Assessment of tolerance to NaCl salinity of five olive cultivars, based on growth characteristics and Na\(^+\) and Cl\(^-\) exclusion mechanisms

Haifa Kchaou b,1, Ajmi Larbi a,1,*, Kamel Gargouri a, Mohamed Chaieb b, Fermín Morales c, Monji Msallema a

a Institut de l’Olivier, BP 208 Tunis, Cité Mahrajène 1082, Tunisia
b Faculté de Science, Sfax 3000, Tunisia
1 Department of Plant Nutrition, Experimental Station of Aula Dei-CSIC, Apdo. 13034, E-50080 Zaragoza, Spain

1. Introduction

In a large agricultural Tunisian area, olive is the most grown fruit tree species, with approximately 60 million trees covering 1611.2 thousand hectares of land. Olive has traditionally been grown under rain-fed conditions, since it is a crop well adapted to the semi-arid and arid Mediterranean region, and able to overcome periods of intense drought, while still producing a reasonable yield. However, the surface of irrigated olive orchards has increased considerably during the last years, motivated by the improvement in the yield when irrigated, and also by the spectacular increase of the price of olive oil. Actually, the irrigated olive orchards are grown under rain-fed conditions, since it is a crop well adapted to the semiarid and arid Mediterranean region, and able to overcome periods of intense drought, while still producing a reasonable yield.

Water used for irrigation in olive orchards is often saline water, especially in the coastal area, centre and southern Tunisia. Furthermore, cultivars used in these orchards are often foreign varieties, mainly ‘Arbequina I18’, ‘Arbosana’ and ‘Koroneiki’. Indeed, during the last years more than 3 millions of olive plants have been imported from Spain. However, little is known about their behavior under Tunisian conditions, and especially about their tolerance to salinity. Thus, little literature is available on the degree of tolerance of ‘Arbequina’ and ‘Koroneiki’ olive varieties (Marin et al., 1995; Chartzoulakis et al., 2002), no work has been done on the tolerance of ‘Arbosana’ olive variety to salinity, and also no much research has been done on the tolerance of Tunisian olive cultivars to salinity (Bouaziz, 1990; Marin et al., 1995).

Olive is moderately tolerant to salinity (Rugini and Fedeli, 1990), although significant differences in salt tolerance have been reported among cultivars (Benlloch et al., 1991, 1994; Tattini et al., 1990, 1992; Tattini, 1994; Marin et al., 1995; Chartzoulakis et al., 2002; Chartzoulakis, 2005). Many studies have focused on physiological
aspects of salinity in plants (Long and Baker, 1986; Morales et al., 1992, 2006; Belkhodja et al., 1994, 1999; Abadia et al., 1999). Thus, plant growth (i.e., root and shoot length, total leaf area, and dry weight) is inhibited by moderate and high salinity (Marin et al., 1995; Tattini et al., 1995; Chartzoulakis et al., 2002; Vigo et al., 2005). The extent of reduction showed, however, significant variation according to the cultivar type and the duration of salt exposure (Chartzoulakis, 2005; Vigo et al., 2005).

Plants grown under NaCl salinity show an increase in sodium and chloride concentrations, and a decrease in potassium, calcium and magnesium (Bartolini et al., 1991; Tattini et al., 1995; Chartzoulakis et al., 2002; Vigo et al., 2002). Nutrient uptake by plants can be reduced by excessive salts in the soil solution, either by direct competition between ions or by increased osmotic potential of the solution reducing the mass flow of mineral nutrients to the root surface (Grattan and Grieve, 1999). Salinity can directly affect nutrient uptake, such as sodium reducing potassium uptake or by chloride reducing nitrate uptake (Grattan and Grieve, 1999). Salinity can also cause a combination of complex interactions that affect plant metabolism, susceptibility to injury or internal nutrient requirement (Grattan and Grieve, 1999). Salt-tolerant plants differ from salt-sensitive ones in having a low rate of sodium and chloride transport to leaves, and the ability to compartmentalize these ions into vacuoles in order to prevent their build up in cytoplasm or cell walls and thus avoid salt toxicity (Munn, 2002). Previous works have demonstrated that salt tolerance in olive cultivars is associated with effective mechanisms of ion exclusion and retention of sodium and chloride in the roots (Benlloch et al., 1991; Tattini, 1994; Tattini et al., 1995; Chartzoulakis et al., 2002), limiting the accumulation of these ions into actively growing shoots. Tattini (1994) reported that the resistance mechanism of salt-tolerant olive cultivar is probably related to sodium exclusion by roots and the ability to maintain an appropriate K/Na ratio in actively growing tissues. Ion exclusion and compartmentation at the root level regulates ion concentration in the xylem sap preventing accumulation of potentially toxic ions in the aerial part (Flower and Yeo, 1989; Drew et al., 1990).

