Volume IABC(12). Published January, 31, 2016
www.jnsciences.org
ISSN 2286-5314

Assessment of salinity tolerance among Doubled Haploid lines of barley (*Hordeumvulgare* L.)

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Abstract-Barley (*Hordeumvulgare* L.) is an important food and fodder cereal crop in Tunisia. While it's highly affected by abiotic stress such us salinity. The latter restricts the use of agricultural land and limits the yield of barley. The development of salinity-tolerant crops is an important management option for maintaining production in salinity-deposited soils. 77 Doubled Haploid lines (DHs) produced from the cross between local accession 1a and Rihane were tested to evaluate the genetic variation and to identify salinity tolerant DHs in two contrasting environments; rain fed and irrigated with high water salinity (8.06 ms/cm). The trial was conducted in an augmented design model with six blocks and five modern cultivars as checks. The phenotypic evaluation was performed at anthesis and maturity stages. The agronomic traits performed at anthesis stage are: height (HA), leaf Area (LA), growth stage (GS) and dry matter of plants (DM). Number of spike (SN), thousand kernel weight (TKW), percentage of infertile spikelet's (PIS) and biological yield (BY) are measured at maturity stage. Significant differences were observed among barley DH lines for all traits at salinity conditions.There was a wide range of variation in the response to salinity stress among the DHs. We observed a significant negative correlation between PIS and all others traits. The correlation coefficient between PIS and TKW is -0.56**. Whereas, significant positive correlations between DMand SN (r= 0.31). LA and DMare good indexes for screening for salinity tolerance at anthesis, and TKW and PIS at maturity stage.5 DHs showed a high TKW more than the parent Rihane. The difference in response to salinity stress among DH lines could offer elite alleles conferring salinity tolerance.

Keywords:*Hordeumvulgare* L., Contrasting Environments, Anthesis, Maturity, PIS, TKW.

1. Introduction

Salinity in soil and water are affected by 2 factors: Natural factor related to season and changeable rainfall and surface water, and by human effects using a low quality of water for irrigation and by the excess of use of fertilizer (Munns 2005,Munnsand Tester2008).It was estimated that salinity affected nearly 950 million ha (6%) in the world (Babuet al. 2007). 20 % of agricultural lands are affected by salinityand 50% of irrigated lands are damaged by the water used for irrigation (Sairan and Tayagi 2004). Salinity damage has rapidly extended to broad areas of arable land in the world,and large areas of arable land are in danger of becoming saline, especially in North of Africa (FAO2009).In the other hand, the world production and consumption of grains (barley, maize, millet, oats, rice, rye, sorghum, wheat, and mixed grains) (USDA2015) are not stable.The production is variable and the consumption is continuously increasing with an increase of the world population that will be reach 9 bln in 2050.Variable production was caused by spread of area damaged by abiotic stress such as salinity. To overcome the unbalance and to ovoid the food securityrisk, one way in genetics is to improve salinity tolerance in crops. The development of salinity-tolerant cereals crops is an important management option for maintaining production in salinity-deposited soils. Barley (*Hordeumvulgare* L.) is the fourth most important cereal crop worldwide and is an important source for feed, malt, and human food (Flowers and Yeo 1995).Barley is widely cultivated in saline areas as one of the most salinity-tolerant field crops.The genetic diversification and the adaptability to a broad range of ecological conditions have highly strengthenedthe salinity tolerance in barley (Sbei et al. 2013). Many studies were interested in screeningbarley for salinity tolerance (Chen et al. 2005, Ellis et al. 2002, Mano and Takeda 1997, Taghipour and Salehi 2008). Most of these studies evaluate the salinity tolerance of barley at earlier stages like germination (Mano and Takeda 1997) and seedling stage (Sbei et al. 2014) becausehydroponic system and controlled conditions reduce environmental effects. But researchers discovered that salt tolerance is controlled by different QTLs/genes at different development stages (Mano and Takeda 1997,Xueet al.2009).In the present study, seventy seven doubled haploid lines (DHs) produced from the cross between 1a and Rihane were tested to evaluate the genetic variation and to identify salinity tolerant DHs. The primary objectives for this work were to: 1) determine suitable traits for screening for salinity tolerance in barley at anthesis and maturity stages 2) check the performance of parents under salinity stress and 3) identify superior salinity tolerant DH lines.

