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GENOTYPIC VARIATION FOR SALT TOLERANCE IN MOROCCAN BARLEY LANDRACES AT SEED GERMINATION

ABSTRACT

Experiments were conducted to evaluate the genotypic variation for salt tolerance on seed germination for 24 genotypes (20 landraces and 4 breeding lines) of barley. The genotypes were evaluated by several criteria, at 4 salt concentrations (0, 100 mM, 150 mM and 200 mM) and 4 seawater concentrations (0%, 20%, 30% and 40%).

The results show a large variability within the genotypes for salt tolerance. Genotype × Treatment interaction is significant for root length. The estimates of broad-sense heritability calculated for percentage of germination (PG), percentage of emergence (PE) and radicle length (RL) were quite high for the salt treatment compared with those for the control treatment. Moderate to high Broad sens heritability estimates were observed for the reduction percentage in both experiments. Discriminant analysis arranged the genotypes in two, clearly separate groups that differ in their tolerance to salinity stress.

Key words: Barley, germination, heritability,landraces, salt tolerance

INTRODUCTION

Soil salinity is a major obstacle to crop production in arid and semi-arid regions of the world. Large areas of arable land are subject to actual or potential salinization. One way to exploit the large areas of saline soils and the abundant saline water sources of the world is the improvement of salt tolerance in the cultivated plant species. Crop growth in salt-affected soils may be restricted by a reduction in available soil moisture, toxic effects, or salt-induced nutritional deficiencies.

Many consider barley to be the most drought and salinity tolerant of cereals (Belaid and Morris 1991; Ceccarelli *et al.* 1987). In the infavorable areas of Morocco, barley is mostly grown as landraces by subsistence farmers with no application of fertilizers, pesticides and herbicides. They allow them, in difficult conditions, to ensure a minimum yield of grains and straw. Barley grain is used for humain consumption, and animal feed. The existence of a large amount of genetic diversity in barley landraces have been already reported by many researchers (Lakew *et al.* 1995; Alemahehu and Parlevliet 1997; Attene *et al.*1996; Lakew *et al.* 1997; Sun *et al.* 1999 and Czembor 2000). Some examples of exploiting the genetic diversity of landraces in breeding pro-

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grams have been reported for barley, in Syria (Ceccarelli *et al.*, 1987; 1995) and in Ethiopia (Lakew *et al.* 1997) and for tetraploid wheat in Ethiopia (Tesemma *et al.* 1993).

The major objective of this present study was to examine the genotypic variation for salt tolerance of barley landraces collected in south of Morocco.

MATERIALS AND METHODS

The material for this study comprised: 20 landrace populations and 4 check varieties: Acsad 178 (V_1), Arig 8 (V_2), Rabat 071 (V_3) and Aanaceur (V_4). Table 1 lists the local landraces and their origin. Twenty samples were collected in different localities from south Morocco with the collaboration of the provincial direction of agriculture (DPA). The samples were collected from farms wich had produced their own seed for at least 10 years. Each sample consisted of 30 heads that were randomly taken from one field at maturity. The samples were multiplied and homogenized during the 1994/95 and 95/96 seasons in the experiment farm Melk Azhar of I.N.R.A (Belfaa).

Table 1

List of local landraces used in the experiment and their origin

Local landraces :	Origin	Elevation above sea level	Coordinates
Ab1, Ab2, Ab3, Ab4	Ait baha	550 m	30°05' N, 9°33' W
Ig1, Ig2, Ig3, Ig4	Ighrem	1800 m	30°06'N, 8°27' W
Im1, Im2, Im3, Im4	Immouzer	1200 m	30°40' N, 9°29' W
Td1, Td2, Td3, Td4	Taroudant	235 m	30°28' N, 8°52' W
Tz1, Tz2, Tz3, Tz4	Tiznit	244 m	29°41' N, 9°43' W

Germination experiment

Germination tests were carried out at four NaCl concentrations (0, 100 mM, 150 mM, 200 mM) and at four concentrations of seawater (0,10, 20, 40%).

