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Preliminary results of leaf mineral concentrations for seven pomegranate cultivars grown on calcareous soil

Preliminarni rezultati mineralnog sastava lista sedam sorata šipka uzgajanih na karbonatnom tlu

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ABSTRACT

Pomegranate (*Punica granatum* L.) is one of the traditional fruits cultivated in the Mediterranean part of Croatia with more intensive cultivation in the recent years. The objective of study was to determine leaf nutrient concentrations of seven pomegranate cultivars (five Croatian and two introduced) grown on calcareous soil as leaf nutrient analysis is consider being the best tool for diagnosing fruit tree nutritional status.

Significant differences between cultivars have been found for leaf P, Ca, Mg, Fe and Zn concentrations. All cultivars leaf K, Mg, Fe and Zn concentrations were under literature cited thresholds for possible deficiencies, although no visible deficiency symptoms were found. Cultivar Sladun had highest concentration of all determined nutrients and could be possible option for organic and low input cultivation.

Keywords: *Punica granatum* L., leaf analysis, cultivar differences

SAŽETAK

Šipak (*Punica granatum* L.) je tradicionalna voćna vrsta u mediteranskom dijelu Hrvatske s povećanim zanimanjem za uzgojem posljednjih godina. Cilj rada bio je utvrditi mineralni sastav lista sedam sorata šipka (pet hrvatskih i dvije introducirane) uzgajane na karbonatnom tlu budući da se analiza lista smatra najboljim alatom kod utvrđivanja stanja ishranjenosti voćnih vrsta.

Značajne razlike među sortama utvrđene su za koncentracije P, Ca, Mg, Fe i Zn u listu. K, Mg, Fe i Zn u svim sortama bio je ispod literaturno navedenih granica suficijencije iako vidljivi simptomi deficijencije nisu utvrđeni. Sorta Sladun imala je najviše koncentracije svih hraniva te bi mogla biti mogući izbor za uvjete ekološke i “low-input” proizvodnje.

Ključne riječi*:* *Punica granatum* L., analiza lista, sortna razlika

INTRODUCTION

Pomegranate (*Punica granatum* L.) has been cultivated since ancient times throughout Mediterranean and Middle East what resulted in a large number of genotypes showing huge genetic variability. In the last decades production has been spread all around the world in areas with Mediterranean climate (Holland et al., 2009). The edible part of the pomegranate fruit, called arils, had high levels of sugars, vitamins, polyphenols, and minerals and additionally the health benefits of consuming pomegranates have been attributed to the exceptionally high antioxidant capacity (Gil et al., 2000). Few studies have been done regarding the physical properties, phenolic content, anthocyanin composition and antioxidant capacities of local and introduced pomegranate accessions grown in Croatia (Radunic et al., 2015; Radunic et al., 2018).

Pomegranates are grown in Croatia mostly on calcareous soils where pH is determined by high $CaCO_3$ which buffers the soils in the pH range 7.5-8.5 (Marschner, 1995). Soil pH affect soil nutrient availability. In these soils, P is fixed in insoluble calcium and magnesium phosphates, while iron, zinc, manganese and copper deficiencies are common due to reduced solubility at alkaline pH values (Rashid & Ryan, 2004). High availability of Ca leads to strong decrease in Mg and K uptake, due to each other antagonistic reactions, so Mg and K deficiency are often found in production (Gransee & Führs, 2013).

Plant species and genotypes differ in their capacity for nutrient uptake and translocation as through evolution plants evolved adaptive morphological, physiological, and biochemical mechanisms, what was also found for fruit cultivars (Miljković & Vrsaljko, 2009). Fertilizer recommendations for fruit crops are mostly based on leaf nutrient concentrations and scarce studies on pomegranates showed that concentrations vary among cultivars (Hepaksoy et al., 2000, Chater and Garner, 2018). Little information is available regarding pomegranate leaf nutrient sufficiency ranges and present knowledge differ due to various cultivation practices, cultivars and agro-ecological conditions.

The aim of this work was to determine possible differences between leaf mineral concentrations of selected Croatian and introduced cultivars grown on calcareous soils in a way to provide data for diagnosing tree nutritional status, as a criterion for future fertilization requirements.

