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Lipoxygenase activity and proline accumulation in leaves and roots of olive trees in response to drought stress

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The olive tree (*Olea europaea* L.) is commonly grown in the Mediterranean basin and is able to resist severe and prolonged drought. Levels of proline (PRO) and malondialdehyde (MDA), and the lipoxygenase (LOX) activity were determined in 2-year-old olive plants (cv. 'Coratina') grown in environmental conditions characterized by high temperatures and high photosynthetic photon flux density levels and gradually subjected to a controlled water deficit for 20 days. Before and during the experimental period, leaf and root samples were collected and analysed for PRO and MDA. The levels of PRO increased in parallel with the severity of drought stress in both leaves and roots. Significant increases of LOX activity and MDA content were also observed during the progressive increment of drought stress in both leaf and root tissues. Measurements of transpiration and photosynthetic rate, stomatal conductance and substomatal CO_2 concentration were carried out during the experiment. The accumulation of PRO indicates a possible role of PRO in drought tolerance. The increases of MDA content and LOX activity show that the water deficit is associated with lipid peroxidation mechanisms.

Introduction

The maintenance of plant water potential during water deficit is essential for continued growth and can be achieved by osmotic adjustment mechanisms resulting from the accumulation of compatible solutes (such as proline, glycine betaine, organic acids, sugars as mannitol and sucrose) in the cytoplasm (Ingram and Bartels 1996, Hare et al. 1998). Proline (PRO) acts as a compatible osmolyte since it can accumulate to high concentrations without damaging cellular macromolecules. Proline can also serve as a nitrogen carbon source in the cell (Chiang and Dandekar 1995, Verbruggen et al. 1996). Moreover, PRO has a protective action which prevents membrane damage and protein denaturation during severe drought stress (Ain-Lhout et al. 2001), and replenishes the $NADP^+$ supply in altered redox potentials (Hare and Cress 1997, Hare et al. 1999). It has also been proposed that PRO can act as an electron acceptor, avoiding damage of photosystems due to their photoinhibition by activated oxygen species (Hare et al. 1998).

LOX (EC 1.13.11.12) isoenzymes are nearly ubiquitous in the plant kingdom and are involved in many physiological processes such as flowering (Ye et al. 2000), seed germination (Suzuki and Matsukura 1997), pigment bleaching (Pastore et al. 2000), formation of flavour and aroma in plant products (Williams et al. 2000) and plant growth and development (Hildebrand et al. 1991). Isoenzymes of LOX differing in their substrate specificity and pH optimum, are located in the cytosol (Siedow 1991), microsomes (Feussner and Kindl 1994), plasma membrane (Macri et al. 1994) and oil bodies (Rodríguez-Rosales et al. 1998). LOXs catalyse the dioxygenation of polyunsaturated fatty acids containing a *cis, cis*-1,4-pentadiene backbone, producing hydroperoxy fatty acids, which are highly reactive compounds that are toxic to cells. These fatty acids are

Abbreviations – CP, control plants; LOX, lipoxygenase; LWP, leaf water potential; MDA, malondialdehyde; MR, medium roots; PPFD, photosynthetic photon flux density; PRO, proline; SP, stressed plants; TR, thin roots.

rapidly degraded into metabolites which lead to the production of jasmonates, conjugate dienoic acids and volatile aldehydes, such as malondialdehyde (MDA) (Bird and Draper 1984, Siedow 1991).

Very often MDA levels have been utilized as a suitable marker for membrane lipid peroxidation (Masia 2003). Nonetheless, lipids are not the only targets for MDA activity. In fact MDA can react with DNA to form adducts to deoxyguanosine and deoxyadenosine (Marnett 1999).

The olive tree (*Olea europaea* L.) is a drought-tolerant sclerophyllous species of the Mediterranean basin, where it is subject to regular prolonged seasons of drought. Although this species is adapted to resist severe drought (Lo Gullo and Salleo 1988), little is known about its mechanisms of osmotic adjustment and ability to repair damage caused by drought-induced oxidative stress in cell membranes. Lipid peroxidation, one of the most important causes of cell deterioration during drought stress, generates changes in the composition of fatty acids which affect the structural and functional properties of cell membranes, such as the inactivation of membrane-bound proteins and the increase in membrane permeability (Smirnoff 1993, Asada 1999).

