



## Salt Stress Effects on Seed Germination and Seedling Growth of Barley (*Hordeum Vulgare L.*) Genotypes

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**Abstract.** Salt tolerance of twelve barley genotypes was investigated at the germination and seedling growth stages. Germination percentage, speed of germination, shoots and roots fresh weight, K and Na concentrations in the shoots and roots were measured. The genotypic responses varied among growth stages. At the germination stage root length and germination percentage were the most discriminating traits between sensitive and tolerant cultivars. At the seedling growth stage, shoots fresh weight, K<sup>+</sup>, Na<sup>+</sup> and K<sup>+</sup>/Na<sup>+</sup> ratio were the discriminating traits. The evaluated genotypes were grouped into three clusters. The genotypes of first cluster were salt tolerant at the germination stage, those of the second and the third clusters were sensitive and tolerant, respectively, at both growth stages. The results indicated that high K concentration and K/Na ratio were the main differentiating traits among clusters.

**Key Words:** *Hordeum vulgare L.*, NaCl, tolerance, cluster, index

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### Introduction

Soils of the Algerian high plateaus had high calcareous content, a semi arid to arid climate type and a high evaporative demand. These conditions contributed to the accumulation of various salts in the upper soil layer (Djili, & Daoud, 2000). Salt stress is reported as a serious problem (Nedjimi & Daoud, 2006), even though water deficit is the most limiting factor to cereal production in this region (Chennafi *et al.*, 2006). Increased demand on cereal products and subsidies policy lead cereal crops to be grown even in stressful areas (Bedrani, 2001). Considered as a stress tolerant species, barley (*Hordeum vulgare L.*) is extensively grown under rainfed conditions in this region which is relatively less favorable to durum (*Triticum turgidum sp durum L.*)

and bread wheat (*Triticum aestivum* L.), even though each species can show a large genetic variability in response to drought and salt stresses (Royo & Abio, 2003 ; Steppuhn *et al.*, 2005). Mainly utilized as feed crop, barley is an important component of farming system based on sheep rearing (Oudina & Bouzerzour, 1989; Benmahammed *et al.*, 2010). Breeding salt tolerant genotypes is an alternate way to minimize yield reduction and to sustain crop production in this area.

Screening plant material for stress tolerance is the first step in the plant breeding process to identify tolerant parental lines which may be crossed to generate useful segregating material. However for this screening to be efficient, it is important to understand the mechanisms involved in the response of the crop to drought and salt stresses (Munns *et al.*, 2006). Salt stress affects plant growth at all developmental stages. It decreases seed germination percentage, retards plant development and reduces yield (Munns *et al.*, 2006). The deleterious effect of salt resulted from the combination of both water deficit and ions toxicity. Salt inhibits growth by reducing the plant ability to take up water, subjecting it to a water-deficit effect. Excessive amounts of salt entering the plant tissues injure the cells, leading to further reduced growth, through the ion-excess effect (Munns *et al.*, 2006). Approaches used to screen plant material for salt tolerance include among others relative growth rate of shoots and roots (Meneguzzo *et al.*, 2000), photosynthesis activity (Munns & James, 2003), rate of Na<sup>+</sup> and K<sup>+</sup> accumulation in the shoot and the roots, K<sup>+</sup>/Na<sup>+</sup> discrimination (Colmer *et al.*, 2005 ) and crop yield (El-Hendawy *et al.*, 2005 ). Crop establishment comprises three processes which are germination, emergence and early seedling growth. These early growth stages are very sensitive to salt stress and could be used as criteria to screen for salt tolerance. The present study was undertaken to investigate the response of twelve barley genotypes to four salinity levels at the germination and seedling growth stages.

### **Material and Methods**

The experiment was implemented under laboratory conditions during the 2011/2012 academic year at the Biology Department of Larbi Ben Mhidi University of Oum El Bouaghi (Algeria). Twelve barley genotypes (*Hordeum vulgare* L.) were subjected to four salt stress levels during germination and seedling growth stages. Saida<sub>183</sub>, Tichedrett and Martin are Algerian landraces. Due to their differential

vernalization requirement, Saida<sub>183</sub> is grown in lowland while Tichedrett is more adapted to high altitude areas (Benmahammed *et al.*, 2008). Martin is still grown in Tunisia ( Karim *et al.*, 2009). Beecher is a Californian variety, selected from the cross Atlas/Vaughn. Dahbia (synonym Jaidor) and Hamra (synonym Barberousse) are 6-row French barley varieties. Manel and Rihane<sub>03</sub> are Tunisian cultivars developed by Inrat (Chaabane *et al.*, 2009 ). Arig<sub>8</sub> is a Moroccan variety while Soufara, Alanda, and Assala are advanced breeding lines from Icarda (International Center of Agricultural Research in Dry Areas).The seed was kindly provided by the Agricultural Research Station of the Institute of Field Crops located at Khroub (Algeria). To assess salt tolerance during germination, 25 seeds per genotype, with similar size, were surface sterilized in 1.5% sodium hypo chloride for 5 min, then washed thoroughly with distilled water. The seeds were then placed on towel paper in Petri dishes containing 20 mL distilled water (Check) or NaCl solutions at three concentrations (50, 100, 150 mM). Electrical conductivities of the check and salt solutions were 0.05, 3.30, 7.10, and 10.90 mS/cm, respectively. Seeds were incubated in the dark at 23°C in a randomized complete block design with three replications. Germination counts were made on day 4, 6 and 8 after incubation. Seeds were considered germinated when their radicle was at least 2 mm out. A part from the germination test, 200 seeds per genotype were incubated to germinate under laboratory conditions, using tap water. Fifteen healthy seedlings from the germinated seeds were potted in PVC pots, filled with 800 g of sand, thoroughly washed. Arranged in a randomized complete block design with three replications, the pots were irrigated thrice a week with a nutritive solution prepared as per Hoagland and Arnon (Hoagland & Arnon, 1938) and subjected to the same salt treatments as the germination test. This experiment lasted two weeks after planting.

