Adaptive Strategies of Olive and Pistachio Seedlings Irrigated with Saline Water in Controlled Conditions

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ABSTRACT

This study aimed to detect possible strategies used by olive (Olea europaea L.) and pistachio (Pistacia vera L.) species under saline conditions to be considered in early selection programs of salt-tolerant genotypes. One-year-old seedlings were irrigated alternatively with ¹/₂ Hoagland nutrient solution and distilled water added with 0, 75, or 150 mM NaCl during 3 months under controlled conditions. Growth, leaf toxicity symptoms, and shoot and root mineral compositions were assessed. Salt induced leaf injury and a significant decrease in growth of both species seedlings. The decrease in shoot elongation was more pronounced on pistachio seedlings, whereas leaf formation and plant fresh weight showed greater reduction on olive plants. Pistachio seedlings developed new axillary shoots and leaves at 75 mM NaCl. The root fresh weight was significantly reduced by salinity in olive, but remained unaffected in pistachio. Both saline treatments induced leaf fall with more pronounced effect on olive plants. Olive salt tolerance may be associated to a better control of Na⁺ accumulation in the leaves and its ability to accumulate potassium in shoots in order to achieve osmotic adjustment. Pistachio seedlings seemed to better control Cl⁻ ion ascension from roots to shoots through salt ion dilution mechanism. The increase in root fresh weight of pistachio seedlings at both salt treatments and the higher ability of this species for uptake of calcium (involved in the regulation of stomata movement) seem to support this hypothesis.

Keywords: Olea europaea L., Pistacia vera L., Sodium chloride, Toxicity symptoms, Water salinity.

INTRODUCTION

Soil salinity is one of the main environmental factors limiting plant growth and productivity. Worldwide, over 800 million hectares of lands are affected by salt (FAO, 2008) and around 20 million hectares are lost annually due to soil salinity (Hassani *et al.*, 2008). Besides the appropriate use of water and soil resources, the adoption of new agricultural technologies, and the use of efficient agronomical practices, the genetic selection of salt-adapted varieties and rootstocks could contribute to alleviate the harmful effects of salts on plants, enhance productivity, and ensure sustainable production. Understanding agronomic and physiological responses of plants to salt and the possible mechanisms they adopt to overcome salt stress is needed before establishment of a selection program for salt-tolerance.

The accumulation of salts in the plant is responsible for metabolic process disturbances. Adverse effects of salt on plants are the results of both osmotic and ionic stresses. Osmotic stress affects growth immediately (Munns and Tester, 2008). The increasing salinity in the root-zone reduces water availability, resulting in a loss of cell

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turgor in plant tissues. High concentrations of salts in irrigation water may lead to a decrease of nutrient uptake due to both ionic competitive interactions and dysfunctions of membrane selectivity (Kozlowski, 1997) causing ionic imbalance (Zhu, 2001; Munns, 2002). The accumulation of Na⁺ and Cl⁻ ions to toxic levels in plants decrease the absorption of K⁺, Mg²⁺ and Ca²⁺ and may explain the reduced growth of species subjected to high concentration of NaCl (Daas-Ghrib *et al.*, 2011; Farissi *et al.*, 2014).

Facing salt stress, plants adopt different strategies to maintain active growth and productivity. Woody plants may adapt to salinity by avoidance and/or tolerance mechanisms (Kozlowski, 1997) to minimize osmotic stress or ionic imbalance and alleviate their adverse effects.

Olive (Olea europaea L.) and pistachio (Pistacia vera L.) are classified as welladapted crops to water and salt stress. Both species can develop well in arid conditions. described olive Literature has as a salt moderately tolerant species (Chartzoulakis, 2005) and pistachio as a species moderately sensitive (Picchioni et al., 1990) to highly tolerant (Behboudian et al., 1986) to salinity. Understanding the specific responses of these species toward salt in irrigation water is of fundamental importance in screening for salt tolerant rootstocks. As woody plants are more sensitive to stress at the first stage of development, the use of young plants for early screening may give more information on the strategies adopted by these plants to overcome the negative effects of salt. The aims of the present work were to study the effect of sodium chloride (NaCl) in the irrigation water on olive (Olea europaea cv. Chemlali) and pistachio (Pistacia vera cv. seedlings in controlled Mateur) environmental conditions and to detect possible specific mechanisms adopted by these species against salt accumulation in their tissues.