Severe drought stress predisposes the photosynthetic system of olive leaves to photoinhibition, resulting in a light-dependent inactivation of the primary photochemistry associated with photosystem II, which persists after rewatering (Angelopoulos et al. 1996). Although olive plants show a higher specific transpiration with respect to other fruit tree species, assimilation and transpiration rates of this species decrease with increasing stress (Xiloyannis et al. 1988, Nogués and Baker 2000). Moreover, under conditions of high vapour pressure deficit of the air, olive plants can reduce an excessive water loss by closing their stomata (Fernández et al. 1997, Moriana et al. 2002).

The relationships among drought stress and variations in the activity of photosynthetic apparatus and water-related parameters in olive trees are sufficiently clear (Xiloyannis et al. 1988, Angelopoulos et al. 1996, Moriana et al. 2002). Very little is known, however, about the linkages between drought stress, PRO and MDA levels, and LOX activity. The aim of this study was to investigate the mechanisms of PRO accumulation and to evaluate the MDA levels and LOX activity in olive plants under drought stress. We hypothesize that there would be correlations between these parameters under different levels of drought stress. It is possible that PRO could be used as an index of drought stress in olive trees.

Materials and methods

Study site and experimental design

Trials were conducted on own-rooted cuttings of 2-year-old *Olea europaea* L. plants, cv. 'Coratina', measuring 130–150 cm in height. The study site was located at the 'Pantanello' Agricultural Experiment Station in Metaponto (Southern Italy – Basilicata Region – 40°24′ N, 16°48′ E). The experimental period started on 3 July and ended on 23 July 2001.

The olive plants grew uniformly outdoors in 0.016 m^3 containers filled with a mixture of loam, peat and sand (in proportion of 1 : 1 : 1). The pots were covered with plastic film and aluminium foil in order to avoid evaporation from the soil surface and to minimize temperature increases inside the containers. All plants were weighed each evening in order to calculate the amount of water transpired daily by weight difference. The mean transpiration value of all the plants was 0.84 l day^{-1}. Soil water content was maintained at a constant value of around 85% of water-holding capacity of the pot by integrating the amount of water lost through transpiration during the day. Plants were fertilized at 25-day intervals throughout the period of vegetative growth with 3.5 g of slow-release nitrogen complex fertilizer (Nitrophoska Gold 15N−9P−16K + 2Ca + 7Mg; Compo Agricoltura, Cesano Maderno, MI, Italy).

The plants were divided into two groups: 14 non-stressed control plants (CP) and 36 stressed plants (SP). In CP, the amount of water added daily during the whole experimental period was equal to the amount transpired. During the first 10 days of the experimental period, the SP were subjected to a gradual controlled water depletion, applying a daily reduction of 10% less than the total transpired water. In the subsequent 10 days, irrigation ceased. Values of leaf water potential (LWP described below) between −0.5 and −2.4 MPa, corresponding to 4 and 8 days from the beginning of the drought-stress period, respectively, were defined as 'mild' drought stress. LWP values between −2.5 and −4.9 MPa (12 and 16 days from the beginning, respectively) were described as 'moderate' drought stress, and those between −5.0 and −6.3 MPa (20 days from the beginning) as 'severe' drought stress.

Environmental and physiological parameters

Environmental parameters were monitored by a weather station placed within 20 m of the experimental plot. Measurements of maximum air temperature, minimum relative humidity, vapour pressure deficit (VPD) and photosynthetic photon flux density (PPFD) for each day of the experimental period were taken. PPFD was recorded at 1-min intervals and daily integrated values were logged.