Seeds of each genotype were surface sterilized with 5% sodium hypochlorite solution for 10 min, rinsed with sterile distilled water several times and placed on Whatman's Grade 182 filter in 50 mm diameter Petri dishes. Each Petri dish (one replication) contained 30 seeds. Petri dishes were arranged in a completely randomized design with four replicates of each salt treatment. 5 ml of the solution treatment were added on alternate day to each Petri dish. Seeds were germinated in an incubator at 25°C. Every day the germinated and emerged seeds were counted. The percentage of germination (PG), the percentage of emergence (PE) and radicle length (RL) of each genotype and treatment was calculated on day 7. Radicle length was mesured from 10 seedlings randomly chosen of each Petri dish.

$$RPG\ or\ RPE = \left(1 - \frac{N_X}{N_C}\right) \times 100$$

The reduction percentage of germination (RPG) and the reduction percentage of emergence (RPE) were calculated according to the following formula:

N_X is the number of germinated (or emerged) seeds under salt treatment, and N_C is the number of germinated (or emerged) seeds in control.

$$RPL = \left(1 - \frac{\lambda_x}{\lambda_c}\right) \times 100$$

For each replicate, the reduction percentage of radicle length (RPL) was calculated, according to the following formula:

Where λ_x and λ_c are the mean values of radicle length recorded in the stressed and control treatment, respectively.

Data analysis and estimation of broad-sense heritability

All ratios were arcsine transformed and analysed in a one-way and two-way analysis of variance. The estimates of broad-sense heritability were based on between and within genotype variances Falconer (1981) and Gallais (1989). Between genotype variances comprised genetic ($V_{G)}$ and environmental component ($V_{E)}$ and within genotype variances comprised environmental component ($V_{E)}$ only, because

$$h = \frac{V_G}{\left(V_G + V_E\right)}$$

barley is largely self-pollinated. The broad-sense heritability (h) was estimated according to the following formula:

Phenotypic and genotypic correlation coefficients were computed from the variance and covariance components. For each treatment and for each experiment, the genotypes were classified in growing order from the best to the worst according to their percentage of reduction for each variable. Agglomerate hierarchical cluster analysis using Euclidean distance was employed in order to assort genotypes according to their salt tolerance. The discriminant function analysis (DFA) was used in order to test the validity of genotypes separation in different groups.

RESULTS AND DISCUTION

The two-way analysis of variance for the percentage of reduction (Table 2), allowed to put in evidence a highly significant difference between genotypes and between treatments in both experiment with NaCl and with sea water. The interaction genotype x treatement is highly significant for the reduction percentage of the radicle length (RPL), significant for the the reduction percentage of emergence in the experiment with NaCl, but it is not significant for the other analysed variables.

Separate analysis of variance of the data for percentage of germination, percentage of emergence and radicle length (Table 3) clearly show that differences among the genotypes were highly significant for both the variables under different salt treatments. Broad-sens heritability estimates for all variables at the control were

relatively low compared with those for the salt treatments. The h varied in the control from 0.28 and 0.49 recorded for the percentage of emergence (PE) in the experiment with sea water and radicle length (RL) in the experiment with NaCl, respectively. For the salt treatments, the lowest value of h was recorded for the percentage of emergence (PE) in the experiment with sea water at 100 mM, and the

Table 2

Analysis of vaiance: F values and significance levels of each variable

Source of variation	DF	R.P.G[1]	R.P.E[1]	R.P.L[1]	R.P.G[2]	R.P.E[2]	R.P.L[2]
Genotypes	23	18.51***	3.46**	9.63***	10.32***	13.45***	17.40***
Treatement	3	27.56***	57.12***	81.30***	39.45***	32.36***	73.90***
Interaction (G x T)	69	1.56	2.91*	5.23***	1.82	1.97	3.89**

*, ** , *** : significant at $P_{0.05}$, $P_{0.01}$ and $P_{0.001}$ levels, respectively; [1] — Experiment with NaCl; [2] — Experiment with sea water

