

Salinity tolerance of hull-less barley genotypes in germination stage

Saeed Aharizad¹, Mohammad Zaefizadeh¹, Mehdi Mehdipour¹

1. Department of Agronomy and Plant Breeding, Ardabil branch, Islamic Azad University, Ardabil, Iran.

***Corresponding author:** Mohammad Zaefizadeh

ABSTRACT: The germination experiments were conducted to evaluate salinity (sodium chloride) tolerance of 20 hulls-less barley genotypes in Ardabil Islamic Azad University water and soil laboratory in 2009-2010. The germination response of genotypes to three levels of salinity (1, as control, 7 and 12 des/m) was studied in this experiment using a factorial completely random design in, Petri-dishes, considering ISTA principles in 3 replications. Statistic analysis revealed that there were significant differences among complete of traits. This shows that there is genetic variation among the genotypes of the trails studied. Results obtained about the measurements of germination related traits and also results of cluster and environmental sensitivity analysis that genotypes: 28618, 28623, 28631, 28631, 28640, 28682, 28686 and 28687 are more saline tolerate that the other genotypes studied.

Keywords: Ardabil, hull-less barley, germination, salinity

INTRODUCTION

Barley is one of the most cereals widely cultivated in the world. It has high concentration of starch, protein, vitamin and other important nutritive compounds. The utilization of barley in poultry feeds is limited because of low energy value as well as high concentration of non Starch Polysaccharides (NSPs) and phytate (the important phosphorus stock resource in plants). glucans are the major NSP in barley cause reduction in protein and carbohydrate digestibility by increasing intestinal viscosity and also by reducing contact of enzymes and substrate (Annison, 1993; Fuente et al., 1998).

Abiotic stresses including drought and salinity are currently the major factors which reduce crop productivity worldwide. Excessive amounts of salts in the soil severely reduced the seed germination and further seedling growth and this has been well documented in the literature (Ashraf, 2004; Munns, 2005; munns, 2002). This has been ascribed due to salt-induced osmotic stress or due to its toxic effects or combination of both of these (Munns, 2005; munns, 2002).

More than 900 million hectares of land world-wide, approx. 20 % of the total agricultural land (FAO, 2007), are affected by salt, accounting for more than 6% of the world's total land area. NaCl is the predominant salt causing salinization, and it is unsurprising that plants have evolved mechanisms to regulate its accumulation (Munns and Tester, 2008). Seed germination is an important and vulnerable stage in the life cycle of terrestrial angiosperms and determines seedling establishment and plant growth. Despite the importance of seed germination under salt stress (Ungar, 1995), the mechanism (s) of salt tolerance in seeds is relatively poorly understood, especially when compared with the amount of information currently available about salt tolerance physiology and biochemistry in vegetative plants (Hester *et al.*, 2001; Garthwaite *et al.*, 2005; Hu *et al.*, 2005; Ren *et al.*, 2005; Kanai *et al.*, 2007).

Cellular NaCl concentration is a function of the fluxes into, and out of, the cell. Glycophytic plants tend to have limited regulation of NaCl entry, whereas halophytes typically have good regulation with ion-gated channels regulating the entry of NaCl into cells (Glenn *et al.*, 1999). Export of Na⁺ from the cell requires specialized plasma-membrane Na⁺/H⁺ antiporters (Horie and Schroeder, 2004). Some halophytic species accumulate Na⁺ and Cl⁻ in the vacuole, mediated by tonoplast Na⁺/H⁺ anti-porters, allowing the cytoplasm to be maintained at substantially lower ion concentrations while avoiding metabolic inhibition (Serrano and Gaxiola, 1994). Over-expression of tonoplast Na⁺/H⁺ antiporter genes (NHX genes), or the proton pumps (ATPase) supplying them with energy, has been shown to increase the salt tolerance of the very glycophytic *Arabidopsis* (Gaxiola *et al.*, 2001). However, all these mechanisms of salt tolerance – excretion, osmotic adjustment using organic solutes and compartmentalization of Na⁺ – are metabolically energetic and compete with plant growth for resources (i.e. sugars). Thus, if these mechanisms, elucidated in vegetative plant cells, also operate within the cells of seeds germinating in saline environments, it suggests conflicting demands for

carbon reserves, both osmotic balance and growth, the balance between which must determine the likelihood of successful germination.

