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Comparative effect of salinity on ion accumulation, grain yield and stability salt tolerance degree of barley (*Hordeum vulgare* L.) in different growth stage

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Abstract: In arid and semi-arid regions of the world, excess salts in agricultural land can limit crop production. Barley (*Hordeum vulgare* L.) is one of the most salt tolerant crop species. This study was conducted to determine the effects of salinity on seed germination, mineral content and yield production of 14 barley accessions from two regions in the Southern Tunisia. First experiment was conducted in laboratory to test the effect of nine levels of NaCl concentrations in different germination parameter. Second experiments wereconducted out in natural conditions. The accessions were grown in soil and exposed to three salinity levels. Salinity decreased significantly the germination rate and the germination rate index for all accessions. Na^+ content, total dry matter and grain yield vary significantly with increasing salinity levels. The degree of tolerance varies between accessions in the different growth stage. A significant correlation was observed between ranking using grain yield and multivariate parameter (r =0,817** at 13 dS/m) and (r = 0,816** at 20.5 dS/m). The tolerance degree based in germination rate and Na^+ content don't present significant correlation with yield ranking. Therefore, ranking using multivariate parameter can be the appropriate method to analysis the tolerance degree of barley under saline conditions. The differences response between accessions of local population of barley "Ardhaoui" reflected an important internal genetic variability against the salinity. This variability could be more explored and used for the barley breeding program. The accessions Ettalah, Chneni Tatouine and Elhezma showed more salt tolerance at 13 dS/m as indicated by the multivariate ranking using germination rate, Na^+ content and grain yield.

Keywords: barley; grain yield; Na^+ *content; salinity tolerance, cluster analysis*

Introduction

The barley (*Hordeum vulgare* L.) is widely cultivated in the semi-arid regions for pasture and grain production (Oueslati, Ben-Hammouda, Ghorbal, Guezzah, and

Kremer, 2005). In the southern Tunisia, barley constitutes the only cereal which could tolerate the severe climatic conditions. In wet years (autumn rainfall), barley is cultivated in large area and the finale yield is dependent to the precipitation during the growth stage of plants. Whereas, during drought years (insufficient autumn rainfall) the sowed areas decreased drastically and barley is cultivated only in the irrigated zones. 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).

Soil salinity is a major factor limiting plant productivity and affecting about 95 million hectares worldwide (Szabolcs, 1994).Tunisia is concerned by this 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; Kalaji and Nalborczyk, 1991). The study of the effect of salt stress has always presented a real problem for researchers. Some found that the salinity tolerance can be detected during the germination stage, but others suggest that the mechanism of salt tolerance cannot be analyzed in each growth stage due to the variation in the degree of tolerance among the cycle life of plant. In the world, many studies have been undertaken in order to solve this problem (Kurt *et al*., 1986; Botia *et al*., 1998; Komori *et al*., 2000). Shannon (1985) and Mano and Takeda (1997) confirmed that the salinity tolerance can be controlled by groups of genes specific to each stage of development. Salt stress affects plant growth and development at different levels of plant organization (Munns, 2002).Seed germination is an important stage in the life cycle of plants and determines seedling establishment and plant growth (Chapman, 1974; Ungar, 1995). Germination percentage of most plant species is inhibited by salt (Mayer and Poljakoff-mayber, 1989; Nichols *et al*., 2009). Indeed, the effects of salinity on seed germination may be caused by the delay of taking water by the seed due to low osmotic potential of the soil water and there may be toxic ion effects on the embryo (Hayward and Wadleigh, 1949). Therefore, 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 (Marschner, 1995; Mengel and Kirkby, 2001; Zhu, 2001; Krouma, 2009). More than the osmotic effect, salinity induces an ionic stress. The existence of some ions in harmful and toxic concentration, constitute a limiting factorfor the normal growth of the plants. Thus, the presence of Na^+ and Cl^- ions at very high concentrations affects the growth of the plants by its useless absorption inside the plant tissue and by its interference with the absorption of other important and essential elements for the plant growth (Hajji & Grignon, 1985). The cereal plants are most sensitive to salinity during vegetative and early reproductive stages and less sensitive during flowering and the grain filling stage (Mass & Poss, 1989). 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).Salinity stress is a complex phenomenon, and involves not just an osmotic effect, but also toxic ion effects, and nutritional imbalance. Thus, the objectives of this work were: (i) to study the effects of salinity stress at the germination stage, the accumulation of nutrient and the yield production (i.e grain yield and total dry matter) in order to understand the adaptation mechanism of 14 barley accessions (*Hordeum vulgare* L.) to salinity. (ii) To compare the stability of tolerance degree of barley in the different growth stage by the classification in each stage using the germination rate , Na^+ content and grain yield and finally the classification using this multivariate parameter in the same time.

