Photosynthesis parameters and activities of enzymes of oxidative stress in two young 'Chemlali' and 'Chetoui' olive trees under water deficit

M. GUERFEL[*,+], Y. OUNI[*], D. BOUJNAH[**], and M. ZARROUK[*]

Laboratoire Caractérisation et Qualité de l'Huile d'Olive, Centre de Biotechnologie de Borj Cédria, B.P. 901, 2050 Hammam-Lif, Tunisia[*]
Institut de l'Olivier, Station de Sousse, Rue Ibn Khaldoun, B.P. 40, 4061 Sousse,Tunisia[**]

Abstract

The effects of water deficit on photochemical parameters and activities of superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase were investigated in two olive cultivars differing in drought tolerance – 'Chemlali' and 'Chetoui'. After 30 days without irrigation, leaf water potential fell to −5.5 MPa that was accompanied by a marked decrease in net photosynthesis in 'Chetoui' olive cultivar. Maximal efficiency of PSII photochemistry (F_v/F_m) decreased slightly in 'Chemlali' (28 %) and substantially in 'Chétoui' (47 %). Both cultivars showed a similar decline (about 25 %) in the photochemical quenching coefficient, but only the drought-sensitive olive cultivar exhibited an enhancement (31 %) of non-photochemical fluorescence quenching under water deficit conditions. The quantum yield of electron transport decreased in both olive cultivars. 'Chemlali' showed a higher protection against oxidative stress, as judged from the lower levels of the malondialdehyde production. Catalase activity was higher in 'Chetoui'. Glutathione reductase activity was increased similarly in both olive cultivars under water stress. Ascorbate peroxidase activity was enhanced in 'Chemlali' under water stress, but was unaffected in 'Chetoui'. While, superoxide dismutase activity was inhibited in both cultivars under water stress, but higher activity was detected in 'Chemlali'. Thus, the ability to increase ascorbate peroxidase and a higher superoxide dismutase activity might be an important attribute linked to the drought tolerance in 'Chemlali' olive cultivar.

Additional keywords: antioxidant system; chlorophyll fluorescence; *Olea europaea* L.; water deficit.

Introduction

Water deficit has been shown to influence various physiological and biochemical processes in plants. Plants can avoid drought stress by maximizing water uptake (*e.g.* tapping ground water by deep roots) or minimizing water loss (*e.g.* stomatal closure, small leaves, *etc.*) (Kozlowski and Pallardy 2002).

A common effect of drought stress, similarly as of other environmental stresses, is to cause oxidative damage (Smirnoff 1998). Water deficit stress induces an oxidative stress because of the inhibition of the photosynthetic activity due to imbalance between the light capture and its utilization (Foyer and Noctor 2000). Changes in the photochemistry of the chloroplasts in the leaves of drought-stressed plants result in a dissipation of excess light energy, thus, generating active oxygen species (AOS). When the accumulation of AOS under water stress conditions exceeds the removing capacity of the antioxidant system, the effects of oxidative damage arise, including peroxidation of membrane lipids, destruction of photosynthetic pigments and inactivation of photosynthetic enzymes (Smirnoff 1993).

Plant cells need to have protective mechanisms by which they respond to oxidative stress: non-enzymatic antioxidants and enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) (Navabpour *et al.* 2003; Zimmermann and Zentgraf 2005).

Malondialdehyde (MDA), a decomposition product of

Received 24 October 2008, *accepted* 15 May 2009.
[+]Corresponding author: fax: +216 71 430 934, e-mail: Guerfel_mk@yahoo.fr
Abbreviations: A – CO_2 assimilation rate; AOS – active oxygen species; APX – ascorbate peroxidase; CAT – catalase; F_0 – initial fluorescence; F_0' – light-adapted F_0; F_m – maximum fluorescence emission; F_m' – maximum fluorescence; F_s – steady-state fluorescence yield; F_v – variable fluorescence; F_v/F_m – maximal photochemical efficiency of PSII; F_v'/F_m' – efficiency of excitation capture by open PSII reaction centers; FM – fresh mass; g_s – stomatal conductance; GR – glutathione reductase; MDA – malondialdehyde; NPQ – non-photochemical quenching; q_p – photochemical quenching; SOD – superoxide dismutase; Φ_{PSII} – quantum yield of PSII electron transport; Ψ_L – leaf water potential.

polyunsaturated fatty acids hydroperoxides, has been utilized very often as a suitable biomarker for lipid peroxidation (Bailly *et al.* 1996), which is an effect of oxidative damage. Nonetheless, lipids are not the only targets for MDA action; in fact MDA damages DNA, forming adducts to deoxyguanosine and deoxyadenosine (Marnett 1999).

