Comparative Study on Gas Exchange, Water Relations and Leaf Anatomy of Two Olive Cultivars Grown under Well-Irrigated and Drought Conditions

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The effect of water stress on gas exchange, water relations and leaf anatomical characteristics have been studied in two olive cultivars (*Olea europea,* L. cv. *'Koroneiki'* and cv. *'Mastoidis'*). Photosynthetic rate as well as stomatal conductance were decreased in stressed plants. Osmotic potential (π) declined rapidly in stressed plants indicating their ability for osmoregulation. Bulk modulus of elasticity (ε) was significantly higher in stressed compared to well irrigated plants. The volume fraction of intercellular spaces of the upper palisade parenchyma, the spongy parenchyma as well as the lower palisade parenchyma were significantly lower in stressed compared to well irrigated plants. On the other hand, the density of mesophyll cells in the upper palisade parenchyma, spongy parenchyma and lower palisade parenchyma increased significantly in stressed plants.

Introduction

Olive tree, native in Mediterranean basin, is usually during summer subjected to drought stress due to high temperatures, high vapour pressure deficit and limited water availability, as a result of lack of precipitation or insufficient irrigation. The importance of stomatal regulation on leaf gas exchange under drought has been recently emphasized (Chaves, 1991).

The maintenance of turgor (P) during a change in the plant water status is supposed to preserve metabolic processes of the plants and aim in its growth and productivity (Hsiao, 1973; Morgan, 1984). Lowering of osmotic potential (π) due to net solute accumulation as well as changes in cell wall elasticity (ε) are well established ecophysiological mechanisms which contribute in turgor maintenance in plants under drought conditions (Morgan, 1984; Patakas and Noitsakis, 1997).

On the other hand, water stress can also affect leaf anatomical parameters by causing changes in the number, density of mesophyll cells and/or cellular dimensions. These result in alterations of internal leaf area being available for the CO_2 absorption per unit leaf surface area and thus influence photosynthetic rate (Nobel, 1991) .

In the Mediterranean region and especially in Crete, two cultivars – *'Koroneiki'* and *'Mastoidis'* – are widely cultivated because of their economical significance and they are both concerned as well adapted to drought conditions.

The aim of this study was to evaluate the ecophysiological mechanisms contributing in turgor maintenance in these two olive cultivars grown under water stress conditions as well as to elucidate the leaf anatomical alterations caused by drought in these cultivars.

Materials and Methods

The experiment was conducted at the Institute of Subtropical Plants and Olive Tree, Chania, Crete. Three year old own-rooted olive trees (*Olea europea,* P. cv. *'Koroneiki'* and cv. *'Mastoidis'*), were used. Twenty plants of each cultivar were grown outdoors in 50 l pots, containing freely drained light soil (sandy loam). Different soil water regimes were imposed to both cultivars during the dry season (April to October). Stress cycle was induced in ten selected plants of each cultivar at the beginning of July 1995 by withholding irrigation while the other ten plants (control) continued to receive daily irrigation. Tensiometers and Buy-

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oucos blocks placed at 20 cm depth were used for monitoring soil water tension in stressed plants. These plants were irrigated only when soil water tension reached −1.5 MPa, about nine days after irrigation was stopped. The experiment was repeated in 1996. No significant differences were obtained between measurements on the two years and hence the results following represent the measurements of the first year (1995).

Predawn water potential (ψ) and osmotic potential (π) were measured every day during the drought cycle using thermocouple psychrometers (Wescor HR-33). Measurements of water and osmotic potential were made on six leaf discs (0.38 cm^2) per treatment obtained from the $3-4^{th}$ fully expanded leaf from the tip at 0600 a.m. Turgor potential (P) – the positive internal pressure to the cell walls – was calculated as:

$$P = \psi - \pi.$$

Concomitant measurements of relative water content (RWC) were made on six leaf discs obtained from the same leaves that were used for the determination of water potential components (Patakas and Noitsakis, 1997). The bulk modulus of elasticity ε-an index of the elastic properties of cell walls- was calculated in both cultivars using the equation (Koide *et al.*, 1991):

$$\varepsilon = (\Delta P/\Delta RWC) \times 100.$$

Osmotic potential due to net solute accumulation (π_s) was calculated using the equation:

$$\pi_s = \pi - [(\pi_{100} \times RWC_{100})/RWC],$$

where π and RWC are the osmotic potential and the relative water content respectively at a measured ψ value; π_{100} and RWC_{100} are the osmotic potential and relative water content at full turgor with $(\pi_{100} \times RWC_{100})/RWC$ being the osmotic potential due to passive solute concentration with water loss. The values of π_{100} were derived from the linear regression equation that was used to describe the relationship between π and ψ in each cultivar during the drought cycle (Wright *et al.*, 1997). Similarly, the RWC_{100} values for each cultivar derived from the relationship between RWC and ψ.

Gas exchange measurements were made daily, between 9 and 10 a.m, on six leaves per treatment using a portable gas exchange system (Li-6200, Li-Cor Inc).

For anatomical studies pieces from 12 leaves per each treatment, taken at the end of the experiment (October 1996), were fixed for 3h in 5% glutaraldehyde buffered with 0.025 M sodium phosphate to pH 7.2. Samples were then washed in the respective buffer and post-fixed 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. Cross as well as paradermal sections for light microscopy (1ìm thick) were obtained in a Reichert Om U2 ultramicrotome, stained with 1% toluidine blue O in borax, and examined with a Zeiss III photomicroscope.

Results and Discussion

Photosynthetic rate as well as stomatal conductance decreased in stressed plants of both cultivars during the drought cycle (Fig. 1). Water relations

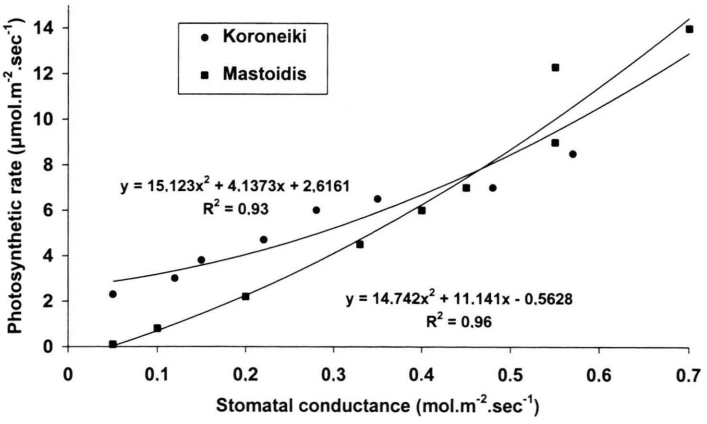

Fig. 1. Changes in photosynthetic rate in relation to stomatal conductance in the two olive cultivars during the drought cycle.

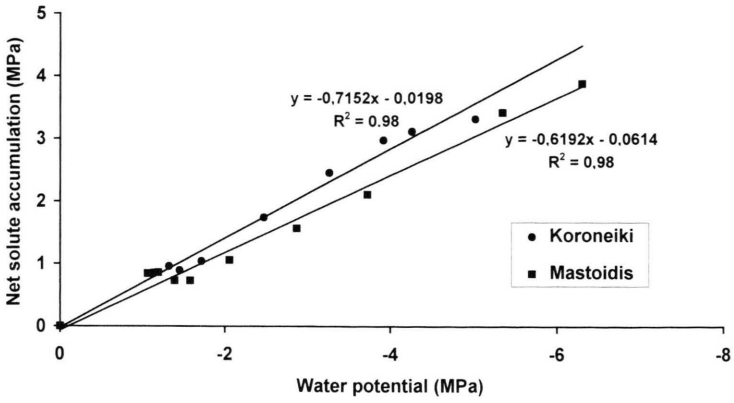

Fig. 2. Relationship between net solute accumulation and leaf water potential in the two olive cultivars during the drought cycle.

Table I. Leaf water potential parameters as well as bulk modulus of elasticity (ε) in well irrigated and stressed plants of the two olive cultivars. (n = 6, ±SD).