The effectiveness of exclusion mechanism depends on the salinity level. Chartzoulakis et al. (2002) reported that the exclusion mechanism works effectively at low and moderate levels of salinity (up to 50 mM NaCl), while at high salinities, sodium was transported and accumulated in the aerial parts, resulting in toxicity symptoms.

The aim of this work was to investigate and compare the effect of salinity on growth parameters and ions accumulation of five olive cultivars grown under controlled conditions, in order to assess NaCl salinity tolerance, to order cultivars with respect to their response to the salt stress and to determine the level of NaCl at which the plants start showing the salinity symptoms.

2. Materials and methods

2.1. Plant material

Main Tunisian olive (Olea europaea L.) cultivars (‘Chemlali’ and ‘Chetoui’) and three foreign olive cultivars imported from Spain, which have recently been grown extensively in Tunisia (‘Arbequina I18’, ‘Arbosana I43’ and ‘Koroneiki’), were studied. One-year-old plants, which were uniform and pruned to only one shoot, were used in this work. Before transplanting plants into pots of 8 l containing sand–perlite mixture (1:1), roots were washed with water to eliminate residues of substrate. Plants were irrigated daily (except Sunday) with a half-strength Hoagland nutrient solution with the following composition (mM) (2.5 KNO₃, 1 MgSO₄, 1 KH₂PO₄, 2.5 Ca(NO₃)₂·4H₂O and 0.5 NaCl and (µM) (4.6 MnCl₂, 23.1 H₂BO₃, 0.06 Na₂MoO₄, 1.2 ZnSO₄ and 0.19 CuSO₄). Each two weeks, iron was added as NaFe(III)-EDTA (45 µM) (Sigma, St Louis, MO, USA) to avoid the appearance of iron deficiency symptoms. Plants were grown in these conditions during two months. Each plant received daily 50 ml of nutrient solution. Salinity treatments were imposed after the two months period of adaptation. Three levels of salinity and the control were used in this experiment. Control plants were irrigated with half-strength Hoagland nutrient solution, which included 0.5 mM NaCl (1.2 dS m⁻¹). For salinity treatments, NaCl was added to the half-strength Hoagland nutrient solution at 50, 100 and 200 mM. The respective electrical conductivities (EC) of the solutions were 6.6, 11.8 and 20.8, respectively. Salinity treatments were applied incrementally over a 10 days’ period to avoid osmotic shock to the seedlings. After application of salt treatment, 200 ml of de-ionized water was applied weekly to each plant to enhance ions, mainly sodium and chloride, absorption. It must be pointed out that de-ionized water addition did not influence substrate EC. Indeed, only a slight and insignificant decrease of substrate EC was found after each addition (data not shown). Substrate EC was also controlled regularly. 20 g of substrate (sand + perlite) was sampled every 10 days at the beginning (from 0 to 60 days) and every month at the end (from 60 to 150 days). The former sampling was used to determine the time required to reach a substrate EC’s similar to that of applied water. Samples were taken from every container, and the samples of each treatment (5 replicates, each one corresponding to one variety) were mixed together in order to get a representative sample. Then, the saturated paste was prepared and, after 4 h, the rest of the water extracted by centrifugation at 3500 rpm for 15 min. EC was measured on the obtained aqueous extract.

During the first 14 days, EC substrate increased highly from 1.59, 3.47, 5.53 and 7.45 dS m⁻¹ at 10 days to 1.80, 5.89, 14.6 and 16.64 dS m⁻¹ at 40 days after salt application for control, 50, 100 and 200 mM NaCl, respectively. From 40 days until the end of the experiment, a little increase was found and average final substrate EC after 150 days for all tested varieties was 2.88, 10.92, 18.23 and 22.71 dS m⁻¹ for control, 50, 100 and 200 mM NaCl, respectively.

