Genotypic differences in root and shoot growth of barley (Hordeum vulgare L.) grown under different salinity levels

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In water-limited areas of Tunisia, more than 30% of subsurface water used for irrigation is saline water, leading to a long-term salinization and degradation processes. To prevent the problems and to minimize the negative impact of using saline water, selection of proper germplasm as well as integrated soil and water management are essential. Understanding the diversity for salt tolerance in barley (Hordeum vulgare L.) genotypes will facilitate their use in genetic improvement. Our objectives were to evaluate, in green, house-salt tolerance for 14 barley cultivars under three different salinity levels: (tap water with an Ec = 0.73 dSm⁻¹, tap water with 102 mM of NaCl, Ec = 10.76 dSm⁻¹ and tap water with 151 mM of NaCl, Ec = 15.38 dSm⁻¹), and quantify genetic variation based on salt tolerance index, morphological traits, molecular and factorial analysis of correspondence (FAC). The study was conducted in a randomized complete block design arranged as a split plot. The results indicate a great genetic variability to salt tolerance among used barley genotypes. Consequently, it is possible to identify superior cultivars, and evaluate the genotypic performance under salinities conditions. In addition, results obtained were confirmed by clustering made by SSR tool on the base of DNA analysis, which is compatible with the arrangement obtained with statistical method.

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Tolerance to salinity stress can be defined as the capacity of the plant to take up sufficient quantities of water from the soil despite a low water potential, and to tolerate sodium toxicity and deficiency in other minerals antagonistic to sodium and chloride (ELLIS et al. 2002).

Salinity stress remains one of the world’s oldest and the most serious environmental problems, which substantially hampers crop productivity in many arid and semi arid regions (CLARK and DUNCAN 1993). Several reclamation measures involving additions of gypsum, sulphur and sulphuric acid to soils and construction of wells and drainage canals have been applied in Tunisia to reduce concentrations of salts in root zone (CHIONG and YUN 2007). However, these practices were discontinued due to escalating energy costs. The development of salt tolerant crop cultivars presents an alternative to expensive approaches to bring saline marginal lands under cultivation (HOLLINGTON 1998). Understanding the diversity for salt tolerance in barley (Hordeum vulgare L.) landraces will facilitate their use in genetic improvement. Indeed, there is a potential for improving salt tolerance through selection and breeding in cultivated species such as barley (MAAS and HOFFMAN 1977; KOVAL 2000).

Powerful new molecular tools for manipulating genetic resources are becoming available (MUNNS 2005). A locus for the low-Na⁺ trait was mapped to the long arm of chromosome 2A using a quantitative trait locus (QTL) approach (LINDSAY et al. 2004). The same authors identified several markers linked to a gene at a QTL designated Nax1 (Na⁺ exclusion). MUNNS et al. (2006) showed that also a region on the long arm of chromosome 1 contain a quantitative trait locus (QTL) for Na⁺ exclusion and K⁺/Na⁺ discrimination. Major increases in salt tolerance would be possible by introducing new genes either by crossing with new donor germplasm or by transformation with single genes.

The plant-breeding approach requires methods for efficient screening and identification of salt-tolerant genotypes. Based on current knowledge of the physiological mechanisms of salt tolerance, identifying traits such as grain yield (GY) for screening in early generations has not been possible. Recently, ROYO et al. (2000) concluded that barley genotypes with deeper root system and highest yield in nonsaline conditions were also the most productive at medium and high salinity levels. However, unless these results can be confirmed and generalized in further trials and for other crops, we still need to measure GY and some roots parameters under saline conditions to reliably identify salt-tolerant barley genotypes (ISLA et al. 1998; SAYAR et al. 2007).

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Barley (*Hordeum vulgare* L.) is a classical ‘salt excluder’, characterized by low rates of Na⁺ transport to the shoot, thus keeping mesophyll cells as Na⁺-free as possible (Colmer et al. 2005; James et al. 2006). Indeed, barley is generally more salt-tolerant than durum wheat (*Triticum turgidum* ssp. *durum*) and this is thought to be due to a more vigorous root growth (Jaradat et al. 2004). Accordingly, most work on improving salt tolerance has focused on increasing root growth (Sayar et al. 2007), although no commercial varieties based on this approach have been released (Flowers 2004; Munns et al. 2006).

