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ARTICLE

Exogenous Proline Effects on Photosynthetic Performance and Antioxidant Defense System of Young Olive Tree

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The ability of exogenous compatible solutes, such as proline, to counteract salt inhibitory effects in olive plants (*Olea europaea* L. cv. Chemlali) was investigated. Two-year-old olive trees were subjected to different saline water irrigation levels supplied or not with exogenous proline. Leaf water relations (relative water content, water potential), photosynthetic activity, and leaf chlorophyll content decreased under either saline water level. The proline supplement mitigated the reduction of growth and photosynthetic activity under salt stress, and the mitigating effect of proline was different among treatments. The increment rate of leaf relative water content (RWC) in the presence of 25 and 50 mM proline was 4.45 and 6.67%, respectively, in comparison to values recorded in SS1-treated plants (plants irrigated with water containing 100 mM NaCl). In SS2 (200 mM NaCl) plus proline-treated plants, this increase was 1.14 times for 25 mM proline and 1.19 times for 50 mM proline higher than those recorded in severe salt stress treatment (SS2). In response to salt stress, Chemlali olive plants seem to activate a complex antioxidative defense system that was displayed via the increase of activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) and the decrease of polyphenol oxidase (PPO) under either salt stress treatment. The exogenous application of proline improved the antioxidative enzyme activities of salt-stressed olive plants. Indeed, in young or old leaf tissues, the highest levels of these antioxidant enzymes activities were recorded in (SS2 + P2)-treated plants (plants irrigated with water containing 200 mM NaCl plus 50 mM proline). In young leaves, this increase was 2.11, 2.96, and 2.76 times, respectively, for SOD, APX, and CAT enzyme activities in comparison to their respective activities in control plants (nonstressed plants irrigated with fresh water). In old leaves, this increase was 2, 2.41, and 2.48 times, respectively, for the various enzymes. If compared to high water salinity-treated plants (SS2), this increase was 1.1, 1.3, and 1.4 times in young leaves, respectively, for SOD, APX, and CAT activities. From these results, the proline supplements seem to improve olive salt tolerance by amelioration of some antioxidative enzyme activities, photosynthetic activity, and, so, plant growth and the preservation of a suitable plant water status under salinity conditions. More to the point, the decrease of soluble sugars contents in proline treated-plants revealed the important osmoprotectant effect played by the added proline in such a way that limited the need of salt-stressed plants for soluble sugars synthesis.

KEYWORDS: *Olea europaea*; salt stress; antioxidant defense system; proline supplement; water relations; photosynthetic performance

INTRODUCTION

One of the important biochemical changes occurring in plants subjected to biotic or abiotic stresses is the production of reactive oxygen species (ROS) (*1*). Under stressed conditions, the accompanying suppression of photosynthesis is attributed mainly to stomatal closure (*2*, *3*). However, as the stress progresses, photosynthetic CO_2 fixation may be limited more directly by biochemical constraints (*4*, *5*). This limitation causes the over-reduction of the photosynthetic electron chain. This excess of reducing power redirects photon energy into processes that favor the production of ROS.

The involvement of antioxidants in protection against oxidative stress has been demonstrated using transgenic plants with enhanced levels of certain antioxidative enzymes (*6*). According to Smirnoff (*7*), low water availability often is associated with increased levels of ROS such as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$), and singlet oxygen ($^1O_2$). ROS are highly reactive and can seriously disrupt the

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normal metabolism of the plant in the absence of any protective mechanism.

Chloroplasts, mitochondria, and peroxisomes are the major intracellular generators of ROS. In these organelles, ROS can be generated by direct transfer of excitation energy from chlorophyll to produce singlet oxygen or by univalent oxygen reduction of photosystem I (*5*).

Plants use enzymatic and nonenzymatic antioxidative defense mechanisms to scavenge ROS. According to Kuwabara and Katoh (*8*), the enzymatic system includes superoxide dismutase (SOD; EC 1.15.1.1), which is the major scavenger of superoxide. With catalase (CAT; EC 1.11.1.6), SOD catalyzes the dismutation of superoxide to H_2O_2 and O_2. The ascorbate peroxidases (APX; EC 1.11.1.11) allow the detoxification of H_2O_2. Polyphenol oxidase (PPO; EC 1.30.3.1), involved in the metabolism of phenols, oxidizes ortho-diphenolic substrates to *o*-quinones. Several reports have signaled changes in the content and activity of different components of the antioxidant defense system in plant responses to salt stress (*9*−*11*). Within a species, salt-tolerant cultivars should have a better antioxidative system for effective avoidance of oxidative damage and removal of ROS (*12*).

