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Effects of Salinity on Germination and Early Growth of Barley (*Hordeum vulgare* L.) Cultivars

SAID EL MADIDI[1], BRAHIM EL BAROUDI AND FOUZIA BANI AAMEUR
Laboratoire de Recherche sur la Variabilité Génétique, Department of Biology, Faculty of Science, Agadir, Morocco
[1]Corresponding author's e-mail: elmadidi@hotmail.com

ABSTRACT

This study was conducted to determine the effects of salinity on seed germination and early vegetative growth of nine genotypes of barley (5 landraces & 4 breeding lines). The genotypes were evaluated by several criteria, at four salt concentrations (0, 100, 150 & 200 mM) and four seawater concentrations (0, 20, 30 & 40%). The results revealed a large variability within the genotypes for salt tolerance at the two early growth stages. Genotype x treatment interaction was significant for root length and highly significant for the dry weight of roots. The levels of salt tolerance in some barley landraces were higher than those found in breeding lines, particularely for seed germination. Our results indicate that there was no relationship between salt tolerance during seed germination and during early vegetative growth.

Key Words: Barley; Salt tolerance; Landraces; Germination; Early vegetative growth

INTRODUCTION

In arid and semi-arid regions of the world, excess salts in agricultural land can limit crop production. In North Africa, large areas of arable land are subjected to actual or potential salinization. One way to exploit the large areas of saline soils and the saline water sources is the improvement of salt tolerance in the cultivated plant species.

In view of many researchers, barley is considered to be the most drought and salinity tolerant among cereals (Ceccarelli *et al.,* 1987; Belaid & Morris, 1991). In the infavorable areas of Morocco, barley is mostly grown as landraces by subsistence farmers without application of fertilizers, pesticides and herbicides. Barley grain is used for human 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 & Parlevliet, 1997; Attete *et al.,* 1996; Lakew *et al.,* 1997; Sun *et al.,* 1999; Czembor, 2000; Dakir *et al.,* 2002). Some examples of exploiting the genetic diversity of landraces in breeding programs 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 determine salt tolerance in some barley cultivars at two early growth stages.

MATERIALS AND METHODS

The material for this study comprised of 5 landraces populations and 4 modern bred barley cultivars i.e. Arig 8 (V_1), Acsad 178 (V_2), Rabat 071 (V_3) et Laânaceur (V_4). Barley landraces were collected in different localities from South Morocco with the collaboration of the provincial direction of agriculture (DPA). The samples were collected from farms which had produced their own seed for the 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 two years in an experimental farm Melk Azhar of I.N.R.A Belfaa (Latitude 30°6'N, Longitude 9°36'W, Latitude 75 m above sea level). Table I lists the local landraces and their origin.

Table I. List of local landraces used in the experiment and their origin

Landrace	Origin	Elevation	Coordinates
Ld_1	Ait baha	550 m	30°05' N, 9°33' W
Ld_2	Ighrem	1800 m	30°06'N, 8°27' W
Ld_3	Imouzzer	1200 m	30°40' N, 9°29' W
Ld_4	Taroudant	235 m	30°28' N, 8°52' W
Ld_5	Tiznit	244 m	29°41' N, 9°43' W

Germination tests were carried out at four NaCl concentrations (0, 100, 150, 200 mM) and at four concentrations of seawater (0, 10, 20, 40%). Firstly, 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 paper in 90 mm diameter Petri dishes (30 seeds per Petri dish). In each Petri dish, 5 mL of the apporopriate solution were added on alternate day. Seeds were germinated in an incubator at 25°C, and all the dishes were arranged in a completely randomized design with four replicates of each salt treatment. 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 of each Petri dish. The reduction percentage of germination (RPG) and reduction percentage of emergence (RPE) were calculated according to the following formula

$$RPG \text{ (or } RPE) = \{1 - (N_X / N_C)\} \times 100$$

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

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

$$RPL = \{1-(\ell_x/\ell_c)\} \times 100$$

Where ℓ_x and ℓ_c are the mean values of radicle length recorded in the stressed and in unstressed treatment, respectively.

To determine the effects of salinity on seedling growth, seeds of the nine genotypes were germinated in distilled water and placed in an incubator at 25°C. After 2 days of emergence, the seedlings were transplanted into plastic pots (six seedlings per pot) containing sand. The Hoagland nutrient solution with macro-nutrients (5 KNO_3, 5 $Ca(NO_3)_2$, 2 Mg SO_4, 1 KH_2 PO_4) (mmol/L) and the following micro-nutrients (50 Fe-EDTA, 25 H_3BO_3, 2 $MnSO_4$, 2 $ZnSO_4$ et 2 $CuSO_4$) (µmol/L) was provided to each pot and renewed after 5 days interval. The seedling were watered daily, at a rate of 100 mL per pot. Salinity treatments were imposed by adding NaCl to the Hoagland nutrient solution. NaCl treatments (150 & 200 mM), were introduced to the nutrient solutions in gradual daily increments (50 mM every 24 h) after the plants were 6 days old. To avoid increase in salt concentration in the soil, all plants were watered with tap water, one week after irrigation with saline water. Pots were arranged in a green-house at 26±2°C day temperature and 18±1°C night temperature. A split-plot design with 3 replicates was adopted, with salinity treatments in main-plots and the genotypes in sub-plots. After 45 days of sowing, shoots and roots were separated, dried at 80°C in an oven for 48 h and dry weight were recorded. Samples of roots and shoots were taken in order to determine dry weight. Three plants constituted one sample and three samples per treatment were taken.