Seed germination percentage (G%) was calculated as follow :  $G\% = 100 (GS/TS)$ , where GS = germinated seeds and TS = total seeds incubated. Seed germination speed (SG) was determined according to Maguire (1962):  $SG = 1/4(n_1) + 1/6 (n_2 - n_1) + 1/8 (n_3 - n_2)$ , where  $n_1$ ,  $(n_2 - n_1)$  et  $(n_3 - n_2)$  are counts of germinated seeds at day 4, 6 and 8<sup>th</sup> after the beginning of the germination test. Seminal roots number was counted on 10 randomly sampled germinated seeds. Maximum root length per germinated seed was also recorded on the same sample, and the total root length (TRL) was derived as:  $TRL (mm) = RN \times RL$ , where RN = root number per germinated seed, and RN = root number per germinated seed (average of 10 germinated seed). The length of the

coleoptile was also measured. Shoot and root fresh weights were determined per seedling. Relative water content was determined from 10 leaf segments, 10 mm length each. The segments were weighed to obtain the fresh (FW) and then immediately floated on double distilled water in a Petri dish, in the dark, at low temperature (5°C) to reach saturation. After 24 h of incubation the turgid weight (TW) was measured. Dry weight (DW) was measured after drying at 70°C for 48 h. The relative weight content (RWC) was calculated as:  $RWC = (FW - DW) / (TW - DW)$ . Shoot and roots were gently washed, dried to a constant dry weight at 70°C for 48 h and ground to pass a 0.5 mm sieve in a laboratory mill. The ground material was mixed thoroughly and samples were ashed for 5 h at 550°C in a muffle furnace. The ash was then dissolved in 2 M HCl for [K] and [Na] determination, using a flame photometer (model M410, Corning Ltd., Essex, UK). Stress tolerance index (STI) was derived for the measured variables, as any decrease or increase relatively to the mean values of the check treatment, as follow:  $STI = 100(1 - S/C)$ , where S and C represent the mean value of the salt stressed (stressed) and unstressed (check) treatments, respectively. Data were statistically analyzed using the balanced analysis of variance subroutine of CropStat7.2.3 (2009). When ever the analysis of variance *F-test* showed significance, salt treatment effect was tested using the linear and quadratic contrasts and the significant difference between treatment means was determined using Duncan multiple rang *test* at the 0.05 probability level. Discriminating traits between the tested varieties for the response to 150mM NaCl were identified through cluster analysis of the tolerance indices data matrix. The cluster analysis was based on Ward's method implemented in the Past free software package (Hammer et al. 2001).

## Results and Discussion

### Seed germination test

Salt affected all the measured traits as indicated by the significant salinity main effect and the linear contrast of the analysis of variance (Tab. 1). The germination percentage decreased from 86.0 to 50.9%, the speed of germination from 16.2 to 8.3% day<sup>-1</sup>, the coleoptile length from 2.5 to 1.5 cm, the roots length from 35.4 to 8.3 cm and the number of roots from 5.1 to 3.1 roots per germinated seed as salinity increased from none to 150mM NaCl treatment (Table 2). Averaged over genotypes, the germination percentage decreased linearly, fitting the following regression equation:

$G\% = -0.2234 [\text{NaCl}] + 85.53$  ( $R^2 = 0.9795$ ). This equation indicated that at 160.0 mM NaCl, the mean germination percentage will decrease by 50%. Maas and Grieve (1991) mentioned that, compared to wheat crop, barley is a salt tolerant species which may germinate even under the supply of 250 mM NaCl, which corresponds to 50% sea water salinity.

**Table 1.** Mean squares of the analysis of variance of seed germination, roots number, speed of germination, coleoptile length and root length

| Sources      | df  | G%       | RN     | SG     | CL     | RL     |
|--------------|-----|----------|--------|--------|--------|--------|
| Genotype (G) | 11  | 1456.4** | 10.8** | 32.3** | 1.4**  | 5.9**  |
| Salinity (S) | 03  | 1529.1** | 11.4** | 54.3** | 3.7**  | 12.6** |
| Linear       | 1   | 19.4**   | 19.3** | 19.3** | 32.6** | 71.9** |
| Quadratic    | 1   | 0.01ns   | 0.3ns  | 0.3ns  | 1.3ns  | 23.4** |
| G x S        | 33  | 657.4**  | 3.1**  | 26.9** | 0.7**  | 2.7ns  |
| Error        | 144 | 96.0     | 0.7    | 4.8    | 0.05   | 1.8    |

%G = percent of seed germination, RN = roots number, SG = speed of seed germination, CL= coleoptile length, RL = root length.