The accumulation of osmolytes such as proline and sugars is a well-known adaptive mechanism in plants against stressed conditions (*5*, *11*, *13*−*15*). It has also been suggested that proline accumulation can serve as a selection criterion for the tolerance of most species to stressed conditions (*16*, *17*). The same authors have stated that these compounds, in addition to sugars, act as osmolytes facilitating the retention of water in the cytoplasm. Moreover, proline has a protective action that prevents membrane damage and protein denaturation during severe drought stress (*18*). However, the improvement of stress tolerance due to proline accumulation is species-dependent (*17*).

The protecting roles of proline in plants under salinity conditions already have been reported in several species (*19*−*21*). Indeed, with melon plants, Kaya et al. (*21*) have demonstrated that exogenous proline mitigated the detrimental effects of salt stress. Similarly, Okuma et al. (*22*) have signaled that proline mitigated the inhibition of the growth of tobacco cells and reduced the oxidation of lipid membranes under saline conditions. Likewise, Khedr et al. (*19*) reported that severe salt stress inhibited the activities of antioxidant enzymes catalase and peroxidase in sea daffodil plants, but the activities of these enzymes were significantly higher in the presence of proline than in its absence. It was expected that up-regulation of the components of the antioxidant system offered by proline protects plants against NaCl-induced oxidative damage. Recently, Hoque et al. (*23*) showed that proline improves salt tolerance in tobacco plants by increasing the activity of enzymes involved in the antioxidant defense system.

Similarly, it has been reported that proline protects higher plants against osmotic stresses not only by adjusting osmotic pressure but also by stabilizing many functional units such as complex II electron transport, membranes, and proteins and enzymes such as Rubisco (*24*), by protecting the photosynthetic apparatus (*4*), by functioning as an oxygen radical scavenger (*25*), and by displaying an antioxidant activity (*22*).

The specific involvement of proline in tolerance to stress, the inconsistency of the response to its exogenous application, the fact that it was mainly tested on bacteria, calli, or isolated cell lines or from foliar application, and the socioeconomic importance of cultivated olives were the leading decisive factors to carry out this research. In fact, there is no study, to our best knowledge, on the effects of exogenous proline on olive tree responses to salt stress, and very little is known about the linkage between proline level and antioxidants in olive tree under saline conditions, on the other hand. Therefore, it was of particular interest to investigate the effects of exogenous supplementation of proline on photosynthetic performance and antioxidant defense system of olive cv. Chemlali conducted under different water salinity levels. A second objective of our study was to assess the effectiveness of supplemental proline to mitigate the adverse effects of salinity stress in young olive plants with respect to some physiological and biochemical aspects.

MATERIALS AND METHODS

Plant Material and Treatments. Trials were conducted at the Olive Tree Institute of Sfax, Tunisia (34° 43′ N; 10° 41′ E), from September 2008 to May 2009. Uniform 2-year-old self-rooted olive trees (*Olea europaea* L. cv Chemlali) were transplanted into 10 L pots filled with sand and perlite (3:1; v/v). The pots were kept under ambient environmental conditions with natural sunlight and temperature and were covered with plastic film and aluminum foil to reduce evaporation from the soil surface and to minimize temperature increases inside the containers.

During the first 6 months of the trial period (September 2008−February 2009), all olive plants were irrigated with half-strength Hoagland solution. When plants developed shoots of 15−25 cm length, they were subjected to the following treatments: (i) control (CP), plants receiving nutrient solution; (ii) moderate salinity (SS1), plants receiving nutrient solution plus 100 mM NaCl; (iii) moderate salinity plus 25 mM proline (SS1 + P1); (iv) moderate salinity plus 50 mM proline (SS1 + P2); (v) high salinity (SS2), plants receiving nutrient solution plus 200 mM NaCl; (vi) high salinity plus 25 mM proline (SS2 + P1); and (vii) high salinity plus 50 mM proline (SS2 + P2). The amount of water used for irrigation daily during the experimental period for the different treatments was equal to the amount lost by transpiration and determined as described by Ben Ahmed et al. (*15*). Each treatment consisted of three blocks of four plants each (84 plants in total).

Leaf Water Relations, Shoot Growth, and Gas Exchange Measurements. At the end of the experiment, measurements of leaf relative water content (RWC) were determined using the equation

$$\mathrm{RWC}\ (\%) = 100\times(\mathrm{Fw}-\mathrm{Dw})/(\mathrm{Tw}-\mathrm{Dw})$$

where Fw is the fresh weight, Dw the dry weight, and Tw the turgid weight of leaf samples. Leaves were excised before dawn, weighed fresh (Fw), and placed in distilled water to rehydrate in the dark for 24 h. The following morning, leaf turgid weight (Tw) was measured, and then leaves were dried at 80 °C for 48 h and dry weight (Dw) was determined.

