photosynthesis is also reduced by the effect of salinity in most plants (Loreto et al., 2003; Tatar et al., 2010). This reduction is more pronounced when plants are exposed to salinity stress during their young age. In this situation, plants decrease the synthesis of protein and increase their hydrolysis, which leads to the reduction of the protein content of plants (Mengel and Kirkby, 2001). Reduction of photosynthesis affects the plant growth as biomass production depends on net photosynthesis (Munns, 2005). Nutritionally, Drevon et al (1998) showed that *nitrogen fixation* is dramatically affected under salt stress.

All these negative effects of salinity curtail growth, photosynthesis, protein synthesis, energy storage, and lipid metabolism. Indeed, cereal plants are most sensitive to salinity during vegetative and early reproductive stages and less sensitive during flowering and the grain filling stage (Mass and Poss, 1989). Many studies made on wheat (James et al., 2002) and other species (Brugnoli and Lauteri, 1991) in photosynthesis activity under salt stress show that the most informative parameter for discriminating salinity-sensitive and - tolerant genotypes is the stomatal conductance (gs) (Jiang et al., in press). Massacci (1996) showed a significant relationship between gs and net photosynthesis (A) to the response of plants to drought or salinity.

The objectives of this work were: (i) to study the effects of salinity stress on the photosynthetic activity, the accumulation of nutrient and the production of total dry matter in order to understand the adaptation mechanism of 14 barley accessions (*Hordeum vulgare* L.) to salinity. (ii) To compare the useful of stomatal conductance (gs) and Na^+ contents as a selection criterion in barley for tolerance to salinity.

Materials and Methods

Plant materials

Fourteen barley accessions from several regions of Southern Tunisia known by the farmers under the name "Ardhaoui" are used in this study; seven accessions from coastal zones (C) and seven from mountainous zones (M) (table 1).

Growth conditions

The trial was carried out in greenhouse under natural conditions at the experimental site of the Institute of Arid Lands of Medenine (Institut des Régions Arides: IRA Latitude 33°29'3''N, Longitude 10º38'46''E, Altitude 184 m) in the South-East of Tunisia, which is characterized by an arid bio-climate of Mediterranean type with a mild winter. The sowing has been achieved in plastic pots of 12 liters each. Every pot contains 10 kg of soil with the following texture: clay 5.38%, loam 6.72%, thin sands 4.15%, very thin sands 6.72% and coarse sands 40.88%.

To maintain a constant level of salinity in the pots along the test and to avoid the progressive accumulation of salts, we used a non-draining pot and the salts have been added before the sowing. Every pot received a known quantity of a mixture of NaCl: $CaCl_2$ (1:1, w/w) that has been mixed with the soil at the beginning of the experiment. The final electric conductivities of the 3 treatments were 5 dS/m, 13 dS/m and 20.5 dS/m. The treatment 5 dS/m (the initial electric conductivity of the soil) was chosen as a control. All pots have received the equivalent of 100 Kg of N/ha, 250 Kg/ha P_2O_5 and 150 Kg/ha K_2O.

Twenty-five grains were sowed in each pot. After emergence, all pots were thinned to 5 seedlings per pot. Every 2 days, 40 pots (10 by treatment) were weighted and the water loss replaced by tap water to reach the level of 80 % field capacity, to avoid drought or flooding of plants seedlings. Pots were arranged in completely randomized design with two factors (salinity and accessions) and four replications, which gives a total of 168 pots.

Gas exchange

Measurements were taken between 10 and 11 am on the upper surface of leaves on sunny days at 60 DAS. Net photosynthesis assimilation (A, $\mu mol.m^{-2}.s^{-1}$), stomatal conductance (gs, $mmol.m^{-2}.s^{-1}$), transpiration rate (E, $mmol.m^{-2}.s^{-1}$) and the intercellular CO_2 concentration (Ci, $\mu mol.m^{-2}.s^{-1}$ were measured on fully mature and sun-exposed leaves using a portable LCi IRGA (LCi, IRGA; ADC Bioscientific Ltd.) with four replications per treatment. The leaf chamber temperature was kept at 25 °C with a photosynthetic photon flux density (PPFD) of 1200 mmol photons $m^{-2}s^{-1}$ and a relative humidity of 60-70 %. Moreover, instantaneous water use efficiency (iWUE) was calculated from the gas exchange measurements. It is the ratio between A and E (Condon et al., 2002):

iWUE = A/E.

Mineral analysis

Samples were totally dried at 100 ± 5 °C to constant weight. Then, 1g of each dry sample was incinerated during 4 h at 550 °C. Ashes were mixed with 4 ml of distilled water and 1 ml of concentrated HCl. The solution was heated until boiling, then filtered and adjusted to 100 ml with distilled water. This solution will be used for mineral analysis.