Plants were placed in a plastic greenhouse. Salinity treatments were applied on March. Each treatment was applied to five plants.

2.2. Growth parameters

The length of the main-shoot (in cm), trunk diameter (cm) and internodes length (cm) were measured just before application of treatments and monthly after treatment application until the end of the experiment.

2.3. Leaf area and leaf thickness

During the experiment, leaf area was determined using the equation established by Tattini et al. (1995). The length and width of five marked leaves were measured monthly. The area of each leaf was calculated according to the following regression equations:

\[ Y = 0.735X + 0.125 \quad (R^2 = 0.987) \]

where \( Y \) is leaf area and \( X \) is the product length \( \times \) width. It must be pointed out that this equation had been previously verified for each variety employed in this work. Very good correlations were obtained between leaf area calculated by using this equation and leaf area calculated from the weight of leaves. A leaf disk with known surface area was cut with a calibrated cork borer and then weighed. The rest of the leaf was also weighed and leaf area was determined. Significant linear correlations (\( r = 0.997^{**}, 0.998^{**}, 0.998^{**}, 0.999^{**} \) and \( 0.996^{**} \) ) were obtained between those two methods of calculating leaf area for ‘Arbequina I18’, ‘Arbosana I43’, ‘Koroneiki’, ‘Chetoui’ and ‘Chemlali’, respectively. At the end of the experiment, the total number and area of leaves of each plant were measured. Leaf thickness was also measured monthly on five marked leaves and, by the end of the experiment, leaf thickness average of the totality of leaves were determined.
2.4. Dry weight

At the end of the experiment, each plant was separated into leaves, stem, and roots. Each part was weighted and then washed once with tap water and once with distilled water, dried at 60 °C for 72 h, and the dry weight of each plant part was determined.

2.5. Mineral nutrient analysis

The mineral composition of each plant organ (roots, shoot and leaves) was determined at the end of the experiment. Leaves, shoots and roots were harvested and analyzed for sodium, chloride and potassium. Roots were intensively washed with de-ionized water. Samples were processed using standard procedures (AOAC, 1990). Sodium and potassium were determined by flame emission spectrophotometer (JENWAY), and chloride by the Mohr method. Results were expressed as percentage of dry weight (DW). Drooped leaves were also collected during the experiment and then analyzed for sodium, chloride and potassium.

2.6. Data analysis

Data were analyzed using analysis of variance (ANOVA) procedures, means separated by Duncan’s multiple range test, and Pearson correlation with the SPSS (Statistical Package of the Social Sciences) base 13.0 software (Chicago, IL, USA).

3. Results

3.1. Effect of salinity on plant growth

After 150 days of salinity treatment, plant growth (total shoot length, trunk diameter) and total plant dry weight were significantly reduced in all cultivars (Table 1). The effect of salinity on these parameters showed a significant genotypic variation. The highest reduction in main-shoot length was found in ‘Arbequina I18’, which reached by 69% at 50 mM NaCl, whereas the lowest one was obtained in ‘Arbosana I43’ with a 15.5% decrease when compared to control plants. At 200 mM NaCl, main-shoot elongation was more depressed in all cultivars. This reduction ranged from 64% for ‘Chemlali’ to 85% for ‘Arbequina I18’.

Trunk diameter was also significantly reduced by salinity treatments. At 50 mM NaCl, the reduction of trunk diameter ranged from 39% for ‘Arbosana I43’ and ‘Chemlali’ to 56% for ‘Arbequina I18’. At 200 mM NaCl, trunk diameter was severely reduced, and it reached 65% for ‘Arbosana I43’, 72% for ‘Chemlali’ and ‘Koroneiki’, and 84% for ‘Arbequina I18’, with respect to the control plants (Table 1).

Total plant dry weight was significantly reduced in all studied cultivars from the concentration of 50 mM NaCl, except in ‘Chetoui’. At 50 mM NaCl, the highest decrease of total plant dry weight was observed for ‘Arbequina I18’, which was about 52% with respect to the control plants. At 200 mM NaCl, total plant dry weight reduction was more severe, and it ranged from 25% for ‘Arbosana I43’ to 74% for ‘Koroneiki’. The decrease in total plant dry weight could be ascribed essentially to the decrease of the dry weight of the aerial part (shoot + leaves). Indeed, salinity treatments affected the shoot mass more than the root mass, resulting in an increased root/shoot ratio with the increase of NaCl concentration (Table 1).

