Effects of Different Salinity Levels on Growth, Yield and Physiology on Durum Wheat (*Triticum turgidum var. durum*)

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ABSTRACT

Comparing the effects of salinity on durum wheat cultivars differing in their salt tolerance is of interest to physiologists investigating traits associated with adaptation to saline conditions and to breeders to develop cultivars for salt infected areas. This study was conducted to determine the effects of salinity on germination percentage, seedling growth, yield components, and physiological traits of two improved durum wheat cultivars (*Triticum turgidum var. durum*) developed by ICARDA durum breeding program namely, Belikh2 and Omrabi5. Plants were grown in greenhouse in sand cultures and irrigated with Hoagland's solution. Six salinity treatments were imposed by NaCl to the base nutrient solution. The treatments were 50, 100, 150, 200, 250, and 300 mM NaCl, along with a control treatment. Germination percentage and seedling growth were tested for both cultivars at the same mentioned treatments using a growth chamber. Germination percentage, seedling growth, vegetative growth, grain production, exclusion of Na+ and Cl-, and K+/Na+ ratio were higher in Belikh2 than Omrabi5 at 50, 100, 150, and 200 mM NaCl treatments. All seedlings of Omrabi5 did not survive more than 250 mM NaCl treatments whereas, Belikh2 survived indicating a high degree of salt tolerance. Therefore, these concentrations; 250 and 300 mM NaCl could be used efficiently to discriminate salt-tolerant varieties. These results showed that salinity tolerance in Belikh2 is largely attributable to the maintenance of higher K+/Na+ ratio, and less Na+ and Cl- accumulation.

Keywords: Durum Wheat, K+/Na+ Ratio, NaCl, Salinity, Salt Tolerance.

INTRODUCTION

Salinity is one of the most serious environmental stresses that threaten crop yields, and soil fertility in irrigated areas of arid and semi-arid regions in the world (Mohammed et al., 1998). This problem in these areas may be a result of limited water availability, unsuitable irrigation practices, improper drainage, and high evaporation (Abd Alrahman et al., 2005), and the problem becomes more acute due to uses of uncultivable saline/sodic soils to fulfill the demand of the increasing population all over the world (Munns, 2002).

Genetic improvement has not been highly successful in improving grain yield under saline conditions. One reason may be that the genetic variability for this trait in the genetic pool being used by wheat breeders is low (Wyn Jones and Gorham, 1991); or it may be that not enough attention is being given to the salinity resistance *per se* without confounding with drought tolerance in wheat improvement programs.

Concerning the effect of salt stress on wheat plant
growth and development, it inhibited germination and seedling growth (Almansouri, et al., 2001), reduced grain yields (Maas and Poss, 1989) by accelerating apex development (Grieve et al., 1992; Katerji et al., 2005), shortening the spikelet development, reducing number of spikelets per spike (Frank et al., 1987), kernels per spike, and the number of spike tillers (Maas and Grieve, 1990; Katerji et al., 2005) by acting on different parameters in relation to water uptake and ion accumulation.

Salinity tolerance in cereals is associated with the capacity to restrict the rate of Na⁺ entry into the shoots (Munns, 2002). In wheat, there is variation for leaf Na⁺ concentrations and for selectivity of K⁺ over Na⁺ (Gorham et al., 1990; Munns and James, 2003). For the Na⁺ that does enter shoots of cereals, it accumulates mainly in old leaves, while of K⁺ is transported to young leaves (Yeo and Flowers, 1984; Wolf et al., 1991). The K⁺/Na⁺ ratio has been considered as an index of salt tolerance and shows higher values in tolerant genotypes than in the susceptible ones (Dvorak and Gorham, 1992).

The present study was, therefore, undertaken in order to compare the effects of salt stress gradually induced by increasing concentration of NaCl on growth, development and agronomic and physiological responses in two durum wheat cultivars contrasting in their salt tolerance.

