Growth, photosynthesis and pollen performance in saline water treated olive plants under high temperature

Georgios C. Koubouris, Nikolaos Tzortzakis, Nektarios N. Kourgialas, Marina Darioti, Ioannis Metzidakis

Hellenic Agricultural Organization "Demeter", Institute for Olive Tree and Subtropical Plants, Chania, Greece

Abstract

Olive cultivation in hot arid areas is hindered by the scarcity of irrigation water. The exploitation of saline water has been proposed as a solution to partially cover plant water demands. This paper presents the effects of salinity [0, 60 and 120 mM sodium chloride (NaCl)] on physiological and reproductive functions of cultivars *Koroneiki* and *Amphissis* in a closed hydroponic system. Shoot growth was markedly reduced in high salinity dose in *Amphissis* (–81%) and *Koroneiki* (–75%). The photosynthetic rate was significantly reduced at 120 mM NaCl for both cultivars, as well as chlorophyll and carotenoids content (43% and 44%, respectively). The Na+ content in all plant parts increased in both salinity doses especially in *Amphissis* while K concentration decreased for both cultivars. Inflorescences in *Amphissis* were severely damaged due to salinity. Consequently, pollen sampling and *in vitro* germination study was only feasible for *Koroneiki*. Indeed, *Koroneiki* pollen germination was reduced at 60 mM NaCl (–42%) and at 120 mM NaCl (–88%). Pollen tube length was also reduced by 15% and 28% for the middle and high salinity dose, respectively. The results of the present study indicate that *Amphissis* is more sensitive in high salinity doses compared to *Koroneiki* and that reproductive functions are severely affected by salinity.

Introduction

Agriculture in hot arid areas is hindered by the scarcity of irrigation water. The exploitation of saline water has been proposed as a solution to partially cover plant water demands. Most of the world's olive production is situated in the Mediterranean region and the olive is considered to be the major tree crop in this area.[1] Compared to other tree crops, the olive tree is moderately tolerant to salinity. Olive cultivation often occurs in locations that are unsuitable for other crops, due to summer drought and lack of good quality water for irrigation, which leads to salinity building up in the soil. The conflicting demands for water between agriculture, civil use and tourism, all reaching higher levels, in late spring, summer, and early autumn, when water is less abundant; lead to an over-pumping of groundwater which in turn generates saltwater intrusion in several agricultural areas. Taking into consideration that olive cultivation is more and more supplemented with irrigation, salinity due to saltwater intrusion is becoming a major problem on the yield of olive crop.[2]

In general, salinity is an environmental stress that limits growth and development in plants. Various effects of salinity on olive tree have been demonstrated.[3] Shoot growth is affected more than root growth under a saline environment, resulting in an increased root-shoot ratio.[4] There are also several genotypic variations for salt tolerance among the cultivars.[5]

In recent years, many studies employ hydroponic culture to study the effects of salinity on crops,[6] in which the experimental process can be controlled in a more appropriate way than in field applications, excluding the plant-soil interaction interference. However, according to our best knowledge, there are limited studies dealing with olive trees in hydroponics and indeed no studies employed NFT system, which is considered suitable method for nutrition studies.[7] Nevertheless, a number of unresolved issues regarding the impact of salinity on olive trees still exist.

Under Mediterranean conditions, salinity stress commonly occurs simultaneously with other environmental constrains such as high temperature and high solar irradiance.[8] High temperature reduces photosynthetic and pollen performance of olive,[9,10] while the impact of solar irradiance is considered as more complex due to qualitative components *i.e.* spectrum composition and light intensity. Aim of the present study was to investigate the effects of two NaCl salinity levels under enhanced temperature on physiological and reproductive functions of two olive cultivars of major importance for Greece – olive oil cv. *Koroneiki* and table olive cv. *Amphissis*.