3.2. Effect of salinity on leaf area and leaf thickness

After the application of salt treatments, leaf area decreased progressively in all cultivars with the exception of ‘Koroneiki’ at 50 mM NaCl. These decreases were already very marked 30 days after salinity application for all applied treatments, and the highest reductions were observed at the highest concentrations of NaCl (100 and 200 mM) (Fig. 1A). In contrast to the leaf area, leaf thickness increased gradually during the salinity treatment period (Fig. 1B). Theses increases were observed in all studied varieties (Fig. 1B).

Table 1

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Shoot elongation (cm)</th>
<th>Trunk diameter (cm)</th>
<th>Roots dry weight (g)</th>
<th>Stem dry weight (g)</th>
<th>Leaves dry weight (g)</th>
<th>Total plant dry weight (g)</th>
<th>Roots/aerial part</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemlali</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14.80a</td>
<td>0.87a</td>
<td>4.48a</td>
<td>7.50a</td>
<td>5.24a</td>
<td>17.20a</td>
<td>0.35</td>
</tr>
<tr>
<td>50</td>
<td>9.20b</td>
<td>0.53b</td>
<td>3.84b</td>
<td>5.73b</td>
<td>3.88b</td>
<td>13.40b</td>
<td>0.39</td>
</tr>
<tr>
<td>100</td>
<td>8.93b</td>
<td>0.27c</td>
<td>3.89b</td>
<td>5.91b</td>
<td>3.92c</td>
<td>13.70b</td>
<td>0.39</td>
</tr>
<tr>
<td>200</td>
<td>5.20c</td>
<td>0.25</td>
<td>3.50b</td>
<td>5.16b</td>
<td>2.05c</td>
<td>10.70c</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>Chetoui</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>23.60a</td>
<td>0.66a</td>
<td>4.68a</td>
<td>11.41a</td>
<td>5.75a</td>
<td>21.85a</td>
<td>0.27</td>
</tr>
<tr>
<td>50</td>
<td>10.07b</td>
<td>0.36b</td>
<td>5.27a</td>
<td>11.0a</td>
<td>4.86a</td>
<td>21.14a</td>
<td>0.33</td>
</tr>
<tr>
<td>100</td>
<td>9.27b</td>
<td>0.28b</td>
<td>4.17a</td>
<td>6.34b</td>
<td>3.95b</td>
<td>14.46b</td>
<td>0.40</td>
</tr>
<tr>
<td>200</td>
<td>5.97c</td>
<td>0.22b</td>
<td>3.11b</td>
<td>5.83b</td>
<td>2.42c</td>
<td>11.36c</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Koroneiki</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>27.10a</td>
<td>1.25a</td>
<td>8.12a</td>
<td>12.7a</td>
<td>6.89a</td>
<td>27.71a</td>
<td>0.41</td>
</tr>
<tr>
<td>50</td>
<td>16.70b</td>
<td>0.73b</td>
<td>6.19b</td>
<td>8.37b</td>
<td>3.92b</td>
<td>18.50b</td>
<td>0.50</td>
</tr>
<tr>
<td>100</td>
<td>10.15c</td>
<td>0.40c</td>
<td>2.36c</td>
<td>3.32c</td>
<td>2.10c</td>
<td>7.78c</td>
<td>0.43</td>
</tr>
<tr>
<td>200</td>
<td>10.07c</td>
<td>0.41c</td>
<td>2.50c</td>
<td>3.73c</td>
<td>0.91d</td>
<td>7.14c</td>
<td>0.53</td>
</tr>
<tr>
<td><strong>Arbequina I18</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>15.90a</td>
<td>1.36a</td>
<td>3.50a</td>
<td>5.00a</td>
<td>5.94a</td>
<td>14.45a</td>
<td>0.32</td>
</tr>
<tr>
<td>50</td>
<td>4.92b</td>
<td>0.60b</td>
<td>1.56b</td>
<td>2.93b</td>
<td>2.43b</td>
<td>6.92b</td>
<td>0.29</td>
</tr>
<tr>
<td>100</td>
<td>3.75b</td>
<td>0.26c</td>
<td>1.60b</td>
<td>1.75c</td>
<td>1.57c</td>
<td>4.92b</td>
<td>0.48</td>
</tr>
<tr>
<td>200</td>
<td>2.33b</td>
<td>0.22c</td>
<td>1.36b</td>
<td>1.61c</td>
<td>1.69c</td>
<td>4.66b</td>
<td>0.41</td>
</tr>
<tr>
<td><strong>Arbosana</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6.27a</td>
<td>0.66a</td>
<td>3.57a</td>
<td>3.42a</td>
<td>2.57a</td>
<td>9.56a</td>
<td>0.59</td>
</tr>
<tr>
<td>50</td>
<td>5.30b</td>
<td>0.40b</td>
<td>3.14a</td>
<td>3.32a</td>
<td>1.86a</td>
<td>8.32b</td>
<td>0.60</td>
</tr>
<tr>
<td>100</td>
<td>3.95c</td>
<td>0.34b</td>
<td>3.19a</td>
<td>3.15a</td>
<td>2.17a</td>
<td>8.51b</td>
<td>0.61</td>
</tr>
<tr>
<td>200</td>
<td>2.60c</td>
<td>0.22c</td>
<td>2.98a</td>
<td>2.26b</td>
<td>1.93a</td>
<td>7.17c</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Means within the column followed by the same letter are not significantly different at p = 0.05 level, using Duncan’s test.
By the end of the 150-days salinity treatments, we measured the total leaf area and leaf thickness. Results obtained show that the total leaf area per plant was reduced significantly in all applied salinity treatments. Leaf area reduction was already marked at 50 mM NaCl, mainly in ‘Arbequina I18’ (77% compared to the controls) (Fig. 2A). The increase of NaCl concentration caused a further decrease of total leaf area per plant in all investigated varieties, which reached 87% in ‘Arbequina I18’ with respect to the controls. The lowest decrease was found in ‘Chetoui’ (48.5% compared to the controls) (Fig. 2A). We investigated the correlation between the leaf sodium concentration and total leaf area per plant. Decreases in total leaf area per plant were linearly correlated with increases of leaf sodium concentration ($r = -0.74^{**}$, Fig. 3A).

