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OLIVE TREE (*OLEA EUROPAEA* L. cv. "CHEMLALI") UNDER SALT STRESS: WATER RELATIONS AND IONS CONTENT

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Abstract

An experiment was conducted to evaluate the effects of salt stress on water relations and ions content of the Chemlali olive cultivar (*Olea europaea* L.) grown under field conditions in Sfax, Tunisia. Twelve-year–old olive trees were subjected to two drip irrigated treatments. The first is fresh water (EC of 1.2 dS m^{-1}, control plants, CP) and the second is saline water (EC 7 dS m^{-1}, stressed plants, SP). Leaf water relations were decreased due to salt stress. At predawn, it is of -1.4 MPa in SP and only -0.6 MPa in CP. The concentrations of Na^+ and Cl^- ions were higher in tissues of SP. However, both Na^+ and Cl^- in salt stressed roots were higher than in salt stressed leaves. Salinity in root zone led to a significant decrease in K^+ concentration in both leaf and root tissues of stressed plants. The Chemlali olive tree tends to cope with salt stress conditions by decreasing enormously its leaf water potential; and excluding the major part of salt ions at the root level. These mechanisms are developed in order to activate water uptake and to avoid salt ions accumulation in actively growing tissues.
Key words: ions content, *Olea europaea*, salt exclusion, salt stress, water relations.

Introduction

The limited water availability in arid and semi arid regions and the increased need for good water quality for urban use restrict the use of fresh water for irrigation. In Tunisia, due to its socio- economic importance, olive cultivation is continuously being extended to irrigated land. So, large quantities of saline water are used for olive tree irrigation, since olive is considered as moderately tolerant to salinity (Rugini & Fedeli, 1990). Several researches reported that olive tree is characterized by its limited water requirement (Loreto *et al.,* 2003) and can be irrigated with water containing 3200 ppm of salts (Al-Saket & Aesheh, 1987). In comparison with other Mediterranean- grown tree crops, olive is more tolerant than citrus but less tolerant than the date palm (Ayers & Westcot, 1976). However, salt tolerance in olive tree is cultivar dependent (Gucci *et al.,* 1997).

Water potential becomes more negative with an increase in salinity (Kan, 2001; Khan *et al.*, 1999; Meloni *et al.*, 2001) which causes detrimental effects on plant growth. However, Olive trees can tolerate extremely low water potential and relative water content induced either due to salt or water stress (Lo Gullo & Salleo, 1988). Salt stress changes water relations of higher plants (Greenway & Munns, 1980). Salt tolerance in olive tree cultivars is associated with effective mechanisms of ion exclusion and retention of Na^+ and Cl^- in the root (Tattini *et al.*, 1995; Chartzoulakis *et al.*, 2002). Long ago, Benlloch *et al.,* (1991) and Tattini (1994) suggested that the mechanism is located within

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the roots and prevents salt translocation to aerial parts. However, little information is available on interactive changes in water relations of the Chemlali olive cultivar adapted to arid climate in the south of Tunisia. The principal objective of the present study was to investigate the effects of salt stress on the Chemlali olive tree in arid region in Tunisia and to assess up to what extent salt exclusion mechanism has a role in the tolerance of the studied variety to salt stress conditions.

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% $CaCO_3$; 1.3% N and a pH of 7.6. The region is characterized by an arid climate with a rainfall average of 250 mm of water annually and a temperature average of 23°C. Based on data collected at the experimental station in the last few years, water irrigation regime, considering the local environment conditions (average of temperature, photon 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:

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

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

Where, ETc is the crop evapotranspiration; ET_o 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 ETc, the reference evapotranspiration was corrected by a crop coefficient Kc of 0.6 (Vermeiren and Jobling, 1980) and a reduction coefficient Kr of 0.9 (Masmoudi *et a.*, 2004). The Kr applies to orchards more than 50 % ground cover and is described as:

$$Kr = 2 * C / 100$$

where; C is percent canopy cover.

Total water supplied to mature olive tree, taking rainfall into account, was 4000 m^3/ha/year.

