Comparative Physiological Analysis of Salinity Effects in Six Olive Genotypes

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Abstract. Changes caused by NaCl salinity on growth, gas exchange, chemical composition, and oxidative stress symptoms have been measured in six olive (Olea europaea L.) cultivars (Casta Cabra, Cornicabra, Frantoio, Ocal, Picual, and Picudo) grown in a growth chamber pot experiment. Six-month-old plants were transplanted to a sand–perlite culture and irrigated with half-strength Hoagland's nutrient solution containing 0 and 200 mM NaCl for 12 weeks. Salinity significantly depressed growth and leaf gas exchange, but to a different degree in each cultivar. Picudo was the cultivar that showed less growth inhibition. The effectiveness of Na\(^+\) exclusion mechanism in the roots differed significantly among studied cultivars, working effectively in ‘Ocal’ and ‘Picudo’ and being less efficient in ‘Picual’. Furthermore, ‘Picudo’ showed the ability to maintain the concentration of leaf K\(^+\) under the stress condition. ‘Ocal’ accumulated phenolic compounds and did not reduce carotenoid or total thiol concentration under saline stress. Between the cultivars studied, ‘Picudo’ and ‘Ocal’ were the most tolerant.

In the Mediterranean region, the olive is considered to be the most important fruit tree in the area (International Olive Oil Council, 2003). In this region, olive tree cultivation is being extended to irrigated land where salinity is becoming a major problem as a result of a high rate of evaporation and insufficient leaching (Calero et al., 2013; Chartzoulakis, 2005). In addition, water scarcity in the Mediterranean basin restricts the availability of fresh water for crop irrigation. To overcome water shortages and to satisfy the increasing water demand for agricultural development, the use of saline water may become an unavoidable necessity.

Olive is considered moderately tolerant to salinity (Demiral, 2005), although the response of plants to saline stress is a genotypic-dependent characteristic (Chartzoulakis et al., 2002; Weissenbein et al., 2008). The olive tree’s ability to acclimate to saline stress includes morphological, anatomical, and physiological alterations at the leaf level (Tattini et al., 1995). However, tolerance to NaCl in olive is mostly related to the salt exclusion mechanism at the root level, which prevents sodium (Na\(^+\)) accumulation in leaf tissue as well as the ability of the olive to maintain an essential potassium (K\(^+\))/Na\(^+\) ratio (Chartzoulakis et al., 2002).

Salt stress inhibits photosynthesis in olive trees, attributable mainly to stomatal closure (Loreto et al., 2003) and salt ion accumulation (Melgar et al., 2008), whereas the ensuing limitation of CO\(_2\) assimilation triggers the over-reduction of the photosynthetic electron chain. As a reaction, to avoid photoinhibition, electron transfer along the photosynthetic chain is directed to oxygen acceptors other than water (Munns and Tester, 2008). This excessive reducing power prompts the production of reactive oxygen species (ROS) that triggers lipid peroxidation, DNA damage, inhibition of photosynthesis, and disturbance in mineral nutrient status (Cordovilla et al., 2014; Turan and Tripathy, 2012).

The accumulation of low-molecular-weight osmolytes such as carbohydrates and amino acids is a well-known adaptive mechanism in plants against saline stress (Iqbal et al., 2014; Munns and Tester, 2008). Moreover, it has been reported that proline protects higher plants against osmotic stress not only by facilitating the retention of water in the cytoplasm, but also by functioning as an oxygen radical scavenger and by displaying an antioxidant activity (Iqbal et al., 2014).

The fresh water scarcity in the Mediterranean basin for crop irrigation, the socioeconomic importance of cultivation olive, and the lack of studies comparing olive cultivars of less than a year in controlled conditions were the leading decisive factors to carry out this research. In fact, olive plants of less than 1 year in the nurseries could be irrigated with saline water to help overcome water shortages. Therefore, the main aim of this study is to compare the salinity tolerance of six olive genotypes of great socioeconomic importance in the Mediterranean region. In an effort to elucidate the adaptive strategies of young olive plants to salinity salt accumulation (Na\(^+\), K\(^+\)) in roots and leaves, leaf concentration of photosynthetic pigments (chlorophylls, carotenoids), free amino acids, free proline, total soluble carbohydrates, total phenols, and total thiols were measured. Also, the effects of salinity on growth and gas exchange rates [net CO\(_2\) assimilation rate and stomatal conductance (gs)] were assessed.

