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Leaf Ion Concentration and Biomass Allocation of Domestic and Introduced Olive Cultivars as Affected by NaCl Stress

Slavko PERICA, Smiljana GORETA

Institute for Adriatic Crops and Karst Reclamation – Split, Put Duilova 11, 21000 Split, Croatia
(e-mail: slavko@krs.hr)

Abstract

Seedlings of olive cultivars from Croatia, Italy, and Spain were exposed to NaCl stress (0, 33, 66, 100, or 166 mM NaCl) during three months. Leaf Na^+ concentration was increased and K^+:Na^+ ratio decreased as salinity of substrate was increased. At 166 mM NaCl 'Oblica' accumulated less Na^+ and was able to keep a higher K^+:Na^+ ratio than 'Leccino' and 'Manzanillo'. The root portion of plant increased and shoot portion decreased with salinity for all cultivars but Lastovka. At shoot level, as salinity increased leaf ratio increased at the expense of stem ratio. These results were not confirmed by allometric analysis, yet direct effect of cultivar on shoot biomass allocation pattern was observed.

Key words: allometry, K:Na ratio, *Olea europaea*, salinity, tolerance

Koncentracija iona u listu i raspodjela biomase domaćih i introduciranih kultivara masline pod utjecajem NaCl

Sažetak

Sadnice masline iz Hrvatske, Italije i Španjolske uzgajane su tri mjeseca pri povišenim koncentracijama NaCl-a (0, 33, 66, 100 i 166 mM). Porastom slanosti supstrata porasla je koncentracija Na^+ u listu i opao K^+:Na^+ omjer. Pri 166 mM NaCl-a 'Oblica' je nakupila manje Na^+ i imala viši K^+:Na^+ omjer u odnosu na 'Leccino' i 'Manzanillo'. Udio korijena u masi biljke je porastao, a udio izboja se smanjio s porastom slanosti supstrata kod svih kultivara osim 'Lastovke'. Na nivou mase izboja, s porastom NaCl-a povećao se udio lista a smanjio udio stabljike. Alometrijska analiza nije potpuno potvrdila sve rezultate, no zabilježen je izravan utjecaj kultivara na raspodjelu biomase izboja.

Ključne riječi: alometrija, K:Na omjer, *Olea europaea*, slanost, toleratnost

Introduction

Olive is one of the most cultivated fruit trees in Croatia, particularly in its Mediterranean part – Dalmatia and Istria. The olive production is often placed at locations unsuitable for other crops, where plants can suffer summer drought and lack of quality water for irrigation, both leading to salinity build up in soil. In Croatia an average yield is less than 10 kg/tree, and is considered very low and mostly attributed to summer drought. The Croatian coast and particularly the islands are areas scarce in high-quality water resources. On the other hand, a limited supply of somewhat saline groundwater is available for irrigation but has not been used so far.

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In comparison to other fruit trees, the olive is considered as moderately tolerant to salinity (Kozlowski, 1997). The reduction of growth in olive trees exposed to salinity depends on the duration of exposure and salt concentration, with an earlier growth decline in sensitive cultivars (Tattini, 1992; Perica et al., 2004). Tolerance to NaCl in olive is mostly related to the salt exclusion mechanism at the root level, which prevents Na^+ accumulation in leaf tissue, as well as to the ability of the olive to maintain essential K^+:Na^+ ratio (Chartzoulakis et al., 2002; Tattini et al., 1992). Shoot growth of olive cultivars is more sensitive to salinity than root growth, and general observation in many studies is that raise of salinity causes increased root:shoot ratio (Chartzoulakis et al., 2002; Tattini et al., 1992). However, it is not clear, whether salinity changed the allocation pattern in olive or increased root:shoot ratio was a consequence of an indirect effect of salinity on growth.