Water relations 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 down, 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:

$$RWC = [(F_W - T_W)/ (F_W- D_W)]* 100.$$

Predawn leaf water potential (Ψ_{pd}) was determined at down at approximately 15 -day- interval on six to eight leaves per treatment with a Scholander pressure chamber (pms.1000).

Ions content: Leaf samples used for ions concentrations were harvested from the same shoots served for relative water content and leaf water potential measurements. Root tissues were harvested at a depth of 80cm. after harvest, leaf and root tissues were washed with distilled water to eliminate the dust, oven dried at 70°C for 72h and then ground to a fine powder. One gram of the ground tissue material were placed in an oven at 250°C for 3 hours and then transferred to 100ml of dilute nitric acid. Cations concentrations (Na^+ and K^+) were determined from digested material using a flame photometer (JENWAY, PEP-7) and the chloride (Cl-) using the chloride meter (JENWAY, PCLM-3).

Data analysis: Statistical analyses were performed using analysis of variance (SPSS.10 Windows). Significant differences were determined at $p \leq 0.05$ according to Duncan multiple range test.

Results

Time course of changes in water relations characteristics: Relative water content and leaf water potential were adversely affected due to increasing salinity stress. Reduction in both leaf water potential and RWC were apparent two months after the beginning of measurements. Along the experimental period, control plants showed higher values of RWC than those of SP with statistically significant differences between them ($p= 0.0107$). Relative water content of SP ranged from 72 to 80% (Fig.1) Furthermore, these deleterious effects of salt stress on both RWC and Ψw was more pronounced during summer due to severe climatic conditions i.e., high air temperature and high light intensity. In fact, the minimums values of RWC were recorded in July – August period, not only in SP but also in CP. Besides, stressed plants tend to maintain high values of RWC (Fig.1).

Differences in plant water status between the two treatments were more revealed via leaf water potential characteristics. Indeed, during the experimental period, the average of leaf water potential in SP ranged from -1.2 to -2.6 MPa, during winter and summer seasons, respectively, with statistically significant differences between control and stressed plants ($p= 0.0067$). However, this salt induced reduction in leaf water potential was less in spring (70%) than that in summer (50%), if compared to control plants (Fig.2).

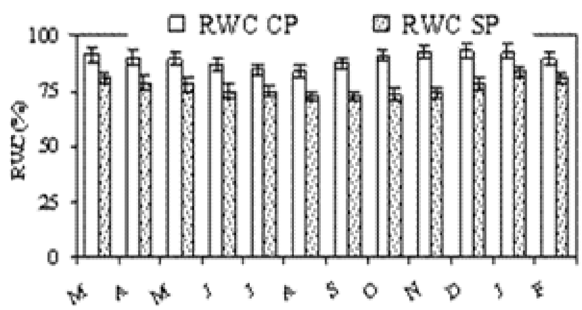

Fig. 1. Relative water content (RWC) of CP and SP for 12 months. Values are means of six replicates ±S.E.

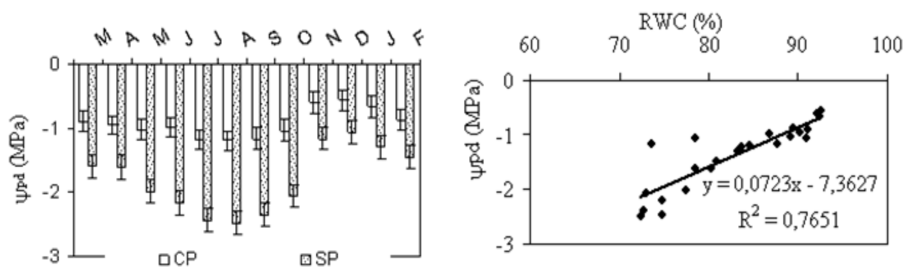

Fig. 2. Predawn leaf water potential (Ψ_{pd}) (on the left) and relationship between Ψ_{pd} and RWC (on the right) of CP and SP. Values are means of six replicates ±S.E.

The similarity between the patterns of relative water content and leaf predawn water potential let us assume that Ψpd depends on the level of water status previously reached which can be confirmed by the high relationship determined between both parameters when control and stressed plants were pooled together (Fig. 2).