Many investigators have reported retardation of germination and growth of seedlings at high salinity (Bernstein, 1961). However plant species differ in their sensitivity or tolerance to salts (Torech and Thompson, 1993). Wheat is a major staple food crop for more than one third of the world population and is the main staple food of Asia (Shirazi *et al.*, 2001). It is originated in South Western Asia and has been a major agricultural commodity since pre historic times. The total production area in Pakistan is 8.2 mha and the average yield is 2170 kg/hectare (Anonymous, 1999). The wheat crop is mainly cultivated under rain fed conditions where precipitation is less than 900 mm annually. Wheat is grown both as spring and winter crop. Winter crop is more extensively grown than spring. The possible cause of varietal difference most likely evolves ion transport properties and cellular compartmentation (Munns., 1988). Schachtmann and Munns (1992), reported that sodium exclusion was a general characteristic of salt tolerance in wheat lines; where as, salt tolerant display much higher shoot sodium level than sensitive lines. Few studies have been carried out on the relative salt tolerance of various cultivars of agricultural crops of Pakistan (Mer *et al.*, 2002). The screening of salt tolerant lines/cultivars has been attempted by many researchers on various species at seedling growth stage (Ashraf, 1999). The relation of various seedling growth parameters to seed yield and yield component under saline conditions are important for the development of salt tolerant cultivar for production under saline conditions.

MATERIAL AND METHODS

This study was carried out with Seeds of 20 barley genotypes (Twelve six-row genotypes and eight two-row genotypes). The genotypes profiles and line numbers are in table 1.

Factorial experiment used on the basis of completely randomized design in the three replications. Salinity effects on germination stage by applying three salinity levels (1, 7 and 12 ds/m) were evaluated. Salt solutions for the preparation of sodium chloride salt were used. The PH and EC of solutions are in table 2.

All the experiments were conducted in 9 cm Petri plate on filter paper beds in growth chambers. 20 seeds were sown in 9 cm diameter Petri plate on filter paper beds, irrigated with 5 ml solution of respective treatment and incubated at 25°C. Each treatment was replicated thrice. The filter paper beds were irrigated daily with 5 ml solution of the respective treatment. The filter beds were changed after 48 hours in order to avoid salt accumulation. Final germination percent measured after 8 days with ISTA standards (ISTA, 1993) and the following traits were measured:

Final germination percent, Germination rate, Number of days until germination, Seminal root lengths, Coleoptiles length and Coleoptiles length/ seminal root lengths.

Table 1. Genotypes profiles and line numbers

No.	Line	Profile	Rows
1	28609	CI10590/CEDRO//OLMO/3/CHINA/4/LINO	SIX
2	18618	MOLA/ALELI//MORA/3/CONDOR-BAR/4/CORI/...	TWO
3	18623	MOLA/SHYRI//ARUPO*2/JET/3/CONDOR-BAR/...	TWO
4	28629	ZARZA/BEREMEJO/4DS4931//GLORIA-BAR/...	SIX
5	28631	RABANO/4/DS4931//GLORIA-BAR/COPAL/3SEN...	SIX
6	28636	ELDO/BERMEJI/5/CM67-B/CENT ENO//CAM-B/3/...	SIX
7	28640	AMAPA/3/ROBUR-BAR/EGYPT20//GLORIA-BAR/4...	TWO
8	28644	MOLA/ALELI//MORA/3/CONDOR-BAR/4/CORI/...	SIX
9	28665	MORA/NB1054/3/MOLA/SHYRI//ARUP*/JET	TWO
10	28682	LION//ALISON/C13909.2/4/CEDRO//MATNAN/...	SIX
11	28686	REGENT-BAR/CONDOR-BAR/3/MOLA/SHYRI//...	TWO
12	28687	MOLA/BERMEJO//NISPERO/5/CM67-B/CENTENO/...	SIX
13	28698	RHODS//TB-B/CHZO/3/GLORIA-BAR/COPAL/4/...	SIX
14	28702	CERAJA/3/AGAVE/BERMEJO//HIGO	SIX
15	28709	MOLA/SHYRI//ARUPO*2/JET/ATACO/4/ALELI	TWO
16	28714	CHAN:B3/AYAROSA	SIX
17	28722	CERRAJA/3/ATACO/ACHIRA//HIGO	SIX
18	28730	MOLA/SHYRI//ARUPO*2//JET/3/ALELI/4/MOLA/...	TWO
19	28731	MOLA/SHYRI//ARUPO*2//JET/3/ALELI/4/MOLA/...	TWO
20	28735	LION/HIGO/4/CEDRO//MATNAN/EH165L/3/MATTCO	SIX