MATERIALS AND METHODS

Plant Material and Treatments

Olive and pistachio seeds were harvested from 'Chemlali' variety of O. europaea L. and 'Mateur' variety of P. vera L., respectively, in the orchards of the experimental station of the Olive Tree Institute, situated in Sfax area (Tunisian Southeast, 34° 44 N, 10° 44 E). Germination of seeds was performed in vitro for olive and in laboratory environmental conditions for pistachio. One-year old seedlings having a shoot length of 40±2 cm (for olive) and 14±2 cm (for pistachio) were used for this experiment. First, they were transferred in 300 mL volume plastic pots filled with perlite and previously autoclaved at 120°C for 30 minutes, and irrigated bi-weekly alternating 1/2 Hoagland solutions (Hogland and Arnon, 1938) or distilled water for one month. Then, sodium chloride was added or not to both solutions at two concentrations: 75 mM (4.38 g L^{-1}) and 150 mM (8.77 g L^{-1} ¹). Solutions with each NaCl concentration were considered as a salt treatment while NaCl-free solutions were used as the control treatment. To avoid osmotic shock, each salt concentration was gradually achieved by steps of 25 mM NaCl increases. The pH of the solutions was adjusted to 5.5 before measuring electrical conductivities the using а Conductimeter (WTW Series Cond. 720). The respective Electrical Conductivities (EC) of the 0, 75, and 150 mM NaCl solutions were 0.62, 7.09, and 13.21 mS cm⁻¹.

Plantlets were placed in a growth room under controlled conditions at a temperature of $24\pm2^{\circ}$ C and a relative humidity of $75\pm2\%$. Luminosity was provided by cool white lamps (Philips TLD 58 w/830). The light intensity ranged between 39 and 44 µmol m⁻² s⁻¹ during a 12 h photoperiod. The experiments were conducted during 3 months. For both species, seedlings were arranged in completely randomized design with three replicates of 10 plants per treatment. The lengths (cm) of the stem and axillary shoots having a minimum length of 1 cm, the number of axillary shoots developed, and the number of existing leaves were fortnightly determined. The stem diameter (mm) was measured monthly at 1 cm above the level of the soil. Fresh weights of shoots and roots (after being thoroughly washed) were determined at the end of the experimental period.

Toxicity symptoms were fortnightly monitored by counting the fallen and damaged leaves. These latter were classified according to the extent of necrotic area: healthy leaves; leaf necrosis < 50% of leaf area; leaf necrosis $\geq 50\%$ of leaf area, and totally necrotic leaf (100%).

Mineral Analysis

At the end of the experimental period, fresh shoots and roots were dried at 65-70°C for approximately 72 hours (until weight stabilization) to calculate the Dry Weight (DW). Then, they were calcinated firstly at 200°C for 30 minutes before gradually raising temperature to 450 °C until the appearance of gray or white ash. Nitric acid (HNO₃) 1N was used for mineralization. Sodium (Na⁺) and potassium (K⁺) contents were determined using a flame emission photometer (Jenway). Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) contents were determined using an atomic absorption spectrophotometer (A Analyst 300). Chloride (Cl⁻) content was determined using a pH meter Ionimeter (pH/ISE) provided by a specific ion electrode.

Statistical Analysis

Data were submitted to one–way ANOVA using the SPPS software for Windows version 20. Duncan's multiple range tests were used for mean comparison at $P \le 0.05$.