Materials and Methods

Plant material and growth conditions. This study was conducted with five autochthonous Spanish cultivars (Casta Cabra, Cornicabra, Ocal, Picual, and Picudo) and one Italian cultivar (Frantoio). Uniform, 6-month-old rooted plants (Viveros Laserplant CB, Córdoba, Spain) were transplanted to 1.5-L pots containing a sand–perlite mixture (1:3, v/v). Plants were well established by watering three times per week with 100 mL of half-strength Hoagland’s solution (Hoagland and Arnon, 1950). After 4 weeks, salt treatments were started by daily applying 25 mM NaCl in the nutrient solution to reach the final NaCl concentration of 200 mM, whereas the salt-free control plant (0 mM) continued to receive only nutrient solution. The salinity treatments lasted 12 weeks, the experiment ending when the plants were 10 months old. The experimental design was a six × two factorial (six cultivars × two salt treatments) with six replicate plants in each treatment using a complete random design in a growth chamber with a 16-8 h light–dark cycle, 25 to 20 °C day–night temperature, relative humidity 55% to 75%, and photosynthetic photon flux density (400 to 700 nm) of 500 µmol·m\(^{-2}\)·s\(^{-1}\).

Plant growth and leaf parameters. At the end of the experiment, all plants were gently removed from the substrate, roots were washed with distilled water, and partitioned into different organs. After the measurement of fresh weight, shoot length, and total leaf area (LA) of each plant, roots, stems, and leaves were dried at 65 °C for 72 h in a forced-d-air oven, and the dry weight (DW) was determined. LA was determined using the method established by Tattini et al. (1995). The area of each leaf was calculated according to the following regressions equations:

For ‘Casta Cabra’:

\[ Y = 0.695X + 0.139(r^2 = 0.980) \]

For ‘Cornicabra’:

\[ Y = 0.732X - 0.061(r^2 = 0.984) \]

For ‘Frantoio’:

\[ Y = 0.641X + 0.383(r^2 = 0.986) \]
For ‘Ocal’:
\[ Y = 0.662X + 0.257 \quad (r^2 = 0.978) \]

For ‘Picual’:
\[ Y = 0.639X + 0.304 \quad (r^2 = 0.987) \]

For ‘Picudo’:
\[ Y = 0.688X + 0.014 \quad (r^2 = 0.986) \]

where \( Y \) is leaf area and \( X \) is the product length \( \times \) width.

The concentrations of Na\(^+\) and K\(^+\) in leaf and root were measured with an emission-absorption spectrophotometer (Perkin Elmer AAnalyst 800) after the tissue had been dry-ashed for 24 h at 450 °C and suspended in 37% HCl.

Biochemical analysis. Net CO\(_2\) assimilation rate \((P_n)\) and \(g_S\) were measured at saturating light photosynthetically active radiation greater than 800 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) on the youngest fully expanded leaves of six plants per treatment using a portable photosynthesis system (LI-COR 6400; LI-COR Inc., Lincoln, NE). Measurements were made between 1 and 4 h after the beginning of the photoperiod. Leaf sections were homogenized in 80% acetone (Arnon, 1949) for chlorophyll (Chl),