Salinity tolerance in olive is cultivar related as in many other species (Chartzoulakis et al., 2002; Perica et al., 2004; Tattini et al., 1992). There is lack of information on tolerance to salinity of the most widespread Croatian autochthonous olive cultivars especially in comparison to cultivars from other Mediterranean regions. Therefore, we have studied the influence of five levels of NaCl on leaf Na^+ and K^+ accumulation and pattern of biomass allocation of seven olive cultivars originating from Croatia, Italy and Spain.

Material and methods

Plant material was obtained from olive collection maintained at the Institute for Adriatic Crops, Split, Croatia. The study included four Croatian autochthonous olive cultivars, two Italian cultivars (Frantoio and Leccino) and one Spanish cultivar (Manzanillo). One year old, self-rooted plants of all cultivars, were transplanted to 1.5 L plastic pots filled with quartz sand:perlite substrate blended at a 1:1 v/v ratio. During three months plants were irrigated daily with half strength Hoagland nutrient solution. Thereafter, a salt stress was induced by adding 0, 33, 66, 100 or 166 mM of NaCl in the nutrient solution. Salinity of the irrigation solution was gradually increased by 33 mM NaCl per day, until the target levels were reached. Irrigation rate with a leaching fraction of 20-30% ensured a stable salinity level in pots of each treatment during the course of experiment.

The experiment was terminated after three months of exposure to salinity and plants were removed from pots and separated into roots and shoots. Shoots were further separated to leaves and stems and weighed after drying at 65 °C for at least 72 hours. Leaves developed after beginning of exposure to salinity were ground for measurements of Na^+ and K^+ concentrations using flame photometer.

The experiment was set up as a factorial with cultivar and salinity as factors. To examine data trends for NaCl concentrations, linear and quadratic coefficients for unequally spaced treatments were generated using proc IML. Effects of NaCl and cultivar on partitioning of biomass were examined by determining ratios between plant components. The pattern of biomass allocation under effect of cultivar and NaCl was also tested by allometric analysis. The general equation: $\log_e y = b_0 + b_1 \log_e x$ was applied, where x and y were examined plant components and the b_1 represented the relative change in allocation between examined components due to the applied treatments. Stepwise analysis of covariance (ANCOVA) was undertaken to distinguish the size-dependent shift in allocation from direct response to applied treatments (Gebauer et al., 1996). The significant interaction between NaCl or cultivar and $\log_e x$ indicated significant differences between slopes or direct influence of treatments on allometric coefficients. Differences among the slopes were tested by Tukey-Kramer test at $P \leq 0.05$.

Results and discussion

After three months of salinity exposure, Na^+ concentration in leaves developed under salinity treatment, ranged from 0.122 to 8.775 g kg^{-1} dry weight being dependent on cultivar and salinity (Table 1). Leaf Na^+ concentration of all cultivars increased linearly and quadratically as salinity increased with the highest increment reached when the salinity of nutrient solution was raised from 100 mM to 166 mM NaCl. Averaged overall salinity levels 'Frantoio' and 'Oblica' accumulated significantly less Na^+ in leaves ($P \leq 0.05$) than other cultivars, whereas there was no difference among 'Leccino', 'Manzanillo', 'Drobnica' and 'Lastovka'. At 166 mM the highest leaf Na^+ concentration was found for 'Leccino' followed by 'Manzanillo' and it was significantly higher ($P \leq 0.05$) than in 'I. bjelica' and 'Oblica'. The growth of olive is reduced and onset of leaf damage could be expected at leaf Na^+ concentration higher than 0.4% of dry matter (Tattini et al., 1992), which was measured in this experiment for all cultivars at 166 mM NaCl.

Metabolic toxicity of Na^+ is largely a result of its ability to compete with K^+ for binding sites essential for cellular functions (Tester and Davenport, 2003), thus the capacity of plants to maintain a high cytosolic K^+:Na^+ ratio is likely to be one of the key determinants of plant salt tolerance. Application of 33 mM NaCl resulted in significant decrease of K^+:Na^+ ratio in all

tested cultivars, with continued negative trend as salinity was increased (Table 1).