Plant Growth

Effects of salt on growth parameters were previously reported on olive (Gucci and Tattini, 1997; Kchaou et al., 2010; Bader et al., 2015) and pistachio (Picchioni et al., 1990; Benmahioul et al., 2009). This study showed variable responses of olive and pistachio seedlings to salt treatments. Salt in irrigation water induced a reduction and even an inhibition of growth. Shoots started to elongate before showing a significant reduction and even an inhibition of growth (Figure 1-A). Reduction of shoot elongation was higher in *P. vera* than in *O. europaea*. Despite this negative effect, pistachio seedlings irrigated with 75 mM NaCl water resumed a slight growth at the end of the experimental period (Figure 1-B). Overall, shoot diameter and number of axillary shoots of both species were not significantly affected by salt (Figures 1-E and -F). Leaf formation suffered a more pronounced decrease in olive than in pistachio. The total number of olive seedling leaves decreased significantly compared to the control after 45th and 31st days at 75 and 150 mM NaCl, respectively (Figure 1G and H). For P. vera, this decrease was lower and occurred later (from the 59th and 45th days of treatment, respectively). Moreover, 20 and 2% of pistachio seedlings treated with 75 and 150 mM NaCl, respectively, formed new leaves and shoots at the end of the treatment period (Figure 1H and D). Similar results were reported by Chelli-Chaabouni et al. (2010) for *P. atlantica in vitro*. This species seemed to have saved energy for its further use to resume growth. Both salt treatments had no effect on the number of axillary shoots of pistachio but resulted in a significant decrease of this parameter in olive (Figures 1-C and -D). Only 2% of olive seedlings maintained their growth at 75 mM NaCl.

For all studied seedlings, the fresh weight of shoots was more affected by salt than that

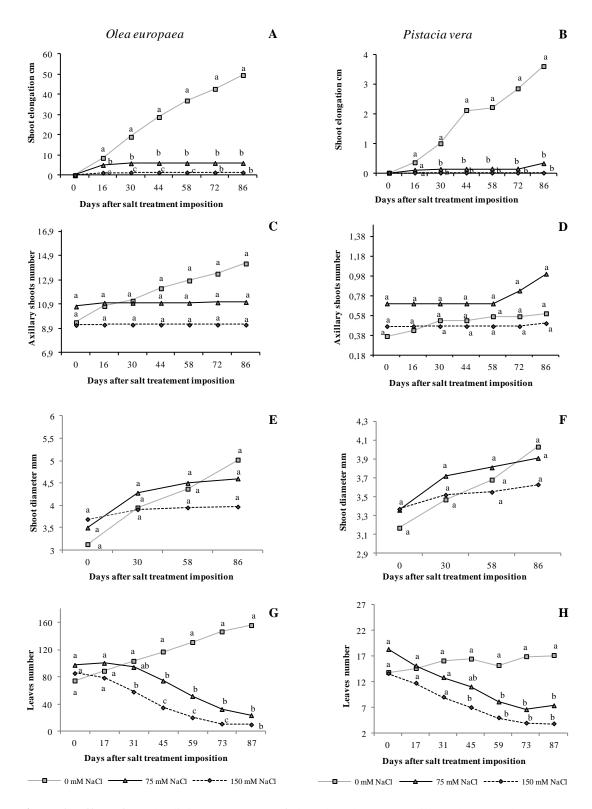


Figure 1. Effect of NaCl salinity on averages of shoot length (A, B), axillary shoot number (C, D), shoot diameter (E, F), number of leaves (G, H) of *O. europaea* and *P. vera* during salt treatment.

of roots (Figure 2). These results are in agreement with those previously described on O. europaea (Chartzoulakis et al., 2002), P. vera (Karimi et al., 2009) and other species (Rochdi et al., 2005). Fresh weight of shoots (stems and leaves) decreased significantly by salt. Compared to the control, this decrease was 87% in olive and 71% in pistachio at 150 mM NaCl. The response of the two studied species was different in terms of root fresh weight that was reduced by salt in olive while it remained unaffected and even recorded a slight increase in pistachio (Figure 2). Similar results were reported on P. vera (Benmahioul et al., 2009) and P. atlantica cultured in vitro (Chelli-Chaabouni et al., 2010). Benmahioul et al. (2009) thought that pistachio species may stimulate root growth under salinity to limit negative salt effects.