2. Material and methods

2.1. Plant material

A barley population of 77 doubled-haploid lines derived from a cross between Rihane, a Tunisian cultivar derived from an ICARDA nursery, and the local line '1a' was used. DH lines were produced from F1 using anther culture (Don Palmer et al. 2005).Parents Rihane and 1a were produced at National Institute of Agronomic at Tunis. DH lines were evaluated in two contrasting environments; rain fed (Mateur) and irrigated (Medenine) with high water salinity (8.06 ms/cm). Five modern cultivars were used as checks (Roho, Tej, Manel, Martin, Rihane).

2.2. Methods

DH lines with parents were sown in the field with 2 contrasting environments: South (Medenine) and North of Tunisia (Mateur) on December 2010.The trial was conducted in mixed model augmented design with six blocks and five checks. Each accession was sown in a square meter area (1 x1 m) ie 5 lines spaced 25 cm. Barley sown in site of Medenine is irrigated with saline water with a drip system. The water irrigation contains 6 gr/l of salt (8.06 ms/cm). The salinity water was supplied from seedling stage to maturity stage. Barley plants have been monitored at 2 development stages: anthesis and maturity. The phenotypic evaluation was performed on 8 agronomic parameters: height (HA), leaf Area (LA), dry matter (DM), growth stage (GS), spike number (SN), biological yield (BY), thousand kernel weight (TKW) and percentage of infertile spikelet's (PIS). At anthesisstages, five plants were taken randomly from each experimental unit to perform the analysis of HA, LA and DM. Height (HA) is measured for the main axis from the basis of tillering to the end of the spike in cm. The F3 leaf of main axis of five plants was scanned measurement of leaf area was calculated by an application "Mesurim_pro_29_03_10". To determine the growth stage, we used the Zadoks scale. We noted the growth stage when 50% of the lines reach the growth stage 69. The growth stage varied from GS 50 to GS 75. For biomass measure, 5 plants per line were dried for 48 hours at 80°C, then weighted (g). At maturity stage, a random 0.25 m was used for measurement of yield and yield components. Spikes and straw were dried for 48 hours at 80°C. 20 spikes per lines were used to determine the percentage of the infertile spikelet's according to the formula below:PIS (%) = infertile spikelet's number/total infertile spikelet's *100.

2.3. Statistical analysis

Error variance was based on variance of the checks between plots and the values were adjusted regarding fixed model augmented design using SAS software(SAS Inst. 2004).Frequency of distributions and pearson correlation analysis were performed using SPSS software (SPSS Inc. 2010).

3. Results and discussion

3.1. Characterization of the DH population

Different traits were measured for the screening for salinity tolerance. At rain fed conditions (Mateur), results show that there is a non-significantvariation among barley DHs for all measuring traits except Height (HA) at anthesis stage (Table 1). While, significant variation were observed for most of traits under salinity conditions.Non-significant variations were observed between blocks under both conditions except for GS under salinity conditions. The highest coefficient variations between DH lines were observed for the spike number per plant (45.29 - 35.75%) and DM (23.18 - 41.43%) under rain fed and salinity conditions, respectively (Table 1). PIS and LA also showed a highest coefficient variation with a non-significant variation between checks. According to the statistical analysis and the wide variation among barley population, we selected five traits for screening for the salinity tolerance.LA andDMare good indexes for screening for salinity tolerance at anthesis stage. SN, PIS and TKW are suitable traits for barley screening at maturity stage. The selected traits showed a large coefficient variation amongDH lines. The coefficient of variation among barley lines is 41.43 and 35.75 % for DM and SN,respectively.

Table 1: Analysis of variance for traits investigated in the 77 DHs at rain fed and salinity conditions

Source of variation	Df	Mean Square							
		HA	**DM**	**LA**	**GS**	**SN**	**BY**	**PIS**	**TKW**
Rain fed									
Block	5	20.9NS	7.72NS	36176NS	41.21NS	28671NS	28.35NS	52.88NS	20.65NS
DHs	76	93.08**	15.84NS	100175NS	16.11NS	12437NS	76939NS	39.96NS	30.97NS
Checks	4	427.4**	24.89NS	324862*	16.73NS	55721NS	83760NS	16.32NS	164.82**
CV %		5.56	23.18	16.64	5.57	45.29	18.48	20.95	12.61
Salinity									
Block	5	73.3NS	2.29NS	41820NS	63.45**	10697NS	68853NS	45.9NS	15.51NS
DHs	76	100.7**	4.9**	136237**	1856**	15411*	123964**	287.6**	33.63**
Checks	4	136*	19.15*	100889NS	271.9**	18988NS	1481233**	503.0NS	69.44NS
CV %		10.48	41.43	21.1	5.85	35.75	22.6	16.26	17.75

NS: non-significant; *Significant at P<0.05 level; **significant at P<0.01 level;CV: coefficient of variation; Df: degree of freedom; HA: height, DM:dry matter of plants;LA:leaf Area, GS:growth stage; SN: spikenumber, BY: biological yield, TKW:Thousand kernel weight, PIS: percentage of infertile spikelet's.

