ACTIVE AND PASSIVE OSMOTIC ADJUSTMENT IN OLIVE TREE LEAVES DURING DROUGHT STRESS

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Abstract:

We evaluated the osmotic adjustment capacity of two-year-old olive trees (*Olea europaea* L.) grown in pots in a greenhouse during a period of drought stress. Total osmotic adjustment increased with increasing severity of drought stress. Trees in the high stress treatment showed a total osmotic adjustment of 2.1 MPa and 2.8 MPa for the cultivars 'Meski' and 'Koroneiki', respectively, 30 days after imposing drought stress. Osmotic adjustment allowed the leaves to reach total water potentials of -4.3 and -6.0 MPa for 'Meski' and 'Koroneiki', respectively. Osmotic adjustment (OA) in olive trees was associated with active (AOA) and passive (POA) osmotic regulation mechanisms. Using a regression analysis with some of the key osmoregulatory compounds (i.e. proline, soluble sugars and potassium), we found that 'Koroneiki' tends to adopt a passive strategy (POA) to tolerate progressive drought stress (confirmed by a reduction in leaf water content), while 'Meski' used an active strategy (AOA) and was able to maintain its leaf water content.

Keywords: Drought stress, Olea europaea, osmotic adjustment, water potential, proline, carbohydrates, potassium

Introduction

Drought stress has been shown to influence various plant physiological and biochemical processes. The majority of studies in olive trees (*Olea europeae* L.) under drought stress have primarily investigated physiological responses such as stomatal reactions and photosynthesis, but information on osmotic adjustment is rather scarce (Morgan 1984, Lakso 1985, Dichio et al. 2005).

Osmotic adjustment is used by plants to tolerate temporary or prolonged periods of water shortage (Chaves et al. 2003). Plants subjected to drought stress may indeed synthesize and accumulate amino acids (e.g., proline, aspartic acid, …), proteins, sugars (e.g., sucrose, glucose, mannitol, …), methylated quaternary ammonium compounds (e.g., glycine betaine, alanine betaine, …) and organic acids (Ingram and Bartels 1996). High concentrations of these compatible solutes contribute to the lowering of the osmotic potential (Ψ_π) and allow water to move into the cells, thereby maintaining turgor (Ψ_p) and increasing tissue tolerance to low soil water potentials (Tyree and Jarvis 1982, Bray 1993). These solutes also sequester water molecules, protect cell membranes and protein complexes and allow the metabolic machinery to continue functioning (Chaves et al. 2003). The lowering of Ψ_π as a result of the net accumulation of compatible solutes is defined as "active osmotic adjustment" (AOA) and can be determined by measuring the osmotic potential at full turgor ($\Psi_{\pi 100}$) (Girma and Krieg 1992). The mechanism by which a net loss of symplastic water of plant tissues causes a reduction in cell volume and an increase in solute concentration is defined as "passive osmotic adjustment" (POA) (Lakso 1985). A correlation between total osmotic adjustment and drought stress has been found in several tree species, including *Ziziphus rotundifolia* Lamk. (Arndt et al. 2001), *Vitis vinifera* L. (Patakas et al. 2002), *Eucalyptus* (Ngugi et al. 2003), *Populus tremula* L. and *Tilia cordata* Mill. (Aasamaa et al. 2004).

In olive trees, many of the responses to drought are well documented (Lo Gullo and Salleo 1988, Chartzoulakis et al. 1999), but few studies have focused on the mechanisms of osmotic adjustment. Little is therefore known about the osmoregulatory capacity of this species during periods of water shortage; periods that are common during the dry season in the Mediterranean basin when temperature and vapour pressure deficit are high. To obtain a better insight in the responses of olive trees to progressing drought stress, we determined the total osmotic adjustment (OA = AOA + POA) for young trees of two olive tree cultivars 'Meski', a native cultivar of Tunisia, and 'Koroneiki', a foreign Greek cultivar.

The main objectives of this research were to investigate the active and passive osmotic adjustment used by the olive trees in response to drought

stress and to relate these mechanisms to an accumulation of potassium, proline and soluble sugars. In addition, the relative contributions of AOA and POA to OA were evaluated and relationships were established between AOA and POA and the accumulated potassium, proline and soluble sugars. This allowed us to unravel the specific drought-adaptive strategies used by both olive tree cultivars.

Materials and methods

Plant material and experimental design

Two-year-old olive trees ('Koroneiki' and 'Meski') were grown in 10L plastic pots in a greenhouse at the Tunisian Olive Institute (Tunisia, 35 49′N, 10 38′E) under normal day-light conditions. Prior to the start of the experiment, trees with a height of about 1.2 m were selected and lifted from a soil mix of organic material, sand and clay. Roots were washed and plants were transplanted into a substrate mixture of sand and peat (1/2 volume ratio). Trees were watered daily to field capacity for a period of 8 weeks with a full-strength Hoagland solution.

