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Leaf mineral concentration of five olive cultivars grown on calcareous soil

Mineralni sastav lista pet sorata masline uzgajanih na karbonatnom tlu

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ABSTRACT

There are limited numbers of scientific publication regarding genotypic differences which exist among olive cultivars concerning nutrient uptake and translocation. For that purpose, the object of our study was to determine possible differences between leaf mineral content of five selected olive cultivars since leaf nutrient analysis is consider being the best method for diagnosing olive tree nutritional status. Plant material was obtained from an olive collection, grown on calcareous soil maintained at Institute of Adriatic Crops and Karst Reclamation, Split, Croatia. The study was conducted with two Croatian autochthonous olive cultivars ("*Istarska bjelica*", "*Lastovka*"), two Italian cultivars ("*Pendolino*", "*Leccino*") and one Spanish cultivar ("*Hojiblanca*"). Completely randomized design was applied. This study has shown questionably low Mg concentration in all olive cultivars with exception for "*Hojiblanca*" cultivar. Also, only Croatian cultivars "*Istarska bjelica*" and "*Lastovka*" as well as Spanish cultivar "*Hojiblanca*" recorded sufficient levels of iron leaf mineral content. Regarding other elements studied (P, K, Ca, Zn, Mn, Cu) all cultivars were above literature cited thresholds for possible deficiencies. Selected olive cultivars in our experiment demonstrated different nutrient leaf concentration, which is of particular importance for fertilization requirements and fertilization practice in Croatian orchards grown on calcareous soil.

Key words: calcareous soil, iron, magnesium, mineral nutrition, olive

PROŠIRENI SAŽETAK

U alkalnim, karbonatnim tlima koja pokrivaju više od od 30 % površine Zemlje pH je određen prisutnošću $CaCO_3$ koji puferira tlo u rasponu od 7.5-8.5. Upravo na takvim tlima moguće su deficijencije P, Fe, Zn, Mn i Cu ali i Mg zbog antagonističkog odnosa Ca i K prema Mg.Kod masline postoji ograničeni broj znanstvenih publikacija vezanih