|                   | Shoot length (cm/plant) | Total DW (g/plant) | Rt/Sh (cm\(^2\)/plant) | \(P_n\) (mmol m\(^{-2}\) s\(^{-1}\)) | \(g_S\) (mmol m\(^{-2}\) s\(^{-1}\)) | Chl\(_{a+b}\) (mg g\(^{-1}\) DW) | Car (mg g\(^{-1}\) DW) |
|-------------------|-------------------------|--------------------|-------------------------|-------------------------------------|-------------------------------|---------------------------------|---------------------|
| Casta Cabra       | 7.85 de                 | 1.96 bc            | 0.33 g                  | 77.11 d                             | 11.12 a                       | 157.23 b                        | 3.90 b                  |
| 200                | 2.98 e                  | 0.87 f             | 0.97 d                  | 25.73 e                             | 8.92 b                        | 24.50 d                         | 1.91 ef                 |
| Cornicabra        | 31.60 a                 | 3.06 a             | 0.45 f                  | 122.15 ab                           | 8.01 bc                       | 120.88 bc                       | 2.79 c                  |
| 200                | 7.20 de                 | 1.31 ef            | 1.07 c                  | 42.35 e                             | 5.65 de                       | 27.25 d                         | 2.13 ef                 |
| Frantoio          | 24.42 b                 | 3.23 a             | 0.66 e                  | 92.93 cd                            | 11.31 a                       | 270.02 a                        | 2.95 c                  |
| 200                | 9.54 d                  | 1.71 cde           | 1.39 ab                 | 31.80 e                             | 4.87 de                       | 64.89 cd                        | 1.97 e                  |
| Ocal              | 16.00 c                 | 2.88 a             | 0.46 f                  | 102.37 bc                           | 7.64 bc                       | 119.76 bc                       | 2.71 cd                 |
| 200                | 3.47 e                  | 1.02 f             | 1.30 b                  | 20.38 e                             | 4.33 de                       | 18.29 d                         | 2.17 e                  |
| Picual            | 21.55 b                 | 2.94 a             | 0.39 fg                 | 136.00 a                            | 8.60 b                        | 160.03 b                        | 4.70 a                  |
| 200                | 6.12 de                 | 1.43 def           | 1.05 cd                 | 42.63 e                             | 6.46 cd                       | 15.71 d                         | 2.03 e                  |
| Picudo            | 5.86 de                 | 2.04 b             | 0.69 e                  | 74.09 d                             | 8.81 b                        | 128.69 bc                       | 2.25 de                 |
| 200                | 3.10 e                  | 1.10 f             | 1.41 a                  | 25.83 e                             | 5.34 de                       | 20.42 d                         | 1.41 f                  |

*Values are mean of six replicates for shoot length, total DW, Rt/Sh, LA, \(P_n\), and \(g_S\), and four replicates for photosynthetic pigments. Within each column, means followed by the same letters are not significantly different at 5%.

Fig. 1. Percentage of leaf sodium (Na\(^+\)) concentration (percent dry weight) as compared with root sodium (Na\(^+\)) concentration (percent dry weight) at 200 mM NaCl in the six cultivars investigated. Values are mean of three replicates. Bars marked with the same letter were not significantly different at 5%.
carotenoid, and phenol determination (Singleton and Rossi, 1965; Wellburn, 1994). Carbohydrate content was measured as described by Irigoyen et al. (1992). Free proline was quantified according to Bates et al. (1973) and free amino acids were estimated by using the method of Rosen (1957). Total thiol content (SH) was assayed according to Ellman (1959) using 5,5'-dithiobis (2-nitrobenzoic acid).

Statistical analysis. All data were subjected to a two-way analysis of variance (effects of cultivar and NaCl treatments as fixed factors with its interaction factor). Significantly different means were compared using Tukey’s test (P < 0.05). All calculations, including statistical analysis, were computed using IBM SPSS Statistics 19 (SPSS Inc., Chicago, IL).

