Evaluating Salt Tolerance of 14 Barley Accessions from Southern Tunisia Using Multiple Parameters

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Abstract

Barley "Ardhaoui" (*Hordeum vulgare* L.) is a local landraces cropped in southern Tunisia characterized by its resistance to drought and salinity. Salt tolerance of barley changes according to the growth stages. In order to study the salinity tolerance of (*Hordeum vulgare* L.), fourteen barely accessions, were grown in soil and exposed to four salinity levels (5, 7, 13 and 20 dS/m).

To evaluate its salt tolerance using multiple parameters, fourteen barley accessions from two regions in the southern Tunisia (costal and mountainous regions) were grown in soil and exposed to four salinity levels (5, 7, 13 and 20 dS/m). Plant growth parameter, total biomass at final harvest, straw yield and yield compound associated with salt tolerance during the different growth stages were determined. The results showed negative effects of salinity on the growth and the development of the barley. However, the salt sensitive accessions showed a greater reduction in tiller number (e.g. by about 80 %) than tolerant ones (e.g. by about 57 %) at 20 dS/m. These decrease on the growth of leaves, tillers and the aerial part constitute a strategy developed by the barely in vegetative phase to reduce the salt stress. Tiller and spikelet numbers were more affected by the salinity at 13 and 20 dS/m the different results showed significant correlation between the spikes numbers and the tiller number ($r = 0.872$). The salinity affected negatively these two parameters, which both initiate during early growth stages. So, the salinity has a greater influence on final grain yield than on yield components in the later stages. The studied parameters showed significant differences ($p<0.05$) between the barely accessions.

The cluster analysis using agronomic parameters at all growth stage showed that the accessions "Ettalah" and "Elhezma" were ranked as the most tolerant to salinity. However, "Boughrara" and "Edwiret Elgdima" accessions were ranked as moderate and presented a change of their degree of tolerance with the different growth stage. The remaining accessions showed the lowest tolerance to salinity. A highest tolerance was observed on the accessions from plain zones compared at those of mountainous zones.

The response differences between accessions of local population of barley "Ardhaoui" reflecting an important internal genetic variability against the salinity. This variability could be more explored and used for the barley breeding program.

Keywords: barley, "Ardhaoui", salinity variability, salt tolerance, cluster analysis

1. Introduction

Salinity is one of the major factors reducing plant growth and productivity in worldwide. It affects about 7% of the world's total land area (Flowers, Garcia, Koyama, & Yeo, 1997). Approximately 930 million ha of cultivated land affected are currently threatened by salinity. It does not include the secondarily salinized soils in cultivated areas (Munns, 2002; Kefu, Hai, & Ungar, 2002).

In the Mediterranean area, with an arid or semi-arid climate, cereal culture depends mainly on irrigation. However, due to their intensive exploitation, water resources become increasingly rare, therefore saline water is used in agriculture.

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 invaded by salinity. In the arid and semi-arid regions, the salinity of waters and soils are among the main factors limiting the plant productivity (Ferchichi, 1997).

The barley (*Hordeum vulgare* L.) is widely cultivated in the semi-arid regions for pasture and grain production (Oueslati, Ben-Hammouda, Ghorbal, Guezzah, & 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 & Mdimagh, 2005). The production of barley was about 0.53Mt in 2007. This average is lower than the record on 2004 (0.60 Mt), but it's upper to the average of the previous five years. The cultivated surfaces are crossed from 450.000 to 602.000 hectares in 2004. It contented 494.000 hectares in (INS, 2007).

In the South of Tunisia and during the favorable years, the accession "Ardhaoui" is cultivated in dry. But, with the succession of the drought years, barley is the more one cultivated in irrigated system around the wells. Those irrigated zones are mainly characterized by the salinity of water. However, few studies have tested the capacity of this accession to tolerate the salinity caused by the irrigation waters. Moreover, this accession seems to present high genetic variability.

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). However, a difference in the salt tolerance among genotypes may also occur at different growth stages.

It has been reported that various responses of different rice genotypes to salt tolerance exist at different growth stages (Zeng, Shannon, & Grieve, 2002). Similarly, Kingsbury and Epstein (1984) found that individual lines from 5000 accessions of spring wheat showed differing tolerance during their life cycle. Generally, in most plants, the response to salinity appears as a depressive effect on growth and development. The reduction in growth is a response to dehydration. Furthermore, it is not considered as a consequence of the osmotic disturbances, but as a strategy that allows the plant to limit water loss by transpiration.

Therefore, the salt tolerance of different barley genotypes must be evaluated at different growth stages using an appropriate statistical method to analyze multiple agronomic parameters simultaneously to facilitate ranking genotypes for salt tolerance (Zeng et al., 2002).

The cluster analysis method used as multivariate statistic has been suggested for comparisons of genotypes means (Jollife, Allen, & Christie, 1989). This method was effective in screening for salt tolerance of same potato genotypes (Khrais, Leclerc, & Donnelly, 1988) and rice (Zeng et al., 2002) based on agronomic parameters. By using cluster methods, genotypes would be assessed by multiple parameters simultaneously. Therefore, there is no need to set scoring boundaries because the genotypes were grouped on the variances basis of the analyzed characters.

The objectives of this work were to identify the relative importance of agronomic parameters associated with salt tolerance, to screen the different barley accessions "Ardaoui" for their salt tolerance at different growth stages from different regions of southern Tunisia, and to rank salt tolerance by using multivariate analysis of multiple agronomic parameters at different growth stages.