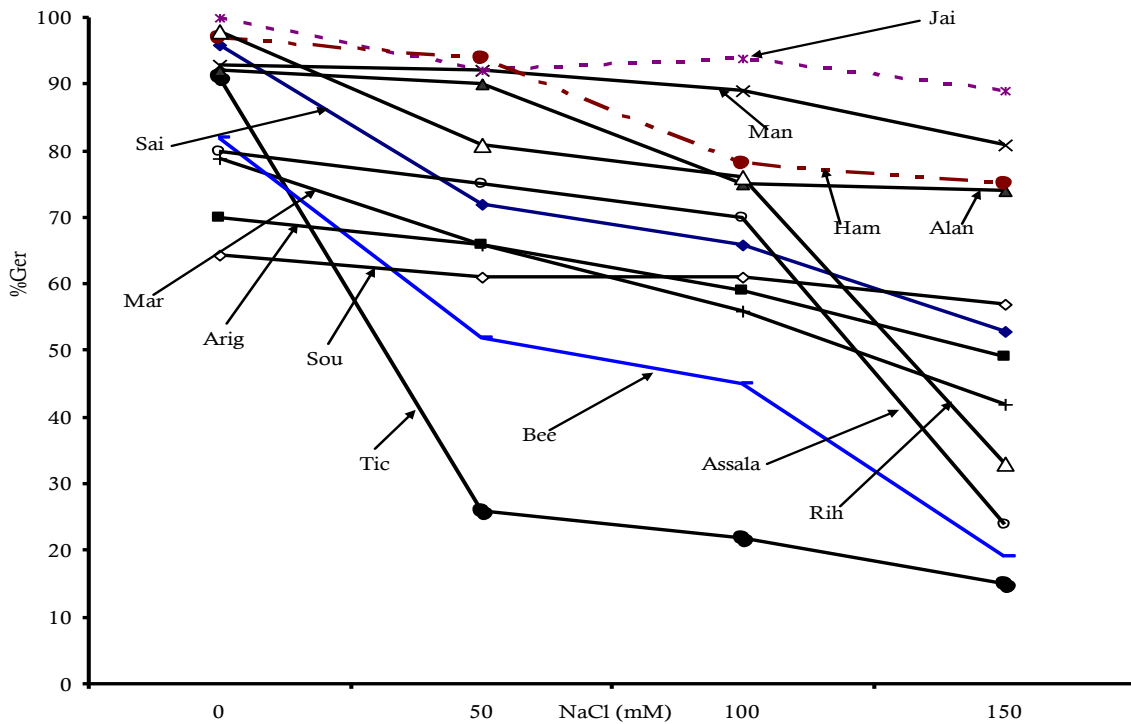
**Table 2.** Salinity main effects of seed germination, speed of germination, coleoptile length, roots length and roots number

| Salinity (mM) | G%   | SG   | CL  | RL   | RN  |
|---------------|------|------|-----|------|-----|
| 0             | 86.0 | 16.2 | 2.5 | 35.4 | 5.1 |
| 50            | 72.3 | 12.1 | 2.3 | 23.9 | 5.0 |
| 100           | 65.9 | 10.9 | 2.0 | 12.3 | 4.2 |
| 150           | 50.9 | 8.3  | 1.5 | 8.3  | 3.1 |
| Average       | 68.8 | 11.9 | 2.1 | 20.0 | 4.3 |

G% = percent of seed germination, SG = speed of seed germination, CL= coleoptile length, RL = roots length, RN = roots number.

The significant interaction of the germination percentage indicated that the responses of the tested set of genotypes varied among salt treatments (Fig. 1). In the absence of salinity, the differences between genotypes were related to seed quality. Salt tolerant genotypes were Dahbia, Manel, Hamra, Alanda and to a lesser extend Soufara and Arigs which exhibited low germination percentage even under the check treatment due probably to poor seed quality. Alam *et al.*, (2005) reported low seed germination in the absence of salt and related this to seed quality. The decrease in the germination percent, varied among the salt tolerant cultivars, between -7 and -12%,

over the three salt treatments (Fig. 1). Genotype main effect indicated that Tichedrett and Beecher are highly sensitive to salinity, based on both the percentage and the speed of germination while Manel and Dahbia were tolerant (Tab. 3). The sensitivity of Tichedrett to salinity was also indicated by the decrease in the coleoptile length and the number of roots, for which Manel and Assala exhibited high tolerance. Based on these traits Soufara, Martin and Saida<sub>183</sub> were sensitive (Tab. 3). High salt treatment was more discriminating between salt tolerant and salt sensitive genotypes than the low salt treatment (Fig. 1). These results corroborated those of Khan *et al.* (1997) and Alam *et al.* (2005), who reported that germination rate decreased as salinity increased and differences between tolerant and sensitive genotypes were observed for this trait at high salt concentration. These results indicated too that the sensitivity/tolerance of the tested genotypes varied among the measured traits. The genotype x salinity interaction of the roots length indicated that the differences between genotypes were higher in the absence of stress and declined under salinity treatments. Assala and Beecher exhibited high root length in the absence of salinity, but they loosed this ability under high salt stress, where Tichedrett and Soufara showed the lowest root length. This trait was less efficient in discriminating between tolerant and sensitive genotypes under high salinity treatment (Fig. 2).