RESULTS

Analysis of variance

Results of analysis of variance given in Table 3. Based on this table between genotypes in all traits, significant differences are observed in 0.01 percent probability level, which indicated that genetic variation in tolerance to salinity is studied genotypes. The effect of salinity was significant in all traits in 0.01 percent probability level that show the impact of salinity on the genotypes, but the interaction in all traits except

germination rate characteristics and germination percent was not significant in these two traits in genotypes with increased salinity.

Mean Comparison

Results of mean Comparisons given in Table 4. In this table genotype 28623 in all traits was tolerant to salinity. Salinity levels Comparison for Seminal root lengths, Coleoptiles length and days until germination showed that with increasing salinity these traits were reduced That show adverse effects of salinity on these attributes. Salinity levels Comparison for Coleoptiles length/ seminal root lengths showed that increasing salinity, affected longitudinal growth of roots more than shoots longitudinal growth.

Table2. PH and EC of solutions

Salinity levels	PH	EC .ds/m)
control	7.15	1
Second level	7.16	7
Third level	7.12	12

Table3. Analysis of variance results in invitro

S.O.V	df	MS					
		Final germination percent	days until germination	Germination rate	Coleoptiles length/ seminal root lengths	Seminal root lengths	Coleoptiles length
Salinity	2	2.378**	2.734**	6.433**	.442**	38.894**	9.227**
Genotypes	19	1.574**	0.22**	1.595**	0.144**	5.743**	2.128**
S×G	38	0.381**	0.103 ^{ns}	0.358**	0.064 ^{ns}	1.911 ^{ns}	0.666 ^{ns}
Error	120	0.164	0.095	0.188	0.055	1.873	0.628
CV .%)	-	16.99	7.76	24.38	24.45	25.77	16.56

** And * significant at the 0.01 and 0.05 levels

Table 4. Results of mean Comparison

Line No.	Final germination percent .%)	days until germination .day)	Germination rate	Coleoptiles length/ seminal root lengths	Seminal root lengths .mm)	Coleoptiles length .mm)
28609	40	17.337	3.167	1.007	23.09	19.58
28618	70	16.672	4.481	0.644	43.83	25.42
28623	100	18.556	8.463	0.646	45.97	27.72
28629	40	15.633	2.426	0.681	25.56	18.59
28631	80	17.024	5.199	0.836	38.37	29.25
28636	60	15.901	3.556	0.770	31.76	23.88
28640	90	15.379	4.458	0.736	35.41	16.01
28644	40	14.866	2.009	1.058	18.69	14.28
28665	30	16.778	2.171	1.492	28.22	27.05
28682	70	16.567	4.366	0.743	37.57	25.17
28686	90	15.809	4.907	0.931	36.37	29.03
28687	90	15.612	4.796	0.710	38.62	21.19
28698	60	15.400	3.236	0.814	29.80	22.72
28702	60	14.520	2.708	0.928	31.17	23.94
28709	60	16.461	3.306	1.064	35.54	30.30
28714	50	14.693	2.444	0.979	25.91	22.94
28722	40	14.833	1.694	1.053	27.80	22.32
28730	60	15.765	3.551	1.137	20.76	21.29
28731	50	13.144	2.167	2.054	19.37	17.75
28735	40	16.798	2.676	1.284	23.66	22.36
LSD .5%)	10	0.776	0.513	0.219	4.83	2.22

Cluster analysis

20 genotypes cluster analysis based on ward method, divided genotypes in two Resistant and Sensitive groups that resistance group included 28618, 28623, 28631, 28640, 28682, 28686 and 28687 genotypes and these genotypes were completed with excellence in traits related to germination. Sensitive groups also were divided into two subgroups, that the genotypes 28609, 28665, 28636, 28698, 28730, 28709 and 28735 in resistance group and other genotypes were in Semi-sensitive group.