Toxicity Symptoms

Seedling growth reduction was associated with leaf toxicity symptoms such as necrosis and leaf drop (Figure 3). Our results showed that both saline treatments induced leaf fall with more pronounced effect on *O*.

europaea. The rate of fallen leaves from total existing leaves was significantly higher than that of the control for this latter species. Leaf fall under salt conditions was previously described on olive and pistachio species (Karimi et al., 2009; Kchaou et al., Munns (2002)explains 2010). this phenomenon as a result of the building up of salt in the leaves to toxic levels, leading to inhibition of photosynthesis and activation of senescence processes. Leaf fall was thought to be an effective mechanism adopted by olive trees to reduce the concentrations of Na⁺ and Cl⁻ in the cytoplasm of active leaves (Gucci and Tattini. 1997). At the end of the experimental period, the level of necrotic leaves reached 88.5% in pistachio and 70.3% in olive at 150 mM NaCl. The observed symptoms were leaf chlorosis, rolling, and browning. Generally, the completely necrotic leaves of pistachio seedlings remained attached to the stem. This was not the case for olive seedlings that showed higher numbers of necrotic leaf fall.

Mineral Analyses

Salinity induced a significant increase in sodium and chloride contents in organs of all seedlings (Table 1). The Na^+ and Cl^- ions concentrations were proportional to the level

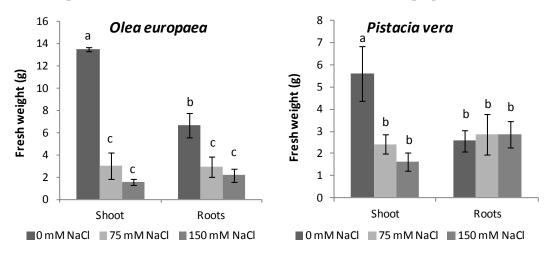


Figure 2. Effect of NaCl on averages of shoot and root fresh weights of *O. europaea* and *P. vera*. Bars having the same letter are not significantly different at $P \le 0.05$.

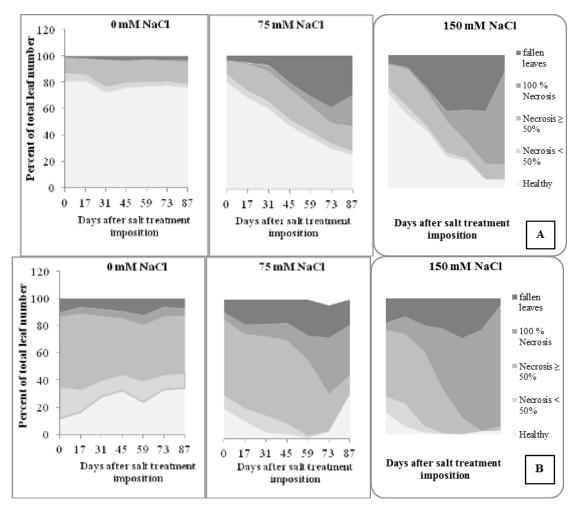


Figure 3. Symptoms on leaf morphology of O. europaea (A) and P. vera (B) for the 3 treatments.

of salinity in irrigation water. However, seedlings accumulated more Na⁺ than Cl⁻ ions in their tissues. At 150 mM NaCl, the Na⁺ content reached 11 and 5 fold of the controls, respectively, in olive and pistachio shoots. In the roots, these respective proportions were 8 and 4 fold of the controls. The increase in chloride content in response to salt treatments did not exceed 2.6 and 2.2 fold in shoots and roots, respectively. For both species, the Na⁺ ions were uniformly distributed in shoots and roots. This was not the case for Cl⁻ ions, where accumulation was found to be higher in shoots than in roots. These results are similar to those of Hokmabadi et al. (2005) and Chelli-Chaabouni et al. (2010) on pistachio. The accumulation of salt ions in the shoots indicates the inclusive trait of the studied species. At the high salt concentration, Na⁺ was transported and accumulated in the aerial parts, which would be the cause of the observed symptoms of toxicity. The accumulation of salt ions in the leaf vacuole may be used by these species as a mechanism of salt tolerance (Bongi and Loreto, 1989; Chartzoulakis, 2005; Bader et 2015). This accumulation ensures al., osmotic adjustment required to maintain leaf turgor and activity of metabolic sites in the cytoplasm. The comparison of olive and pistachio responses revealed a higher tendency of olive seedlings to accumulate salt ions in the roots at 150 mM NaCl. At this salt concentration, olive plants appeared to start controlling the amount of Na⁺