Plants were subjected to drought stress from 12 March 2006 till 12 April 2006. The drought stress treatments were gradually imposed by withholding water, while control plants were irrigated daily to field capacity (saturation). Three drought stress levels were considered and compared to the control treatment: 10 days without watering, 20 days without watering and 30 days without watering. During the drought stress experiment, the mean day and night temperature was 32°C and 18°C and the mean day and night air humidity was 65% and 85%, respectively.

Control and drought-stressed trees were arranged in a complete randomized design with six replications for each cultivar. Drought stress (i.e., four levels, including the control treatment) and cultivars (two) were considered as treatments. In total, 48 olive trees were used.

Plant water status

Throughout the drought stress treatment, plant water status was determined by measuring the total leaf water potential (Ψ_w) and the osmotic potential (Ψ_π) on fully expanded sunlit leaves (taken from the mid-section of the shoots). Three plants per treatment were measured at predawn (6 am) and at midday (12 am) with a thermocouple psychrometer (sample chambers type C52; Wescor, Logan, Utah, USA) following the method described by Chazen et al. (1995). For each plant and at each measurement event, two leaf disc samples were taken. One sample (surface area of the leaf disc = 0.25 cm^2) was used to measure Ψ_w, whereas the second one, taken from the same leaf, was wrapped in aluminium foil and kept in a freezer at -20°C for 24 h in order to disrupt the cell membranes. After thawing, the second leaf disc was used to measure Ψ_π.

At specified days during the treatment (i.e., 10, 20 and 30 days after the start of the treatment), osmotic adjustment (OA) was calculated for both cultivars by taking the difference between midday Ψ_π measured in control and drought-stressed plants.

Active osmotic adjustment (AOA) was defined as the difference between Ψ_π at full turgor measured at predawn in control ($\Psi_{\pi 100}$ (control)) and drought-stressed ($\Psi_{\pi 100}$(stressed)) plants.

The contribution of passive osmotic adjustment (POA) to OA via the loss of symplastic water was determined as:

$$POA = OA - AOA \quad (4.1)$$

Concomitant values of leaf water content (LWC, %) were calculated for fully expanded leaves taken from the mid-section of shoots from four plants per treatment and cultivar using the following equation:

$$LWC = (FW- DW)/FW \times 100 \quad (4.2)$$

Where FW is the fresh weight (g) and DW the dry weight (g) of the leaf sample. The values were, respectively, determined before and after oven-drying at 80°C for 48 h and were also used to determine the leaf DW/FW ratio.

Proline content

Free proline content was determined after Dreier and Göring (1974) for both current year (young) and previous year (old) olive tree leaves. To this end, for each leaf age class, eight leaves were harvested from four plants in each treatment and for each cultivar and combined into a composite leaf sample. A 5.0 ml sample of methanol 40% was added to 0.5 g of the fresh leaf material, homogenized and boiled in a water bath at 80°C for 30 min in glass tubes covered at the top. 1.0 ml of the extract was mixed with 2.0 ml of the reagent mixture (120 ml distilled water, 300 ml acetic acid and 80 ml orthophosphoric acid) and 1 ml of ninhydrine (25 mg/ml), and boiled at 100°C for 1h. After cooling the reaction mixture, 5.0 ml toluene was added. The chromophore containing toluene was separated and A528 was read, using a spectrophotometer (Jenway, England) and toluene as a blank. Proline content (μmol (g^{-1} DW) was calculated using L-proline as standard curve.

Mineral analysis

Potassium (K) content (% DW) of previous year (old) olive leaves was determined according to Martin-Prével et al. (1984). Eight leaves were harvested from four plants in each treatment and for each cultivar and combined into a composite sample, dried at 70°C for 48 h and then ground. One-half gram of that leaf material was transferred to nitric acid after calcination. The K content was determined using a flame photometer (Jenway, England).

Soluble carbohydrate determination

Soluble carbohydrates were extracted according to the method described by Bartolozzi et al. (1997). Briefly, the soluble carbohydrates from the same composite leaf sample described for proline were extracted twice in 80% ethanol at 70°C. Extracts were dried and converted into trimethylsilyl ethers with a silylation mixture made up of pyridine, hexamethyldisilazane and trimethylchlorosilane. Soluble carbohydrates were analysed using a gas chromatograph mass spectrometer (Hewlett-Packard 5890 series II, Calif) equipped with a flame ionisation detection system and a HP-5MS capillary column (30 m x 0.25 mm) as described by Bartolozzi et al. (1997). Identification of individual carbohydrates was achieved by use of the relative retention times (i.e., in comparison to that of the standard). These were compared to those identified earlier by gas chromatography-mass spectrometry.

Statistical analysis

Means and standard errors (SE) of the investigated parameters for each drought stress level and each cultivar were calculated. Analysis of variance (ANOVA) was performed on the data using SPSS 16.0. When significant differences occurred, means were separated by the Duncan's multiple range test at $p < 0.05$.