The olive tree (*Olea europaea* L.) is a sclerophyllous species of the Mediterranean area presenting a high degree of drought tolerance (Lo Gullo and Salleo 1988) and a higher specific transpiration rate in comparison to other fruit tree species in both ideal and water shortage conditions (Nogués and Baker 2000, Sofo *et al*. 2004).

Tunisia is a very important country in the olive oil producing world. It is the largest African exporter and fourth worldwide after Spain, Italy and Greece, with an average annual export of over 10 000 t (IOOC 2004). The olive tree (*Olea europaea* L.) is present in practically every region of the country, up to the border of the southern dessert. Many olive varieties are present in Tunisia, but there are two that stand out. The 'Chemlali' cultivar accounts for 80 % of the national olive oil production and is grown in central and southern Tunisia, areas characterized by a low rainfall (< 250 mm per year). The Chétoui cultivar is widespread in the north of the country, occurring in plains as well as in mountainous regions. It covers an area of 176,000 ha and accounts for about 20 % of the olive oil produced in Tunisia (Ben Temime *et al.* 2006).

'Chemlali' is considered to be more drought tolerant than 'Chetoui'. Therefore, our working hypothesis in this study was that 'Chemlali' should exhibit an increased ability to protect against photooxidation following periods of water deficit. To test this hypothesis, the drought-induced effects on photochemical events and on the activities of some antioxidant enzymes were examined in the two main Tunisian olive cultivars by experimentally withholding irrigation.

Materials and methods

Plants and experimental design: The experiment was conducted at the Olive Tree Institute of Sousse, Tunisia (35°50'N; 10°37'E). One-year-old own-rooted olive trees (*Olea europaea* L. cv. Chemlali and cv. Chetoui) were transplanted in 3-L plastic pots containing freely drained light soil with a pH of 7.6, a field capacity of 22 % (–0.02 MPa), and a permanent wilting point of 6.2 % (–1.5 MPa). The pots were kept under environmental conditions with a temperature between 30 °C and 35 °C; there was no rainfall during the experiment. The soil of the pots was covered with plastic film and aluminum foil to reduce evaporation from the soil surface and to minimize temperatures inside of the containers. Plants were irrigated daily with half-strength Hoagland's solution for 6 months before the experiment. The two different cultivars were exposed to different water regimes during July of 2006. Ten plants of each cultivar were used as controls (well watered) and irrigated every 2 days to maintain a soil water content close to field capacity. At the beginning of the experiment, all pots were saturated with water and allowed to drain freely from the holes in the bottoms of the containers. Additional ten plants from the two cultivars were stressed by withholding water during one month until the soil water content almost reached the wilting point (6.2 %).

Leaf water potential (Ψ_L) was measured using a *Scholander* pressure chamber (*SKPM 1400, Skye Instruments*, Powys, UK). Two leaves per plant were used with six plant replicates for each treatment from 9:00 to 10:00 a.m. After cutting, the leaf was immediately enclosed in a plastic bag and the determination of Ψ_L was started in less than 1 min. These measurements were carried out at the end of the drought stress cycle.

Photosynthetic gas exchange and chlorophyll (Chl) fluorescence measurements: CO_2 assimilation rate (A), stomatal conductance (g_s) and Chl fluorescence were measured on two leaves per plant with six replicate trees for each treatment from 9:00 to 10:00 a.m. with a portable porometer (*LCA-4, ADC, BioScientific Ltd*. Hoddesdon, UK) which was equipped with leaf chamber fluorometer (*LI-6400-40 LCF*) at the end of the drought stress cycle. These measurements were made with the following specifications: ambient CO_2 concentration 350 μmol mol^{-1}, temperature of leaf chamber varied from 31.5 to 37.6 °C, PAR at leaf surface was 1050 μmol m^{-2} s^{-1}. Chl fluorescence parameters were measured on the adaxial surface on the same leaves as CO_2 assimilation. Leaves, dark adapted for 30 min, were illuminated with a weak modulated measuring beam to obtain the initial fluorescence (F_0). A saturating white light pulse of 6000 μmol m^{-2} s^{-1} was applied for 1 s to ensure maximum fluorescence emission (F_m). In dark adapted samples, the maximum photochemical efficiency of PSII was estimated from the variable to maximum fluorescence ratio, $F_v/F_m = (F_m - F_0)/F_m$. The samples were then illuminated for 480 s with a continuous actinic PPF (900 μmol m^{-2} s^{-1}) to obtain a steady-state fluorescence yield (F_s). Subsequently, saturating white light pulses were applied to achieve the maximum fluorescence (F_m'). Actinic light was then turned off and a far-red illumination was turned on to measure light-adapted F_0 (F_0'). The capture efficiency of excitation energy by open PSII reaction centers (F_v'/F_m') was estimated as $F_v'/F_m' = (F_m' - F_0')/F_m'$. The coefficient for photochemical quenching (q_p) was calculated as $q_p = (F_m' - F_s)/(F_m' - F_0')$ and that for non-photochemical quenching (NPQ) was determined as $NPQ = F_m/F_m' - 1$ (Krause and Weis, 1991). The actual quantum yield of PSII electron transport

(Φ_{PSII}) was computed as $\Phi_{PSII} = (F_m' - F_s)/ F_m'$ (Genty *et al*. 1989).

