Changes in Water Relations, Proline Content and Leaf Anatomy Induced by Drought in *Olea europaea* (L.) cv. Picual

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**Abstract**

Water relations parameters, proline content as well as leaf anatomical characteristics were studied in olive cultivar (*Olea europaea* (L.) cv. picual) grown under different levels of available water. The results clearly show that total osmotic adjustment increased and the relative water content decreased with increasing severity of drought. Drought stress resulted in an increase of the upper and lower epidermis and significantly decreased the palisade parenchyma, spongy parenchyma and the total leaf thickness with a parallel increase of the free proline content.

**Key words:** *Olea europaea*, water relations, proline, leaf anatomy.

**Introduction**

In arid and semi-arid regions, like Egypt, water stress is often the most limiting factor for agricultural production. Plants grown in such conditions have evolved a series of adaptations, which confer tolerance to water stress. Lowering of osmotic potential in response to water stress is a well established mechanism where by many plants adjust to low soil water availability (Morgan, 1984; Nabli and Coudret, 1995; Dichio *et al*., 2003). One of the most common stress responses for osmotic adjustment is overproduction of different types of compatible organic solutes (Serraj and Sinclair, 2002; Sánchez-Blanco *et al*., 2004). Proline appears to be the most widely distributed metabolite accumulated under stress conditions (Bellinger *et al*., 1991; Delauney and Verma, 1993). Changes in leaf anatomical characteristics are known to alter the CO₂ conductance diffusion components from the substomatal cavities to sites of carboxylation and thus contribute to maintenance of photosynthetic rates despite the low stomatal conductance (Evans *et al*., 1994). The olive tree (*Olea europaea* L.) is well known for its resistance to severe and prolonged drought and is traditionally grown under drought conditions (Logullo and Salleo, 1988; Larsen *et al*., 1989; Gimenez *et al*., 1997; Giorio *et al*., 1999; Sofo *et al*., 2004). It possesses adaptive mechanisms to allow it to tolerate quite severe drought (Larcher *et al*., 1981). Its leaves show several sclerophyllous characteristics such as small size and thick cuticle and trichome layers (Bacelar *et al*., 2004). However, Moriana and Orgaz (2003) recorded that the yields of mature olive orchards are often affected by water deficit. Some differences among olive cultivars have been observed concerning their ability for adaptation and production under drought conditions.

To select drought-resistant cultivars, breeders commonly evaluate several traits. High water-use efficiency and net photosynthesis rates under drought conditions are often sought (Moriana *et al*., 2002). Although several active olive breeding programmes exist in many countries, old cultivars still dominate olive orchards worldwide. Part of the reason could be the slow-growing nature of olive, and the length of its juvenile phase, which make field trials time-consuming and costing (Bongi and Palliotti, 1994). It is, therefore, useful to take advantage of anatomical and physiological traits relevant to drought tolerance to facilitate the selection process.

The main purposes of this work are (1) to investigate the effects of available water on the Picual olive cultivar (*Olea europaea* L.) in arid region in Egypt and (2) to improve the knowledge about its adaptive strategies at low water availability.

**Materials and Methods**

**Plant material and growth conditions**

The experiment was conducted at the Horticulture Research Station at Seds, Beni Suef, Egypt. One year-old olive Transplants, (*Olea europaea* L., cv ‘Picual’) were used. The plants were grown outdoors in pots (25cm diameter and 30cm depth) with three holes in the bottom to regulate drainage. Each pot filled with 6Kg of loamy sand soil taken from a farm located at southwest Beni Suef Governorate. Table (1) showing the chemical and physical properties of the soil used. with EC (1:1) of 1.48dS m⁻¹, pH 7.5, field capacity 13% and permanent wilting point 4.5%. Different soil water regimes were imposed to olive plants during the dry season (from May to October) of the years 2005 and 2006.

Olive plants were divided into four groups (treatments). Each of three replicates of five plants. The four irrigations levels were: (1) Irrigation after 20% depletion of the available soil water, (2) Irrigation after 40% depletion of the available soil water, (3) Irrigation after 60% depletion of the available soil water, and (4) Irrigation after 80% depletion of the available soil water.

**Measurement of leaf growth**

For leaf growth determination, leaves of three plants per treatment were collected and the dry weight per each was measured at the end of each season.

