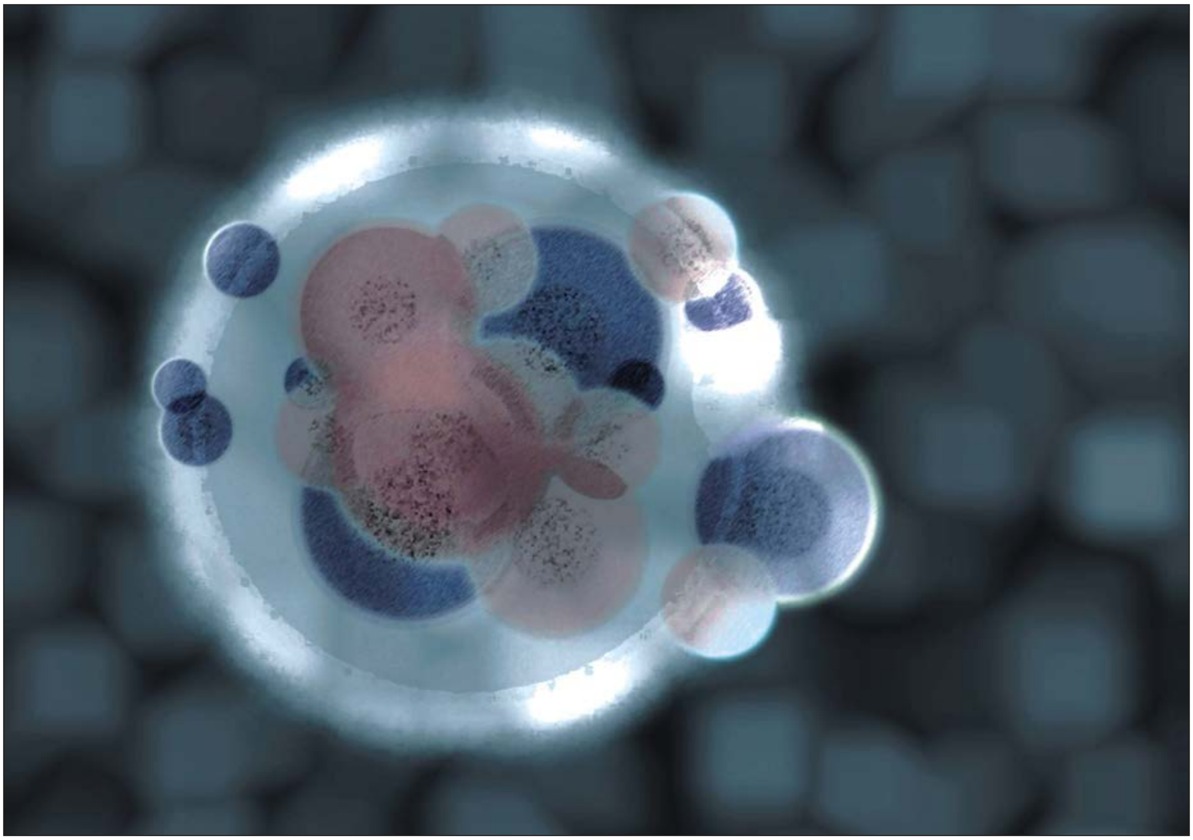

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Analysis of Genetic Diversity to Salt Stress of South Tunisian Barley Cultivars Using Agronomic Parameters

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Abstract: In the present research eighty barley cultivars from South Tunisia were evaluated. The markers used are: leaf number, length and width at vegetative and reproductive stage, tiller number and grain number per spike at maturity. Analyses of histogram's showed that salinity had significant effect on all the growth parameters at different growth stages. However, vegetative stage was affected more adversely than reproductive and grain filling stage. At vegetative stage, the average reduction of the number of leaf is 38%, wile this reduction don't exceed 27% at reproductive stage. The salinity induce also an important reduction in tiller number 24%. At reproductive stage, the results showed that, average length was affected more by salinity 30% than the tiller number 24%, width of leaves 27% and leaf number 27%. At maturity, number of grain was least affected by salinity. The reduction is 4.7% compared with the control treatment. Thus, at the vegetative stage, Cultivars Ksar Oun, Elbagbag 1 and 2, Amdi, Oued el Kil (Tataouine) were ranked at the more salt tolerant. At reproductive stage the result show that cultivars Mgitt, El Kir, Tlalite, Ksar Ouled Boubaker and El Ferch (Tataouine) were ranked as the most tolerant genotypes.

Key words: Barley, salinity, South Tunisia, genetic diversity

INTRODUCTION

Salinity is a global problem that limits crop production, especially on irrigated area of the world. It is one of the major factors reducing plant growth and productivity world wide and affects about 7% of the world's total land area (Flowers *et al*., 1997). The percentage of cultivated land affected by salt is even greater. Twenty three percent of the cultivated land being saline and 20% of the irrigated land suffering from secondary Stalinization. Furthermore, there is also a dangerous trend of increase in the saline throughout the wold (Ponnamierumo, 1984).

Salt tolerance of crops may vary with their growth stage (Mass and Grieve, 1994; Azevedo Neto *et al*., 2004). In general, cereal plants are the most sensitive to salinity during the vegetative and flowering and during the grain filling stage (Mass and Poss, 1989).

