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Intoduction and Evaluation of Salt- Tolerant Barley Gentypes

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ABSTRACT

Drylands are extensively spread in southern Tunisia where there are sizeable underground saline water that can to be used for sustainable production of salt-tolerant plants. Barley is a relatively salt-tolerant plant, compared to other Poaceae species. In order to identify salt-tolerant barley genotypes, we evaluated and compared the effect of salinity on eight genotypes. We have used two levels of saline irrigation water (6 and 12 g NaCl.L^{-1}) along with a non-saline control. Our results showed that for all genotypes, plant height was significantly reduced at 12 g NaCl.L^{-1}. However at this level of stress, leaf surface was reduced in some genotypes. It is worth to note that high salinity did not reduce leaf surface in both Tunisian varieties Manel and Rihane as well as in the introduced PK30118. These improved genotypes showed a strong decrease in their chlorophyll content, assessed by SPAD measurement. Yield parameters analysis showed that the local population Ardhaoui, Pak1, PK30109 and PK30046 exhibit a high number of ears/m^2, independently of the salinity level of the irrigation water. The Tunisian improved cultivars as well as PK30118 and PK30130 were severely affected when irrigated with a 12 g NaCl.L^{-1}. The thousand grains weight parameter was affected for all study genotypes at severe salinity except the local genotype Ardhaoui. The global yield analysis showed that in the absence of salinity, improved cultivars Manel, Rihane and the Pakistani lines PK30118 and PK30130 showed the highest yields. The lowest yield reduction at moderate stress was observed with Pak1 and with Ardhaoui with the severe stress. In general, our results showed that, only a severe salinity stress significantly affected barley lines confirming the salt tolerance of this specie. Moreover, our results indicated that salt sensitive genotypes are unable to restrict leaf expansion when submitted to salinity. On the whole, Manel and Rihane can be used only under non-saline conditions. The genotypes Pak1 and PK30046 can be also used under moderate salinity, while Ardhaoui can be used under both moderate and severe saline conditions.

Key words: Salinity tolerance, Barley genotypes, NaCl salinity, Leaf surface, SPAD, Yield components

INTRODUCTION

Salinity is a major abiotic stress affecting agriculture in the world. According to FAO, the area of land affected by salinity reached ca. 400 million hectares worldwide. Of the 230 million hectares of irrigated land, 45 million hectars are affected by salinity (i.e. 19.5%) while within 1500 million hectars of agricultural arid land, there are 32 million hectars (i.e. 2%) that became saline [1]. In North Africa salinity and drought are the major factors hampering crop production [2]. In Tunisia 10% of the total surface area are saline, i.e. 1.8 million hectares, mainly in the central and southern regions.

Various factors are related to the phenomenon of soil salinization. There are basically a primary salinization of natural origin, which is due to the proximity of the sea or the existence of geological salt deposits, and a secondary salinization due to human activities, particularly in poorly managed irrigated areas. Generally, soil is classified saline when the electrolytic conductivity (EC) exceeds 4 dS/m or the equivalent osmotic potential of approximately 0.2 MPa. Beyond this threshold of the EC, the majority of plants undergo a reduction of growth [1].

Salinity induces two major stresses on plant tissues. (i) Osmotic stress which is a water deficit resulting from the relatively high solute concentrations of the soil solution, and (ii) ion imbalance which mainly induced and alteration in the K^+/Na^+ ratio and a concentration of the Na^+ and Cl^- to toxic levels. As a consequence of these primary effects, secondary stresses such as oxidative damage often occur. As a consequence, cellular functions are disrupted, such as photosynthesis. Plants can tolerate salt by developing several mechanisms. These include osmotic adjustment, ion exculsion and tissue tolerance.