Materials and Methods

Plants of *Koroneiki* and *Amphissis* were developed in a hydroponic system (Nutrient Film Technique-NFT) in an unheated glasshouse with a north-south orientation of the Institute of Olive Tree and Subtropical Plants of Chania. The average minimum and average maximum temperature in the greenhouse were 18 and 38.8ºC, respectively. Each plot (three replicate plots per salinity treatment per cultivar) consisted of a separated (twin trough with a 3% slope) NFT system containing at least 10 plants. Two-year-old olive trees were fed with a complete nutrient solution.[11]

Plants were grown for 6 months with a basic nutrient solution. Plants were subjected to 60 mM and 120 mM of NaCl which were added to the basic solution starting on the 2nd week after transplanting. To avoid osmotic shock NaCl was applied in stepped up 4-days increments of approximately 20 mM until the final level was reached. The nutrient solution was renewed regularly to avoid phenomena of toxicity or deficiency. The setpoints for pH and EC were 6 and 6 $dS \cdot m^{-1}$, respectively.

During the cultivation period (6 months), the consumption of nutrient solution from the cultivars was recorded on a monthly basis. For the determination of Na and K, pooled samples of roots, young shoots, old shoots, young leaves and old leaves from 3 plants were taken, dried at 65ºC for 48h, and then grounded. After extraction with diluted nitric acid for 24 h, Na

Correspondence: Georgios Koubouris, Hellenic Agricultural Organization "Demeter" (formerly, NAGREF - National Agricultural Research Foundation), Institute for Olive Tree and Subtropical Plants, Agrokipion, 73100 Chania, Greece.
E-mail: koubouris@nagref-cha.gr

Key words: *Olea europaea L.*; photosynthesis; pollen germination; salinity.

Acknowledgements: this work was supported by General Secretariat for Research and Technology in the framework of Greek-Egyptian Bilateral Cooperation 2006-2008 (contract no. GSRT-271-e).

Contributions: GCK, experimental plan, experimental work, writing the paper; NT, experimental work, writing the paper; NNK, manuscript writing, statistical analysis; MD, experimental work; IM, experimental work supervision.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 26 May 2015.
Revision received: 22 June 2015.
Accepted for publication: 22 June 2015.

International Journal of Plant Biology 2015; 6:6038
doi:10.4081/pb.2015.6038

and K were determined using flame photometry (PFP-7, Barloworld Scientific T/As Jenway, Gransmore Green, UK). Regarding the plant physiology parameters that were studied, new vegetation (cm/plant) and consumption of nutrient solution (Lt) were measured for comparing the tolerance of the two cultivars in the various salinity treatments.

Additionally, gas exchange measurements were made at the end of the experimental period. Ten leaves for each treatment were used to measure photosynthetic rate, stomatal conductance and intercellular CO_2 concentration (*Ci*) using a portable gas exchange system (LI-6400, Li-Cor Biosciences, Lincoln, NE, USA).

In order to assess pollen performance of plants grown in saline culture medium, pollen was collected from freshly opened flowers and subsequently incubated at room temperature (~22°C) in the dark for 24 h, in a growth chamber (Kottermann 2770, D3162; Hanigsen, Germany) before counting pollen germination and pollen tube length. Throughout the experiment, pollen was cultured on solid medium consisting of 0.8% (w/v) agar, 15% (w/v) sucrose, 100 ppm boric acid and 60 ppm tetracycline hydrochloride, according to Koubouris *et al.*[10] Pollen germination was evaluated on five petri dish fields containing over 50 pollen grains for each treatment. Pollen tube length was measured for approximately 60 pollen tubes for each treatment.

Data were analyzed using SPSS (SPSS Inc., Chicago, USA) and were subjected to one-way analysis of variance (ANOVA). Significantly different means were calculated at $P \leq 0.05$ using least significant difference (LSD) test.