MDA determination: The level of lipid peroxidation products in leaf samples was expressed as MDA content and was determined (Heath and Packer 1968). Fresh tissue was ground in 0.25 % 2-thiobarbituric acid (TBA) in 10 % trichloro-acetic acid (TCA) using a mortar and pestle. After heating at 95 °C for 30 min, the mixture was quickly cooled in an ice bath and centrifuged at 10.000 × *g* for 10 min. The absorbance of the supernatant was read at 532 nm and corrected for unspecific turbidity by subtracting the absorbance of the same at 600 nm. The blank was 0.25 % TBA in 10 % TCA. The concentration of lipid peroxides together with oxidatively modified proteins of plants were thus quantified in terms of MDA level using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} fresh mass (FM).

Antioxidant enzymes: Extracts for determination of antioxidant enzymes SOD, APX, CAT and GR activities were prepared from leaf discs homogenized with a mortar and pestle in 1.5 cm^3 of ice cold 50 mM potassium-phosphate buffer (pH 7.4), containing 0.1 mM EDTA and 0.2 % polyvinyl polypyrrolidone. CAT activity was assayed according to Fadzilla *et al.* (1997). APX activity was assayed according to Nakano and Asada (1981). The reaction mixture (3 cm^3) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA, 1 mM hydrogen peroxide and 0.2 cm^3 of enzyme extract. SOD activity was assayed according to Beauchamp and Fridovich (1971). The reaction mixture (1 cm^3) consisted of 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM nitro blue tetrazolium (NBT), 0.05 mM xanthine, 0.025 unit of xanthine oxidase and 0.05 cm^3 of enzyme extract. The enzyme extract was prepared as described by Dhindsa *et al.* (1981). One unit of SOD activity is equivalent to 50 % decline in the control rate of NBT reduction. GR activity was assayed as described by Hodges *et al.* (1997). The reaction mixture (3 cm^3) contained 50 mM potassium phosphate buffer (pH 7.8), 3 mM EDTA, 0.15 mM NADPH, 0.2 mM oxidised glutathione and 0.3 cm^3 of enzyme extract. Protein concentration was determined according to Bradford (1976).

Statistics: A two-way analysis of variance was used to examine cultivar and water availability treatment effects on all studied parameters using statistical software (*Stat Plus 2007, AnalystSoft*, Washington, USA). Significant different means were separated using the Fisher's Least Significant Difference test (p<0.05).

Results

Water status and gas exchange: Differences in Ψ_L were not significant between the two olive cultivars ('Chemlali', drought-tolerant; and 'Chetoui', drought-sensitive) under well-watered conditions (Table 1). After 30 days of withholding water, 'Chétoui' showed lower values of Ψ_L (–5.5 MPa) compared to 'Chemlali' (–4 MPa). Under well-watered conditions, 'Chemlali' had lower values of CO_2 assimilation and stomatal conductance (Table 1). Moreover, as shown in Table 1, the greatest reduction in g_s (67 %) was observed in 'Chetoui' plants compared to 'Chemlali' (55 %) in response to water deficit. In addition, 'Chemlali' revealed less reduction in A (64 %) than Chétoui (73 %) (Table 1).

Photochemical parameters, evaluated through modulated Chl *a* fluorescence technique, are presented in Table 2. Exposure of leaves to water stress decreased maximal efficiency of PSII photochemistry (F_v/F_m). This decrease was slight for 'Chemlali' (28 %) and substantial for 'Chétoui' (47 %).

Under well-watered conditions, 'Chemlali' had lower values of Φ_{PSII} (Table 2). After 30 days of withholding water Φ_{PSII} decreased by 24 % in 'Chemlali' and by 34 % in 'Chetoui'. In 'Chemlali', decline in Φ_{PSII} arose from a decrease in the photochemical quenching coefficient (q_p) (26 %), while in 'Chetoui' decreases in both q_p (24 %) and F_v'/F_m' (14 %) contributed to the overall reduction in Φ_{PSII}. In this cultivar, the decrease in F_v'/F_m' led to a 33 % increase in NPQ, suggesting a rise in thermal energy dissipation in the antennae. By contrast, F_v'/F_m' and NPQ did not change in 'Chemlali', indicating a lesser degree of thermal dissipation than 'Chetoui'. Moreover, 'Chemlali' showed higher value of NPQ under well-watered conditions (Table 2).