**MATERIALS AND METHODS**

**Germplasm**

Two durum wheat (Triticum turgidum var. durum) cultivars differing in their salt tolerance were used in this work. Seeds were obtained from the International Centre for Agricultural Research in the Dry Areas (ICARDA; Aleppo, Syria). The Omrabi5 durum cultivar is a cross between the landrace Haurani and the improved cultivar Jori-C69; Omrabi5 and Belikh2 were developed for the Mediterranean dryland conditions (Nachit, 1998). Omrabi5 is released in Jordan, Turkey, Algeria, Iran, and Iraq for commercial production; it combines drought tolerance with high and stable yields. Belikh2 is released in Algeria, Lebanon and Syria, it was bred at ICARDA (Crane/Stork) and developed for rainfed areas. It is early heading and maturing and shows good protein quality for pasta processing.

**Seed Germination and Seedling Growth**

For each cultivar, 350 hand-selected seeds of uniform size were surface sterilized with 1% sodium hypochlorite for 15 min and then rinsed with sterile deionized water for three times (each time for 5 minutes) under a laminar airflow cabinet. These seeds were then transferred to sterile Petri dishes (10 seeds per Petri dish) containing two Whatman No. 1 filter paper moistened with 10 ml of Hoagland solution NaCl (control). NaCl was used in six concentrations of 50, 100, 150, 200, 250, or 300 mM. Four replicates of 50 seeds were used in each treatment. In order to avoid water losses, edges of Petri dishes were tightly sealed with an impermeable colourless parafilm. Seeds were allowed to germinate in a growth chamber at 25 °C under a 12 h photoperiod (120 µmol m⁻² s⁻¹) fluorescent; germination percentages were recorded after 6 days using radicle extrusion (≥ 2 mm long) as a criterion. Seedling growth was determined by measuring coleoptile length, seminal root number and length, and seedling height after 6 days of germination.

**Growth and Osmotic Potential**

Seeds of the two durum wheat cultivars were germinated using peat moss trays. After 10 days at two leaves stage, seedlings were transferred into each pot at a rate of three equidistantly spread seedlings per pot. Seedlings were watered initially with tap water, then quarter strength Hoagland nutrient solution was
introduced two days after transplanting and increased to full strength at three weeks after transplanting. The salinity concentration was increased stepwise in aliquots of 50 mM every day until the required concentration was reached. Salinity treatments were begun 14 days after the start of the experiment. The experiment was conducted in a glasshouse using plastic pots filled with washed sandy soil, each containing 10 kg soil (dry wt. basis). The salinity treatments were 0.2 (control), 50, 100, 150, 200, 250, and 300 mM NaCl supplemented in Hoagland nutrient solution, tap water was used. A total of 28 pots were used for each genotype with four replicates for each treatment.

At maturity the plants were harvested and data for plant height, biological yield per plant, grain yield per plant, productive tiller number per plant, spikelets number per spike, seeds number per spike, and a hundred-kernel weight were recorded on plant basis. All the above parameters were measured with three sample plants per genotype and four replicates (pot) per treatment. We have opted for screening under controlled conditions (greenhouse) to have repeatable results; as in the field screening land heterogeneity is usually very high.

**Mineral Content**

Approximately, 0.25 g dry and ground plant material from leaves, from each replicate (pot) was placed in a digestion tube and 3.0 ml of the digestion mixture (H$_2$SO$_4$–salicylic acid) was added. After mixing, the tube was allowed to stand for 2 h and was then placed into a heating block and heated for 2 h at 100 °C. After cooling, 3 ml aliquots of HClO$_4$ were added. The contents of the tube were thoroughly mixed. Then the tube was placed in the block and heated to 220 °C. The digestion was complete in about 5 h. The cooled-clear digest was diluted to 100 ml with distilled water, and aliquots were taken for analyses. Potassium and sodium content of these plant samples were measured using a flame photometer (Genway PFP7). For determining chloride in leaves the procedure recommended by Piper (1947) was followed using dry ashing with calcium oxide. The ashed samples were extracted with hot water and the chloride in the extract is titrated with standard silver nitrate.