**Figure 1.** Genotype x salinity interaction of the percentage of germination

**Table 3.** Genotype main effects of seed germination, speed of germination, coleoptile length, roots length and roots number

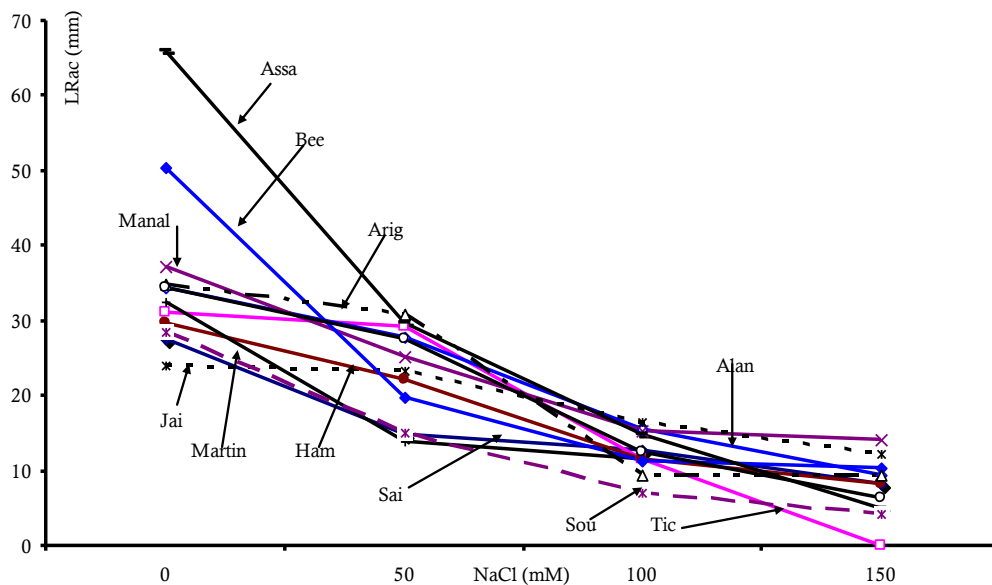
| Genotype             | G%                 | SG                  | CL                 | RL                  | RN                |
|----------------------|--------------------|---------------------|--------------------|---------------------|-------------------|
| Alanda               | 82.8 <sup>d</sup>  | 16.4 <sup>fg</sup>  | 2.0 <sup>b</sup>   | 21.8 <sup>ef</sup>  | 4.2 <sup>ab</sup> |
| Arigs                | 61.0 <sup>bc</sup> | 11.2 <sup>cd</sup>  | 2.4 <sup>cd</sup>  | 21.2 <sup>de</sup>  | 4.6 <sup>ab</sup> |
| Assala               | 62.3 <sup>bc</sup> | 9.5 <sup>b</sup>    | 2.0 <sup>b</sup>   | 28.8 <sup>g</sup>   | 4.3 <sup>ab</sup> |
| Beecher              | 49.5 <sup>a</sup>  | 6.9 <sup>ab</sup>   | 2.1 <sup>bc</sup>  | 22.9 <sup>f</sup>   | 4.1 <sup>ab</sup> |
| Dahbia               | 93.8 <sup>d</sup>  | 19.7 <sup>g</sup>   | 2.2 <sup>bcd</sup> | 17.9 <sup>abc</sup> | 4.9 <sup>b</sup>  |
| Hamra                | 86.0 <sup>d</sup>  | 15.6 <sup>efg</sup> | 2.2 <sup>bcd</sup> | 19.0 <sup>bcd</sup> | 4.5 <sup>ab</sup> |
| Manel                | 88.8 <sup>d</sup>  | 14.8 <sup>def</sup> | 2.5 <sup>d</sup>   | 23.0 <sup>f</sup>   | 5.2 <sup>b</sup>  |
| Martin               | 60.8 <sup>b</sup>  | 11.3 <sup>cde</sup> | 2.1 <sup>bc</sup>  | 16.7 <sup>ab</sup>  | 3.9 <sup>a</sup>  |
| Rihane <sub>03</sub> | 72.0 <sup>c</sup>  | 12.5 <sup>cde</sup> | 2.1 <sup>bc</sup>  | 20.1 <sup>cde</sup> | 4.4 <sup>ab</sup> |
| Saida <sub>183</sub> | 63.7 <sup>bc</sup> | 10.8 <sup>c</sup>   | 2.0 <sup>b</sup>   | 15.8 <sup>a</sup>   | 4.2 <sup>ab</sup> |
| Soufara              | 60.9 <sup>bc</sup> | 10.1 <sup>bc</sup>  | 1.6 <sup>a</sup>   | 18.0 <sup>abc</sup> | 3.6 <sup>a</sup>  |
| Tichedrett           | 38.8 <sup>a</sup>  | 3.6 <sup>a</sup>    | 1.4 <sup>a</sup>   | 18.0 <sup>abc</sup> | 3.6 <sup>a</sup>  |
| Average              | 68.8               | 11.9                | 1.9                | 20.0                | 4.3               |

%G = percent of seed germination, SG = speed of seed germination, CL= coleoptile length, RL = root length, RN = roots number, means followed by the same letter are not significantly different based on Duncan New Multiple Range Test

