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Increasing salt tolerance in Olive, *Olea europaea* L. plants by supplemental potassium nutrition involves changes in ion accumulation and anatomical attributes

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Abstract

The effects of supplemental potassium were studied on growth, ion concentration and anatomical parameters in one year old olive trees, *Olea europaea* L., grown in sand culture in greenhouse at different levels of NaCl for 80 days. The experiments were conducted in a completely randomized design as a factorial. Factor one was salinity (0, 40 or 80 mM NaCl) and the second factor was potassium levels of 4 and 8 mM. Salinity caused a significant decrease in the growth of plants; however, the supplemental potassium could partly ameliorate the adverse effects of salinity on growth. Due to salinity Na^+ and Cl^- ions accumulated and the K^+ /Na^+ ratio decreased in the plants. The lower relative water content and the higher cortex /stele ratio in plants under salinity indicate water deficit. Under salinity, however, the supplemental potassium led to lower Na^+ and higher K^+ and P concentration which probably reduced the toxicity. The ratio of cortex to stele became normal as salt-grown plants were supplemented with potassium. Supplemental potassium increased palisade cell layer thickness in leaves under salinity that may be accompanied with increased potential for photosynthesis. The results indicate that supplemental potassium can be useful in ameliorating salinity stress effects in olive plants.

Keywords: Anatomical characters; Ions accumulation; Olive; Potassium; Salinity

Introduction

High salt concentration in soils inhibits crop growth and yield and is one of the major constraints in agricultural production in arid regions (Malash et al., 2008). It is estimated that approximately a third of the world's irrigated lands and half of the lands in semiarid and costal regions are affected by salinization. Improving plant resistance to salinity may provide yield stability in subsistence agriculture (Flowers and Yeo, 1995; Flowers, 1999; Asghari, 2008). Plants face two basic problems in saline environments. First, excess salt in soil lowers the osmotic potential of soil water and leads to decreased water uptake and consequently water deficit in plants. This in turn leads to perturbations in cell division

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and/or extension and influences the integrity of metabolic reactions in plants. Second, increased uptake and accumulation of Na^+ and Cl^- ions decreases the absorption of essential minerals and imposes toxicity to plants (Munns, 1993; Tester and Davenport, 2003). Accordingly, salt stress brings about many harmful changes such as reduction of enzyme activity, cellular membrane disorganization/dysfunction, reduced photosynthesis and finally decreased plant growth (Tester and Davenport, 2003; Paridaa and Das, 2005). Furthermore in response to salinity plants display morpho-anatomical changes involving leaf thickness, number and size of stomata, diameter and number of xylem vessels. These changes, depending on the species, may either represent adaptive responses of plants or be symptomatic of damage due to salinity (Pouaakoff-Mayber, 1975; Longstreth and Nobel, 1979; Baum et al., 2000).

Potassium is an important macronutrient and the most abundant cation in higher plants. An optimal K^+ concentration is essential for protein synthesis, enzyme activation, and photosynthesis. Potassium has an important role in osmoregulation during cell expansion, stomatal movements and turgor-driven movements (Marschner, 1995). Furthermore, K^+ is necessary for phloem solute transport and for the maintenance of cation: anion balance and pH stat between apoplast, cytosol and vacuole. Potassium supply from soil can be rate limiting for agricultural production (Shabala, 2003; Marschner, 1995). Although closely related to K^+, sodium dose not generally fulfill potassium physiological functions in plants. Certain halophytes require Na^+ for growth, but in the majority of plants including most crops, Na^+ is toxic at high millimolar concentrations (Flowers, 1999; Maser, 2002). It is well known that salinity induces potassium deficiency, e.g., in tomato (Satti and Al-Yahyai, 1995; Kaya et al., 2001b) cucumber and spinach (Chow et al., 1990). Maintenance of adequate potassium levels is essential for plant survival in saline habitats (Tester and Davenport, 2003). In fact, supplemental potassium nutrition plays a role in increasing tolerance to salinity of plants such as rice (Bohra and Doerffling, 1993), tomato (Kaya et al., 2001b; Satti and Lopez, 1994; Yurtseven et al., 2005), cucumber and pepper (Kaya et al., 2001a), lettuce and Chinese cabbage (Feigin et al., 1991) and strawberry (Kaya et al., 2003).