**Statistical Analysis**

The experiment was set in split plot design with 4 replicates. Salt concentrations were the main plots, and genotypes were the sub-plots. Collected data were subjected to Analysis of Variance (ANOVA) using SAS program (SAS, 1999). Probability of significance was used to indicate significant treatments and interaction effects and means were separated according to a Duncan's Multiple Range Test at 0.05 levels of probability.

**RESULTS AND DISCUSSION**

**Seed Germination and Seedling Growth**

Seed germination percentage was significantly reduced in response to increased NaCl stress in comparison to control solution in both cultivars (Figure 1). Germination percentages declined sharply with 200, and 250. At these NaCl concentrations, differences among the genotypes were significant. Belikh2 had germination percentages higher than 50% even with the 200 mM NaCl treatment, while Omrabi5 had lower germination percentages with the same NaCl treatment (Figure 1). No germination occurred at the highest external osmotic potential induced by 300 mM NaCl concentration in Omrabi5 while the germination percentage was 10% in Belikh2; 300 mM is 50% of salt in the sea.

Table 1 shows for both genotypes, seminal root number, seminal root length, coleoptile length, and total seedling length which decreased in response to increased concentrations of NaCl with a drastic effect at the highest...
NaCl concentration (300 mM). Using 250, 300 mM NaCl concentration had a significant depressive effect on seminal root number, seminal root length, coleoptile length, and total seedling length of both cultivars.

According to Dodd and Donovan (1999), salt stress could reduce germination and seedling growth either by limiting water absorption by the seeds, by affecting the mobilization of stored reserves (Bouaziz and Hicks, 1990; Lin and Kao, 1995; Prakash and Prathapasenan, 1988) or directly by affecting the structural organization or synthesis of proteins in germinating embryo (Ramagopal, 1990). Moreover, little or no correlation has been found between genotypic differences in germination and later growth in salinity (Ashraf and McNeilly, 1998; Francois et al., 1986; Kingsbury and Epstein, 1984).

Figure 1: Effect of salinity on germination percentage of two durum wheat cultivars (Omrabi5 and Belikh2). All means followed by the same letter are not significantly different at the 5% probability level according to Duncan Multiple Range Test.
Table 1: Effect of salinity on seminal root number, seminal root length, coleoptile length, and total seedling length of two durum wheat cultivars (Omrabi5 and Belikh2).

<table>
<thead>
<tr>
<th>Salinity level</th>
<th>Seminal root number (cm)</th>
<th>Seminal root length (cm)</th>
<th>Coleoptile length (cm)</th>
<th>Total seedling length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Omrabi5</td>
<td>Belikh2</td>
<td>Omrabi5</td>
<td>Belikh2</td>
</tr>
<tr>
<td>Control</td>
<td>4.7 aA</td>
<td>5.2 aA</td>
<td>8.8 aA</td>
<td>7.3 aA</td>
</tr>
<tr>
<td>50 mM</td>
<td>4.5 aA</td>
<td>4.8 abA</td>
<td>6.7 bA</td>
<td>6.5 aA</td>
</tr>
<tr>
<td>100 mM</td>
<td>3.8 aA</td>
<td>4.2 bcA</td>
<td>5.1 cA</td>
<td>3.9 bA</td>
</tr>
<tr>
<td>150 mM</td>
<td>3.7 aA</td>
<td>3.6 cdA</td>
<td>3.1 dA</td>
<td>2.6 bcA</td>
</tr>
<tr>
<td>200 mM</td>
<td>1.9 bA</td>
<td>2.9 dA</td>
<td>1.4 eA</td>
<td>1.6 cdA</td>
</tr>
<tr>
<td>250 mM</td>
<td>0.0 cB</td>
<td>0.9 ea</td>
<td>0.1 fb</td>
<td>0.7 edA</td>
</tr>
<tr>
<td>300 mM</td>
<td>0.0 cA</td>
<td>0.3 eA</td>
<td>0.0 fA</td>
<td>0.3 eA</td>
</tr>
</tbody>
</table>

All means followed by the same letter are not significantly different at the 5% probability level according to Duncan Multiple Range Test.