Environmental Sensitivity

As you know some of the interaction between genotype differences in salinity can be attributed to the sensitivity of different genotypes. With this explains the effect of a particular operating environment (salty environment) on some varieties more than others. Because the genotypes was too more, the Environmental Sensitivity was not calculated for each genotypes. About germination trait (Fig 2) Can be said that resistance group ($R^2=0.759$, $B=0.511$) and then semi-sensitive group ($R^2=0.982$, $B=1.106$) according to genotypes Value amounts were in better status and these results approved Cluster analysis and properly category. For final

germination percent (Fig 3) also with genotypic value, R^2 and Slope lines, is correct Confirmation of the grouping. So, can be said for germination stage resistance group were include genotypes 28618, 28623, 28631, 28640, 28682, 28686 and 28687 And set the genotypes as salinity resistant varieties to introduce development.

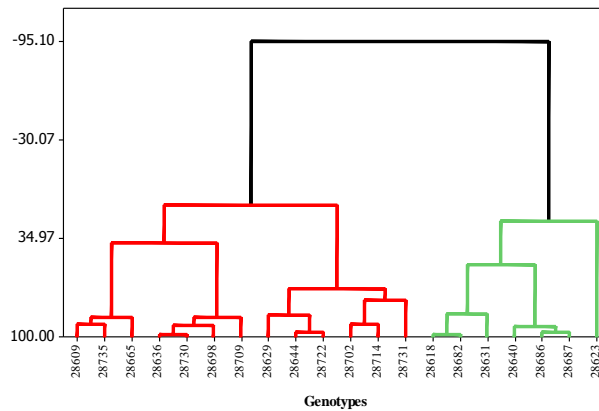


Fig1. Cluster analysis based on germinations traits

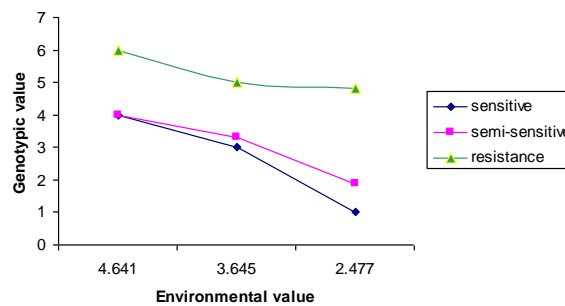


Figure 2. Environmental sensitivity of three barley genotypes for germination rate

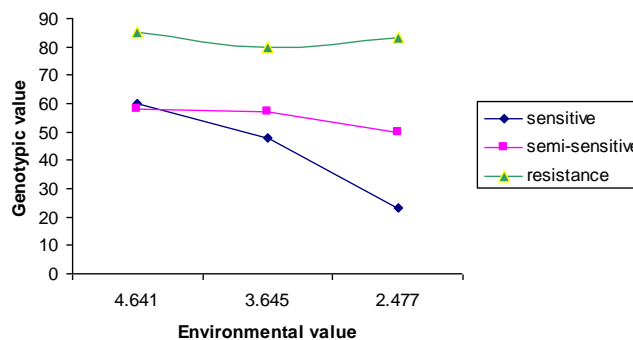


Figure 3. Environmental sensitivity of three barley genotypes for germination percent

DISCUSSION

Using salt as an osmoticum in saline environments appears to allow seeds to germinate more rapidly, and at lower osmotic potentials than they might otherwise be able to. This may have functional ecological effects, increasing these plants' ability to compete temporally with other species, germinating faster and shading seedlings which do not germinate as rapidly. Salt has been shown to be an order of magnitude metabolically 'cheaper' than sugars for generating osmotic potential in vegetative plants (Raven, 1985), and may be similarly used in seeds, provided toxicity issues can be resolved. In vegetative plant cells, toxicity is resolved by vacuolar compartmentalization (Qiu *et al.*, 2007), and recent reports suggest that Na^+ can be bound in starch granules (Kanai *et al.*, 2007). In seeds, however, this compartmentalization would be energy intensive,

while binding sodium to starch would prevent the starch from being used to provide energy for germination. Thus, it would seem metabolic tolerance to salt would be more important in seeds than at other life stages, due to their limited carbohydrate reserves.