Table 1. Leaf water potential (Ψ_L), stomatal conductance (g_s) and CO_2 assimilation rate (A) in the two studied olive cultivars subjected to water deficit (n = 12, mean ± SD). Means with different letters (a, b, c) are significantly different at p<0.05.

Parameters	Chemlali well watered	water stressed	Chetoui well watered	water stressed
Ψ_L [MPa]	–1.41±0.12a	–4.10±0.11b	–1.51±0.12a	–5.51±0.11c
g_s [mol m^{-2} s^{-1}]	0.38± 0.01b	0.17± 0.01a	0.56± 0.01c	0.18± 0.02a
A [µmol m^{-2} s^{-1}]	17.51±0.66b	6.12±0.67a	24.51±0.65c	6.71±0.56a

Table 2. Photochemical efficiency of PSII (F_v/F_m). quantum yield of PSII electron transport (Φ_{PSII}). efficiency of excitation capture by open PSII reaction centers (F_v'/F_m'). photochemical quenching coefficient (q_p) and non-photochemical quenching (NPQ) of the two studied olive cultivars subjected to water deficit (n = 6, mean ± SD). Means with different letters (a, b, c, d) are significantly different at p<0.05.

Parameters	Chemlali well watered	water stressed	Chetoui well watered	water stressed
F_v/F_m	0.53±0.01c	0.37±0.01b	0.51±0.02c	0.27±0.02a
Φ_{PSII}	0.16±0.03c	0.12±0.01a	0.22±0.01d	0.15±0.01b
F_v'/F_m'	0.29±0.03b	0.29±0.01b	0.28±0.01b	0.24±0.01a
q_p	0.31±0.03a	0.23±0.01b	0.42±0.03c	0.32±0.04a
NPQ	0.53±0.15c	0.54±0.15c	0.34±0.14a	0.45±0.17b

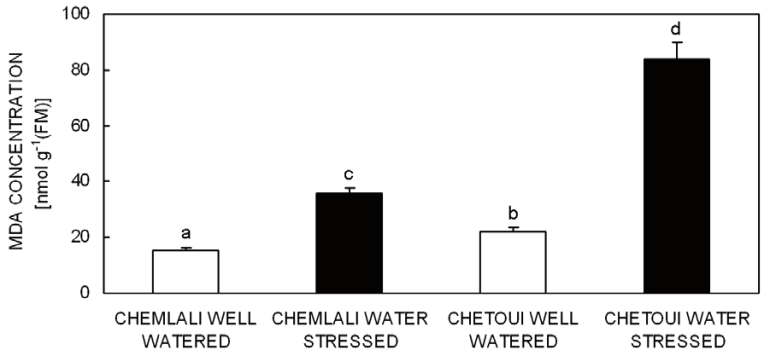

Fig. 1. Malondialdehyde (MDA) levels of leaves in olive leaves of the two cultivars. Results (n=6, mean ± SD) are expressed as nmol g^{-1}(FM) from three independent treatments. Different letters represent significant difference compared to control (p<0.05 by two-way *ANOVA*). Means with different letters (a, b, c) are significantly different at p<0.05.

Effect of drought on lipid peroxidation: Drought-induced increases in lipid peroxidation, as estimated through MDA production, were 140 % in 'Chemlali' against 280 % in 'Chetoui' (Fig. 1).

Effect of drought on antioxidative defense systems: When subjected to water deficit, CAT activities in both olive cultivars were increased compared to control plants (Fig. 2). The increase in CAT activity in 'Chétoui' was higher (50 %) compared to 'Chemlali' (27 %). APX activity in water stressed 'Chemlali' plants was markedly increased compared to control plants (44 %) (Fig. 2), while APX activity in 'Chétoui' was not significantly affected by water stress. Overall, when subjected to water stress, APX activity in 'Chemlali' was higher compared to 'Chétoui' (Fig. 2). Water stress inhibited SOD activity in both cultivars (Fig. 2), however, higher SOD activity was detected in 'Chemlali'. GR activity was enhanced in both cultivars in a similar way. In stressed plants, the increase in GR activity was 55 % and 53 %, in 'Chemlali' and 'Chétoui', respectively (Fig. 2). It should be stressed that enzyme activities were expressed on a total protein basis. Such a basis appeared to be adequate since the overall leaf protein concentration remained largely unaffected under drought conditions. Further, very similar trends in changes in enzyme activities when expressed on a leaf fresh mass basis were also found (data not shown).