The reduction percentage of dry weights (roots & shoots) was calculated, according to the following formula :

$$RPD = (1 – (Y/X)) \times 100$$

Where Y and X are the mean values of dry weights recorded in the stressed and in unstressed treatment, respectively.

All ratios were arcsine transformed and analysed in a two-way and a split-plot analysis of variance. Differences between the means were compared using the Newman-Keuls Test. Relationships among variables were determined using Spearman's correlation test. All statistical procedures were carried out using the SAS computer programme.

RESULTS AND DISCUSSION

The results obtained in all genotypes (Table II), show that the percentage of reduction for all variables increase with the increase in concentration of the salt. The average percentages of reduction was variable with the variable measured and the stage of development. The most important percentages of reduction were recorded at the stage of germination by comparison to those obtained at the early growth stage. This agrees with the results put forward by other studies, and which showed that the stage of germination is the most affected stage of developement by salt stress (Baldwin *et al.,* 1996; Katembe, 1998).

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

There was a highly significant difference between the various concentrations of NaCl and between genotypes, for the reduction of the dry weight of roots, as well as for the dry weight of shoots (Table IV). The interaction was significant for the reduction percentage of dry weight of roots.

The lowest reduction percentage of germination (R.P.G) was recorded in Ld4 (12,59%) witch was statistically at par with that of Ld3 and Ld5 while highest R.P.G was recorded in V1 (50,59%) under NaCl saline conditions (Table IV). During experiment with sea water, lowest R.P.G. was observed in Ld4 while highest R.P.G was observed in V1 which statistically at par with that of génotypes V2, V3, Ld1 and Ld2. For the reduction percentage of emergence, the genotype Ld4 had the lowest percentage of reduction and V3 had the highest percentage of reduction. For the reduction percentage of the radicle length, Ld1 was the most tolerant genotype, and V3 and V1 were the least tolerant genotypes in experiment with NaCl and experiment with sea water respectively. The reduction percentage of dry weight of roots ranged from 10, 82% recorded in Ld2 to 59, 14% recorded in Ld1. The data regarding dry weight of shoots, Ld2 had also the lowest percentage of reduction which was statistically similar to all the genotypes except Ld4 while Ld4 had the highest percentage of reduction which was statistically similar with the genotypes (Ld1, Ld5, V1 and V2). In order to appreciate the performence of different genotypes to the salinity tolerance, we classified the genotypes according to their percentage of reduction for every studied variable.

All these results 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

Table II. Mean values of the percentages of reductions

NaCl	R.P.G	R.P.E	R.P.L	R.P.D Shoots	R.P.D Roots	Sea water	R.P.G.	R.P.E	R.P.L
100 mM	18.20	25.50	27.60	-	-	20 %	13.90	18.20	28.10
150 mM	23.20	54.10	41.50	11.30	25.82	30 %	37.10	59.40	45.10
200 mM	45.50	86.50	59.50	28.52	44.60	40 %	62.20	88.80	62.80

R.P.G : Reduction percentage of germination; R.P.E : Reduction percentage of emergence; R.P.L : Reduction percentage of radicle length; R.P.D : Reduction percentage of dry weight

Table III. Results of the two-way analysis of variance

		NaCl concentrations			Sea water Concentrations		
Source of variation	**d.f**	**R.P.G**	**R.P.E**	**R.P.L**	**R.P.G**	**R.P.E**	**R.P.L**
Genotypes	8	22.11***	5.69***	10.58***	10.02***	14.35***	23.59***
Treatments	3	70.15***	145.02***	209.33***	13.9.29***	322.84***	374.39***
Interaction :(Gent. x Treat.)	24	1.30N.S	0.61N.S	4.82***	1.58N.S	1.75N.S	3.41**

N.S : not significant ($p > 0.05$), ** : ($p < 0.01$), *** : ($p < 0.001$)

Table IV. Results of the split-plot analysis of variance

Source of variation	d.f	F (R.P.D.) Shoots	F (R.P.D) Roots
Main-plots :			
Treatements	2	22.5***	12.20***
Error (a)	4		
Sub-plots :			
Genotypes	8	7.84***	3.42**
Interaction (g x t)	16	1.05 ^{NS}	1.92*
Error (b)	48		
Total	80		

N.S : not significant ($p > 0.05$), * : ($p < 0.05$), ** : ($p < 0.01$), *** : ($p < 0.001$).

identify genotypes with a high degree of tolerance to salt. Numerous researchers indicated a large variability for salinity tolerance in crop plants (Norlyn & Epstein, 1984; Allen *et al.,* 1986; Ashraf, 1993). The use of this variability for improvement of salinity tolerance, can be achieved only with a good knowledge of the physiological mechanisms allowing the plant a better tolerance to the salt.