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).

Experimental design and treatments

First experiment

The germination of barley seeds is achieved in Petri dishes (25 seeds /dishes and 4 dishes by treatment and accession). Each Petri dishes was covered with two sheets of filter paper moistened with distilled water and were placed in an incubator for 7 days

Table 1 - Different sites of seeds collection, code and zone

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

under controlled conditions (20 ± 2°C in dark). Nine levels of NaCl concentrations have been tested: 0, 25, 50, 100, 150, 200, 250, 300 and 350 mM of NaCl. Petri dishes were arranged in completely randomized design with two factors (NaCl concentration and accessions) and four replications, which gives a total of 504 dishes. Barley seeds were considered germinated when the radicles reached 1.5 mm (Côme, 1970).

Measurements

It concerned: (i) the germination rate: is the number of grains that germinate for each 24 h during seven days.

$$GR\ (\%) = \text{Number of seeds germinated x 100}$$

(ii) The germination rate index (GRI), it is calculated according to the formula proposed by Emmerich and Hardegree (1990).

$$GRI\ (\%/days) = \sum \left[\frac{(G_i - G_{i-1})}{i} \right]$$

With i = Day of measurement, G_i = Germination rate (%) on the day i and G_{i-1} is the germination rate on the day i-1.

Second experience

The trial was carried out under natural conditions at the experimental site of the Institute of Arid Lands of Medenine (IRA: 33°29'3"N, 10°38'46"E, Altitude 184m 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 % and sands 85%. 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, 30 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.

Mineral analysis

At maturity, three samples (3g) of roots and shoots from each treatment 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 flame photometer (Sherwood 410; England). 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).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.

Tolerance degree

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

Analysis of variance (ANOVA) was performed using the GLM Procedure to calculate the effects of salinity level and genotype. Means were compared by the LSD test ($P<0.05$). Data were analyzed using the SPSS statistical package (SPSS Inc., Chicago,IL, USA). The relationship between variables was measured using Person correlation test. Figures were created using a Sigma-Plot11.0 program.

Table 2 - Mean squares and F test of main affect on salinity, accession and their interaction for the daily germination rate, germination rate index (GRI%) under the effect of salinity, accessions and their interaction.

Source of variation	MSS (103)	d.f	F	Sig.
GR%				
Salinity (S)	3840.080	8	15612.480	0.000**
Accession (A)	25.465	13	63.712	0.000**
Days	967.647	6	5245.505	0.000**
*S * A*	41.770	104	13.063	0.000**
*S * Days*	306.110	48	207.424	0.000**
*A * Days*	19.324	78	8.058	0.000**
*S * A * Days*	67.464	624	3.517	0.000**
GRI%				
Salinity (S)	358.284	8	1719.836	0.000**
Accessions (A)	5.931	13	17.522	0.000**
S x A	12.045	104	4.448	0.000**

*** significant at 1%, * significant at 5%.*

Results

First experiment

Effects of salt stress on the germination of 14 accessions of barley "Ardhaoui"

Germination is a critical stage in the development cycle of barley plants. Its process is inhibited by several environmental stresses like soil salinity. The salinity, the accessions, the time of experiment and the different interactions affected significantly the germination rate (GR %) as shown in table 2. Moreover, the germination rate index (GRI %) vary under the effect of salinity levels, the accessions and their interaction (S*A) (table 2).

The final germination rate recorded after seven days varies between 100% for the control and the low levels of salinity (25, 50 and 100 mM NaCl) and between 1 and 6% for the highest salt concentration (350 mM NaCl). Differences among barley accessions were found. thus, according to the Lsd test (5%) the accessions 1,10, 12 and 14 represent the group having the most elevated germination rates but the accessions 3, 4, 5, 7 and 11 represent the group having the lowest rates (table 3). The comparison between the accessions showed the absence of significant differences at the concentrations lower than 100 mM NaCl and for the level of 350 mM NaCl (table 3). Though, at 150, 200, 250 and 300 mM, the differences are highly significant. So these treatments can be used effectively to identify moderately and highly resistant genotypes, respectively.