Recent studies on barley have suggested that the magnitude of NaCl-induced efflux from the roots of young seedlings shows a strong negative correlation with salt tolerance as measured by grain yield under greenhouse conditions (Chen et al. 2005, 2007). These results suggest that, although salinity is a complex multigenic trait (Flowers 2004), over 70% of the genetic variability in barley is attributed to just one physiological trait which is the root ability to exclude salt. The latter trait shows high heritability (Chen et al. 2005, 2008). Thus, measurements of growth of roots and aerial parts upon the application of NaCl could be used as a screening tool for tolerance to salt. Whether, this is true for some other crops needs to be validated.

In Tunisia, barley is commonly grown on marginal soils under rainfed (natural and non-irrigated) conditions. It often suffers from drought and salt stress. It is worth noting that most of the high-yielding barley varieties that are being introduced into the country from various sources are not sufficiently salt tolerant, hence, there is a need for the development of a specific barley breeding programme for salt tolerance. The present study was conducted with the objective of identifying lines to be used in a breeding programme to develop salt-tolerant barley varieties. The underlying hypothesis of the study is that salt tolerance correlates positively with the number of primary roots as well as negatively with aerial parts of the plant (Sayar et al. 2007). This relationship may be exploited as a selection tool in barley breeding. Thus, measurements of root, salt tolerance index and some yield components might be useful as ‘physiological markers’ for salt tolerance in cereal breeding programmes.

**MATERIAL AND METHODS**

*Greenhouse experiment and plant material*

Whole plant responses to salinity were studied in a greenhouse experiment without supplemental lighting. Relative humidity was maintained at about 70% (± 5), and the day/night temperature was 24/16°C (± 2). Seeds of each genotype were previously sterilized with 5% calcium hypochlorate for 10 min and thoroughly washed with sterile deionized water. Five seeds of the fourteen barley (*Hordeum vulgare* L.) cultivars (Table 1) were sown in soil-filled polyethylene tubes (20×133 cm) containing 70% vertisol and 30% sand. All seeds were irrigated with tap water (0 mM NaCl) until 15 days after sowing (DAS). Plants were thinned to one per tube 14 DAS. Plants were irrigated with the assigned saline solution (Ec = 0.73 dSm⁻¹, Ec = 10.60 dSm⁻¹, Ec = 15.38 dSm⁻¹) at 15 DAS. Irrigation occurred every 5 d and involved wetting the soil to beyond field capacity (0.51 plant⁻¹). The 0.73 dSm⁻¹ saline solution was used to simulate natural field conditions. The 10.60 dSm⁻¹ salinity level was chosen to represent the predominant salinity level of saline water aquifers in Tunisia (Ben Naceur et al. 2005). Thus, more than 65% of the water used for cereal irrigation has a salinity varying from 4.7 to 10.94 dSm⁻¹. The 15.38 dSm⁻¹ was used to discriminate the most tolerant genotypes to salt stress.