Means of selected traits with standard deviation were showing in the table 2. Leaf area, dry matter, percentage of infertile spikelet's and the thousand kernel weight were affected by salinity. The dry matter was reduced from 13.53 to 4.33 g/ $m^2$when plants are irrigated with saline water. The means of PIS was 22.34 %under rain fed condition and increased to 26% under salinity condition. Salinity affected the yield production of barley starting by the reduction of the dry matter and surface area at anthesis stage and the stressed continued at maturity stage by reducing the spike number per plant and the infertility ofspikelet's per plant.

Table 2: Means (± standard error) for traits of parents and 77 DH lines grown at rain fed and salinity conditions

Parameter	Leaf Area	Dry Matter	PIS	Spike Number	TKW
Rainfed					
P1: 1a	2469±136.90	15.85±2.35	13.41±1.96	268±100.24	38.11±2.67
P2: Rihane	1949.00±286.72	14.30±2.80	21.54±2.00	327.41±72.08	35.69±4.11
DHs	1983.53±335.88	13.537±4.07	22.34±6.77	357.29±111.24	37.13±5.8
Salinity					
P1: 1a	834.97±131.90	1.32±0.36	75.52±3.94	76.600±11.69	34.37±5.4
P2: Rihane	1415.65±164.32	4.43±1.21	28.61±5.72	366.00±96.39	48.61±2.37
DHs	1136.14±390.71	4.33±1.90	26.00±11.86	238.96±122.0	46.77±5.7

P1, P2: Parent 1 (1a) and parent 2 (Rihane); DHs: doubled haploid lines; PIS: percentage of infertile spikelet's; TKW: thousand kernel weight.

Underrain fed conditions, parent 1 (1a) had higher LA, DM and TKW with the lowest PIS compared to parent 2 (Rihane) and DH lines (Table 2). The means values of traits of Rihane look similar to the DH lines under rain fed conditions. Under salinity treatment, parent 1 (1a) had the lowest LA, DM and TKW compared to DH lines and Rihane. The PIS for the parent 1 was 75% while it was 26 and 28.61% for the DH lines and Rihane respectively. Parent 1 (1a) is susceptible and highly affected by salinity compared toRihane and compared to the DH lines.

3.2. Correlation analysis

Significant positive correlation was observed between SN and the traits DM and LA with r = 0.31 and 0.22 respectively. Also, we observed significant negative correlation between PIS and LA which means that the seed production is significantly correlated to the vegetal biomasses produced at anthesis stage. A significant positive correlations between TKW and the traits LA and SN with r = 0.27 and 0.34 respectively, whereas significant negative correlation between TKW and PIS with r = -0.56**. The seed production is affected by the infertility of spikelet's.

Table 3: Pearsan correlation analysis between traits measured under salinity conditions

	HA	**DM**	**LA**	**GS69**	**SN**	**BY**	**PIS**	**TKW**
HA	1.00							
DM	0.41**	1.00						
LA	0.16	0.12	1.00					
GS69	0.16	0.25**	0.28**	1.00				
SN	0.17	0.31**	0.22*	0.32**	1.00			
BY	0.34**	0.25*	0.18	0.22*	0.79**	1.00		
PIS	-0.24*	-0.08	-0.21*	-.36**	-0.22*	-0.20*	1.00	
TKW	0.12	0.08	0.27**	0.42**	0.34**	0.26**	-0.56**	1.00

*Significant at P <0.05 level; **significant at P<0.01 level, HA: Height, DM: dry matter of plants, LA:leaf Area, GS: growth stage, SN: spike number, BY: biological yield, TKW: thousand kernel weight, PIS: percentage of infertile spikelet's.

3.3. Frequency distributions

Frequency distributions among the 77DHs lines and parents positions were showed in the figure 1 for rain fed and salinity conditions. There were a wide variation among DH lines under salinity conditions and the parents have a different response under salt stress and not at rain fed conditions. The parent 1a is susceptible to salt. Rihane is moderate to tolerate to salt. The distributions of frequency of DHs follow a normal distribution for the traits PIS and TKW.