Problem of soil salinity can be combated through two approaches. One is to make use of available technology for reclaiming these soils, while other is based on biological exploitation of such soils through cultivation of salt tolerant plant species (Apse and Blumward, 2002). The first approach, due to certain limitations such as insufficient supply of irrigation water and high reclamation and drainage cost, present difficulties in his application. While the later approach, does not involve so such investment, is feasible (Flowers and Hajibaghri, 2001).

Barley (*Hordeum vulgare* L.) is one of the most important crop species in the world and has been subject to considerable genetic study. It is a diploid (2n = 2 x = 14) largely self fertilizing species (Bennett and Heun, 1995).

Barley grains are used to manufacture a variety of human foods and are also valuable as livestock feed, but the most economically important use is for malting and brewing.

Improving salt tolerance of barley genotypes has been inhibited by a number of factors, such as the lack of effective evaluation methods for salt tolerance to screen the genotypes in breeding programs, low selection efficiency using overall agronomic parameters and a complex phenomenon involving morphological, physiological and biochemical parameters among genotypes (Zeng *et al*., 2002).

Barley can tolerate extreme environmental and edaphic conditions (Karim *et al*., 1994). Thus, in order to have effective utilization of salt affected soils, it is important to select barley genotypes, which may tolerate salinity and produce substantial yields under adverse soil environment (Flowers and Hajibagheri, 2001). With this objective in view, this experiment was conduced using 80 barley genotypes.

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The objectives of this study were to identify the relative importance of agronomic parameters associated with salt tolerance and to screen the different barley genotypes for their salt tolerance at different growth stage.

MATERIALS AND METHODS

Plant materials: Eighty cultivars of barley (*Hordeum vulgare* L.) from different regions in South Tunisia were used in this study (Table 1).

Growth conditions and sampling strategy: This study was carried out in experiment field of Institut des Régions Arides de Medenine. Tunisie.

Seeds of different genotypes were sowed in January and the measurement was carried in May.

Tow salt levels were applied: 1.5 g L^{-1}: control, it represent the minimum level of salinity in south Tunisia and 7 g L^{-1} NaCl.

For each cultivar of Barley and each salt level treatment, 6 rows (2 m long) were sowed.

During experiment, the irrigation is conduced once every ten days. The quantity of water of irrigation is the equivalent of 20 mm per irrigation

The measurements were carried out at vegetative, reproductiveand grain maturity stages.

Measurements at the vegetative stage were conduced at 45 days after sowing. The parameters used are the number, length and width of leaves (3 leaves are measured/plant).

The reproductive stage, reached at 100 days, is characterized by the number of tiller, the number, length and width of leaves (3 leaves are measured/plant).

Productivity was evaluated by the number of grain.

For each treatment 6 plants were randomly selected and measured.

Statistical analysis of data: Data were analysed using SPSS version 12. Cluster group were obtained using average linkage (between groups) and Euclidian distance.

Table 1: Studied Barley cultivars with their origin

Name	Origin
Tataouine ejdida	Tataouine
Oued el khil 2	Ben keddache
Gasbett gomri	Gomrassen
El bagbag 3	Tataouine
lamaat	Tataouine
Ksar ouled boubaker	Tataouine
Mareth	Gabes
Labyar 2	Medenine
Bir ezwai 3	Medenine
tlalite	Tataouine
Oued el khil	Tataouine
Dkilet toujene	Gabes
Amadi	Tataouine
Elmejni	Gabes
Belkir 1	Gafsa
Mgitt 2	Tataouine
Belkhir 3	Gafsa
Missawa	Tataouine
Gomrassen 2	Tataouine
Gattouffa	Tataouine
Manzel mgor 3	Ben khddache
Manzel mgor 2	Ben khddache
Bir lahmer 2	Tataouine
Essaidane	Ben Guerdane
Matmatta jdida 1	Gabes
El bhira 1	Medenine
El bhira 2	Medenine
El bag bag 1	Tataouine
Matmata jdida 2	Gabes
Labyar 1	Ben khddache
Hjar	Medenine
Errssifett	Zarzis
El ferch 2	Tataouine
Chehbania 2	Tataouine
Tarf ellil	Medenine
Oued el khil 3	Tataouine
Elmdon	Gabes
Ben khddache centre	Medenine
Ksar oun	Tataouine
Bir ezzwai	Medenine
Grager 2	Tataouine
Bniri	Ben guerdane
Oued erbaii	Ben guerdane
Zmorten	Matmata
Essolb	Zarzis
Gormassa	Tataouine
Ferjania 2	Medenine
Ksar oun 1	Tataouine
Bir addim	Medenine
Oued elhalouf	Medenine
Thahret el gbour 2	Medenine
Ksar ouled dbab	Tataouine
Ksar ejdid	Medenine
El mawouna	Tataouine
Ezzahra 2	Tataouine
Elmziraa	Medenine
Lagrabette	Medenine
Bonzrida	Tataouine
Echahbania	Tataouine
Gormassa 2	Tataouine
Layhet mars	Medenine
Aiin tounine	Matmata
Jellal	Ben guerdane
Thahret elgbour	Medenine
Elbagbag 2	Tataouine
Mazreet ben slama	Gabes
Gragre 1	Tataouine
Oued el khil 1	Tataouine
Switir 1	Medenin
Chenenni	Tataouine
Bir 30 -2	Tataouine
Bir ezzwai 2	Medenine
El werssania	Ben guerdane
Ramtha	Tataouine
Eskir 1	Tataouine
Bir lahmer 1	Tataouine
Essmar 1	Tataouine
Sidi mesbeh	Tataouine
Matmata elkdima	Gabes
Elmorchdiya	Ben guerdane

RESULTS

All the data were converted to salt tolerance indices before cluster analysis to allow comparisons among barley cultivars for salt tolerance by using multiple agronomic parameters.