Olive (*Olea europaea* L.) tree cultivation is mostly conducted in semiarid regions where the scarcity of water in hot and dry climate requires the use of brackish water, which inevitably results in salt accumulation in soil followed by limited yield of the trees. Olive is considered as having a medium tolerance to salt while the young plants are able to produce new growth in the presence of 150 mM NaCl (Therios and Misopolinos, 1988). It has been shown that three-year old olive plants can withstand salt stress at NaCl concentrations lower than 80 mM during a 90-day culture period (Tattini et al., 1992). Drip irrigation of 18-years-old olive trees with low saline water did not lead to any changes of growth parameters and yield (Melgar et al., 2009). Chartzoulakis et al., (2002) reported significant effects of NaCl salinity on olive cultivars where high salinity (100 and 200 mM) resulted in significant reduction of total biomass. On the other hand, 100 mM supplemental potassium reduced the concentration of Na^+ and increased the concentrations of K^+ in olive leaves (Chartzoulakis et al., 2006).

In the present study, growth parameters, changes in ion concentrations and anatomical changes were studied in *Olea europaea* plants following exposure to salinity in the

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presence of 4 and 8 mM potassium to evaluate salt tolerance of the plant as affected by supplemental potassium.

Materials and Methods

Plant material and growth condition

All of the experiments were conducted in the greenhouse of Gorgan University of Agricultural Sciences and Natural Resources during 2004-2005. One-year old olive *(Olea europaea* L. *var zard roghani*) cuttings were cultivated in pots filled with acid washed sand and irrigated twice per day with 200 ml Hoagland nutrient solution. Salinity treatments were started two weeks after transfer of plants to pots. The experiment was carried out in a completely randomized factorial design. Factor one was salinity in the form of 0, 40 and 80 mM NaCl and factor two was potassium nutrition of 4 mM (in the form of Hoagland solution) and 8 mM (Hoagland solution plus 4 mM KCl). Salinity treatments were started with 20 mM NaCl and increased in 3 steps to 80 mM to avoid osmotic shock. The averages of daily maximum and minimum temperatures in the greenhouse during the growing period were 25 and 18 °C respectively and relative humidities between 60 to 78% were recorded. Plants were harvested after 80 days and used for analyzing growth parameters, ionic contents and anatomical studies.

Concentration of ions

The harvested plants were excised into stems, leaves and roots and the components weighed, dried in an oven for 3 days at 70 ºC, re-weighed and ground for determination of ion composition. Sodium and potassium content was measured by flame photometry (model JENWAY, PEP-7) after digesting about 100 mg of plant material in a mixture of concentrated nitric and perchloric acid (3: 1) at 175 ºC. Chloride content was measured at 450 nm according to the method of Diatloff and Rengel (2001) using Hg $(SCN)_2$ and $FeNO_3$. Total phosphorus was assayed using a UV-Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan) using vanado-molybdate method (AOAC, 1975).

Light Microscopy

Leaf discs (7 mm diameter) were cut with a paper punch along the mid-rib at the central part of mature leaves. Stem segments (1 cm long) were cut about 10 cm above the plant crown. The segments were fixed for 8 h in FAA (37% formalin, acetic acid, 50% alcohol; 10:5:85; v/v/v). The materials were then dehydrated through a TBA (tertiary butyl alcohol) solution series and embedded in paraffin blocks, cross sectioned (15 µm thickness) using a rotary microtome (HM 335 E) and stained with 1% Safranin O and 0.5% Fast-Green (Ruzin, 1999). All sections were examined using an Olympus microscope and photographed. The thicknesses of leaves, spongy and palisade layers and the diameters of cortex and stele were measured using an ocular micrometer.

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Statistical analyses

Statistical analyses of data were carried out using SAS statistical software (SAS Institute Inc., 2001). All data were subjected to analysis of variance and comparisons of means were performed using LSD test.

Result

Growth

Plant growth and dry matter partitioning were significantly inhibited by salinity treatments (Figure 1). Additional potassium treatment could alleviate the detrimental effects of high salinity on plant growth. The higher fresh and dry weights in all plant organs particularly in roots was observed in 8 mM potassium as compared to 4 mM under salinity. Shoot dry weight was more sensitive to salinity than root dry weight and consequently the shoot to root ratio decreased due to salt stress. The 8 mM potassium did not change this ratio markedly. The tissue relative water content was lower in plants grown at high salinity as compared to control treatment and this was increased by additional potassium nutrition.