Table 3

Variance components, and broad-sense heritability estimates for percent germination (PG), percent emergence (PE) and radicle lenght (RL) under different NaCl and Sea water concentrations

	NaCl concentrations											
F-Values (genotypes)	1	2	3	4	1	2	3	4	1	2	3	4
	PG				PE				RL			
Mean	3.45*	11.84***	8.91***	4.85**	2.84*	4.56**	9.84***	7.31***	4.69**	12.06***	16.22***	10.62***
Vg	0.06	0.20	0.53	0.11	0.07	0.13	0.17	0.09	0.20	1.02	2.01	0.88
Vp = Vg + Ve	0.13	0.26	0.80	0.20	0.19	0.23	0.24	0.13	0.41	1.92	2.71	1.23
h = Vg / Vp	0.46	0.77	0.66	0.58	0.37	0.56	0.70	0.69	0.49	0.53	0.74	0.71
	Sea water concentrations											
F-Values (genotypes)	1	2	3	4	1	2	3	4	1	2	3	4
	PG				PE				RL			
Mean	3.36	6.44	8.50	5.65	2.70	4.74	4.66	7.91	4.20	14.89	12.34	5.41
Vg	0.12	0.133	0.05	0.11	0.14	0.26	0.13	0.97	0.42	0.921	0.41	0.23
Vp = Vg + Ve	0.29	0.21	0.08	0.19	0.50	0.52	0.23	1.52	0.96	1.35	1.90	0.42
h = Vg / Vp	0.41	0.62	0.63	0.57	0.28	0.50	0.57	0.64	0.44	0.68	0.74	0.55

Experiment with:
NaCl: 1 — control, 2 — 100 mM, 3 — 150 mM 4 — 200 mM
sea water: 1 — control, 2 — 20 %, 3 — 30 %, 4 — 40 %

highest value was recorded for the percentage of germination (PG) in the experiment with NaCl at 100 mM, 0.50 and 0.77, respectively.

In Table 4, the estimates of broad-sens heritability for the percentages of reduction are reported for each treatment. The estimates of broad-sense heritability were moderate to high for both treatments and ranged between 0.46 and 0.78 for the reduction percentage of germination (RPG), between 0.38 and 0.81 for the reduction percentage of emergence (RPE) and between 0.39 and 0.84 for the reduction percentage of radicle length (RPL). Phenotypic and genotypic correlation coefficients among variables in experiment with NaCl and experiment with sea water are shown in Table 5. In most cases the magnitude of phenotypic and genotypic correlations was nearly the same. This indicates the minimal influence of environment on the re-

lationships. A highly significant and positive correlation coefficients were found between RPG and RPE compared with those observed between RPG and RPL and

Table 4

Broad-sense heritability estimates for the percentage of reduction

Salt treatment	R.P.G.	R.P.E.	R.P.L.
100 mM	0.46	0.38	0.39
150 mM	0.78	0.56	0.43
200 mM	0.76	0.78	0.84
20%	0.48	0.38	0.34
30%	0.62	0.81	0.68
4 %	0.63	0.69	0.54

Table 5

Phenotypic (r_p) and genotypic (r_g) correlation coefficients among different salt treatments for the percentage of reduction

Salt treatment	Phenotypic (rp) and genotypic (rg) correlation coefficients	R.P.G. and R.P.E.	R.P.G. and R.P.E.	R.P.G. and R.P.E.
100 mM	rp	0.91	0.12	0.23
	rg	0.83	0.07	0.08
150 mM	rp	0.66	0.37	0.31
	rg	0.54	0.33	0.20
200 mM	rp	0.83	0.90	0.77
	rg	0.70	0.64	0.60
20 %	rp	0.82	-0.04	0.12
	rg	0.72	-0.06	0.09
30 %	rp	0.48	0.17	0.52
	rg	0.43	-0.23	0.37
40 %	rp	0.52	0.09	0.71
	rg	0.50	0.06	0.56

between RPE and RPL.