A salt tolerance index was defined as the observation at salinity divided by the average of the controls ‘auteur).

Analyses of histogram’s (Fig. 1) showed that salinity had significant effect on all the growth parameters at different growth stages. However, vegetative stage was affected more adversely than reproductive and grain filling stage.

At vegetative stage, the maximum leaf number was 19.33 at control treatment but at salinity 7 g L^{-1} the maximum don’t exceed 11.17. The maximum average length and width leaves were, respectively 16.43 and 1cm at control treatment but at salinity 7 g L^{-1} the maximum don’t excess 11.68 and 0.67 cm (Fig. 1).

At reproductive stage, the maximum leaf number was 37 at control treatment but at salinity 7 g L^{-1} the maximum don’t excess 22.67 (Fig. 2), the number of tiller was 6 at control and 3.83 at salinity 7 g L^{-1}, the maximum average length and width of 3 leaves were, respectively 19.38 and 1.51 at control treatment but at salinity 7 g L^{-1}, the maximum don’t excess 12.64 and 1.07 (Fig. 2).

At maturity, the grain number was not affected by salinity (Fig. 3), the maximum number of grain observed at tow treatment is 48.

The more adverse effect of salinity was found at vegetative stage where salinity reduced the number of leaf by 38%, whereas this reduction does not exceed 27% at reproductive stage.

Figure 1 the maximum number of leaf (11.7) and the minimum (5.17) was observed respectively in cultivar Erremtha 2 (Tataouine) and El wersania (Ben Guerdane). At reproductive stage, the results showed that, average length was affected more by salinity (30%) than the tiller number (24%), width of leaves (27%) and leaf number (27%).

The maximum number of tillers (3.83) was observed in cultivar Oued erbaii (Ben Guerdane) and the minimum (1.5) was observed in cultivar Thahret El Gbour (Medenine).

At maturity, number of grain was least affected by salinity. The reduction is 4.7% compared with the control treatment.

At different growth stage, the relative salt tolerance indices for all the measured parameters varied among cultivars (Table 2).

When a large number of genotypes have to be evaluated in salt tolerance breeding by using multiple agronomic parameters, cluster analysis can be used to facilitate the ranking of the genotypes for salt tolerance.

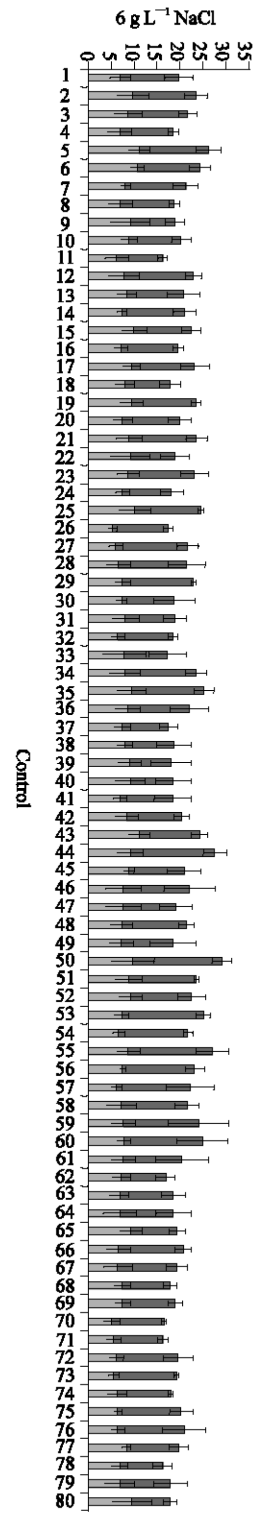

Fig. 1a: Effect of salinity levels at vegetative stage (at day 45) estimated by leaf number for different Barley cultivars

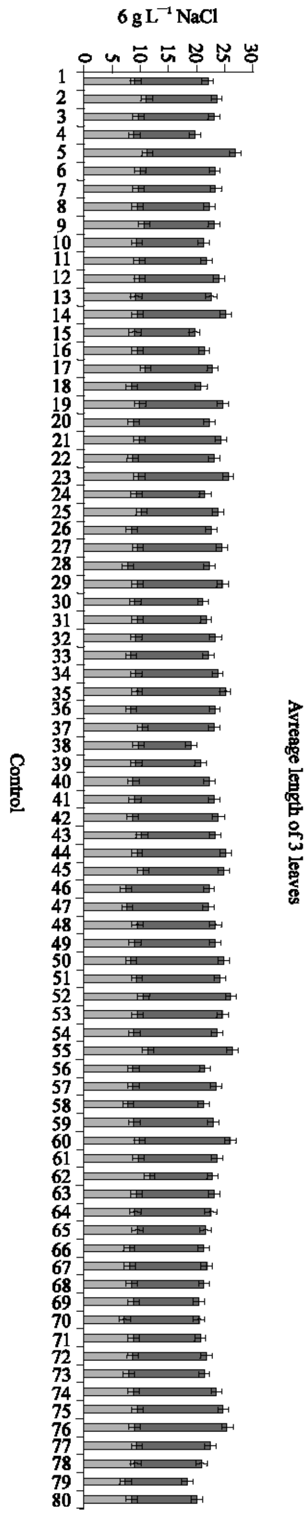

Fig. 1b: Effect of salinity levels at vegetative stage (at day 45) estimated by length for different Barley cultivars