Table 3 - Variation of the germination rate (%) for 14 accessions of barley under 9 levels of saline stress (0-25-50-100-150-200-250-300 and 350 mM)

Accessions	Germination rate (%) mM NaCl									Means
	0	25	50	100	150	200	250	300	350	
1	100	100	100	100	98 ab	83 abc	45 ab	22 a	6	72.6 7 AB
2	100	100	100	99	95 abcd	83 abc	41 abc	19 a	3	71.11 C
3	100	100	100	97	91 d	74 d	31 e	9 b	2	67.11 D
4	100	100	100	98	92 cd	77 bcd	33 de	8 b	1	67.67 D
5	100	100	99	97	92 cd	74 d	36 cde	9 b	1	67.56 D
6	100	100	99	98	94 bcd	83 abc	44 ab	18 a	3	71.00 C
7	100	100	100	99	93 cd	75 cd	34 cde	10 b	2	68.11 D
8	100	100	98	98	95 abcd	82 abcd	39 bcd	18 a	2	70.22 C
9	100	99	100	98	96 abc	81 abcd	40 bcd	19 a	3	70.67 C
10	100	100	99	99	99 a	89 a	48 a	23 a	5	73.56 A
11	100	100	99	98	92 cd	76 bcd	31 e	9 b	1	67.33 D
12	100	100	100	100	99 a	84 ab	45 ab	22 a	5	72.78 AB
13	100	100	100	99	96 abc	86 a	40 bcd	19 a	2	71.33 BC
14	100	100	100	99	99 a	88 a	44 ab	23 a	6	73.22 A
Means	*100 A*	*99,93 A*	*99,57 AB*	*98,5 B*	*95,07 C*	*81,07 D*	*39,36 E*	*16,29 F*	*3,0 G*	

The averages followed by the same letters are not significantly different at 5%. The letters in lower-case letters (a,b) for the comparison of accessions in each level of salinity. The capital letters (A, B, C) for the global averages of accessions. The capital Italic letters compares the global averages by salinity.

Table 4 - Effects of 9 salinity levels (0-25-50-100-150-200-250-300 and 350 mM) on the germination rate index (GRI%) of 14 barley accessions

Accessions	mM de NaCl									Means
	0	25	50	100	150	200	250	300	350	
1	74.50 b	55.25b	62.35b	70.88a	40.67bc	27.97c	14.73cd	6.71ab	2.1	39.5 BCD
2	75.33 b	75.33ab	54.42b	41.55c	41.17bc	29.60c	15.62c	5.27ab	1.0	37.7 CD
3	85.00 a	78.50a	73.34a	56.80b	38.63c	28.90c	13.28cd	1.88c	0.7	41.9B
4	69.16 b	61.42b	64.16ab	52.48bc	44.33bc	31.35b	12.92d	1.36c	0.3	37.5CD
5	68.16 b	64.75b	63.33ab	45.19c	38.64c	27.69c	12.40d	2.88bc	0.5	35.9D
6	74.17 b	68.37ab	62.06b	57.80b	44.90b	33.78b	14.58cd	5.18b	1.0	40.2BC
7	71.25 b	77.16ab	63.41ab	53.31bc	40.42bc	31.82bc	12.26d	3.24bc	0.7	39.3C
8	69.50b	62.34b	57.72b	53.79b	42.18bc	29.88bc	14.42cd	4.02bc	1.0	37.2CD
9	71.67b	65.90b	42.48c	42.38c	40.42bc	29.40c	13.60cd	4.82c	1.3	34.7D
10	74.83b	77.83a	67.08ab	73.48a	42.63bc	34.37a	23.00a	7.46ab	1.3	44.7A
11	86.50a	59.67b	73.35a	58.12b	45.47b	32.14bc	10.98d	2.73bc	0.5	41.1BC
12	86.33a	72.45ab	70.37ab	70.13a	43.35bc	32.38bc	19.78b	7.12ab	2.3	44.9A
13	74.00b	72.66ab	61.65b	52.45bc	42.25bc	36.32ab	14.92cd	4.07bc	0.6	39.9BC
14	74.75b	80.83a	63.12ab	74.95a	60.50a	38.25a	20.17ab	8.36a	2.2	47.0A
Means	*75.4 A*	*69.5 B*	*62.8C*	*57.4 D*	*43.3 E*	*31.7 F*	*15.2 G*	*4.7H*	*1.1I*	

The averages followed by the same letters are not significantly different at 5%. The letters in lower-case letters (a.b) for the comparison of accessions in each levels of salinity.The capital letters (A. B. C) for the global averages of accessions. The capital Italic letters compares the global averages by salinity.