**Table 1. Origin or pedigree of used barley cultivars.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>JND1</td>
<td>Local cultivar from Jendouba region in the north west of Tunisia (subhumid)</td>
</tr>
<tr>
<td>JND2</td>
<td>Local cultivar from Jendouba region in the north west of Tunisia (subhumid)</td>
</tr>
<tr>
<td>KLA</td>
<td>Local cultivar from Kalaa region in the north east of Tunisia (semiariad)</td>
</tr>
<tr>
<td>KSR</td>
<td>Local cultivar from Kasserine region in the central west of Tunisia (semiariad)</td>
</tr>
<tr>
<td>KBL1</td>
<td>Local cultivar from Kebili region in the south west of Tunisia (saharian)</td>
</tr>
<tr>
<td>KBL3</td>
<td>Local cultivar from Kebili region in the south west of Tunisia (saharian)</td>
</tr>
<tr>
<td>KLB2</td>
<td>Local cultivar from Kebili region in the north east of Tunisia (costal)</td>
</tr>
<tr>
<td>MNL</td>
<td>Line527/5/As54/Tra/2/Cer/Tol1/3/Avt/Tol1/1CB81-607-1Kf-12Bj-1BJ-1BJ-0Bjselected 1996</td>
</tr>
<tr>
<td>MRT</td>
<td>Six row variety, late, selected from an Algerian population in the high plateau since 1931</td>
</tr>
<tr>
<td>RHN</td>
<td>Atlas 46 /Arivat //Athenais ICB76-2L-1AP-0AP selected at ICARDA (1976)</td>
</tr>
<tr>
<td>SBZ</td>
<td>Local cultivar from Sidi Bouzid region in the central of Tunisia (semiariad)</td>
</tr>
<tr>
<td>SWH</td>
<td>Local cultivar from Sahel region in the central of Tunisia (costal)</td>
</tr>
<tr>
<td>TZ1</td>
<td>Local cultivar from Kebili region in the south west of Tunisia (saharian)</td>
</tr>
<tr>
<td>TZ2</td>
<td>Local cultivar from Kebili region in the south west of Tunisia (saharian)</td>
</tr>
</tbody>
</table>

Abbreviation: Jendouba 1 = JND1, Jendouba 2 = JND2, Kalaa = KLA, Kasserine = KSR, Kebili 1 = KBL1, Kebili 3 = KBL3, Kebili 2 = KLB2, Manel = MNL, Martin = MRT, Rihane = RHN, Sidi Bouzid = SBZ, Swihli = SWH, Tozeur 1 = TZ1, Tozeur 2 = TZ2.
Root system, biomass and yield measurements

At maturity (Zadoks scale, Z9.9), aerial parts of the plant were cut at the soil line and its relative yield components recorded for each tube. The plants were removed from the tubes and the roots were carefully washed to remove soil and laid flat and root characteristics were recorded. Primary root number (RN) and dry matter (RDM) was measured. Plant organs which were used for analysis of morphological traits were dried at 60°C in a Unitherm Dryer (Birmingham, UK) to a constant weight and then weighted again. GY and its components were recorded.

Relative variation (D) due to salinity stress was calculated as the difference between value under saline (Xs) and non-saline condition (Xt) divided by the value under saline condition:

\[ D = \frac{(X_s - X_t)}{X_s} \]

with: D = deviation (%); Xs = value under saline condition; Xt = value under non-saline conditions.

Factorial analysis of correspondence (FAC)

FAC was utilized to group all measured and derived variables into the minimum number of components that can account for the maximum variance available in the multivariate data set (Fig. 2).

Genetic variability analyses

Genetic variability among genotypes was done using the SSR (simple sequence repeat) method. DNA extraction, purification, quantification and then PCR amplification were performed as described by Abdelraouf et al. (2007).

Statistical analysis was carried out using MSTATC (ver. 1.2) and Minitab (ver. 12.1) at the 5% level. Genetic diversity distance is calculated using UPGMA procedure according to Nei and Li (1979). Variables were further analyzed based on Duncan multiple range tests at the 0.01 significance level. Mean values of the variables were compared with those at the lowest salt level as the controls.

Experimental design

The study was conducted in a randomized complete block design arranged as a split plot with salinity level as the main plot factor and accession (genotype) as the subplot factor. The total number of plots sown was 126 (14 cultivars × 3 replications × 3 salinity treatments). Data for each variable from all replicates within a salinity treatment were combined for statistical analyses. Correlations between two traits were evaluated using linear correlation analysis. The positive and significant correlation coefficients (r < 0.92, P < 0.01) found among replicates of a certain treatment, were considered as indicators of repeatability of the experiment.