### Seedling growth

The F-tests of the analysis of variance indicated significant genotype and salinity main effects and significant genotype x salinity interaction for the measured traits (data not shown). Averaged over genotypes, the mean shoot fresh weight varied from 718.2 mg for the check to 520.0 mg seedling<sup>-1</sup> under the supply of 150mM NaCl. The mean of the roots fresh weight varied from 642.5 mg to 210.78 mg/seedling, from zero to 150mM NaCl treatments, indicating a global reduction in both traits due to salt effects. The reduction of the shoots and the roots fresh weight varied among genotypes in response to salinity. Arigs, Beecher, Dahbia, Rihane<sub>03</sub> and Soufara were sensitive over the whole range of salt treatments tested, showing a shoot fresh weight reduction varying from -10 to -29% under 50 mM NaCl and -16 to -39% under 150 mM NaCl. Assala, Hamra, Manel and Saida<sub>183</sub> were tolerant under 50 mM NaCl stress and sensitive under 100 mM and 150 mM NaCl treatments. Alanda, Martin and Tichedrett were sensitive under 150 mM NaCl treatment only (Tab. 4). The roots fresh weight showed a similar pattern of responses. Alanda, Arigs, Assala, Beecher, Manel

and Martin were sensitive to salt stress while the remaining varieties showed tolerance only at 50 mM NaCl. The relative decline of the roots fresh weight varied from -13 to -55% in the first group and from -11 to -63% in the second group (Tab.4). The leaf relative water content, average over genotypes, goes down from 73.7%, in the check treatment to 61.8% under the supply of 150 mM NaCl. This decline exhibited a quadratic pattern fitted by the following regression equation:  $RWC (\%) = -0.0005 [NaCl]^2 + 0.0022[NaCl] + 73.66$ , ( $R^2 = 0.9996$ ). The relative water content measured at 50mM Nacl was almost similar to that of the check treatment for all the tested entries, suggesting tolerance to this salt stress level. The genotypes responded differently at 100 and 150 mM NaCl treatments (Tab. 4). Arigs, Beecher Manel and Soufara were sensitive under 100 and 150mM NaCl, while Alanda, Dhahbia, Martin, Saida<sub>183</sub> and Rihane<sub>03</sub> exhibited sensitivity under 150mM Nacl. Assala, Hamra and Tichedrett were tolerant to the salt treatments tested, since their relative water content decreased by less than 10% from the values measured in the check (Tab. 4). Averaged over the tested genotypes, the mean values of the accumulated  $K^+$  in the shoot were 4.39, 4.06, 3.80 and 3.42 mg g<sup>-1</sup> dry weight, under the supply of 0, 50, 100 and 150 mM NaCl, respectively. The corresponding means of the  $K^+$  accumulated the roots were 2.10, 1.95, 1.83 and 1.69 mg g<sup>-1</sup> dry weight. In both organs,  $K^+$  concentration declined as salt stress increased. The decline was higher in the shoots (-22.2%) than in the roots (-19.7%).



**Figure 2.** Genotype x salinity interaction of root length



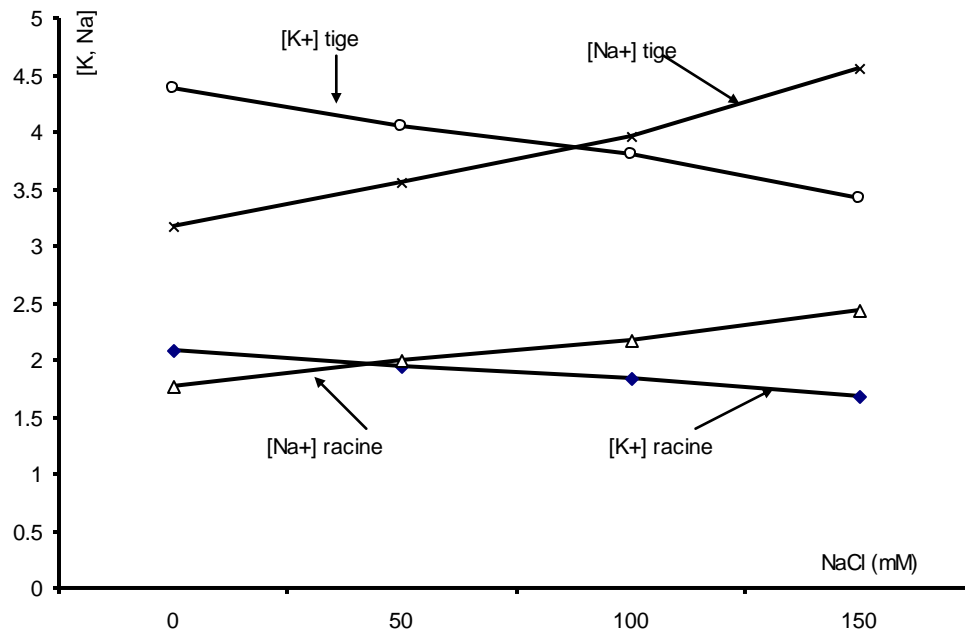
**Table 4.** Stress tolerance indices of the shoots and the roots fresh weight (SFW, RFW), the relative water content (RWC) and the K<sup>+</sup> concentration in the shoots [K<sup>+</sup> shoot] of the tested genotypes.

