# Evaluation of performance of different barley genotypes irrigated with saline water in South Tunisian Saharan conditions

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#### Abstract

Barley (*Hordeum vulgare* L.) is an important crop in Tunisia, especially in the south, which is characterized by an arid Saharan climate. Water resources, when available, are saline. In this study, 26 barley genotypes were evaluated in Saharan conditions. Plants were irrigated with saline water. Germination percentage, height, leaf area, tiller number, percentage of senescing leaves, leaf chlorophyll content, shoot dry weight and grain yield varied significantly between genotypes. This implies that important intraspecific genetic variation in this germplasm exists in response to salinity and aridity. The estimated grain yield of the introduced cultivar '113/1B' and a local landrace 'Ardhaoui 'reached 1.1 t ha<sup>-1</sup>, despite these saline and arid conditions, and exceeded the overall national average yield of about 0.75 t ha<sup>-1</sup>. Selection of these genotypes can be profitable when grown in marginal areas using brackish water. The soil electric conductivity did not increase at the experimental location following saline irrigation: 2.30 dS m<sup>-1</sup> at sowing and 2.37 dS m<sup>-1</sup> at harvest, indicating that there is no risk of salt accumulation in sandy soil.

#### Key words: barley, Saharian environment, salinity, yield.

**Abbreviations:** CC, total chlorophyll conten; DAS, days after sowing; EC, electrical conductivity; GP, germination percentage; GY, grain yield; LA, leaf area; OM, organic matter; PCA, principal component analysis; PDL, percentage of dead leaves; PH, plant height; SDW, shoot dry weight; TN, tiller number.

### Introduction

Agriculture is a major sector of the Tunisian economy. However, Tunisia is represented by a Saharan arid and semiarid regions. Crops in these regions cannot be irrigated by rainfall, which is rare. In addition, salt concentration in the water of reservoirs and wells is 3 to 8.5 dS m<sup>-1</sup> and 6 to 10 dS m<sup>-1</sup>, respectively (Slama 2004). In some cases, water in wells can reach 14 dS m<sup>-1</sup>, particularly in arid and Saharan areas. In this region, salinity in soil or irrigation water is the major limiting factor to crop growth (Ashraf et al. 2008; Kausar et al. 2013) and is a major abiotic stress affecting agricultural production.

One way to exploit these areas and saline water sources is to improve the salt tolerance of cultivated species. This would be useful for local communities, including cereals, especially barley (*Hordeum vulgare* L.). However, despite its importance as food and feed in these areas (El Felah, Medimagh 2005), barley culture is relatively marginalized by farmers and the average grain yield in Southern Tunisia never exceeds 0.5 t ha<sup>-1</sup> (GDPA 2009). This low productivity is due to environmental limitations such as desertification and salinity as well as socio-economic constraints. On the other hand, the importance of barley derives from its ability to grow and produce in marginal environments, which are often characterized by drought, high temperature and salinity (Slama 2005; Al-Dakheel et al. 2012). The salinity tolerance of barley is 8.0 dS m<sup>-1</sup> (Maas, Hoffman 1997; Tabatabaei, Anagholi 2013). Barley is also considered a model species for cereals due to its widely available genetic information (Hayes et al. 2002). Consequently, the improvement of abiotic stress tolerance in barley depends largely on exploiting the available genetic variation. In addition, using cultivars tolerant to salinity allows the conservation of freshwater and its conservative use, mainly in arid areas (Keating et al. 2010).

The objectives of the present investigation were to study the possibility of extending and ameliorating the barley crop in marginal, Saharan areas. To achieve this, we examined the effects of irrigation with saline water (EC =  $13 \text{ dS m}^{-1}$ ) on the dynamics of salinization and evolution of soil pH in an experimental plot (Chamsa-Tozeur) during the 2009– 2010 cropping season, and to determine variability between 26 barley cultivars in response to severe abiotic stress.

# **Materials and methods**

# Plant material

In total, 26 varieties of barley were used, consisting of three local landraces ('Ardhaoui', 'Arbi Abidh' and barley 'Mednine'), four improved Tunisian barley cultivars ('Rihane', 'Tej', 'Konouz', 'Manel') and 19 introduced cultivars (Pakistain cultivars: 'PK 30109', 'PK 30046', 'PK 30163', 'PK 30118'; Batini landraces from Oman: '113/1B', '100/1B', '186 AD', 'AD/87', '111/4A', '16/2A'; 'Furat 1' from Syria, 'Giza 125' from Egypt; IPA7 from Iraq; 'Alanda-01' from ICARDA; 'Rihane-03' from ICARDA; 'Barjouj' from Libya; 'ICARDA 20' from ICARDA; and 'Saudi' from Saudi Arabia) obtained from the National Research Institute for Rural Engineering, Water and Forestry, Ariana, Tunisia in collaboration with the International Center for Biosaline Agriculture, Dubai, United Arab Emirates (Table 1).