Ion	NaCl (mM)	Olive		Pistachio	
		Shoots	Roots	Shoots	Roots
[Na ⁺]	0	$0.25\pm0.07g^*$	0.36±0.16g	0.54±0.15g	0.61±0.03g
	75	$2.27 \pm 0.87 cd$	2.09±0.14de	1.70±0.32ef	1.43±0.02f
	150	$2.65 \pm 0.08 abc$	3.03±0.12a	2.83±0.13ab	2.43±0.07bcd
[Cl ⁻]	0	$0.08\pm0.01g$	0.09±0.01ef	0.10±0.01ef	$0.08 \pm 0.00 g$
	75	$0.13\pm0.01\text{d}$	0.10±0.00e	0.11±0.00e	0.09±0.00fg
	150	$0.21\pm0.01b$	$0.20 \pm 0.00 b$	0.22±0.00a	0.18±0.01c
$[K^+]$	0	$1.50\pm0.06b$	1.05±0.09c	2.08±0.27a	1.24±0.08c
	75	$1.18\pm0.07c$	0.51±0.19d	1.21±0.15c	0.62±0.03d
	150	$1.27 \pm 0.24c$	0.39±0.03d	1.21±0.02c	0.58±0.06d
K ⁺ /Na ⁺	0	6.20 ±1.31a	3.51±2.21b	4.12±1.48b	2.03±0.17c
	75	0.56 ±0.17cd	0.25±0.10d	0.72±0.06cd	0.43±0.01cd
	150	0.48±0.09cd	0.13±0.01d	0.43±0.03cd	0.24±0.03d
[Ca ²⁺]	0	0.43±0.02bcd	0.43±0.05bcd	0.68±0.05a	0.63±0.06a
	75	0.33±0.04e	0.39±0.07cde	0.48±0.05bc	$0.50 \pm 0.02b$
	150	0.02±0.01g	$0.19 \pm 0.06 f$	0.35±0.03de	0.41±0.05cde
Ca ²⁺ /Na ⁺	0	1.78±0.39a	1.44±0.90b	$1.34 \pm 0.41b$	1.03±0.13b
	75	0.15±0.04c	0.19±0.03c	0.29±0.04c	0.35±0.02c
	150	0.01±0.00c	0.06±0.02c	0.12±0.01c	0.17±0.01c
[Mg ²⁺]	0	0.11±0.01f	0.33±0.08a	0.25±0.03bc	0.22±0.01cd
	75	0.11±0.02f	0.28±0.02ab	0.13±0.02ef	0.18±0.00de
	150	0.17±0.08de	0.31±0.05a	0.16±0.03ef	0.16±0.00de

Table 1. Effect of NaCl on the sodium, chloride, potassium, calcium and magnesium content (% dry weight), K^+/Na^+ and Ca^{2+}/Na^+ ratios in shoots and roots of *O. europaea* and *P. vera.*^{*a*}

^{*a*} Values having the same letter are not significantly different at P < 0.05 according to Duncan's multiple range tests.

transported to the leaves. This control seems to be less effective in pistachio seedlings which showed higher accumulation of Na⁺ ions in shoots than in roots at 150 mM NaCl.

Chloride content of shoots and roots of *P. vera* seedlings treated with 75 mM NaCl was not influenced by saline treatment; this species seems to better control Cl⁻ ion ascension from root to shoot. This control may be possible through salt ion dilution mechanism that allows *P. vera* to maintain a normal water absorption and dilute chlorides to avoid reaching toxic levels in the aerial part. In the case of olive seedlings, statistical data analysis revealed higher chloride content in shoots than in roots at 75 mM NaCl, but, at 150 mM NaCl, this ion was equally distributed in both plant parts.

Chartzoulakis et al. (2002) and Kchaou et al. (2010) described a higher accumulation of chlorides in the roots than in the shoots of olive plants after 150 days of imposing salt treatments. They suggest an activation of exclusion mechanism (to limit the transport of toxic ions to the shoots) that operates only at low and moderate levels of salinity (up to 50 mM NaCl) and was ineffective at 150 mM NaCl (Chartzoulakis et al., 2002). The high rate of leaf fall observed in olive seedlings may be the result of high level of salt ion accumulation in leaves. This suggestion is also supported by Kchaou et al. (2010) who found high concentrations of Cl⁻ and Na⁺ in fallen olive leaves.