Table VI shows genotypic ranking order of different measurements, where genotypes are classified in growing order from the best to the worst during various experiments. The ranking order was different from one variable to another. The ranking order of the barley landrace Ld4 was the 1st in R.P.G and in R.P.E, 3th and 4th in P.R.L, 4th in R.P.D of roots and 9th in R.P.D. of shoots. In the germination experiment, the varieties V1 and V3 were ranked highest while the barley landraces Ld4, Ld3 and Ld5 were ranked lowest.

The rank correlation coefficients between the classifications of genotypes are presented in Table VII. One notices the existence of highly significant correlation between the germination and the emergence, so much for

Table V. The results of means comparison of the percentage of reduction

Genotype	R.P.G[1]	R.P.G[2]	R.P.E[1]	R.P.E[2]	R.P.L[1]	R.P.L[2]	R.P.D.r	R.P.D.s
V1	50.59 f	52.31 d	59.29 bc	71.60 de	48.52 c	54.82 f	36.62 abc	29.28 ab
V2	25.43 bc	39.40 bcd	62.03 bc	62.89 cd	38.90 ab	44.62 bcd	51.42 bc	24.82 ab
V3	45.36 ef	48.15 cd	73.42 c	77.77 f	56.34 d	53.59 ef	35.47 abc	13.81 a
V4	33.83 cd	36.78 bc	57.11 bc	48.31 ab	44.59 bc	49.64 def	17.78 ab	11.13 a
Ld1	26.21 bc	41.62 bcd	57.24 bc	46.92 ab	36.73 a	29.97 a	59.14 c	17.09 ab
Ld2	38.02 de	45.14 cd	53.59 b	53.06 bc	41.97 abc	41.55 b	10.82 a	10.10 a
Ld3	14.98 ab	29.55 b	46.67 ab	53.35 bc	35.03 a	48.52 cde	17.45 ab	10.24 a
Ld4	12.59 a	15.56 a	35.43 a	38.48 a	37.60 ab	43.41 bc	22.64 ab	34.92 b
Ld5	14.84 ab	30.63 b	53.93 b	44.91 ab	42.16 abc	41.55 b	57.72 c	27.81 ab

Within column, the means with the same letter are not significantly different (Newman-Keuls test at level of 0.01 P) 1 : Experiment with NaCl and 2 : Experiment with sea water

Table VI. Classification of genotypes in growing order according to their percentage of reduction

	R.P.G[1]	R.P.G[2]	R.P.E[1]	R.P.G[2]	R.P.L[1]	R.P.L[2]	R.P.D Roots	R.P.D Shoots
V1	9	9	7	8	8	9	6	8
V2	4	5	8	7	4	5	7	6
V3	8	8	9	9	9	8	5	4
V4	6	4	5	4	7	7	3	3
Ld1	5	6	6	3	2	1	9	5
Ld2	7	7	3	5	5	2	1	1
Ld3	3	2	2	6	1	6	2	2
Ld4	1	1	1	1	3	4	4	9
Ld5	2	3	4	2	6	3	8	7

[1] Experiment with NaCl; [2]Experiment with sea water

Table VII. Rank Correlation coefficients between the classification of genotypes

Variable	R.P.G[1]	R.P.G[2]	R.P.E[1]	R.P.E[2]	R.P.L[1]	R.P.L[2]	R.P.D.s	R.P.D.r
R.P.G[1]	1	0.93**	0.63	0.73*	0.73*	0.48	-0.30	-0.12
R.P.G[2]		1	0.73*	0.70*	0.62	0.30	-0.13	0.15
R.P.E[1]			1	0.71*	0.58	0.42	0.07	0.50
R.P.E[2]				1	0.47	0.68*	-0.27	-0.12
R.P.L[1]					1	0.60	0.10	0.03
R.P.L[2]						1	0.10	-0.20
R.P.D.s								0.60
R.P.D.r								1

[1] Experiment with NaCl; [2]Experiment with sea water

the treatment with NaCl, as for that with sea water. On the other hand, negative correlations was observed among classification of the genotypes for the three criteria at the stage of germination and the classication of genotypes at the early growth stage. This seems to be indicate that there was no relation between the classification of the various genotypes for the salinity tolerance at the stage of germination with the classification at the early growth stage. This confirms the results put forward 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 developement (Shannon, 1985; Mano & Takeda, 1997). On the other hand, it is known that the inhibition of the germination and the emergence by salinity is mainly due to an osmotic effect (Allen *et al.,* 1986; Houle *et al.,* 2001), while during the growth, the salinity inhibts especially the absorption and the transport of the major elements, which limits the supply of the plant in essential elements for its growth (Lynch & Läuchi, 1985; Soltani *et al.,* 1990).

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