Table	1.	Categories	and	origin	of 1	the	barley	genotypes	used	in
the stu	ıdy	,								

Code	Genotype	Breeding	Origin
		status	
1	'Rihane'	Cultivar	Tunisia
2	'Tej'	Cultivar	Tunisia
3	'Konouz'	Cultivar	Tunisia
4	'Manel'	Cultivar	Tunisia
5	'Arbi Abidh'	Landrace	ICARDA/Syriea
6	'Barley Mednine'	Landrace	ICARDA/Tunisia
7	'Ardhaoui'	Landraces	Tunisie
8	'PK 30046'	Landrace	Pakistan
9	'PK 30109'	Landrace	Pakistan
10	'PK 30163'	Landrace	Pakistan
11	'PK 30118'	Landrace	Pakistan
12	'100/1B'	Landrace	Oman
13	ʻ111/4A'	Landrace	Oman
14	ʻ113/1B'	Landrace	Oman
15	'AD/87'	Landrace	Oman
16	'186 AD'	Landrace	Oman
17	ʻ16/2A'	Landrace	Oman
18	'Rihane-03'	Cultivar	ICARDA
19	'IPA7'	Cultivar	Iraq
20	'Furat 1'	Cultivar	Syria
21	'Giza 125'	Cultivar	Egypt
22	'Alanda-01'	Cultivar	ICARDA
23	'Rihane 3'	Landraces	ICARDA
24	'ICARDA 20'	Cultivar	ICARDA
25	'Saudi'	Landrace	Saudi Arabia
26	'Barjouj'	Cultivar	Libya

# Experimental site

The Tunisian oasis covers approximately 40 800 ha (Sghaier 2010), about 13% of the irrigated area of the country (Hajji 1997). We selected a site near an oasis due to the ability to draw on brackish water. The experimental site near the Chamsa oasis (Fig. 1) is characterized by a Saharan bioclimate, high temperature and little, irregular rainfall (< 90 mm year<sup>-1</sup>). Evapotranspiration is high (1800 to 2000 mm year<sup>-1</sup>) and insulation is very high (3061 h year<sup>-1</sup>). In Tunisia, continental winds are dry and cold during winter, and dry and hot during summer, with an average of 120 days year<sup>-1</sup> of sandstorms and 40 days year<sup>-1</sup> of sirocco (National Institute of Meteorology, Tunis, Tunisia, 2010).

Weather conditions during the 2009/2010 growing season are detailed in Table 2. The total accumulated precipitation during this season was 35 mm. Mean and maximum temperatures recorded during November 2009 to May 2010 were 22.3 and 45.2 °C, respectively.

Field experiments were conducted during the 2009/2010 cropping season near the Chamsa oasis ( $33^{\circ}58'00.67''N$ ;  $8^{\circ}02'05.78''E$ ). The experiment was designed as a randomized complete block with three replications. Each cultivar was sown in a 2 m<sup>2</sup> area ( $2 \times 1$  m), in 10 lines spaced 20 cm apart. The cultivation density was determined based on 200 seeds m<sup>-2</sup>, the weight of 1000 seeds and the germinability of each cultivar. Plots were irrigated using a flood irrigation system with natural groundwater that had an EC equal to 13 dS m<sup>-1</sup> (i.e., saline, brackish water). The EC of water was measured with a conductiviometer (Consort C830, Turnhout, Belgium).



Fig. 1. Bioclimatic zones of Tunisia, including the study area.