Results and Discussion

Vegetative growth of both olive cultivars was significantly affected by high salinity dose (120 mM NaCl). Indeed, overall new shoot growth was reduced both for *Koroneiki* (–75%) and *Amphissis* (–81%) (Figure 1A). However, the results of the present study indicated that *Koroneiki* may grow sufficiently at mild salinity as there were no differences among 60 mM NaCl and control treatments. In contrast, a major reduction of shoot growth (–65%) even at mild salinity (60 mM NaCl) indicates higher sensitivity of *Amphissis* under salinity conditions. Shoot elongation was reduced by salinity (up to 80 meq l^{-1} NaCl) in *Koroneiki* but was unaffected in *Amphissis* in a previous study.[12] In fact, different salinity doses were then tested and shoot elongation was measured in four selected shoots per plant, in contrast with the present study where total plant shoot growth was monitored. Shoot elongation was also affected by salinity in other olive varieties.[2]

Absorption of nutrient solution from the plants was studied as an indicator of plant nutrition functionality, since it is generally well established that saline conditions limit the vegetative development of olives, mainly as a result of interference with the osmotic balance in the root system zone.[13] Significant reduction in the absorption of nutrient solution from the plants was recorded at high salinity dose (120 mM NaCl) both for *Koroneiki* (-86%) and *Amphissis* (–85%) (Figure 1B). Mild salinity dose (60 mM NaCl) had no effect for *Koroneiki* but suppressed nutrient absorption for *Amphissis* (–64%), which is directly related with the reduced shoot elongation and/or increased sensitivity to salinity. Reduced water absorption by olive plants grown in sand-perlite (1/1) culture in a saline medium was also reported by Therios & Misopolinos.[12]

The antagonistic role of Na^+ and K^+ and the negative effect of salinity on plant nutrition were confirmed by plant tissue analysis. The Na^+ content increased in both salinity doses compared to the control plants (Table 1). Indeed, Na accumulation was higher in *Amphissis* compared to *Koroneiki* in all plant parts. It was previously shown that salinity induces detrimental effects by specific toxic accumulation of chloride and sodium ions in the leaves.[14] In the present study, the Na accumulation was increased in salinity treatments in all plant tissues – roots, stems, shoots, leaves – in both cultivars, in agreement with Melgar *et al.*[15] For both *Koroneiki* and *Amphissis*, it was observed that the higher the salinity dose the higher the reduction of K concentration in plant tissues (Table 1). Similar effect was reported by several studies (*e.g.* Chartzoulakis *et al.*[16]) while, in contrast, higher K accumulation in salt-stressed olive leaves was reported by Melgar *et al.*[15]