At the beginning of the experiment, two shoots per plant × three plants per treatment were selected. The initial length of each shoot was measured. The shoot elongation rate (SER) was determined two times per month as the difference between final and initial measurement for each time.

Using a portable gas exchange system (Li-Cor Inc. 6200, Lincoln, NE) (*11*), gas exchange measurements were taken from 10:00 to 11:00 a.m. on well-exposed and fully expanded leaves from the median part of the shoot from three plants per treatment.

At the end of the experiment, leaf water potential (Ψ_{LW}) was measured on the same leaves used for gas exchange parameters by a Scholander pressure chamber (pms-1000, Corvallis, OR) (*11*). We took care to minimize water loss during the transfer of the leaf to the chamber by enclosing it in a black plastic bag immediately after excision.

Measurements of gas exchange parameters were also taken on young leaves (fully expanded leaves that developed soon after the onset of the different treatments) and old leaves (fully expanded leaves that developed soon before the imposition of different treatments).

Proline and Soluble Sugars Content Determinations. Leaf and root samples used for proline content determination were frozen immediately in liquid nitrogen. Free proline was determined according to the method of Bates et al. (*26*). A total of 0.5 g of frozen powder was mixed with a 5.0 mL portion of 3% (w/v) sulfosalicylic acid in covered glass tubes and boiled in a water bath at 100 °C. The mixture was centrifuged at 2000*g* for 5 min at 25 °C. A 200 μL portion of the extract was mixed with 400 mL of distilled water and 20 mL of the reagent mixture (30 mL of glacial acetic acid, 20 mL of distilled water, and 0.5 g of ninhydrin) and boiled at 100 °C

Table 1. Relative Water Content (RWC), Leaf Water Potential (Ψ_{LW}), Shoot Elongation Rate (SER), Net Photosynthesis (Pn), Stomatal Conductance (Gs), and Transpiration Rate (*E*) in Young Chemlali Olive Plants Subjected to Different Treatments[a]

treatment	RWC (%)	Ψ_{LW} (MPa)	SER (cm month^{-1})	Pn (μmol of CO_2 m^{-2} s^{-1})	Gs (mmol of H_2O m^{-2} s^{-1})	*E* (mmol of H_2O m^{-2} s^{-1})
CP	95.2 ± 3.1 a	−1.2 ± 0.1 a	3.4 ± 0.1 a	21.3 ± 3.5 a	192.4 ± 4.2 a	12.3 ± 2.0 a
SS1	85.4 ± 3.6 b	−2.5 ± 0.6 b	1.6 ± 0.2 b	12.1 ± 2.2 bc	131.6 ± 2.6 b	6.4 ± 1.0 b
SS1 + P1	89.2 ± 2.8 c	−3.1 ± 0.5 b	2.1 ± 0.1 c	14.5 ± 1.4 b	145.6 ± 4.4 c	7.2 ± 1.5 b
SS1 + P2	91.1 ± 3.7 c	−3.6 ± 0.6 b	2.4 ± 0.1 c	15.6 ± 2.1 b	153.7 ± 3.3 c	8.4 ± 1.5 b
SS2	75.5 ± 2.5 d	−2.9 ± 0.3 c	1.2 ± 0.2 b	10.8 ± 1.3 c	118.7 ± 4.7 d	4.2 ± 0.9 c
SS2 + P1	86.6 ± 2.2 b	−3.7 ± 0.4 b	1.9 ± 0.2 c	12.3 ± 1.3 c	132.5 ± 3.3 b	6.5 ± 1.5 b
SS2 + P2	90.4 ± 3.0 bc	−4.2 ± 0.4 b	2.3 ± 0.2 c	14.7 ± 2.0 b	144.8 ± 3.8 c	7.7 ± 2.5 b

[a] Values represent means of at least three replications per treatment ± SE. Means within each column followed by different letters are significantly different (p = 0.05).

for 1 h. After cooling the mixture, we added 6.0 mL of toluene. The chromophore-containing toluene was separated, and absorption at 520 nm was read, using toluene as a blank. Proline concentration was calculated using L-proline for the standard curve.

Soluble sugar content was determined according to the method of Robyt and White (*27*). A total of 0.3 g of fresh tissue (leaf + root) was mixed with a 5.0 mL portion of methanol (80%) in covered glass tubes and boiled at 70 °C for 30 min. After the mixture had cooled, a 1.0 mL portion of the extract was mixed with 1.0 mL of phenol and 5.0 mL of concentrated sulfuric acid. After agitation and cooling of the reagent mixture, A_{640} was read using methanol as a blank. Soluble sugar concentration was calculated using glucose solutions to develop a standard curve.