2.1. Plant Materials

Fourteen accessions from several regions of Southern Tunisia known by the farmers under the name "Ardhaoui" are used in this study. Seven accessions: "Ettalah", "Bir Echefa", "Bloul Mareth", "Boughrara", "Twaiel Ali Ben Said", "Mengar Ben Chaaban" and "Elhezma" were obtained from the costal zones. The other accessions: "Edwiret", "Toujan", "Zaafran Gomrassen", "Chneni Tataouine", "Edwiret Elgdima", "Matmata-Toujan" and "Matmata Ejdida" were obtained from the mountainous zones.

2.2. Growth Conditions

The trial was carried out in greenhouse under natural conditions from January 2008 to June 2008 in the experimental site of the Arid Lands Institute of Medenine (Institut des Régions Arides: IRA) in the South-East of Tunisia, which is characterized by an arid bioclimate 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 4 treatments were 5.5 dS/m, 7.11 dS/m, 13.3 dS/m and 20.5 dS/m. The treatment 5.5 dS/m (the initial electric conductivity of the soil) was chosen as a control. Most soils, in Southern Tunisia, have an electric conductivity close to 7 dS/m for this raison this level of treatment was tested. 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. Each 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 224 pots.

2.3. Sampling Strategy

The effect of salinity and the salt tolerance of crops may vary with their growth stage (Mass & Grieve, 1994). Therefore, the measurements were carried out at vegetative and grain maturity stages. The grain maturity was visually estimated according to the complete loss of green color from grumes. Measurements at the vegetative stage were concerned the tiller number by plant, the leaves number by plant and the plant height at 80 days after sowing (DAS). At grain maturity, the five plants were harvested. Main spikes were separated from the others of the plants, and the spikelet numbers were recorded. Ears were threshed. The grain number, the total biomass at final harvest and the straw yield were also determined.

2.4. 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 were converted to salt tolerance indices before cluster analysis. A salt tolerance index was defined as the observation at salinity divided by the average 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 two methods. Ward's minimum variance cluster analysis of the salt tolerance index averages of three parameters at vegetative stage (i.e. height of plants, tiller number and leaves number) and two parameters at maturity stage (i.e. spikelet number per plant and grain number). Single-Link cluster analysis of salt tolerance indices were computed for total biomass at final harvest, straw yield and grain yield. 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.

2.5. Statistics Analyses

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. We conducted the variance analyses and the multiple comparisons of means by the LSD test at 1% and 5%. 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 parameters measured.

3. Results

3.1 Growth Parameters: Height in the Aerial Part, Tiller Number and Leaves Number

The mean height in the aerial part, the tiller and the leaves number decreased significantly under the effect of salinity, accessions and their interaction ($p < 0.001$). Therefore, the different results linked to the growth parameters showed that the salinity affects negatively the vegetative development of barley.

At 7, 13 and 20 dS/m, the plants height in 80 DAS and the leave numbers were reduced respectively by 2%, 21% and 40.7% and by 0.9%, 42% and 78%, respectively. The tiller number was increased by 0.5% at 7dS/m and decreased by 42%, 79% at 13 and 20 dS/m.

The relative salt tolerance indices for all measured parameters varied according to the accessions and to the salinity levels (Table 1). The salt tolerance indices of plant height ranged from 0.872 (Twaiel Ali Ben Said) to 1.062 (Elhezma) at low salinity (7 dS/m) and from 0.400 (Bir Echefa) to 0.790 (Chneni Tataouine) at high salinity (20 dS/m) among accessions. In the same way, the salt tolerance indices ranged from 0.779 (Boughrara) to 1.333 (Elhezma) for tiller number and from 0.745 (Boughrara) to 1.385 (Elhezma) for leaves number at law salinity. However, at high salinity they ranged from 0.133 (Toujan) to 0.426 (Elhezma) and from 0.142 (Toujan) to 0.230 (Bloul Mareth), respectively (Table 1).

Table 1. Salt tolerance indices of agronomic parameters in barely accessions under different salinity levels at different growth stages