Discussion

The largest reductions in Ψ_L and gas exchange which were found in 'Chetoui' compared to 'Chemlali' corroborate our previous results under field conditions (Guerfel *et al.* 2007). In fact, after studying the seasonal changes in gas exchange in leaves of these two olive cultivars under rain-fed, or rain-fed plus a moderate level of a supplemental irrigation, we have found that CO_2 assimilation rates and stomatal conductances showed substantial seasonal variation, but were similar in the two olive cultivars, with higher values during the spring and lower values during the summer. The difference in gas exchange between the two studied olive cultivars under well watered conditions could be explained by the differences in the leaf anatomical characteristics (data not shown). Several experiments have demonstrated some level of genetic variation in gas exchange responses to water stress between olive cultivars (*e.g.* Chartzoulakis *et al*. 1999; Tognetti *et al*. 2002).

The reduction in photosynthetic performances of the stressed olive tree could be considered as a dehydration avoidance mechanism, which minimizes water loss by transpiration, enabling plants to maintain hydration. Since A was strongly decreased under drought conditions then the photochemical energy could not be dissipated through CO_2 assimilation. Therefore, surplus excitation energy must be dissipated in alternative ways. Increases in NPQ occurred only in 'Chetoui' cultivar (Table 2), which should have contributed to its lesser excess energy more

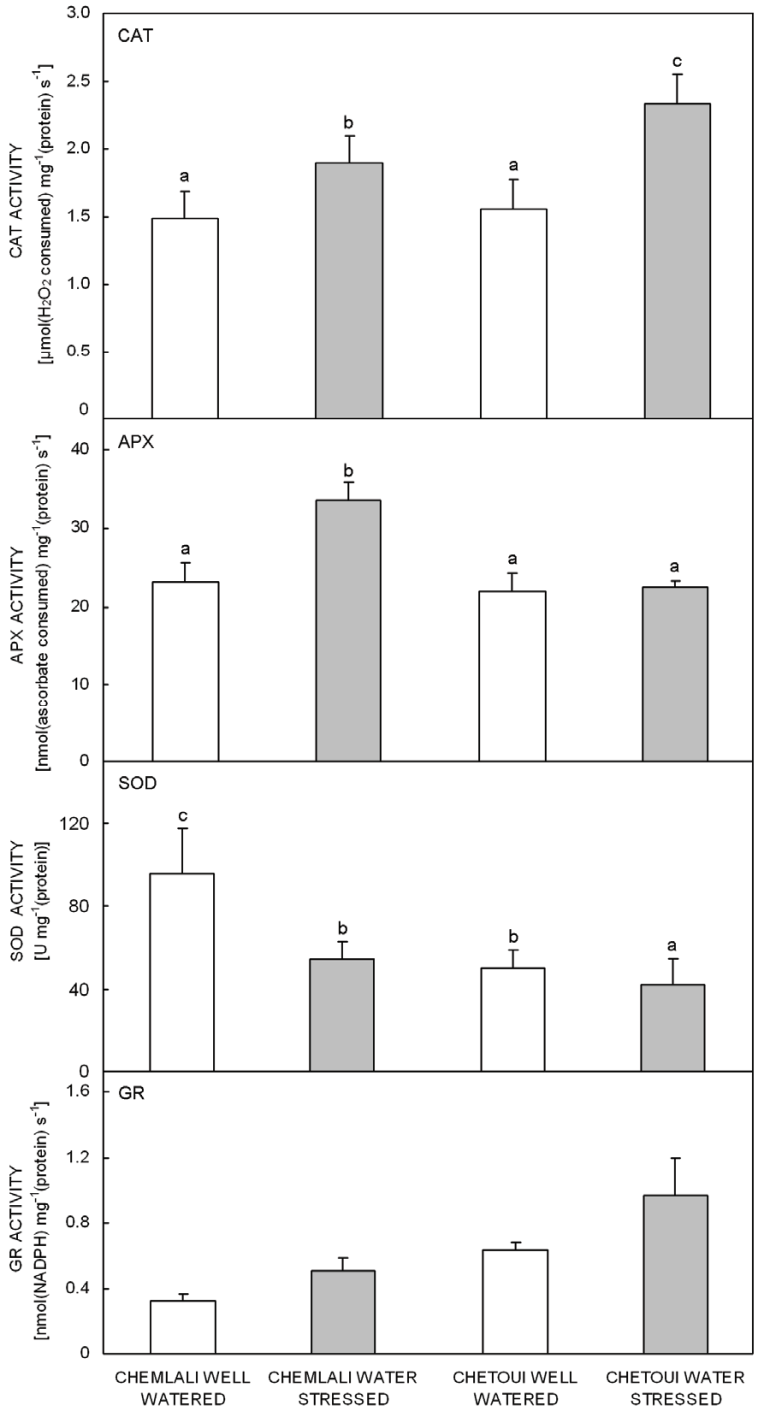

Fig. 2. Changes in the activities of catalase (CAT) [µmol(H_2O_2 consumed) mg^{-1}(protein) s^{-1}], ascorbate peroxidase (APX) [nmol(ascorbate consumed) mg^{-1} (protein) s^{-1}], superoxide dismutase (SOD) [U mg^{-1} (protein)], and glutathione reductase (GR) [nmol(NADPH) s^{-1} mg^{-1}(protein)] in olive leaves of the two cultivars (n=3, mean ± SD)

