

WATER RELATIONS, PROLINE ACCUMULATION AND PHOTOSYNTHETIC ACTIVITY IN OLIVE TREE (*OLEA EUROPAEA* L. CV “CHEMLALI”) IN RESPONSE TO SALT STRESS

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Abstract

This study was conducted to evaluate the effects of salt stress on photosynthetic capacity and proline accumulation in the Chemlali olive cultivar (*Olea europaea* L.) grown under field conditions at Sfax, Tunisia. Twelve year-old- olive trees were subjected to two drip irrigated treatments. The first was fresh water (EC = 1.2 dS m⁻¹, control plants CP) and the second was saline water (EC = 7 dS m⁻¹, Stressed plants SP). Each treatment contained twenty uniform plants. Salt stress adversely affect plant water status, net CO₂ assimilation rate, stomatal conductance and transpiration rate of stressed plants. However, the most important reduction of these activities was observed in summer season. Maximum photosynthetic activity was recorded during vegetative growth phase as well in CP as in SP. The severe reduction in photosynthetic performances in CP during summer season was clear evidence that photosynthesis of olive tree is not only related to water quality treatment but is also environmental conditions dependent. Salt stress has also led to the increase of proline content in both leaves and roots of salt stressed plants. Salt stressed olive trees tend to activate osmotic adjustment mechanism by the accumulation of proline, in order to activate water uptake to actively growing tissues to accomplish their photosynthetic activity even in low rates. Likewise, a possible protective role of proline in improving photosynthetic capacity is suggested.

Key words: photosynthetic performances, proline accumulation, salt stress, water relations

Introduction

Availability of good quality water for irrigation is limited worldwide and thus agriculture is likely to be forced to make increasing use of brackish water. In view of recent advances in crop management, irrigation and drainage methods, it becomes feasible to use low quality water for irrigation with minimum adverse impacts on crop growth and productivity (Chartzoulakis *et al.*, 2005). In Mediterranean region, where olive (*Olea europaea* L.) is one of the major tree crops, salinity is becoming a major constraint for agriculture due to high rates of evapo-transpiration and insufficient leaching. On the other hand, in these areas large quantities of saline water is available for olive tree irrigation. Thus, in Tunisia like other Mediterranean countries, due to shortage of good quality water, saline water is promising alternative for olive tree irrigation. Olive tree is considered to be drought tolerant because under water deficit conditions, leaves of olive can maintain turgor at very low values of leaf water potential and relative water content (Lo Gullo & Salleo, 1988; Larsen *et al.*, 1989). In addition, olive leaves often

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experience a diurnal osmotic stress due to high light and temperature during day particularly in dry seasons. Furthermore, the high hydraulic resistance of the stem also causes the development of water deficit in leaves (Larsen *et al.*, 1989). In view of large number of studies, it has been found that salt-induced osmotic stress caused a severe reduction in photosynthesis of olive tree (Chartzoulakis *et al.*, 2002). It has been pointed out that salt-induced reduction in photosynthesis is attributable to stomatal limitation (Loreto *et al.*, 2003). Furthermore, they suggested that the sensitivity of olive photosynthesis to salt stress is not dependent on Na⁺ and Cl⁻ content (Loreto *et al.*, 2003). However, Allakhverdiev *et al.* (1999) were of view that the reduction in photosynthesis under saline conditions is mainly due to salt induced osmotic stress. In response to drought and salt stress conditions, plants, generally accumulate compatible solutes to maintain ion homeostasis (Ashraf, 2004; Ashraf & Harris, 2004; Ashraf & Foolad, 2007). Proline was known as the main important osmolyte accumulated under salt stress (Ashraf & Foolad, 2007). These osmolytes play a great role in facilitating water retention in the cytoplasm and to activate water uptake to the growing tissues.

In view of all these reports, the present study was aimed to find out the possibility of safe use of saline water for olive tree cultivation. Furthermore, it was investigated how far continuous use of saline water adversely affect plant photosynthetic capacity of olive over whole year; and up to what extent accumulation of proline has a role in protecting photosynthetic capacity.