Acessions	Salinity levels (by dS/m)	Plant Height	Tiller number	Leaves number	Grain yield	Straw yield	Total biomass at final harvest	Spikelet number	Grain number
Ettalah	7	1.039	0.908	0.825	0.831	1.049	0.986	0.863	1.123
	13	0.784	0.997	0.921	0.771	0.685	0.709	0.703	0.968
	20	0.520	0.307	0.271	0.055	0.150	0.123	0.281	0.158
Bir Echefa	7	0.977	0.866	0.895	0.860	1.063	1.008	0.881	1.373
	13	0.754	0.530	0.516	0.487	0.258	0.320	0.371	0.931
	20	0.400	0.175	0.184	0.063	0.053	0.048	0.242	0.232
Edwiret	7	0.949	1.177	1.147	1.435	1.041	1.156	1.192	1.083
	13	0.720	0.396	0.360	0.456	0.338	0.373	0.365	0.818
	20	0.604	0.244	0.251	0.027	0.081	0.065	0.221	0.436
Toujan	7	1.065	0.843	0.871	1.099	1.107	1.105	0.967	1.510
	13	0.696	0.316	0.308	0.283	0.309	0.302	0.327	0.696
	20	0.638	0.133	0.142	0.060	0.108	0.094	0.142	0.438
Bloul Mareth	7	0.926	0.983	0.982	1.024	0.885	0.929	0.896	0.933
	13	0.689	0.427	0.431	0.253	0.350	0.319	0.352	0.727
	20	0.583	0.199	0.230	0.052	0.115	0.095	0.136	0.370
Zaafran Gomrassen	7	0.951	0.965	1.037	0.856	1.061	0.999	1.363	0.685
	13	0.728	0.526	0.531	0.374	0.617	0.543	0.738	0.601
	20	0.510	0.167	0.159	0.064	0.077	0.073	0.272	0.224
Chneni Tataouine	7	0.962	1.107	1.029	1.028	1.411	1.279	1.567	0.826
	13	0.935	0.932	0.833	0.735	0.753	0.747	0.857	0.547
	20	0.790	0.170	0.147	0.030	0.124	0.092	0.284	0.406
Boughrara	7	0.952	0.779	0.745	0.996	0.839	0.888	0.919	1.381
	13	0.736	0.399	0.409	0.500	0.324	0.379	0.527	1.016
	20	0.690	0.221	0.222	0.093	0.159	0.138	0.248	0.630
Edwiret Elgdima	7	0.953	1.226	1.146	0.978	0.959	0.964	1.152	0.864
	13	0.771	0.976	0.938	0.379	0.514	0.478	0.679	0.796
	20	0.493	0.240	0.229	0.065	0.057	0.059	0.127	0.367
Twaiel Ali Ben Said	7	0.872	0.981	0.981	0.936	1.257	1.140	1.041	1.233
	13	1.050	0.520	0.552	0.690	0.557	0.605	0.672	1.223
	20	0.678	0.216	0.217	0.055	0.134	0.150	0.218	0.675
Matmata-Toujan	7	1.058	0.876	0.849	1.229	0.989	1.054	0.867	0.796
	13	0.839	0.515	0.484	0.528	0.334	0.386	0.546	0.477
	20	0.626	0.188	0.195	0.049	0.095	0.082	0.177	0.359
Mengar Ben Chaaban	7	0.968	1.117	1.200	1.124	1.160	1.147	1.185	1.079
	13	0.817	0.786	0.796	0.580	0.588	0.585	0.843	0.939
	20	0.596	0.246	0.239	0.097	0.102	0.101	0.178	0.374
Elhezma	7	1.062	1.333	1.385	1.168	1.452	1.352	1.258	1.219
	13	0.853	1.036	1.075	0.682	0.924	0.838	0.926	0.927
	20	0.668	0.426	0.438	0.114	0.277	0.219	0.282	0.696
Matmata Ejdida	7	0.981	1.135	1.046	1.145	1.302	1.252	1.183	1.231
	13	0.753	0.606	0.491	0.419	0.308	0.343	0.585	0.764
	20	0.621	0.206	0.161	0.058	0.105	0.090	0.165	0.415

The results at vegetative stage were illustrated in Figure 1 and shows that “Bir Echefa”, “Toujan” and “Bloul Mareth” accessions are more affected by the salinity at 20 dS/m. Therefore, the averages decreased respectively by 60.04%, 36.25% and 41.69% for the plant height (Figure 1a), 82.54%, 86.71% and 80.12% for the tiller number (Fig. 1b), and 81.56%, 85.76% and 77.01% for the leaves number per plant (Figure 1c), as compared with the control treatment**.**