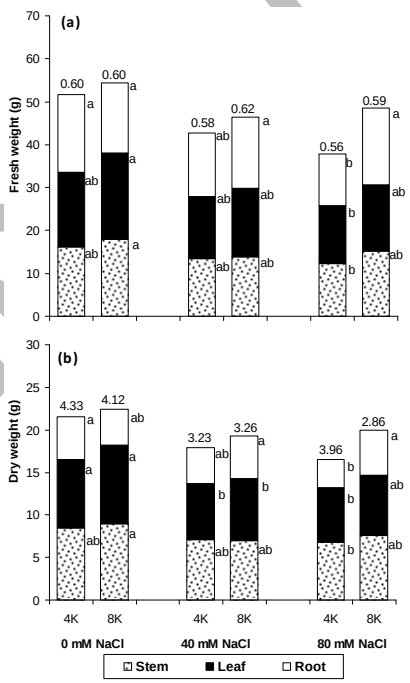

Figure 1. Effect of salinity on fresh (a) and dry (b) weights of leaves, roots and stems of olive plants grown for 80 days salinity treatments with or without application of 4 mM supplementary potassium. Numbers above each column indicate relative water content (a) and shoot to root ratio (b). Columns labeled with different letters indicate significant differences between treatments according to the LSD test ($P<0.05$).

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Concentration of ions in tissues

The concentration of Na^+ and Cl^- was very low in control plants, but increased significantly under salinity in all plant organs. Compared to that of stem and leaves roots displayed higher accumulation of Na^+ and Cl^- ions in all treatments (Figure 2). Under salinity, supplemental potassium treatment decreased the accumulation of Na^+ ion in leaves and stems of plants so that at 8 mM potassium, the concentration of Na^+ in leaves of 40 mM NaCl treatment was about 25% lower compared to 4 mM potassium treatment. Similarly, the concentration of Cl^- ions in leaves decreased significantly by supplemental potassium nutrition.

In the presence of NaCl in the nutrient solution, the concentrations of both P and K^+ decreased in all plant organs particularly in roots (Figure 3). Supplemental potassium nutrition (8 mM) under salinity led to increased accumulation of K^+ ions in roots, stems and leaves. The K^+/Na^+ ratio decreased markedly due to salinity, however supplemental potassium nutrition recovered this ratio to some extent. The observed decrease in P content of leaves and stems under salinity was also alleviated by 8 mM potassium nutrition. The P/Cl^- ratio reduction in tissues due to salinity was affected in the same way by supplemental potassium.

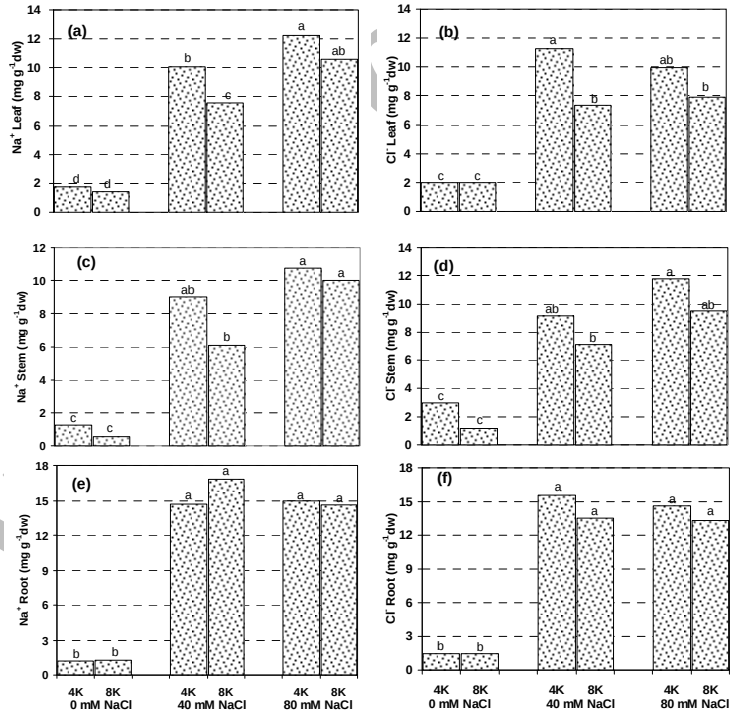

Figure 2. Changes in the concentration of Na^+ and Cl^- in leaves (a, b), stems (c, d) and roots (e, f) of olive plants grown for 80 days salinity treatments with or without application of 4 mM supplementary potassium. Columns labeled with different letters indicate significant differences between treatments according to the LSD test ($P<0.05$).