Values followed by different small letter in column represent significant difference of treatment levels within cultivar at 5% level.

Values followed by different capital letter in row represent significant difference among cultivars at 5% level.

**Plant height, Yield, and Yield Components**

Plant height, biological yield, grain yield, and harvest index were significantly reduced in response to increased concentration of NaCl in comparison to control solution in both cultivars (Table 2). The biological yield per plant in Omrabi5 was decreased by 70, 81, and 100% at 150, 200, and 250 mM NaCl concentrations, respectively; the corresponding decreases in grain weight per plant were 92, 96, and 100%, respectively. Table 2 showed at 250 mM NaCl, Omrabi5 was succumbed at this concentration, whereas Belikh2 even at 300 mM NaCl concentration did give biological and grain weight per plant although they were decreased by 89 and 97%, respectively. In both cultivars, the decreases in grain yield per plant with increased salinity were due to significant decreases in the number of spikes per plant, number of spikelets per spike, number of kernels per spike and mean kernel weight (Table 3). This in agreement with studies made by Frank et al. (1987), Maas and Grieve (1990), and Katerji et al. (2005). Belikh2, which produced 5.7 productive tillers under nonsaline conditions, produced 4.7, 3.3, 2.7 and 2.0 productive tillers at 50, 100, 150, and 200 mM, respectively, and only 1.0 productive tiller at 250 and 300 mM NaCl concentration. The percentage reduction was even greater for Omrabi5, where salinity reduced the average number of spikes from 4.3 in control plants to 0.0 at 250 mM. The mean number of spikelets per spike, number of kernels per spike, and weight of a hundred kernel weight of Belikh2 at 250 mM NaCl concentration were 14.2, 12.7, and 0.9, respectively. For Omrabi5, all mentioned characters were 0.0 at similar NaCl concentration.

Salt tolerance in Belikh2 as compared with Omrabi5, was evident in the present study by better maintenance of growth and grain production when exposed to 250 and 300 mM NaCl treatments. For example, when grown to maturity at 250 mM NaCl concentration, for Belikh2,
the harvest index was reduced by 78%; whereas Omrabi5 did not produce any grain. However, at high salt concentrations, the genotype Belikh2 tended to have foliar salt-excretion by secreting small drop of salt on the tip of leaves whereas, the genotype Omrabi5 did not show this phenomenon (Data not shown).

Growth and yield reduction could arise from a number of reasons, this could be impeded by the osmotic effects of salt in the soil solutions, that causes accelerated senescence due to leaf water deficit or hormonal effects arising from root signals. Also, there could be nutrient imbalances resulting in deficiencies or excesses of other ions. Moreover, there could be toxic effects of salts on leaves, due to excessive salt build up in cytoplasm or cell wall (Sairam and Tyagi, 2004).

<table>
<thead>
<tr>
<th>Salinity level</th>
<th>Plant height (cm)</th>
<th>Biological yield (gm)</th>
<th>Grains yield (gm)</th>
<th>Harvest index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Omrabi5</td>
<td>Belikh2</td>
<td>Omrabi5</td>
<td>Belikh2</td>
</tr>
<tr>
<td>Control</td>
<td>67.8 aA²</td>
<td>67.6 aA</td>
<td>8.1 aA</td>
<td>9.8 aA</td>
</tr>
<tr>
<td>50 mM</td>
<td>64.2 aA</td>
<td>61.9 bA</td>
<td>6.3 bB</td>
<td>8.2 bA</td>
</tr>
<tr>
<td>100 mM</td>
<td>43.7 bA</td>
<td>49.5 cA</td>
<td>3.8 cB</td>
<td>4.5 cbA</td>
</tr>
<tr>
<td>150 mM</td>
<td>38.5 cA</td>
<td>40.2 cdA</td>
<td>2.4 cdB</td>
<td>3.0 cdA</td>
</tr>
<tr>
<td>200 mM</td>
<td>21.4 dB</td>
<td>39.6 dA</td>
<td>1.5 cdB</td>
<td>2.1 dA</td>
</tr>
<tr>
<td>250 mM</td>
<td>-</td>
<td>34.2 e -</td>
<td>1.4 d -</td>
<td>0.3 e -</td>
</tr>
<tr>
<td>300 mM</td>
<td>-</td>
<td>32.8 e -</td>
<td>1.1 d -</td>
<td>0.1 e -</td>
</tr>
</tbody>
</table>