Data analyses

All statistical analyses were conducted with several modules in the statistical packages MSTATC (ver. 1.2) and Minitab (ver. 12.1). Genetic diversity distance is calculated using UPGMA procedure according to Nei and Li (1979). Data for each variable were plotted to test for normality, and the homogeneity of variances among cultivars was verified by a Bartlett’s test (Zar 1996). Factorial analysis of correspondence (FAC) was done with BIOMECO package (ver. 12.2). Data are separated into three equal classes (Fig. 2). Each character is then subdivided in low level (class 1) medium (class 2) or high (class 3). The first axis (horizontal) represents the axis of GY. The parameters contributing to GY production are represented to the second axis (vertical).

RESULTS

Knowledge of a plant’s response to salinity, which may or may not involve a unique physiological response, is important in determining the most efficient method of increasing yield under salt stress. Screening for salt tolerance under controlled conditions is more efficient and less expensive than screening under field conditions (Shannon and Noble 1990).

Whole plant responses

Differences among cultivars for all measured parameters within each salinity level were highly significant (P < 0.01). In addition, the highest yielding genotypes at the low or medium salinity levels were not the highest yielding at the high salinity level. The genotypic responses to salinity also depended on the intensity of salinity.
were not necessary the highest RN at the high salinity level. On the other hand, KBL1 ranked first, fourth and fourth at 0.73, 10.60 and 15.38 dSm$^{-1}$. At 0.73 dSm$^{-1}$, RN varied from 136 (TZ2) up to 260 (KBL1). However, at 10.76 dSm$^{-1}$, it varied from 115 (RHN) to 289 (KBL3). The same variations were observed at 15.38 dSm$^{-1}$, where RN varied from 143 (MNL) to 271 (KLB2) (Table 3).

Surprisingly, RN was stimulated by salinity stress for KLA, KSR, KLB2, SBZ and TZ2 ecotypes. Although, RN increased for KBL3 and SWL ecotypes at 10.76 dSm$^{-1}$ but it decreased at 15.38 dSm$^{-1}$ for the same genotypes.

### Responses on the number of primary roots (RN)

The number of primary root (RN) varied widely among cultivars in response to salinity treatments (Table 3). Differences among genotypes for RN within each salinity level were highly significant (P < 0.01). In addition, the highest RN cultivars at the low or medium salinity levels were not necessary the highest RN at the high salinity level. On the other hand, KBL1 ranked first, fourth and fourth at 0.73, 10.60 and 15.38 dSm$^{-1}$ (Table 3). The overall means of RN were around 194, 198 and 206 per plant at 0.73, 10.76 and 15.38 dSm$^{-1}$. At 0.73 dSm$^{-1}$, RN varied from 136 (TZ2) up to 260 (KBL1). However, at 10.76 dSm$^{-1}$, it varied from 115 (RHN) to 289 (KBL3). The same variations were observed at 15.38 dSm$^{-1}$, where RN varied from 143 (MNL) to 271 (KLB2) (Table 3).

**Table 2. Analysis of variance for physiological traits.**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Treatment</th>
<th>Genotype</th>
<th>Treatment × genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary root number (RN)</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Root dry matter (RDM)</td>
<td></td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>Spike number (SN)</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Kernel number by spike KNS$^{-1}$</td>
<td></td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Grain yield production (GY)</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

* P < 0.05, ** P < 0.01, NS: not significant.

The effect of salt stress on root dry matter (RDM) was influenced by ecotype and salt stress intensity. It was highly significant (P < 0.01) for genotype effect, whereas, interaction genotype × salinity was not significant for RDM (Table 2). Depending on the salinity stress, RDM reached 2.89, 2.15 and 1.74 g plant$^{-1}$ respectively at 0.73, 10.76 and 15.38 dSm$^{-1}$. Within each salinity level, RDM increased four-fold. It varied from 0.81 (TZ1) to 4.38 (JND 1) at 0.73 dSm$^{-1}$. Even at 10.76 dSm$^{-1}$, it was 1.08 (TZ 1) and 3.94 (KLB2). The same trend was observed at 15.38 dSm$^{-1}$ where RDM ranged between 0.89 (MNL) and 3.15 g plant$^{-1}$ (KLB2) (Table 3).
values showed a decrease of 35 and 38% when salinity level increased from 0.73 to 10.76 and from 0.73 to 15.38 dSm⁻¹ respectively.