In this study different tolerance to salinity variation in the germination stage was observed.

Test results showed that salinity levels are imposed their destructive effects on the roots of most plants and the air sector show response to salinity less than their.

Salinity was applied through the various levels of sodium chloride germination stage, causing a negative effect on the decline associated with the traits and characteristics of germination. As the genotypes 28618, 28623, 28631, 28640, 28682, 28686 and 28687 were in resistance group.

Water stress due to salinity and drought is probably the most significant abiotic factor limiting plant and also crop growth and development (Hartmann *et al.*, 2005). Drought stresses is physiologically related, because induce osmotic stress and most of the metabolic responses of the affected plants are similar to some extent (Djibril *et al.*, 2005). Water deficit affects the germination of seed and the growth of seedlings negatively (Van den Berg and Zeng, 2006).

In conclusion, barley seeds appear to acquire Na⁺ from the saline environment prior to and during germination, which has the net effect of allowing them to absorb more water and germinate faster than seeds in an isotonic PEG solution, and to be able to germinate under osmotic conditions in which they would otherwise not be able to. Seed sodium concentration increased with time and with increases in environmental salt concentration. Temperature had a significant effect, with a general increase in germination rate and seed sodium concentration with increasing temperature, and a decrease in germination percentage and seed dry mass. It is unclear whether the germinating seeds germinate because they are more able to regulate sodium levels in their cytoplasm, perhaps by compartmentalization into the vacuole, whilst also being able to generate significant turgor, or whether their sodium concentrations are merely lower as a result of the decreased duration in which they are in the sodium solution.