The germination rate index (GRI) is considered at the same time a representative of the germination speed and the final germination rate. The analysis of variance shows significant differences between the accessions for the germination rate index in the different levels of salinity except in 350 mM NaCl (table 4). In the same way, the GRI decreases with raising salinity. Thus, in presence of salt, the lowest GRI is observed for the most sensitive and the highest for the tolerant accession. The accessions 3, 6, 10, 11 12 and 14 recorded the highest GRI (>40%), the lowest values were registered for the accessions 5 and 9 (table 4).

Second experiment

Effects of salinity on total dry matter and grain yield

The effect of salinity and the variability among the 14 accessions of barley were evident for most parameters (table 5). Effects due to salinity treatments were detected for the Total dry matter (TDM on g/pot) and grain yield (GY on g/pot). The interaction between salinity treatments and barley accessions were significant for the K^+ content in shoots and roots (table 5).

Table 5 - Analysis of variance for Na^+ and K^+ content, the total dry matter (TDM) and the grain yield (GY) under the effects of accession, salinity and their interaction

Parameter	Salinity (S)	Accessions (A)	Interaction (S*A)
Na^+ content	***	***	***
Na^+ shoots	***	***	ns
Na^+ roots	***	***	ns
K^+ content	***	***	***
K^+ shoots	***	***	*
K^+ roots	***	***	***
TDM	***	***	***
GY	***	***	***

**** Significant at 1%, * significant at 5%. ns: non significant*

The variation of total dry matter and grain yield for the 14 accessions and between the 3 levels of salinity was illustrated in Figure 1. The data results presented by figure 1 show the variation of the dry weight between the 14 accessions in the 3 treatments tested (fig. 1b). Increasing salinity resulted in a significant decrease in the production. At the treatment 13 dS/m we observe an important fall of the TDM about 50%. At 20.5 dS/m, the total dry matter is decreased by about 80% and 90% compared to the treatments 5 and 13dS/m respectively. The highest averages are observed for the accessions 9, 1 and 2 with 30.93 g/pot, 30.51 and 25.22 g/pot. Whereas, the lowest averages are recorded for the accessions 3 and 14 with 19.63 and 17.98 g/pot (fig. 1a).

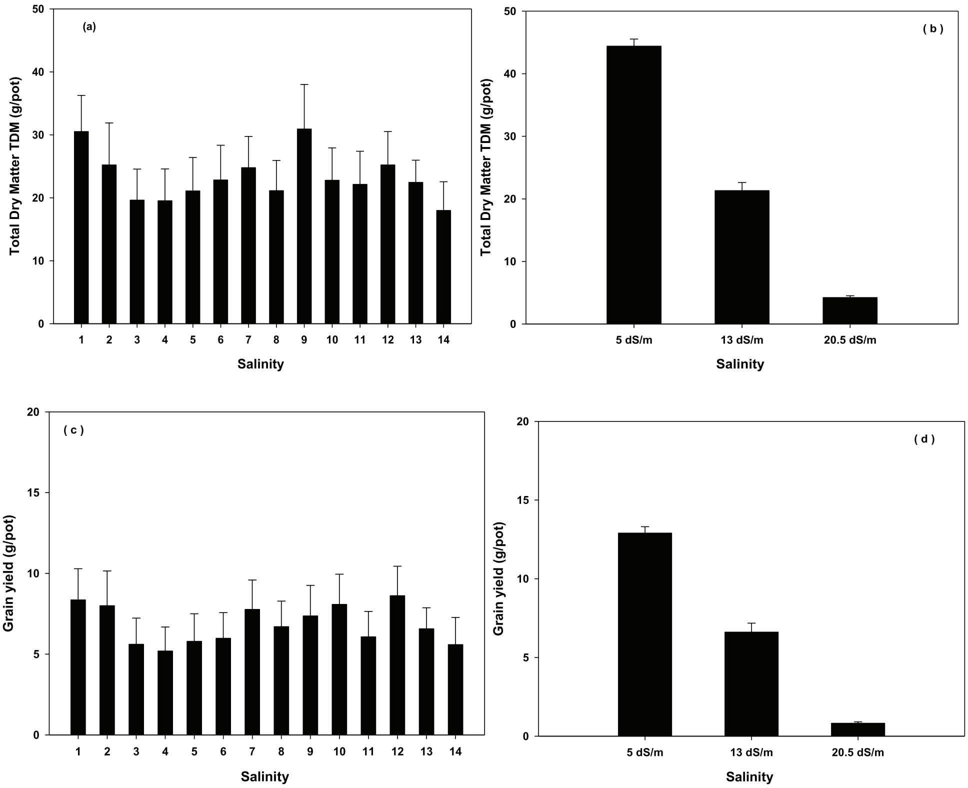

Figure 1 - Variation of the total dry weight (g/pot) and grains yield (g/pot) by accession (a) and by level of salinity (b). Vertical bars indicate standard errors of means