The sodium and potassium contents were determined with a Sherwood 410 flame photometer regulated on the filter of sodium or potassium. The contents of sodium (% Na^+) or of potassium (% K^+) in the dry matter plant were calculated as:

$$\% Na^+ \text{ or } \% K^+ = (C * DF) / (100 * m),$$

Where C is the concentration of sodium or potassium (mg/l), DF is the factor of dilution and m is the mass of the extract (g).

Ranking of genotypes for salt tolerance

The ranking of genotypes for salt tolerance using multiple agronomic parameters was determined using the method of Zeng et al., (2002). That's why all the data was converted to salt tolerance indices before cluster analysis. A salt tolerance index was defined as the observation at salinity divided by the mean of the controls.

Cluster group ranking numbers can be assigned to the cluster groups based on cluster means, and were used to score genotypes. Cluster group rankings were obtained based on Ward's minimum method. The cluster group rankings were obtained from the average of the multiple parameters in each cluster group. The genotypes were finally ranked based on the sums, such those with the smallest and largest sums were ranked respectively as the most and least tolerant genotypes in terms of relative salt tolerance. A sum was obtained by adding the number of cluster group rankings at each salt level in each accession.

Statistical analysis

All measurements were taken on four replicates. Salinity susceptibility index (SSI) for each of several parameters was calculated for each accession in each treatment as:

$$SSI (\%) = (X_s/K_c)* 100\%$$

Where X_s is the mean value (computed over five plants) of the parameter measured under saline conditions and X_c is the mean value of the parameter measured under control conditions. The Statistical Package for the Social Sciences (SPSS 18.0) software was used in order to compare the averages obtained to the different treatment levels. The variance analyses and the multiple comparisons of means by the LSD test at 5% were also conducted. Results are significant when $p < 0.05$. The ANOVA test has been achieved to determine the effect of the different applied treatments on the variation of the different measured parameters

Results and Discussion

Effect of salt stress on the gas exchanges

Effects due to salinity treatments were detected for net photosynthesis (A), transpiration rate (E) and stomatal conductance (gs). Differences among barley accessions were found only for A and E. interaction between salinity treatments and barley accessions were significant for all parameters except intercellular CO_2 concentration (Ci) where $P = 0.853$.

Under control conditions, accessions 4, 10 and 13 exhibited the highest A and E while 6 and 14 had the lowest values (table 3). In 20.5 dS/m saline treatment, the accessions 2, 7 and 14 and 2, 5, 6 and 7 exhibited, respectively, the highest A and E values. Five accessions (2, 5, 6, 7 and 14) were the least affected by saline treatments for A (figure 1) and 7 (1, 2, 4, 5, 6, 7, 9 and 14) for E (SSI > 30%).

Salinity affected negatively stomatal conductance (gs) of all accessions, SSI were 35% and 22%, respectively for 13 and 20 dS/m (figure 1). Under control condition 4, 12 and 13 had the highest gs values while 14 presented the lowest one (table 3). At 20.5 dS/m, 4 and 7 had the highest gs values and 11, 12 recorded the lowest values as shown in table 3.

The Ci values did not differ statistically with the salinity. Their averages are 131, 130 and 125 µmol.m^{-2}.s^{-1} in 5, 13 and 20.5 dS/m, respectively (table 3). These values are particularly low for the non-stressed plants cultivated (5 dS/m). Therefore, for the C_3 species, the report ci/ca (ca: ambient concentration 360 µmo.m^{-2}.s^{-1}) varies from 0.6 to 0.7 in absence of stress. In our case the report ci/ca is about 0.3 to 0.4 that is very low and indicates a limitation of photosynthesis by the availability of CO_2.

Instantaneous water use efficiency (iWUE)

Salinity significantly affected Instantaneous water use efficiency (iWUE) (*P* ≤ 0.001). However, the analysis of variance shows an absence of effect among accessions (*P* = 0.061) and the interaction salinity * accessions (*P* = 0.053), indicating that all accessions responded similarly to salt stress. Increasing salinity reduced SSI for iWUE by 15% and 9% for the treatments 13 and 20 dS/m respectively (figure 1). Under control treatment, the accessions 1, 2, 6 and 10 are the most efficient with an iWUE higher than 6 µmol CO_2 / H_2O mmol. At 20 dS/m, the accessions 12 and 14 show the highest iWUE and the accession 3 recorded the lowest value (table.3).

Sodium content (Na^+) and K^+/Na^+ selectivity

Salinity, barley accessions and their interaction affected significantly Na^+ content in shoots and K^+/Na^+ selectivity (*P* ≤ 0.0001) (table 2). The concentration of Na^+ ions increased (table 4) significantly in response to salt treatments (from 117.336 to 1038.023 µmol.g^{-1}DM). The accession 3 had the highest Na+ value while 5 and 12 had the lowest ones under salt condition (table 4). Relative effects of salt stress differed among barley accessions. 10, 13 and 14 were the most affected by salinity (figure 2).