The leaf photosynthetic rate was signifi-

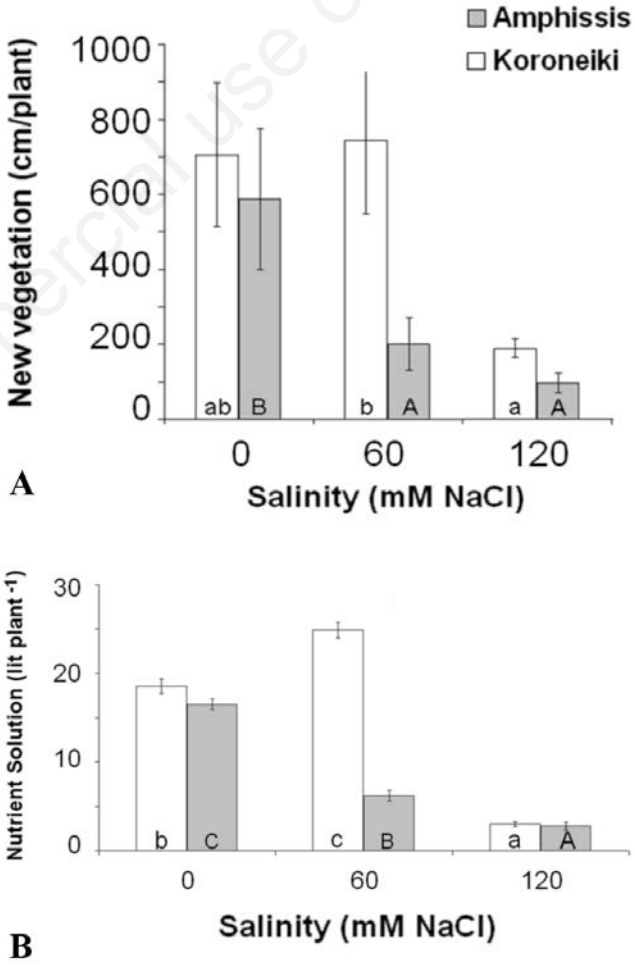

Figure 1. Influence of NaCl salinity (60 mM and 120 mM) on shoot growth of hydroponically grown olive tree *Koroneiki* and *Amphissis* in NFT (A) and on total consumption of nutrient solution of hydroponically grown olive tree *Koroneiki* and *Amphissis* in NFT (B). Each bar is mean ± standard error for each treatment. Bars with the same letter were not significantly different at P<0.05 [LSD test; n=10 (A) and n=3 (B)].

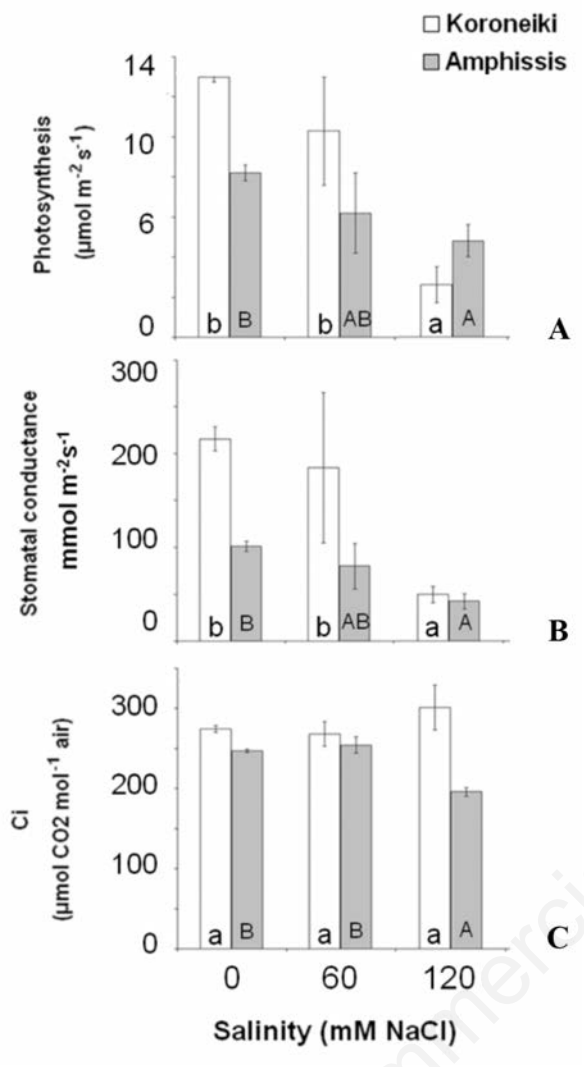

Figure 2. Influence of NaCl salinity (60 mM and 120 mM) on (A) photosynthesis rate, (B) stomatal conductance, and (C) substomatal CO_2 concentration (Ci) of hydroponically grown olive tree *Koroneiki* and *Amphissis i*n NFT. Each bar is the mean ± standard error for each treatment. Bars with the same letter were not significantly different at P<0.05 (LSD test, n=10).

cantly reduced at 120 mM NaCl for both cultivars with greater effects observed in *Koroneiki* (Figure 2A). This result is in agreement with Loreto *et al.*[17] who reported that when olive trees exposed to salt stress, cultivars with inherently high photosynthesis showed the highest photosynthetic reductions. However, sufficient carbon assimilation was retained at intermediate salinity level (60 mM) for both cultivars compared to reference values reported for olive at normal conditions.[18,19]