Results

Drought stress development

Table 1 shows the progressive effects of drought stress on the water status. Ψ_w showed an important decrease in the drought-stressed olive trees, whereas the control trees maintained a rather constant value. The decrease was more pronounced in 'Koroneiki' compared to 'Meski' and became more important with increasing drought stress: values for 'Koroneiki' were 12%, 18% and 38% lower compared to 'Meski' for, respectively, 10, 20 and 30 days of drought stress. Ψ_π showed a similar trend as Ψ_w. The resulting OA was 0.38, 1.18 and 2.10 MPa for 'Meski' and 0.34, 0.85 and 2.84 MPa for 'Koroneiki', respectively.

In addition to OA, POA and AOA increased with decreasing Ψ_w in all drought-stressed olive trees (Table 1). Values of POA in stressed 'Koroneiki' trees increased from 0.01 MPa after 10 days of drought stress to 0.90 MPa after 30 days of drought stress, corresponding with 0 and 32% of total OA, respectively (Table 1). Values of AOA increased from 0.33 MPa at the beginning of the drought stress up to 1.94 MPa at the end of the experiment, corresponding to 100 and 68% of total OA, respectively (Table 1).

For stressed 'Meski' trees, POA and AOA contributed, respectively, 26 and 74% to OA 10 days after the start of the drought treatment and this

contribution changed to 10 and 90%, respectively, at the end of the experiment (Table 1).

Table 1. Total midday leaf water potential (Ψ_w; MPa), midday osmotic potential (Ψ_π; MPa), osmotic adjustment (OA; MPa), osmotic potential at full turgor ($\Psi_{\pi 100}$; MPa), active osmotic adjustment (AOA; MPa) and passive osmotic adjustment (POA; MPa) measured in leaves of control and drought-stressed olive trees ('Meski' and 'Koroneiki') 10, 20 and 30 days after the start of a drought stress treatment. Each value is the mean ± SE of three measurements.

		'Meski'			'Koroneiki'		
		10 days	20 days	30 days	10 days	20 days	30 days
Ψ_w	Control	-2.85±0.40[a]	-2.72±0.08[a]	-2.64±0.04[a]	-3.47±0.19[a]	-3.07±0.80[a]	-3.27±0.15[a]
	Stressed	-2.99±0.30[b]	-3.58±0.32[b]	-4.34±0.22[b]	-3.35±0.12[a]	-4.22±0.32[b]	-6.00±0.16[b]
Ψ_π	Control	-2.90±0.09[a]	-2.83±0.04[a]	-2.72±0.13[a]	-3.64±0.40[a]	-3.71±0.15[a]	-3.77±0.23[a]
	Stressed	-3.27±0.30[b]	-4.01±0.32[b]	-4.82±0.30[b]	-3.98±0.10[b]	-4.56±0.30[b]	-6.61±0.14[b]
OA		**0.38±0.19**	**1.18±0.35**	**2.10±0.26**	**0.34 ±0.30**	**0.85±0.70**	**2.84±0.12**
$\Psi_{\pi 100}$	Control	-2.87±0.20[a]	-2.67±0.18[a]	-2.56±0.37[a]	-3.50±0.25[a]	-2.79±0.42[a]	-2.49±0.34[a]
	Stressed	-3.15±0.04[a]	-3.79±0.09[b]	-4.45±0.58[b]	-3.83±0.05[b]	-3.44±0.35[b]	-4.43±0.32[b]
AOA		**0.28±0.17**	**1.12±0.36**	**1.89±0.23**	**0.33±0.30**	**0.65±0.37**	**1.94±0.05**
POA		**0.10±0.07**	**0.06±0.02**	**0.21±0.31**	**0.01±0.01**	**0.20±0.04**	**0.90±0.08**

Effect of drought stress on proline content

Subjected to drought stress, 'Koroneiki' accumulated more proline than 'Meski' for both young and old leaves (Fig. 1). The proline content increased significantly in relation to the severity of the drought stress. This is particularly true for the younger leaves in 'Koroneiki'.