than 'Chemlali' due to the charge separation at the reaction center of photosystem II. According to Demmig-Adams and Adams (1996), the considerable decrease in F_v'/F_m' could be associated with an increase in energy dissipation in the PSII antennae, which was confirmed by our results in 'Chetoui'. The decreases in F_v/F_m and Φ_{PSII} can be ascribed to a down-regulation of PSII that reflects the protective or regulatory mechanism to avoid photodamage of photosynthetic apparatus (Demmig-Adams 1990; Souza *et al*. 2004). Even under well watered conditions both olive cultivars showed lower values of F_v/F_m which could be explained by the genetic characteristics of the two studied olive cultivars.

Altogether, changes in q_p and in F_v'/F_m' ('Chetoui' only) in response to drought were relatively small, thus allowing maintenance of electron transport through photosystems at considerable rates. Under drought the photosynthetic reduction of O_2, *via* photorespiration, has been proposed to provide photoprotection by acting as a sink for excitation energy in the photosynthetic apparatus.

However, this leads to superoxide and H_2O_2 formation (Cornic 1994; Smirnoff 1995). 'Chetoui' showed higher lipid peroxidation compared to 'Chemlali' under water stress. Moreover, it has been shown that changes in the composition of leaf membrane lipids in 'Chemlali', a drought-tolerant cultivar, revealed a more stability of its cellular membranes to drought stress as compared to the drought susceptible olive cultivar (Guerfel *et al.* 2008).

Several studies have shown lipid peroxidation in water-deficit conditions, including studies with *Olea europaea* L. plants (Sofo *et al.* 2004; Bacelar *et al.* 2006). Lipid peroxidation is a natural metabolic process under normal aerobic conditions and is one of the most investigated AOS actions on membrane structure and function (Blokhina *et al.* 2003). It is widely reported that AOS bring about peroxidation of membrane lipids leading to membrane damage (Shalata and Tal 1998).

If attributes for drought tolerance in 'Chemlali' are indeed linked to less oxidative damages, a relatively greater increase in the capacity of its antioxidant system in relation to that of 'Chetoui' was to be expected.

CAT is the principal harmful oxygen species scavenging enzyme in plants, which decomposes hydrogen peroxide and thus maintains the redox balance during oxidative stress (Pereira *et al*. 2002). CAT activities in both olive cultivars were increased under water deficit conditions compared to control plants (Fig. 2). Under water stress, CAT activity in 'Chetoui' was higher compared to 'Chemlali'. Therefore, it is suspected that CAT may not be directly responsible for the better protection against oxidative injury in 'Chemlali'.

APX is a hydrogen peroxide scavenging enzyme (McKersie and Leshem 1994). APX activity in 44 % water stressed 'Chemlali' plants was markedly increased compared to controls ones (Fig. 2). APX activity 'Chetoui', on the other hand, was not significantly affected by water deficit. Overall, when subjected to water stress, APX activity 'Chemlali' was higher compared to 'Chetoui'. Therefore, APX activity may be a more crucial antioxidant defence than CAT in water-stressed olive tree.

Under mild and/or moderate drought stress some adapted species exhibit increases in activities of antioxidant enzymes, such as SOD and peroxidase (Lima *et al.* 2002, Liang *et al.* 2003). However, severe drought stress may cause damage to cells by inducing active oxygen production or by disrupting the scavenging systems that quench active oxygen and eliminate the detrimental effects (Van Breusegem *et al.* 1998). Water stress inhibited SOD activity in both cultivars (Fig. 2), however, higher SOD activity was detected in 'Chemlali'. Higher SOD activity was therefore associated with better protection against water stress-induced oxidative injury in this olive cultivar. Comparable to our finding, decreased SOD activity was detected in water-stressed olive plants cv. Cobrançosa (Bacelar *et al.* 2007).