uz genotipske razlike koje postoje između sorata a vezane su uz usvajanje i translokaciju hraniva. Iz tog razloga, cilj našeg istraživanja bio je odrediti moguće razlike između koncentacije minerala u listovima pet različitih sorata masline. Naime upravo folijarna analiza smatra se najboljim metodom za određivanje mineralnog statusa masline. Biljni materijal uzorkovan je iz kolekcijskog nasada masline uzgajanog na karbonatnom tlu u Institutu za jadranske kulture i melioraciju krša u Splitu. Istraživanje je provedeno na dvije hrvatske ("*Istarska bjelica*", "*Lastovka*"), dvije talijanske ("*Pendolino*", "*Leccino*") i jednoj španjolskoj ("*Hojiblanca*") sorti po shemi potpuno slučajnog rasporeda. Uzorkovano lišće osušeno je na 80°C tijekom 48 h. Nakon digestije osušenog i samljevenog lišća s koncentriranom HNO_3 i $HClO_4$ (Milestone 1200 Mega Microwave Digester) P je određen spektofotometrijski, K plamenofotometrijski, a sadržaj kalcija (Ca), magnezija (Mg), željeza (Fe), cinka (Zn) mangana (Mn) i bakra (Cu) određen je atomskom apsorpcijskom spektrofotometrijom – AAS. Sve sorte imale su koncentracije (P, K, Ca, Zn, Mn, Cu) iznad pragova deficijencije dok su koncentracije Mg i Fe, ovisno o sorti, bile na ili ispod granice deficijencija koje se navode u literaturi. Najvišu koncentraciju fosfora u listu masline imala je Lastovka (0.12 % ST), signifikantno višu ($P \leq 0.05$) od "*Hojiblance*" (0.08 % ST) ali bez statisički signifikantne razlike prema "*Istarskoj bjelici*" (0.11 % ST), "*Pendolinu*" (0.10 % ST) i "*Leccinu*" (0.10 % ST). Također statistički značajna razlika ($P \leq 0.05$) između odabranih sorata uočena je u mineralnom sastavu K, Ca i Mg. Najvišu razinu K (0.77 % ST) imala je sorta "*Pendolino*" a najvišu razinu Ca (2.04 % ST) i Mg (0.15 % ST) sorta "*Hojiblanca*". Koncentracija Mg u ostalim sortama kretala se u graničnom području deficijencije (0.08-0.10 % ST) iz razloga što samo mala promjena u kocentraciji (0.01 %) može izazvati pojavu simptoma kod masline. U našem istraživanju "*Istarska bjelica*" (54.5 $mgkg^{-1}$ ST), "*Lastovka*" (52.7 $mgkg^{-1}$ ST) i "*Hojiblanca*" (50.3 $mgkg^{-1}$ ST) imale su optimalnu koncentraciju Fe u listu dok su sorte "*Leccino*" (44.1 $mgkg^{-1}$ ST) i "*Pendolino*" (38 $mgkg^{-1}$ ST) pokazale relativni nedostatak Fe. Koncentracije cinka u lišću sorte "*Hojiblance*" (30.2 $mgkg^{-1}$ ST) bile su signifikantno više od svih ($P \leq 0.05$) drugih proučavanih sorata dok je koncentracija Mn kod sorte "*Leccino*" (33.4 $mgkg^{-1}$ ST) bila signifikantno viša od svih ostalih sorata (23.8-25.6 $mgkg^{-1}$ ST) izuzev "*Istarske Bjelice*" (27 $mgkg^{-1}$ ST). Koncentracije Cu u listu masline nisu se signifikantno razlikovale između odabranih sorata i kretale su se u rasponu od 6-7 $mgkg^{-1}$ ST. Odabrane sorte u našem istraživanju pokazale su različite koncentracije hraniva u listu, što je posebno važno zbog optimiziranja gnojidbe u skladu s potrebama pojedine sorte masline.

Ključne riječi: karbonatno tlo, magnezij, maslina, mineralna ishrana, željezo

INTRODUCTION

In alkaline, calcareous soils, which cover more than 30% of the earth's surface, pH is determined by the presence of $CaC0_3$, which buffers the soils in the pH range 7.5-8.5 (Marschner, 2003). In calcareous soils soil pH effects soil nutrient availability and chemical reactions that affect the loss or fixation of almost all nutrients. Both native and applied phosphorous is tied up in highly insoluble calcium and magnesium phosphates, rendering the added phosphorous only sparingly available for plant uptake (Imas, 2000). Furthermore, calcium represents more than 80 % of the total amount of exchangeable cations in calcareous soils, while there is less than 4 % of exchangeable magnesium which frequently causes magnesium deficiency in plants

(Gluhić et al., 2009). Specifically, high levels of calcium in the soil reduce the adsorption of Mg and K due to competition between Ca, Mg and K (Marschner, 2003). Iron, zinc, manganese and copper deficiencies are common in soils that have a high $CaCO_3$ due to reduced solubility at alkaline pH values (Imas, 2000). Divalent metals such as Zn, Mn, Cu and reduced Fe decrease in solubility by one hundred-fold for every unit increase in pH, whereas trivalent Fe decreases in solubility by a thousand-fold for each pH unit increase (Rengel et al., 1999).
Differential nutrient uptake and translocation between different plant species or cultivars has been well documented by various authors (White et al., 1978; Shuxin et al., 2000; Damon and Rengel, 2007; Khoshgoftarmanesh et al. 2010). On contrary for the olive tree, although it is considered a species with great capacity to survive and produce under low-fertility soils, there are limited numbers of scientific publication regarding genotypic differences which exist among olive cultivars concerning their nutrient status (Chatzistathis and Threrios, 2009).
For that purpose, five different olive cultivars (Hojiblanca, Leccino, Pendolino, Istarska bjelica and Lastovka) were use in our study.
The aim of this work was to determine possible differences between leaf mineral content of selected olive cultivars since leaf nutrient analysis is consider being the best method for diagnosing tree nutritional status, and represents an important tool for determining future fertilization requirements (Fernandez- Esocbar et al., 1999).