Up to 66 mM NaCl, 'Frantoio' maintained a higher K^+:Na^+ ratio than other cultivars, and at 100 mM, it was followed by 'Oblica'. Variation of the K^+:Na^+ ratio between cultivars was relatively small at 166 mM NaCl (1.7) and it was significantly lower ($P \leq 0.05$) only in 'Leccino' (2.7) and 'Manzanillo' (2.8) compared to Oblica (4.4).

Table 1. Leaf Na^+ content and K^+:Na^+ ratio, and biomass partitioning of seven olive cultivars grown for three months at five levels of salinity.

Cultivar	NaCl (mM)	Na^+ (g kg^{-1})	K^+:Na^+	Root: plant	Shoot: plant	Leaf: shoot	Stem: shoot
Drobnica	0	0.180	161.4	0.165	0.834	0.587	0.413
	33	0.599	56.4	0.195	0.805	0.641	0.359
	66	1.638	18.8	0.204	0.796	0.602	0.398
	100	2.839	10.6	0.225	0.775	0.726	0.274
	166	6.553	4.1	0.245	0.755	0.804	0.196
		*L***Q****	*L***Q***	*L****	*L****	*L****	*L****
Frantoio	0	0.122	267.6	0.155	0.846	0.646	0.354
	33	0.176	185.3	0.141	0.859	0.628	0.372
	66	0.344	155.5	0.189	0.811	0.646	0.354
	100	1.651	16.7	0.210	0.790	0.660	0.340
	166	6.183	3.4	0.260	0.740	0.736	0.264
		*L****	*L***Q**	*L**	*L*****	*L****	*L****
I. bjelica	0	0.157	198.6	0.175	0.825	0.598	0.402
	33	0.897	37.9	0.196	0.805	0.628	0.372
	66	1.613	20.2	0.197	0.803	0.644	0.356
	100	2.863	9.4	0.211	0.788	0.671	0.329
	166	5.211	3.8	0.216	0.784	0.739	0.261
		*L***Q****	*L***Q****	*L****	*L**	*L****	*L****
Lastovka	0	0.135	215.6	0.235	0.765	0.636	0.364
	33	0.663	66.4	0.229	0.771	0.659	0.341
	66	1.663	18.2	0.240	0.759	0.695	0.305
	100	2.454	9.8	0.238	0.763	0.690	0.310
	166	6.063	2.7	0.248	0.752	0.706	0.294
		*L***Q****	*L***Q****	*ns*	*ns*	*L**	*L**
Leccino	0	0.195	157.5	0.129	0.871	0.478	0.522
	33	0.539	100.5	0.132	0.868	0.519	0.481
	66	1.303	23.2	0.170	0.829	0.556	0.444
	100	2.375	12.2	0.159	0.841	0.542	0.458
	166	8.775	2.7	0.217	0.783	0.661	0.339
		*L****	*L****	*L****	*L****	*L****	*L****
Manzanillo	0	0.145	180.4	0.179	0.821	0.607	0.393
	33	0.498	67.4	0.170	0.829	0.614	0.386
	66	0.825	35.3	0.209	0.791	0.615	0.385
	100	3.363	7.5	0.219	0.781	0.697	0.303
	166	7.667	2.2	0.217	0.783	0.722	0.278
		*L***Q**	*L****	*L**	*L**	*L****	*L****
Oblica	0	0.170	186.9	0.219	0.781	0.630	0.370
	33	0.288	91.5	0.226	0.774	0.634	0.366
	66	0.850	34.2	0.229	0.771	0.663	0.337
	100	1.739	14.7	0.207	0.793	0.667	0.333
	166	4.788	4.4	0.289	0.711	0.693	0.307
		L***	L***	L*	L***Q*	L*	L*

[z] The effects of NaCl for each cultivar were non-significant (NS) or significant *, **, *** by polynomial analysis at $P \leq 0.05$, 0.01, 0.001, respectively. Linear (L) or quadratic (Q).