Barley (*Hordum vulgare*) which is considered as a relatively salt tolerant plant can be used with saline irrigation water. Indeed, it was shown that barley yield is unaffected up to about 8 dS/m, but salinity induces a 50% loss at 18 dS/m [3]. In Tunisia, the overall area for the barley crop is ca. 500 000 ha. Much of the lands cultivated with barley are located in semi-arid areas. From 1997 to 2007, the average production reached 39 billion tons. The yields are not only very low but also very variable from year to year due to irregular rainfall. However, barley is considered as an important crop, mainly for small farmers. Irrigation water in Tunisia is characterized by its high salinity. In fact, about 50% of underground water has more than 3 g/l. This brakich water can be used for barley production using salt tolerant genotypes.

In order to identify the more salt-tolerant barley genotypes hat can be used under saline irrigation water in field conditions, we evaluated the impact of salinity on eight genotypes of barley. We used two levels of saline irrigation water (6 and 12 g NaCl/l), along with a non-saline water as a control. Evaluation was done according to morphological and physiological parameters.

MATERIALS AND METHODS

Plant material

We have used a total of eight genotype of barley (*Hordeum vulgare* L.) cultivars. Two local varieties : Manel and Rihane; one local population “Ardhaoui Tataouine” and five varieties introduced from Pakistan : Pak1, PK30118, PK30109, PK30130 and PK30046.

Experimental design

Field experiment was performed at the National Institute of Agriculture of Tunis under a shelter to avoid rainfall interference and salt leaching.

Seed of different genotypes were sown at a density of 250 plants/m^2, in November 2010 according to an incomplete random block model with three replicates. Each replicate is a 0,4 m line. To avoid border and plot edge effect, assays were placed in the center of plots whose borders were sown to the same density with a control barley genotype.

Irrigation was monitored using the program MABIA-Etc as described [4]. Irrigation was done using a control non saline (S0), a 6 g NaCl/l (S1) or a 1 2g/l (S2) water.

Measured parameters

Plant height:

Plant height was measured on the main tiller at grain maturity. Height of the plant was measured to the top of the spike in cm.

Leaf area:

This measurement was made on the flag leaf at grain maturity. Three leaves per repicate were used. Leaf area was determined by scanning the leaves and measuring the surface uising the freesoftware mesurim (http://pedagogie.ac-amiens.fr/svt/info/logiciels/Mesurim2/Telecharge. htm).

Chlorophyll content:

The chlorophyll content was measured at four different stages: tillering stage, booting , head emergence and medium milk.

The mesurement were done using a chlorophyll meter "SPAD-502 Konica Minolta". Measurments were done on 7 last emerged leaves per replicate.

Yield parameters:
At grain maturity, the following parameters were measured on all the plants of each replicate: number of ears/m², number of spikelets/spike, number of grains/spikelets and thousand grains weight (TGW). The reduction in estimated global yield was calculated using the following equation:

Yied reduction (%) = [(yield of control plants- yield of stressed plants)/yield of control plants]* 100

Statistical Analyses
One-way ANOVA was carried out to test for differences between treatments. When appropriate, differences between means were compared using the Duncan Test, SPSS V16.0.

RESULTS

Overall, there are significant differences ($P < 0.05$) beteween the genotypes for the study parameters (Table 1), which might be due to their divergent geaographic origins and/or different genotypic/physiological aptitude to tolerate salinity.

Plant height and leaf area
At moderate stress, all genotypes' height, except the improved cultivar Manel, was unaffected compared to the non saline control. This does confirm the classification of barley as a relatively salt tolerant plant. Under severe stress, the average plant height decline significantly compared to control plants for all study genotypes; up to 69 cm on average in the genotype PK30046 which represents a reduction of 46.61 % (Table 2).

In this study, we found that the leaf area of the local improved genotypes Rihane, Manel, as well as the Pakistani PK30118 and PK30046 cultivars, drops when the salinity of the water of irrigation increases but in a non significant way even in severe stress conditions. This is not the case of genotypes Ardhaoui, Pak1, PK30109 and PK30130. For Ardhaoui and Pak1 lines only a severe stress affects the leaf area (Table 2). Interestingly, among Tunisian cultivars, leaf area was reduced in Ardhaoui genotypes while it was not for the improved cultivars.