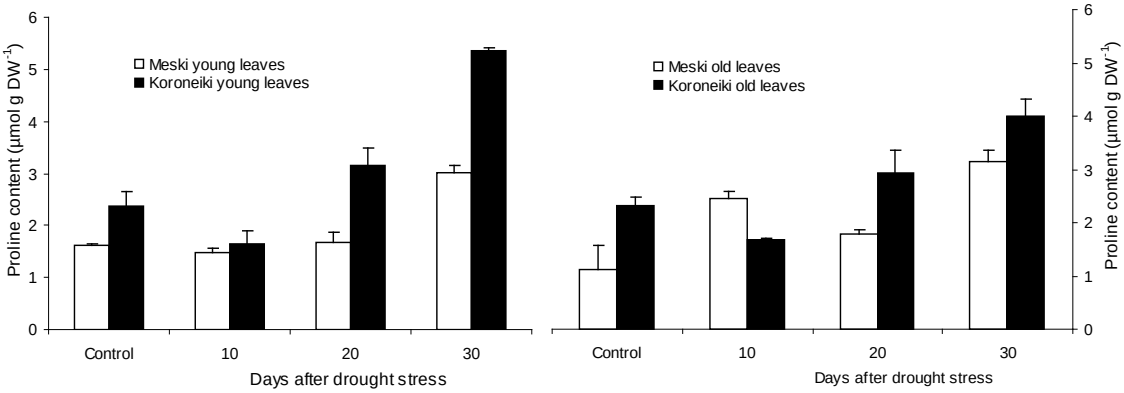

Figure 1. Proline content in old and young 'Meski' and 'Koroneiki' leaves sampled from control trees and trees subjected to drought stress. The effect of drought stress was evaluated 10, 20 and 30 days after the start of the drought stress treatment. Each value is the mean ± SE of three measurements.

Effect of drought stress on soluble carbohydrates

The imposed drought stress caused a clear decrease in soluble carbohydrate content for both cultivars. At the end of the experiment, after 30 days of drought stress, the soluble carbohydrate content dropped to 30 and 27% of the control value for 'Koroneiki' and 'Meski', respectively (Fig. 2).

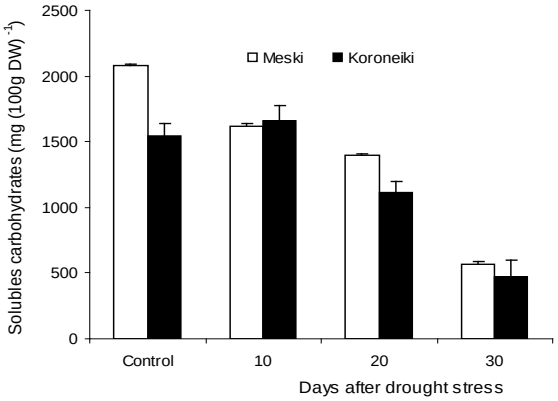

Figure 2. Soluble carbohydrate content in 'Meski' and 'Koroneiki' leaves sampled from control trees and trees subjected to drought stress. The effect of drought stress was evaluated 10, 20 and 30 days after the start of the drought stress treatment. Each value is the mean ± SE of three measurements.

Soluble carbohydrates measured in olive tree leaves were fructose, glucose, sucrose, mannitol, galactose and inositol. Under severe drought, at the end of the experiment, the mannitol fraction (expressed as a percentage of the total carbohydrate content) increased in 'Koroneiki' leaves to 71% and in 'Meski' leaves to 57% (Fig. 3). This increase was statistically significant ($p = 0.001$) in both cultivars. Fructose and glucose fractions decreased in both drought-stressed cultivars, as well as the sucrose fraction in 'Koroneiki'.