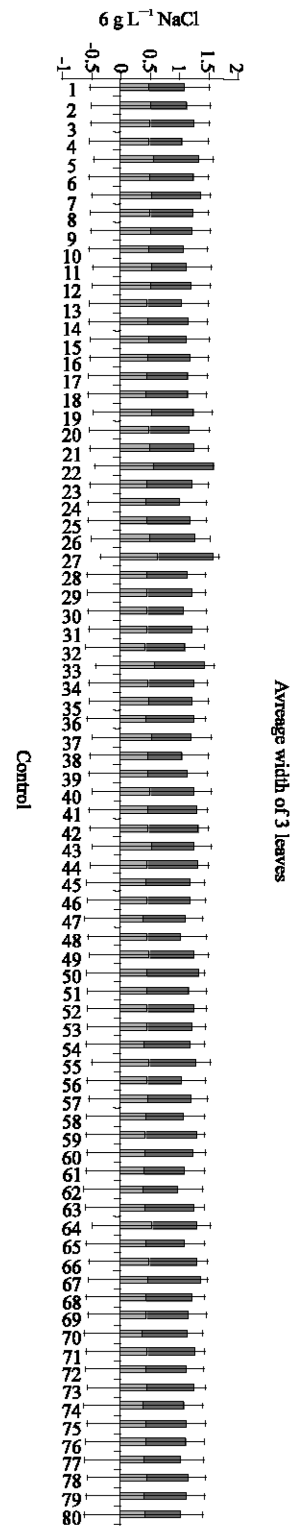

Fig. 1c: Effect of salinity levels at vegetative stage (at day 45) estimated by width for different Barley cultivars

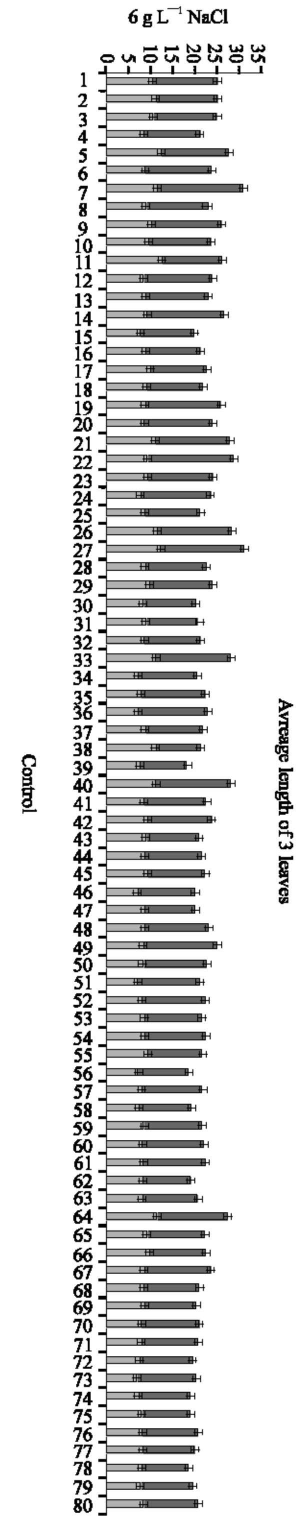

Fig. 2a: Effect of salinity levels at vegetative stage (at day 100) estimated by leaf number for different Barley cultivars

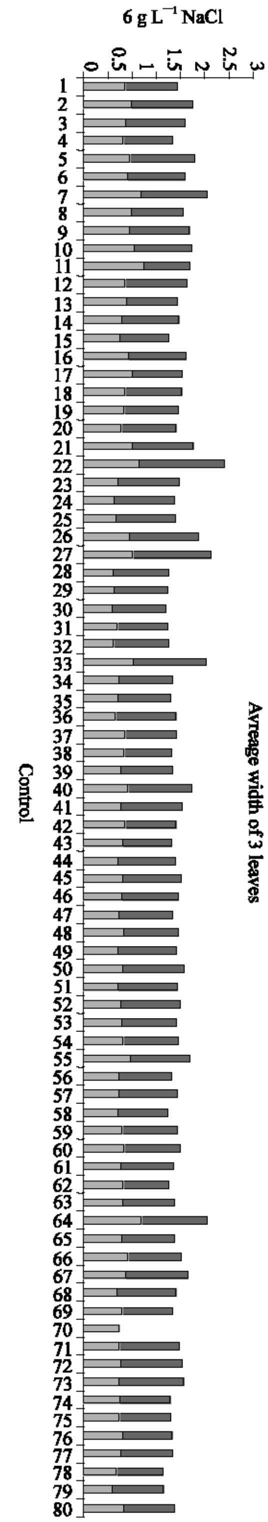

Fig. 2b: Effect of salinity levels at vegetative stage (at day 100) estimated by length for different Barley cultivars