Besides photosynthetic rate, in both cultivars, stomatal conductance was influenced by salinity in a very similar way (Figure 2B). Specifically, the reduction on stomatal conductance declined to high salinity for *Koroneiki* (–78%) and for *Amphissis* (–60%) which is in accordance with previous studies.[16] In the present study, salinity (120 mM) significantly reduced intercellular CO_2 concentration (*Ci*) of *Amphissis*, however, no such effect of either salinity doses was observed for *Koroneiki* (Figure 2C). These results show that the reduction of photosynthesis in salt-stressed *Koroneiki* leaves is attributable to stomatal resistance, while for *Amphissis* both stomatal and mesophyll resistances are involved as it has been also reported previously for other olive cultivars.[20] The simultaneous existence of biochemical limitations to photosynthesis such as carboxylation rate and efficiency is also common in salt-stressed olive leaves.[20] Impairment of the photosynthetic apparatus by salinity was also indicated by a reduction in chlorophyll and carotenoids content (43% and 44%, of the controls respectively at 120 mM NaCl; data not shown).

Inflorescences in *Amphissis* were severely damaged due to salinity. Consequently, pollen sampling and *in vitro* germination study was only feasible for *Koroneiki*. This also highlights the increased sensitivity to salinity damage of *Amphissis* compared with *Koroneiki* as

Table 1. Influence of NaCl salinity (60 mM and 120 mM) on K and Na content in different plant tissues of hydroponically grown olive tree *Koroneiki* and *Amphissis* in NFT. A composite sample from 4 plants was analyzed for each case.

Salinity (mM NaCl)	Variety	Element	% dw				
			Root	Young leaves	Old leaves	Young shoots	Old shoots
0	Koroneiki	K	1.58	2.05	2.00	1.68	0.96
60	Koroneiki	K	0.67	1.73	1.38	1.25	0.99
120	Koroneiki	K	0.70	1.20	1.03	0.92	0.81
0	Amphissis	K	0.31	2.53	2.17	2.72	1.78
60	Amphissis	K	0.49	2.29	1.73	2.47	1.43
120	Amphissis	K	0.35	1.78	1.20	1.20	0.57
0	Koroneiki	Na	0.27	0.24	0.25	0.31	0.20
60	Koroneiki	Na	0.27	0.44	0.57	0.39	0.36
120	Koroneiki	Na	1.70	1.33	1.87	1.56	1.05
0	Amphissis	Na	0.08	0.19	0.16	0.25	0.21
60	Amphissis	Na	0.71	1.19	1.16	1.76	1.09
120	Amphissis	Na	1.01	1.96	2.34	2.28	0.78

the reproductive phase of the tree, being more sensitive and susceptible to stresses, was more damaged compared to the vegetative part of the tree (*i.e.* stems and leaves). Indeed, *Koroneiki* pollen germination was reduced at 60 mM NaCl (–42%) and at 120 mM NaCl (–88%) as presented in Figure 3A. Pollen tube length was also reduced by 15% and 28% for the mild and high salinity dose, respectively (Figure 3B).

Data on olive pollen germination in response to salinity are scarce. In olive, pollen performance is reduced at high temperature,[10] similarly with photosynthetic activity.[9] A number of previous studies are in agreement with the present results highlighting the increased importance and scientific interests on salinity effects on crops. In a previous study on 5 Pistacia species, pollen was found to be more sensitive to salinity compared to seeds.[21] Exposure of 3 *Cicer arietinum* L. varieties to salinity induced reduction of pollen production, germination and tube length, especially at higher doses.[22] Similarly, reduced pollen viability and germination was observed for *Brassica napus* L. plants previously irrigated with sea water solutions.[23] In a relevant study on *B. napus* L., pollen germination was shown to better reflect overall plant sensitivity to salt stress compared to pollen tube length,[24] which also reflects and supports the present findings as the pollen germination provided clearer evidence on salinity effects compared to data pertaining to pollen tube length.