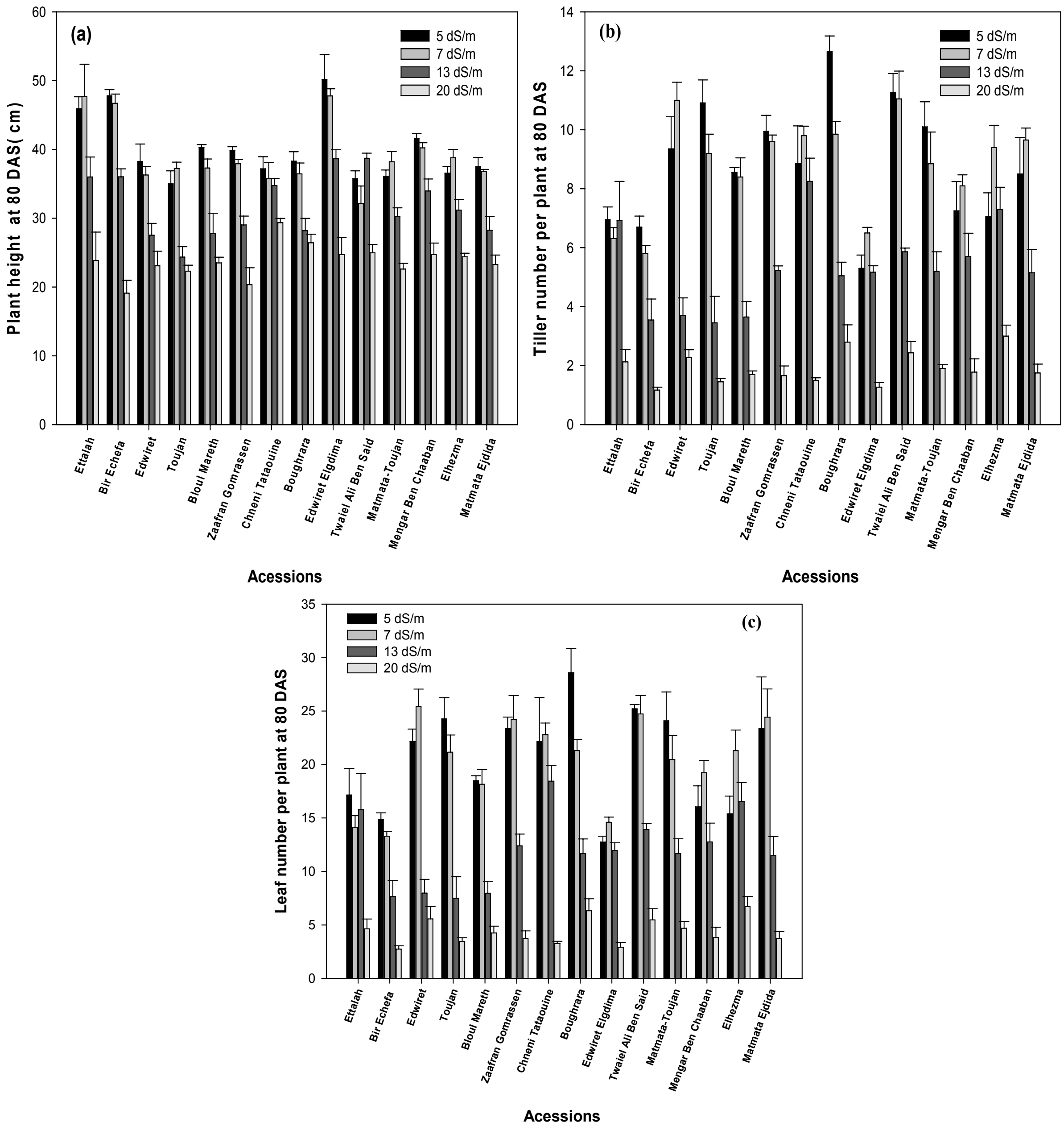

Figure 1. Effect of salinity levels on plant growth parameters (a: height of plant, b: tiller number, c: leaves number) at 80 days after sowing (DAS) for different barley accessions tested. Error bars represent standard deviations

The accessions “Twaiel Ali Ben Said”, “Mengar Ben Chaaban” and “Elhezma” were the least affected by increasing salinity. Plant height, tiller number and leave numbers per plant at 20 dS/m were decreased by 32.25%, 78.44% and 78.27% for “Twaiel Ali Ben Said”, by 40.43%, 75.45% and 76.12% for “Mengar Ben Chaaban” and by 33.24%, 57.45% and 56.21% for “Elhezma” compared at the control treatment.

At the vegetative stage, the accessions were classified using Ward’s minimum variance cluster analysis at the different salinity levels. The accessions were divided into three clusters at low salinity and into four clusters at moderate and high salinity levels (Table 2). “Ettalah”, “Edwiret Elgdima”, “Mengar Ben Chaaban” and “Elhezma” were ranked as the most tolerant accessions. However, “Edwiret”, “Toujan”, “Bloul Mareth”, “Zaafran Gomrassen” and “Matmata Ejdida” were clustering as the most sensitive accessions.

Table 2. Rankings of accessions for their relative salt tolerance in terms of plant growth parameter (i.e. height of plants, tiller number and leaves number) in a cluster analysis used Ward’s minimum variance analysis

Accessions	Salinity levels (dS/m)			Sum	Accessions ranking	Tolerance degree
	7 dS/m	13 dS/m	20 dS/m			
Ettalah	2	1	2	5	2	Tolerant
Bir Echefa	2	3	4	9	6	Sensitive
Edwiret	3	4	2	9	6	Sensitive
Toujan	2	4	3	9	6	Sensitive
Bloul Mareth	3	4	2	9	6	Sensitive
Zaafran Gomrassen	3	3	4	10	7	Sensitive
Chneni Tataouine	3	1	3	7	4	Moderate
Boughrara	2	4	2	8	5	Moderate
Edwiret Elgdima	3	1	2	6	3	Tolerant
Twaiel Ali Ben Said	3	2	2	7	4	Moderate
Matmata-Toujan	2	3	3	8	5	Moderate
Mengar Ben Chaaban	3	1	2	6	3	Tolerant
Elhezma	1	1	1	3	1	Tolerant
Matmata Ejdida	3	3	3	9	6	Sensitive

3.2. Total Biomass at Final Harvest and Straw Production

The results presented in figure 2-a show that the total biomass at final harvest (TBFH) of the different accessions, was significantly affected by different salinity levels ($p<0.001$). The averages increased by 8% at 7 dS/m and decreased progressively by 50.52% and 89.79% at 13 dS/m and 20dS/m, respectively. The highest TBFH (44.94g/pot) was produced in the treatment 7 dS/m and the lowest one (4.11 g/pot) was recorded in the highest salinity level (20 dS/m).