The grain yield averages varies between 5.19 g/pot to 8.61 g/pot for, respectively, the accessions 4 and 12. For the higher salinity, we observe a significant decrease in grain yield (fig 1c, 1d).The highest grains yields were recorded in the treatment 5 dS/m by the accessions 12 and 2 with 15.41 and 14.43 g/pot, respectively (fig. 2). At the treatment 13 dS/m the grain yield represents 51% of the means recorded in the control treatment (5 dS/m). However, this reduction varies from accession to another and it is about 23% for the accession 1 and 75% for the accession 5 (fig. 2). The grain yield is affected severely by the salinity in 20 dS/m and the registered values vary from 3% to 11% in comparison with the control treatment for all accessions.

Accumulation of Na^+ and K^+ ions

Effects due to salinity treatments were detected for all mineral accumulation in shoots and roots (Na^+, K^+). The interaction between salinity treatments and barley accessions were significant for K^+ content but not significant for Na^+ contents in

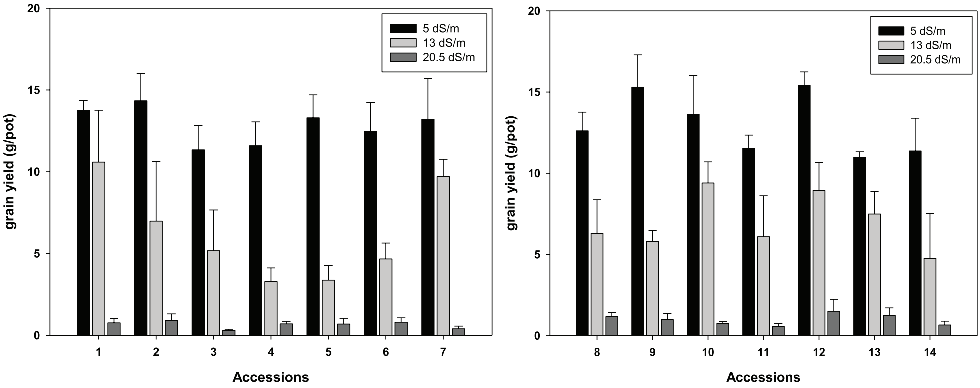

Figure 2 - Variation of the grain yield (g/pot) by accession and by salinity level (5 dS/m, 13 dS/m and 20.5 dS/m). Vertical bars indicate standard errors of means

The treatment 5 dS/m gave high K and low Na concentrations (K varied between 280.16 and 520.84 µmol.g^{-1}DM, Na varied between 88.66 and159.04 µmo.g^{-1}DM in the shoots; K varied between 29.74 and 57.95 µmo.g^{-1}DM, Na varied between60.41and 103.64 µmo.g^{-1}DM in the roots) (table 6).

However, with increases in salt concentrations K uptake decreased while Na uptake increased (fig. 3). The treatment 20.5 dS/m gave K and Na concentrations in the shoots of 200.33-460.60 µmo.g^{-1}DM and 856.12-1752.67 µmo.g^{-1}DM, respectively, while K and Na concentrations in the roots were 10.77-29.74 µmo.g^{-1}DM and 97.02-174.83 µmo.g^{-1}DM, respectively.

Accessions differed significantly in K and Na uptake in both the shoots and roots (table 6).