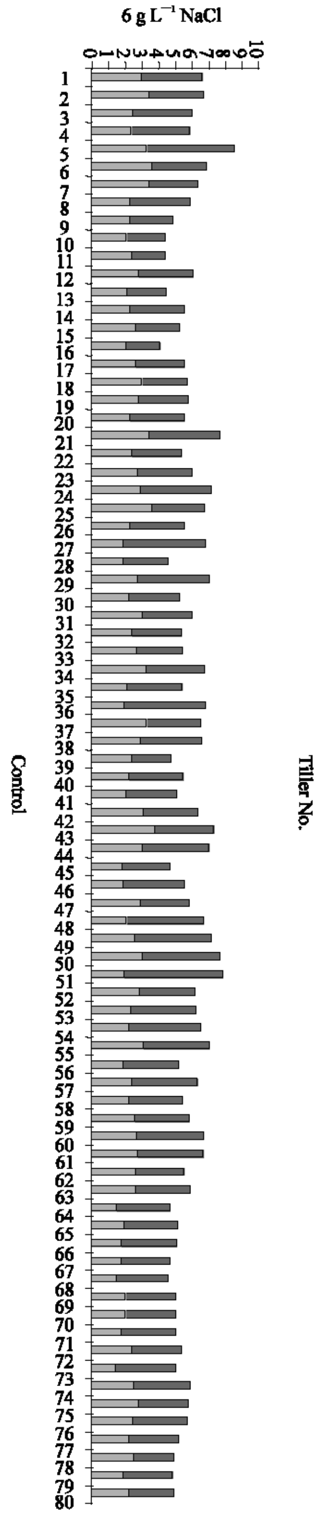

Fig. 2c: Effect of salinity levels at vegetative stage (at day 100) estimated by tiller number for different Barley cultivars

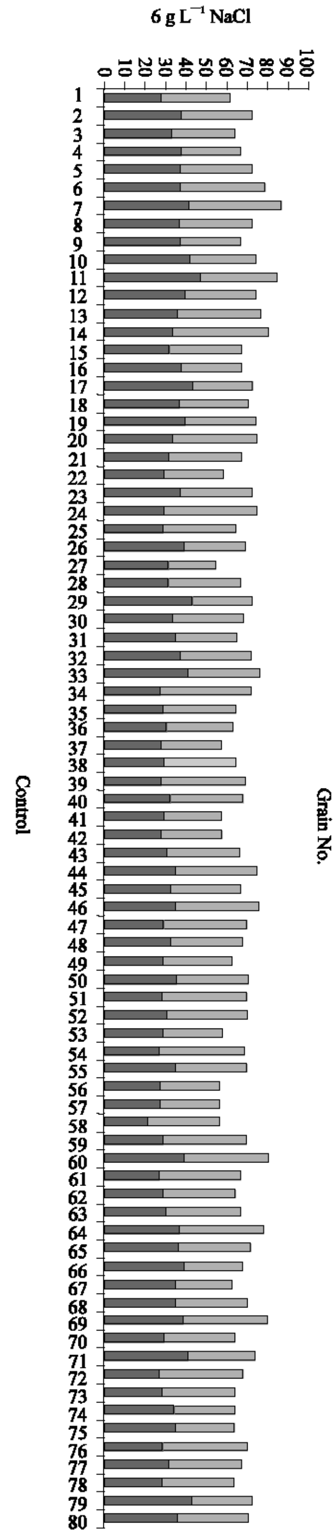

Fig. 3: Effect of salinity levels on grain number at maturity for different Barley cultivars Errors bars represent standard deviation

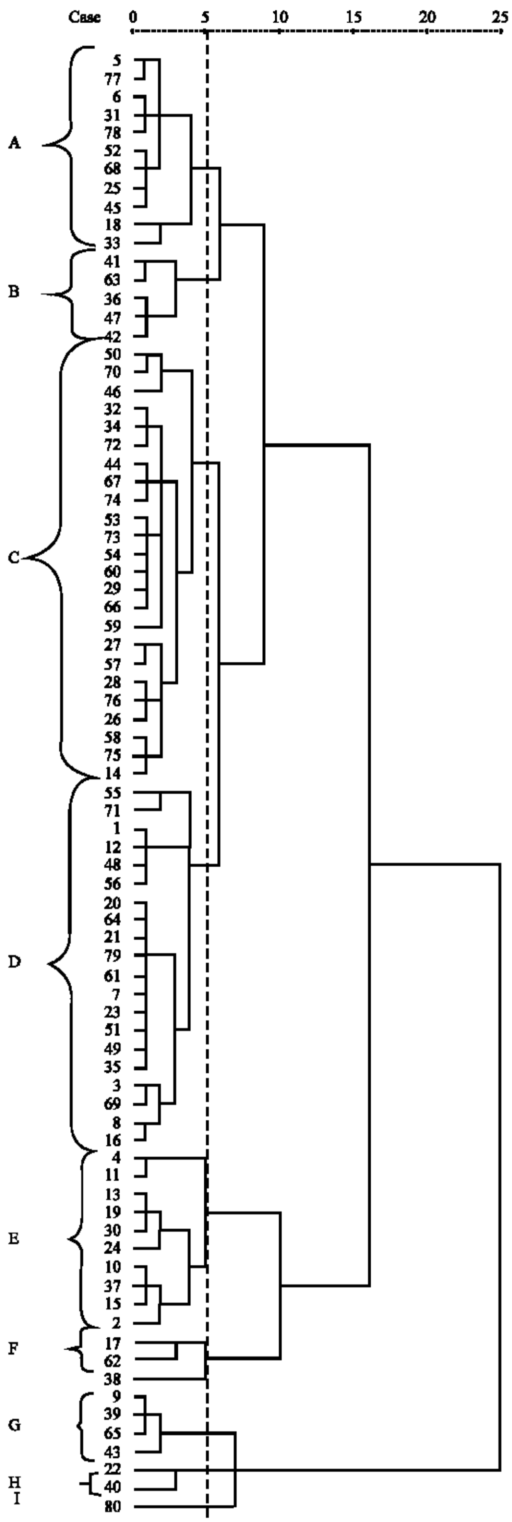

Fig. 4: Dendrogramme of classification of studied barley cultivars using SPSS (average linkage between groups) at vegetative stage (leaf number, width and length of leaves)