3.4. Selected genotypes

Five DHs (30, 37, 57, 63 and 65) had good response according to the combined traits (LA, DM, PIS, SN, TKW) and showed better performance to salinity stress. The behavior of the 5 lines during salinitytreatment showed that these lines could be introduced as salinity tolerance and use for future experiments.

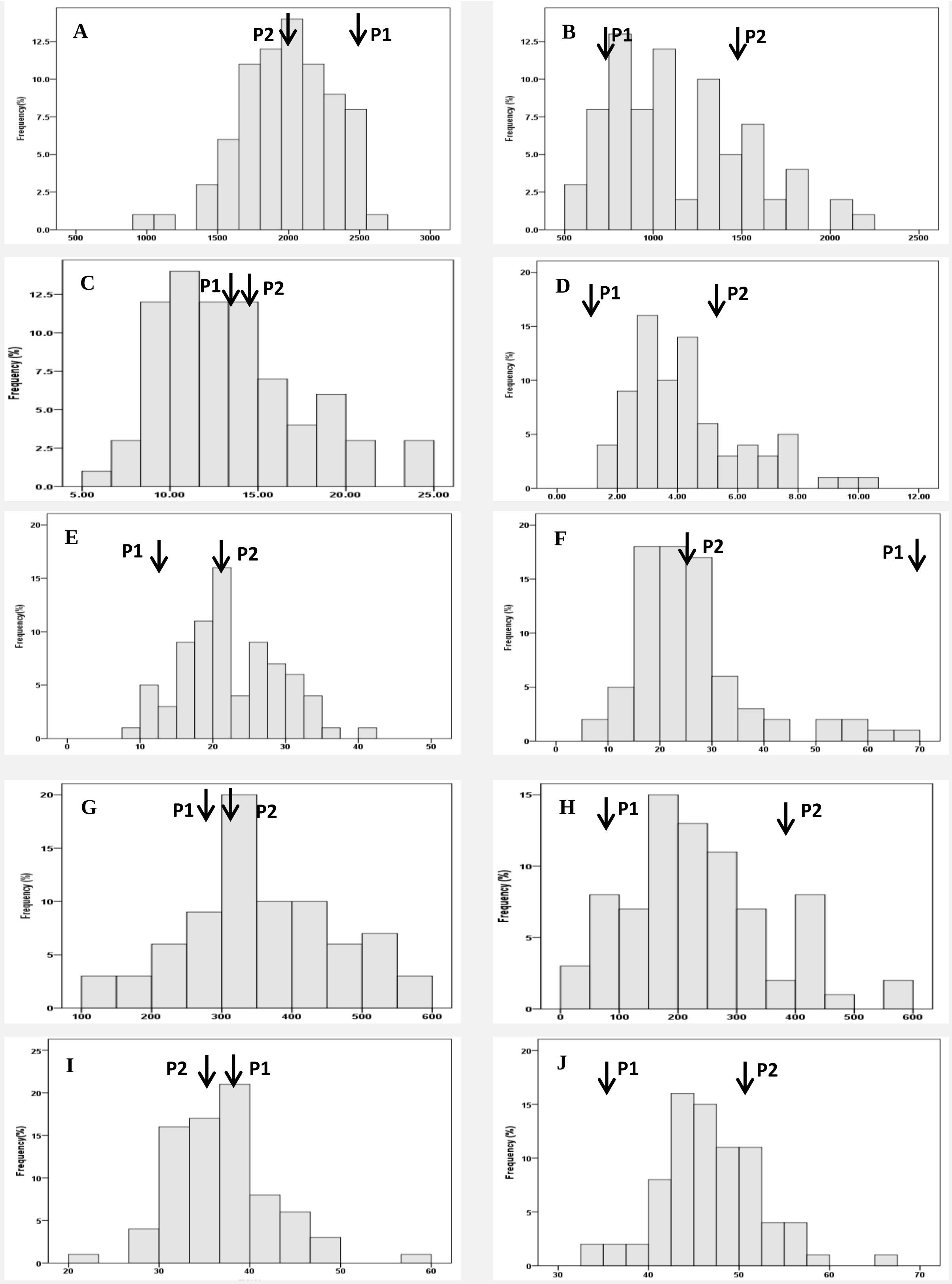

Figure 1: Frequency distribution of LA (A,B), DM (C,D), PIS (E,F), SN (G,H) and TKW (I,J) among the 77 DHs at rain fed (A,C,E,G,I) and salinity (B, D, F, H,J) conditions

Table 4: Salinity tolerance of parents and superior DHs in salinity conditions

Parents/ DHs number	**Leaf Area (cm^2)**	**Dry Matter (g)**	**PIS (%)**	**Spike Number (per plant)**	**TKW (g)**
1a	834.97	1.320	75.52	76.60	34.37
Rihane	1415.65	4.437	28.61	366	48.61
30	1571.00	5.192	18.06	228	52.22
37	1563.00	4.682	21.67	564	50.06
57	1543.67	2.240	18.69	284	50.00
63	2023.50	6.320	22.41	452	44.71
65	2160.67	6.092	20.21	412	51.8

PIS:percentage of infertile spikelet's,TKW: thousand kernel weight.