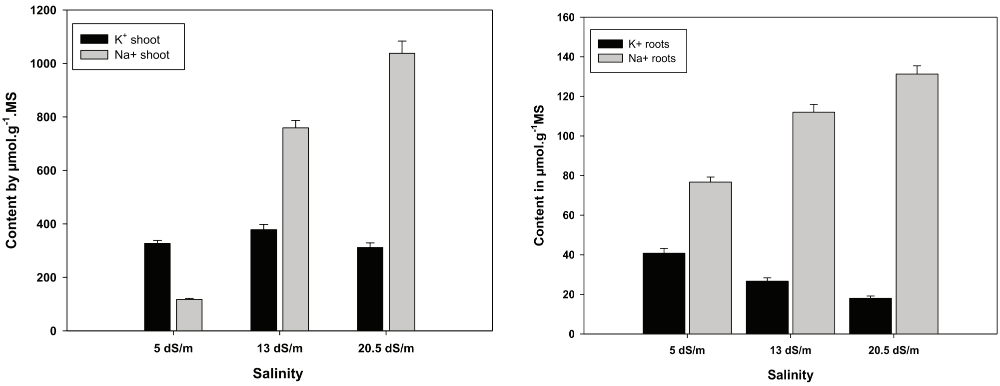

Figure 3 - Variation of the contents in Na+ and in K+ in shoot (a) and in roots (b) according to the salinity levels. Vertical bars indicate standard errors of means

Table 6 - Variation of the shoot content of K^+ and Na^+ in μmol g^{-1}DM for the 14 accessions under the effect of 4 salinity levels (5, 7, 13 and 20.5 dS/m)

Accessions	K						Na					
	Salinity levels						Salinity levels					
	5 dS/m		13 dS/m		20.5 dS/m		5 dS/m		13 dS/m		20.5 dS/m	
	Shoot	Roots	Shoot	Roots	Shoot	Roots	Shoot	Roots	Shoot	Roots	Shoot	Roots
1	360.58	30.77	401.16	30.77	460.60	15.38	119.98	60.41	575.17	71.62	1082.98	115.33
2	520.84	45.13	547.51	37.95	200.33	29.74	90.40	103.64	851.86	142.79	1003.93	147.37
3	320.51	48.20	320.51	37.95	347.22	27.69	113.75	87.87	757.39	124.48	1752.67	138.21
4	280.45	29.74	387.29	17.44	427.35	10.77	88.66	83.07	744.06	115.33	968.18	129.06
5	280.45	53.85	280.45	20.51	333.87	19.49	109.30	83.29	645.31	115.33	856.12	119.91
6	240.38	36.92	427.35	21.02	400.64	18.41	107.53	74.14	677.30	106.18	1192.28	97.02
7	307.16	38.05	400.64	34.87	288.46	11.28	107.52	64.99	770.65	115.33	1024.2	133.63
8	387.29	37.44	397.97	21.54	277.78	14.36	119.98	83.43	744.06	96.79	968.18	119.91
9	304.49	57.95	394.24	37.95	267.10	23.59	119.98	74.14	1076.37	142.79	1024.19	156.52
10	360.58	34.87	253.74	10.77	280.45	14.23	152.87	78.72	744.06	115.33	1024.2	124.48
11	280.16	39.55	387.29	25.64	200.33	13.33	107.53	69.56	796.16	106.18	964.26	133.64
12	347.22	30.77	440.71	19.49	347.22	14.36	113.75	78.72	602.63	94.51	794.72	135.16
13	373.19	34.86	363.25	25.64	232.38	20.00	132.42	64.99	900.70	110.75	908.22	110.53
14	414.00	52.82	293.8	31.79	299.15	19.49	159.04	67.05	744.03	110.75	968.17	174.83
Means	341.23a	40.78a	378.28a	26.67b	311.63a	18.01c	117.336c	76.72b	759.268b	112.01a	1038.021a	131.11a
LSD	200.46	52.58	435.56	14.81	279.67	14.91	47.78	45.43	463.84	60.72	564.81	72.28

Tolerance degree

Classification of barley accessions using cluster analysis based on the different parameters tested in each stage at 150 mM and 250 mM for the germination rate and for 13dS/m and 20.5 dS/m for the other parameters is shown in table 7. The accessions are classified in growing order from the best to the worst during various experiments. Therefore, the ranking allowed us to obtain three groups: Group 1 (tolerant accessions), Group 2 (moderate accessions) and Group 3 (sensitive accessions) for all the parameter except the ranking using germination rate at 150 mM in which we found only two groups.

The ranking of genotypes for salt tolerance using multiple agronomic parameters (multivariate ranking using germination rate GR%, Na^+ content and grain yield) 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.

The comparison between the different ranking shows a large difference between the degree of tolerance of all the accessions. Thus, the ranking order was different from one variable to another and also between the two salinity tested.

The accessions don't respect their degree of tolerance from the germination stage to the finale grain yield.