	October	November	December	January	February	March	April	May	June
Rainfall (mm)	0	2	5	5.1	0	9	0	0	0
Average	22	21.7	18.0	17.8	18.4	21.7	28.2	30.5	22.5
temperature (°C)									
Maximal	33.3	31.4	26.6	25	30.3	39.8	40.2	45.2	37
temperature (°C)									

Table 2. Agro-meteorological parameters of the experimental site

# Soil analysis

A soil auger was used to extract soil samples to a depth of 1 m from the soil surface of the experimental plot. At each grid point (one per block), disturbed soil samples were taken at vertical depth increments (0 to 0.2, 0.2 to 0.4, 0.4 to 0.6, 0.6 to 0.8, and 0.8 to 1 m). Physico-chemical analyses were performed on soil dried for 45 days in the open air and sieved to 2 mm. Total organic nitrogen concentration was determined by the Kjeldahl method (Kjeldahl 1883; Skoog et al. 1997). Granulometric fractions were determined by sedimentation, pipetting after de-carbonation, and destruction of organic matter (OM) (Pansu, Gautheyrou 2006). Phosphorus and labile potassium concentrations were determined by the Olsen method (Baize 2000) on dried soil and were extracted with a solution of sodium bicarbonate. The concentration of these elements in filtrate was determined by a spectrophotometer (UV1800, Shimadzu Corp., Kyoto, Japan) at  $\lambda = 840$  nm. The levels of nitrogen, phosphorus and potassium were evaluated (low or appropriate) by comparison with the standards proposed by Calvet and Villemin (1986). Soil pH and EC were measured in an extract of 1/5, according to the method of Pawels et al. (1992), using a pH meter (Consort C830, Parklaan 36, B2300 Turnhout, Belgium) and conductivimeter, respectively.

# Agromorphological analysis

Agronomic and physiological measurements were made at two growth stages: tillering stage at 100 days after sowing (DAS) and maturation stage at 145 DAS. A morphological evaluation was performed on eight agronomic parameters: germination percentage (GP) (%), plant height (PH) (cm), leaf area (LA) (cm<sup>2</sup>), tiller number (TN) (per plant), percentage of dead leaves (PDL) (%), total chlorophyll content (CC), shoot dry weight (SDW) (g m<sup>-2</sup>) and grain yield (GY) ( $g m^{-2}$ ).

At the tillering stage, three plants were taken randomly from each experimental unit, thus nine plants for each germplasm were used in the analysis. pH was determined using a pH meter (Consort C830, Parklaan 36, B2300 Turnhout, Belgium). SDW was calculated by drying each sample in an oven for 48 h at 80 °C. The third leaf of the main axis of nine plants was scanned and LA was measured by image software Mesurim Pro-02-08 (Madre, Academy of Amiens, France). Chlorophyll content was determined on the third leaf (three readings) of nine plants of each plot, thus 27 plants for each germplasm, using a portable chlorophyll meter (Minolta SPAD 502 Meter, Osaka, Japan). The percentage of dead leaves was determined as the number of dead leaves from base to tip of plants divided by the total number of leaves. At grain maturity (145 DAS), plants from 1 m<sup>2</sup> from each plot were harvested to estimate GY.

# Statistical analysis

Data were statistically analyzed using analysis of variance (ANOVA) (p < 0.05), using SPSS Version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Descriptive statistical analysis and principal component analysis (PCA) were performed with Excel (version 2003, Microsoft, Seattle, WA) add-in XLSTAT-PRO version 5.2 (AddinSoft, New York, NY).

# **Results and discussion**

## Soil

This study assessed whether barley could be extended as a crop to more marginal desert areas (Chamsa oasis region) in south Tunisia by irrigating soils with saline water (EC =  $13 \text{ dS m}^{-1}$ ). Granulometric analysis showed that the soil is sandy loam type. All the soil layers had a low OM content

Table 3. Characteristics of soil at the Chamsa-Tozeur experimental station at different depths. K, potassium ion;  $P_{ass}$ , assimilable phosphorus

Depth (cm)	<b>Clay</b> (%)	Silt (%)	Sand (%)	Organic	Nitrogen (%)	Labile K <sup>+</sup>	P <sub>ass</sub>
				matter (%)		(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )
0-20	0	13.50	85.49	0.89	0.02	120.00	1.74
20-40	0	24.07	75.93	0.25	0.02	113.33	2.00
40-60	0	30.30	69.49	0.59	0.03	106.66	1.62
60-80	0	27.78	72.22	1.40	0.02	153.33	1.63
80-100	0	22.00	77.17	0.12	0.02	120.00	2.25



Fig. 2. Salinization dynamics (A) and evolution of soil pH (B) at the experimental plot (Chamsa-Tozeur) in 2009–2010.

(< 1; Table 3). Generally, in arid regions, OM is low or nonexistent (Mtimet 2001) while barley requires at least 0.5% OM (Brink et al. 2006). The soil had a very low content of nitrogen (0.02%), phosphorus (122 mg kg<sup>-1</sup>) and potassium (1.8 mg kg<sup>-1</sup>). This result was explained, on one hand, by the absence of annual fertilization, and on the other, by the high mobility of these elements in soil, especially nitrogen (Bertschinger et al. 2003), combined with high filteration of sandy loam soil. These elements, which are essential for crop production, are generally provided by fertilizers. Therefore, low levels of these elements in the soils of the Tunisian south can be corrected by suitable fertilization. In addition, nutrients needs of barley are generally very low compared to that of other cereals (Brink et al. 2006; Robert et al. 2007).