4. Conclusion

Salt tolerance is such a complicated trait that different methods and parameters have been used to assess barley germplasm for salt tolerance. In the same context, 77 DH lines were screened for salinity tolerance at 2 development stages. Wide variations among barley population were observed and a difference in response to salinity wasshowedbetween parents. Some traits from the used parameters mentioned a large variation among barley lines. These traits could be used for screening for salinity. LA and DM could be screening traits for salinity tolerance at anthesis stage for barley. TKW and PIS could be considered as salinity indexes for salt tolerant at maturity stage.The behavior of the four lines during salinity treatment showed that these lines could be introduced as salinity tolerance and use for future experiments.The difference in response to salinity stress among DH lines could offer elite alleles conferring salinity tolerance and the identification of QTLs for salinity tolerance is very probable.

Acknowledgements

This research was supported by the research and development action "Characterization and evaluation of salinity tolerance in barley accessions'' funded by the ministry of agriculture, water resources and fisheries.

5. References

Babu S, Sheeba A, Yogameenakshi P, Anbumalarmathi J, Rangasamy P (2007) Effect of salt stress in the selection of salt tolerance hybrids in rice (*Oryza Sativa* L.) under in vitro and in vivo condition. Asian J Plant Sci6: 137-142

Chen Z, NewmanI, ZhouM, MendhamN, ZhangG,ShabalaS (2005) Screening plants for salt tolerance by measuring K$^+$ flux: a case study for barley. Plant Cell Environ 28: 1230-1246

Don Palmer CE, KellerW, KashaK, DevauxP, Pickering R(2005) Haploids in the improvement of Poaceae.*In* Haploids in Crop Improvement II: Springer Berlin Heidelberg. Biotechnology in agriculture and foresty56: 215-242

Ellis RP, ForsterBP, GordonDC, HandleyLL, KeithRP, LawrenceP, MeyerR, PowellW, RobinsonD, ScrimgeourC (2002) Phenotype/genotype associations for field and salt tolerance in a barley mapping populations segregating for two dwarfing genes. J Exp Bot 53: 1163-1176

FAO (2009) High level expert forum – how to feed the world in 2050? Economic and social development department, Food and Agricultural Organization of the United Nations, Rome

Flowers TJ, Yeo AR (1995) Breeding for salinity resistance in crop plants: where next? Australian JPlant Physiol 22:875-884

Mano Y, Takeda K(1997) Mapping quantitative trait loci for salt tolerance at germination and the seedling stage in barley (*Hordeumvulgare* L.). Euphytica 94: 263–272

MunnsR (2005) Genes and salt tolerance: bringing them together. New Phytol167: 645-663

Munns R, Tester M (2008) Mechanisms of salinity tolerance.Annu Rev Plant Biol 59: 651-681

USDA (2010) World agricultural supply and demand estimates. WASDE-547, pp1-40

Sairam RK, Tyagy A(2004) Physiology and molecular biology of salinity stress tolerance in plants. Current Sci 86: 407-421

Sbei H, Shehzad T, Harrabi M, Okuno K (2013) Salinity tolerance evaluation of Asian barley accessions (*Hordeumvulgare* L.) at early vegetative stage. J Arid Land Studies 24: 183-186

Sbei H, Sato K, Shehzad T, Harrabi M, Okuno K (2014) Detection of QTLs for salt tolerance in Asian barley (*Hordeum vulgare* L.) by association analysis with SNP markers. Breed Sci 64: 378-388

SPSS Inc. (2010) GPL reference guide for IBM SPSS Statistics, SPSS Inc. 1989

Taghipour F, SalehiM (2008) The study of salt tolerance of Iranian barley (*Hordeumvulgare* L.) genotypes in seedling growthstages. Am Eurasian J Agric Environ Sci 4: 525-529

XueD, HuangY, ZhangX, WeiK, WestcottS, LiC, ChenM, Zhang G, Lance R(2009) Identification of QTLs associatedwith salinity tolerance at late growth stage in barley. Euphytica169: 187-196