The rank correlation coefficients between the classifications of accessions are presented in Table 7. On the one hand, we notice the existence of highly significant correlation between ranking using grain yield and multivariate parameter (r =0,817** at 13 dS/m) and (r = 0,816** at 20.5 dS/m). On the other hand, the classification of accessions based on grain yield doesn't present any correlation with the germination

Table 7 - Genotypes ranking using germination rate GR% (150 mM and 250 mM), Na^+contents (Na^+ in shoots and roots), and grain yield (13 and 20.5 dS/m) and Multivariate Ranking using (GR%, Na^+ content and Grain yield)

Accessions	Germination rate GR%		Na^+ content		Grain yield		Multivariate Ranking	
	150 mM	250 mM	13 dS/m	20.5 dS/m	13 dS/m	20.5 dS/M	13 dS/m	20.5 dS/m
1	1	1	3	3	1	3	1	2
2	2	2	1	2	2	2	1	1
3	2	3	2	1	2	3	2	2
4	2	3	1	2	3	3	2	3
5	2	3	2	3	3	3	3	3
6	2	1	2	2	3	2	3	1
7	2	3	2	3	1	3	1	3
8	2	2	2	3	2	1	2	1
9	2	2	1	3	3	2	2	2
10	1	1	3	3	2	2	2	1
11	2	3	2	3	2	3	2	3
12	1	1	3	3	2	1	2	1
13	2	2	2	3	1	1	1	1
14	1	1	3	3	2	3	2	2

rate and the Na^+ content. This seems to be indicate that there was no relation between the classification of the various accessions for the salinity tolerance at the germination stage with the classification at the early growth stage and finale yield. Moreover, Na^+ content don't present a criterion for salt tolerance selection.

The ranking using multivariate parameter present a very significant criterion for salt tolerance degree of barley. Therefore, at 20.5 dS/m the accessions 2, 6, 8, 10, 12, and 13 present the tolerant group, the accessions 1, 3, 9 and 14 constitute the moderate group and finally the sensitive group is presented by the accessions 4, 5, 7 and 11.

Discussion

Several studies have been reported that salinity affects negatively the germination rate in many species (Mayer and Poljakoff-Mayber,1989; Nichols *et al*., 2009) such as triticale (Norlyn and Epstein, 1984), alfalfa (Hefny & Dolinski, 1999; Smith and Dobrenz, 1987), lettuce (Coons *et al*., 1990), wheatgrass (Johnson, 1991) and barley (Donovan and Day, 1969). In this study, significant reduction was observed mainly at the higher level of salt concentration compared to control treatment. Therefore, a reduction superior to 90% was observed for all accessions between the control and the treatment of 350 mM NaCl. The results obtained in this trial showed a significant difference between the accessions tested for the concentrations between 150 mM and 300 mM NaCl. Whereas, for the lowest (0-100 mM NaCl) and the highest concentrations (350 mM of NaCl), any significant differences has been observed between the 14 accessions. Thus, the accessions 3, 4, 5, 7 and 11 are the most sensitive to salinity at 250 and 300 mM of NaCl since they recorded the lowest germination rates and speed. On the other side, the accessions 1, 10, 12 and 14 are the most tolerant at the same concentrations. The depressive effect of the NaCl on the normal progress of the germination process could be osmotic (Bliss *et al*., 1986) and/or ionic nature (Huang and Redmann, 1995). Therefore, the presence of the NaCl increases its osmotic pressure of water which decreases the imbibitions of the grains and, consequently, it delay the reserve mobilization and transport of hydrolysis product to the embryo (Gomes-filho *et al*., 2008).

The osmotic effect was observed in our case for the treatments 25, 50, and 100 mM of NaCl where we noticed a delay of germination indicated by the decrease of GRI, but the majority of the grains germinate in the end of the experiment. For the highest salinity levels (150-350 mM NaCl) the osmotic effect that makes the water more difficult to extract (delay of germination) is jointed water with the toxic effect of the Na^+ and Cl^- ions on the progress of the different biochemical reactions of germination. This result corroborate with those obtained by Werner and Finkelstein (1995) and Fricke *et al.* (2006) who found that, in vegetative plants, salt stress causes reduced cell turgor and depressed rates of root and leaf elongation (Werner and Finkelstein, 1995; Fricke *et al.*, 2006), suggesting that environmental salinity acts primarily on water uptake. Furthermore, high intracellular concentrations of both Na+ and Cl_2 can inhibit the metabolism of dividing and expanding cells (Neumann, 1997).