EC increased with soil depth but salinity remained relatively unchanged throughout the cropping season (Fig. 2A). Among cereals, rice (*Oryza sativa*) is the most sensitive and barley is the most tolerant (Munns, Tester 2008). Therefore, barley is considered as an ideal model plant for genetic and physiological studies on salt tolerance. Barley is widely cultivated in saline areas as one of the most salt-tolerant field crops (Munns et al. 2006).

Soil pH of the experimental plot was basic and remained relatively unchanged throughout the experimental period, with a slight increase in deeper horizons (Fig. 2B). Arid soils are generally alkaline with a pH between 8 and 8.5 (Daoud, Halitim 1994). Biological activity of soil and availability of most nutrients are dependent on pH (Bertschinger et al. 2003). Soil in the southern region of Tunisia was basic. Indeed, several studies confirm that in arid regions, soils are generally alkaline with a pH value between 8 and 8.5 (Daoud, Halitim 1994). For wheat culture, the optimal pH value is between 5.5 and 7.5 (Brink et al. 2006). However, pH close to 8 is not considered as a limiting factor for the development of durum wheat (Brink et al. 2006). High pH values (8 to 8.5) are frequently associated with difficulty in plant assimilation of elements like phosphorus, zinc, manganese, copper and iron (Heller et al. 1998). Also, micronutrient deficiencies are common in soil having acid or alkaline pH (FAO 2003), resulting in the formation of insoluble hydroxides (Heller et al. 1998).

#### Yield variation

The average grain yield was 0.38 t ha<sup>-1</sup> (Table 4). This performance was similar to the average national Tunisian production of barley over several years: 0.28, 0.19, 0.41 and 0.22 t ha<sup>-1</sup> in 1994, 1995, 2000 and 2002, respectively (GDAP 2009). These levels were also similar to the southern average yield most of the time. In this study, yield reached 1.16 t ha<sup>-1</sup> under salt stress, better than the national average yield in 2009 and 2008 which was about 0.75 and 0.8 t ha<sup>-1</sup>, respectively (GDAP 2009). All the phenotypic parameters (GP, PH, TN, PDS, CC and LA) varied widely between genotypes, in some cases exceeding 100% variation, since their potential and response to stress differed. Jaradat et al. (2004) estimated genetic variation for salinity tolerance in the Batini landrace to be 73%. Al-Dakheel et al. (2012)

**Table 4.** Descriptive statistical analysis for parameters GP, H, TN, PDL, CC, LA, BY and GY for different barley cultivars. GP, germination percentage; GY, grain yield; LA, leaf area; PH, plant height; PDL, percentage of dead leaves; SDW, shoot dry weight; CC, leaf chlorophyll content; TN, tiller number

	GP (%)	PH (cm)	TN	PDL	CC	LA (cm <sup>2</sup> )	SDW	GY (t ha <sup>-1</sup> )
							(g m <sup>-2</sup> )	
Min.	46.50	18.40	1	30.04	17.11	6.73	39.21	0.064
Max.	89.00	32.72	3	68.61	42.24	16.66	111.78	1.163
General	65.33	26.40	1.8	48.83	33.72	10.60	60.00	0.378
means								

Table 5. Variance analysis of measured traits estimates for 26 barley genotypes. *, **, significant at P = 0.05 level and P = 0.01, respectively;
df, degrees of freedom; GP, germination percentage; GY, grain yield; LA, leaf area; PH, plant height; PDL, percentage of dead leaves; SDW,
shoot dry weight; CC, leaf chlorophyll content; TN, tiller number

Source of	Df	GP (%)	PH (cm)	LA (cm <sup>2</sup> )	SDW	TN	PDL (%)	CC	GY (g m <sup>-2</sup> )
variation					(g m <sup>-2</sup> )				
Cultivars	25	305.68*	124.83**	53.74**	0.14**	1.25*	531.76*	462.19**	1468.85*
R2		0.48	0.38	0.21	0.25	0.16	0.18	0.35	0.62
CV		19.28	19.72	48.68	47.57	49.04	36.17	32.34	71.13

also reported wide genotypic differences by barley cultivars consisting of Batini landrace material, which included 234 entries selected from 2308 entries and international breeding material from ICARDA, differing in salt tolerance after irrigation with saline water in response to salinity stress. However, the observed level of tolerance was 8 dS  $m^{-1}$ .