Photosynthetic activity
The photosynthetic activity as expressed by chlorophyll content is not affected by salinity in barley genotypes during the vegetative (tillering) and reproductive stages (booting, head emergence) (data non shown). At medium milk stage, we have noticed that the chlorophyll content is reduced significantly under stress conditions for some genotypes. Ardhaoui and Pak1 were not affected even at high salinity level. Among the other genotypes, the improved varieties Manel and Rihane were characterized by the largest drop of chlorophyll content, compared to the control (Table 2). This indicates a shortening in the maturation-filling stage under salt stress.

Number of ear/m² and number of spikelets/spike
In the improved varieties Rihane and Manel and Pakistani variety PK30130, the number of ears/m² declined significantly under severe salinity. The percentage of these reductions is about 48.36% in Rihane; 30.74% in Manel and 49.10% in PK30130. PK30118 was even sensitive to the moderate salinity. However, for other genotypes such as Ardhaoui and Pak1 lines, PK30109 and PK30046, the number of ears/m² was stable regardless of the treatment (Table 2).

In the non saline irrigated plants, both improved varieties (Manel and Rihane) showed the highest number of spikelets per spike indicating a high yield potential in non stressed conditions (Table 2). This parameter is sharply affected by salinity even at the moderate salinity. This is also the case for the PK30118, which was sensitive to salinity as its number of eras was also reduced. The addition of 6 g NaCl/l also affects PK30130 although to a lower extent. The other genotypes (Arthaoui, Pak1, PK30109, PK30046) were not affected by salinity indicating a relative tolerance at the early stages of of spikes' initiation.

Number of grains/spikelet and the thousand grains weight
This parameter is an indicator of flower fertility. Only Ardhaoui, Pak1 and PK30109 genotypes were slightly affected by salinity (Table 2). It is interesting to note that these genotypes had stable number of spikelets per spike. These genotypes regulated their production later than the other by aborting some flowers.

With the exception of PK30046 whose thousand grains weight (TGW) is relatively low compared to other genotypes (35.35 g), all other genotypes showed an average TGW of 43.7 g in PK30109 to 47.29 g in PK30118 (Table 2). Ardhaoui was the only genotype whose TGW is stable, regardless of the salinity level. The two improved varieties Manel and Rihane have decreased their TGW under severe salinity compared to control. Both varieties displayed reduced photosynthetic activity under 12g NaCl/l irrigation. Grain filling period was shortened which can explain the decrease in the TGW.

Grain yield and yield reduction

In non-saline conditions, the two Tunisian improved varieties (Manel and Rihane) as well as the introduced varieties PK30118 and PK30130 showed the highest yields (4.31, 48.6, 5.56, 4.89 T/ha, respectively). Moderate and severe salinity had induced a reduction of the grain yield of all studied genotypes. However, it is worth to note that the genotypes that gave the highest grain yield in non-saline conditions showed the sharpest yield drop at both moderate and severe salinity (Table 3).

At moderate salinity, Pak1 and PK30046 showed the lowest yield reduction, suggesting that they are the most tolerant to moderate salinity (Tables 2 and 3). Severe salinity induced more than 50% yield reduction for all the study genotypes. In these conditions, Ardhaoui, Pak1, PK30046, and to a lower extent PK30109 are likely to be the most tolerant genotypes as they had the lowest yield reduction (Table 3).

DISCUSSION

Plant height, leaf area, chlorophyll content and yield paramters showed a significant reduction, mainly under severe salinity. On the whole and at moderate salinity, plant height of the study barley genotypes was not reduced; thus supporting that barley is a relatively salt tolerant plant. Decrease in plant height is due to the shortening of the internodes, mainly as a consequence of the osmotic component of the salt stress [1].