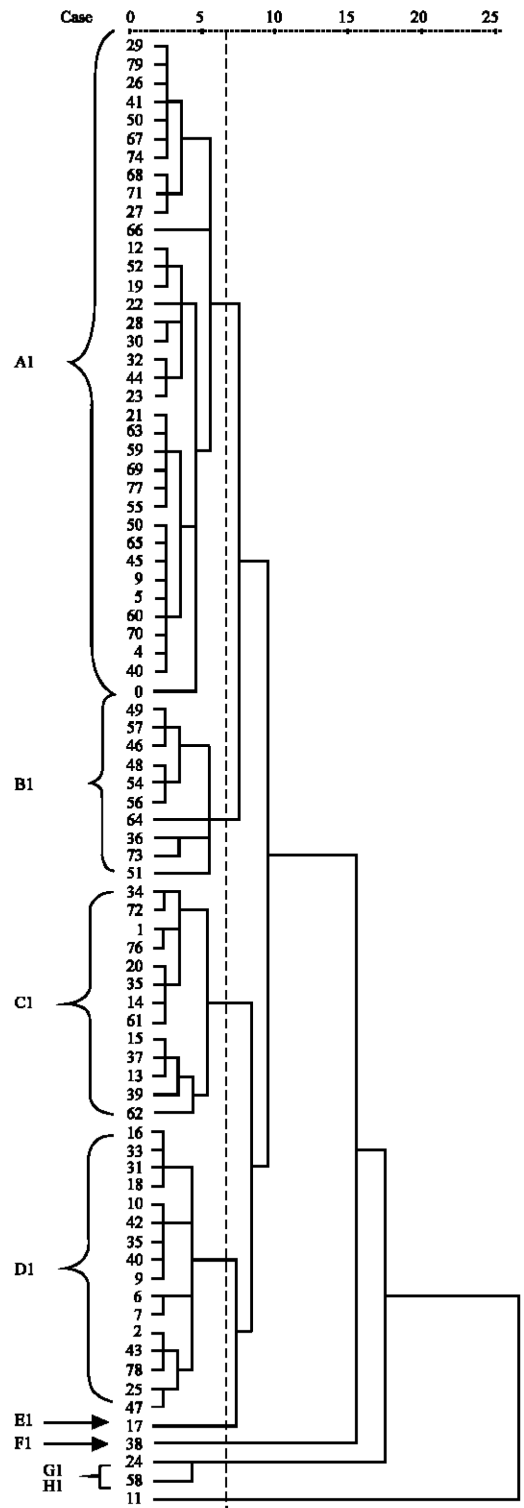

Fig. 5: Dendrogramme of classification of studied barley cultivars using SPSS (average linkage between groups) at reproductive stage (tiller and leaf number, width and length) at day 100

Table 2: Ranking of genotypes for their salt tolerance in terms of plant vegetative stage

Groups	Tolerance degree at leaf No.	Tolerance degree at leaves length	Tolerance degree at leaves width	Average tolerance degree
A	Moderate	Moderate	Moderate	Moderate
B	Moderate	Moderate	Sensitive	Moderate
C	Sensitive	Sensitive	Moderate	Sensitive
D	Moderate	Moderate	Moderate	Moderate
E	Moderate	Tolerant	Tolerant	Tolerant
F	Tolerant	Tolerant	Moderate	Tolerant
G	Tolerant	Tolerant	Tolerant	Tolerant
H	Tolerant	Moderate	Moderate	Moderate
I	Tolerant	Moderate	Moderate	Moderate

Table 3: Ranking of genotypes for their salt tolerance in terms of plant reproductive stage (leaf number, tiller number, width and length of leaves)

Groups	Tolerance degree at tiller No.	Tolerance degree at leaf No.	Tolerance degree at leaves length	Tolerance degree at leaves width	Average tolerance degree
A1	Moderate	Moderate to tolerant	Sensitive to moderate	Moderate to sensitive	Moderate to sensitive
B1	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
C1	Moderate to tolerant	Moderate	Sensitive	Moderate	Moderate
D1	Very tolerant	Very tolerant	Moderate	Tolerant	Tolerant
E1	Tolerant	Very tolerant	Tolerant	Very tolerant	Tolerant to very tolerant
F1	Tolerant	Very tolerant	Very tolerant	Very tolerant	Very tolerant
E1	Moderate	Sensitive	Sensitive	Moderate	Sensitive to moderate
G1	Very tolerant	Very tolerant	Very tolerant	Very tolerant	Very tolerant

Table 4: Salt tolerance indices of agronomic parameters in barley genotypes at different growth stages