In both olive and pistachio seedlings, potassium ion (K^+) contents were higher in

shoots than in roots in all tested treatments. Irrigation with saline water induced a significant decrease in K⁺ content in shoots and roots of both species with a more pronounced reduction in roots (Table 1). These results are in accordance with those found by Chartzoulakis (2005) and Kchaou et al. (2010) on O. europaea and Walker et al. (1987) and Chelli-Chaabouni et al. (2010) on *P. vera*. The decrease of K^+ concentration in olive roots and the maintenance of high K^+ levels in young leaves is a mechanism by which olive plants achieve ionic balance at high Na⁺ concentrations in the roots (Chartzoulakis et al., 2002). This strategy is also adopted by salt-tolerant grapevine genotypes (Hamrouni et al., 2011). This mechanism seemed to be more effective in olive than in pistachio at the tested conditions. The shoots of P. vera were more affected by salt than those of O. *europaea.* Compared to the control, the K^+ content reduction at 150 mM NaCl was of 41.8% in shoots and 53.2% in roots of P. vera. This reduction did not exceed 15.3% in shoots and 62.8% in roots for O. europaea. This result may also be explained by the higher level of necrotic leaf fall recorded in olive compared to pistachio seedlings. Since analyses were only made on leaves that remained on the stem at the end of the treatment period, the obtained data represent more necrotic leaves in pistachio shoots.

As a result of high Na+ uptake, the K+/Na+ ratio decreased sharply with increasing salt concentration to attain values below 1. Under saline conditions, this ratio was no significantly different neither between species nor between the organs of each studied species (Table 1). This ratio reduction below control was 82.5 to 92.2% in shoots and 78.8 to 96.2% in roots. These results indicate the failure of the K–Na selectivity mechanism caused by Na⁺ ions accumulation. Maintaining a high K⁺/Na⁺ ratio in the cytoplasm was found to be a trait of salt–tolerant olive cultivars (Gucci and Tattini, 1997).

Sodium chloride induced a significant decrease in calcium content in shoots and

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roots of both studied species, except that of O. europaea roots treated with 75 mM NaCl that showed no significant change (Table 1). This decrease may be explained by a limitation of the Ca²⁺ ions uptake due to Na⁺ and Cl⁻ accumulations in the plant tissues (Gucci and Tattini, 1997; Hokmabadi et al., 2005; Daas-Ghrib et al., 2011). The comparison of calcium content in shoots and roots showed a higher plant tendency to accumulate this cation in roots. This phenomenon was more pronounced in O. europaea at 150 mM NaCl. It may be a strategy used by plants to ensure ionic balance in the roots since potassium was transported to the shoots. The accumulation of calcium in the roots is related to its role in limiting the toxic effect of Na⁺ on the integrity of the plasma membrane (Chartzoulakis, 2005). Calcium contents of shoots and roots were higher in pistachio than in olive tissues in all treatments. At 150 mM NaCl, Ca²⁺ content was of 95.3 and 55.8% below to the control in olive shoots and roots, respectively. In case of pistachio seedlings, this reduction was 48.5% in the shoots and 34.9% in the roots. Besides, pistachio was more effective than olive in maintaining a high level of calcium in the shoots at 150 mM NaCl. It may be a selfprotecting response of this species to combat detrimental consequences resulting from imbalance of K^+ and Na^+ as suggested by Xiang *et al.* (2008). The involvement of Ca^{2+} ions in the regulation of stomata movement leads to higher water retention (Sanders et al., 1999; Xiang et al., 2008). Salt ions in the cells are diluted and maintained at concentrations below a cytoplasmic toxic level. This may support our findings concerning the increase of root fresh weight pistachio seedlings at both salt of concentrations.

The Ca^{2+}/Na^+ ratio values of the control plant shoots and roots were above 1 at the end of the experimental period with significantly higher value in olive shoots. It dropped significantly with salt treatments to reach similar levels in both species shoots and roots. The shoot ratio values reached 8.5

and 0.6% of the control in olive and 21.7 and 9% of the control in pistachio, respectively, at 75 and 150 mM NaCl. In the roots, this ratio reached 4.2% of the control in olive and 16.6% in pistachio at 150 mM NaCl. The decrease of Ca^{2+}/Na^{+} ratio with increase in Na⁺ content is a common response of many species to salinity (Camara-Zapata *et al.*, 2004; Chelli-Chaabouni *et al.*, 2010). According to these ratio data, pistachio seedlings seemed to be ranked at a little higher salt tolerance level than olive seedlings.