Ions content: Salt stress caused a significant increase in accumulation of Na^+ in both leaves and roots. However, Na^+ accumulation consistently increased in the roots four months after the beginning of measurements, whereas it remained almost same in the salt stressed leaves during first four months. In salt – stressed leaves, Na^+ content ranged from 0.2 to 0.25% dw, however, in stressed roots, it increased from 0.34 to 0.57 from March to June (Fig.3). For both plant tissues, the maximum of Na^+ content was recorded during summer period (June – September). Besides, the Na^+ accumulation was higher in roots than that in leaves. The occurrence of moist seasons (autumn and winter) was accompanied with a decrease in Na^+ content in both leaves and roots.

As well for Na^+ accumulation, both leaf and root Cl^- contents increased significantly under salt stress conditions. The patterns of changes in Cl^- contents were in some ways similar to those of Na^+ concentrations. The maximum of Cl^- contents (0.25 and 0.8% dw, respectively in leaves and roots) were registered during summer period, too (Fig.3). Besides, root Cl^- contents were higher than those of leaves. Although the accumulation of salt ions (Na^+ and Cl^-) in plant tissues to a high levels, no toxicity symptoms such as dead leaf edge, leaf drop or necrosis were observed. However, the salt

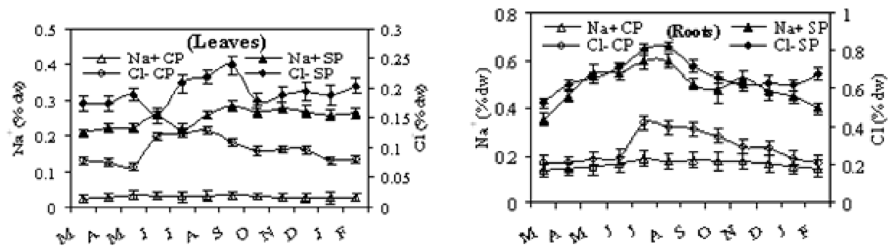

Fig. 3. Sodium (Na^+) and chloride (Cl^-) contents (% d.w) in leaves (on the left) and roots (on the right) of CP and SP for 12 months. Values are means of four replicates ±S.E.

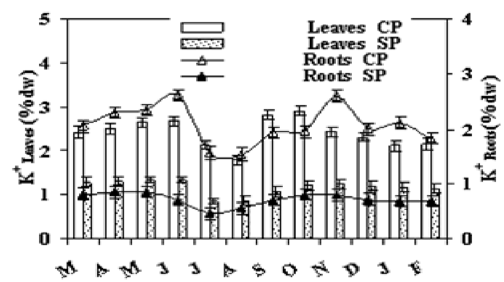

Fig. 4. Potassium (K^+) content (% d.w) in leaves and roots of CP and SP for 12 months. Values are means of four replicates ±S.E.

stress induced resulted in large decrease of K^+ content, as well in leaves as in roots (Fig.4). The enormous decrease of K^+ concentrations was recorded during summer period coinciding with the period of maximum accumulation of potentially toxic ions. After that, a significant increase in K^+ content was recorded, as well in leaves as in roots with statistically significant differences between CP and SP ($p < 0.05$). Besides, the relative reduction of K^+ concentration in SP was about 50 and 60% if compared to CP, respectively in leaves and roots. In contrast to Na^+ and Cl^- accumulations trends, the K^+ accumulation was higher in leaves than that in roots.

Discussion

Salt induced-reduction tissue water content (measured as RWC and leaf water potential) in olive tree as observed in the present study has also been reported by different workers (Tattini *et al.*, 1995; Loreto *et al.*, 2003; Chartzoulakis, 2005). However, the highest reduction was observed during summer suggesting that increasing ambient temperature causes increase in water loss through transpiration; and that the effects of salt stress were reinforced by severe environmental conditions. The immediately response of the Chemlali olive tree to salt stress, as has been reported in other glycophytes plants (Chartzoulakis, 2005), is the reduction in leaf water potential and relative water content. The decrease of Ψpd represent for the Chemlali olive tree an adaptive mechanism to salt stress more than merely a negative consequence of it. The olive tree tends to activate water uptake and retention by decreasing enormously its leaf water potential. These responses allow the salt stressed plants to maintain better level of relative water content ranging from 72 to 80%. The decrease in water uptake by salt stressed olive tree has also been reported by Therios & Misopolinos (1988). The increase of Ψpd and RWC of stressed plants, during autumn period, occurring under more favorable climatic conditions than those characterizing the summer period, testified the ability of olive tree for recovery.