	Mastoidis		Koroneiki	
	Irrigated	Stressed	Irrigated	Stressed
Water potential (MPa)	−0.85 ± 0.04	−5.65 ± 0.10	−0.82 ± 0.06	−5.02 ± 0.15
Osmotic potential (MPa)	−1.95 ± 0.09	−6.32 ± 0.12	−1.85 ± 0.09	−5.19 ± 0.14
ε (MPa)	1.40 ± 0.23	2.78 ± 0.45	1.51 ± 0.25	2.52 ± 0.48

parameters recovered fully overnight after rewatering the plants in the afternoon, while photosynthesis and stomatal conductance showed a partial recovery (data not shown).

Water and osmotic potential decreased significantly in the stressed plants of both cultivars reaching minimum values at the end of the drought cycle (Table I). Net solute accumulation accounted for almost 63% of the changes in osmotic potential in the stressed plants of both cultivars indicating the occurrence of an active osmotic adjustment (Fig. 2). Furthermore, the value of the bulk modulus elasticity (ε) also increased significantly in stressed plants compared to the well irrigated. These results indicate that both osmotic adjustment and changes in tissue elasticity contribute in turgor maintenance in olive plants under water stress conditions.

Table II. Volume fractions (%) of the leaf histological components in the two olive cultivars under well irrigated and stressed conditions (n = 12, ±SD).

	Mastoidis		Koroneiki	
	Irrigated	Stressed	Irrigated	Stressed
Volume fraction of upper palisade parenchyma	22.8 ± 1.6	23.6 ± 0.6	25.8 ± 1.4	26.5 ± 0.8
Volume fraction of upper palisade intercellular spaces	9.2 ± 0.7	6.3 ± 0.4*	12.9 ± 0.9	8.6 ± 0.7*
Volume fraction of the spongy parenchyma	15.4 ± 1.5	27.2 ± 1.0*	19.9 ± 1.6	23.8 ± 1.0*
Volume fraction of the spongy parenchyma intercellular spaces	22.9 ± 1.9	16.4 ± 1.1*	16.0 ± 1.9	15.0 ± 1.1*
Volume fraction of the lower palisade parenchyma	4.5 ± 0.5	6.2 ± 0.6*	5.1 ± 0.8	5.6 ± 0.4*
Volume fraction of the lower palisade intercellular spaces	4.6 ± 0.7	3.2 ± 0.5*	4.5 ± 0.5	3.7 ± 0.4*

* Statistically significant from the control, $P < 0.05$.

Leaves of both stressed olive cultivars appeared in cross sections thinner than the well irrigated leaves (Fig. 3). The volume fraction of intercellular spaces of the upper palisade parenchyma, the spongy parenchyma as well as the lower palisade parenchyma were significantly lower in stressed compared to well irrigated pants (Table II). Furthermore the total volume of cells in the spongy and in the lower palisade parenchyma increased significantly in stressed plants. Comparative observations of the paradermal sections at the levels of upper palisade parenchyma, spongy