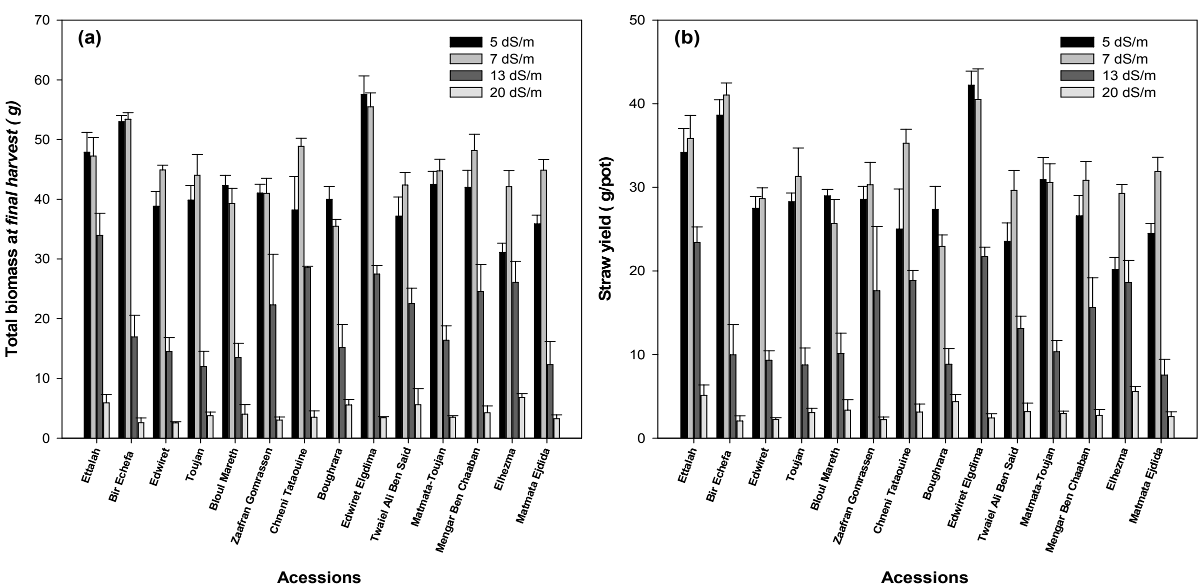

Figure 2. Effect of salinity levels on (a) Total biomass at final harvest (TBFH) and (b) straw yield (SY) for different barley accessions tested. Error bars represent standard deviations

The averages indices of TBFH from all accessions show a high variability under the salinity levels. The indices of all accessions ranged from 0.888 to 1.352 at 7ds/m, from 0.302 to 0.838 at 13dS/m and from 0.048 to 0.219 at the highest salinity level (20 dS/m). The results show also a wide variation among accessions. For instance, the TBFH at 13dS/m was decreased by 69%, 68% and 65% for "Toujan", "Bloul Mareth" and "Matmata Ejdida" and by 29%, 25% and 16% for "Ettalah", "Chneni Tataouine" and "Elhezma".

Under the highest salinity level (20 dS/m), the TBFH of all accessions was decreased by 91% except for "Ettalah", "Twail Ali Ben Said" and "Elhezma" it was decreased by 87%, 85% and 78%, respectively.

The results presented in figure 2-b show the averages of straw production recorded by accessions and by salinity levels. The results present significant differences between salinity levels and accessions. Therefore, "Ettalah", "Bir Echefa" and "Edwiret Elgdima" were more produced than the other accessions. In the same way, a significant increase was observed of the straw production in the treatment 7dS/m compared at the control treatment (figure 2-b). This increase is observed for all the accessions excepting "Bloul Mareth", "Boughrara, Edwiret Elgdima" and "Matmata-Toujan" accessions (Table 1).

At 13dS/m, the average of straw produced is about 49% of the control treatment and varies between 26% for "Bir Echefa" and 92% for "Elhezma". At 20dS/m and in the same way the production was very affected by the salinity and it is average is about 12% of the control treatment and varied between 5% for "Bir Echefa" and 28% for "Elhezma" (Table 1).

The indices of straw yield were decreased with the increase of salinity levels. Indeed, the indices were ranged from 0.839 to 1.452 at 7 dS/m, from 0.258 to 0.924 at 13 dS/m and from 0.053 to 0.277 at 20 dS/m. At the highest salinity level (20dS/m), "Ettalah", "Twail Ali Ben said" and "Elhezma" recorded the highest indices 0.150, 0.134 and 0.277, respectively.

According to the single link cluster analysis for the total biomass at final harvest and straw yield (Table 3), the accessions were divided in three groups at the lowest and the highest salinity levels and in four groups at the moderate salinity. The results show that "Ettalah", "Chneni Tataouine", "Edwiret Elgdima", "Mengar Ben Chaaban" and "Elhezma" were ranked as the most tolerant accessions, whereas "Bir Echefa", "Bloul Mareth" and "Matmata Ejdida" were considered as the most sensitive among all accessions.

Table 3. Rankings of accessions for their relative salt tolerance in terms of total biomass at final harvest and straw yield in a cluster analysis used Single-link cluster analysis.

Accessions	Salinity levels (dS/m)			Sum	Accessions ranking	Tolerance degree
	7 dS/m	13 dS/m	20 dS/m			
Ettalah	3	2	2	7	3	Tolerant
Bir Echefa	3	4	3	10	6	Sensitive
Edwiret	3	4	3	10	6	Sensitive
Toujan	3	4	3	10	6	Sensitive
Bloul Mareth	3	4	3	10	6	Sensitive
Zaafran Gomrassen	3	3	3	9	5	Moderate
Chneni Tataouine	1	2	3	6	2	Tolerant
Boughrara	3	4	2	9	5	Moderate
Edwiret Elgdima	3	3	3	9	5	Tolerant
Twaiel Ali Ben Said	2	3	3	8	4	Moderate
Matmata-Toujan	3	4	3	10	7	Sensitive
Mengar Ben Chaaban	3	3	3	9	5	Tolerant
Elhezma	1	1	1	3	1	Tolerant
Matmata Ejdida	1	4	3	8	4	Sensitive

3.3 Spikelet Number Per Plant, Grain Number and Grains Yield

It's clearely shown in figure 3 that the spikelet number per plant and grain number were significantly affected by salinity levels ($p<0.001$). Indeed, the averages recorded by all accessions for these two parameters were decreased by 42 % and 79 % at 13dS/m, and by 20% and 60% at 20 dS/m, respectively. However, at 7ds/m, the averages were increased by 8% and 5% in comparison with the control.