Total Chlorophyll and Carotenoid Concentrations. At the end of the experiment, leaf disks for each treatment were taken from three fully expanded leaves of plants with comparable leaf water potentials. Leaf sections were ground in 80% acetone. Total chlorophyll (a + b) and carotenoid concentrations were determined according to the method of Arnon (*28*).

Enzyme Determinations. At the end of the experiment, plants used for physiological measurements were removed carefully from the soil in the early morning. Leaf samples collected for enzyme activity determination were treated as were the young and old leaves described above. The roots were separated carefully, washed with distilled water, and divided into two groups: "thin roots", with a diameter < 3 mm, and "medium roots", with a diameter between 3 and 8 mm. Frozen leaf and root samples were ground to a fine powder in liquid nitrogen and extracted with ice-cold 50 nM phosphate buffer (pH 7.0). The extracts were centrifuged at 4 °C for 30 min at 20000*g*, and the resulting supernatants were used for enzyme activity estimations. The total SOD, APX, CAT, and PPO activities were determined as described by Ben Ahmed et al. (*11*). For the different measurements, at least, three replicates were used for each field and laboratory test.

Statistical Analysis. Statistical analyses were performed using the statistical software package SPSS 10 for Windows (*29*). Treatment means were compared using the least significant difference (LSD) test at p < 0.05 (*21*), and plant tissues means were compared using Tukey's test calculated at the $p \leq 0.05$ level (*29*).

RESULTS

Physiological Parameters. Table 1 shows changes in relative water content, leaf water potential, shoot elongation rates, net photosynthesis, stomatal conductance, and transpiration rates in olive plants subjected to the different treatments. The irrigation with saline water at either salinity level resulted in a significant decrease of leaf water relations characteristics (RWC and Ψ_{LW}) and photosynthetic activity, in comparison to plants irrigated with fresh water (control plants). The alterations of photosynthetic activity and water relation characteristics in salt-stressed olive plants were accompanied by the reduction of shoot elongation rates.

The largest reduction in RWC was recorded in SS2-treated plants, being 11 and 21% of CP values for SS1 and SS2 (**Table 1**). Differences in RWC and Ψ_{LW} between both salt stress treatments were statistically significant (p = 0.05). At the end of the experiment, Ψ_{LW} in SS1 and SS2 treatments was 2.08 and 2.41 times lower, in comparison to CP, respectively. Both leaf water relations characteristics (RWC and Ψ_{LW}) were improved significantly in the presence of proline but at different levels among treatments. The increment rate of RWC in the presence of 25 and 50 mM proline was 4.45 and 6.67%, respectively, in comparison to values recorded in SS1-treated plants. In SS2 + proline-treated plants, this increase was 1.14 and 1.19 times higher than those recorded in severe salt stress treatment (SS2), respectively. Nevertheless, these values remained lower than those registered in CP. Similarly, the increment of proline medium supplement was accompanied by a decrease of Ψ_{LW} values.

Table 2. Net Photosynthesis in Young and Old Leaves from Chemlali Olive Plants Subjected to Different Treatments[a]

	net photosynthesis (Pn, μmol of CO_2 m^{-2} s^{-1})	
treatment	young leaves	old leaves
CP	26.67 ± 0.25 a*	19.50 ± 0.18 b*
SS1	13.34 ± 0.26 b	10.42 ± 0.24 b
SS1 + P1	15.78 ± 0.24 bc*	12.27 ± 0.19 b*
SS1 + P2	17.83 ± 0.24 c*	14.58 ± 0.28 b*
SS2	10.56 ± 0.19 d*	7.69 ± 0.27 b*
SS2 + P1	14.67 ± 0.25 d*	10.45 ± 0.22 b*
SS2 + P2	16.39 ± 0.26 c*	12.96 ± 0.23 b*

[a] Values represent means of at least three replications per treatment ± SE. Different letters indicate significant differences (p = 0.05) between treatments within each leaf type treated separately. An asterisk indicates significant difference between young and old leaves within each treatment treated separately ($p \leq$ 0.05, Tukey's test).

The improvement of better plant water status in proline medium was accompanied by increased photosynthetic activity and shoot elongation rates. Furthermore, the higher the proline medium was, the more important the net photosynthesis and SER were. The increase of Pn in both proline media was accompanied with increased stomatal conductance and transpiration rates, but at different extents among treatments (**Table 1**). Besides, the improved photosynthetic activity in both proline media was different between young and old leaves (**Table 2**). Under either NaCl treatment, young leaves showed higher photosynthetic activity than old ones. Similarly, the increment rate of Pn in either proline medium was more important in young than in old leaves. Furthermore, under both water salinity treatments, proline supply at 50 mM seems to be more effective in alleviating salt stress effects than proline supply at 25 mM. Indeed, net photosynthetic rates, plant water status, and shoot elongation rates were more enhanced in the presence of 50 mM proline than in that of 25 mM proline medium.

