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Effect of NaCl on Growth and Mineral Nutrition of Laurel (*Laurus nobilis* L.)

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ABSTRACT: ***Laurel, Laurus nobilis L., is a strict endemic species of natural vegetation of the Mediterranean region which is known for its medicinal, aromatic, forestry, ornamental and culinary properties. This has resulted in the species becoming rare or threatened with risk of extinction in Tunisia. Many factors can affect the production of this valuable plant including salinity, which appears to be most important, mainly in coastal areas. The present work has been carried out to test this hypothesis. The effect of different concentrations of NaCl (0, 50, 100 and 150 mM) of irrigation water on growth, SPAD index, chlorophyll fluorescence and mineral content of laurel was studied. The experiment was carried out in Tunis, in the year 2013. The results revealed that increasing NaCl concentrations significantly reduced fresh and dry weight of certain organs of the plant, SPAD index and chlorophyll fluorescence compared with the control. Application of NaCl induced also an increase in Na, Cl, K, P and N contents in leaves while Ca content decreased with increasing salt stress. Based on the present results, it can be concluded that laurel is an intermediate salt sensitive shrub.***

Keywords: biomass accumulation; chlorophyll fluorescence; salinity stress; SPAD index.

INTRODUCTION

Laurel, Laurus nobilis L., is one of the most important woody perennial species grown in the Mediterranean area, and a member of the Lauraceae family which contains 32 genera and about 2000-2500 species (Barla et al., 2007). It represents one of the most appreciated aromatic herbs with multiple properties. It is widely used in traditional

medicine for the treatment of chronic sinusitis, bronchitis and flus (Barlier, 2014).

Essential oil of laurel has been reported also as a powerful analgesic product indicated in arthritis, polyarthritis, in osteomuscular rheumatism and sprains problems (Gayda, 2013). In the same context, essential oil of laurel can be used on the treatment of diabetes and the prevention of migraine (Duke, 1997). The leaves are widely used and known as condiment and medicinal herbs since the ancient Greek and Roman periods (Demir et al., 2004); furthermore, because of their aromatic properties, leaves (fresh or dried) are considered as an important flavouring agent, commonly used as a condiment to flavour several dishes and sauces throughout the year especially in Western Countries. At last, the biological activities and photochemistry properties of laurel have also been extensively investigated and recognized, so that could be used as bio-pesticide in postharvest crop protection, for instance against spoilage fungi (De Corato et al., 2010) or as repellent against the bean weevil (Acanthoscelides obtectus)(Papachristos and Stamopoulos, 2002). Nevertheless, in Tunisia, wild natural populations of laurel have declined so dramatically that it is considered now as a threatened species (Matallah, 2012). Several factors are involved and water or soil Salinity appears to be one of the main abiotic factors. In fact, salinity is considered one of the most devastating environmental stresses, leading to significant reductions in cultivated area, productivity and crop quality (Yamaguchi and Blumwald, 2005; Shahbaz and Ashraf, 2013).

In Tunisia, more than 47% of water used for irrigation has a salt content between 1.5 and 3 g L^{-1} (Nagazi et al., 2012). Salinity is, at present, one of the most serious environmental problems influencing especially arid climate. Information concerning the effects of salinity on plant growth and mineral content of L. nobilis are rare. Thus, the aims of our study were to evaluate the effects of different NaCl concentrations on plant growth, SPAD index and mineral composition of laurel leaves.

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MATERIALS AND METHODS

Plant material and experimental design

The study was performed at the department of Agronomy and Biotechnology (National Agronomic Institute of Tunisia – INAT) in Tunis (36°49' N, 10°10' E, 8 m a.s.l.), during March 2014 to March 2015, The climate, of the Mediterranean type, has a minimum average temperature of 7.5 °C and a maximum average temperature of 31.7 °C.

In March 2014, a total number of 200 young plants (2 years old) of Laurus nobilis ecotype Sousse (a city in the East-Center of Tunisia) was randomly selected from a commercial nursery. Each plant was transplanted in plastic pot (17 cm diameter). The substrate used is a mixture of equal parts of soil and peat. The physical-chemical characteristics of the soil were: 49% sand, 32% silt, 26% clay, pH 7.9, 0.9% organic matter, 25% total $CaCO_3$, and 0.152 dS m^{-1} electrical conductivity. The peat used had a dry matter content of 25%, an organic matter content of 90%, a pH of 6.4 and an electrical conductivity of 0.4 dS m^{-1}.