Three CP and three SP were randomly chosen to measure physiological parameters at 0, 4, 8, 12, 16 and 20 days from the beginning of the drought-stress period using three fully expanded leaves selected from each plant along the median segment of new-growth shoots and marked at the beginning of the experiment. Selected leaves were not covered. LWPs were measured pre-dawn (at 0400–0500 h) using a Sholander pressure chamber (PMS Instrument Co. Corvallis, OR, USA), according to Turner (1981).

The measurements of transpiration and photosynthetic rate, stomatal conductance, substomatal CO_2 concentration and leaf temperature were carried out using the leaf chamber analyser LCA-4 (ADC, Hoddesdon, Herts.,UK) operated at 200 μmol m^{-2} s^{-1} flow rate,

under natural temperature, relative humidity and full sunlight conditions (at 1300–1400 h) at 0, 4, 8, 12, 16 and 20 days from the beginning of the drought-stress period. The CO_2 concentration inside the leaf chamber was automatically controlled at 355 μmol mol^{-1}. VPD values calculated using the LCA-4 were higher than those measured in the weather station by about 0.7 kPa.

Proline, malondialdehyde and lipoxygenase

In both CP and SP, leaves were collected early in the morning (at 0600–0700 h) at 0, 4, 8, 12, 16 and 20 days from the beginning of the drought-stress period. Three plants having similar LWPs were selected for tissue sampling. Each sample contained eight fully expanded leaves taken from each plant along the median segment of new-growth shoots. Roots (at least 10–15 g), were sampled at 0600–0700 h, after the plant destruction, at 0, 8, 16 and 20 days from the beginning of the drought-stress period, and divided in two groups: 'thin roots' (TR, with a diameter <1 mm) and 'medium roots' (MR, with a diameter between 1 and 5 mm). Leaf and root samples were washed with distilled water, dried with filter paper, immediately frozen in liquid nitrogen and then stored at -80°C.

The frozen tissues were finely ground in liquid nitrogen using a mortar and pestle previously chilled with liquid nitrogen and the frozen powder was immediately used for the extraction of PRO, MDA and LOX. All procedures were carried out at 0°C in an ice bath unless otherwise stated.

The values of LOX activity were expressed as units mg^{-1} dry weight (DW), whereas those of PRO and MDA concentrations as μmol mg^{-1} DW and nmol g^{-1} DW , respectively. Such a basis appeared to be appropriate since the overall dry weight remained relatively unaffected during drought conditions, whereas fresh weight and total soluble protein concentration were more variable (data not shown).

Proline

Free PRO content was determined after Bates et al. (1973) modified. A 5.0-ml aliquot of 3% (w/v) sulfosalicylic acid was added to 0.5 g of powder and boiled in a water bath at 100°C for 30 min in glass tubes covered at the top. The mixture was centrifuged at 2000 *g* for 5 min at 25°C. A 200-μl aliquot of the extract was mixed with 400 μl distilled water and 2.0 ml of the reagent mixture (30 ml glacial acetic acid, 20 ml distilled water and 0.5 g of ninhydrin), and boiled at 100°C for 1 h. After cooling the reaction mixture, 6.0 ml toluene were added. The chromophore containing toluene was separated and A_{520} was read, using toluene as a blank. PRO concentration, in μmol mg^{-1} DW, was calculated using L-proline for the standard curve.

Malondialdehyde

MDA content was measured according to Du and Bramlage (1992) and expressed as nmol g^{-1} DW. A 0.5-g aliquot of frozen powder was added to 5.0 ml 0.1% (w/v) trichloroacetic acid and centrifuged at 10 000 *g* for 5 min. A 1-ml aliquot of supernatant was added to 4.0 ml 0.5% (w/v) thiobarbituric acid in 20% (w/v) trichloroacetic acid. The mixture was heated at 100°C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10 000 *g* for 10 min, the A_{532}, A_{600} and A_{440} values of the supernatant were recorded. The value for aspecific absorption at 600 nm was subtracted and a standard curve of sucrose (from 2.5 to 10 μmol ml^{-1}) was used to correct the results from the interference of soluble sugars in samples, reading A_{532} and A_{440}. MDA content was calculated using its absorption coefficient of 157 mmol $^{-1}$ cm^{-1} and expressed as nmol g^{-1} DW.