Proline, Soluble Sugars, and Photosynthetic Pigments Contents. NaCl stress significantly increased the content of proline and soluble sugars in either leaves or roots of Chemlali olive plants, but at different levels among plant tissues and salinity stress levels (**Table 3**). Leaves of both salt stress treated plants (SS1 + SS2) accumulated higher proline than roots. Salt-stressed leaves

Table 3. Proline and Soluble Sugars Contents in Leaves and Roots from Young Chemlali Olive Plants Subjected to Different Treatments[a]

	proline (μmol mg^{-1} of Fw)		soluble sugars (μmol mg^{-1} of Fw)	
treatment	leaves	roots	leaves	roots
CP	0.62 ± 0.09 a	0.47 ± 0.10 a	0.85 ± 0.10 a	0.27 ± 0.10 a
SS1	2.56 ± 0.10 b	1.45 ± 0.11 b	1.78 ± 0.11 b	0.65 ± 0.11 bd
SS1 + P1	2.78 ± 0.11 b	1.87 ± 0.10 b	1.14 ± 0.07 b	0.41 ± 0.11 bc
SS1 + P2	2.89 ± 0.09 b	2.06 ± 0.12 b	1.09 ± 0.09 b	0.32 ± 0.11 ac
SS2	3.95 ± 0.11 bc	2.16 ± 0.11 bc	2.45 ± 0.09 c	0.88 ± 0.12 d
SS2 + P1	4.67 ± 0.09 c	3.37 ± 0.16 c	1.62 ± 0.05 bc	0.62 ± 0.14 b
SS2 + P2	4.99 ± 0.09 c	3.92 ± 0.19 cd	1.43 ± 0.03 bc	0.54 ± 0.13 b

[a] Values represent means of three measurements (± SE). Different letters indicate significant differences (p = 0.05) between treatments within each plant organelle treated separately.

Table 4. Total Chlorophyll ($a + b$) and Carotenoid Contents and Chlorophyll/Carotenoid Ratio from Young Chemlali Olive Plants Subjected to Different Treatments[a]

treatment	chlorophyll ($a + b$) (mg/g of Dw)	carotenoids (mg/g of Dw)	chlorophyll/carotenoids
CP	9.76 ± 0.09 a	5.08 ± 0.10 a	1.92
SS1	4.17 ± 0.11 b	3.16 ± 0.10 bc	1.32
SS1 + P1	5.62 ± 0.09 bc	3.55 ± 0.12 bc	1.58
SS1 + P2	6.84 ± 0.11 c	3.89 ± 0.11 b	1.75
SS2	2.86 ± 0.09 d	2.15 ± 0.16 c	1.33
SS2 + P1	4.65 ± 0.09 b	2.96 ± 0.19 bc	1.57
SS2 + P2	6.12 ± 0.11 c	3.42 ± 0.20 bc	1.79

[a] Values represent the means of three samples (± SE). Means within each column followed by different letters are significant different (p = 0.05).

accumulated proline at almost 4 and 6 times, respectively, under SS1 and SS2 treatments, than control plants. The proline supplement increased significantly the proline content in olive tissues. The proline content reached 4.99 and 3.92 μmol/mg of Fw, respectively, in leaves and roots of plants conducted under high water salinity plus 50 mM proline medium. As well, the higher the proline medium was, the more important the proline content was. On the other hand, the proline medium led to the decrease of soluble sugars contents in leaves or roots of olive plants grown under either water salinity treatment.

The data in **Table 4** show that 100 and 200 mM NaCl treatments caused a significant decrease of chlorophyll ($a + b$) or carotenoid content, in comparison to control plants. Nevertheless, the decrease of the chlorophyll/carotenoid ratio in either water salinity medium was not significant. The externally supplied proline increased the photosynthetic pigments contents, but at different levels among the proline media (**Table 4**). The highest levels of chlorophyll and carotenoid contents were recorded in (SS1 + P2)-treated plants. These values were 6.84 and 3.89 mg/g of Dw, respectively, for chlorophyll ($a + b$) and carotenoid contents. Although not statistically significant, the chlorophyll ($a + b$)/carotenoid ratio increased under either proline medium.