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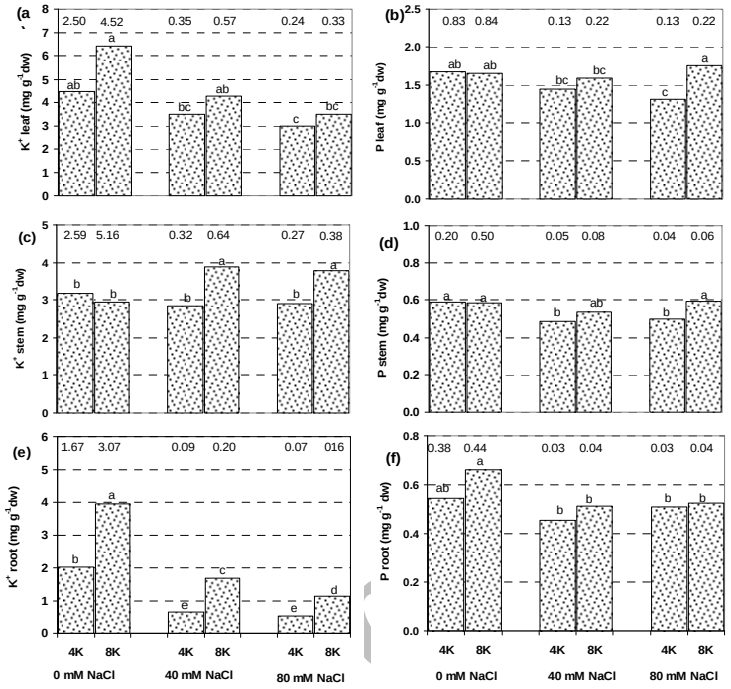

Figure 3. Changes in concentration of K^+ and P in leaves (a, b), stems (c, d) and roots (e, f) of olive plants grown for 80 days salinity treatments with or without application of 4 mM supplementary potassium. Numbers above each column indicate K^+/Na^+ ratio (a, c, e) and P/Cl^- ratio (b, d, e). Columns labeled with different letters indicate significant differences between treatments according to the LSD test ($P<0.05$).

Leaf thickness did not change significantly due to salinity. However, supplemental potassium nutrition increased it by 13% in non-saline conditions. A marked increase in spongy mesophyll thickness due to salt stress was observed after 80 d of treatment (Figure 4 and 6). Supplemental potassium nutrition did not produce any significant changes in spongy mesophyll thickness under non-saline conditions. However, interaction of 8 mM potassium and 80 mM NaCl in the root environment resulted in the reduction of thickness of the spongy mesophyll layer in the leaf. Palisade mesophyll cell length decreased slightly under salinity, but supplemental potassium nutrition increased the palisade mesophyll cell length significantly. Depending on the salinity level, the palisade mesophyll cell length was between 20% to 41% more in 8 mM potassium treatment compared to 4 mM potassium-treated plants (Figure 5 and 6). The ratio of cortex to stele increased in plants grown under 40 and 80 mM NaCl. On the contrary, additional potassium nutrition decreased this ratio in both saline and non-saline conditions.

Discussion

The main objective of this study was to evaluate the possibility of reducing the negative effects of salinity by applications of supplemental potassium in olive plants. Salinity

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decreased plant growth significantly. Supplemental potassium (8 mM K treatment) alleviated the harmful effects of salinity and improved growth parameters. These findings are compatible with those obtained on rice (Bohra and Doerffling, 1993), tomato (Kaya et al., 2001b; Satti and Lopez, 1994), cucumber and pepper (Kaya et al., 2001a) lettuce and chinese cabbage (Feigin et al., 1991) and strawberry (Kaya et al., 2003).