In order to overcome the water deficit due to increased water needs in agriculture, many countries may have to use water of lower quality such as saline water or treated waste water. In order to achieve that, however a detailed study of the effects of salinity on plants must be performed. This study elucidates the response to salinity stress of two major olive cultivars in Greece, *Koroneiki* and *Amphissis*, through several physiological and reproductive indicators. Specifically, the findings of this work indicate that *Amphissis* physiological processes are more sensitive in high salinity doses compared to *Koroneiki*. However, olive tree response to salinity may vary as influenced by agronomic practices *e.g.* proper leaching methodology,[25] soil type and precipitation intensity and distribution in the area of cultivation. The results of the present study also indicate that reproductive functions are severely affected by salinity. Therefore, future salinity studies would benefit from addition of reproductive indicators to investigate whether a plant can not only grow but also produce sufficiently under saline conditions.

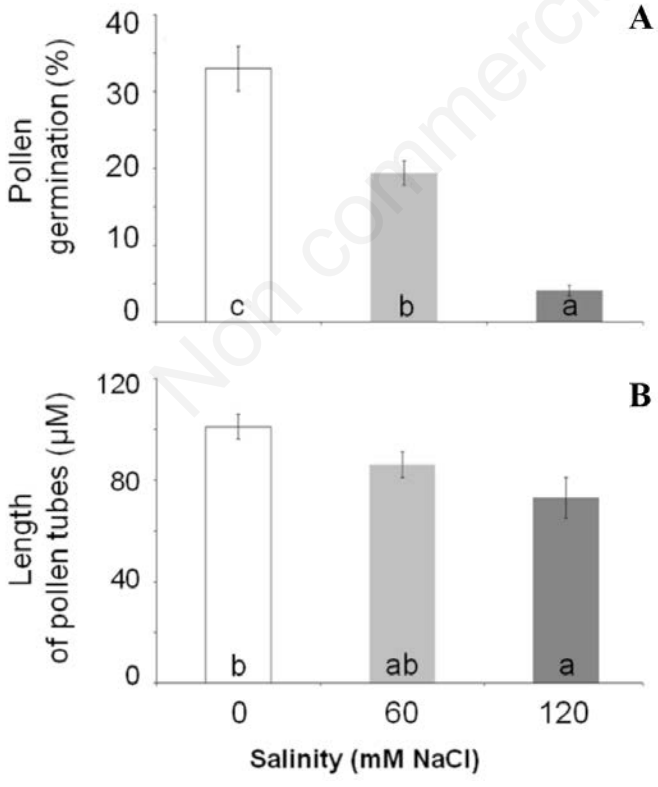

Figure 3. Effect of NaCl salinity (60 mM and 120 mM) on (A) pollen germination (n=250) and (B) length of pollen tubes (n=60) for hydroponically grown olive tree *Koroneiki* in NFT. *Amphissis* pollen was unavailable due to salinity-induced damaged inflorescences. Each bar is the mean ± standard error for each treatment. Bars with the same letter were not significantly different at P<0.05 (LSD test).