Material and Methods

Experimental site and plant material: Trials were carried out in 2004/2005 crop season, at 2 years after the application of salt stress treatment, at the Tunisian Olive Tree Institute plantation (Sfax, 34° 43 N, 10° 41 E). The plant material used consists of 12 – year - old olive trees cv. Chemlali (*Olea europaea* L.) All the trees were spaced 4 x 6m; drip irrigated with the same amount of water and subjected to the same fertilization and common olive cultivation practices. The sandy soil had an organic matter content of 1.1%; 13.4% CaCO₃; 1.3% N and a pH of 7.6.

Climatic conditions of the experimental site were obtained from a meteorological station installed within the Institute. Average of rainfall, air temperature and global solar radiations were recorded. Based on data collected at the experimental station in the last few years, water irrigation regime, considering the local environment conditions (average of temperature, photons flux density, evapotranspiration, plant cover was applied (Masmoudi *et al.*, 2004).

Two plots, of 20 trees each, were used as replicates and subjected to the following treatments: irrigation with fresh water (1.2 dS m⁻¹ EC, control plants CP) and irrigation with saline water (7 dS m⁻¹ EC, stressed plants SP). The water used for irrigation was either that supplied by the Tunisian National Water Carrier (CP) and from the local well established in the area of the Institute (SP). The water use of olive tree (ETc) was calculated as:

$$ETc = ETo * Kc * Kr \text{ (Vermeiren \& Jobling, 1980);}$$

$$ETo = 0.0023 * Ra * (T_{\text{average}} + 17.8) * (T_{\text{max}} - T_{\text{min}})^{0.5} \text{ (Doorenbos \& Pruitt, 1977)}$$

Where; ETc is the crop evapotranspiration; ETo was the reference evapotranspiration;
 Ra: Solar radiation (MJ m⁻² j⁻¹); T_{average}: the average of temperature;
 T_{max}: maximum temperature; T_{min}: minimum temperature

To estimate E_{Tc} , the reference evapotranspiration was corrected by a crop coefficient K_c of 0.6 (Vermeiren & Jobling, 1980) and a reduction coefficient K_r of 0.9 (Masmoudi *et al.*, 2004). The K_r applies to orchards more than 50 % ground cover and was described as $(2 * \text{percent canopy cover}/100)$. Total water supplied to mature olive tree, taking rainfall into account, was 4000 m³/ha/year.

Water relations and gas exchange measurements: Relative water content (RWC) was determined on 36 fully expanded leaves of similar age, divided into 6 blocks of six leaves each per treatment. Leaves were excised before dawn, weighed fresh (F_w) and placed in distilled water in the dark for 24 hours to re-hydrate. The following morning, leaf turgid weight (T_w) was measured and then leaves were dried at 80°C for 48 hours and dry weight (D_w) was determined. The RWC was calculated as:

$$RWC = [(F_w - T_w) / (F_w - D_w)] \times 100$$

Leaf gas exchange measurements were taken 4 days per week from 9:00 to 11:00 am, on well exposed six – one- year- old leaves from each treatment using a portable gas exchange system (Li-CorInc - 6200). It measures net photosynthesis (P_n), stomatal conductance (G_s), transpiration rates (E), air humidity, air and leaf temperature, air CO₂ and intercellular CO₂ concentrations.

Proline content: Proline content was determined following the method of Bates *et al.* (1973). Leaf and root samples served for proline determination were harvested at the same time of collect of leaves for relative water content measurements. Plant tissues were immediately frozen in liquid nitrogen. A 0.5g of frozen powder was mixed with 5.0ml aliquot of 3% (W/v) sulfosalicylic acid in glass tubes covered at the top and boiled in a water bath at 100°C. The mixture was centrifuged at 2000g for 5min at 25°C. A 200µl aliquot of the extract was mixed with 400µl distilled water and 20 ml of the reagent mixture (30ml glacial acetic acid, 20ml distilled water and 0.5g of ninhydrin), and boiled at 100°C for 1h. After cooling the mixture, we added 6.0ml of toluene. The chromophore containing toluene was separated and absorption at 520nm was read, using toluene as a blank. Proline concentration was calculated using L-proline for the standard curve.