Table 2. Allometric coefficients (b_1) for biomass allocation among various plant tissues of of seven olive cultivars grown for three months at five levels of salinity).Values of b_1 were obtained from the relationship: $log_e y = b_0 + b_1 log_e x$, where x and y are dry mass (W) of the tissues under consideration.

	x-y			
Treatment	Wtotal -Wroot	Wtotal -Wshoot	Wshoot -Wleaf	Wshoot -Wstem
Cultivar		b_1		
Drobnica	0.671 a*	1.089 a	0.781 c	1.554 a
Frantoio	0.628 a	1.115 a	0.836 b	1.431 b
I.bjelica	0.716 a	1.084 a	0.839 ab	1.363 bc
Lastovka	0.743 a	1.103 a	0.891 a	1.244 c
Leccino	0.586 a	1.090 a	0.713 d	1.412 b
Manzanillo	0.748 a	1.061 a	0.822 bc	1.354 bc
Oblica	0.759 a	1.086 a	0.833 b	1.318 c
NaCl (mM)				
0	0.748 a	1.056 a	0.759 a	1.398 a
33	0.704 a	1.065 a	0.789 a	1.370 a
66	0.762 a	1.061 a	0.860 b	1.354 a
100	0.713 a	1.078 a	0.851 b	1.375 a
166	0.823 a	1.064 a	0.867 b	1.370 a

*Different letters within column for effect of cultivar or NaCl indicate a difference among slopes (b_1) by Tukey-Kramer test at $P \leq 0.05$.

Balanced growth models assume that biomass is preferentially allocated to the plant part obtaining the resource in growth limiting conditions (Shipley and Meziane, 2002) i.e. to the root system in case of salinity. Analysis of biomass partitioning showed linear increase of root to plant ratio and consequently decrease of shoot to plant ratio as salinity increased for all cultivars but 'Lastovka' (Table 1). At the shoot level, leaf ratio was linearly increased and stem ratio decreased as salinity increased for all cultivars (Table 1). Among tested cultivars less plasticity was observed for 'Lastovka' and 'Oblica'. As salinity increased from 0 to 166 mM NaC, leaf to shoot ratio of 'Lastovka' and 'Oblica' increased only 11% and 10%, and stem to shoot decreased 19% and 17%, respectively, whereas for 'Drobnica' and 'Leccino' the changes were higher than 30%. Influence of salinity and cultivar on biomass allocation tested in conventional way as in Table 1 was not completely confirmed with allometric analysis.

When total plant weight was used as a covariate and differences in plant size adjusted statistically there was no effect of cultivar or salinity on allocation of dry matter between below and above ground plant growth (Table 2).

The slopes of regression (b_1) between total shoot weight and leaf and stem weight indicated direct effect of cultivar on pattern of shoot biomass allocation. 'Lastovka' allocated more biomass in leaves at the expense of stem, whereas different pattern was found for 'Drobnica' (Table 2). Salinity higher than 33 mM NaCl enhanced allocation of dry matter in leaves, but the effect is hardly consistent since no significance was found for stem allocation. Allometric analysis revealed that most of differences observed at the end of experiment at the whole plant level reflected only size-dependent shifts of olive cultivars in allocation patterns. At shoot level, direct effect of cultivar on biomass allocation in leaf or stem tissue was observed, whereas effect of salinity was not consistent.

Conducted study confirmed the genetic variability in salt tolerance among tested cultivars. At 166 mM NaCl 'Oblica' accumulated less Na^+ and was able to keep higher K^+:Na^+ ratio than 'Leccino' and 'Manzanillo'. Salinity changed proportion of dry matter allocation among plant parts for most of the cultivars, however results of allometric analysis confirmed only effect of cultivar on shoot biomass allocation pattern.

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