References

1. Paranychianakis NV, Chartzoulakis KS. Irrigation of Mediterranean crops with saline water: From physiology to management practices. Agricult Ecosyst Environ 2005;106:171-87.
2. Perica S, Goreta S, Selak GV. Growth, biomass allocation, and leaf ion concentration of seven olive (Olea europaea L.) cultivar under increased salinity. Sci Hort 2008;117:123-9.
3. Tattini M, Traversi ML. On the mechanism of salt tolerance in olive (Olea europaea L) under low or high-Ca^{2+} supply.Environ Exper Bot 2008;65:72-81.
4. Chartzoulakis K, Loupassaki M, Bertaki M, Androulakis I. Effects of NaCl salinity on growth, ion content and CO_2 assimilation rate of six olive cultivars. Sci Hort 2002;96:235-47.
5. Benlloch M, Ojeda MA, Ramos J, Rodriguez-Navarro A. Salt sensitivity and low discrimination between potassium and sodium in bean plants. Plant Soil 1994;166:117-23.
6. Chondraki S, Tzerakis C, Tzortzakis N. Influence of NaCl and calcium foliar spray on hydroponically grown parsley in NFT system. J Plant Nutr 2012;35:1457-67.
7. Tzortzakis NG. nfluence of NaCl and calcium nitrate on lettuce and endive growth using nutrient film technique. Int J Veget Sci 2009;15:1-13.
8. Chaves MM, Maroco JP, Pereira JS. Understanding plant responses to drought-from genes to the whole plant. Funct Plant Biol 2003;30:239-64.
9. Koubouris GC, Kavroulakis N, Metzidakis IT, et al. Ultraviolet-B radiation or heat cause changes in photosynthesis, antioxidant enzyme activities and pollen performance in olive tree. Photosynthetica 2015;53:279-87.
10. Koubouris GC, Metzidakis IT, Vasilakakis MD. Impact of temperature on olive (Olea europaea L.) pollen performance in relation to relative humidity and genotype. Environ Exper Bot 2009;67:209-14.
11. Economakis CD, Daskalaki AA, Metzidakis IT. Seasonal variation in water and nutrient uptake by olive plants grown in solution culture. Proceedings of the International Symposium on the Olive Tree and the Environment 2003;85.
12. Therios I.N, Misopolinos ND. Genotypic response to sodium chloride salinity of four major olive cultivars (Olea europaea

L.). Plant Soil 1988;106:105-11.
13. Tattini M. Ionic relations of aeroponically-grown olive genotypes during salt stress. Plant Soil 1994;161:251-6.
14. Hassan MM, Seif SA, Morsi ME. Salt tolerance of olive trees. Egypt J Horticult 2000;27:105-16.
15. Melgar JC, Syvertsen JP, Martinez V, Garcia-Sanchez F. Leaf gas exchange, water relations, nutrient content and growth in citrus and olive seedlings under salinity. Biol Plant 2008;52:385-90.
16. Chartzoulakis K, Psarras G, Vemmos S, et al. Response of two olive cultivars to salt stress and potassium supplement. J Plant Nutr 2006;29:2063-78.
17. Loreto F, Centrito M, Chartzoulakis K. Photosynthetic limitations in olive cultivars with different sensitivity to salt stress. Plant Cell Environ 2003;26:595-601.
18. Sofo A, Dichio B, Xiloyannis C, Masia A. Lipoxygenase activity and proline accumulation in leaves and roots of olive trees in response to drought stress. Physiol Plant 2004;121:58-65.
19. Sofo A, Dichio B, Xiloyannis C, Masia A. Antioxidant defences in olive trees during drought stress: changes in activity of some antioxidant enzymes. Funct Plant Biol 2005;32:45-53.
20. Centrito M, Loreto F, Chartzoulakis K. The use of low [CO_2] to estimate diffusional and non-diffusional limitations of photosynthetic capacity of salt-stressed olive saplings. Plant Cell Environ 2003;26:585-94.
21. Martinez-Palle E, Herrero M, Aragues R, et al. Salt response of seeds and pollen of five *Pistacia* species. Acta Hort 1995;419:49-54.
22. Dhingra HR, Varghese TM. Flowering and male reproductive functions of chickpea (Cicer arietinum L.) genotypes as affected by salinity. Biol Plant 1993;35:447-52.
23. Gul H, Ahmad R. Effect of salinity on pollen viability of different canola (Brassica napus L.) cultivars as reflected by the formation of fruits and seeds. Pak J Bot 2006;38:237-47.
24. Tyagi RK, Rangaswamy NS. Screening of pollen grains vis-à-vis whole plants of oilseeds brassicas for tolerance to salt. Theor Appl Genet 1993;87:343-6.
25. Wiesman Z, Itzhak D, Ben Dom N. Optimization of saline water level for sustainable Barnea olive and oil production in desert conditions. Sci Hort 2004;100:257-66.