**Grain yield and other relative variables**

Based on the analysis of variance, the overall effect of salinity indicated highly significant (P < 0.01) on GY and SN, but it was significant (P < 0.05) on KNS⁻¹. The overall effect of genotype was highly significant (P < 0.01) on grain yield and other relative variables except KNS⁻¹. The interaction between salinity and genotype had no significant effect on RDM and KNS⁻¹. However, a highly significant effect (P < 0.01) was noted on GY and SN (Table 2). However, RN and SN presented a slight increase at 10.76 and 15.38 dSm⁻¹ depending on the genotype. For the three salt treatments, the SN mean was about 19.6. Within each treatment, the SN varied from simple to at least two fold. More than 50% of the tested genotypes increased their SN under saline condition comparatively to the control (0.73 dSm⁻¹). Additionally, KLA, TZ2, JND2 and KSR ecotypes have maintained continuous SN increase under saline condition as compared to non-saline one. Positive deviation was superior at 15.38 dSm⁻¹ than at 10.76 dSm⁻¹ (Table 3).

The KNS⁻¹ overall mean was 32 under the three salinity levels. It varied between 19 and 33 at 0.73 dSm⁻¹, 24 and 39 at 10.76 dSm⁻¹, and finally between 26 and 35 at 15.38 dSm⁻¹ (Table 3). In fact, salinity stress stimulated KNS⁻¹ for JND2, KLB2, MRT, SBZ and TZ1 ecotypes.

The overall means of GY were around 24.80, 25.79 and 22.18 g plant⁻¹ at 0.73, 10.76 and 15.38 dSm⁻¹ respectively. KLB2 produced significantly higher GY at both salinity stress levels, as compared with the remaining genotypes. KLB2 grain yield varied from 32.5 g plant⁻¹ at 10.76 dSm⁻¹ to 38.25 g plant⁻¹ at 15.38 dSm⁻¹. Variation among cultivars (Tukey HSD, P < 0.05) was higher at the three salinity levels. GY was significantly reduced by salinity at 10.76 and 15.38 dSm⁻¹. On average, GY shows a reduction of about of 82% when salinity stress increased from 0.73 to 10.76 dSm⁻¹ and of 72% when salinity stress increased from 0.73 to 15.38 dSm⁻¹ respectively.

**Correlations analyses**

RN was positively correlated with KNS⁻¹ at 0.73 dSm⁻¹ (r = 0.73, 0.78 and 0.75 (P < 0.01) respectively at 0.73, 10.60 and 15.38 dSm⁻¹). Whereas, GY was positively and significantly correlated (r=0.65, 0.65 and 0.68 (P < 0.01)) with RN at 0.73 dSm⁻¹ under 0.73, 10.76 and 15.38 dSm⁻¹ salinity levels respectively. Under all salinity treatments, GY was highly (P < 0.01) positively correlated with all previously studied variables. However, the linear correlation between GY and RDM was not as pronounced or clear as was expected especially at 10.76 dSm⁻¹.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Salinity level</th>
<th>RN</th>
<th>RDM</th>
<th>SN</th>
<th>KNS⁻¹</th>
<th>GY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jendouba 1</td>
<td>0.73 dSm⁻¹</td>
<td>211.3</td>
<td>2.38</td>
<td>4.38a</td>
<td>22.67</td>
<td>32.3</td>
</tr>
<tr>
<td>Jendouba 2</td>
<td>10.76 dSm⁻¹</td>
<td>214.7</td>
<td>3.34</td>
<td>22.67</td>
<td>32.94</td>
<td>26.5</td>
</tr>
<tr>
<td>Jendouba 3</td>
<td>15.38 dSm⁻¹</td>
<td>208.7</td>
<td>2.70</td>
<td>37.56</td>
<td>32.67</td>
<td>23.0</td>
</tr>
<tr>
<td>Kos serine</td>
<td>0.73 dSm⁻¹</td>
<td>176.9</td>
<td>4.25</td>
<td>22.67</td>
<td>32.67</td>
<td>23.0</td>
</tr>
<tr>
<td>Kos serine</td>
<td>10.76 dSm⁻¹</td>
<td>178.7</td>
<td>4.25</td>
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<td>Kos serine</td>
<td>15.38 dSm⁻¹</td>
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<td>32.67</td>
<td>23.0</td>
</tr>
</tbody>
</table>