MATERIAL AND METHODS

Plant material was obtained from an olive collection, grown on calcareous soil ($pH_{(H20)}$=8.35; $CaCO_3$= 60.5 %), maintained at Institute of Adriatic Crops and Karst Reclamation, Split, Croatia (Latitude: 43°31'N, Longitude: 16°27'E). The study was conducted with two Croatian autochthonous olive cultivars ("Istarska bjelica", "Lastovka"), two Italian cultivars ("Pendolino", "Leccino"), and one Spanish cultivar ("Hojiblanca"). Cultivar "Istarska bjelica" is grown at cooler northern part of the country, while "Lastovka" is mostly planted in warmer southen region (Perica et al., 2008). Completely randominized design was applied. Every of five cultivars were represented by tree trees, each tree as one replicant. Total number of plants in experiment was 18. 1 kg of NPK 7:14:21 fertilizer (per tree) was given 2-3 weeks before growing season.
In July from each tree leaf samples were taken. Each leaf sample comprised four subsamples of 25 healthy, fully expanded mature leaves collected from the middle portion of current-season shoots, about 1.8 m above the soil surface, at the four cardinal points around the tree (Perica, 1996). These four subsamples were mixed in paper bags to provide a bulked sample with 100 leaves to ensure that it was representative of the surrounding area. The leaves were taken to the laboratory in portable coolers and washed with deionized water, dried at 80 °C for 48 hours, and finally ground to a fine powder (Gomez-Casero et al., 2007).
A portion of 0.5 g of the fine powder of each sample was weighted. After leaf digestion with concentrated HNO_3 and $HClO_4$ (Milestone 1200 Mega Microwave Digester) P was determined spectrophotometrically, K was determined with a flame photometer and Mg, Ca, Fe, Zn, Mn and Cu were determined by the atomic adsorption spectroscopy (AAS) method (AOAC, 1995).
Statistical data processing was performed by one way analysis of variance.

RESULTS AND DISCUSSION

Phosphorous leaf concentrations are shown in Table 1. "*Hojiblanca*" cultivar has significantly (P≤0.05) lowest P concentration comparing with all other cultivars tested except "*Leccino*" cultivar. Furthermore, "*Lastovka*" cultivar has highest P leaf concentration, significantly higher (P≤0.05) then "*Hojiblanca*" cultivar but without any significant difference with "*Istarska bjelica*", "*Pendolino*" and "*Leccino*" cultivar. P concentrations of all cultivars in our experiment, which ranged from 0.08 % DM to 0.12 % DM (Table 1), according to Bouat (1968, cited in Lasram and Tnani, 1992) can be classified as mean concentrations for Mediterranean countries.

Table 1. P, K, Ca and Mg dry matter leaf mineral content (% DM) of five olive cultivars.
Tablica 1. Koncentracija P, K, Ca i Mg u suhoj tvari lista (% ST) pet sorata masline.

Cultivar	P %		K %		Ca %		Mg %	
Hojiblanca	0,08	b	0,77	c	2,04	a	0,15	a
Leccino	0,10	ab	1,13	ab	1,50	bc	0,10	b
Pendolino	0,10	a	1,24	a	1,14	c	0,08	bc
Istarska Bjelica	0,11	a	1,00	b	1,68	ab	0,09	bc
Lastovka	0,12	a	1,11	ab	1,53	bc	0,08	c
LSD	**0.02**		**0.18**		**0.42**		**0.02**	

Means followed by a different letter differ significantly at P≤0.05 according to LSD test.
Srednje vrijednosti označene različitim slovom značajno se razlikuju za P≤0.05 prema LSD testu.