Plants were subjected to irrigation with saline solutions having different NaCl concentrations (50, 100 and 150 mM NaCl). The control was irrigated with tap water (7.93 meq L^{-1} NaCl). The different irrigation waters had electrical conductivities increasing from 1.4 (control), 5.3, 9.7, and 14.1 dS m^{-1} (150 mM NaCl). Irrigations were conducted to maintain soil moisture near maximum water-holding capacity. In order to save plants from rainfall, plastic films were used during the rainy season. Plants were kept outside and positioned in the experimental site according to a completely randomized design, with 50 plants for each treatment. Fertilization occurred monthly with a modified half-strength Hoagland solution (pH 6.3; N 7.5 mM, P 0.5 mM, Ca 2.0 mM, Mg 1.0 mM, S 1.0 mM, B 23.0 µM, Mn 4.6 µM, Zn 0.38 µM, Cu 0.14 µM, Mo 0.05 µM, Fe 10.0 µM).

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Plant growth, SPAD index and chlorophyll fluorescence

At the end of the experiment, data on plant height, number of leaves and stem diameter were recorded. Furthermore, a chlorophyll meter (SPAD-502, Minolta corporation, Ltd., Osaka, Japan) was used to take readings from the fully expanded functional leaves. Twenty leaves were randomly measured per treatment (Lucini et al., 2015).

Modulated chlorophyll fluorescence was measured in dark adapted (for at least 15 min) leaves of six plants per treatment, using a chlorophyll fluorometer Handy PEA (Hansatech Instruments Ltd, UK) with an excitation source intensity higher than 3000 mol m^{-2} s^{-1} at the sample surface. The minimal fluorescence intensity (F0) in a dark-adapted state was measured in the presence of a background far-red light to favour rapid oxidation of intersystem electron carriers (Lucini et al., 2015). The maximal fluorescence intensities in the dark-adapted state (Fm) were measured by 0.8 s saturating pulses (3000 mol m^{-2} s^{-1}). The maximum quantum yield of open photosystem II (PSII) (Fv/Fm) was calculated as (Fm − F0)/Fm (Maxwell and Johnson, 2000).

Dry biomass and leaf mineral content

At the end of experiment, plants were separated into stems, leaves, and roots. Shoots and roots were separately washed with distilled water to remove any adhering debris. All plant organs were dried in a forced-air oven at 105 °C for 72 h for biomass determination. Sub-samples of leaves of twelve selected plants of each treatment were taken for leaf analysis. After recording their fresh biomass, they were oven-dried at 65 °C for one week and dry weight (DW) was recorded. The mineral analysis was conducted with ICP-OES, Arcos EOP (Spectro A. I. GmbH, Kleve, Germany). Between 0.500 and 0.550 g of dry matter of each sample was weighted and placed in a TFM vessel with 1 mL of 30% hydrogenperoxide and 7 mL of concentrated (65%) nitric acid. Then samples were subjected to a microwave digestion.

After cool down to room temperature, the dissolved sample was diluted with ultrapure water to a final volume of 25 mL. Calibration standards were prepared using multi element and single elements standards solutions (Inorganic Ventures Inc. Christiansburg, VA, USA) in 10% Suprapurnitric acid (Merck Chemicals GmbH, Darmstadt, Germany) to get similar matrix as the samples. Concentrations of 0, 0.005, 0.02, 0.05, 0.2, 0.5, 2 and 5 mg L^{-1} of the analytes were prepared. The concentrations of the calibration solutions for calcium, potassium, magnesium, sodium, phosphorous and sulphur were the same like other analytes plus 20, 50, 200 and 500 mg L^{-1}, respectively.

Statistical analysis

The results were analysed by ANOVA using SPSS 20.0. To separate treatment means, Duncan's multiple range test was performed at P=0.05.