| Traits               | SFW |     |     | RFW |     |     | RWC |     |     | [K <sup>+</sup> ]shoot |     |     |
|----------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------------------------|-----|-----|
|                      | 50  | 100 | 150 | 50  | 100 | 150 | 50  | 100 | 150 | 50                     | 100 | 150 |
| Alanda               | -2  | -2  | -21 | -18 | -23 | -43 | -1  | -1  | -11 | 0                      | -10 | -13 |
| Arigs                | -14 | -27 | -38 | -15 | -34 | -33 | -3  | -14 | -28 | 0                      | -31 | -37 |
| Assala               | -8  | -20 | -20 | -19 | -35 | -38 | -1  | -8  | -9  | -5                     | -26 | -41 |
| Beecher              | -15 | -18 | -21 | -13 | -21 | -32 | -6  | -14 | -14 | -4                     | -38 | -39 |
| Dahbia               | -22 | -27 | -28 | -4  | -23 | -32 | -6  | -6  | -20 | -6                     | -68 | -71 |
| Hamra                | -2  | -13 | -18 | -10 | -16 | -37 | -1  | -1  | -4  | -9                     | -68 | -76 |
| Manel                | -6  | -22 | -21 | -26 | -29 | -41 | -5  | -10 | -14 | -2                     | -36 | -47 |
| Martin               | -2  | -5  | -50 | -18 | -21 | -55 | -5  | -6  | -13 | -17                    | -51 | -54 |
| Rihane <sub>03</sub> | -10 | -16 | -16 | -5  | -13 | -14 | -5  | -5  | -14 | -24                    | -29 | -30 |
| Saida <sub>183</sub> | -8  | -23 | -26 | -7  | -19 | -43 | -8  | -8  | -33 | -5                     | -6  | -7  |
| Soufara              | -29 | -38 | -39 | -8  | -45 | -63 | -5  | -18 | -30 | -16                    | -51 | -63 |
| Tichedrett           | -3  | -6  | -35 | -6  | -11 | -31 | -1  | -2  | -5  | -3                     | -5  | -9  |

The mean values of the accumulated Na<sup>+</sup> in the shoots were 3.17, 3.57, 3.96 and 4.56 mg g<sup>-1</sup> dry weight, under 0, 50, 100 and 150 mM NaCl, respectively. The corresponding means of Na<sup>+</sup> accumulated in the roots were 1.78, 2.00, 2.17 and 2.43 mg g<sup>-1</sup> dry weight. The results indicated that Na<sup>+</sup> accumulated quantitatively more in the shoots than in the roots, and conversely to the pattern of K<sup>+</sup> accumulation, Na<sup>+</sup> concentration increased as salinity increased (Fig. 3). The relative increase of Na<sup>+</sup> was higher in the shoots (+44.0%) than in the roots (+36.0%). For both K<sup>+</sup> and Na<sup>+</sup> elements, their concentrations were almost two fold higher in the shoots than in the roots (Figure 3). Since the accumulation pattern of these two elements diverged with increased NaCl treatments, their K/Na ratio decreased by -16, -27 and -40% in the roots against -18, -31 and -46% in the shoots under the supply of 50, 100 and 150 mM NaCl, respectively. The genotype x salinity interaction of the shoots K<sup>+</sup> indicated that Martin, Rihane<sub>03</sub> and Soufara were highly sensitive to salinity as their K<sup>+</sup> concentration decreased by an average of 19% to 49% at the 50 and 150mM NaCl treatments, respectively (Tab.4).

Salt tolerant genotypes were Saida<sub>183</sub>, Tichedrett and Alanda, reducing their shoot K<sup>+</sup> concentration to less than 10% at the 150 mM NaCl. The remaining genotypes

were all sensitive at 100 and 150 mM NaCl treatments (Table 4). Based on the genotype x salinity interaction of the  $K^+$  accumulated in the roots, Arigs, Assala, Manel, Saida<sub>183</sub> and Tichedrett were classified as salt tolerant.  $K^+$  concentration in the roots of these genotypes was reduced by less than 10% at 150mM NaCl. Sensitive genotypes showing sizeable reduction in the roots  $K^+$  concentration were Alanda, Beecher, Dahbia and Soufara (Tab. 5).  $Na^+$  concentration in the shoots and the roots increased in most genotypes as salinity increased. The main differences between genotypes were the salinity level at which this increase occurred, at 50 mM or at 100 mM NaCl (Tab. 5). Similar pattern of responses was noted also in the K/Na ratios. In this study, several traits related to seed germination and seedling growth were used to assess the salt tolerance of barley genotypes. In general, salinity affected all the measured traits which decreased/increased relatively to the values of the check treatment. The results are in agreement with El-Hendawy *et al.*, (2005) who reported that germination percentage, root and shoot length and fresh weights decreased in barley varieties with increasing salinity level.



**Figure 3.** Pattern of  $K^+$  and  $Na^+$  accumulation in the shoots and the roots of barley at four NaCl salt treatments.

The pattern of cultivar responses varied within each trait and between traits. This leads to the variation of the tolerance among cultivars according to the various traits used as selection criteria. A given cultivar is tolerant or sensitive depending on the trait used for its classification. These results suggested that salinity tolerance is a complex trait, affected by a large number of mechanisms as mentioned by Ashraf & Haris, (2004). Similarity of the cultivar responses was then sought through hierarchical clustering technique to summarize the data measured at both growth stages and to have a clear picture of the response pattern of the tested genotypes. At the 50% similarity, the tested genotypes formed three clusters (Fig. 4). Tichedrett, Beecher, Assala and Rihane<sub>03</sub> clustered together (Cluster<sub>1</sub>). Saida<sub>183</sub>, Hamra and Soufara formed the second cluster (Cluster<sub>2</sub>), while the third cluster (cluster<sub>3</sub>) contained Arigs, Martin, Dahbia, Alanda and Manel (Fig.4).