In barley, in a hydroponic culture, it has been shown that the leaf area can be reduced under saline conditions [5]. However, among Tunisian genotypes, leaf area was reduced in Ardhaoui while it was not for the two improved varieties (Manel and Rihane). This might suggest that leaf area reduction is an efficient mechanism of tolerance to salinity, in accordance with the findings of [6], that have suggested that the reduction in the foliar index can be used as tolerance strategy by reducing the effects of the osmotic component of the salt stress.

The photosynthetic activity is not affected by salinity in barley genotypes during the vegetative and reproductive stages. At medium milk stage, photosynthetic activity is reduced under salt stress conditions for some genotypes, while Ardhaoui and Pak1 were not affected even at high salinity level.

The relative stability of the number of fertile tillers in some genotypes can be related to their relative salt tolerance compared to the other genotypes. Because tillers and ears are developed at early growth stage of barley, these results can also be due, in part, to the soil quenching capacity in the sodium ion. The reduction of tillers was generally assumed to be one of the major effects of salinity in cereals [1].

It has been shown that salinity strongly shorten the duration of the spike differentiation, leading to a reduced number of spikelets per spike in wheat and barley [7-9]. However, the genotypes Ardhaoui, Pak1, PK30109, PK30046 were not affected by salinity, indicating a relative tolerance at the early stages of spikes' initiation. The number of grains per spikelet was slightly affected by salinity in Ardhaoui, Pak1 and PK30109 genotypes. These genotypes seemed to modulate their production process later than the other study genotypes, by aborting some flowers.

Under severe salinity, the highest yield was obtained with the Ardhaoui genotype. This genotype was the only one to maintain a stable TGW associated with a stable chlorophyll content at late stages of development. Leaves are maintained green longer allowing a good translocation of reserves to the grains. This phenomenon called "stay green" is used as a marker of salinity tolerance [10].

Table 1. Statistical analysis of morphological and physiological and yield parameters under NaCl salinity

S.O.V.	ddl	Plant height	<Leaf area	Chlorophyll content	Number of ears / m²	Number of spikelets / spike	Number of grains / spikelets	TGW
Genotypes (G)	7	1,70ns	5,40 **	5,41**	1,69ns	1,57ns	1,69ns	6,93**
Salinity levels (S)	2	85,24 **	17,89 **	26,91**	17,60 **	8,72**	1,69ns	40,78**
Interaction (G) x (S)	14	1,20ns	0,53ns	0,94ns	1,20ns	1,15ns	0,63ns	2,14*
C.V.(%)		9,63	23,66	11,83	23,02	6,78	18,87	11,07
R^2		0,83	0,62	0,62	0,57	0,68	0,53	0,76

*ns : non significant ; * : significant at $p< 0,05$; ** : significant at $p< 0,01$*

Table 2. Mean values of some morphological and physiological and yield parameters under NaCl salinity.