RESULTS

Plant growth

The leaf number and plant height of laurel were significantly affected by salinity (P<0.001). As NaCl concentration increased from 0 to 50 mM, a clear decrease in these parameters was observed (-27.2 and -10.5%, respectively). The shoot diameter showed a significant decrease with the increase of NaCl level (P<0.05) and 50 mM NaCl in the irrigation water reduced the diameter of laurel by 8.8% compared to the control. With a further increase of salinity from 50 to 150 mM, no other difference in plant growth was observed (Table 1).

No significant decrease was observed for the fresh weight of the aerial plant organs (leaves and stem), whereas the laurel root fresh yield was significantly affected by salinity (P<0.001); in fact root fresh weight decreased with the increase of NaCl concentration from 0 to 100 and 150 mM NaCl by about 35.1% and 43.5% respectively.

Concentrations of 100 and 150 mM NaCl reduced stem dry biomass by 35.5% and 28.4%, respectively, compared with control plants. The negative effect of salinity on leaves and roots dry weights was observed already at 50 mM NaCl, with a reduction of 16.5% and 37.8%, respectively; at higher salinity levels no further biomass reduction was recorded (Table 2). As a consequence of different magnitude of effects between above and below ground plant organs, the increase of NaCl concentration in irrigation water lead also to a reduction of root-to-shoot ratio which was significant already at the lower salinity level.

SPAD index, chlorophyll fluorescence

The SPAD index was highly influenced by NaCl application (P<0.001). Concentrations of 50 NaCl decreased the SPAD index by 19.1% and at 150 mM NaCl values were further reduced by 31.6% (Table 3). The maximum quantum use efficiency of PSII in dark-adapted state (Fv/Fm), was significantly affected by salinity and the highest reduction was observed at NaCl 150 mM with a chlorophyll fluorescence that decreased by 15.9 %, compared to the control.

Mineral composition of leaves

Leaf concentration of different minerals (N, P, K, Ca, Mg, Na and Cl) are presented in table 4. The Cl increasing content in leaves increased with NaCl concentration, reaching the highest values in plants subjected to 150 mM (+118%); the highest level of NaCl also increased Na concentration by about 46.4%, compared with untreated plants. At the opposite, a reduction of Ca concentration was observed in leaf tissue only with the highest salinity level (-21.3%). In addition, no significant effect on Mg concentration was observed with increasing levels of salinity. At last, with 100 mM NaCl the highest uptake of K, P and N were observed (about 25.1%, 52.8% and 36.5%, respectively).

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DISCUSSION

Several studies have demonstrated that salinity induces growth inhibition and reduction of biomass production (Munns and Tester, 2008; Cassaniti et al., 2009; Bankaji et al., 2014; Yadav et al., 2011; Zhuo et al., 2015; Luo et al., 2017). Accordingly, in laurel, increasing salinity level lead to a significant reduction of leaf number, plant height and stem diameter of laurel plants (Table 1). Moreover, under salt stress, root fresh and dry weights were greatly affected. No significant effect was observed on leaf and stem fresh weights while a significant decrease was observed on root and stem dry biomass with increasing NaCl level (Table 2).

The significant dropped of plant biomass under salt stress has been attributed to considerable decrease in plant growth, photosynthesis and canopy structure caused by a combination effect of a low potential of soil solution, ion toxicities and/or nutritional imbalance (Munns, 2002; Yadav et al., 2011). O'Leary (1986) explains this reduction of plant growth by the fact that salinity increases the energy needs of the plant necessary to combat the osmotic and ionic stress for normal cellular maintenance and there is relatively less energy available for growth processes.

Root-to-shoot ratio is often altered by salinity stress, and root growth is more often less reduced than shoot growth (Munns and Termaat, 1986; Cheeseman, 1988; Bernstein and Kafkafi, 2002). This has been observed both in herbaceous (Hester et al., 2001; Niu and Rodriguez, 2006) and in woody species (Chartzoulakis et al., 2002; Bernstein et al., 2004; Álvarez and Sánchez-Blanco, 2014). However, in other cases, no effect on root-to-shoot ratio has been observed (Niu and Rodriguez, 2006; Cassaniti et al., 2009; Cirillo et al., 2016) or even, as in the present experiment, the magnitude of biomass reduction was higher in roots than leaves and stem (Cheeseman, 1988; Singla and Garg, 2005; Zulfiqar et al., 2013; Mahmoud et al., 2014). The lowering of the root-to-shoot ratio can increase the salt tolerance by reduction of salt flux to the shoot and, thus, the chloride uptake rate (Moya et al., 1999; Maggio et al., 2001).