The K^+/Na^+ ratio represents the capacity of plants to extract and to accumulate potassium selectively over sodium. With increases in salt concentrations K+/Na+ ratio (figure 2a, b) decreased (from 2.979 to 0.311).

As illustrated in figure 2a and 2b, the accessions 1, 4, 5, 6 and 12 were the least affected by salinity.

Total dry matter (TDM)

Effects due to salinity treatment, barley accessions and their interaction were detected in the total dry matter (*P* ≤ 0.0001). The data results presented by figure 3 show the variation of the total dry matter between the 14 accessions in the 3 treatments tested.

Table.1 Different sites of collection of the seeds

Code	Site of collection	Zone	Code	Site of collection	Zone
1	Ettalah	C	**8**	Boughrara	C
2	Bir Echefa	C	**9**	Edwiret Elgdima	M
3	Edwiret	M	**10**	Twaiel Ali Ben Said	C
4	Toujan	M	**11**	Matmata-Toujan	M
5	Bloul Mareth	C	**12**	Mengar Ben Chaaban	C
6	Zaafran Gomrassen	M	**13**	Elhezma	C
7	Chneni Tataouine	M	**14**	Matmata Ejdida	M

C: costal zone ; M: mountainous zone.

Table.2 Observed significance levels (p-values) for effects of salinity levels, of barley accessions, and of their interaction from analysis of variance for net photosynthesis (A), transpiration rate (E), stomatal conductance (gs), intercellular CO_2 concentration (Ci), instantaneous water-use efficiency (iWUE), Sodium rate (Na^+), selectivity K+/Na+ and total dray matter (TDM).

Parameters	**Salinity**		**Accessions**		**Interaction**	
	F	**Sig**	**F**	**Sig**	**F**	**Sig**
A	408.551	0.000**	1.800	0.050*	1.820	0.000**
E	313.341	0.000**	2.002	0.026*	2.603	0.000**
gs	265.551	0.000**	1.762	0.056ns	2.184	0.002*
Ci	0.159	0.853ns	1.353	0.192ns	0.701	0.853ns
iWUE	6.986	0.001*	1.739	0.061ns	1.571	0.053ns
Na^+	642.222	0.000**	4.749	0.000**	4.609	0.000**
k^+/Na^+	908.251	0.000**	7.909	0.000**	6.996	0.000**
TDM	710.782	0.000**	5.557	0.000**	3.715	0.000**

** significant at 1%, * significant at 5%. ns: not significant.

The highest total dry matters were observed in the treatment 5 dS/m by the accessions 2 and 9 which the values exceed 50 g/pot (figure 3). In the treatment 20.5 dS/m, the accession 1 and 13 were the most productive.

TDM for the accession 13 was less reduced than other accessions (table 5) followed by the genotypes 7 and 1. Their Salinity susceptibility index SSI was about 80.43, 78.66 and 71.65 for the treatment 13 ds/m and about 21.98, 13.71 and 13.04 for 20.5 ds/m.

Tolerance structure

Ranking of barely accessions using cluster analysis based in stomatal conductance (gs), Na^+ content and total dry matter (TDM) for the tow levels of salinity tested is shown in table 6. Hierarchical classifications based on the gs, TDM and Na^+ content did not result in the same grouping for the different accessions. The three rankings allowed us to obtain three groups: Group 1 (tolerant accessions),

Table.3 Observed significance levels (*p-values*) for overall tests of barley accessions differences, and comparison of salinity effects of 14 barley accessions for net photosynthesis (A), transpiration rate (E), stomatal conductance (gs), intercellular CO_2 concentration (Ci).