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Table 1: Some chemical and physical properties of the soil used in the experiment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Measure</th>
<th>Na</th>
<th>48.0</th>
<th>Ca</th>
<th>27.4</th>
<th>Mg</th>
<th>8.8</th>
<th>K</th>
<th>3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCO₃⁻</td>
<td>(meq/L)</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl</td>
<td>(meq/L)</td>
<td>3.71</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>(meq/L)</td>
<td>40.74</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCO₃</td>
<td>(%)</td>
<td>9.66</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Sand</td>
<td>(%)</td>
<td>72.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silica</td>
<td>(%)</td>
<td>9.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clay</td>
<td>(%)</td>
<td>18.12</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Soil Texture**

Loamy sand

**Table (2):** Density of the leaf tissue (D), relative water content (RWC) and water content at saturation (WCS) of O. europea L. cv. picual cultivar under contrasting water availability regimes (n = 4).

<table>
<thead>
<tr>
<th></th>
<th>RWC %</th>
<th>D (gKg⁻¹)</th>
<th>WCS (g H₂O g⁻¹DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1st season)</td>
<td>(2nd season)</td>
<td>(1st season)</td>
</tr>
<tr>
<td>T₁</td>
<td>40.3%±2.51</td>
<td>49.2%±2.81</td>
<td>74.1±17.6</td>
</tr>
<tr>
<td>T₂</td>
<td>44.7±2.62</td>
<td>50.9±2.57</td>
<td>59.2±15.6</td>
</tr>
<tr>
<td>T₃</td>
<td>44.0±3.28</td>
<td>54.9±5.5</td>
<td>55.6±6.98</td>
</tr>
<tr>
<td>T₄</td>
<td>44.5±3.94</td>
<td>52.7±5.19</td>
<td>56.3±6.07</td>
</tr>
<tr>
<td>LSD At 5%</td>
<td>4.53</td>
<td>16.30</td>
<td>14.53</td>
</tr>
</tbody>
</table>

**Water relations measurements**

Several indices of leaf water status were calculated in the same leaves: relative water content (RWC), calculated as RWC = (FM - DM)/ (TM - DM) X 100, water content at saturation (WCS = (TM - FM)/DM) and leaf tissue density (D), calculated as D = (DM/FM) X 100. Where FM is leaf fresh mass, TM is the fresh mass at full turgor and DM is leaf dry mass. Dry mass was determined after drying the leaf samples at 80°C for 24h. For TM determination, leaves were rehydrated by immersing the petiole in distilled water in a beaker sealed with parafilm. Full rehydration was achieved in 24–48h in complete darkness at 2–4°C.

**Proline content and Osmotic potential measurements**

Proline was quantified by the acid-ninhydrin procedure of Bates et al. (1973). Leaf samples (0.5g) were ground with 3% sulphosalicylic acid (10 ml) and clarified by centrifugation. Supernatant (2ml) was mixed with the same volume of acid ninhydrin and acetic acid, the mixture was oven incubated at 100°C for 1h, and the reaction was finished in an ice bath. The reaction mixture was extracted with toluene (4ml) and absorbance was read at 517nm, using toluene as a blank. The proline concentration was determined from a standard curve and calculated on a fresh weight basis. Three replicates were measured for each sample, and mean values are displayed.

For osmotic potential measurements, 4–5 leaf-samples per treatment were collected predawn at 05.00 clock, wrapped in damp paper and enclosed in a plastic bag and stored at −80°C. Before analysis these samples were equilibrated at room temperature for about 15 min. Cell contents were extracted using plastic syringes, to squeeze homogeneously the tissue and to extrude 100/L cell-content samples. Each sample was analyzed by osmometer (Wescor model, 2000).

**Leaf anatomy**

Leaf anatomical measurements were obtained at the end of the experiment (October 2006). Thus, they refer to the long-term effects caused by the repeated cycles of stress during the dry season of both years. Leaf pieces take at the end of the experiment (October, 2006) were fixed for 3h in 5% glutaraldehyde buffered with 0.025M sodium phosphate to pH 7.2. Samples were then washed in the respective buffer and postfixed for 5 h in 1% osmium tetroxide similarly buffered. Tissue dehydration was carried out in an alcohol series followed by infiltration and final embeddent in Spur’s resin. Sections for light microscopy (1?m thick) were obtained in a Reichert Om U2 ultramicrotome, stained with 1% toluidine blue O in borax, and examined with a Zeiss III photomicroscope. Total leaf thicknes, upper and lower epidermis, palisade parenchyma and spongy parenchyma were measured.