Results and Discussion

Plant growth, leaf characteristics, and tissue mineral content. The largest reduction in shoot length, total DW, and LA caused by salinity was detected in ‘Ocal’ (78%, 65%, and 80%, respectively) (Table 1). In contrast, shoot length was unaffected in ‘Picudo’ and ‘Casta Cabra’. Furthermore, ‘Picudo’ showed the lowest inhibition in total DW and LA (46% and 65%, respectively) and the lowest decrease of K+/Na+ in leaf (88%), whereas ‘Picual’, ‘Cornicabra’, ‘Picudo’, and ‘Casta Cabra’, Cornicabra, and Frantoio, which showed the lowest decrease (Table 2). In leaf, the lowest decrease (Table 2). In leaf, the lowest decrease of K+/Na+ in leaf (88%), whereas ‘Picual’, ‘Cornicabra’, ‘Picudo’, and ‘Casta Cabra’, Cornicabra, and Frantoio, which showed the lowest decrease of K+/Na+ in leaf (88%), whereas ‘Picual’, ‘Cornicabra’, ‘Picudo’, and ‘Casta Cabra’, Cornicabra, and Frantoio, which showed the lowest decrease of K+/Na+ in leaf (88%), whereas ‘Picual’, ‘Cornicabra’, ‘Picudo’, and ‘Casta Cabra’, Cornicabra, and Frantoio, which showed the lowest decrease of K+/Na+ in leaf (88%).

Leaf gas exchange parameters. $P_a$ and $g_s$ sharply declined in response to salinity (Table 1). The drop in $g_s$ under salin stress may be an adaptive response to decreased water content. In this regard, Loreto et al. (2003) indicated that photosynthesis was indirectly limited by the lower water availability in salt-stressed olive trees with different sensitivity to salt stress. In addition, photosynthesis could be limited by non-optimal metabolic conditions caused by Na⁺ accumulation (Table 2). Similar results were reported by Kchaou et al. (2013) for other olive cultivars.

Photosynthetic pigments and metabolites in leaves. Cultivar ‘Ocal’ did not significantly inhibit carotenoid content by salinity (Table 1). It has been well documented that carotenoids are involved in the protection of the photosynthetic apparatus against photoinhibitory damage by singlet oxygen, which is produced by the excited triplet state of chlorophyll (Yazici et al., 2008). In fact, ‘Ocal’ was the cultivar that showed the lowest reduction in Chl (a+b) content (20%). In contrast, ‘Picual’ showed the sharpest reduction in the carotenoid and Chl (a+b) Contents.

Salinity induced a decrease in total thiol concentration in ‘Casta Cabra’ (34%), ‘Picual’ (27%), ‘Picudo’ (22%), and ‘Frantoio’ (11%) (Table 3), probably as a result of the oxidation of non-protein -SH groups. Oxidative stress depletion on non-protein thiols enhances the susceptibility to membrane damage by lipid peroxidation and may trigger ROS irreversible negative effects on cellular function (Ali et al., 2005).

The soluble carbohydrate, amino acids, and proline in leaves were not increased by salinity in any cultivars (Table 3). In contrast, Ben Ahmed et al. (2011) reported proline accumulation by salinity in plants of 2 years

### Table 2. Effects of salinity (0 or 200 mM NaCl) on sodium (Na⁺) and potassium (K⁺) content in leaf and root of six olive cultivars 12 weeks after saline treatments.

| Cultivar  | Leaf (%) | Root (%) |
|-----------|----------|----------|
|           | Na⁺ | K⁺ | K⁺/Na⁺ | Na⁺ | K⁺ | K⁺/Na⁺ |
|           | 0   | 200 |       | 0   | 200 |       |
| Casta Cabra | 0.08 g/h | 1.96 a | 25.76 c | 0.75 f | 1.69 c | 2.25 d |
| Cornicabra | 1.67 b | 1.36 e | 0.81 f | 3.58 b | 0.44 g | 0.12 e |
| Frantoio | 0.03 i | 1.38 de | 50.37 a | 0.81 e | 3.58 a | 4.39 b |
| Ocal | 1.11 c | 1.01 f | 1.10 f | 3.63 b | 0.57 f | 0.16 e |
| Picual | 0.04 i | 1.47 c | 34.04 b | 0.46 g | 1.54 d | 3.36 c |
| Picudo | 1.04 d | 1.22 f | 1.17 f | 2.73 d | 0.61 f | 0.22 e |
|           | 0.07 h | 1.38 de | 19.76 d | 0.15 i | 0.84 e | 5.45 a |
|           | 0.53 f | 1.23 f | 2.30 f | 3.28 c | 0.42 g | 0.13 e |
|           | 0.09 gh | 1.52 b | 17.35 de | 0.38 h | 2.06 b | 5.37 a |
|           | 2.62 c | 0.75 g | 0.29 f | 2.74 d | 0.82 e | 0.30 c |
|           | 0.09 g | 1.42 cd | 15.02 e | 0.39 h | 1.67 c | 4.21 b |
|           | 0.84 e | 1.43 c | 1.70 f | 5.03 a | 0.90 e | 0.18 c |