Accessions	**A ($\mu mol.m^{-2}s^{-1}$)**			**E ($mmol.\ m^{-2}\ s^{-1}$)**			**gs ($mmol.\ m^{-2}\ s^{-1}$)**			**Ci ($\mu mol.m^{-2}.s^{-1}$)**		
	5 dS/m	**13 dS/m**	**20.5 dS/m**	**5 dS/m**	**13 dS/m**	**20.5 dS/m**	**5 dS/m**	**13 dS/m**	**20.5 dS/m**	**5 dS/m**	**13 dS/m**	**20.5 dS/m**
p	***0.015***	***0.450***	***0.172***	***0.002***	***0.286***	***0.027***	***0.029***	***0.221***	***0.079***	***0.423***	***0.609***	***0.510***
1	14.14*ab*	4.75*a*	3.26*a*	1.83*ab*	0.95*a*	0.70*a*	0.11*ab*	0.03*a*	0.03*ab*	111.75*a*	108.75*a*	133.50*a*
2	13.07*ab*	2.94*a*	4.70*a*	2.09*ab*	0.82*a*	0.94a	0.11*ab*	0.03*a*	0.03*ab*	132.75*a*	163.50*a*	115.25*a*
3	13.18*ab*	3.10*a*	2.20*a*	2.47*ab*	0.76*a*	0.56*a*	0.12*ab*	0.03*a*	0.02*ab*	157.50*a*	132.75*a*	190.50*a*
4	18.04*a*	5.47*a*	3.15*a*	3.26*a*	1.11*a*	0.65*a*	0.20*a*	0.04*a*	0.02*ab*	137.75*a*	99.25*a*	111.00*a*
5	11.36*ab*	4.87*a*	3.89*a*	2.18*ab*	1.29*a*	0.94*a*	0.10*b*	0.04*a*	0.04*a*	94.00*a*	111.00*a*	127.75*a*
6	10.88*ab*	5.10*a*	3.92*a*	1.75*b*	0.97*a*	0.86*a*	0.10*b*	0.03*a*	0.03*ab*	115.25*a*	120.25*a*	81.00*a*
7	13.23*ab*	4.47*a*	4.22*a*	2.55*ab*	1.58*a*	0.85*a*	0.12*ab*	0.06*a*	0.03*ab*	137.00*a*	184.00*a*	136.00*a*
8	13.72*ab*	5.58*a*	3.54*a*	2.72*ab*	1.20*a*	0.65*a*	0.12*ab*	0.05*a*	0.02*ab*	136.50*a*	136.25*a*	102.50*a*
9	12.15*ab*	4.49*a*	2.76*a*	2.55*ab*	0.92*a*	0.79*a*	0.11*ab*	0.03*a*	0.03*ab*	143.50*a*	101.00*a*	194.75*a*
10	16.18*ab*	5.44*a*	3.40*a*	2.66*ab*	1.07*a*	0.69*a*	0.13*ab*	0.04*a*	0.02*ab*	116.67*a*	121.00*a*	98.50*a*
11	13.83*ab*	4.88*a*	2.62*a*	2.89*ab*	0.91*a*	0.50*a*	0.12*ab*	0.04*a*	0.01*b*	128.75*a*	115.75*a*	145.00*a*
12	13.96*ab*	5.99*a*	1.99*a*	3.02*ab*	1.01*a*	0.31*a*	0.13*ab*	0.04*a*	0.01*b*	120.25*a*	91.25*a*	80.50*a*
13	14.43*ab*	5.80*a*	2.29*a*	2.67*ab*	1.38*a*	0.42*a*	0.12*ab*	0.05*a*	0.02*ab*	135.00*a*	166.75*a*	94.00*a*
14	10.84*ab*	2.80*a*	4.35*a*	2.12*ab*	0.78*a*	0.75*a*	0.09*ab*	0.03*a*	0.03*ab*	166.75*a*	174.75*a*	141.50*a*
Means	***13.50***	***4.69***	***3.31***	***2.48***	***1.05***	***0.69***	***0.12***	***0.04***	***0.02***	***130.96***	***130.45***	***125.13***

Values are the means of 8 replications for A, E, gs and Ci. Mean values with the same letter were not significant different (LSD test mean comparison at α=0.05)

Table.4 Observed significance levels (*p-values*) for overall tests of barley accessions differences, and comparison of salinity effect of 14 barley accessions for sodium content in shoot (Na+) (µmo.g-1DM) , ratio potassium- sodium (K^+/Na^+) and instantaneous water use efficiency (iWUE) (µmol CO2 / H2O mmol).