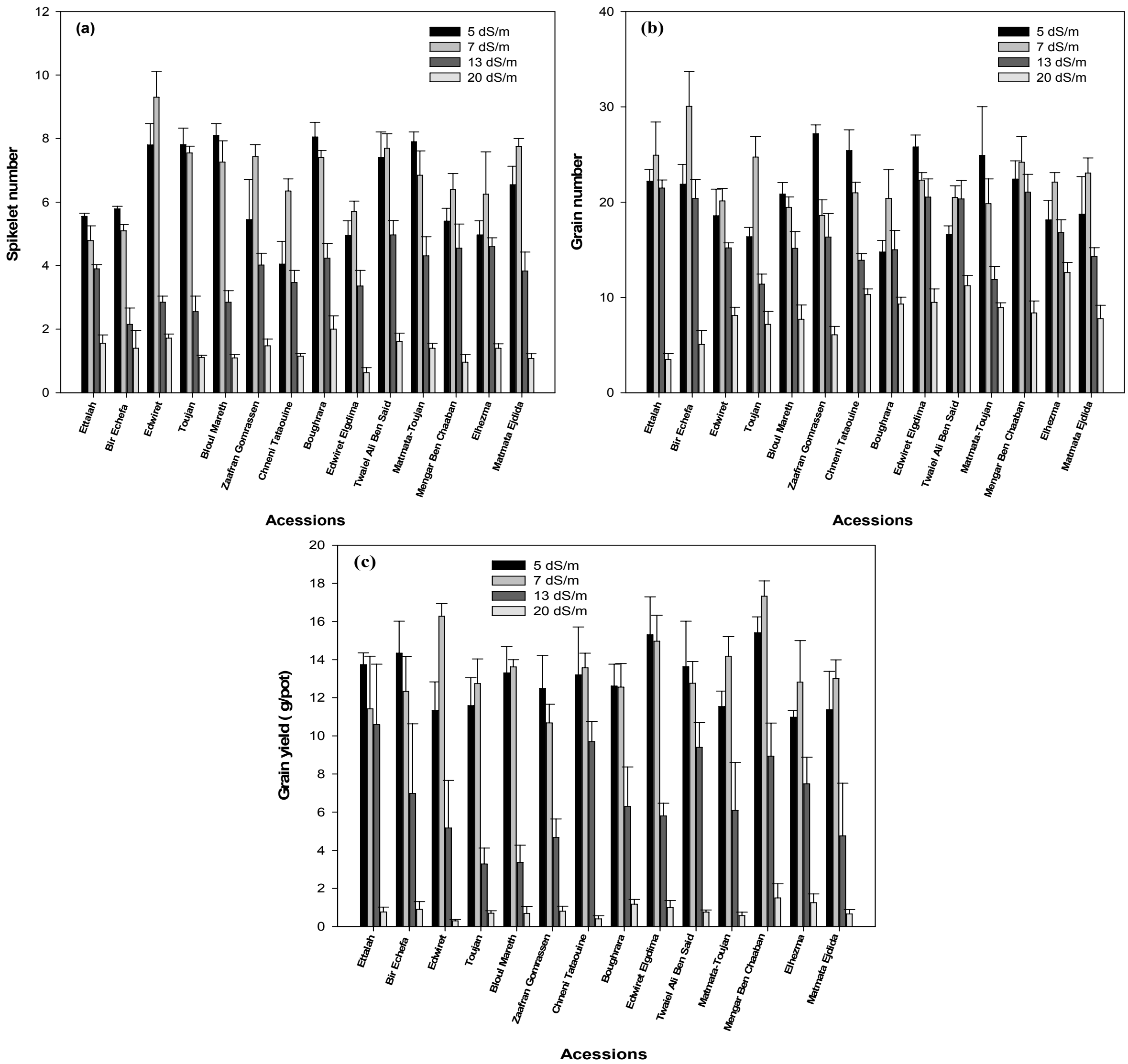

Figure 3. Effect of salinity levels on spikelet number per plant (a), grain number (b) and grain yield per pot (c) for different barley accessions tested. Error bars represent standard deviations.

The variation of the indices among accessions increased from low to high salinity. For instance, salt tolerance indices for spikelet number and grain number are ranged from 0.863 to 1.567 and from 0.685 to 1.510 at 7ds/m, from 0.127 to 0.284 and from 0.158 to 0.630 at high salinity level (20 dS/m). "Twaiel Ali Ben Said" and "Elhezma" recorded the highest salt tolerance indices at 20 ds/m.

According to the Ward's minimum variance cluster analysis, the accessions were divided into three groups at low salinity and into four cluster groups at moderate and high salinity (Table 4). The results show that "Ettalah", "Chneni Tataouine", "Boughrara", "Mengar Ben Chaaban" and "Elhezma" were ranked as the most tolerant accessions. Whereas, "Toujan", "Bloul Mareth" and "Edwiret Elgdima" were ranked as the least tolerant among all accessions. "Bir Echefa", "Edwiret", "Zaafran Gomrassen", "Matmata-Toujan" and "Matmata Ejdida" were intermediate between the most and the least tolerant accessions.