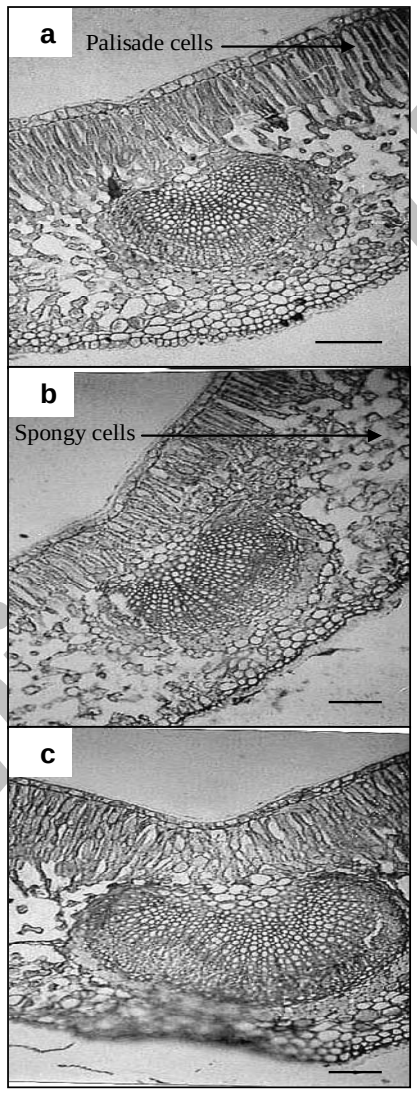

Figure 4. Light microscopic micrographs showing cross sections from mature leaves of olive plants grown for 80 days salinity treatments with or without application of supplementary potassium. Control (a), 80 mM NaCl without supplementary potassium (b) and 80 mM NaCl with 4 mM supplementary potassium (c). Bar =100 µm.

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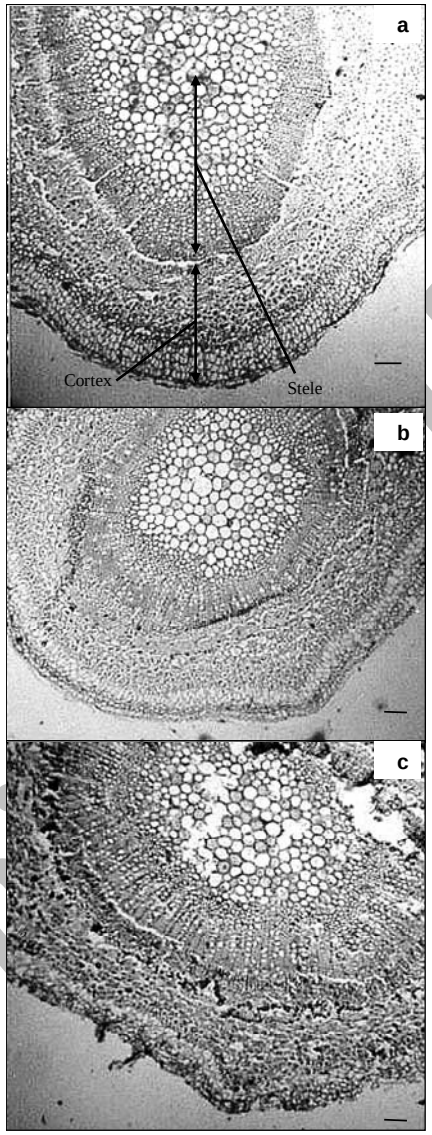

Figure 5. Light microscopic micrographs showing cross sections from stems of olive plants grown for 80 days salinity treatments with or without application of supplementary potassium. Control (a), 80 mM NaCl without supplementary potassium (b) and 80 mM NaCl with 4 mM supplementary potassium (c). Bar =160 µm.

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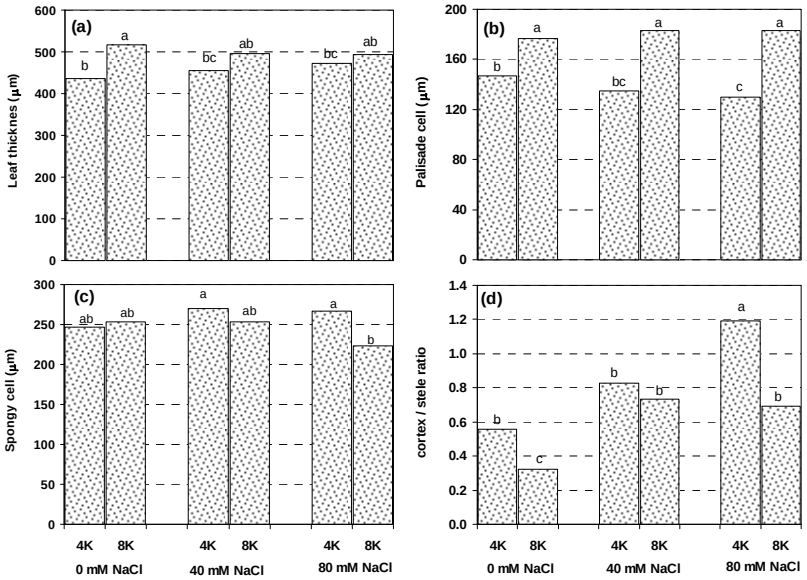

Figure 6. Changes in leaf thickness (a), palisade mesophyll cell length (b), spongy mesophyll thickness (c) and the ratio of stem cortex to stele (d) of olive plants grown for 80 days salinity treatments with or without application of 4 mM supplementary potassium. Columns labeled with different letters indicate significant differences between treatments according to the LSD test ($P<0.05$).