Accessions	Na^+ (µmo.g^{-1}DM)			K^+/Na^+			iWUE(µmol CO2 / H2O mmol)		
	5 dS/m	**13 dS/m**	**20.5 dS/m**	**5 dS/m**	**13 dS/m**	**20.5 dS/m**	**5 dS/m**	**13 dS/m**	**20.5 dS/m**
p	***0.012***	***0.035***	***0.012***	***0.383***	***0.315***	***0.033***	***0.009***	***0.100***	***0.424***
1	119.980*ab*	575.170*bc*	1082.980*b*	3.000*a*	0.730*a*	0.430*a*	7.800*a*	5.080*a*	4.850*a*
2	90.400*bc*	851.860*ab*	1003.930*bc*	5.740*a*	0.650*a*	0.200*a*	6.530*ab*	4.010*a*	5.090*a*
3	113.750*ab*	757.390*ab*	1752.670*a*	2.830*a*	0.450*a*	0.200*a*	5.350*ab*	4.270*a*	3.870*a*
4	88.660*bc*	744.060*ab*	968.180*bc*	3.210*a*	0.520*a*	0.450*a*	5.620*ab*	5.220*a*	4.870*a*
5	109.300*bc*	645.310*ab*	856.120*bc*	2.590*a*	0.450*a*	0.390*a*	5.260*ab*	3.970*a*	4.020*a*
6	107.530*bc*	677.300*ab*	1192.280*ab*	2.230*a*	0.630*a*	0.350*a*	6.830*ab*	5.420*a*	5.040*a*
7	107.520*bc*	770.650*ab*	1024.200*a*	2.860*a*	0.520*a*	0.280*a*	5.030*ab*	2.770*a*	5.160*a*
8	119.980*b*	744.060*ab*	968.180*bc*	3.250*a*	0.530*a*	0.280*a*	5.050*ab*	4.860*a*	5.400*a*
9	119.980*ab*	1076.370*a*	1024.190*bc*	2.550*a*	0.390*a*	0.270*a*	4.750*ab*	5.290*a*	3.470*a*
10	152.870*ab*	744.060*ab*	1024.200*bc*	2.360*a*	0.340*a*	0.280*a*	6.150*ab*	5.000*a*	5.460*a*
11	107.530*bc*	796.160*ab*	964.260*bc*	2.610*a*	0.490*a*	0.210*a*	4.760*ab*	5.620*a*	5.150*a*
12	113.750*ab*	602.630*bc*	794.730*bc*	3.050*a*	0.730*a*	0.440*a*	4.630*b*	5.990*a*	6.310*a*
13	132.420*a*	900.700ab	908.230*bc*	2.820*a*	0.400*a*	0.260*a*	5.470*ab*	4.000*a*	5.630*a*
14	159.040*ab*	744.030*ab*	968.170*bc*	2.600*a*	0.390*a*	0.310a	5.380*ab*	3.900a	5.890*a*
Means	**117.336**	**759.268**	**1038.023**	**2.979**	**0.516**	**0.311**	**5.615**	**4.671**	**5.015**

Values are the means of 8 replications for Na^+, k^+/Na^+ and iWUE. Mean values with the same letter were not significant different (LSD test mean comparison at α=0.05)

Table.5 Observed significance levels (*p-values*) for overall tests of barley accessions differences, and comparison of salinity effect of 14 barley accessions for Total dry matter Salinity susceptibility index SSI of TDM (%).

Accessions	TDM Salinity susceptibility index (SSI)	
	13 dS/m	20.5 dS/m
p	***0.01***	***0.01***
1	71.65*a*	13.04*ab*
2	33.23*a*	5.07*bc*
3	35.71*a*	6.82*bc*
4	30.88*a*	9.40*ab*
5	31.63*a*	9.26*ab*
6	53.33*a*	7.44*bc*
7	78.66*a*	10.52*ab*
8	38.30*a*	13.71*ab*
9	48.36*a*	5.91*bc*
10	57.34*a*	8.08*bc*
11	38.18*a*	9.51*ab*
12	58.05*a*	10.36*ab*
13	80.43*a*	21.98*a*
14	35.23*a*	8.89*bc*
Means	49.36	10.00

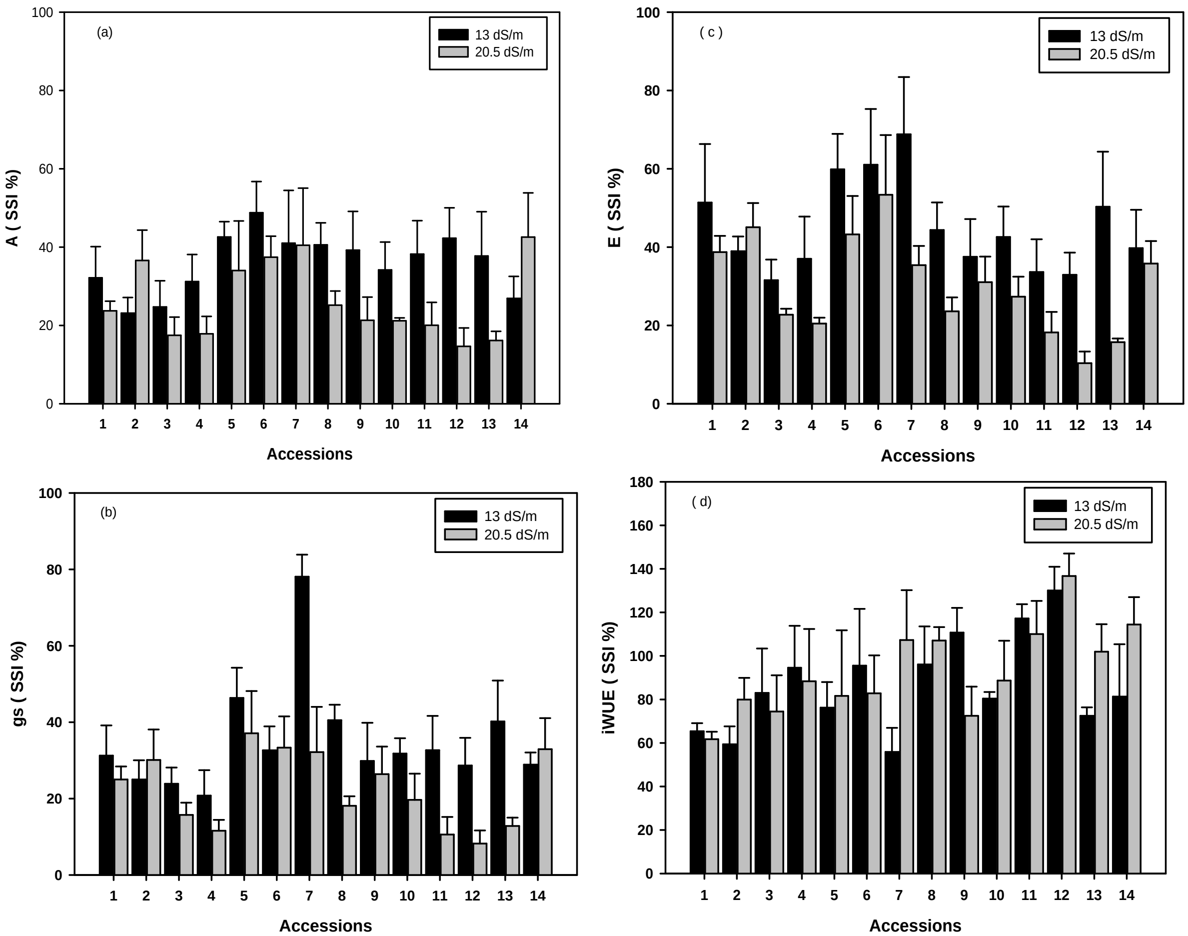
Mean values with the same letter were not significant different (LSD test mean comparison at α=0.05).