In the other way, the results show that the two factors; accessions and salinity have significant effects on the variation of the grains yield (Figure 3). The averages recorded by all accessions increased by 5% at 7 dS/m and decreased with the severity of salinity by 49% and 94% at 13 dS/m and 20 dS/m, respectively. The averages indices of grain yield from all accessions show high variability under the effect of salinity levels ($p<0.05$). The indices of all accessions ranged from 0.831 to 1.435 at 7ds/m, from 0.253 to 0.771 at 13dS/m and from 0.027 to 0.114 at the highest salinity level (20 dS/m). The results show also a wide variation among accessions. For instance, “Boughrara”, “Mengar Ben Chaaban” and “Elhezma” recorded the highest salt tolerance indices at 20ds/m. Whereas, “Edwiret” and “Chneni Tataouine” were recorded the lowest salt tolerance indices (Table 1).

Clustering based on analysis of the means of salt tolerance indices in grain yield using in the single linked cluster analysis show that the accessions were divided into three cluster groups at low salinity and four cluster groups at moderate and high salinity levels. “Ettalah”, “Mengar Ben Chaaban” and “Elhezma” were ranked as the most tolerant accessions and “Bir Echefa”, “Toujan”, “Bloul Mareth” and “Zaafran Gomrassen” as the least tolerant among all accessions (Table 4).

Table 4. Rankings of accessions for their relative salt tolerance in terms of spikelet number per plant and grain number in a cluster analysis used Ward’s minimum variance analysis

Accessions	Salinity levels (dS/m)			Sum	Accessions ranking	tolerance degree
	7 dS/m	13 dS/m	20 dS/m			
Ettalah	2	2	3	7	3	Tolerant
Bir Echefa	2	4	3	9	4	Moderate
Edwiret	2	4	4	10	5	Moderate
Toujan	2	4	5	11	6	Sensitive
Bloul Mareth	3	4	5	12	7	Sensitive
Zaafran Gomrassen	3	3	3	9	4	Moderate
Chneni Tataouine	1	3	1	5	1	Tolerant
Boughrara	2	2	2	6	2	Tolerant
Edwiret Elgdima	3	3	5	11	6	Sensitive
Twaiel Ali Ben Said	2	2	2	6	2	Tolerant
Matmata-Toujan	3	3	4	10	5	Moderate
Mengar Ben Chaaban	2	1	4	7	3	Tolerant
Elhezma	2	1	2	5	1	Tolerant
Matmata Ejdida	2	3	5	10	5	Moderate

3.4 Finale Ranking of Accessions Based on Their Relative Salt Tolerance

The clustering based on the combination of the principal parameters in the different stages classified the studied accessions in three different groups according to their tolerances degrees to the salinity (Table 5). “Ettalah”, “Chneni Tataouine”, “Twaiel Ali Ben Said”, “Mengar Ben Chaaban” and “Elhezma” were ranked as the most tolerant accessions. “Bir Echefa”, “Edwiret”, “Toujan”, “Bloul Mareth”, “Zaafran Gomrassen” and “Matmata-Toujan” were ranked as the least tolerant salinity (sensitive) and finally, Boughrara and Edwiret Elgdima were intermediate between the most and the least tolerant accessions (moderate).

Table 5. Finale ranking of accessions for their relative salt tolerance in terms of plant growth, straw yield and total biomass at final harvest, yield components and grain yield

Accessions	Ranking GP	Ranking SY and TBFH	Ranking YC	Ranking GY	Sum	Final ranking	Tolerance degree
Ettalah	2	3	3	2	10	3	Tolerant
Bir Echefa	6	6	4	5	21	9	Sensitive
Edwiret	6	6	5	4	21	9	Sensitive
Toujan	6	6	6	5	23	10	Sensitive
Bloul Mareth	6	6	7	5	24	11	Sensitive
Zaafran Gomrassen	7	5	4	5	21	9	Sensitive
Chneni Tataouine	4	2	1	2	9	2	Tolerant
Boughrara	5	5	2	3	15	6	Moderate
Edwiret Elgdima	3	5	6	4	18	7	Moderate
Twaiel Ali Ben Said	4	4	2	2	12	4	Tolerant
Matmata-Toujan	5	7	5	4	21	9	Sensitive
Mengar Ben Chaaban	3	5	3	2	13	5	Tolerant
Elhezma	1	1	1	1	4	1	Tolerant
Matmata Ejdida	6	4	5	4	19	8	Moderate

GP : plant growth parameter , SY: Straw yield ; TBFH: total biomass at final harvest; YC: Yield components and GY: grain yield.

4. Discussions

At the vegetative state, barley is characterized by its high tillering capacity. *Therefore, the three agronomic* parameters (i.e. plant height, tiller number and leaf number) were used to evaluate the accessions for salt tolerance. The different results linked *to* the growth parameters showed that the salinity affects negatively the vegetative development of barley. Thus, we observed a significant decrease of the mean height in the aerial part, the tiller number and the leaves number according to the applied stress level. However, salt sensitive accessions showed a greater reduction in tiller number (e.g. by about 80%) than tolerant ones (e.g. by about 57%) at 20 dS/m. These decrease on the growth of leaves, tillers and the aerial part constitute a strategy developed by the barely in vegetative phase to reduce the salt stress.