Data analysis: The data were subjected to analysis of variance (ANOVA, 10.Windows). Significant differences between values of all parameters were determined at $p \leq 0.05$ according to Duncan'S Multiple Range Tests.

Results

Environmental parameters of the experimental site: The rainfall pattern in the trial year was characterized by a scant rain in spring (from March to May) and in autumn (37 and 66mm of rain, respectively) and a dry summer (from June to August). The most important quantity (103mm) was recorded during winter season (December – February). The experimental period was characterized by a moderate temperature average (23°C) during spring season, a high temperature during summer and autumn periods (32 and 27°C, respectively) and an average of 16.9°C during winter. Table 1 summarizes the most important environmental parameters recorded during the trial year.

Table 1. Environmental parameters of the experimental site during the trial period.

	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
T	19.4	22.7	27.5	30.7	33.6	32.5	30.4	27.4	22.7	17.1	16.7	16.8
P	11	12	14	5.2	0	2.4	12	10	44	52.9	38.5	11.2

T = Temperature (°C); P = Precipitation (mm)

Time course of changes in water status: Along the experimental period, control plants showed higher values of RWC than those of SP with statistically significant differences between them ($p = 0.0107$). For both treatments, the minimums of RWC were recorded in July – August period (Fig.1). The plant water status in olive tree is not only related to water quality treatment but is also dependent on environmental conditions. Indeed, the deleterious effects of salt stress on plant water level were reinforced by environmental conditions characterizing the arid climate in the south of Tunisia. During the trial period, relative water content in SP ranged from 72 to 80%.

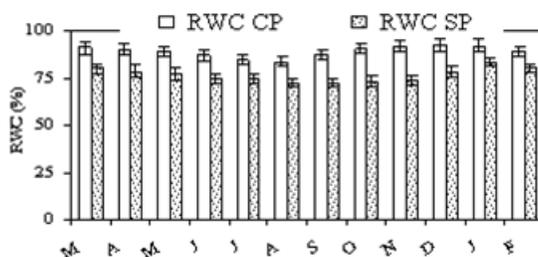


Fig. 1. Relative water content (RWC) of CP and SP for 12 months. Values are means of six replicates \pm S.E.

The occurrence of more moist times (autumn period) was accompanied with an increase in RWC. However, values registered in SP remained lower than those of CP. This increase of leaf water status testified the tendency of salt – stressed olive tree to restore better level of plant water status when climatic conditions become more favorable.

Time course of changes in net photosynthesis: Salt stress has enormously altered photosynthetic activity of olive tree (Fig.2). Furthermore, for both treatments, the maximums of photosynthesis were noticed during June period coinciding with the intense vegetative growth phase of the olive tree. These values were of 28 and 13 $\mu\text{mol m}^{-2}\text{s}^{-1}$, respectively in CP and SP with a relative reduction of Pn in SP of 55%. Furthermore, during the experimental period, differences between the two treatments were statistically significant ($p = 0.007$). Besides, minimums of Pn, as well in CP as in SP, were registered during summer season, coinciding with the minimum level of plant water status. During August, Pn was of only 3.38 and 5.55 $\mu\text{mol m}^{-2}\text{s}^{-1}$ in SP and CP, respectively.

The occurrence of more clement climatic conditions during autumn period has induced to the increase in CO_2 assimilation rate, as well in CP as in SP. As well as, net photosynthesis is enormously related to plant water status. These tendencies are justified by the relationship determined between net photosynthesis and minimum leaf water potential when control and stressed plants were pooled together (Fig. 2). However, values

of Pn remained lower than those noticed during the intense vegetative growth phase occurring in June. Furthermore, the comparison of Pn of SP to those of CP showed that the lowest relative reduction of net photosynthesis in SP were recorded during summer and winter seasons coinciding with the plant rest phases, adapted by the olive tree in order to avoid damaging its survival mechanism.