Means followed by the same letter(s) are not significantly different at P=0.05.
Molecular analysis

The constructed dendrogram (Fig. 1) separates the genotypes into two different groups (A and B). The first group (A) is subdivided into two subgroups A1 and A2, the genetic dissimilarity (GD) does not exceed 36.84%. In the subgroup A1, KLB2 is the best yielder. JND1 shows a proportional decrease in GY as salinity level increase. SWH ecotype produces high grain yield even under severe salinity stress. The second subgroup (A2) is composed of the ecotypes JND2, KLA and MRT, which were genetically related with each other at a GD < 37%. Based on morpho-physiological characters, these three genotypes responded differently to salt stress. The most important differences are shown under severe salt stress conditions where KLA shows an increase in RDM while JND2 shows an increase in KNS⁻¹. Even these differences in response to salt stress, the ultimate result (grain yield) is similar under mild salt stress.

The second group (B) is subdivided into five subgroups, for which RHN, MNL and KBL3 ecotypes constitute three separately subgroups. Genetic dissimilarity (GD) among these ecotypes is higher than 54%, 50% and 42% respectively comparatively to other genotypes. They share the same RDM at 10.76 dSm⁻¹. The fourth subgroup is composed by KBL1 and TZ1 ecotypes (GD=20%). They have a great similarity between them on the base of yield production and yield components under saline-conditions. The last subgroup contains KSR, SBZ and TZ2. No significant differences between these ecotypes were observed for agronomic traits. The only difference was observed in RDM at high stress salinity (15.38 dSm⁻¹), in which KSR’s ecotype presents the lowest RDM but has good agronomic traits.

DISCUSSION

Barley genotypes more often are confronted with higher salinity at the germination and early seedling growth stages than at later stages when plants are vigorously growing because germination and early seedling growth occur in surface soils where there is higher salt accumulation due to evaporation and capillary rise of water (Almansouri et al. 2001). Considering the physiological complexities of plant growth under salinity stress (Alvarado and Bradford 2002), the positive phenotypic correlations between GY and some morphological traits attributed under stress conditions (Table 4), can be exploited through phenotypic selection to increase salt tolerance at the adult plant level (Ashraf et al. 1986). Moreover, if salt tolerance is controlled by independent genes, it will be possible, upon genetic analysis, and by marker-assisted selection, to develop more resistant germplasm by combining genes for salt tolerance (Mano and Takeda 1997). For susceptible cultivars, salinity reduces the ability of plants to take up water, and this quickly causes reductions in growth rate, along with a suite of metabolic changes identical to those caused by water stress (Munns 2002). The reduction in shoot growth is probably due to hormonal signals generated by the roots (Miroslaw and Szarejko 2006).

There may be salt-specific effects that later have an impact on growth; if excessive amounts of salt enter the plant, salt will eventually rise to toxic levels in the older transpiring leaves, causing premature senescence, and reduce the photosynthetic leaf area of the plant to a level that cannot sustain growth (Widodo et al. 2009). Salt-tolerant genotypes differ from salt-sensitive ones in having a low rate of Na⁺ and Cl⁻ transport to leaves, and the ability to compartmentalize these ions in vacuoles to prevent their build-up in cytoplasm or cell walls and thus avoid salt toxicity (Mano et al. 2006). One of these mechanisms depends on the capacity for osmotic adjustment, which allows growth to continue under saline conditions.