The magnesium content of olive and pistachio seedlings differed significantly between the two studied species, in all treatments. Whereas shoot Mg^{2+} content was significantly lower than that of roots in olive seedlings, pistachio Mg²⁺ content was similarly partitioned in shoots and roots. Salt treatments induced stabilization (at 75 mM NaCl) and a significant increase (at 150 mM NaCl) of this ion content in olive shoots. Inversely, magnesium content in pistachio shoots showed a significant reduction at both salt treatments. In the roots of all seedlings, the Mg^{2+} content was not significantly influenced by salinity of the irrigation solution. These results corroborate those reported by Grattan and Grieve (1999), who described different effects of salt on leaf Mg content according to species. As magnesium plays a major role in chlorophyll biosynthesis metabolic and process unfolding of photosynthesis (Cakmak and Yazici, 2010), the significant reduction of Mg²⁺ content in *Pistacia vera* shoots at 75 and 150 mM NaCl may be the result of functional disturbance of the photosynthetic apparatus. As supported by other authors (Grattan and Grieve, 1999; Hamrouni et al., 2011) magnesium may not play a critical role in the adaptation of plants to salt stress.

In conclusion, both olive and pistachio species responded to salinity by growth reduction. This decrease was less important in pistachio seedlings, which showed higher ability to maintain water absorption necessary for salt ion dilution and resumption of growth capacities.

ACKNOWLEDGEMENTS

The authors thank the Ministry of Agriculture and the Ministry of Higher Education and Scientific Research for their financial support of this study. They are grateful to Mr. Nabil Soua for his technical assistance.

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راهبردهای ساز گاری نهال زیتون وپسته آبیاری شده با آب شور در شرایط کنترل شده

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چکیدہ

هدف این یژوهش شناسایی راهبردهایی بود که در شرایط شور مورد استفاده گونه های زیتون (Olea europaea)و يسته (Pistacia vera)قرار ميگيرند و ميتوان آن ها را در مراحل اوليه برنامه گزینش ژنو تیپ های مقاوم به شوری به کار بست. به این منظور، نهال های یکساله این دو گیاه در شرایط کنترل شده و طی سه ماه به طور یک در میان با محلول غذایی نیم-هو گلند و آب مقطر حاوی مقادیر صفر، ۷۵، و ۱۵۰ میلی مول NaCl آیباری شدند. در این دوره، رشد، علایم مسمومیت در برگ و محتوای کانی های شاخساره و ریشه اندازه گیری شد. شوری باعث صدمات برگ و کاهشی معنادار در رشد هر دو نهال شد. کم شدن طول شاخساره در نهال يسته مشهود تر بود، در حاليکه تشکيل برگ و وزن تر گیاه در نهال زیتون کاهش بیشتری داشت. در تیمار ۷۵ میلی مول NaCl، روی نهال های یسته برگ ها و ساقه های جانبی جدیدی پدیدار شد. وزن تر ریشه زیتون در تیمار شوری به طور معناداری کم شد ولي در نهال يسته تغييري نکرد. هر دو تيمار شوري باعث ريزش برگ شدند و اين اثر در زيتون شدیدتر بود. تحمل به شوری در زیتون ممکن است به علت کنترل بیشتر تجمع ⁺Na در برگ ها و توانایی زیتون در انباشت پتاسیم در شاخساره به منظور موفقیت در تنظیم اسمزی باشد. به نظر می رسد که نهال های پسته، فرایند بالارفتن بون ⁻CIاز ریشه به شاخسار را با استفاده از سازو کار رقبق کردن بون بهتر کنترل کردند. این فرضیه با در نظر گرفتن افزایش وزن تر ریشه در نهال های یسته در هر دو تیمار شوري و توانايي بيشتر اين نهال براي جذب كلسيم (كه در تنظيم حركات روزنه ها نقش دارد) تقويت مي شود.