Table.6 Rankings of accessions for their relative salt tolerance in terms of stomatal conductance (gs), sodium content (Na^+) and Total dry matter (TDM) at 13dS/m and 20.5 dS/m in a cluster analysis used Ward's minimum variance analysis.

Accessions	gs^A	Na^{+A}	TDM^A	*Sum*	$Ranking^B$
1	2	3	1	6	2
2	2	1	3	6	2
3	3	1	3	7	2
4	3	1	3	7	2
5	1	2	3	6	2
6	2	2	2	6	2
7	1	2	2	5	1
8	2	2	2	6	2
9	2	2	2	6	2
10	3	3	2	8	3
11	3	2	3	8	3
12	3	3	2	8	3
13	2	2	1	5	1
14	2	3	3	8	3

*[A:] Sum of ranking at 13 dS/m and 20.5 dS/m, [B:] Ranking of the sum of cluster (1: Tolerant, 2: moderate 3: sensitive).

Figure.1 Salinity susceptibility index (SSI) for the 14 barley accessions in 13 dS/m (black bars) and 20.5 dS/m (gray bars). Net photosynthesis assimilation (A) on panel a; stomatal conductance (gs) on panel b; transpiration rate (E) on panel c; instantaneous water-use efficiency (iWUE) on panel d. Vertical bars represent standard errors



Group 2 (moderate accessions) and Group 3 (sensitive accessions). Ranking based in gs were similar to the ranking based in TDM. Six accessions presented the same degree of tolerance. The accessions 3, 4 and 11 were ranked as sensitive accessions and 6, 8 and 9 were classified in the moderate group. 1, 2, 13 and 14, which belong to the 2nd Group are part of the 1st group (1 and 13) or 3rd Group (2 and 14) in the groupings based on TDM data. The accessions 10 and 12 change from sensitive to moderate, Chneni Tataouine (7) from tolerant to moderate and only one accession (5) changed from tolerant in gs ranking to sensitive in TDM ranking. Comparison between Na^+ and TDM ranking show that five accessions (6, 7, 8, 9 and 14) were grouped in the same way. Four accessions change between sensitive to moderate (5, 10, 11 and 12); 13 from tolerant to moderate; 2, 3 and 4 from sensitive to tolerant and 1 from tolerant to sensitive (table 6). Ranking based on stomatal conductance (gs) gives a comparable ranking as the total dry matter than that of Na^+ content. Therefore, stomatal conductance (gs) presents a significant indicator of stress under salinity conditions. Same results are

Figure.2 Selectivity K^+/Na^+ for 14 barley accessions in three salinity treatments 5 dS/m, 13 dS/m and 20.5 dS/m (figure a and b).Salinity susceptibility index (SSI%) for 14 barley accessions in 13 dS/m (black bars) and 20.5 dS/m (gray bars) for sodium content Na^+ (µmol.g-1DM) (figure c) . Vertical bars represent standard errors.