Lipoxygenase

LOX activity was assayed after Williams et al. (2000) with some modifications. A 1.0-g aliquot of frozen powder was suspended in 10 ml acetone at -20°C for 30 min, centrifuged at 10 000 *g* for 15 min and then the supernatant was discarded. This extraction was repeated three times. The pellet was re-suspended in 10 ml of 50 m*M* HEPES, pH 7.2, containing 3 m*M* dithiothreitol 10 m*M* MgCl and 1 m*M* EDTA, for 1 h, and then centrifuged at 10 000 *g* for 10 min. The resultant supernatant was desalted on a Sephadex® G-25M column and used for the enzyme activity assay. Linoleate hydroperoxidation activity was photometrically determined at 25°C by monitoring the increase of A_{234}, due to the conversion of linoleate into the corresponding hydroperoxide. LOX activity was measured in a reaction mixture consisting of 120 μl 10 m*M* linoleate, 1.25 ml 100 m*M* sodium-phosphate buffer, pH 6.5, 0.1% (w/v) Tween 80 and 25 μl of enzyme extract. One unit of enzyme was defined as the quantity that generates 1 μmol of conjugate diene per minute at 25°C, according to Axelrod et al. 1981.

Statistical analysis

The physiological parameters were represented as a single average measurement from each selected plant (three measurements per plant), whereas the values of PRO and MDA concentrations and LOX activity were expressed as means of three measurements (± SE) from three plants having a similar level of drought stress (one measurement per plant and three replications of each measurement).

Statistical analysis was performed using analysis of variance (ANOVA). Significant differences between values of enzyme activity in CP and SP were determined at $P \leq 0.05$, according to Duncan's multiple range test.

Results

Environmental conditions and physiological parameters

The highest value of maximum temperature was 33.8°C on 12 July and the mean of all the daily maximum values was 31.2°C (Fig. 1). Minimum RH pattern showed the highest value (40.8%) on 5 July with a mean of 30.1%. The VPD range was between 2.2 (on 5 July) and 4.1 kPa (on 22 July), with a mean value of 3.2 kPa. The PPFD

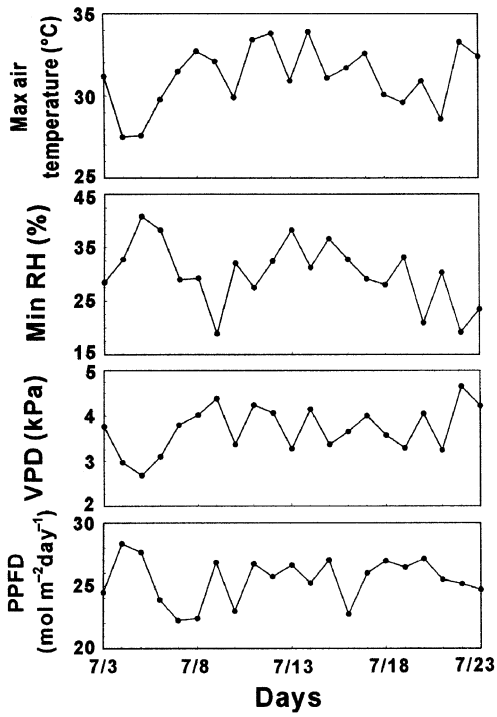

Fig. 1. Maximum air temperatures, minimum relative humidity (RH), vapour pressure deficit (VPD) and photosynthetic photon flux density (PPFD) at the field site during the experimental period.