All means followed by the same letter are not significantly different at the 5% probability level according to Duncan Multiple Range Test.

x Values followed by different small letter in column represent significant difference of treatment levels within cultivar at 5% level.

y Values followed by different capital letter in row represent significance among cultivars at 5% level.

- Absent reading due to the death of plants during seedling stage.
Table 3: Effect of salinity on productive tiller number per plant, spikelet number per spike, seeds number per spike, and a hundred kernel weight of two durum wheat cultivars (Omrabi5 and Belikh2).

<table>
<thead>
<tr>
<th>Salinity level</th>
<th>Productive tillers/plant</th>
<th>Spikelets/spike</th>
<th>Kernels/spike</th>
<th>100-kernel weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Omrabi5</td>
<td>Belikh2</td>
<td>Omrabi5</td>
<td>Belikh2</td>
</tr>
<tr>
<td>Control</td>
<td>4.3 aA</td>
<td>5.7 aA</td>
<td>14.4 aA</td>
<td>16.3 aA</td>
</tr>
<tr>
<td>50 mM</td>
<td>2.3 bB</td>
<td>4.7 bA</td>
<td>12.8 bB</td>
<td>16.0</td>
</tr>
<tr>
<td>100 mM</td>
<td>1.0 cB</td>
<td>3.3 cA</td>
<td>12.1 bB</td>
<td>15.9</td>
</tr>
<tr>
<td>200 mM</td>
<td>1.0 cB</td>
<td>2.0 cA</td>
<td>10.3 bB</td>
<td>14.7</td>
</tr>
<tr>
<td>250 mM</td>
<td>-</td>
<td>1.0 e</td>
<td>-</td>
<td>14.2 b</td>
</tr>
<tr>
<td>300 mM</td>
<td>-</td>
<td>1.0 e</td>
<td>-</td>
<td>14.0 b</td>
</tr>
</tbody>
</table>

All means followed by the same letter are not significantly different at the 5% probability level according to Duncan Multiple Range Test.

z Values followed by different small letter in column represent significant difference of treatment levels within cultivar at 5% level.
y Values followed by different capital letter in row represent significance among cultivars at 5% level.
- Absent reading due to death of plants during seedling stage.

K⁺, Na⁺, and Cl⁻ Concentrations and K⁺/Na⁺ Ratio

The control treatment gave high K⁺ and low Na⁺ and Cl⁻ concentrations in the leaves and in both cultivars Omrabi5 and Belikh2: K⁺ 0.84-0.53%, Na⁺ 0.41-0.68%, Cl⁻ 1.3-1.2%, and K⁺/Na⁺ ratio 74.8-83.0%, respectively. However, with increases in salt concentrations K⁺ uptake decreased while Na⁺ and Cl⁻ uptake increased rapidly (Table 4). The significant differences obtained for K⁺, Na⁺, and Cl⁻ values could not explain genotypic differences regularly. However, differences in tolerance can be seen clearly when the K⁺, Na⁺, and Cl⁻ values of Belikh2 are compared with those of Omrabi5 at the 250 and 300 mM NaCl treatments. All seedlings of Omrabi5 did not survive 250 and 300 mM NaCl treatments; contrarily, Belikh2 did survive the levels. At the same concentrations, the genotype Belikh2 tended to have lower Na⁺ and Cl⁻, and higher K⁺ and K⁺/Na⁺ ratio with 200 mM NaCl concentration than Omrabi5. These results indicate that K⁺, Na⁺, and Cl⁻ concentrations and their distributions may be important in terms of salt tolerance screening; however, it cannot be applied for all genotypes, as salt tolerance may have a close relationship with tissue tolerance.