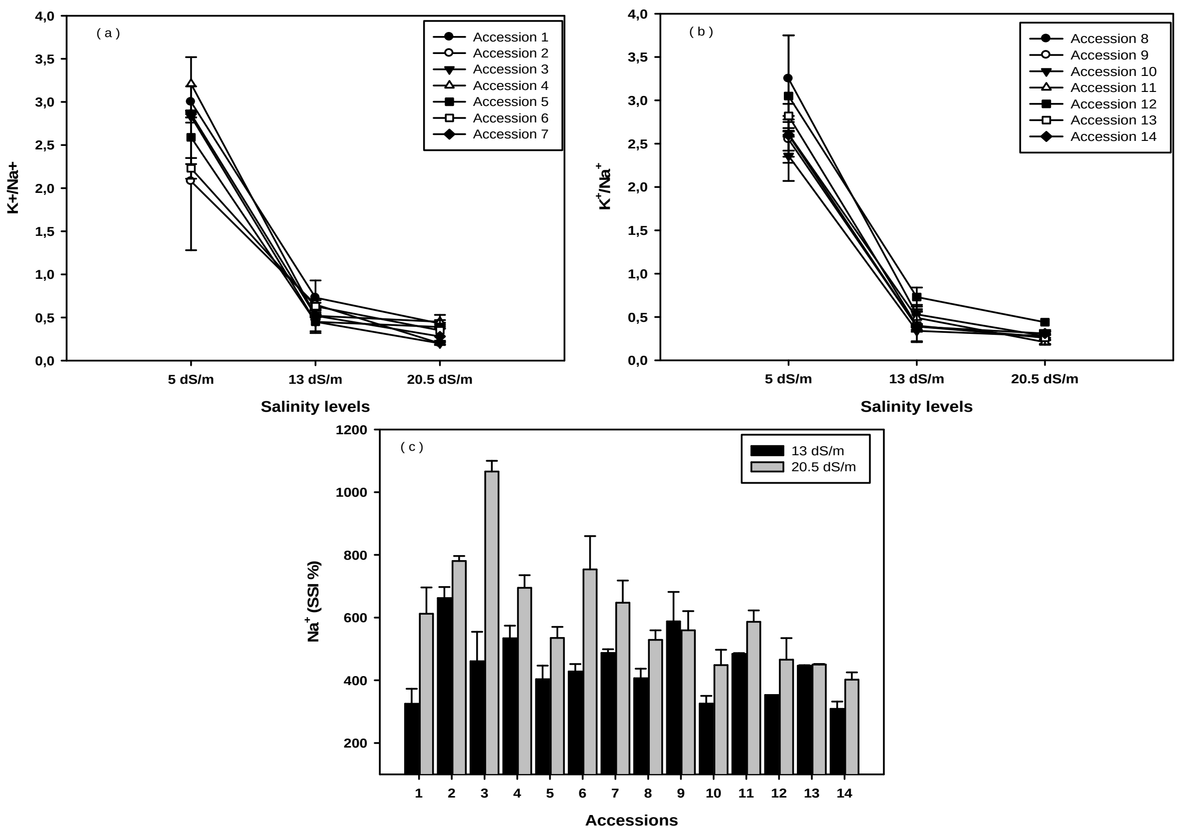
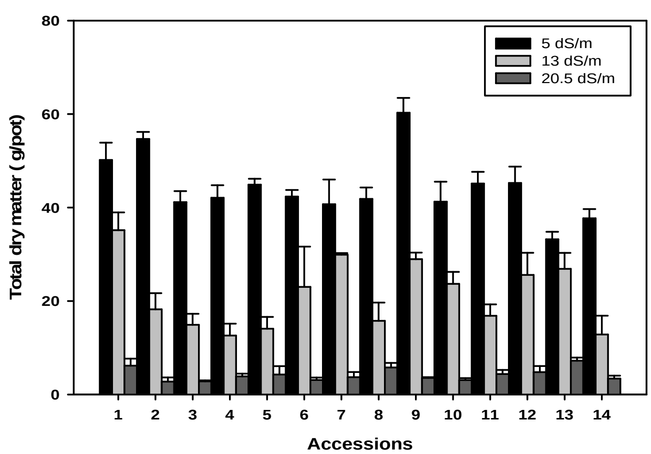


Figure.3 Total dry matter TDM (g/pot) for 14 barley accessions in control (black bars), 13dS/m (gray bars) and 20.5 dS/m (brown bars). Vertical bars represent tow standard errors based on variability among 4 pots and 20 plants. Barley accessions are abbreviated by code from 1 to 14.



obtained by James et al (2002) and Netondo et al (2004) which found that stomatal conductance (gs) reported as one of the most sensitive indicators of stress under salinity for wheat and sorghum.