Zeng *et al.* (2002), has reported that various responses of different rice genotypes to salt tolerance exist at different growth stages. Similarly, Kingsbury and Epstein (1984) found that individual lines from 5000 accessions of spring wheat showed differing tolerance during their life cycle. Therefore, the salt tolerance of different barley genotypes must be evaluated at different growth stages (Zeng *et al*., 2002).

The research has shown that the total dry matter and the grain yield are negatively affected by salinity. Thus, in control treatment (5 dS/m) we recorded a grain yield equal to 12.92g/pot; whereas in the conditions of moderate stress (13 dS/m) the grain yield 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 showing a reduction of the barley yield under salt stress were founded by Sohrabi *et al.* (2008), Taffouo *et al.* (2009) and Gill (1979).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 *et al.*, 2002).

Rawson *et al.* (1988), Schactman and Munns (1992) 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. Other than the osmotic effect, salinity induces an ionic stress. The existence of some ions in harmful and toxic concentration, constitute a limiting factorfor the normal growth of the plants. Thus, the presence of

Na^+ and Cl^- ions at very high concentrations affects the growth of the plants by its useless absorption inside the plant tissue and by its interference with the absorption of other important and essential elements for the plant growth (Hajji and Grignon, 1985). Results show the accumulation of high quantities of Na+ in the plant tissue particularly in shoot rather than roots. Therefore, we have recorded a high level of Na^+ in shoots equivalent to 100 $\mu g.g^{-1}$ DM at 20 dS/m. This high Na^+ content was to the expense of the K^+ absorption, an essential element for the plant. Indeed, potassium participates in a 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. Also, our results show negative and highly significant correlations between the Na^+ content and the grain yield (r = -0.763 **) and the total dry matter (r = -0.75 **). Salinity stress is a complex phenomenon, and involves not just an osmotic effect, but also toxic ion effects, and nutritional imbalance. In view of many researchers, barley is considered to be the most drought and salinity tolerant among cereals (Ceccarelli *et al.*, 1987; Belaid and Morris, 1991). Therefore, there are many research groups working to identify criteria for salt-tolerant of barley at different stages (i.e seedling, vegetative and reproductive stages).this study show that the accessions varies in tolerance degree from stage to another and under different salinity levels (table 7). This shows that species or varieties can never be selected simply on the basis of higher germination rate. According to Mass and Grieve (1990), the ability of seed to geminate and emerge in saline soil not only depends upon the concentration of salts, but also upon various other biological factors i.e. viability of seed, seed age, dormancy, seed coat permeability, internal inhibitors and genetic makeup. Thus, there was no relation between the classifications of the various genotypes for the salinity tolerance at the germination stage with the classification at the early growth stage. This confirms the results founded by other studies (Maas *et al.*, 1983; Kurt *et al.*, 1986; Botia *et al.*, 1998; Komori *et al.*, 2000), and suggests that the salinity tolerance can be controlled by groups of genes specific at every stage of development (Shannon, 1985; Mano and Takeda, 1997). Morover, the sodium contents in shoots don't present a significant correlation with grain yield ranking. Our results disagrees with those founded by Poustini and Siosemardeh (2004) who found that Na^+ contents and the ratio Na^+/K^+ can be used as a criterion of selection in salinity tolerance. The differences response between accessions of local population of barley "Ardhaoui" reflected an important internal genetic variability against the salinity. This variability could be more explored and used for the barley breeding program.

Conclusion

This study was conducted to determine the effects of salinity on seed germination, mineral content and yield production of 14 barley accessions from two regions in the Southern Tunisia. First experiment was conducted in laboratory to test the effect of nine levels of NaCl concentrations in different germination parameter. Second experiments were conducted out in natural conditions. The accessions were grown in soil and exposed to three salinity levels. Firstly, the different Tunisian barley accessions responded differently to salinity stress at different growth stages (i.e. germination stage, vegetative stage and yield production). Secondly, the seed germination was decreased significantly with salt concentration. Moreover, salinity affected negatively the plant growth and the grain production. Thirdly, it can be concluded that selection of accessions/cultivars for better salt stress tolerance at germination stage don't present a significant criteria of selection in comparison with ranking based in grain yield. Furthermore, the Na^+ content don't present also a significant correlation with grain yield ranking. Finally, the degree of salt tolerance varies between accessions and between the different growth stages. Therefore, ranking using multivariate parameter (at different growth stage) can be the appropriate method to analysis the tolerance degree of barley under salinity conditions.

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