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Salt stress interferes with several aspects of plant biochemistry, including photosynthesis and pigment synthesis (Colla et al., 2010) and Chlorophyll fluorescence is an important indicator reflecting that plant growth is limited by salt stress (Luo et al., 2017). The photosynthetic apparatus and particularly PSII may be temporarily affected by environmental stresses before any irreversible structural damage becomes apparent (Force et al., 2003). In the present study, the maximum quantum use efficiency of PSII in dark-adapted state (Fv /Fm) decreased under salt stress (Table 3), suggesting that salinity induced an inhibition of PSII electron transport (Lucini et al., 2015). Similar results have been obtained with Beta vulgaris L. (Dadkhah, 2011). Our results are in accordance also with Ferrante et al. (2011) who reported a significant decrease on Fv/Fm ratio of treated plants showing a high (Acacia cultriformis and Gaura lindheimeri) and intermediate sensitivity (Callistemon citrinus, Carissa edulis and Jasminum sambac) to salt stress, in contrast to Westringia fruticosa which is much more resistant to salinity stress. This resistance was due to the photochemical efficiency (Fv/Fm) of PSII in dark-adapted leaves, which did not change after 77 days of stress application (Ferrante et al., 2011). These results suggest that reduced plant pigment may represent a possible mechanism to protect PSII against photo inhibition through a reduction in the number of light-harvesting antennae and thus affecting the photochemical efficiency of PSII (Ferrante et al., 2011). In addition to reduced Fm/Fv ratio, the SPAD index, which is indicative of chlorophyll content, decreased in salt treated plants (Table 3). This reduction can be considered as a part of senescence response occurring under salinity (Hörtensteiner, 2006).

Salt stress disturbs the plant uptake and accumulation of essential nutrients (Shalan et al., 2006). Content of Na increased with increases in NaCl levels and reached the highest value in plants treated with 150 mM NaCl. Generally, K and Ca decreased in plant cells under saline conditions due to the antagonism of Na and K at uptake sites in the roots, the effect of Na on K transport into the xylem or the inhibition of uptake processes (Al-Harbi, 1995).

However, in our study, we observed a significant increase of K with increases in NaCl levels, reaching the higher significant value in plants subjected to 50 or more mM NaCl. In agreement with previous studies on Carissa edulis var. microphylla and Callistemon citrinus, which are considered as an ornamental shrub with intermediate tolerant to salt stress (Ferrante et al., 2011). Chow et al. (1990) classified spinach also as a moderately salt-sensitive plant because of the high leaf content of K under salt stress. Moreover, Ca content of 50 and 100 mM treatment was statically similar to the control. A significant decreasing of Ca content occurred only at the increasing of NaCl content to 150 mM. Similar results have been obtained on Jasminum sambac which showed an intermediate behaviour to salt stress (Ferrante et al., 2011). Concentration of Mg in plant may increase or decrease in response to salinity depending on species or plant organ (Marosz and Nowak, 2008; Keutgen, 2008). In this work no significant effect on Mg content was observed with increasing levels of salinity which is in line with what has been reported on other aromatic plants exposed to salt stress such as Trachyspermum ammi (Ashraf and Orooj, 2006) and Mentha piperita (Tabatabaie and Nazari, 2007).

The ability of plants to maintain a higher K/Na ratio is a key feature for salt tolerance of plants (Shabala and, Cuin, 2008; Abbasi et al., 2015). For instance Dvorak et al. (1994) and Gorham et al. (1997) found that with wheat salt tolerance is associated with an enhanced K/Na discrimination trait. Similarly in Agropyron spp., the high salt tolerance of A. elongatum relative to A. intermedium, is associated with its higher uptake of K+ under saline conditions (Elzam and Epstein 1969). We also observed a significant gradual increase in K/Na ratio, with increasing NaCl concentration, reaching the highest value at 100 mM NaCl after which a reduction was observed. This confirms that laurel (Laurus nobilis L.) is a species having intermediate sensitivity against salt stress with a salt tolerance threshold of about 100 mM NaCl. Thus, as for several other species, it appears that also for laurel K/Na ratio could be a useful screening criteria to improve salt tolerance.