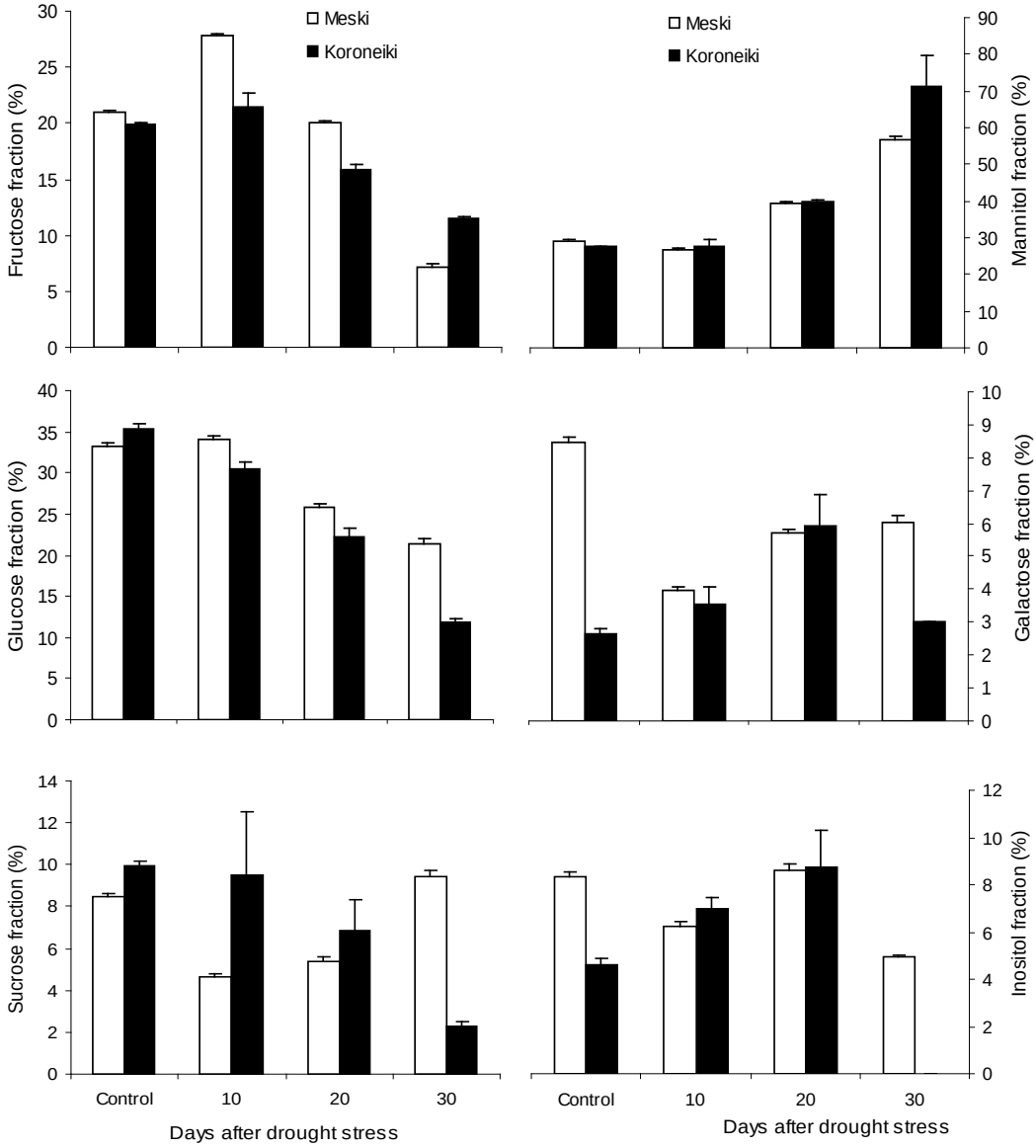

Figure 3. Soluble carbohydrate fraction (expressed as a percentage of the total soluble carbohydrates) in 'Meski' and 'Koroneiki' leaves sampled from control trees and trees subjected to drought stress. The effect of drought stress was evaluated 10, 20 and 30 days after the start of the drought stress treatment. Each value is the mean ± SE of three measurements.

To be able to investigate the effects of drought stress on the soluble carbohydrate concentration, a conversion of the content (expressed per 100 g DW) through LWC and DW/FW ratio (Fig. 4) was performed.

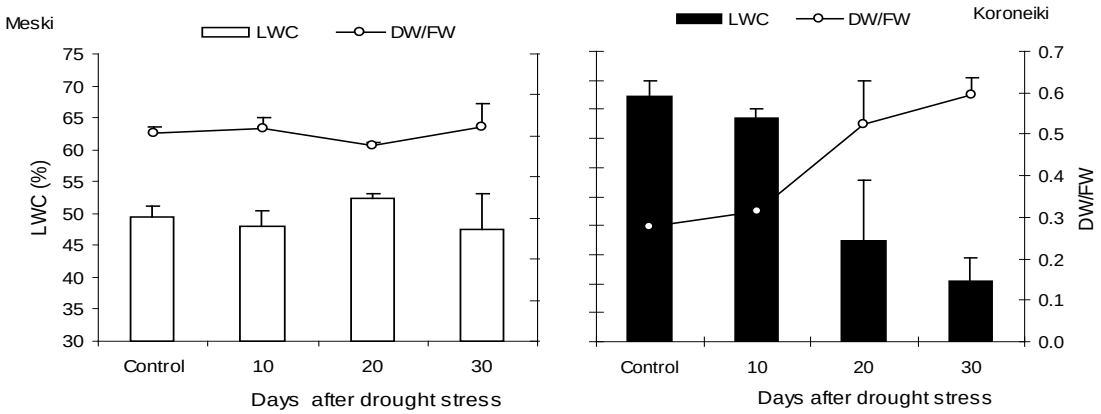

Figure 4. Leaf water content (LWC) and dry weight over fresh weight ratio (DW/FW) of 'Meski' and 'Koroneiki' leaves sampled from control trees and trees subjected to drought stress. The effect of drought stress was evaluated 10, 20 and 30 days after the start of the drought stress treatment. Each value is the mean ± SE of four measurements.

The results (displayed in Fig. 5) show for 'Koroneiki' an interesting increase in soluble carbohydrate concentration of 86% 20 days after drought stress, and a slight increase of 14% (compared to control values) 30 days after drought stress. This observation is in correspondence with a decrease in LWC of 35 and 44%, respectively. In contrast, the soluble carbohydrate concentration in 'Meski' decreased gradually (Fig. 5), because of the stable LWC values for this cultivar (Fig. 4).