Barley accessions displayed distinct responses to salinity (Table 3). In this sense, genetic variability within species offers a valuable tool for studying mechanisms of salt tolerance. High RN in salinity tolerant cultivars may be induced by high thickness or high specific root length which helps to explore more soil volume and more water reserve (Bchini et al. 2002; Sayar et al. 2007). According to Ben Naceur et al. (2004) and Abdelkouri et al. (2007), salt tolerance is attributed to an adaptation phenomenon. RDM reduction is due to the sensitivity of root hair to salt (Shabala et al. 2003). These results are consistent with Wu and Cogreve (2000) and Tester and Leigh (2001) who affirm that roots growing under saline conditions released chemicals, which modified the root environment and increased nutrient solubility allowing the roots to continue their elongation in low water potential while aerial part was stopped.

Traits related to salinity resistance are those correlated with yield production under stress. Correlations between SN and root variables showed that RN and RDM were determinant for the genotypic capacity to produce spike (r=0.78, 0.83, P < 0.01) for RN and (r=0.74, 0.80, P < 0.01) for RDM respectively at 10.76 and 15.38 dSm⁻¹.

The relationships between measured variables are of particular interest and permit to predict the RN via KNS⁻¹ and GY traits. Consequently, at maturity stage accessions with high yield under high salinity stress levels could be identified by selection simultaneously for high RN and/or high KNS⁻¹. Among yield components and roots parameters, SN and RN were the highly correlated under all salinity treatments (Fig. 2). Therefore, they may be considered as the best predictors of GY since they had on average a determination coefficient (R²) of 56% and 43% respectively (Table 4).

The level of GY production is related to the level of RN and SN (Fig. 2). SN can be considered as a direct criterion
of selection to salinity resistance. Salt tolerant genotypes are more able to promote RN than sensitive ones. Compensatory effects appears between SN and KNS\(^{-1}\) at the medium and high GY where KNS\(^{-1}\) is related to RDM level under saline treatments (Fig. 2). KNS\(^{-1}\) has compensatory effect dependence. It helps sensitive genotypes to promote their GY under saline conditions. Improvement of barley tolerance to saline conditions can be achieved by promoting some characters linked to yield production. Agronomic interventions to promote root characteristics under saline conditions could be useful ways.

Molecular analysis using the SSR (simple sequence repeats) method is also an important tool for screening barley genotypes. The constructed dendrogram (Fig. 1) separates the used cultivars into two groups. The first group (A) is mostly originated from the same ecogeographical region the north district of Tunisia where the rainfall is more than 450 mm. Except for the improved varieties (RHN and MNL), all the remainder ecotypes originated from the same ecogeographical region: the center (arid) and the south (desert) zones of Tunisia, where the rainfall does not exceed 250 mm. A study of the differentiation origin of these two groups (A and B) on the basis of molecular analysis, using GB318 primer, shows a clear geographical differentiation between them (Fig. 3). For example, results of GB318 show a different transition of alleles from the north to the south of Tunisia. This hypothesis based on morpho-physiological and molecular marker data is supported by reports of other researchers who evaluated these both characters in autochthonous cereal in Tunisia which constitute the second center of origin of cereal in the world (Hamza et al. 2004; Abdellaoui et al. 2007).

Results obtained with SSR method are consistent with those obtained with agronomic traits under the three water qualities. However, some behaviour appears among group A (A1 and A2) originate from the well rainfed zone of Tunisia. In this group, JND1 and JND2 ecotypes showed a decrease in GY production with increasing salt stress, while the other genotypes maintained or increase their GY production with increasing salt stress. Two hypotheses can be presented to explain this behaviour: 1) 37% of the GD between JND2 and MRT which constituted the same group (A2) is insufficient to explain different behaviour; even of the final grain yield is similar. 2) 15% of GD between MRT and KLA normally cannot explain their different physiological behaviour, but due to long adaptation period in coastal saline soil, KLA accession showed an increase in RN and RDM compared with MRT. This may be an adaptation phenomenon of this ecotype as it was suggested by Abdellaoui et al. (2007). But, adaptation phenomena does not explain for all types of reactions toward stress salinity. In fact, TZ1 and KBL1 genotypes are not genetically different (Fig. 1), but have contradictory reactions to salt stress. While KBL1 is salt tolerant, TZ1 remains in the group of low level production (Fig. 2). Genetic potential of production can be involved.