Genotypes	Salt levels	Plant height (cm)	Leaf area (cm²)	Chlorophyll content (SPADvalues)	Number of ears / m²	Number of spikelets / spike	Number of grains / spikelets	TGW (g)
		25,08	4,99	6,9	87	3,92	0,17	17,35
Rihane	**S0**	123^{a}	19,36^{a}	45,12^{a}	275^{a}	21,01^{a}	1,66^{a}	44,87^{a}
	S1	119^{a}	17,73^{a}	40,63^{a}	188^{a}	14,43^{b}	2,06^{a}	42,72^{a}
	S2	82,5^{b}	16,62^{a}	24,48^{b}	142^{b}	12,35^{b}	2,04^{a}	33,77^{b}
Manel	**S0**	133,8^{a}	21,61^{a}	44,78^{a}	283^{a}	21,78^{a}	1,73^{a}	46,16^{a}
	S1	108,7^{b}	16,46^{a}	39,06^{a}	258^{a}	14,90^{b}	1,79^{a}	45,56^{a}
	S2	88,3^{c}	14,86^{a}	28,69^{b}	196^{b}	13,74^{b}	1,67^{a}	28,91^{b}
Ardhaoui	**S0**	135^{a}	19,00^{a}	44,98^{a}	246^{a}	14,72^{a}	2,38^{a}	46,80^{a}
	S1	130,3^{a}	15,91^{a}	40,59^{a}	242^{a}	14,23^{a}	1,92^{b}	44,93^{a}
	S2	88^{b}	8,52^{b}	33,36^{a}	188^{a}	16,56^{a}	1,72^{b}	44,25^{a}
Pak1	**S0**	123^{a}	18,76^{a}	39,20^{a}	258^{a}	14,72^{a}	1,88^{a}	41,97^{a}
	S1	125,5^{a}	18,14^{a}	41,19^{a}	208^{a}	14,23^{a}	1,71^{a}	38,48^{a}
	S2	89^{b}	10,92^{b}	32,76^{a}	192^{a}	16,56^{a}	1,63^{b}	32,51^{b}
PK30118	**S0**	127,8^{a}	23,13^{a}	45,36^{a}	363^{a}	18,59^{a}	2,16^{a}	47,30^{a}
	S1	125,7^{a}	21,68^{a}	37,30^{b}	208^{b}	12,91^{b}	2,04^{a}	39,91^{a}
	S2	95^{b}	19,71^{a}	32,19^{b}	213^{b}	12,63^{b}	2,11^{a}	34,10^{b}
PK30109	**S0**	132,3^{a}	18,77^{a}	37,25^{a}	225^{a}	18,05^{a}	1,98^{a}	43,70^{a}
	S1	129,5^{a}	12,73^{b}	36,62^{a}	179a	17,35^{a}	1,83^{a}	36,69^{b}
	S2	-	10,47^{b}	29,38^{b}	188^{a}	16,31^{a}	1,50^{b}	33,02^{b}
PK30130	**S0**	137^{a}	20,67^{a}	47,02^{a}	279^{a}	15,89^{a}	2,28^{a}	48,11^{a}
	S1	122,7^{a}	15,68^{b}	40,12^{b}	258^{a}	11,97^{b}	2,04^{a}	31,41^{b}
	S2	89,5^{b}	10,88^{c}	34,75^{b}	142^{b}	13,59^{b}	2,01^{a}	30,09^{b}
PK30046	**S0**	128^{a}	27,15^{a}	41,32^{a}	238^{a}	21,38^{a}	1,97^{a}	35,35^{a}
	S1	120^{a}	22,25^{a}	34,71^{a}	258^{a}	16,05^{a}	1,93^{a}	33,52^{a}
	S2	69^{b}	19,24^{a}	27,39^{b}	213^{a}	14,65^{a}	1,90^{a}	28,02^{b}

- Missing data ; letters indicates different classes at $P<0.05$

Table 3. The grain yield of barley genotypes in tons per hectare and the yield reduction expressed in %.

Genotype	Rihane	Manel	Ardhaoui	Pak1	PK30118	PK30109	PK30130	PK30046
Global yield (S_0) (T/ha)	4,31	4,86	4,19	3,34	5,56	3,49	4,89	3,48
Yield reduction (S1)	45,48	35,90	29,15	18,73	60,79	41,59	56,08	26,25
Yield reduction (S2)	71,21	73,77	45,43	51,85	63,22	57,77	73,25	51,70

CONCLUSION

We can conclude that saline irrigation water differently affected the study eight barley genotypes. The two Tunisian improved varieties (Manel and Rihane) seemed to be the best candidates but only under non-saline conditions. The genotypes Pak1 and PK30046 can be used under moderate salinity, while the Tunisian Arthaoui genotype can be used under both moderate and severe saline conditions. All of the study genotypes are now screened under field conditions where they are irrigated with different levels of saline water.

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