The deleterious effects of salinity on plants are attributed to reduction of water absorption by roots, ion toxicity and disturbed ionic balances (Greenwey and Munns, 1980; Tester and Davenport, 2003). The drastic increase in the concentration of Na^+ and Cl^- in tissues following plant exposure to salinity led to toxicity as it was evidenced by reduced plant growth. It is well documented that salt tolerance in glycophytes is associated with the ability to limit the uptake and/or transport of salt ions (mainly Na^+ and Cl^-) from root to shoot (Greenway and Munns, 1980; Tester and Davenport, 2003). Plants fed with 8 mM potassium had significantly lower Na^+ and Cl^- concentrations in leaves. The obtained results are in agreement with those reported by Satti and Al-Yahyai (1995) for tomato and Asch et al., (1999) for rice. The contents of P and K^+ decreased in both roots and leaves in the presence of NaCl in the root medium that might have increased the stress severity. It has been reported that leaf K^+ concentration is declined by increasing NaCl concentration in the nutrient solution or soil in plants as diverse as maize and barley (Benes et al., 1996) and olive (Tattini et al., 1992; Chartzoulakis et al., 2002). Leaf P concentration, also decreased in tomato (Adams, 1991), pepper and cucumber (Kaya et al., 2003) with increasing NaCl concentration in the nutrient solution. However, supplemental potassium could alleviate the deficiencies of both P and K^+ in all plant organs. This finding is in agreement with Satti and Al-Yahyai (1995) and Kaya et al., (2003) who showed that additional potassium in the nutrient solution corrected P and K^+ deficiencies. Chartzoulakis et al., (2006) have reported potassium supplements (100 mM) reduced the concentration of Na^+ and increased the concentrations of K^+ in leaves under salinity, but decreased photosynthesis on olive.

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However, 100 mM K^+ treatment (compared with 8 mM in our experiment) is too much and may impose potassium toxicity that intensifies the effects of NaCl stress. While the accumulation of toxic ions (Na^+ and Cl^-) did not change in roots, the reduced levels of toxic ion in the leaves and the increase of P and K^+ content by supplemental potassium under moderate salinity resulted in lower toxicity and probably better ionic balances and consequently enhanced growth.

Relative water content was reduced in plants grown under saline conditions and this parameter was partially corrected partly by supplemental potassium. Similarly, Kaya et al., (2003) have reported that leaf relative water content was lower in cucumber and pepper plants grown at high salinity compared to control treatments and it was increased by supplementary P and K^+. In addition, the ratio of cortex to stele in stem increased gradually due to salinity which is indicative of the adverse effects of salt stress on sap transportation via the stele. The size and number of xylem vessels and the stele diameter affect water uptake and transport in plants. Decrease in xylem diameter and area and transpiration rate due to salinity have been reported by several investigators (Baum et al., 2000; Hilal et al., 1998). However, supplementary potassium could recover this ratio and improve sap transport in plants. Additional potassium, probably, could recover relative water content and ameliorate water deficit observed by growing olive plants in saline environment via the decrease of ratio of cortex to stele in stem.

An increase in spongy mesophyll thickness due to salt stress was observed along with a slight reduction in palisade mesophyll thickness. In contrast, additional potassium increased palisade mesophyll thickness drastically. Leaf thickness and the kinds of mesophyll cell influence photosynthetic rates. Increase in palisade mesophyll thickness with higher numbers of chloroplasts may recover reduction in photosynthesis ability due to salinity (Hwang and Chen, 1995; Longstreth and Nobel, 1979). Increased chlorophyll contents due to supplying soil with P and K^+ have been reported in cucumber and pepper plants grown at high salinity (Kaya et al., 2003).

In conclusion, the present study confirms the potential of supplementary potassium to alleviate NaCl-induced growth reduction in olive plant. Obviously, potassium-enriched nutrient solution can significantly improve the parameters affected by high salinity (e.g., fresh and dry weights, relative water content, ionic balances and anatomical parameters).

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