In conclusion, this study shows that (a) all cultivars exhibit variability in their tolerance to salinity; (b) root number (RN) and grain yield (GY) and its relative components were efficient predictors of barley varieties salt-tolerant. These results are useful to breeders and plant

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**Table 4. Simple regressions between grain yield (GY) and the other traits. Primary root number (RN), root dry matter (RDM), spike number (SN) and kernel number per spike (KNS\(^{-1}\)).**

<table>
<thead>
<tr>
<th>$\text{0.73 dSm}^{-1}$</th>
<th>$\text{10.76 dSm}^{-1}$</th>
<th>$\text{15.38 dSm}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{GY} = -2.60 + 0.140 \text{RN}^{**}; R^2 = 42%$</td>
<td>$\text{GY} = 4.61 + 0.109 \text{RN}^{**}; R^2 = 42%$</td>
<td>$\text{GY} = -3.02 + 0.121 \text{RN}^{**}; R^2 = 46%$</td>
</tr>
<tr>
<td>$\text{GY} = 12.6 + 4.23 \text{RDM}^{**}; R^2 = 31%$</td>
<td>$\text{GY} = 12.9 + 5.94 \text{RDM}; R^2 = 27%$</td>
<td>$\text{GY} = 8.19 + 7.98 \text{RDM}^{**}; R^2 = 52%$</td>
</tr>
<tr>
<td>$\text{GY} = 2.98 + 1.20 \text{SN}^{**}; R^2 = 65%$</td>
<td>$\text{GY} = 3.99 + 1.09 \text{SN}^{**}; R^2 = 51%$</td>
<td>$\text{GY} = 0.70 + 1.06 \text{SN}^{**}; R^2 = 51%$</td>
</tr>
<tr>
<td>$\text{GY} = 3.08 + 0.671 \text{KNS}^{***}; R^2 = 20%$</td>
<td>$\text{GY} = -3.26 + 0.896 \text{KNS}^{***}; R^2 = 24%$</td>
<td>$\text{GY} = -6.20 + 0.922 \text{KNS}^{***}; R^2 = 27%$</td>
</tr>
</tbody>
</table>

\(* P < 0.05, \text{NS: not significant, } R^2 = \text{coefficient of determination.}\)
physiologists. RN and SN, could be used as an efficient
tool for screening new or existing cultivars for their
salt tolerance and (c) this work may explain the limited
success of some breeding programs which use simple
selection protocols. In fact, research has consistently
shown that salt tolerance is a complex character controlled
by several genes or groups of genes and involves a num-
ber of component traits which are likely to be quantitative
in nature.

Nevertheless, our results should be further confirmed
with a field study with a larger number of cultivars. Recent
progress in marker technology and genetic transformation
seems to offer considerable promise for the development
of salt tolerance in the near future.

Certainly extensive work is needed to elucidate well the
genetics, biochemical, and physiological basis of barley
salt tolerance. Future knowledge of components of salt
tolerance and the identification and cloning of target genes
may allow the transfer of multiple genes to produce highly
salt-tolerant transgenic cultivars. With the current advances
in genetic transformation technology, it seems possible to
transfer multiple genes that may act in combination to
improve barley salt tolerance. Further improvements in
salt tolerance will undoubtedly result from close interactions
between molecular geneticists, physiologists, breeders and
agronomists.

Acknowledgements – The authors would like to thank the MERST
(Ministry of Education, Scientific Research and Technology of
Tunisia) for funding this work. We also thank Dr. Habib Halila
and Dr. Mongi Zekri for critically reviewing the manuscript.

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