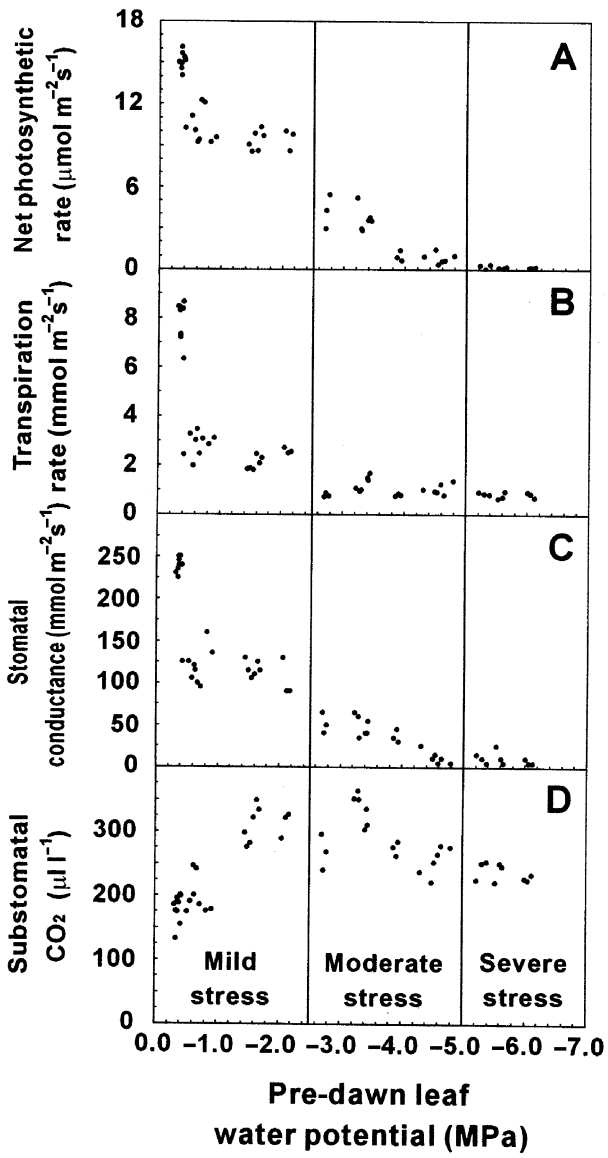

Fig. 2. Relationships between leaf water potential, measured at 0400–0500 h, and net photosynthesis (A), transpiration (B), stomatal conductance (C) and substomatal CO_2 concentration (D), measured at 1300–1400 h, in drought-stressed plants at 0, 4, 8, 12, 16 and 20 days from the beginning of the stress period.

levels fluctuated within a range from 22.21 to 28.34 mol m^{-2} day^{-1} (on 7 and 4 July, respectively).

The mean pre-dawn leaf water potential in CP was −0.34 MPa, whereas that in SP declined during the whole period of water deficit, reaching a mean minimum of −5.81 MPa after 20 days of drought stress (Fig. 2). During measurements, leaf temperatures varied between a mean value of 32 and 37°C in CP and SP, respectively.

SP showed a gradual decrease of net photosynthesis rate within mild stress levels, followed by a rapid decline at moderate stress levels to almost zero at severe stress levels (Fig. 2A). Transpiration rate and stomatal conductance patterns in SP showed a similar pattern, as both displayed a rapid drop at mild levels of stress followed by a gradual decrease at moderate and severe drought stress (Fig. 2B and C). Substomatal CO_2 concentration initially increased at mild stress levels and subsequently reached a plateau phase during moderate and severe drought-stress conditions (Fig. 2D). Midday net photosynthetic rate, transpiration rate, stomatal conductance and substomatal CO_2 concentration of CP remained relatively stable during the 20-day experimental period (data not shown).

Proline and malondialdehyde content

PRO content increased significantly in relation to the severity of drought stress, in particular in leaves and medium roots (Table 1). There was a rapid increase of PRO at leaf water potentials lower than −3.09 MPa. The increase of PRO level in thin roots was less at 1.6 times the CP value at severe drought stress. MDA levels increased slightly at mild stress levels and subsequently rose with water deficit in leaf tissues at 3.3 times the CP value, at severe drought stress. Significant increases of MDA levels of about three times greater than those of CP were observed in TR and MR at maximum level of drought stress (Table 2). CP did not show any significant changes in PRO and MDA concentration during the 20-day experimental period.