In contrast to the total leaf area, the leaf thickness increased in response to salt stress. However, the extent of this increase differed among studied varieties. ‘Chemlali’ and ‘Chetoui’ developed thicker leaves under high salinity levels (100 and 200 mM), which were 1.6–2-fold thicker than the control leaves (Fig. 2B). Leaf thickness increase in ‘Arbequina I18’, ‘Arbosana I43’ and ‘Koroneiki’ in response to salinity was less important, and leaves were 1.2–1.6-fold thicker when compared to the controls (Fig. 2B). A positive linear relationship was found between leaf thickness increase and increases of leaf sodium concentration ($r = 0.82^{**}$, Fig. 3B).

3.3. Internodes length

Significant decrease of internode length of all tested varieties was observed at the end of the experiment. This decrease was significant even at moderate salinity (50 mM) in ‘Chemlali’ (Fig. 2C). At high salinity levels (100 and 200 mM), internode
length decrease was more accentuated, and the highest decrease (38%) was obtained in 'Koroneiki' whereas the lowest one was obtained in 'Chemlali' (19%) (Fig. 2C).

3.4. Tissue mineral content

Both sodium and chloride concentration in the different plant parts (leaves, shoot and roots) increased gradually with all salinity treatments. In most cases, root sodium concentration was higher than that of shoot and leaves (Fig. 4A). The root sodium concentration showed a similar trend, increasing proportionally with the increase of NaCl concentration in the nutrient solution up to 100 mM NaCl and remaining fairly constant at 200 mM, except in ‘Chemlali’ that continued increasing (Fig. 4A). Concerning chloride concentrations, ‘Chetoui’ (at 100 mM NaCl) and ‘Arbequina I18’ (at 100 and 200 mM NaCl) showed a chloride exclusion mechanism more effective than ‘Arbosana I43’ and ‘Koroneiki’ (Fig. 5B and D).

Salinity induced a decrease of potassium concentration in the different plant parts. Salinity treatment led to the inhibition of the absorption of this cation in all tested varieties. The same tendency was observed for all tested varieties with a pronounced decrease when NaCl concentration increased in the nutrient solution. The decrease of potassium concentration at 200 mM NaCl comparing to the controls plants was between 3 and 59% in the roots, between 20 and 49% in the shoots, and between 34 and 63% in the leaves (Fig. 4C).