REFERENCES

- Annisson G .1993 . The role of wheat non- starch polysaccharides in broiler nutrition. Aust. J. Agric. Res., 44: 405-422.
- Anonymous .1999 . Agricultural statistics of Pakistan: Ministry of Food, agriculture and livestock, economics wing, Islamabad, pp: 3-4.
- Ashraf M .1999 . Interactive effect of salt .NaCl and Nitrogen form of growth, water relations and photosynthesis capacity of sunflower *.Helianthus annuus* L. . Ann. Appl. Biol., 135: 509-513.
- Ashraf M .2004 . Some important physiological selection criteria for salt tolerance in plants. Flora, 199 .5 : 361-376.
- Bernstein L .1961 . Osmotic adjustment of plants to saline media. I. Steady state. Am. J. Bot., 48: 909-918.
- Djibril S, Mohamed OK, Diaga D, Diégane D, Abaye BF, Maurice S, Alain B .2005 . Growth and development of date palm *.Phoenix dactylifera* L. seedlings under drought and salinity stresses. Afr J. Biotechnol, vol.4, no. 9, pp. 968-972.
- FAO .2007 . FAO Agristat, www.fao.org .accessed on 10 June 2010 .
- Fuente JM, Perez de Ayala P, Flores A, Villamide MJ .1998 . Effect of storage time and dietary enzyme on the metabolizable energy and digesta viscosity of barely-based diets for poultry. Poul. Sci., 77: 90-97.
- Garthwaite AJ, von Bothmer R, Colmer TD .2005 . Salt tolerance in wild *Hordeum* species is associated with restricted entry of Na⁺ and Cl⁻ into the shoots. Journal of Experimental Botany 56: 2365–2378.
- Gaxiola RA, Li JS, Undurraga S .2001 . Drought- and salt-tolerant plants result from overexpression of the AVP1 H⁺-pump. Proceedings of the National Academy of Sciences of the USA 98: 11444–11449.
- Glenn EP, Brown JJ, Blumwald E .1999 . Salt tolerance and crop potential of halophytes. Critical Reviews in Plant Sciences 18: 227–255.
- Hartmann T, College M, Lumsden P .2005 . Responses of different varieties of *Lolium perenne* to salinity". Annual Conference of the Society for Experimental Biology, Lancashire.
- Hester MW, Mendelssohn IA, McKee KL .2001 . Species and population variation to salinity stress in *Panicum hemitomon*, *Spartina patens*, and *Spartina alterniflora*: morphological and physiological constraints. Environmental and Experimental Botany 46: 277–297.
- Horie T, Schroeder JI .2004 . Sodium transporters in plants. Diverse genes and physiological functions. Plant Physiology 136: 2457–2462.
- Hu L, Lu H, Liu QL, Chen XM, Jiang XN .2005 . Overexpression of mtID gene in transgenic *Populus tomentosa* improves salt tolerance through accumulation of mannitol. Tree Physiology 25: 1273–1281.
- international seed testing association .ISTA .1993 . The germination rules for seed testing. Seed science and technology, 27:152.
- Kanai M, Higuchi K, Hagihara T .2007 . Common reed produces starch granules at the shoot base in response to salt stress. New Phytologist 176: 572–580.
- Mer, RK, Prajith PK, Pandya DH, Pandey AN .2000 . Effect of salts on germination of seeds and growth of young plants of *hordeum vulgare*, *triticum aestivum*, *cicer arietinum* and *brassica juncea*. J. Agron. Crop Sci. 185: 209-217.
- Munns R .1988 . Causes of Varietal differences in Salt tolerance. In: International congress of plant physiology, New Delhi, India, pp: 960-968.
- Munns R .2002 . Comparative physiology of salt and water stress. Plant Cell Environ. 25: 239–250.
- Munns R .2005 . Genes and salt tolerance: bringing them together. New Phytol. 167: 645-663.
- Munns R, Tester M .2008 . Mechanisms of salinity tolerance. Annual Review of Plant Biology 59: 651–681.
- Pandey AN, Thakrar NK .1997 . Effect of chloride salinity on survival and growth of *Brassica juncea*. J Agronomy and Crop Sciences, 185: 209-217.
- Qiu NW, Chen M, Guo JR, Bao HY, Ma XL, Wang BS .2007 . Coordinate up-regulation of V-H⁺-ATPase and vacuolar Na⁺/H⁺ antiporter as a response to NaCl treatment in a C-3 halophyte *Suaeda salsa*. Plant Science 172: 1218–1225.
- Rahman M, Kayani SA, Gul S .2000 . Combined effects of temperature and salinity stress on corn cv. Sunahry, Pak. J. Biological Sci., 3.9 : 1459-1463.
- Raven JA .1985 . Regulation of pH and generation of osmolarity in vascular plants – A cost–benefit analysis in relation to efficiency of use of energy, nitrogen and water. New Phytologist 101: 25–77.

- Ren ZH, Gao JP, Li LG .2005 . A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nature Genetics* 37: 1141–1146.
- Schachtman DP, Munns R .1992 . Sodium accumulation in leaves of *Triticum* species that differ in salt tolerance. *Aust. J. Plant Physiol.*, 19: 331-340.
- Serrano R, Gaxiola R .1994 . Microbial models and salt stress tolerance in plants. *Critical Reviews in Plant Sciences* 13: 121–138.
- Shirazi MU, Asif SM, Khanzada B, Khan MA, Mohammad A .2001 . Growth and ion accumulation in some wheat genotypes under NaCl stress. *Pak. J. Biol. Sci.*, 4: 388-391.
- Torech FR, Thompson LM .1993 . *Soils and soil fertility*. Oxford University Press, New York.
- Ungar IA .1995 . Seed germination and seed-bank ecology of halophytes. In: Kigel J, Galili G. eds. *Seed development and germination*. New York: Marcel Dekker, 599–627.
- Van den Berg L, Zeng YJ .2006 . Response of South African indigenous grass species to drought stress induced by polyethylene glycol .PEG 6000. *Afr. J. Bot.*, vol. 72, pp. 284-286.