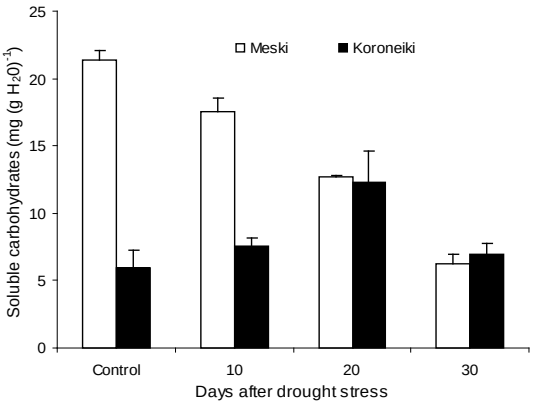

Figure 5. Soluble carbohydrate content in 'Meski' and 'Koroneiki' leaves sampled from control trees and trees subjected to drought stress. The effect of drought stress was evaluated 10, 20 and 30 days after the start of the drought stress treatment. The values obtained at 30 days after drought stress are excluded from the 'Koroneiki' correlations shown in Fig. 7. Each value is the mean ± SE of three measurements. The concentration is calculated using the results shown in Fig. 2 and 4.

Relationship between osmotic adjustment and organic and inorganic compounds

The analyzed organic compounds (i.e., proline and sugars) contributed to OA. Proline in both young and old 'Koroneiki' leaves showed a significant correlation with POA and AOA, and, hence, with OA (Fig. 6). In case of 'Meski', a strong correlation between proline and AOA could only be detected in young leaves (Fig 6).

The soluble carbohydrates showed a clear correlation with both POA and AOA in 'Koroneiki', but with AOA only in 'Meski' (Fig. 7).

The inorganic compound potassium (K) was only correlated with AOA in 'Meski' (Fig. 8).

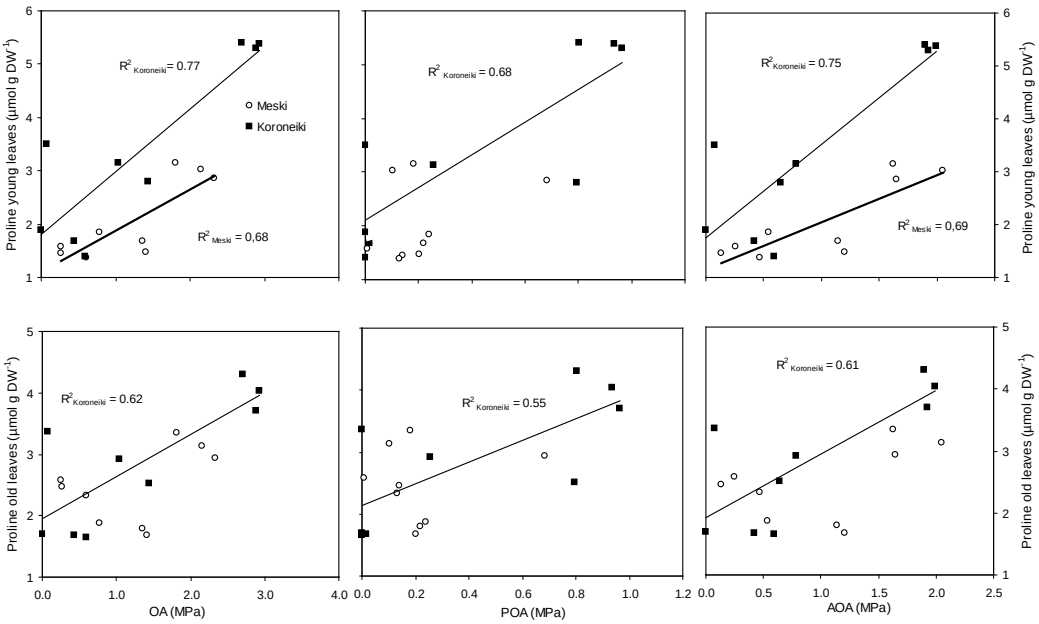

Figure 6. Relationship between proline accumulation, active (AOA), passive (POA) and total (OA) osmotic adjustment in young and old 'Meski' and 'Koroneiki' leaves. Thin and thick lines indicate significant linear regressions for 'Koroneiki' and 'Meski'. Only significant correlations (R^2) are shown at p = 0.001.

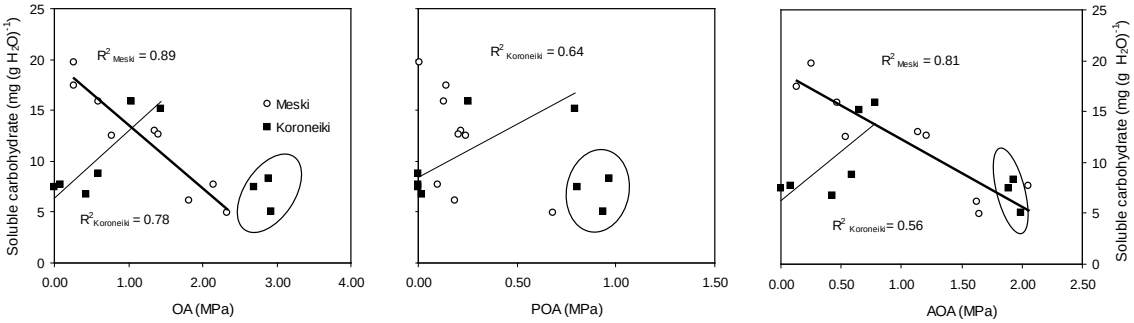

Figure 7. Relationship between soluble carbohydrate accumulation and active (AOA), passive (POA) and total (OA) osmotic adjustment in 'Meski' and 'Koroneiki' leaves. Thin and thick lines indicate significant linear regressions for 'Koroneiki' and 'Meski'. Only significant correlations (R^2) are shown at p = 0.001. The values obtained at 30 days after drought stress (Fig. 5) are excluded from the correlations (see circles).