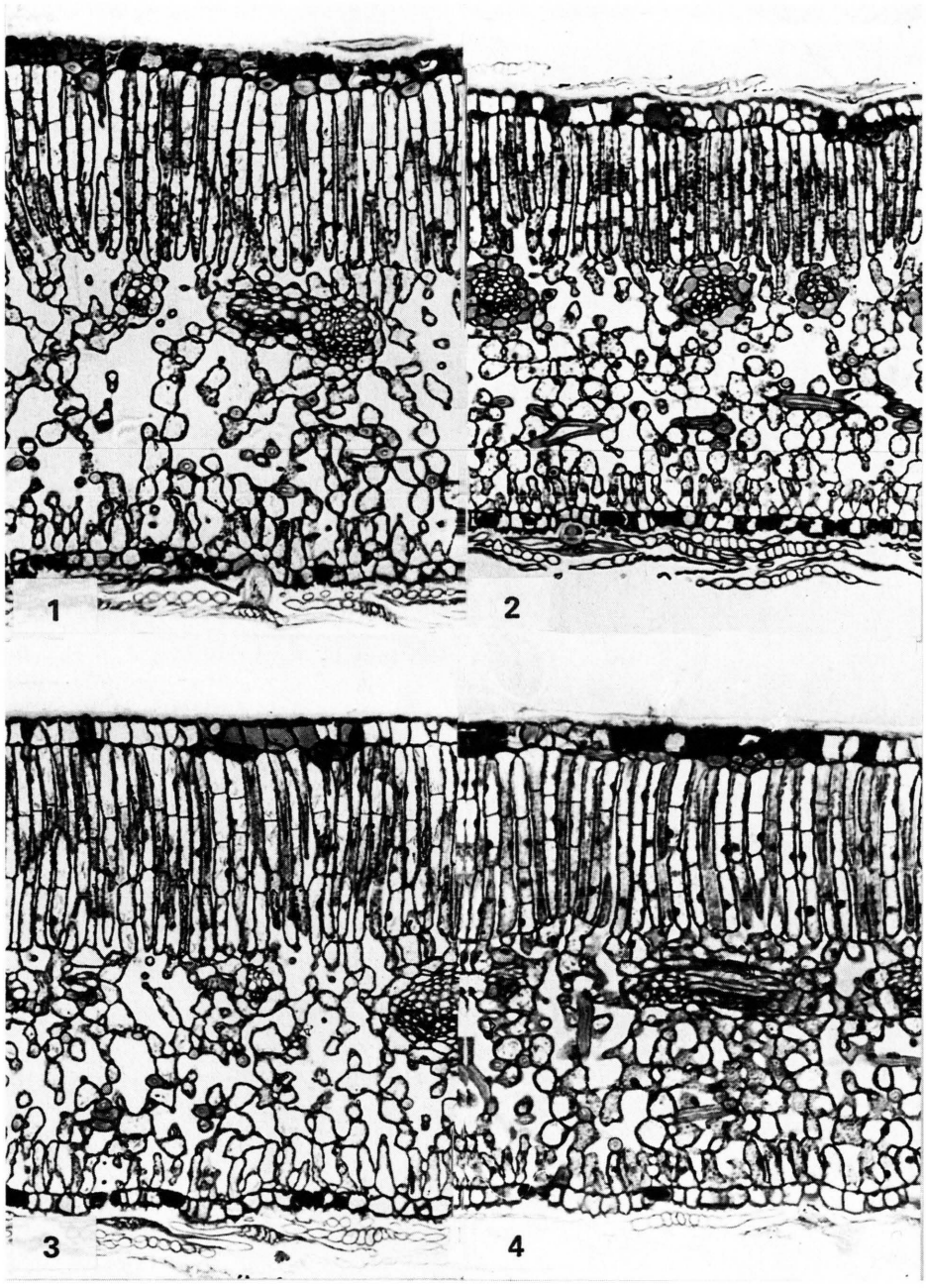

Fig 3. Comparative leaf anatomy in blade cross sections of *'Mastoidis'* (1, 2) and *'Koroneiki'* (3, 4) leaves grown under well irrigated (1, 3) and water stress conditions (2, 4). (X200).

Table III. Density (No/mm^2) of mesophyll cells in paradermal sections of the two olive cultivars grown under well irrigated and water stressed conditions (n = 12, ±SD).

	Mastoidis		Koroneiki	
	Irrigated	Stressed	Irrigated	Stressed
Upper palisade parenchyma	6657 ± 566	8761 ± 707*	7075 ± 586	9049 ± 903
Spongy parenchyma	1081 ± 122	1239 ± 104*	1268 ± 135	1398 ± 211*
Lower palisade parenchyma	3646 ± 464	5231 ± 381*	3761 ± 485	5476 ± 506*

* Statistically significant from the control, $P < 0.05$.

parenchyma and lower parenchyma between irrigated and stressed plants showed that in the latter the density of cells (No/mm^2) in all of the leaf histological components significantly increased in both cultivars (Table III). These changes on anatomical characteristics would lead to an increase in the mesophyll surface area per unit leaf area (Ames/A) which in turn could facilitates CO_2 uptake and thus maintain photosynthetic rate although stomatal conductance presented low values under drought conditions. On the other hand, the decrease in volume fraction of intercellular spaces occurred under water stress conditions is expected to decrease the diffusion component of CO_2 conductance through the intercellular spaces from the substomatal cavity to the outer surface of the mesophyll cells. It seems that this component of the total internal conductance of CO_2 (Syvertsen *et al.*, 1995) might be considered of less importance in olive trees. A more detailed studies as well as an estimation of the components of the total internal CO_2 conductance in olive leaves are needed.

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