Time course of changes in transpiration rates: For both treatments, as well for photosynthesis activity, the maximums of E rate were registered in June. These values were of 10.1 and 5.3 mmol m⁻² s⁻¹ in CP and SP, respectively (Fig.2). After that, E rates decreased steadily until the end of August when it marked the lowest values which were statistically lower (p < 0.05) than those recorded during the intense vegetative growth phase of olive tree. This decrease was in parallel with the increase in air temperature and the decrease in RWC and Ψ_{min}. At the beginning of autumn (September), an appreciable recovery of E rate was observed for both treatments. However, the recovery was better in CP than in SP. In fact, during this season, the averages of transpiration rates were of 6.2 and 3.76 mmol m⁻² s⁻¹, respectively in CP and SP.

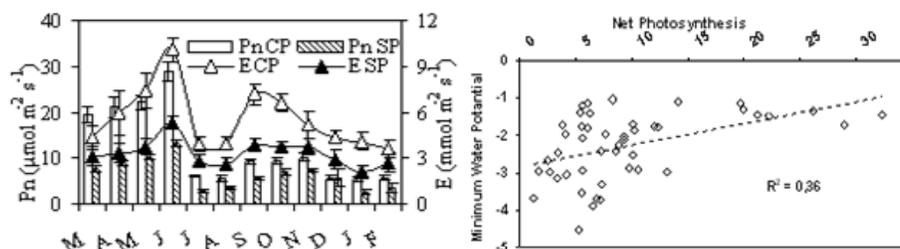


Fig 2: Time course of net photosynthesis (Pn) and transpiration rate (E) of CP and SP during the trial period (on the left) and relationship between Pn and Ψ_{min} (on the right). Values are means of six replicates ±S.E.

Time course of changes in stomatal conductance: The patterns of stomatal conductance were in some ways similar to those of Pn and E rate. For both treatments, the highest values of Gs (173 and 103 mmol m⁻² s⁻¹, respectively in CP and SP) were observed in June. However, the lowest values (103 and 64 mmol m⁻² s⁻¹, respectively) were registered in August period (Fig. 3). The effects of salinity conditions on photosynthetic performances were reinforced by severe environmental conditions characterizing the arid climate in the south of Tunisia.

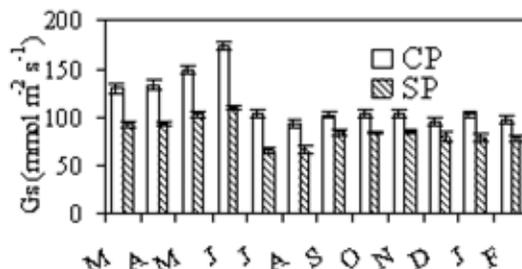


Fig. 3. Time course of stomatal conductance of CP and SP during the trial period. Values are means of six replicates ±S.E

In autumn, G_s increased to statistically higher values ($p < 0.05$) than those recorded at the end of summer period, not only for CP, but also for stressed ones. However, the time taken for recovery depended enormously on the plant's water status. The lower the RWC values were, the slower the stomatal recovery was. Besides, autumn rainfall allowed SP to restore better stomatal conductance values under appreciable relative water content level. Indeed, high positive relationships were determined between the components of photosynthetic performances (G_s and E rate) and RWC when control and stressed plants were pooled together (Fig. 4). These kinds of relationships suggest that a hydraulic feedback mechanism exist between water relations characteristics and physiological parameters of olive tree, as well under stressed as under non stressed conditions. Furthermore, the similarity between the patterns of P_n , E rates and G_s revealed the correlation between all these parameters expressed via the high relationships determined between them (Fig. 4).

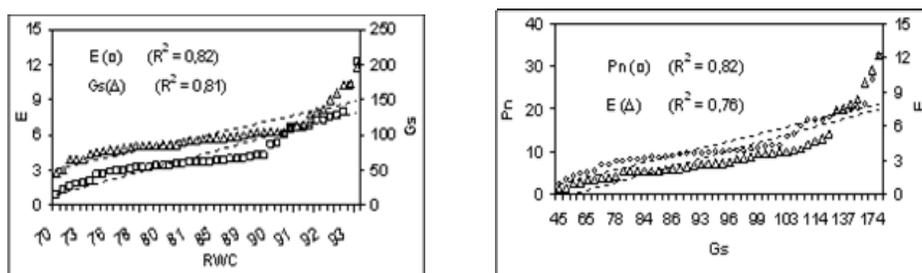


Fig. 4. Relationships between RWC and E and G_s (on the left) and between G_s and P_n and E rate (on the right) ($n = 48$).