However, Lopez-Granados et al. (2004) reported leaf P concentration in Spanish orchards from 0.06 % DM to 0.09 % DM with 0.07 mean values. Although soil chemical characteristic (pH $_{(H20)}$ =8.4; 41.6 % $CaCO_3$) in 200 d pot study for "*Leccino*" cultivar conducted by Paskovic et al. (2012) was similar to our experiment, results revealed lower P leaf concentration (0.06 % P DM) in comparation to our results (Table 1). This difference, beside different olive age and different growing conditions, is probably due to lack of phosphorous fertilization in previously mentioned study.
Regarding K, Ca and Mg leaf concentration in cultivars used in our experiment significant difference between cultivars (P≤0.05) can be observed (Table 1).
In accordance with our results Chatzistathis and Therios (2009) reported significant differences between "*Kothreiki*" and "*Koroneiki*" cultivars and their Ca and Mg leaf concentration but without any differences in K leaf concentration for cultivars studied. However, similar with our data, Yang et al. (2004) noted that two K-efficient rice genotypes had two-fold higher K concentration in lower leaves, and 30% higher K concentration in the upper leaves compared with K inefficient rice genotype at the booting stage.
According to Bouat (1968, cited in Lasram et Tnani, 1992) our results for K and Ca are in normal ranges found in Mediterranean countries for selected nutrients (Table 1).
Nevertheless, for all cultivars studied in our experiment, with exception of "*Hojiblanca*", Mg leaf concentrations are according to Bouat (1968, cited in Lasram et Tnani, 1992) near minimum ranges (0.08 % DM) or in magnesium deficiency range (0.06–0.10 % DM) due to Hartman and Brown report (1953) (Table 1).
Under field conditions magnesium deficiency in olive would be expected to cause chlorotic leaves, with light-green area advancing from tip toward the base with general defoliation of younger shoots followed by death of terminal twigs (Hartmann

and Brown, 1953). Although in our experiment no leaf symptoms occurred these results show that it can be fairly close to the point where deficiency might be expected, because, only very narrow margin (about 0.01 %) exist between the magnesium deficient range and adequate magnesium range (Hartmann and Brown, 1953).

A significant part of the fruit tree industry in Europe and especially in the Mediterranean area is located on calcareous or alkaline soils, which favour the occurrence of Fe chlorosis (Tagliavini and Rombola, 2001). Benitez et al (2002) reported that beside increasing use of marginal highly calcareous soil in last decades in Spain, iron deficiency chlorosis in olive occurs due to introduction of intensive management practices, particularly irrigation, that have increased tree nutrient demands, use of bicarbonate-rich irrigation waters, and substitution of Fe chlorosis-tolerant for susceptible varieties. According to Therios (2009), Fe concentrations between 50-150 $mgkg^{-1}$ DM are considered to be sufficient, while values between 20-50 $mgkg^{-1}$ DM are defined as relatively deficient for olive's tree mineral nutrition. Toplu et al. (2010) reported "*Hojiblanca*" olive leaf concentration between 71.9 and 75.8 $mgkg^{-1}$ DM for samples taken in December in calcareous soil (pH =7.5-7.6; 25-35 % $CaCO_3$). Fe concentration in our study for "*Hojiblanca*" cultivar is lower than data reported from above cited authors (Table 2). Pasković et al. (2012) reported 41.0 $mgkg^{-1}$ DM leaf concentration for "*Leccino*" cultivar which is similar to our data for same cultivar (Table 2). In our study "*Istarska bjelica*", "*Lastovka*" and "*Hojiblanca*" cultivar have optimum olive leaf Fe concentration while "*Leccino*" and "*Pendolino*" cultivar are showing relative lack of Fe leaf concentration (Table 2).

Table 2. Fe, Zn, Mn and Cu dry matter leaf mineral content (% DM) of five olive cultivars.
Tablica 2. Koncentracija Fe, Zn, Mn i Cu u suhoj tvari lista (% ST) pet sorata masline.