*ZValues are mean of three replicates. Within each column, means followed by the same letters are not significantly different at 5%.*

### Table 3. Effects of salinity (0 or 200 mM NaCl) on soluble carbohydrates, free proline, free amino acids, total phenol, and total thiol concentrations of leaf of six olive cultivars 12 weeks after saline treatments.

| Cultivar  | Soluble carbohydrates (mg g⁻¹ DW) | Proline (µg g⁻¹ DW) | Amino acids (µg g⁻¹ DW) | Total phenol (mg g⁻¹ DW) | Total thiol (nmol g⁻¹ DW) |
|-----------|----------------------------------|--------------------|------------------------|-------------------------|--------------------------|
|           | 0                                | 200                |                        |                        |                          |
| Casta Cabra | 28.21 cdef | 81.71 abc | 185.50 ab | 24.79 f | 172.04 a |
| Cornicabra | 21.16 g | 47.25 de | 146.92 def | 19.93 g | 113.01 c |
| Frantoio   | 52.40 a | 82.79 abc | 175.88 abc | 23.39 f | 108.23 d |
| Ocal       | 35.59 bcd | 74.84 bc | 119.57 fgh | 25.08 f | 108.04 d |
| Picual     | 25.76 fg | 56.20 cd | 160.04 cde | 32.55 d | 103.58 e |
| Picudo     | 25.71 fg | 25.40 e | 125.56 fg | 41.37 a | 92.59 g |
|           | 44.33 ab | 64.42 cd | 99.68 gh | 35.69 e | 90.38 g |
|           | 35.34 cd | 75.18 bc | 65.91 i | 38.30 b | 92.55 g |
|           | 31.61 cde | 100.64 ab | 198.63 a | 14.67 h | 145.02 b |
|           | 27.17 def | 103.75 a | 137.17 ef | 19.75 g | 105.40 dc |
|           | 36.84 bc | 70.56 cd | 102.69 gh | 33.96 cd | 96.86 f |
|           | 27.34 def | 68.28 cd | 91.38 hi | 28.28 e | 75.61 h |

*Values are mean of four replicates. Within each column, means followed by the same letters are not significantly different at 5%.*

DW = dry weight.
of the salt-tolerant olive cultivar Chemlali. However, phenolic compound was increased in ‘Picual’ (34%), ‘Frantoio’ (27%), and ‘Ocal’ (7%) (Table 3). This is important because phenolic compounds can act as compatible organic solutes and as molecular antioxidants through their ability to destroy free radicals (Blokhina et al., 2003). A similar result was described by Remorini et al. (2009) for the olive cultivar Cipressino.

In conclusion, between the cultivars studied, ‘Picudo’ and ‘Ocal’ were the most tolerant. Those cultivars showed the most effective mechanism of ion exclusion and retention of saline ions in the root. In addition, ‘Picudo’ and ‘Ocal’ showed an ability to maintain the most appropriate K+/Na+ ratio in actively growing tissues. In contrast, ‘Picual’ was the least salt-tolerant K+/Na+ ratio in root and leaf and the highest decrease of K+/Na+ ratio in leaf. Furthermore, between the compatible osmo-lytes studied, only phenolic compounds were accumulated in ‘Ocal’ and ‘Picual’.

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