Cultivars	Leaf No. at day 45	Tiller No. at day 100	Average length of 3 leaves at day 45	Average width of 3 leaves at day 45	Grain No.	Leaf No. at day 100	Average length of 3 leaves at day 100	Average width of 3 leaves at day 100
1	0.55	0.82	0.72	0.79	0.84	0.93	0.70	0.75
2	0.71	1.05	0.89	0.82	1.06	0.78	0.77	0.78
3	0.67	0.68	0.73	0.67	1.04	0.64	0.73	0.71
4	0.59	0.64	0.83	0.89	1.27	0.70	0.69	0.79
5	0.74	0.63	0.72	0.72	1.06	0.69	0.82	0.71
6	0.78	1.10	0.76	0.67	0.90	0.74	0.58	0.72
7	0.63	1.17	0.71	0.65	0.91	0.71	0.60	0.86
8	0.60	0.64	0.75	0.69	1.04	0.67	0.63	0.93
9	0.95	0.88	0.87	0.71	1.27	0.56	0.64	0.78
10	0.78	0.93	0.79	0.78	1.24	0.63	0.71	0.91
11	0.62	1.25	0.83	0.95	1.26	0.75	0.93	1.28
12	0.52	0.85	0.71	0.76	1.11	0.68	0.56	0.68
13	0.68	0.93	0.69	0.81	0.86	0.94	0.65	0.88
14	0.54	0.70	0.62	0.71	0.71	1.00	0.54	0.68
15	0.81	1.00	0.86	0.81	0.89	0.91	0.64	0.79
16	0.58	1.08	0.81	0.70	1.27	0.55	0.73	0.77
17	0.70	0.89	0.92	0.67	1.47	0.45	0.81	0.99
18	0.83	1.19	0.69	0.65	1.12	0.58	0.72	0.73
19	0.68	0.94	0.68	0.79	1.11	0.71	0.51	0.73
20	0.61	0.70	0.66	0.73	0.81	0.90	0.55	0.73
21	0.60	0.81	0.68	0.65	0.89	0.73	0.66	0.83
22	0.95	0.83	0.60	0.59	1.00	0.59	0.49	0.65
23	0.60	0.85	0.62	0.67	1.06	0.64	0.62	0.56
24	0.70	0.69	0.77	0.86	0.65	1.32	0.50	0.53
25	0.71	1.10	0.75	0.63	0.83	0.75	0.68	0.57
26	0.44	0.70	0.60	0.68	1.33	0.51	0.67	0.64
27	0.38	0.40	0.65	0.71	1.33	0.54	0.67	0.65
28	0.45	0.75	0.54	0.67	0.89	0.75	0.64	0.55
29	0.49	0.65	0.64	0.61	1.47	0.42	0.72	0.62
30	0.65	0.78	0.76	0.77	0.94	0.82	0.69	0.53
31	0.77	1.06	0.78	0.64	1.20	0.54	0.78	0.67
32	0.54	0.83	0.66	0.66	1.06	0.62	0.71	0.57
33	0.84	1.06	0.61	0.69	1.17	0.59	0.70	0.71
34	0.52	0.91	0.64	0.64	0.61	1.05	0.55	0.68
35	0.61	0.65	0.60	0.71	0.83	0.85	0.55	0.66
36	0.65	0.40	0.57	0.58	1.00	0.58	0.47	0.56
37	0.75	1.00	0.82	0.83	0.96	0.86	0.69	0.81
38	0.75	0.82	1.04	0.86	0.83	1.03	1.07	0.92
39	1.00	1.07	0.81	0.71	0.67	1.06	0.73	0.77
40	0.98	0.70	0.65	0.71	0.93	0.77	0.67	0.71