Antioxidant Enzyme Activities. SOD, APX, CAT, and PPO activities of young or old leaves of Chemlali olive plants grown under the different treatments are shown in **Table 5**. The results show that SOD, APX, and CAT activities increased significantly in water salinity treated plants (SS1 + SS2), compared to values recorded in control plants. This increase was reinforced by exogenous proline supplement, but to different extents according to the applied proline medium level. Indeed, in both leaf tissues, the highest levels of these antioxidant enzyme activities were recorded in (SS2 + P2)-treated plants. In young leaves, this increase was 2.11, 2.96, and 2.76 times, respectively, for SOD, APX, and CAT enzyme activities in comparison to values recorded in control plants. In old leaves, this increase was 2, 2.41, and 2.48 times, respectively. In comparison to high water salinity-treated plants (SS2), the increases of SOD, APX, and CAT activities in young leaves were 1.1, 1.3, and 1.4 times, respectively. In old leaves, these increment rates were 1.1, 1.4, and 1.4 times, respectively, for the various enzymes.

The variation of the different enzyme activities in thin and medium roots of the different treatments showed the same pattern as in young and old leaves (**Table 6**). In thin roots of (SS2 + P2)-treated plants, the SOD, APX, and CAT activity increases were 1.44, 1.97, and 1.5 times, respectively, in comparison to high water salinity-treated plants (SS2). In medium roots, this increase was 1.4, 2.1, and 1.4, respectively. From these results, it appears that the higher the proline supply was, the more important the activities of antioxidant enzymes were.

The PPO was the only enzyme clearly suppressed by both saline water levels, and a significant decline of PPO activity was noted in all tissues of the different treatments (**Tables 5** and **6**). Furthermore, for all analyzed tissues, the decrease of PPO activity was more important in salt-stressed plants supplied with exogenous proline, but at different levels among plant tissues and treatments. The relative reduction of PPO activity in leaves of SS1-treated plants, compared to well-irrigated ones (CP), was 20 and 26%, respectively, for young and old leaves. In moderate water salinity plus 50 mM proline treatment (SS1 + P2), the decrease of PPO activity was 25 and 15%, respectively, in young and old leaves, in comparison to SS1 treatment. In (SS2 + P2)-treated plants, this decrease was 18 and 14%, respectively, if compared to SS2-treated plants. In root tissues, for all treatments, PPO activity of medium roots was higher than that of thin roots.

DISCUSSION

Both water salinity levels (SS1 and SS2) significantly affected leaf water relations and photosynthetic performances of olive plants. The decrease in RWC and Ψ_{LW} indicated a loss of turgor that resulted in limited water availability for cell expansion processes. Nevertheless, no toxicity symptoms (leaf necrosis, leaf drop) were recorded. Previous papers have correlated the decrease of net photosynthetic rate in salt-stressed plants mainly to lower stomatal conductance (Gs) and to salt ion accumulation in the different plant tissues (*30*). Our data confirmed this hypothesis, whereas nonstomatal limitation on photosynthetic activity might have occurred also in leaves of either water salinity treated-plants. For instance, the lowered leaf chlorophyll content in SS1- and SS2-treated plants might have contributed to the decrease of net photosynthesis (Pn). Nevertheless, faced with such damage, the Chemlali olive plants tend to maintain higher photosynthetic rates in young leaves than in old leaves, in such a way to preserve their growth and development even in low rates. In fact, the differential pattern of water status and photosynthetic activity between young and old leaves of saline water treated plants may result from higher RWC in young tissues and high salt ion accumulation in the old ones, actions that seem to play a protective role for the young ones against salt ion damage as reported previously (*30*, *31*).

The inhibition of photosynthetic activity by high water salinity would induce oxidative stress resulting from the imbalance between light capture and its utilization (*32*). In this experiment, the decrease of leaf chlorophyll content and the increase of SOD, CAT, and APX activities in leaves of stressed plants exhibit the oxidative stress induced by NaCl salinity stress and suggest that the antioxidant defense system would play an important role in the salt tolerance of the olive tree. Either young or old leaves of NaCl-treated olive plants showed a considerable increase of APX

Table 5. Antioxidative Enzyme Activities of Young and Old Leaves from Chemlali Olive Plants Subjected to Different Treatments[a]