Table 6 shows the mean of the percentage of reduction and genotypic ranking order of different measurements, where genotypes are classified in growing order according to their percentage of reduction from the best to the worst during the various experiments. The ranking order of the 24 genotypes was different; the ranking order of V4 was 19th in $R.P.G^1$ and 11th in $R.P.E^1$. The ranking order of the barley landrace Ig1 was the 2nd in $R.P.G^1$ and $R.P.E^2$, and the 4th in $R.P.L^1$ and $R.P.L^2$. The mean rank ranged from 3.50 in Ig1 to 21.16 in Im2.

Hierarchical cluster analysis assorted the genotypes into two main groups according to their salt tolerance (Fig. 1). The first group included 9 genotypes and the second group included 15 genotypes. The discriminant function analysis (DFA) was used in order to test the validity of this separation. The discriminant analysis is higly significant, the value of F (Pseudo F) equal to 15.33, the squared Mahalanobis

distance between groups is equal to 23.39 and the percentage of well classified equal to 100%. The canonical scores (table 6) ranged from 1.48 for V4 to 3.89 for Td1 in the group I and from 0.41 to -3.67 for Td2 and Im2 in group II respectively. The mean of the reduction percentage (Table 7) are smaller in group I for all variables compared with those observed in group II. This leads to the conclusion that

Table 6

Mean of the percentage of reduction, ranking order and canonical scores of the 24 barley genotypes

Group	R.P.G.^{1}	R.P.G.^{2}	R.P.E.^{1}	R.P.E.^{2}	R.P.L.^{1}	R.P.L.^{2}	Mean rank	Canonical scores
Ab1 I	25.45	33.412	50.214	53.9315	17.93	32.69	9.66	2.24
V4 I	43.619	38.616	46.711	49.112	25.38	34.311	12.83	1.48
Td3 II	31.78	44.720	62.121	59.319	36.413	38.112	15.50	-0.53
Tz1 I	36.210	32.19	46.310	44.76	22.57	20.33	7.50	3.06
Ab2 I	38.114	31.78	48.913	45.27	26.39	29.27	9.66	2.17
Ig1 I	14.82	29.36	38.43	36.82	18.34	21.74	3.50	3.61
Ig2 I	25.24	19.82	35.21	41.34	20.45	25.66	3.66	3.42
Tz4 II	45.620	38.315	53.716	56.117	34.711	43.315	15.66	-0.67
V1 II	33.49	36.714	43.28	48.711	51.320	49.719	13.50	-2.16
Tz2 II	39.416	41.219	67.424	62.823	48.418	45.216	19.33	-2.06
Im2 II	50.422	48.523	56.118	59.721	51.319	62.424	21.16	-3.67
Td1 I	29.56	26.95	39.95	48.310	17.42	13.81	4.83	3.89
Td2 II	36.812	29.97	42.37	45.79	35.612	41.713	10	0.41
Im1 II	56.724	40.318	57.619	62.924	37.215	57.422	20.33	-1.62
Ab3 II	41.717	39.117	53.415	49.613	54.122	51.820	17.33	-2.50
V2 II	42.818	47.322	65.223	54.116	60.324	48.317	20	-3.33
Ig3 II	36.411	33.813	44.99	45.38	52.321	43.414	12.66	-1.52
Ig4 II	37.313	32.411	62.722	58.518	42.917	59.323	17.33	-1.84
Im4 II	48.321	44.921	48.612	51.114	36.914	48.718	16.66	-0.91
Tz3 II	38.415	32.310	54.517	59.420	57.223	51.921	17.66	-3.00
Im3 I	14.93	18.91	42.16	36.41	22.36	31.78	4.16	3.14
V3 II	52.623	51.124	57.720	60.722	42.716	33.910	19.16	-1.17
Ab4 I	13.61	25.74	35.42	38.33	28.410	15.22	3.66	3.01
Td4 II	14.93	35.610	68.82	17.43	63.29	46.615	7	-1.42