Samples of new leaves formed after salt treatment application (50 and 100 mM) were analyzed for sodium, chloride and potassium (only ‘Chemlali’ and ‘Chetoui’ cultivars had formed new leaves at 200 mM). Sodium concentration in new leaves was significantly lower than that of old leaves at 50 and 100 mM NaCl, except for ‘Koroneiki’ at 100 mM. This difference was ca. 44 and 21% at 50 mM for ‘Chemlali’ and ‘Koroneiki’, whereas at 100 mM
NaCl the difference was ca. 24 and 30% for ‘Chemlali’ and ‘Chetoui’, respectively (Fig. 6A and B).

Chloride concentration in new leaves was also significantly lower than that found in old leaves, especially at 100 mM NaCl (Fig. 6C and D). Decrease of chloride concentration in neo-formed leaves as compared to old leaves was ca. 60% in ‘Koroneiki’ at 50 mM NaCl. At 100 mM NaCl, reduction of chloride concentration in newly formed leaves as compared to old leaves was about 51, 22 and 21% in ‘Chemlali’, ‘Chetoui’ and ‘Koroneiki’, respectively.

Potassium concentration of leaves formed after salinity treatments application only changed in ‘Chetoui’ (17% decrease at 50 mM NaCl), and in ‘Chemlali’ and ‘Koroneiki’ (10–27% increase at 100 mM NaCl) when compared to the old leaves (Fig. 6E and F).

3.5. Toxic symptoms

Toxicity characteristic symptoms resulted from salinity stress are characterized by leaf edge death and leaf drop in all tested cultivars. However, the severity of these symptoms differed significantly among tested varieties. Leaf drop phenomenon was observed essentially in ‘Arbequina I18’, and ‘Arbosana I43’ from the concentration of 50 mM NaCl. Indeed, leaf drop phenomenon was observed at 80 days after salt application for these varieties and was more pronounced later. At 100 mM NaCl, leaf drop phenomenon was observed in all tested varieties but to different extent. The most affected cultivar was ‘Arbequina’ followed by ‘Arbosana’ with an important leaf drop at 60 days after salt application. However, for the rest of cultivars this phenomenon starts to be observed at 80 days after salt application and was more important at 120 days for ‘Chemlali’ and ‘Koroneiki’. The less affected cultivar was ‘Chetoui’. At 200 mM, ‘Arbequina I18’ and ‘Arbosana I43’ start to show symptoms of leaf drop from 45 days after salt application. This phenomenon was also very important in ‘Chemlali’ and ‘Koroneiki’ from 80 days after salt application. Leaf drop phenomenon was followed by withering and death of some plants, and it was mainly observed at 100 and 200 mM NaCl (data not shown).

Dropped leaves were collected from ‘Arbequina I18’, ‘Chemlali’ and ‘Arbosana I43’, and then analyzed for chloride, sodium and...
Fig. 5. Percentage of leaf sodium (A and C) and chloride (B and D) concentration (% dry weight) as compared to root sodium and chloride concentrations (% dry weight) at 100 mM (A and B) and 200 mM (C and D) NaCl in the five olive cultivars investigated. Data are means ± SE of 3–5 replicates. Bars marked with the same letter were not significantly different (Anova) at the $p < 0.05$ probability level.

Fig. 6. Effect of NaCl salinity at 50 mM (A, C and E) and 100 mM (B, D and F) on young and old leaf sodium, chloride and potassium concentration (% dry weight) of three olive cultivars Chemlali, Chetoui and Koroneiki. Data are means ± SE of 3–5 replicates. Bars marked with the same letter were not significantly different (Anova) at the $p < 0.05$ probability level.
potassium. Results obtained show that dropped leaves sodium concentration was significantly higher than that of attached leaves. This increase was ca. 31, 46 and 33% for ‘Arbequina I18’, ‘Chemlali’ and ‘Arbosana I43’, respectively (Table 2). Dropped leaves chloride concentration was also significantly higher than that found in attached leaves. The chloride concentration in dropped leaves increased by 30.5, 34 and 19% as compared to attached leaves in ‘Arbequina I18’, ‘Chemlali’ and ‘Arbosana I43’, respectively (Table 2). Potassium concentrations were also higher in dropped leaves when compared with the respective attached leaves (Table 2). It must be taken in consideration that dropped leaves were collected at different times during the experiment.