Plants submitted to the salt stress are exposed in the same time to water deficit and ionic stress. According to Ykhlef (2001) and Nouri (2002), to palliate the effects of the water deficit, the plant tried to limit the water loss by transpiration by decrease on stomatal opening. However, a prolonged closing of the stomata affects negatively the photosynthesis activity and therefore the growth and the productivity of the plants. Many authors suggested that the biomass production, the growth attributes in seedling stage and the K^+/Na^+ ratio are the main criteria for improvement of salt tolerance in barley (Chen et al., 2005; Leonova et al., 2005). However, limited information is available on other physiological characters as related to salt tolerance in this important cereal crop such as photosynthesis which is an important parameter used to monitor plant response to abiotic stress (Munns et al., 2006; Huang et al., 2006). Our results show that a stress of 13 dS/m or 20 dS/m influences considerably the stomata behavior. Thus, all the accessions show a reduced stomatal conductance (gs), limited photosynthesis assimilation (A) and a low transpiration rate (E). The reductions in gs and E might be a response of plant to decreased water potential of environment (Koyro, 2006).

The low values of the intercellular CO_2 concentration (Ci) and the maintenance of a steady content in chlorophyll (data non shown), indicate the stomatic effect in the photosynthesis assimilation. However, the stomatal effect doesn't explain alone the reduction of the photosynthesis activity observed in this study. Therefore, for the same values of Ci registered in the treatments 5 dS/m, 13 dS /m or 20 dS/m, we observe different values of A.

Many authors indicated an improvement of the instantaneous water use efficiency (iWUE) in salt stress condition following an important reduction of the transpiration in relation to the photosynthesis activity (Quariani et al., 2000). However, other independent mechanisms of transpiration reduction intervened in the regulation of the relative water content. It is the suction capacity developed by the roots that maintain good water potential in leave of plants submitted to a water or saline stress. Other than the osmotic effect, salinity induces an ionic stress. The non-stomatal effects seem to be in relation with the accumulation of Na^+ in the plant tissue. Our results show the accumulation of high quantities of Na^+ in the plant tissue. This high content of Na^+ was to the expense of the K^+ absorption, an essential element for the plant. Indeed, potassium participates in several physiological processes and particularly those in relations with cell turgor, as the cellular elongation, maintains the plant’s erect shape port erected and the stomato control (rapid opening and closing) an influx/efflux mechanism at the level of guard cells. The K^+/Na^+ selectivity is an important characteristic of tolerance to the salt stress in the glycophyte. Therefore, if the K^+/Na^+ selectivity is high the species is more able to maintain regular potassium nutrition and therefore a normal growth. Our results indicate that some accessions are characterized by a selectivity (K^+/Na^+) higher than the others. The accessions 1, 4, 5, 6 and 12 were the least affected by salinity.

The total dry biomass was negatively

affected by salinity, barley accession and their interaction. Thus, in control treatment (5 dS/m) we recorded a total dry matter equal to 12.92g/pot; whereas in the conditions of moderate stress (13 dS/m) the TDM decreases by 49% in comparison with the control treatment and in the treatment 20 dS/m the average of reduction is about 94%. Similar results of a reduction of the barley dry matter under salt stress were founded by Sohrabi et al (2008) and Taffouo et al (2009).The depressive effects of the salinity on the growth and the productivity of the plants are the results of the difficulties in water uptake, mineral nutrition and the toxicity of the ions accumulated within plant tissue (Xiong and Zhu, 2002).

The results show negative and highly significant relationship between the Na^+ content and the TDM (r = -0.859 **). In the same way the accumulation of Na^+ has a negative effect on the physiological parameters as the net photosynthesis assimilation (A) (r = -0,850**), the transpiration rate (E) (r = -0,838 *) and the stomatal conductance (gs) (r=-0,795 **). Kumar et al (1994) and Wahid et al (1997) showed that the reduction of the barley productivity under salt stress is due to an important reduction of the physiological activity of the plant. Indeed, the TDM showed positive correlations with the net photosynthesis assimilation (A) (r= 0.809 **), the transpiration rate (E) (r= 0.780 **) and the stomatal conductance (gs) (r= 0.765 **) (figure 4). A significant correlation was also found between growth and photosynthetic rate in sunflower (Ashraf and O'Leary, 1999) and wheat genotypes (El-Hendaway et al 2005) differing in salt tolerance.

Salt stress had a negative effect in all studied parameters. The comparison of ranking based on gs and on Na+ content to that obtained from TDM data (mainly used by the farmers to select the most tolerant accessions) shows that the stomatal conductance (gs) is the main indicator of stress under salinity conditions. The analysis of results allows distinguishing some genotypes. Three accessions could be recommended under salt stress conditions (Ettalah, Chneni Tatouine and Elhezma), as indicated by their relatively high SSI for TDM and a relatively low impairment of gs, A and E (Figure 1 and Table 5). Furthermore, these accessions are usually classified as tolerant or moderate in all parameters.

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