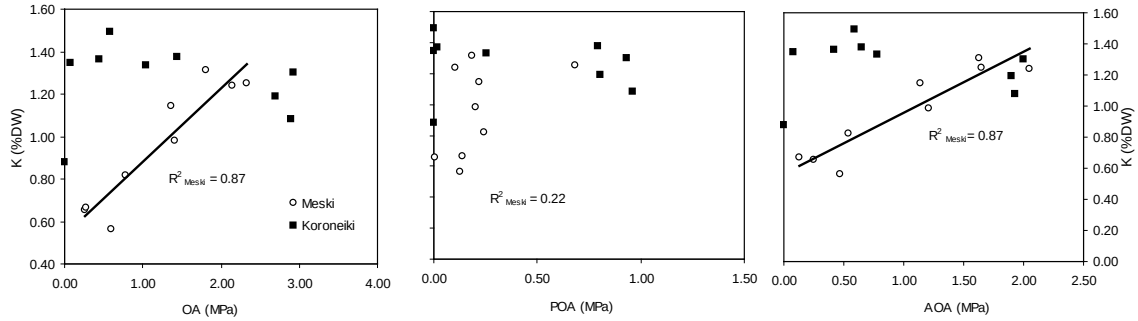

Figure 8. Relationship between potassium (K) accumulation and active (AOA), passive (POA) and total (OA) osmotic adjustment in 'Meski' and 'Koroneiki' leaves. The % is expressed per g dry weight (DW). Thick lines indicate significant linear regressions for 'Meski'. Only significant correlations (R2) are shown at p = 0.003.

Discussion

The ability of olive tissues to lose water to the transpiration stream caused the concentration of cell solutes to increase and Ψ_w to decrease with increasing drought stress (Table 1) (cf. Chartzoulakis et al. 1999). Lo Gullo and Salleo (1988) showed that predawn $\Psi_{\pi 100}$ in drought stressed 20-year-old wild olive trees (*Olea oleaster*) grown in a semi-arid environment fluctuated between -1.95 and -2.50 MPa. These values are higher (less negative) than those measured in our investigation (Table 1). This difference is probably associated with the difference in OA mechanism adopted by cultivated (*Olea europaea* L.) and wild (*Olea oleaster*) olive trees. Three cultivars of carob (*Ceratonia siliqua* L.) subjected to seasonal drought also had higher midday Ψ_π values than our olive trees, ranging from -1.80 to -1.89 MPa (Correia et al. 2001). .Furthermore, $\Psi_{\pi 100}$ in two eucalyptus species (Ngugi et al. 2003)

and in cherry trees (Ranney et al. 1991) subjected to drought were higher than in our olive trees, ranging from -1.50 to -1.77 MPa and from -1.55 to -2.00 MPa, respectively. These findings confirm the greater ability of cultivated olive trees to tolerate severe drought stress through regulation of Ψ_π compared to other tree species.

In our experiment, both Ψ_w and Ψ_π significantly decreased for olive seedlings subjected to 10, 20 and 30 days of drought stress. This is caused by an accumulation of compatible solutes at cellular level (AOA) and/or by a net loss of symplastic water causing a reduction in cell volume and an increase in solute concentration (POA). Both the active and the passive process determine total OA (Table 1), which has been correlated to drought stress in several tree species. In accordance with the results reported for pistachio trees (Ranjbarfordoei et al. 2000), almond trees (Romero et al. 2004, Rouhi et al. 2005), apple trees (Sircelj et al. 2005) and olive trees (Braham 1997, Chartzoulakis et al. 1999, Kasraoui et al. 2005, Dichio et al. 2005), an increase in total OA with increasing drought stress was detected.

To compare both cultivars and to distinguish between AOA and POA, the contribution to total OA was calculated across the drought stress experiment. Ten days after the start of drought stress, 'Koroneiki' used only AOA (100% contribution to OA), while after 30 days of drought, the contribution of AOA and POA changed to 68% and 32%, respectively. In contrast, 'Meski' gradually increased the contribution of AOA to OA from 74 to 90% with increasing level of drought stress and the contribution of POA to the total OA decreased from 26 to 10%. Therefore, we can conclude that 'Koroneiki' tends to adopt a passive strategy to tolerate progressive drought stress (confirmed by a reduction in LWC). In contrast, 'Meski' uses an active strategy and kept its LWC at a constant level (Fig. 4).