Cultivar	Fe $mgkg^{-1}$		Zn $mgkg^{-1}$		Mn $mgkg^{-1}$		Cu $mgkg^{-1}$
Hojiblanca	50,30	a	30,17	a	25,57	b	7,17
Leccino	44,13	b	18,20	b	33,40	a	5,80
Pendolino	38,00	c	18,47	b	25,40	b	6,00
Istarska Bjelica	54,50	a	20,37	b	26,97	ab	6,67
Lastovka	52,70	a	17,80	b	23,80	b	6,10
LSD	**4.53**		**3.83**		**7.51**		**ns**

Means followed by a different letter differ significantly at P≤0.05 according to LSD test.
Srednje vrijednosti označene različitim slovom značajno se razlikuju za P≤0.05 prema LSD testu.

Zinc concentration in "*Hojiblanca*" cultivar leaves was significantly higher then other cultivars studied (P≤0.05) (Table 2).

With strong negative correlation coefficient (r=- 0.502, P=0.057) our data (Table 2) may be in accordance with Marshner's (2003) statement that zinc deficiency increase P uptake by the roots, by increasing the permeability of the plasma membrane of root cells to phosphorus, and its translocation to the aboveground plant parts, although in olive zinc deficiency is defined with concentration lower than 10 $mgkg^{-1}$ DM (Panagiotopoulos, 2001, cited in Chatzistathis et al., 2010).

According to Bouat (1968, cited in Lasram and Tnani, 1992) mean concentration of Zn in olive plant material is 23.5 $mgkg^{-1}$ DM which is higher than our mean data range for "*Lastovka*", "*Leccino*", "*Pendolino*" and "*Istarska bjelica*" cultivar by 15.5% to 32.0 % (Table 2). Dimassi et al. (1999) reported that olive cultivars in Greece contain 10-

16 $mgkg^{-1}$ DM Zn, while Jardao et al. (1999) study demonstrated higher values of Zn concentration (16.9-30.3 $mgkg^{-1}$ DM) in olive leaf. For "*Leccino*" cultivar Pasković et al. (2012) data are higher (28.6 $mgkg^{-1}$ DM) then data submitted by our experiment (Table 2).

"*Leccino*" cultivar Mn concentration showed significant difference ($P \leq 0.05$) from "*Hojiblanca*", "*Lastovka*" and "*Pendolino*" cultivar (Table 2). The lowest average Mn concentration in our experiment is recorded in "*Lastovka*" cultivar but with no significant difercence to "*Hojiblanca*", "*Pendolino*" or "*Istarska bjelica*" (Table 2). According to Panaggiotopulos (2001, cited in Chatzistathis et al., 2010) when Mn concentration of mature leave is less than 20 $mgkg^{-1}$ DM the olive trees suffer from mild deficiency symptoms, and severe Mn deficiency only occurred at less then 5 $mgkg^{-1}$ DM In our experiment all cultivars selected have recorded Mn concentration higher than $mgkg^{-1}$ DM (Table 2).

There weren't any significant difference for Cu leaf concentration between cultivar studied (Table 2). Threshold for relative lack of Cu according to Therios (2009) is beyond 5 $mgkg^{-1}$ DM all Cu data recorded in our experiment were sufficient (Table 2).

CONCLUSION

Olive cultivars selected in our experiment demonstrated different nutrient leaf concentration, which is of particular importance for fertilization requirements and fertilization practice in Croatian orchards grown on calcareous soil. Our study demonstrated questionably low Mg concentration in all olive cultivars with exception for "*Hojiblanca*" cultivar. Also, it is important to notice that Croatian cultivars "*Istarska bjelica*" and "*Lastovka*" as well as Spanish cultivar "*Hojiblanca*" recorded sufficient levels of iron leaf mineral content. Regarding other elements studied (P, K, Ca, Zn, Mn, Cu) all cultivars were above literature cited thresholds for possible deficiencies.

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