**Statistical analysis**

All measurements were expressed as means of three measurements (± SE) from four plants per treatment. Significant differences were detected at P = 0.05, according to Snedecor and Cochran (1980).

**RESULTS**

**Effect of available water on leaf growth**

Leaf dry weight per plant decreased by increasing water deficiency (Fig. 1a), recording 45% and 40% reduction at high stress (80% available water depletion) plants at the first and the second season, respectively.

**Effect of available water on water relations**

Relative water content (RWC) decreased by about 9% and 20% at 40% depletion of the available soil water in the 1st and 2nd season respectively and still nearly constant at the other two higher levels of water stress around 44% (Table 2). WCS increased with
decreasing available water content recording 24% and 20% increasing in the highest stress plants in the first and second seasons respectively. Leaf density showed slightly increasing with increasing water stress (Table 2).

**Effect of available water on osmotic potential and proline content**

Measurements using the osmometer method showed that osmotic potential decreased by increasing water shortage recording 69% and 50% reduction in the highest stress plants at the first and second season respectively (Fig. 1b). In contrast proline content increased by decreasing water availability. High water stress (80% depletion of the available soil water) increased proline content by more two folds than those at 20% depletion of the available soil water in the two seasons (Fig. 1c).

**Effect of available water on leaf anatomical characters**

Anatomical characteristics of leaves showed palisade parenchyma in both leaf sides, which considered as an indicator for xeromorphy. There were changes in leaf anatomical characteristics induced by water stress. The obtained results (Table 3) showed that water stress slightly increased the thickness of the upper and lower epidermis by about 5.5% and 6% respectively and significantly (P = 5%) decreased the palisade parenchyma, spongy parenchyma and the total leaf thickness by about 7.8%, 9% and 7.5% respectively.

**DISCUSSION**

The plants which grow under water deficit have developed strategies allowing photosynthesis to proceed. Water stress affect leaf growth of the studied cultivar, as it has been observed in other plant species (Pita and Pardos, 2001; Sánchez-Blanco et al., 2002; Anyia and Herzog, 2004). (Maroco et al. (2000) stated that under water stress a different strategy imposes a different pattern of allocation of assimilates, resulting in the decrease of investment in leaves relative to other organs, or the alteration of the relative amounts of photosynthetic and non-photosynthetic tissues.

Water stress can increase leaf density since reductions in turgor pressure and cell expansion result in the same dry mass within a smaller leaf area (Pena-Rojas et al., 2005). According to Witkowski and Lamont (1991), variations in leaf density, manifested as variations in the dry mass to fresh mass ratio, may be the result of differences in thickness and density of the cuticle and cell walls, inclusions in the cells (starch grains and crystals) and abundance of air spaces, sclereids, fibre groups and vascular bundles. Mediavilla et al (2001) stated that leaves with high density (D) are better able to survive a severe drought because of a higher resistance to physical damage by desiccation. The leaves with high D are also mechanically more stable than leaves with low D, and this may be the fundamental cause for their longer life-span (Niinemets, 2001). RWC measurement describe the internal water status of plant tissues and is also a convenient parameter for following changes in tissue water content without errors caused by continually changing tissue dry weight (Erickson et al., 1991). The stability of high RWC in the studied cultivar at high water shortage (T3 and T4) can be considered drought resistance rather than drought.
Changes induced by drought in *Olea europea* (L.)