GR catalyses the reduction of oxidised glutathione (GSSG) to reduced glutathione (GSH), an important endogenous antioxidant (McKersie and Leshem 1994). GR activity was enhanced in both cultivars in response to water deficit (Fig. 2); however, higher GR activity was detected in 'Chetoui'. Activity of APX, which catalyses the first step of the ascorbate-glutathione cycle, was generally not affected in water-stressed 'Chetoui' plants. Lack of concerted action between GR and APX may account for the higher level of oxidative injury in 'Chetoui' despite its higher GR activity compared to 'Chemlali'. Noctor and Foyer (1998) pointed out that efficient destruction of reactive oxygen species requires the actions of several antioxidant enzymes acting in synchrony.

In conclusion, results fit well with the hypothesis presented. Water deficit caused alterations in the photochemical parameters evaluated herein. This study demonstrated that oxidative stress tolerance differs between the two studied olive cultivars. Compared to 'Chetoui', 'Chemlali' was more tolerant to water stress-induced oxidative damage as evidenced by its lower MDA content. Enzymatic antioxidants responded differently in the two olive cultivars under water stress. Nevertheless, higher APX and SOD activities in 'Chemlali' were associated with its better protection against water stress-induced oxidative damage.

References

Bacelar, E.A., Santos, D. L., Moutinho-Pereira, J. M., Gonçalves, B. C., Ferreira, H.F., Correia, C.M.: Immediate responses and adaptative strategies of three olive cultivars under contrasting water availability regimes: Changes on structure and chemical composition of foliage and oxidative damage. – Plant Sci. **170**: 596-605, 2006.

Bacelar, E.A., Santos, D.L., Moutinho-Pereira, J.M., Gonçalves, B.C., Ferreira, H.F., Correia, C.M.: Physiological behaviour, oxidative damage and antioxidative protection of olive trees grown under different irrigation regimes. – Plant Sci. **292**: 1-12, 2007.

Bailly, C., Benamar A., Corbineau, F., Dôme, D.: Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seed as related to deterioration during accelerated aging. – Physiol. Plant. **97**: 104-110, 1996.

Beauchamp, C., Fridovich, I.: Superoxide dismutase: Improved assay and an assay applicable to acrylamide gels. – Anal. Biochem. **44**: 276-287, 1971.

Ben Temime, S., Campeol, E., Cioni P.L., Daoud, D., Zarrouk, M.: Volatile compounds from Chétoui olive oil and variations induced by growing area. – Food Chem. **99**: 315-325, 2006.

Blokhina, O., Virolainen, E., Fagerstedt, K.V.: Antioxidants, oxidative damage and oxygen deprivation stress: a review. – Ann. Bot. **91**: 179-194, 2003.

Bradford, M.M.: A rapid and sensitive method for the quantita-

tion of microgram quantities of protein utilizing the principle of protein-dye binding. – Ann. Biochem. **72**: 248-254, 1976.

Chartzoulakis, K., Patakas, A., Bosabalidis, A.M.: Changes in water relations, photosynthesis and leaf anatomy induced by intermittent drought in two olive cultivars. – Environ. Exp. Bot. **42**: 113-120, 1999.

Cornic, G.: Drought stress and high light effects on leaf photosynthesis. – In: Baker, N.R., Bowyer, J.R. (ed.): Photoinhibition of Photosynthesis from Molecular Mechanisms to the Field. Pp. 297-313. Bios Sci. Publ., Oxford 1994.

Demmig-Adams, B.: Carotenoids and photoprotection in plants. A role for xanthophyll zeaxanthin. – Biochem. Biophys. Acta **1020**: 1-24, 1990.

Demmig-Adams, B., Adams, W.W., III.: Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. – Planta **198**: 460-470, 1996.

Dhindsa, R.S., Plumd-Dhindsa, P., Thorpe, T.A.: Leaf senescence - correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide-dismutase and catalase. – J. Exp. Bot. **32**: 93-101, 1981.

Fadzilla, N.M., Finch, R.P., Burdon, R.H.: Salinity, oxidative stress and antioxidant responses in shoot cultures of rice. – J. Exp. Bot. **48**: 325-331, 1997.

Foyer, C.H., Noctor, G.: Oxygen processing in photosynthesis: regulation and signaling. – New Phytol. **146**: 359-388, 2000.

Genty, B., Briantais, J.M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence. – Biochim. Biophys. Acta **990**: 87-92,1989.

Guerfel, M., Baccouri, B., Boujnah, D., Zarrouk, M.: Seasonal changes in water relations and gas exchange in leaves of two Tunisian olive (*Olea europaea* L.) cultivars under water deficit. – J. Hortic. Sci. **82**: 721-726, 2007.

Guerfel, M., Baccouri, O., Boujnah, D., Zarrouk, M.: Changes in lipid composition, water relations and gas exchange in leaves of two young 'Chemlali' and 'Chetoui' olive trees in response to water stress. – Plant Soil. **311**: 121-129, 2008.