CONCLUSIONS

Our results showed that NaCl concentrations caused a reduction in the growth of laurel plants especially at 150 mM. Moreover, SPAD index and chlorophyll fluorescence decreased with increasing NaCl concentration and reached the lower values at 150 mM NaCl. The effect of salinity is pronounced at higher NaCl concentration (150 mM) suggesting that Laurus nobilis L. is an intermediate salt sensitive crop. Concerning the mineral composition of different plant organs, our study showed a specific behaviour of laurel plants, then the increase of the concentration of salt leads to an increase in the foliar contents specially K, Na and Cl which are the highest at the higher concentrations of NaCl (150 mM NaCl). These results are similar to those obtained by Ferrante et al. (2011) who arrived to classify Carissa edulis, Callistemon citrinus and Jasminum sambac as a plants with an intermediate behaviour to salt stress, through their attitude against salt stress by increasing K content.

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Table 1. Salinity effect on leaves number, length and diameter of Laurus nobilis plants.

NaCl (mM)	Leaves number	Plant height (cm)	Stem diameter (mm)
0	79.8 a	68.8 a	7.04 a
50	58.1 b	60.5 b	6.42 b
100	57.9 b	62.1 b	6.38 b
150	54.4 b	61.6 b	6.30 b
Significance[a]	***	***	*

[a] *, *** = significant at P ≤ 0.05 and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P≤ 0.05).*

Table 2. Salinity effect on fresh and dry weight of leaves, stems and roots (g per plant), and root-to-shoot ratio (R/S) of Laurus nobilis plants.

NaCl (mM)	Fresh weight				Dry weight				
	Leaves	Stems	Roots	Whole plant	Leaves	Stems	Roots	Whole plant	R/S
0	17.7	15.4	174.4 a	207.5 a	9.16 a	10.5 a	65.6 a	85.3 a	3.33 a
50	16.0	13.9	145.6 ab	175.5 ab	7.65 b	9.80 a	40.8 b	58.2 b	2.39 b
100	15.3	12.9	113.2 b	141.4 b	7.13 b	6.77 b	29.3 b	43.2 b	2.11 b
150	14.5	11.6	98.6 b	124.7 b	7.50 b	7.52 b	30.1 b	45.1 b	1.99 b
Significance[a]	n.s	n.s	***	***	***	***	***	***	**

[a] n.s., **, *** = not significant or significant at P≤0.05, 0.01 and 0.001 respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P≤0.05).*

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Table 3. Effect of salinity on SPAD index, maximum quantum use efficiency of PSII in dark-adapted state (Fv /Fm) in Laurus nobilis plants.

NaCl (mM)	*SPAD index*	*Fv/Fm*
0	*43.9 a*	*0.797 a*
50	*35.5 b*	*0.767 b*
100	*34.6 b*	*0.725 b*
150	*30.0 c*	*0.670 c*
Significance[a]	***	***

[a] *** = *significant at P≤ 0.001. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P≤0.05).*

Table 4. Mineral contents of N, P, K, Ca, Mg, Na and Cl in leaves of Laurus nobilis plants cultivated at different concentrations of NaCl (0, 50, 100 and, 150 mM).

NaCl (mM)	Mineral elements (mg kg^{-1} DM)						
	N	P	K	Ca	Mg	Na	Cl
0	1.21 c	853 c	4507 b	10469 a	1188	525 b	793 bc
50	1.45 b	959 bc	5247 a	10239 a	1249	582 b	769 c
100	1.64 a	1303 a	5639 a	10013 a	1201	583 b	1049 b
150	1.53ab	1143 ab	5150 a	8252 b	1143	768 a	1729 a
Significance[a]	***	**	**	**	n.s.	**	***

[a] n.s., **, *** = not significant or significant at P≤0.05, 0.01 and 0.001 respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P≤0.05).