Table 1. Proline (PRO) levels of leaves, thin roots and medium roots from drought-stressed (SP) and irrigated (CP) plants. Samples were collected, at 0600–0700 h, at 0, 4, 8, 12, 16 and 20 days from the beginning of the stress period. Each value represents the mean of three measurements (± SE) from three plants having a similar level of drought stress. [a]Significant differences at the 5% level between values obtained under control and the respective drought-stressed plants ($P \leq 0.05$, according to Duncan's multiple range test).

		PRO (μmol mg^{-1} DW)					
		Leaves		Thin roots		Medium roots	
Degree of drought	Days	SP	CP	SP	CP	SP	CP
Control	0	0.53 ± 0.01	0.56 ± 0.02	0.50 ± 0.01	0.48 ± 0.01	0.30 ± 0.01	0.30 ± 0.01
Mild	4	0.56 ± 0.02	0.59 ± 0.02	–	–	–	–
Mild	8	0.73 ± 0.02[a]	0.50 ± 0.01	0.63 ± 0.02[a]	0.50 ± 0.01	0.44 ± 0.01[a]	0.29 ± 0.01
Moderate	12	1.09 ± 0.02[a]	0.47 ± 0.02	–	–	–	–
Moderate	16	1.22 ± 0.03[a]	0.54 ± 0.01	0.63 ± 0.01[a]	0.40 ± 0.01	0.45 ± 0.02[a]	0.27 ± 0.01
Severe	20	1.59 ± 0.03[a]	0.65 ± 0.01	0.73 ± 0.03[a]	0.45 ± 0.02	0.54 ± 0.02[a]	0.28 ± 0.01

Lipoxygenase activity

In SP, moderate drought stress caused marked increase in LOX activity in leaves, TR and MR, which then rose slightly until reaching the maximum level of drought stress. In particular, leaves were the most affected by water deficit, showing a three-fold increase in LOX activity at severe drought stress, with respect to CP. LOX activities in TR and MR at the highest level of drought stress were 1.7 and 1.6 times the CP values, respectively (Table 3). In CP, LOX activity did not change significantly during the 20 day the experimental period.

Discussion

PRO within the cell can act as an osmolyte with high compatibility for enzymes and other cell macromolecules, therefore protecting them from drought-stress-induced damage (Hare et al. 1998). Osmotic adjustment produced by PRO causes a drop of the osmotic potential in plant tissues (Hare and Cress 1997, Nanjo et al. 1999) and is a common response in many woody and herbaceous plant species following an osmotic stress induced by water deficit, both in vivo (Gzik 1996, Hernandez et al. 2000) and in vitro (Martinez et al. 1996, Watanabe et al. 2000). Lower osmotic potentials allow leaves to withstand a greater evaporative demand without loss of turgor (Holbrook and Putz 1996). The capacity for osmotic adjustment via net solute accumulation during drought-stress imposition in olive trees has been reported by Larcher et al. (1981), and has also been found in leaves of grapevine (During 1984, Schultz and Matthews 1993), apple trees (Lakso et al. 1984, Wang et al. 1995) and cherry trees (*Prunus*) (Ranney et al. 1991). This process seems to be species related, in fact PRO accumulation in two Mediterranean shrubs (*Halimium halimifolium* L. and *Pistacia lentiscus* L.) during increasing water deficit was twice the amount found in olive tree (Ain-Lhout et al. 2001).

The role of proline in response to osmotic stress includes a very important part in the biosynthesis of cell-wall matrix proteins, such as extensins, that have important roles in cell morphology and provide mechanical support for cell under stressed conditions (Nanjo et al. 1999). A very neglected aspect in proline metabolism concerns its importance during the stress relief phase. In fact its rapid oxidation is equally important in recycling the free iminoacid accumulated during the stress conditions with the production of reducing power, amino nitrogen and energy, all needed in the restoration of cellular homeostasis during the recovery from osmotic stress (Verbruggen et al. 1996). Finally its unique capacity to consume high levels of reductants during its biosynthetic pathway, paralleled by high-energy output during its degradation justify the main role played in many different species as a great resource either in the acclimation to stress or in plant recovery upon relief from it (Hare and Cress 1997).