Proline accumulation: Salt stress-induced caused a significant increase in proline content in both leaves and roots of stressed plants (Fig. 5). Along the experimental period, it ranged from 0.84 to 2.04 and from 0.62 to 1.56 $\mu\text{mol mg}^{-1}$, respectively in leaves and roots of SP. Furthermore, it increased progressively with time. However, the proline accumulation was more intense in stressed leaves than in roots. For both plant tissues, the highest accumulation was observed during summer season coinciding with lowest values of RWC and Ψ_{min} . The autumn and winter rainfall did not induce the decrease of proline content which is increasing with time. Indeed, the more severe the salt stress was, the more important the proline accumulation was. Averages of proline content in leaves of SP were at almost three times higher than those in CP; and they have similar patterns as those in roots.

Discussion

The salt stress reduced the leaf water status of olive measured as RWC which may have been due to salt induced osmotic effect resulting in dehydration at tissue level (Ashraf, 2004). Accompanying these changes in leaf water relations, imposition of salt stress also reduced the photosynthetic capacity of olive, which is in agreement with earlier findings (Bongi & Loreto, 1989; Tattini *et al.*, 1995; Chartzoulakis *et al.*, 2002). However, higher reduction in photosynthetic rate in both non-stressed and salt stressed plants during summer (July – August) can be clearly linked to climatic conditions. This is

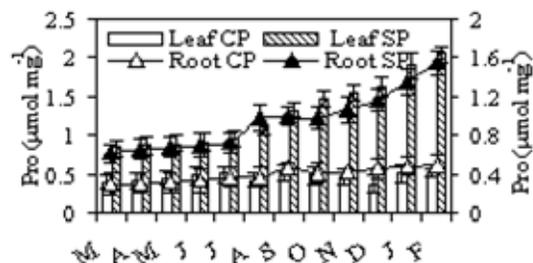


Fig. 5. Proline content in leaves and roots of CP and SP during the trial period. Values are means of four replicates \pm S.E.

a clear evidence that water supply (even with fresh water) will be without a great benefits for photosynthetic performances if applied under harsh environmental conditions inducing the rest phase of olive tree (Ben Ahmed *et al.*, 2007). Furthermore, the decrease in Gs and E rates in salt stressed plants supported this view. Indeed, as soon as olive tree perceived water deficit (resulting from salt ions accumulation or high temperature), it adjusted rapidly its water flux by stomatal closure to avoid water loss by transpiration. The reduction in photosynthetic rate is also due to the reduction in stomatal conductance resulting in restricted availability in CO₂ for carboxylation reactions. In fact, stomatal closure minimizes the loss of water by transpiration and this affects chloroplast light harvesting and energy conversion (Iyengar & Reddy, 1996). The severe reductions in photosynthetic performances in stressed plants can represent adaptive mechanisms for olive tree to cope with salinity conditions more than merely a negative consequence of it.

Several papers showed that the effects of salinity on CO₂ assimilation rates in olive tree are cultivar dependent (Tattini *et al.*, 1995; Gucci *et al.*, 1997; Loreto *et al.*, 2003). Besides, Loreto *et al.* (2003) noticed that the most sensible cultivars are those with inherently high photosynthetic and stomatal conductance. In the Chemlali olive tree, the most sensitive period to salt stress is the intense vegetative growth phase during which the relative reductions of Pn, Gs and E rates were the most important. The less sensitive periods coincide with the rest phases adapted by the olive tree to avoid damaging its survival mechanisms by harsh environmental conditions. Even under irrigation with fresh water, the photosynthetic activity was not maintained at a stable rate during the experimental period. Indeed, the olive tree responses to salt stress are not only related to water quality treatment, but are also dependent on environmental conditions and olive growth cycle.