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**Table (3): Effect of water stress on leaf thickness and thickness of leaf anatomical component of *O. europea* L., cv. Picual.**

<table>
<thead>
<tr>
<th></th>
<th>Upper epidermis</th>
<th>Palisade parenchyma</th>
<th>Spongy parenchyma (with lower palisade)</th>
<th>Lower epidermis</th>
<th>Total leaf thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T1</strong></td>
<td>16.5±0.09</td>
<td>130.3±2.4</td>
<td>210.2±5.3</td>
<td>16.1±0.87</td>
<td>372.9±5.4</td>
</tr>
<tr>
<td><strong>T2</strong></td>
<td>16.5±1.12</td>
<td>125.2±2.5</td>
<td>201.3±4.4</td>
<td>16.3±1.03</td>
<td>359.3±7.3</td>
</tr>
<tr>
<td><strong>T3</strong></td>
<td>17.1±1.02</td>
<td>120.3±3.1</td>
<td>195.5±4.6</td>
<td>16.9±1.05</td>
<td>349.8±5.9</td>
</tr>
<tr>
<td><strong>T4</strong></td>
<td>17.2±1.01</td>
<td>120.1±2.1</td>
<td>190.3±2.3</td>
<td>17.1±1.21</td>
<td>344.7±3.6</td>
</tr>
<tr>
<td><strong>LSD at 5%</strong></td>
<td>1.85</td>
<td>12.10</td>
<td>8.75</td>
<td>1.90</td>
<td>11.42</td>
</tr>
</tbody>
</table>

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escape mechanism. It is consequence of adaptive characteristics such as osmotic adjustment and/or bulk modulus of elasticity (Grashoff and Ververke, 1991). The increase by about 20% only of WCS in the high stress plants means that this cultivar has a greater capacity to withstand arid environments (Abd-El-Rahman et al., 1966; Bacelar et al., 2004).

The increase of proline content in leaves under drought stress associated with lowering cell osmotic potential, can be consider a drought tolerant strategy of the studied cultivar (Boggess et al., 1976; Morgan, 1984; Wright et al., 1997). In recent study Rejskova et al. (2007) observed an increase in the amount of proline in salt stressed plants; however, the increase was not greater than two-fold. The increase of proline concentration in response to water deficit is a well-documented fact (Hanson et al., 1977; Ferreira et al., 1979; Hasegawa et al., 1994), and a large body of data indicates a positive correlation between proline accumulation and enhanced tolerance to drought and salt stress (Van Rensburg and Krüger, 1994; Kishor et al., 1995). Proline has been suggested to play multiple roles in plant stress tolerance. It acts as a mediator of osmotic adjustment (LeRudulier et al., 1984; Hu et al., 1992; Delauney and Verma, 1993; Kishor et al., 1995; Yoshiba et al., 1997), protects macromolecules during dehydration (Yancey et al., 1982), and serves as a hydroxyl radical scavenger (Smirnoff and Cumbes, 1989; Alia et al., 1995). Sofo et al. (2004) reported that olive trees under drought-stress conditions activate osmotic adjustment mechanisms not only in leaves, but also in roots, in such a way increasing their capacity to extract water from dry soil.

Leaf anatomy was reported to be modified significantly with drought, which showed a significance increase in epidermal cells thickness, while the total thickness and palisade tissues was found to decrease as water available decreased. The increase in upper and lower epidermis (including upper cuticle) under water deficiency in this cultivar may enhance survival and growth under low available water conditions by improving water relations and providing higher protection for the inner tissues (Bacelar et al., 2004). The reduction in cell size under water stress conditions may be considered as drought adaptation mechanism (Cutler et al., 1977; Steudle et al., 1977).

The above anatomical characteristics are in accordance with relevant physiological observations and contribute to the interpretation as to how olive cv. Picual is drought-tolerant.

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**الملخص العربي**

تم دراسة معايير العلاقات المائية ومحتوى البرولين وتشريح الورقة الناتج عن الجفاف في نبات الزيتون صنف بكاوال. عادل جوده

أقسام النبات، كلية العلوم، جامعة بني سوف، بني سوف، مصر

محطه البحوث الزراعية، سدس، بني سوف، مصر

**المستقبل الهندي**

تم دراسة تغيرات في العلاقات المائية ومحتوى البرولين وتشريح الورقة الناتج عن الجفاف في نبات الزيتون صنف بكاوال بالحقل النامي تحت مستويات مختلفة من الماء المائي. وأظهرت النتائج أن الضغط الأسمرزي والمحتوى المائي النسبي قلل من زيادة شدة الجفاف، وأن الجزء الجافي تسبب في زيادة سمك خلايا البشرة العلوية والبشرة السفلية ونقص البارنشيميا الذراعية والباراشيميا الإسفنجية والسماك الكلي للورقة مع زيادة في محتوى البرولين.