	enzyme activity (units mg^{-1} of Dw)							
	young leaves				old leaves			
treatment	SOD	APX	CAT	PPO	SOD	APX	CAT	PPO
CP	14.42 ± 1.22 a	3.81 ± 0.71 a	5.53 ± 1.01 a	32.54 ± 3.02 a	15.62 ± 1.21 a	4.32 ± 0.95 a	5.61 ± 0.99 a	30.20 ± 3.01 a
SS1	24.13 ± 2.36 bc	5.53 ± 0.99 b	8.27 ± 1.26 b	26.19 ± 3.14 b*	25.38 ± 2.45 b	6.31 ± 1.02 b	8.73 ± 1.03 b	22.51 ± 2.95 b*
SS1 + P1	23.61 ± 2.56 b*	6.37 ± 1.21 bc	10.26 ± 1.37 bc	22.37 ± 2.11 c	27.29 ± 2.38 bc*	7.83 ± 1.12 b	10.44 ± 1.25 b	20.42 ± 2.97 bc
SS1 + P2	25.13 ± 2.68 c*	7.71 ± 1.13 cd*	11.47 ± 2.31 c	19.82 ± 2.17 d	28.46 ± 2.89 cd*	9.22 ± 1.34 c*	12.32 ± 1.76 c	19.34 ± 1.79 c
SS2	27.61 ± 2.61 d	8.46 ± 1.51 de	10.81 ± 2.44 c	22.37 ± 2.89 c	27.72 ± 2.18 c	7.41 ± 1.23 b	9.61 ± 1.25 b	20.14 ± 2.12 c
SS2 + P1	28.73 ± 2.03 d	9.71 ± 1.72 e	13.28 ± 2.18 d*	19.16 ± 2.01d	29.53 ± 2.38 d	8.34 ± 1.21 c	11.52 ± 1.45 c*	18.33 ± 1.93 cd
SS2 + P2	30.45 ± 2.89 e	11.29 ± 1.92 f	15.29 ± 1.34 e	18.47 ± 1.98 d	31.24 ± 2.19 e	10.44 ± 1.76 d	13.93 ± 2.18 d	17.43 ± 1.98 d

[a] Values represent the means of three measurements (± SE). Different letters indicate significant differences ($p = 0.05$) between treatments within each leaf type treated separately. An asterisk indicates significant differences between young and old leaves within each treatment treated separately ($p \leq 0.05$, Tukey's test).

Table 6. Antioxidative Enzyme Activities of Thin and Medium Roots from Chemlali Olive Plants Conducted under Different Treatments[a]

	enzyme activity (units mg^{-1} of Dw)							
	thin roots				medium roots			
treatment	SOD	APX	CAT	PPO	SOD	APX	CAT	PPO
CP	6.22 ± 0.89 a	1.04 ± 0.22 a	2.67 ± 0.67 a	25.61 ± 2.16 a*	7.81 ± 1.01 a	1.01 ± 0.22 a	1.98 ± 0.59 a	33.25 ± 2.19 a*
SS1	10.24 ± 1.21 b	2.88 ± 0.56 ab	5.42 ± 1.01 b*	21.26 ± 2.17 b*	9.62 ± 1.54 b	2.56 ± 0.45 b	3.42 ± 0.32 b*	30.51 ± 2.78 b*
SS1 + P1	12.37 ± 1.67 d	3.96 ± 0.82 b	6.51 ± 1.14 b	19.35 ± 2.56 b*	14.56 ± 2.01 c	3.44 ± 0.56 bc	4.88 ± 0.71 bc	28.76 ± 3.01 bc*
SS1 + P2	14.62 ± 2.07 c	4.92 ± 1.01 b	8.23 ± 1.65 c*	18.92 ± 2.61 bd*	16.82 ± 2.31 cd	4.99 ± 0.91 c	4.91 ± 0.49 c*	27.31 ± 2.61 c*
SS2	11.26 ± 1.89 bd	3.57 ± 0.95 b	6.34 ± 1.38 b*	20.78 ± 2.87 b*	12.62 ± 2.05 d	3.21 ± 0.81 b	3.99 ± 0.61 bc*	27.67 ± 2.78 c*
SS2 + P1	15.23 ± 2.06 c	5.91 ± 1.01 bc	7.89 ± 1.87 c*	17.36 ± 2.01 d*	15.95 ± 2.08 c	5.48 ± 0.76 cd	4.65 ± 0.56 bc*	26.54 ± 2.78 c*
SS2 + P2	16.29 ± 2.12 c	7.05 ± 1.21 c	9.62 ± 2.01 c*	16.72 ± 2.16 d*	17.98 ± 2.18 d	6.87 ± 1.01 d	5.71 ± 0.85 c*	25.71 ± 3.01 c*

[a] Values represent means of three measurements (± SE). Different letters indicate significant differences ($p = 0.05$) among treatments within each root type treated separately. An asterisk indicates significant difference between thin and medium roots within each treatment treated separately ($p \leq 0.05$, Tukey's test).

activity with higher levels than those recorded in roots. This tendency could be attributed to the higher APX biosynthesis in leaf than in root tissues.

The establishment of better plant water status under salinity plus proline medium conditions revealed the capacity for osmotic adjustment, which allows the growth to continue under saline conditions (*25*). Comparison of plant activity performances under the two proline medium levels showed that application of proline at 50 mM was more effective than that at 25 mM in mitigating the alterations induced by both water salinity levels.