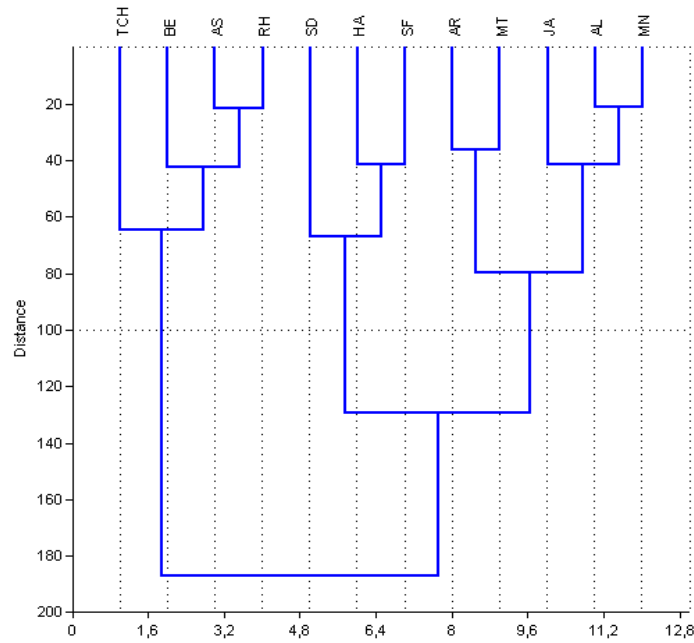
**Table 5.** Stress tolerance indices of the K<sup>+</sup> roots, Na<sup>+</sup> shoot, Na<sup>+</sup> roots, K/Na shoots, and K/Na<sup>+</sup> roots of the tested genotypes

| Traits               | [K <sup>+</sup> ]roots |     |     | [Na <sup>+</sup> ]shoots |     |     | [Na <sup>+</sup> ]roots |     |     | K/Na shoots |     |     | K/Na |     |     |
|----------------------|------------------------|-----|-----|--------------------------|-----|-----|-------------------------|-----|-----|-------------|-----|-----|------|-----|-----|
|                      | 50                     | 100 | 150 | 50                       | 100 | 150 | 50                      | 100 | 150 | 50          | 100 | 150 | 50   | 100 | 150 |
| Alanda               | -11                    | -14 | -20 | +19                      | +23 | +31 | +8                      | +39 | +45 | -14         | -27 | -34 | -5   | -33 | -41 |
| Arigs                | -7                     | -7  | -6  | 4                        | 16  | 28  | 6                       | 10  | 18  | -5          | -22 | -35 | -12  | -15 | -20 |
| Assala               | -1                     | -5  | -7  | 3                        | 31  | 42  | 3                       | 14  | 40  | -8          | -29 | -49 | -3   | -17 | -34 |
| Beecher              | -9                     | -21 | -25 | 7                        | 43  | 71  | 18                      | 20  | 21  | -11         | -37 | -48 | -23  | -34 | -38 |
| Dahbia               | -18                    | -23 | -28 | 13                       | 19  | 38  | 18                      | 25  | 32  | -17         | -35 | -49 | -31  | -38 | -45 |
| Hamra                | -5                     | -16 | -41 | 83                       | 83  | 86  | 14                      | 30  | 41  | -50         | -53 | -65 | -17  | -36 | -58 |
| Manel                | -3                     | -5  | -8  | 4                        | 20  | 26  | 5                       | 11  | 42  | -6          | -28 | -44 | -8   | -15 | -35 |
| Martin               | -4                     | -14 | -16 | 26                       | 27  | 43  | 11                      | 15  | 25  | -34         | -36 | -46 | 0    | 0   | -22 |
| Rihane <sub>03</sub> | -3                     | -3  | -13 | 28                       | 41  | 43  | 4                       | 4   | 34  | -40         | -52 | -53 | -6   | -7  | -35 |
| Saida <sub>183</sub> | -0                     | -6  | -7  | 7                        | 6   | 57  | 16                      | 38  | 73  | -14         | -13 | -47 | -14  | -32 | -46 |
| Soufara              | -10                    | -16 | -21 | 3                        | 17  | 73  | 42                      | 45  | 54  | -19         | -33 | -66 | -37  | -42 | -48 |
| Tichedrett           | -1                     | -5  | -9  | 17                       | 25  | 35  | 13                      | 18  | 30  | -17         | -23 | -31 | -13  | -19 | -30 |