Table 4: Continued

Cultivars	Leaf No. at day 45	Tiller No. at day 100	Average length of 3 leaves at day 45	Average width of 3 leaves at day 45	Grain No.	Leaf No. at day 100	Average length of 3 leaves at day 100	Average width of 3 leaves at day 100
41	0.62	0.72	0.64	0.56	1.05	0.54	0.65	0.60
42	0.69	0.95	0.57	0.61	0.96	0.64	0.67	0.86
43	0.86	1.05	0.80	0.74	0.87	0.85	0.74	0.84
44	0.50	0.79	0.60	0.59	0.90	0.66	0.70	0.62
45	0.74	0.61	0.73	0.58	0.93	0.63	0.73	0.66
46	0.53	0.55	0.51	0.62	0.84	0.74	0.57	0.69
47	0.68	1.00	0.54	0.55	0.71	0.76	0.78	0.64
48	0.52	0.46	0.68	0.81	0.93	0.87	0.61	0.75
49	0.63	0.57	0.63	0.66	0.88	0.75	0.52	0.57
50	0.50	0.68	0.51	0.53	1.00	0.53	0.60	0.65
51	0.60	0.33	0.63	0.67	0.70	0.95	0.51	0.61
52	0.69	0.90	0.69	0.61	0.80	0.76	0.59	0.63
53	0.42	0.63	0.63	0.62	0.98	0.64	0.70	0.72
54	0.44	0.54	0.61	0.58	0.67	0.87	0.67	0.75
55	0.47	0.79	0.77	0.70	1.00	0.70	0.80	0.83
56	0.48	0.60	0.69	0.81	0.93	0.86	0.68	0.66
57	0.39	0.63	0.60	0.69	0.93	0.74	0.59	0.61
58	0.50	0.70	0.58	0.75	0.61	1.22	0.67	0.72
59	0.47	0.80	0.64	0.52	0.71	0.73	0.69	0.72
60	0.46	0.71	0.62	0.60	0.95	0.63	0.62	0.74
61	0.61	0.71	0.69	0.69	0.72	0.97	0.66	0.70
62	0.74	0.89	1.05	0.70	0.83	0.84	0.84	0.89
63	0.59	0.80	0.68	0.56	0.82	0.68	0.70	0.79
64	0.59	0.45	0.68	0.73	0.90	0.81	0.73	0.89
65	0.93	0.60	0.78	0.69	1.04	0.67	0.73	0.70
66	0.48	0.55	0.61	0.63	1.33	0.47	0.78	0.90
67	0.51	0.61	0.60	0.56	1.26	0.44	0.62	0.73
68	0.73	0.56	0.67	0.58	1.00	0.58	0.72	0.60
69	0.66	0.72	0.76	0.71	0.95	0.75	0.80	0.81
70	0.46	0.72	0.55	0.54	0.83	0.65	0.66	0.79
71	0.52	0.55	0.75	0.59	1.24	0.47	0.65	0.63
72	0.46	0.83	0.67	0.67	0.67	1.00	0.65	0.65
73	0.40	0.41	0.60	0.62	0.81	0.76	0.54	0.53
74	0.54	0.64	0.60	0.62	1.20	0.52	0.66	0.78
75	0.48	0.94	0.63	0.74	1.23	0.60	0.75	0.73
76	0.45	0.75	0.55	0.69	0.71	0.97	0.68	0.81
77	0.74	0.78	0.72	0.73	0.93	0.79	0.77	0.76
78	0.73	1.14	0.79	0.67	0.81	0.82	0.83	0.73
79	0.62	0.67	0.67	0.67	1.47	0.45	0.69	0.63
80	1.14	0.88	0.74	0.69	1.06	0.66	0.72	0.79

Thus, at the vegetative stage, cultivars were classified into 9 cluster (Fig. 4). Cultivars Ksar Oun, Elbagbag 1 and 2, Amdi, Oued el Kil (Tataouine) were ranked at the more salt tolerant. By contrast, Oued el Halouf, El Bhira 1, El Bhira 2 and Thahret El Gbour (Medenine) were ranked as the most sensitive (Table 3).

At reproductive stage studied cultivars can be divided into 8 cluster groups (Fig. 5), the result show that cultivars Mgitt, El Kir, Tlalite, Ksar Ouled Boubaker and El Ferch (Tataouine) were ranked as the most tolerant genotypes, whereas cultivars Bir Addim, Lagrabett, Thahret El Gbour and El Mgiraa (Medenine) were ranked as the least tolerant among all genotypes (Table 4). The others cultivars present intermediate tolerance.

DISCUSSION

Salt tolerance among barley cultivars was evaluated in this study using agronomic parameters at vegetative and reproductive stages.

Improving the vegetative and the reproductive stage and grain yield of barley is always the main target in plant breeding. Therefore the evaluation of vegetative stage and a final yield is a critical aspect of breeding programs.

The results at the vegetative stage showed that leaf number was more infected by salinity. These results are in agreement with those of Senin *et al.* (1985) and Francois *et al.* (1994) who also reported that salinity in the growth stage decreased the number of leaf per plant in barley.

Nicolas *et al.* (1994) found that salt stress during leaf emergence can inhibit their formation and can cause their abortion at later stages.

In view of Shoe and Gale (1983), the lesser number of leaves at salinity may be due to decreased amount of photosynthates reaching the growing region because of inhibition of photosynthesis due to stomatal closure or by direct effects of salts on the photosynthetic apparatus.

The decrease in plant growth could be attributed to the toxic effects of Na^+ or Cl^- on plant metabolism, nutritional imbalance or osmotic reduction in water availability in the growth medium (Greenway and Munns, 1980).

Reduction in leaf area under salt stress may have been due to suppressed cell division or fewer number of cells (Ashraf, 2002; Malibari *et al.*, 1993). These results support the earlier findings of El Kady *et al.* (1980), Yeseen *et al.* (1987) and Kalaji and Nalborczyk (1991) who found that increasing salinity of the growth medium decrease the leaf area in barley cultivars.

Because spikelets initiate at the vegetative stage, the negative effect of salinity on spikelet number indicates that the number of spikelets per spike together with the number of tillers per plant are sensitive parameters at the vegetative stage. This suggests that evaluation for salt tolerance among genotypes can be based on the genetic diversity in tiller number. Another advantage is that the tiller number can again be used as a simple measurement to evaluate large number of barley genotypes in breeding programs, especially, because this parameter can be determined at early growth stages (Grando and Ceccarelli, 1991).

The comparison between studied genotypes showed that cultivars Belkir 3 (Gafsa), Oued Erbaii (Ben Guerdane) and Oued ElKil (Tataouine) were the most tolerant genotypes, whereas, Lagrabett, Thahret Elgbour, Elbhira 1 (Medenine) were more sensitive at all growth stages.

Because cultivars from Tataouine were identified as the most salt tolerant genotypes in the cluster analysis, they can be utilized through appropriate selection and breeding programs for further improvement in salt tolerance of Tunisian barley genotypes.

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