It is, hence, important to advance the compounds that contribute to this phenomenon. It is expected that some sugars, proline and some inorganic compounds accumulate and function as osmolytes to help the olive trees to tolerate the imposed drought stress. A decrease in soluble carbohydrate content was, however, found for both cultivars during 30 days of drought stress (Fig. 2) due to a substantial decline in maximum net assimilation rate (see Boussadia et al., 2008). This decline was, however, accompanied with a decrease in LWC for 'Koroneiki'. 'Meski', on the other hand, maintained its LWC, suggesting that OA was highly effective. The concentration of soluble sugars, calculated from the soluble carbohydrate content using LWC and the DW/FW ratio, showed that 'Koroneiki' accumulated soluble carbohydrates as osmoregulator which contributed to both the active and the passive osmotic adjustment (Fig. 7). After 30 days, 'Koroneiki' appeared to be severely affected by drought. Hence, the contribution of carbohydrates to OA decreased. This finding demonstrates

that 'Koroneiki' lost its ability to tolerate this level of stress towards the end of the experiment.

In contrast, 'Meski' was able to keep its LWC and DW/FW ratio fairly constant (Fig. 4), which caused the calculated concentration of soluble carbohydrates to decrease (Fig. 5). This finding explains that soluble carbohydrates did not substantially contribute to the osmoregulation in 'Meski'.

In order to find the main sugar that contributed to OA, it is essential to know that the soluble carbohydrate composition in the leaves of cultivated olive trees differ from that of other fruit species in which sucrose and/or sorbitol are the main components (Moing et al., 1992; Lo Bianco et al., 2000). Previous findings of Priestley (1977) and Drossopoulos and Niavis (1988) indicated that for olive leaves mannitol represented about 70% of the total soluble carbohydrates, followed by sucrose (20%) and glucose (10%). Our results show that drought stress induced an increase in the mannitol fraction in 'Meski' (28%) as well as in 'Koroneiki' (44%) (Fig. 3). In fact, the accumulation of mannitol indicates that mannitol acts as an important osmolyte and compatible soluble compound, particularly in 'Koroneiki'. The accumulation of mannitol in cell organs, cytosol and vacuole seems to be a necessary and important mechanism to balance the extracellular water potential (Stoop et al. 1996) and is considered to be an important contributor in maintaining growth and metabolism in olive leaf tissue (Tattini et al., 1996). With its hydroxyl group, mannitol also seems to stabilize the water molecular structure, limits the peroxidation of lipids and protects cells from plasmolysis (Shen et al., 1997). For the cultivar 'Meski', the sucrose fraction was maintained in response to drought stress and, consequently, contributed to the osmotic potential at full turgor. The drought stress imposed in this experiment also seemed to induce and activate fructose transformation in mannitol (Fig. 3) by a specific enzyme (i.e. mannitol-1-phosphate dehydrogenase; Abébé et al. (2003)), or by enzymatic reactions using phospho-mannose isomerases, mannose-6-phosphate reductase and mannose-1-phosphate phosphatase to transform fructose into mannose-6-phosphate, mannitol-1-phosphate and mannitol, respectively (Stoop et al., 1996). The increased sugar fractions (mainly mannitol) in the leaves of 'Meski' and 'Koroneiki' in response to drought stress supports the idea that sugars can play an active role in the process of osmotic adaptation to drought stress (Chimenti et al., 2006).

The soluble sugar concentration in 'Koroneiki' contributed equally to AOA and POA, while in 'Meski' the soluble sugars were only correlated with AOA (Fig. 7). The negative slope observed for the latter was attributed to the constant LWC that could be maintained by OA, and which was mainly driven by other osmoregulatory compounds.

Another response that is frequently observed during drought stress is the accumulation of intracellular solutes such as proline. Proline is thought to play a multifunctional role in defence mechanisms. A strong correlation between the accumulation of proline and OA was, hence, found (Fig. 6). Proline in young and old 'Koroneiki' leaves contributed to both AOA and POA, but proline was correlated with AOA only in young 'Meski' leaves. The distinction between both cultivars seems to be related to the difference in the mechanisms of tolerance to drought stress.

In addition to the organic compounds, the inorganic element K contributed to OA. K accumulated substantially in 'Meski', apparently to help the tolerance mechanism. K is indeed known to be an activator for enzymes related to photosynthesis and respiration and promotes osmoregulation and stomatal regulation. This theory is confirmed by the strong correlation between AOA and K in 'Meski' leaves (Fig. 8).

Conclusions

Assessment of the contribution of AOA and POA to total OA revealed some interesting differences between the two cultivars 'Koroneiki' and 'Meski'. Proline and sugars contributed equally to AOA and POA in 'Koroneiki'. In 'Meski', only strong relationships were found with AOA. Our results demonstrate the role and the contribution of some organic and inorganic compounds to AOA and POA and the strategy used by 'Meski' and 'Koroneiki' to cope with drought stress.

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