Relative salinity tolerance was also related to restricted shoot Na⁺, and Cl⁻ levels, in conjunction with maintenance of high shoot K⁺ concentrations. At high salinity, Belikh2 accumulated significantly less shoot Na⁺, and Cl⁻ than did Omrabi5, resulting in a higher K⁺/Na⁺ ratio. Salinity
tolerance among several species was associated with exclusion of both Na⁺ and Cl⁻ from shoot (Schachtman et al. 1989; Colmer et al., 1995; Colmer et al., 2006). Storey and Wyn Jones (1979) suggested that the capacity to maintain high shoot K⁺/Na⁺ is an important element of salt tolerance, especially in species which lack foliar salt-excretion mechanisms. However, concentrations of Na⁺ above 100 mM NaCl concentration usually start to inhibit most enzymes as shown by Leopold and Willing (1984) and when the tissue concentrations are over 100 mM, NaCl, the compartmentation of Na⁺ in vacuoles will be induced.

Table 4: Effect of salinity on Na⁺, K⁺, and Cl⁻ concentrations (percentage of dry weight) and K⁺/Na⁺ ratio in leaves of two durum wheat cultivars (Omrabi5, and Belikh2).

<table>
<thead>
<tr>
<th>Salinity level</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>K⁺/Na⁺</th>
<th>Cl⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Omrabi5</td>
<td>Belikh2</td>
<td>Omrabi5</td>
<td>Belikh2</td>
</tr>
<tr>
<td>Control</td>
<td>0.4 dA</td>
<td>0.7 cA</td>
<td>0.80 aA</td>
<td>0.53 aB</td>
</tr>
<tr>
<td>50 mM</td>
<td>5.5 cA</td>
<td>2.8 cA</td>
<td>0.18 bB</td>
<td>0.50 aA</td>
</tr>
<tr>
<td>100 mM</td>
<td>8.5 cB</td>
<td>4.7 cA</td>
<td>0.15 bB</td>
<td>0.49 aA</td>
</tr>
<tr>
<td>150 mM</td>
<td>14.6 bB</td>
<td>5.9 cA</td>
<td>0.14 bB</td>
<td>0.25 bA</td>
</tr>
<tr>
<td>200 mM</td>
<td>21.7 Ab</td>
<td>11.0 bA</td>
<td>0.11 cA</td>
<td>0.22 bA</td>
</tr>
<tr>
<td>250 mM</td>
<td>-</td>
<td>21.7 a cB</td>
<td>0.10 c</td>
<td>-</td>
</tr>
<tr>
<td>300 mM</td>
<td>-</td>
<td>24.3 a</td>
<td>0.09 c</td>
<td>-</td>
</tr>
</tbody>
</table>

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* Values followed by different small letter in column represent significant difference of treatment levels within cultivar at 5% level.

y Values followed by different capital letter in row represent significance among cultivars at 5% level.

- Absent reading due to death of plants during seedling stage.

CONCLUSIONS

In this paper, we have documented differential growth, development, and agronomic and physiological responses of Belikh2 and Omrabi5 durum wheat genotypes under various salinity levels. Differences in plant response and salinity tolerance between cultivars were attributed largely to maintenance of higher K⁺/Na⁺ ratio, less Na⁺, and Cl⁻ accumulation in shoots. These criteria might effectively be utilized in breeding programs to develop salt resistant durum wheat cultivars. Future research to confirm the results under field conditions is recommended and also identification of molecular markers as required to be used in Marker-assisted selection.

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REFERENCES


ICARDA, Aleppo, Syria.
دراسة تأثير الدورة الربيعية والأسمدة المختلفة على نمو الأصناف المختلفة على مANDLE حديثة 

**دورنسون، سوزان* 
**دورنر، محمد* 
**دورنر، نشتي، وميملد* 

**التحضير ويوقعه: ** 
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