During autumn period, the olive tree cv. Chemlali established better photosynthetic activity. This response testified the rapid response of the olive tree to better climatic conditions, even if it was stressed. Furthermore, it displayed the important role played by olive leaves to maintain photosynthetic activity during a long period of the year (even in low rates). These characteristics let us to consider the olive tree as an arid active specie which can survive long period of drought and harsh environmental conditions without impairing its photosynthetic system. Besides, the olive tree was able to restore its activity when conditions become more favorable. A study to verify the recovery of photosynthesis in the Chemlali olive tree during a relief period after long term irrigation with saline water is on the way.

Furthermore, our results are similar to those of Koyro (2006) reporting that gas exchange properties such as net photosynthesis and water use efficiency were strongly

affected by salt stress. The author has also attributed the reduction in net photosynthesis in salt stressed *Plantago coronopus* to stomatal limitations. As has been reported by Hasegawa *et al.* (2000); and in order to accommodate the ionic balance in the vacuoles, the salt stressed olive trees tend to accumulate proline in its cytoplasm to high level to improve water uptake to actively growing tissues by osmotic adjustment effects. These results confirmed the idea of Ashraf and Foolad (2007), who has stated that proline is known to accumulate in large quantities in higher plants in response to environmental stresses. The same reports have been noticed by several papers indicating that many plants accumulate proline as a non toxic and protective osmolyte under saline conditions (Lee & Liu, 1999; Khatkhar & Kuhad, 2000). As observed in *Zea mays* (Abd El Baki *et al.*, 2000), the accumulation of proline in the Chemlali olive tree constitutes another adaptive mechanism to harmful stress. The capacity for osmotic adjustment, via the accumulation of proline, during stress imposition in olive tree has been also found in leaves of grapevine (Shultz & Matthews, 1993) and in apple trees (Lakso *et al.*, 1984). However, accumulation of proline compound under stress conditions seems to be species and cultivar dependent. In fact, in many plants, under stress conditions, Ashraf & Foolad (2007) have stated that proline accumulation has been correlated with stress tolerance and its concentration is generally higher in stress tolerant than in stress sensitive plants. Furthermore, Ain Lhout *et al.* (2001) have shown that proline content in two Mediterranean shrubs (*Halimium halimifolium* L. and *Pistacia lentiscus* L.), during increasing water deficit, was twice the amount found in olive tree. Under stress conditions, the Chemlali olive tree tend to accumulate, added to proline, other osmolyte compounds such as sugar and starch in both leaves and roots; however, the chlorophyll concentrations in stressed leaves tend to decrease under salt stress (data not shown).

As well for photosynthesis, Proline content was not maintained at a stable rate during the trial period, as well in CP as in SP. These patterns testified the effects of environmental conditions on osmolyte compounds in the olive tree, even under stressed or non stressed conditions. The increase of proline content in both leaves and roots of control and stressed plants, even during moist times, let us suggest a possible protective role of proline in improving photosynthetic activity by activating osmotic adjustment along the stress period, in order to maintain hydration status of the olive tree. However, Ashraf & Foolad (2007) have noticed that there are great variations in different plant species in response to exogenous application of proline; and that not all plant species are responsive to external application of the compound. A study to verify the role of proline on photosynthetic performances of salt stressed Chemlali olive tree by the exogenous application of proline during and after the stress imposition is on the way.

Under stress conditions, the olive tree tends to reduce its stomatal conductance and thus its photosynthetic activity by stomatal closure control to avoid water loss by transpiration and to maintain its hydration status. Furthermore, in order to activate osmotic adjustment mechanism, the salt – stressed olive trees tend to accumulate, at high level, the proline, not only in leaves but also in roots, in such a way increasing water uptake and retention to actively growing tissues. In so doing, the stressed olive trees were able to maintain their photosynthetic activities and thus growth during a long period of the year, even in low rates. These tendencies justified the relationships determined between Pn and RWC when control and stressed plants were pooled together.

Overall, results discussed here demonstrated that different physiological and biochemical mechanisms are developed by salt stressed olive trees. These mechanisms seem to act additively or synergistically. Furthermore, these results support the hypothesis that proline accumulation during salt stress plays a protective role in improving photosynthetic activity of stressed olive trees.

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