The results on the depressive effect of salinity on the growth and the development of plants corroborate with previous study (Zeng & Shannon, 2000; Binzel, Hess, Bressan, & Hasegawa, 1988; Munns, Richard, & Lauchli , 2006). Similar studies were also conducted on other species such as wheat (Bhatti, Ali, Bakhsh, Razaq, & Jamali, 2004), oat (Zhao, Ma, & Ren, 2009) and rice (Gain et al., 2004; Razzaque, Talukder, Islam, Bhadra, & Dutta, 2009) and showed that the tiller and the leaves numbers decreased under the effect of salt stress. Several works, using split root system approaches on glycophyte and halophyte species, have demonstrated that nutritional disturbances caused by salt are implicated in the restriction of the growth under salinity stress (Messedi, Labidi, Grignon, & Abdelly, 2004; Attia, Karray, Mokded, & Lachaâl, 2008). According to Majd and Shahbazi (1996), this reduction is due to the effect of Na^+ and Cl^- ions on the cellular division in the shoot apical meristem which affecting the formation of the floral and the auxiliary buds, and then on the final number of leaves and tillers produced. This may indicate that, under salinity, tiller number and their behavior would be used as simple and non-destructive measurement to evaluate barley accessions in breeding programs. Moreover, Nicolas, Munns, Samarakoon and Gifford (1994) found that the salt stress during tiller emergence could inhibit their formation and cause their abortion at later stages. Therefore, increasing the salinity tolerance in barely may require an increase in the tillering capacity (Islam & Sedgley, 1981).

The final harvest of total biomass can be divided in two components: the grain yield and the straw yield. Indeed, these two components reflect the different aspects of plant development. Thus, the major part of the straw production takes place during the early stage of growth cycle and it is essentially related to the vigor and the vegetative growth as the tiller number, the leaves number, the height of plants and the accumulation of the reserves during the vegetative stage. The negative effect of salinity on spikelet number and on tiller number indicates that they are sensitive parameters at the vegetative stages. This suggests that the evaluation of salt tolerance among accessions can be based on the genetic diversity in tiller and spikelet number at the early growth stages. Whereas, the grains yield takes place mainly during the productive phase and it is essentially influenced by the spike number, the spike fertility, the grain-filling period and the efficiency of the reserve mobilization.

The grain yield and the straw yield are the principal criteria used by the farmers to choose the tolerant accessions under salt stress. Therefore, improving the grain yield of barley is the main target in plant breeding.

The yield components (i.e spikelet number, grain number and grain weight) determined the final grain yield. Furthermore, the different results showed significant correlation between the spikes numbers and the tiller number ($r = 0.872$). The salinity affected negatively these two parameters, which both initiate during early growth stages. So, the salinity has a greater influence on final grain yield than on yield components in the later stages (Mass, Hoffman, Chaba, Poss, & Shannon, 1983; Mass & Poss, 1989).

The barley accession "Ardhoui" is characterized by its tolerance to the salinity and according to FAO it can tolerate up to 8 dS/m. This affirms the increase in certain parameters (growth or production parameter) at 7 dS/m. In southern Tunisia Soils have an electric conductivity within 5 and 7 dS/m. Therefore, the variability observed between accessions would be explained by the genetic factor.

The salt tolerance of the fourteen studied barley was evaluated in this study using cluster analysis. This method was involved in potato (Khrais et al., 1988), in rice (Zeng et al., 2002) and in Wheat (El-Hendawy et al., 2005). The main advantage to use the multivariate analysis, in the evaluation of salt tolerance, is the ranking of the accessions even when plants are evaluated at different salt levels; indeed the salt tolerance varies with the salinity levels. In this study we used as this advantage to classify the barely accessions according to their salt tolerance under different salinity levels.

The cluster analysis makes possible to distinguish some accessions. "Ettalah", "Chneni Tataouine", "Twaiel Ali Ben Said", "Mengar Ben Chaaban" and "Elhezma" present a similar salt tolerance at different growth stages. These accessions present more tillers, taller and higher leaves number compared to the others. The grain yield and the yield components are the less affected by the different salinity levels. We noted that the salt tolerance of these accessions remained almost unchanged at the different growth stages. In addition, “Ettalah” and “Elhezma” were the most tolerant and they have the same salt tolerance characters.

“Boughrara” and “Edwiret Elgdima” were intermediate between the most and the least tolerant accessions. Thus, a change in salt tolerance on the growth stage was observed and they were ranked as having poor salt tolerance based on the parameters at the vegetative stage and straw yield. Therefore, to improve the salt tolerance of these accessions we must increase the tillering ability and/or the frequency of irrigation which allows the improvement of the soil salt stress at early growth stages. “Bir Echefa”, “Edwiret”, “Toujan”, “Bloul Mareth”, “Zaafran Gomrassen” and “Matmata-Toujan” were ranked as the slightest tolerant salinity at all growth stages.

In conclusion, the accessions of plain zones are most tolerant than those of mountainous zones using multiple agronomic parameters. A possible hypothesis is that the accessions seeds of the coastal zone have been probably cultivated one or several times and irrigated with saline waters and therefore they developed several strategy to support this stress. Probably, their development under saline stress had a positive impact on the tolerance of these accessions. However, “Ettalah”, “Chneni Tataouine”, “Twaiel Ali Ben Said”, “Mengar Ben Chaaban” and “Elhezma” were identified as the most salt tolerant accessions in the cluster analysis, they can be investigated through appropriate selection and breeding programs for improving salt tolerance of Tunisian barley genotypes. “Bir Echefa”, “Edwiret”, “Toujan”, “Bloul Mareth”, “Zaafran Gomrassen” and “Matmata-Toujan” were more sensitive to salinity at early growth stages than at later stages; therefore their salt tolerance can be improved by developing strategies for agronomic management according to the different growth stages.

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