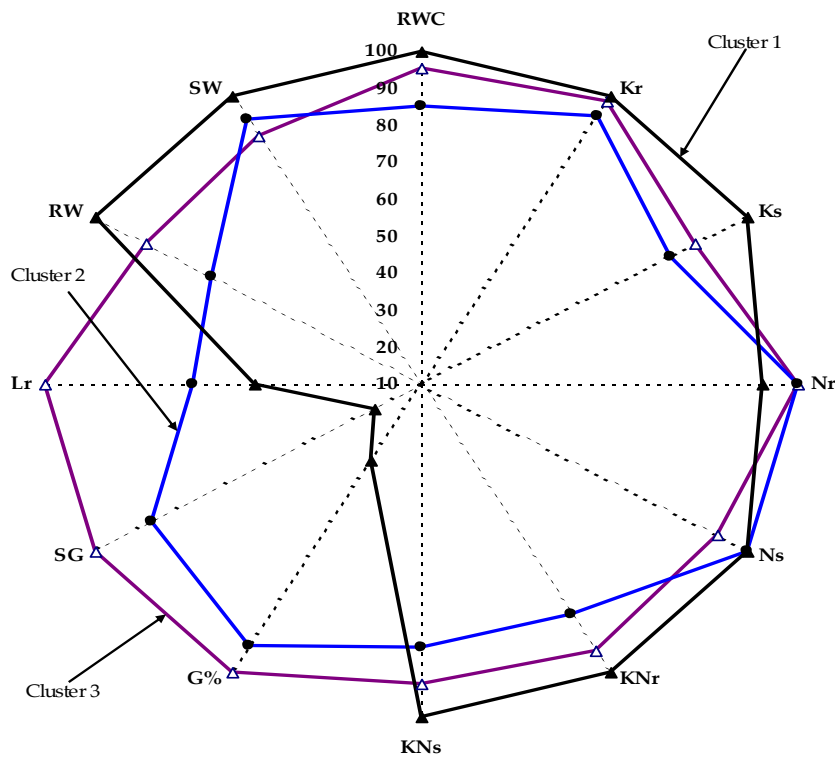
The average of the stress tolerance indices per cluster indicated that genotypes belonging to cluster<sub>1</sub> decreased more their germination percentage, speed of germination and roots length under salt treatment compared to the genotypes of clusters 2 and 3. Tichedrett, Beecher, Assala and Rihane<sub>03</sub> are thus salt sensitive at

the germination growth stage (Figure 5). Germination percentage, speed of germination and roots length discriminated efficiently between genotypes as far as tolerance to salinity is concerned. At the seedling stage the rate of accumulation and the partitioning of the  $K^+$  and  $Na^+$  ions as well as the accumulated biomass in the shoots and the roots were the best discriminating traits among clusters (Fig.5). Based on these variables, genotypes of cluster<sub>1</sub> and cluster<sub>3</sub> are classified as salt tolerant while those of cluster<sub>2</sub> were salt sensitive. Genotypes of cluster<sub>2</sub> was characterized by high accumulation of  $Na^+$  in the roots and the shoots, leading to a decrease in  $K^+/Na^+$  ratio of both organs. Genotypic behaviour at both growth stages, under 150 mM NaCl, indicated that genotypes of cluster<sub>1</sub> behaved as sensitive at the germination stage and as tolerant at the seedling stage. Genotypes of cluster<sub>2</sub> were sensitive at both growth stages and those of the cluster<sub>3</sub> were tolerant at both growth stages.

The present work focused on the effects of NaCl at germination and seedling growth stages of 12 barley genotypes. The results showed decreases in the measured traits at both growth stages as salt levels increased. According to Munns & James, (2003) these decreases are brought about by modification of ionic balance, water status, mineral nutrition, and carbon allocation and utilization. The results indicated also that the responses of the cultivars varied according to the trait used for classification as well as the growth stage considered. This is in agreement with Munns & James, (2003) who mentioned that genotypic tolerance to salinity varied with the growth stage. Based on data collected at of both growth stages, the tested genotypes were grouped into three clusters, with differential abilities to germinate and to accumulate  $K^+$  and  $Na^+$  ions under salt. Since high  $Na^+$  accumulation hampers  $k^+$  uptake, and impacts enzymatic processes, these results are consistent with the idea of the important role played by a high  $K^+/Na^+$  ratio in plant salt tolerance as mentioned Moller *et al.*, (2009).



**Figure 4.** Genotypes clustering based on germination test and seedling growth data of the 150 NaCl treatment expressed as tolerance indices (TICH= Tichedrett, BE= Beecher, AS= Assala, RH = Rihane03, SD= Saida183, HA= Hamra, SF= Soufara, AR = Arig8, MT= Martin, JA = Dabha, AL= Alanda, MN = Manel)



**Figure 5.** Behaviour of the three clusters of genotype at the germination and seedling growth stages under 150 mM NaCl (G% = germination percent, SG = speed of germination, Lr = root length, SW = shoot fresh weight, RW = roots fresh weight, RWC = relative water content, Kr = K roots, Ks = K shoot;

Nr = Na roots, Ns = Na shoots, KNr = K/Na roots ratio ; Cluster<sub>1</sub>= Tichedrett, Beecher, Assala, Rihane<sub>03</sub> ; Cluster<sub>2</sub> = Saida, Hamra, Soufara ; Cluster<sub>3</sub> = Arigs, Martin, Jaidor, Alanda, Manel).

## Conclusions

This study was conducted to investigate the effect of salinity on germination and seedling growth of 12 barley genotypes. The genotypic responses to salinity varied between traits and growth stages. An overall decrease was noted for all traits as salt levels increased associated with an increased concentration of Na<sup>+</sup>, decreased concentration K<sup>+</sup>, and decreased K<sup>+</sup>: Na<sup>+</sup> ratio. Resistant varieties at seedling growth stage maintained K<sup>+</sup> uptake in the presence of Na<sup>+</sup>, thus alleviating the toxic effect of Na<sup>+</sup>. No relation was found between salt tolerance at the germination stage and that at the seedling stage.

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