Table 7

Mean of the percentage of reduction in Gr. I and Gr. II for different variables

	R.P.G.^{1}	R.P.G.^{2}	R.P.E.^{1}	R.P.E.^{2}	R.P.L.^{1}	R.P.L.^{2}
Gr I	26.81	28.48	42.56	43.77	22.08	24.93
Gr II	40.42	39.74	55.88	53.35	46.96	48.11

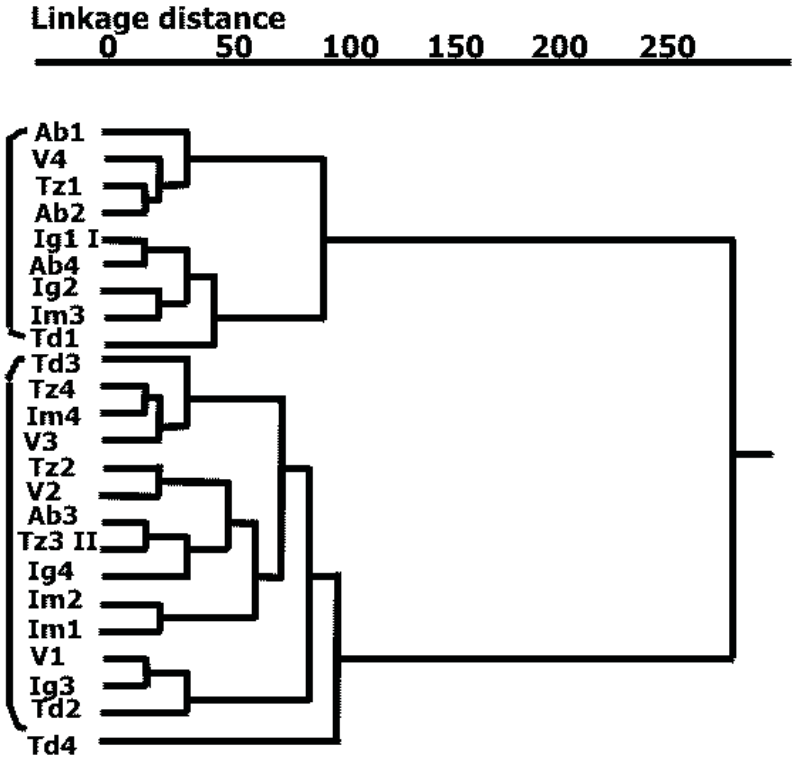

Fig 1. Dendogram of cluster analysis of 24 barley genotypes classified according of six variables

genotypes belonging to group I performed better than genotypes from group II. They are characterised by low percentage of reduction under salt stress conditions.

All these results revealed the existence of a genetic variability for the salinity tolerance and suggest that variability in tolerance to salinity stress exist among Moroccan barley landraces and there is a need to screen a large number of landraces to identify genotypes with a high degree of tolerance to salt. Numerous researchers indicated the presence of a large variability for the tolerance of the salinity (Allen *et al.* 1986; Norlyn and Epstein, 1984 et Ashraf 1993). The use of this variability for improvement of salinity tolerance, can be achieved only with a good knowledge of the physiology and the mechanisms allowing the plant a better tolerance to the salt. A superiority of landraces over modern cultivars for root growth in early stages has been observed in wheat (Jaradat and Duwayri, 1981) and barley (Khaldoun *et al.*, 1990). Grando and Ceccarelli (1995) reported that barley landraces have more vigorous seminal root system than modern cultivars. Some Ethiopian barley landraces and Syrian selection were found to be most drought resistant by maintenance of a relatively high photosynthetic activity of the uppemost leaves (Gorny, 2001). Tichedrett, a landrace genotype with a very extensive root development, exhibited a capacity to maintain its photosynthetic activity under water stress (Arnau and al. 1997).

It is known that salt tolerance varies with the stage of plant growth, as has been observed in a number of study (Kurt *et al.*, 1986; Maas *et al.*, 1983; Botia *et al.*, 1998; Komori *et al.*, 2000). Nonetheless, information regarding genetic variation and heritability at germination is important for screening the most promissing genotypes for inclusion in barley-breeding programme aimed at developing varieties with a high level of salinity tolerance.

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