4. Discussion

The aim of the present study was to assess the salinity tolerance of five olive cultivars (‘Arbequina I18’, ‘Arbosana I43’, ‘Koroneiki’, ‘Chemlali’ and ‘Chetoui’), based on the effects of salinity on growth characteristics and on the ability of the different cultivars to avoid transport to leaves of the toxic ions sodium and chloride. Both criteria have been reported to have genotypic variability in response to salt stress (Tattini, 1994; Chartzoulakis et al., 2002; Chartzoulakis, 2005; Vigo et al., 2005), and could be used to assess the degree of salinity tolerance. Thus, Tabatabaei (2006) indicated that salt tolerance in olive trees is associated with the ability to reduce uptake and/or transport of salinity (Therios and Misopolinos, 1988; Tattini et al., 1992, 1995; Aragués et al., 2004) reported that the sodium exclusion mechanism in olive trees (one internodes length and leaf thickness) ‘Arbequina’ and ‘Chemlali’ to exclude this ion from the aerial part. The less effective mechanism at high salinity was found in ‘Chemlali’ variety, which was characterized by an important accumulation of sodium in roots and shoot and leaves. In moderate salt tolerance (50 mM NaCl) the sodium exclusion capacity was not effective in all studied cultivars, except in ‘Arbosana I43’ cultivars. However, the effectiveness of this mechanism was more important at 100 and 200 mM NaCl. The most effective mechanism at high salinity was found in ‘Chemlali’ variety, which is characterized by an important accumulation of sodium in roots and an important inhibition of translocation of this element to the aerial part. However, ‘Arbosana I43’ and ‘Koroneiki’ accumulated sodium at high concentrations in roots, but are less effective than ‘Chemlali’ to exclude this ion from the aerial part. The less effective mechanism was found in ‘Arbequina’ and ‘Chetoui’ varieties, which showed similar sodium concentrations in the different plant parts. Similar results were found by Chartzoulakis et al. (2002), who reported that the sodium exclusion mechanism in olive trees (one-year-old) seems to work effectively at moderate levels of salinity up to 50 mM NaCl. The same authors indicated that at high salinities in most cultivars, sodium was transported and accumulated to the aerial parts, resulting toxicity symptoms except in salt-tolerant genotypes. Differences in effectiveness of sodium exclusion mechanism among cultivars at high salinity reflect differences in salt tolerance (Tattini, 1994; Chartzoulakis et al., 2002).

Grattan and Grieve (1999) reported that numerous studies with a wide variety of horticultural crops have shown that potassium concentration in plant tissue declines as the salinity in the root media increases. Our results show that salinity treatment in olive trees decreased leaf and root potassium concentrations. This may be due to a direct effect of sodium, displacing potassium, and/or causing loss of potassium from the root tissue.

Salinity-mediated growth changes and the ability of the different cultivars to avoid transport to leaves of the toxic ions

<table>
<thead>
<tr>
<th>Olive cultivars</th>
<th>Salinity (mM)</th>
<th>Na⁺ (% dry weight)</th>
<th>Cl⁻ (% dry weight)</th>
<th>K⁺ (% dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Attached leaves</td>
<td>Dropped leaves</td>
<td>Attached leaves</td>
<td>Dropped leaves</td>
</tr>
<tr>
<td>Arbequina I18</td>
<td>100</td>
<td>0.55 ± 0.03a</td>
<td>0.78 ± 0.02b</td>
<td>0.38 ± 0.03a</td>
</tr>
<tr>
<td>Arbequina I18</td>
<td>200</td>
<td>0.58 ± 0.04a</td>
<td>0.91 ± 0.03b</td>
<td>0.41 ± 0.06a</td>
</tr>
<tr>
<td>Chemlali</td>
<td>200</td>
<td>0.45 ± 0.03a</td>
<td>0.83 ± 0.02b</td>
<td>0.35 ± 0.09a</td>
</tr>
<tr>
<td>Arbosana</td>
<td>200</td>
<td>0.51 ± 0.02a</td>
<td>0.76 ± 0.02b</td>
<td>0.38 ± 0.08a</td>
</tr>
</tbody>
</table>