Besides, olive tree, characterizing the Mediterranean landscape, is well known for its resistance to severe and prolonged water stress (Lo Gullo & Salleo, 1988; Larsen *et al.,* 1989; Giorio *et al.,* 1999). The same results have been reported by Ben Ahmed *et al.,* (2007) suggesting that irrigation of olive tree during summer period will be without benefits for photosynthetic activity and growth, since the olive tree has to enter in a rest phase to avoid damaging its survival mechanism by severe environmental conditions. Indeed, the Chemlali olive tree is considered as an arid active species which can survive long period of water stress; and that its activity is established when climlatic conditions become favorable (Ben Ahmed *et al.,* 2007).

The decrease of RWC in salt – stressed plants has been considered as the result of the high salt concentration of the external solution (Greenway & Munns, 1988). The salt – induced decrease of Ψpd was accompanied with an increase in potentially toxic ions (Na^+ and Cl^-), as well in leaves as in roots. The large increase of these ions contents in salt stressed plants was observed during summer period in coincidence with low levels of RWC and Ψpd. Furthermore, increasing temperature causes also a substantial increase in Na^+ concentration in both leaves and roots under both stressed and non stressed conditions. The higher levels of both Na^+ and Cl^- contents recorded in roots than those in leaves confirm the findings of Chartzoulakis (2005) who noticed the existence of an increasing gradient in Na^+ and Cl^- ions from the roots to the apical parts of the olive tree. The ion exclusion and ion compartmentation at the root level regulates ion concentration in the xylem in order to prevent the accumulation of potentially toxic ions in the aerial parts (Tattini *et al.,* 1995; Gucci *et al.,* 1997; Chartzoulakis, 2005). This mechanism seems to work effectively in the Chemlali olive tree, since this cultivar tends to accumulate salt ions at the root level with low delivery to shoots; and that no toxicity symptoms were detected after a long period of salt stress treatment.

However, salt accumulation in root zone causes the development of an osmotic stress and disrupts the cell ion homeostasis by inducing an inhibition in the uptake of essential nutrients in a number of crops, e.g., in wheat (Raza *et al.,* 2007) and in sunflower (Akram *et al.,* 2007). Chartzoulakis (2005) has showed that the greatest decrease in K^+ concentration occurs in old leaves and roots; and that the olive tree is able to maintain high level of K^+ in young leaves. These tendencies seem to be similar with those of the salt - stressed Chemlali olive tree. In fact, even stressed, this cultivar was able to maintain its photosynthetic activity, even at low rates (data not shown). A comparative study of photosynthetic performances of young and old olive leaves of different cultivars in Tunisia under salt stress conditions is on the way.

Overall, the salt tolerance of the Chemlali olive tree is associated with its ability to exclude the major part of incoming salts at the root level, and to avoid Na^+ and / or Cl^- accumulation in actively growing tissues. Indeed, highest accumulation of Na^+ with concomitant decrease in K^+ in the leaves of salt stressed plants during August suggested that Chemlali olive cultivar maintained low cytoplasmic Na^+ concentrations through vacuolar compartmentation; and thus protected photosynthetic tissue from Na-induced damages. Thus, tolerance to salt toxicity through maintaining ion homeostasis seems to be achieved by ion exclusion at both root and leaf level is suggested.

As well as our results were evaluated after a long period of salt stress, and despite the fact that we did not evaluate the effects of salt stress on growth or oil quality; importantly, this study has confirmed the hypothetically moderate tolerance of the Chemlali olive tree to salt stress, since no toxicity symptoms were detected. Furthermore, in view of scarcity of water available for irrigation, the use of saline water for olive tree irrigation is suggested to enhance olive cultivation.

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