As the olive tree is well adapted to the high temperatures and irradiance levels in summer conditions of Mediterranean climate, we hypothesize that this species

Table 2. Malondialdehyde (MDA) levels of leaves, thin roots and medium roots from drought-stressed (SP) and irrigated (CP) plants. Samples were collected, at 0600–0700 h, at 0, 4, 8, 12, 16 and 20 days from the beginning of the stress period. Each value represents the mean of three measurements (± SE) from three plants having a similar level of drought stress. [a]Significant differences at the 5% level between values obtained under control and the respective drought-stressed plants ($P \leq 0.05$, according to Duncan's multiple range test).

		(MDA (nmol g^{-1} DW)					
		Leaves		Thin roots		Medium roots	
Degree of drought	Days	SP	CP	SP	CP	SP	CP
Control	0	74.36 ± 5.32	77.11 ± 4.03	37.51 ± 1.33	31.18 ± 0.57	33.28 ± 0.64	38.18 ± 1.92
Mild	4	73.78 ± 2.39	68.33 ± 5.30	–	–	–	–
Mild	8	119.96 ± 5.77[a]	69.00 ± 7.66	57.12 ± 1.65[a]	27.37 ± 0.84	65.72 ± 2.11[a]	36.27 ± 1.01
Moderate	12	116.83 ± 6.01[a]	74.31 ± 6.90	–	–	–	–
Moderate	16	176.95 ± 9.21[a]	73.62 ± 5.34	98.20 ± 4.90[a]	34.58 ± 0.64	77.30 ± 2.28[a]	33.05 ± 1.45
Severe	20	256.06 ± 13.27[a]	78.00 ± 8.39	102.67 ± 7.88[a]	34.60 ± 0.55	92.67 ± 3.48[a]	35.63 ± 1.20

Table 3. Lipoxygenase (LOX) activity of leaves, thin roots and medium roots from drought-stressed (SP) and irrigated (CP) plants. Samples were collected, at 0600–0700 h, at 0, 4, 8, 16 and 20 days from the beginning of the stress period. Each value represents the mean of three measurements (± SE) from three plants having a similar level of drought stress. [a]Significant differences at the 5% level between values obtained under control and the respective drought-stressed plants ($P \leq 0.05$, according to Duncan's multiple range test).

		LOX activity (units mg^{-1} DW)					
		Leaves		Thin roots		Medium roots	
Degree of drought	Days	SP	CP	SP	CP	SP	CP
Control	0	149.93 ± 7.35	152.38 ± 8.02	59.49 ± 2.13	64.30 ± 2.84	50.82 ± 2.73	54.45 ± 2.57
Mild	4	151.65 ± 8.27	159.82 ± 5.24	–	–	–	–
Mild	8	240.10 ± 9.12[a]	173.11 ± 10.37	89.56 ± 6.29[a]	76.32 ± 5.39	64.22 ± 7.39[a]	59.27 ± 1.20
Moderate	12	336.87 ± 18.36[a]	161.59 ± 11.41	–	–	–	–
Moderate	16	461.35 ± 28.20[a]	145.65 ± 5.02	110.40 ± 3.84[a]	73.16 ± 4.51	74.67 ± 7.57[a]	55.21 ± 2.54
Severe	20	492.43 ± 34.29[a]	168.80 ± 8.29	112.50 ± 4.11[a]	68.02 ± 2.39	79.51 ± 9.84[a]	50.79 ± 1.42

probably possesses some other mechanisms of drought tolerance in addition to the solely PRO accumulation, such as stomata closure and leaf tolerance to dehydration (Fernández et al. 1997, Dichio et al. 2002). In fact olive trees gradually close their stomata to avoid water loss by transpiration, as observed by Fernández et al. (1997) and Moriana et al. (2002). These observations are confirmed by our data, which show gradual decreases in transpiration rate and stomatal conductance throughout the experiment (Fig. 2B and C).