Values marked with the same letter were not significantly different (Anova) at the p < 0.05 probability level.
sodium and chloride are not variables completely independent. For instance, total leaf area per plant was significantly reduced in all cultivars by salt treatment, which was due to both a decrease of leaf growth per se and a leaf abscission phenomenon during the salt treatments. At high salinity levels (100 mM), old leaf sodium and chloride concentrations were higher than those of young leaves in most of the cultivars studied, indicating preferential accumulation of sodium and chloride in old leaves that avoids deleterious effect of salinity on young leaves and apical stem. Leaf abscission, which occurred preferentially in old leaves, can be considered an additional tolerance mechanism of olive varieties allowing the elimination of leaves that had accumulated sodium and chloride ions. The extent of total leaf area per plant reduction varied among tested varieties, and this reduction was highly correlated with leaf sodium concentration. Previous studies have indicated the depression of leaf area by salt treatment in olive trees (Tattini et al., 1995; Chartzoulakis et al., 2002) and in other plant species such as citrus (Camara-Zapata et al., 2004). Contrary to what was observed in total leaf area per plant, leaf thickness increased by salt treatments, and the extent of this increase differed between studied varieties. This increase was also linearly correlated with leaf sodium concentration (r = 0.82**). Some authors have indicated that the increase of leaf thickness is a common response of plants grown under saline conditions, and is due to osmotic adjustment (Nastou et al., 1999; Sotiropoulos et al., 2002).

Christodulakis and Bazos (1990) indicated that the increase of leaf thickness seems to increase the internal surface per unit of leaf area in which CO2 and water vapor diffusion takes place, with the consequent reduction of internal resistance to CO2 assimilation and keeping water potential at high levels. Vigo et al. (2005) indicated that Chondrorolia Chalikidikis olive cultivar subjected to high seawater concentration developed thicker leaves, with a thicker palisade and spongy parenchyma, and denser hairs than did control plants, adapting them to the salinity conditions.

The level of NaCl beyond which salinity significantly affect the olive plants’ behavior varied significantly among studied cultivars. Based on observation of typical symptoms of salinity (leaf drop), we suggest that water containing 50 mM NaCl is the threshold value for Arbequina I18 and Arbosana I43. It must be taken into account that substrate EC when this NaCl dose is employed was approximately (11 dS m⁻¹) at 150 days after salt application. Similar results has been reported by Aragüés et al. (2004), who indicated that survival of ‘Arbequina’ is compromised when grown in a soil with EC of 10 dS m⁻¹. For ‘Koroneiki’, ‘Chemlali’ and ‘Chetoui’, the suggested level is 100 mM NaCl with a substrate EC about 18 dS m⁻¹ at 150 days after salt application. The maximum salt residue in irrigation water tolerated by olive has been estimated at 137 mM NaCl (Rugini and Fedeli, 1990).

In conclusion, tolerance to salinity stress in the five cultivars investigated was as follows: ‘Chemlali’ (because it was the best one excluding sodium and chloride (at 200 mM NaCl) from the aerial part, and showed a moderate growth reduction at 50 mM NaCl), ‘Chetoui’ (because growth is not significantly affected at 50 mM NaCl), ‘Arbosana I43’ and ‘Koroneiki’ (between both criteria, both excluded similarly sodium although ‘Arbosana’ excluded a slightly better chloride and the growth in presence of salt is better in ‘Arbosana’ than in ‘Koroneiki’), and ‘Arbequina I18’ (because of the large growth reductions even at moderate (50 mM NaCl) salinity, and because it is within the cultivars that exclude worse sodium). The tolerance of ‘Chemlali’ to salinity was not due only to the effectiveness of the exclusion mechanism in the root system and moderate salinity-mediated growth reduction but also due to the mechanism by which plants eliminate leaves accumulating excessively sodium and chloride. In ‘Chetoui’, in spite of the high accumulation of sodium in leaves, growth parameters were not severely affected in presence of salt when compared to ‘Arbosana I43’, ‘Koroneiki’ and ‘Arbequina I18’. Furthermore, no leaf toxicity or intense leaf drop was observed during the five months of the experiment, and therefore ‘Chetoui’ should be considered tolerant to salinity.

Acknowledgements

This work was supported by grants from Olive tree Institute to Ajmi Larbi and Monji Msallem. Fermín Morales wishes to thank Gobierno de Aragón (A03 research group) for financial support. Authors gratefully acknowledge the technical assistance to Nabil Soua in mineral analysis techniques.

References