Heath, R.L., Packer, L.: Photoperoxidation in isolated chloroplasts. I. Kinetic and stoichiometry of fatty acid peroxidation. – Arch. Biochem. Biophys. **125**: 189-198, 1968.

Hodges, D.M., Andrews, CJ., Johnson, DA., Hamilton, R.I.: Antioxidant enzyme responses to chilling stress in differentially sensitive inbred maize lines. – J. Exp. Bot. **48**: 1105-1113, 1997.

IOOC, International Olive Oil Council. Olive oil exportations. http://www.internationaloliveoil.org, 2004.

Kozlowski, T.T., Pallardy, S.G.: Acclimation and adaptive responses of woody plants to environmental stresses. – Bot. Rev. **68**: 270-334, 2002.

Krause, G.H, Weis, E.: Chlorophyll fluorescence and photosynthesis: The basics. – Annu. Rev. Plant Physiol. **42**: 313-349, 1991.

Liang, Y.C., Hu, F., Yang, M.C., Yu, J.H.: Antioxidative defenses and water deficit-induced oxidative damage in rice (*Oryza sativa* L.) growing on non-flooded paddy soils with ground mulching. – Plant Soil **257**: 407-416, 2003.

Lima, A.L.S, DaMatta, F.M., Pinheiro, H.A., Totola, M.R., Loureiro, M.E., Photochemical responses and oxidative stress in two clones of *Coffea canephora* under water deficit conditions. – Environ. Exp. Bot. **47**: 239-247, 2002.

Lo Gullo, A.M., Salleo, S.: Different strategies of drought resistance in three Mediterranean sclerophyllous trees growing in the same environmental-conditions. – New Phytol. **108**: 267-276, 1988.

Marnett, LJ.: Lipid peroxidation – DNA damage by malondialdehyde. Mut. Res. **424**: 83-95, 1999.

McKersie, B.D., Leshem, Y.Y.: Stress and Stress Coping in Cultivated Plants. – Kluwer Academic Publ., Dordrecht 1994.

Nakano, K. Asada,K: Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. – Plant Cell Physiol. **22**: 867-880,1981.

Navabpour, S., Morris, K., Allen R, Harrison, E., Mackerness, S.A.H., Buchanan-Wollaston V.: Expression of senescence-enhanced genes in response to oxidative stress. – J. Exp. Bot. **54**: 2285-2292, 2003.

Noctor, G., Foyer, C.H: Ascorbate and glutathione: Keeping active oxygen under control. – Annu. Rev. Plant Physiol. **49**: 249-279, 1998.

Nogués, S., N.R. Baker, Effects of drought on photosynthesis in Mediterranean plants grown under enhanced UV-B radiation. – J. Exp. Bot. **51**: 1309-1317, 2000.

Pereira, G.I.G., Molina S.M.G., Azevedo R.A.: Activity of antioxidant enzymes in response to cadmium in *Crotalaria junce*. – Seedling and Soil **239**: 123-132, 2002.

Shalata, A., Tal, M.: The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon penellii*. – Physiol. Plant. **104**: 169-174, 1998.

Smirnoff, N.: Tansley review. 52. The role of active oxygen in the response of plants to water deficit and desiccation. – New Phytol. **125**: 27-58, 1993.

Smirnoff, N.: Antioxidant systems and plant response to the environment. – In: Smirnoff, N. (ed.), Environment and Plant Metabolism. Flexibility and Acclimation. Pp. 217-243. Bios Sci. Publ., Oxford, 1995.

Smirnoff, N.: Plant resistance to environmental stress. – Current Opinion in Biotechnology **9**: 214-219, 1998.

Sofo, A., Dichio, B., Xiloyannis, C., Masia, A.: Effects of different irradiance levels on some antioxidant enzymes and on malondialdehyde content during rewatering in olive tree. – Plant Sci. **166**: 293-302, 2004.

Souza, R.P., Machado, E.C., Silva, J.A.B., Lagôa, A.M.M.A, Silveira, J.A.G.: Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. – Environ. Exp. Bot. **51**: 45-56, 2004.

Tognetti, R., Costagli, G., Minnocci, A., Gucci, R.: Stomatal behaviour and water use efficiency in two cultivars of *Olea europaea* L. – Agric. Med. **132**: 90-97, 2002.

Van Breusegem, F., Van Montagu, M., Inze, D., Engineering stress tolerance in maize. – Outlook Agr. **27**: 115-12, 1998

Zimmermann, P., Zentgraf, U.: The correlation between oxidative stress and leaf senescence during plant development. – Cell. Mol. Biol. Lett. **10**: 515-534, 2005.