A response to abiotic stress generally occurs in most crops by affecting morphological parameters (Jaradat et al. 2004). Results obtained from ANOVA indicated significant differences between cultivars for all traits (Table 5). However, barley is considered to be the most salt-tolerant cereal (Jiang et al. 2006, Megan et al. 2013). Our results revealed large variability within the 26 genotypes for salt stress. Thus, for similar salinity stresses, the levels of tolerance in barley vary depending on the genotype (Munns et al. 2006). The variation in plant height between genotypes was the most commonly observed effect of salt stress, as confirmed by several studies (Kadri et al. 2009, Samah et al. 2013). In response to water deficit caused by salinity, plants reacted by reducing biomass. In particular, the reduction of leaf area was observed in local genotype 'Ardaoui' and the two Batini landraces '113/1B' and '100/1B'. Parida et al. (2005) showed that salinity can reduce dry weight. The significant effect of genotype on leaf chlorophyll content showed that abiotic stress, especially salinity, affects nutrient uptake and metabolic activities in plants to different degrees (Othman et al. 2006). Barley genotypes showed also variation in tolerance to salinity at germination stage. Many authors have observed that germination percentage varies between genotypes when irrigated with salinity solution (Naseri et al. 2012). Emam (2011) indicated that seed germination and seedling establishment are the periods when barley is most sensitive to salinity, as was observed for introduced cultivars 'Rihane-03' and 'IPA7' and improved Tunisian genotypes.

Principal component analysis (PCA) based on all variables was used to discriminate between genotypes (Fig. 3). PCA1 axis, which explained 33% of total variability, is a linear combination of CC, LA and GY. PCA2 axis represents 25% of total variability and is influenced by PH, PDL and SDW. PCA could distinguish four groups, one of which was adapted to abiotic stress, shown by good GY.

The first group was composed of genotypes with the best morphological performance. The second group was a

subgroup of the first group and was composed of genotypes in which the best GY was observed, indicating successful completion of the life cycle, and included some introduced varieties such as the two Batini landraces '113/1B', '100/1B' and 'Giza 125'. This result was similar to the result of evaluations for salt tolerance by Al-Dakheel and Belhaj Fraj (2012) and Jaradat et al. (2004) for Batini, and by Noaman (1995) and Jiang et al. (2006) for 'Giza 125'. In addition, the Tunisian landraces Arbi Abidh and Ardhaoui fall into this second group. El Faleh et al. (1991) showed that under abiotic stress, the local landraces barley 'CRG134' and 'CRG334' performed better than improved barley such as 'Ceres', 'Martin', and 'Faiz Rihane'.

The third group was composed of genotypes with poor GY, including improved Tunisian genotypes ('Konous', 'Rihane' and 'Manel') and two introduced accessions, 'IPA7' and 'Alanda-01'. Najar et al. (2010) indicated that improved Tunisian genotypes ('Manel', 'Rihane' and 'Martin') were developed for favourable growing conditions in northern Tunisia. The fourth group was composed of genotypes with the highest percentage of dead leaves, which was associated with very poor biological performance and GY. The percentage of dead leaves has been used in several studies to screen tolerance to abiotic stress, especially salt stress, such as in bread wheat (Michael et al. 2011). Avoiding leaf senescence allows the plant to maintain transpiration and increase photosynthates, which accumulate during the



Fig. 3. Principal component analysis of 26 barley accessions

crop's life cycle (Borrell et al. 2001). This was also observed in our study since, according to PCA, this parameter was inversely correlated with the leaf chlorophyll content (Fig. 3). According to Munns et al. (2006), acceleration of senescence was due to either high leaf Na<sup>+</sup> concentrations or to low tolerance of the accumulated Na<sup>+</sup>.

## Conclusions

Introduced cultivars ('113/1B,'100/1B' and 'Giza 125') and local genotypes ('Arbi Abidh' and 'Arthaoui') showed the highest tolerance at a high level of salinity under a Saharan climate. These genotypes can be profitable in marginal areas using brackish water and, through appropriate selection and breeding programs, can be utilized for further improving salt tolerance of Tunisian barley genotypes. The study of the dynamics of soil salinization showed no risk of salt accumulation in sandy soils of the Chamsa region, suggesting the sustainability of barley production when irrigated with saline water.

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