On the other hand, the decrease of soluble sugars content in plants conducted under salinity plus proline medium revealed the significant effects of proline on photosynthetic activity, in such a way to maintain the metabolic and photosynthetic products, necessary for plant growth process, at appropriate levels. More to the point, this decrease revealed the important osmoprotectant effect played by the added proline in such a way to limit the need of salt-stressed plants for soluble sugars synthesis and, thus, relaxing the pressure on the photosynthetic chain. The proline medium at 50 mM had a more important osmotic effect on the stressed plants than the 25 mM proline medium. The increase of photosynthetic activity of salt-stressed plants in the presence of proline could be associated with the improved salt tolerance of olive plants by the exogenous proline supply. Similarly, Lopéz-Climent et al. (*33*) correlated the higher salt tolerance of FA5 citron seedlings to their ability to keep a higher photosynthetic activity under elevated saline conditions.

SOD, CAT, and APX activities in NaCl-stressed olive plants were significantly higher than those registered in nonstressed ones, as has been reported by Gomez et al. (*34*) in pea and by Ben Ahmed et al. (*11*, *15*) in adult olive tree irrigated with saline water and young plants grown under drought conditions, whereas Khedr et al. (*19*) and Mittova et al. (*35*) indicated a decrease of antioxidant enzyme activities under salinity conditions. Other investigations signaled a decrease of antioxidant enzyme activities in salt-sensitive plants and an increase in salt-tolerant ones (*12*). It seems that activities of antioxidant enzymes under stressed conditions are species- and cultivar-dependent. Besides, the extent to which the Chemlali olive tree increases its antioxidant enzyme activities under salt stress conditions seems to be plant age-dependent (*15*).

Proline has been considered to act as a compatible solute, osmoprotectant, and hydroxyl radical scavenger (*5*). Under salinity conditions, the increase in SOD, APX, and CAT activities has been enhanced in the presence of proline, as expected. These results are consistent with the findings of Khedr et al. (*19*), who noted that the activities of catalase and peroxidase in sea daffodil plants increased under salt stress in the presence of proline. These findings suggest that exogenous proline improved olive salt tolerance by enhancing the activities of antioxidant enzyme activities under salinity conditions. More to the point, the positive evolution of CAT, SOD, and APX activities and proline accumulation in leaves or roots of saline water-treated plants reinforces the hypothesis developed by Khedr et al. (*19*) and Ben Ahmed et al. (*11*) suggesting a positive relationship among the antioxidative enzyme activities and proline accumulation under salinity conditions. The same authors suggested that proline can act as a free radical scavenger to alleviate salt stress as has been developed by Kohler et al. (*29*). The decrease of PPO activity in salt-stressed olive tissues could be developed by the olive tree to improve the antioxidative action of phenols. Either proline medium added to the saline solution led to a significant decrease of PPO activity in plant tissues. Indeed, the improved reduction of PPO activity by proline could be developed for the maintenance of phenol compound contents at acceptable levels (*36*).

In this study, the increase of photosynthetic activity, the growth rate, the activities of CAT, SOD, and APX enzymes, and the preservation of appropriate leaf water status of salt-stressed olive plants revealed the improvement of Chemlali olive salt tolerance by exogenous proline application. Therefore, we

could conclude that the exogenous proline supply mitigates the detrimental effects of salt stress and reinforces the antioxidant defense system developed by the olive tree to tolerate stressed conditions. On the other hand, the accumulation of proline in salt-stressed olive plants was accompanied by the increase of some antioxidative enzyme activities (APX, CAT, and SOD) as has been found in salt-sensitive citrus rootstock by Arbona et al. (*37*). Hence, it appears that the increment of the analyzed antioxidative enzyme activities and proline content under salinity circumstances is among the olive salt tolerance traits, at least under the described experimental conditions. More to the point, the proline supply seems to have an important relaxing effect on the photosynthetic chain of salt-stressed olive plants by limiting the need for soluble sugars synthesis.

ABBREVIATIONS USED

APX, ascorbate peroxidase; CAT, catalase; CP, control plants; Ψ_{LW}, leaf water potential; MR, medium roots; OL, old leaves; PPO, polyphenol oxidase; ROS, reactive oxygen species; RWC, leaf relative water content; SOD, superoxide dismutase; SS1, plants irrigated with water containing 100 mM NaCl; SS2, plants irrigated with water containing 200 mM NaCl; SS1 + P1, plants irrigated with water containing 100 mM NaCl plus 25 mM proline; SS1 + P2, plants irrigated with water containing 100 mM NaCl plus 50 mM proline; SS2 + P1, plants irrigated with water containing 200 mM NaCl plus 25 mM proline; SS2 + P2, plants irrigated with water containing 200 mM NaCl plus 50 mM proline; TR, thin roots ; YL, young leaves.

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