The MDA content in SP increased more than three-fold in leaves and about three-fold in TR and MR when compared with the values of CP during drought stress (Table 2). Accumulation of MDA as a consequence of lipid peroxidation, the main effect of oxidative damage, has also been observed during the imposition of other abiotic stresses such as those responsible for seed deterioration in sunflower (Bailly et al. 1996) and in chill-stressed roots of *Coffea arabica* (Queiroz et al. 1998). MDA levels in water-stressed olive trees appear to be higher than those observed in herbaceous species grown in the same environmental conditions (Sairam et al. 1997/98).

Drought-stress conditions can activate specific LOX isoenzymes, as observed by Ye et al. (2000) in *Arabidopsis* flowering, leading to membrane lipid peroxidation. This can explain the observed three-fold increase of LOX activity in olive leaves and the less marked increment in TR and MR during the progression of drought stress (Table 3). Our results on LOX activity and MDA levels provide evidence of a higher lipid peroxidation in SP in comparison to CP. Although olive trees are very resistant to drought stress, drought conditions produced a marked decrease in LWP paralleled by a substantial decrease in the net photosynthetic rate (Fig. 2A) probably derived from lipid peroxidation. This hypothesis was supported by MDA accumulation and high LOX activities determined in other drought-stressed species, as recently confirmed by Lima et al. (2002) in *Coffea canephora*. The observed high degree of lipid peroxidation could produce lipid derivatives acting as secondary messengers capable to activate some drought-stress-associated genes by means of specific transcription factors, in such a way starting the response of plant to desiccation (Ingram and Bartels 1996, Shinozaki and Yamaguchi-Shinozaki 1997). Jasmonic acid, another final product of hydroperoxy fatty acids catabolism, can act as a regulatory molecule with growth-inhibitory properties, analogous to those of abscisic acid (Siedow 1991), and it may be responsible for reduced olive tree growth during drought-stress conditions (Dichio et al. 2002).

Our results demonstrate that the cell membrane's damage is a direct consequence of water deficit. In drought-stress conditions, olive tree chloroplasts become more sensitive to oxidative damage and this could be the main cause of the observed loss of photosynthetic functionality (Fig. 2A), confirmed also by the results of previous findings (Angelopoulos et al. 1996, Nogués and Baker 2000). The increase of MDA in olive tissues under drought-stress conditions suggests that the repairing mechanisms do not keep pace with damage and that water deficit can influence the composition and turnover of membrane lipids. In particular, a peroxidation of thylakoid glycolipids and the subsequent production of diacylglycerol, triacylglycerol and free fatty acids occur (Smirnoff 1993).

The dynamics of LOX activity and the contents of PRO and MDA in root tissues highlighted a different response of TR and MR against drought stress: TR, involved in water absorption, appeared to be more sensitive and reactive to water content changes, whereas MR, in which the carbohydrates produced in leaves are stored and presenting a higher degree of lignification, showed less marked changes.

In conclusion, the results discussed here support the hypothesis that PRO accumulation during drought stress is a part of a physiological response of olive tree to the imposition of an intense drought stress. Under drought-stress conditions, olive tree activates osmotic adjustment mechanisms not only in leaves, but also in roots, in such a way increasing its capacity to extract water from dry soil (Holbrook and Putz 1996). Our results support a direct correlation between the degree of drought stress and PRO content. As a consequence, PRO concentrations could be used as a biochemical marker of drought-stress level in olive plants. We can also conclude that there is a direct correlation between MDA and drought stress, particularly at severe degrees of stress. Finally, the

gradual increase of LOX activity during the progression of water deficit suggests a strict relationship of this enzyme with drought-stress conditions. The results obtained in this investigation may be important for a more complete understanding of the behaviour of the vegetative growth of olive tree plants in semi-arid regions.

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