MATERIAL AND METHODS

The study was conducted with five Croatian pomegranate cultivars (Sladun, Konjski zub, Barski slatki, Ciparski rani and Glavaš) and two introduced well-known world cultivars (Hicaznar –Turkey; Wonderful - USA).

Plant material was obtained from a collection maintained at Institut za jadranske kulture i melioraciju krša (Institute of Adriatic Crops and Karst Reclamation), Split, Croatia (latitude: 43°31'N, longitude: 16°27'E) grown on calcareous rendzina leptosol soil (pH(H_20) 8.4; pH(1M KCl) 7.50; $CaCO_3$ 56.0%; active lime (CaO) 17.32%; organic matter 2.61%; available P (Troug method, extraction with ammonium sulphate; Bolland et al., 1994) 8.7 mg/100 g soil; available K (extraction with 1M ammonium acetate; Benton Jones, 2001) 46 mg/100 g soil). All trees were 8 years old, trained to single trunk and had uniform vigor. Orchard was maintained with standard cultivation practices for region that include winter application of NPK fertilizers (200 – 300 g/tree).

Leaf samples were taken in July and each sample comprised of 40-60 fully expanded mature leaves collected from the middle portion of new season shoots, about 1.5 m above the soil surface, all around the tree (Marathe et al., 2017). The leaves were washed with deionized water in the laboratory, dried at 80 °C for 48 hours and finally ground to a fine powder. Subsequently, 0.5 g powdered material was subjected to dry ashing in a muffle furnace at 550 °C for 5 h, and after dissolving in 2 mL HCl, used to extract P, K, Ca, Mg, Zn, Mn and Cu, the solution was diluted to 50 ml with deionized water. P was determined spectrophotometrically at 420 nm by the vanadate-molybdate yellow colour method (Chapman and Pratt, 1961). K was measured using the flame photometer (Model 410; Sherwood), while concentrations of Ca, Mg, Zn, Mn, Fe and Cu were determined by flame atomic absorption spectrometry (Spectraa 220; Varian). The total leaf N concentration were measured by the micro-Kjeldah digestion system (1026, Foss). (AOAC, 1995).

Completely randomized design was applied. Each of seven cultivars were represented by tree trees, each tree as one replicant. The data obtained were compared between cultivars using one-way ANOVA ($P \leq 0.05$; Tukey post hoc test). Statistical analysis was carried out using StatView software (SAS Institute, 1999).

RESULTS AND DISCUSSION

Significant differences between cultivars have been found for P, Ca, Mg, Fe and Zn (Table 1, Table 2). There were not significant difference for N leaf concentration between cultivars and varied from 1.41 to 1.67% DM (Table 1). These values were on the lower level of threshold for sufficiency or even deficient compared to values of 1.8 to 2.5% DM as proposed by Agehara (2017). Charter and Garne (2018) reported for cultivar „Wonderful“

different N levels regarding soil type, and Marathe et al. (2017) found N values similar to ours in unfertilized plants, so it seems that leaf N is more under influence of cultural practices than cultivars.

Table 1 Leaf macronutrient concentrations in seven pomegranate cultivars

Tablica 1. Koncentracija makrohraniva u listu sedam kultivara šipka

	N	P	K	Ca	Mg
Cultivar	% DM				
Barski slatki	1.41	0.17 b	0.57	1.01 b	0.13 b
Ciparski rani	1.55	0.16b	0.59	0.79b	0.13 b
Glavaš	1.46	0.13b	0.55	1.24 ab	0.17 b
Konjski zub	1.55	0.19 b	0.47	1.13 ab	0.16 b
Sladun	1.67	0.40a	0.76	1.69 a	0.23 a
Hicaznar	1.54	0.12b	0.55	0.85 b	0.13 b
Wonderful	1.41	0.17 b	0.57	1.09 ab	0.13 b

Different letters within columns indicate significant differences by Tukey HSD test at $P \leq 0.05$.
Različita slova unutar stupaca označavaju značajne razlike s Tukey HSD testom pri $p \leq 0.05$

Table 2 Leaf micronutrient concentrations in seven pomegranate cultivars

Tablica 2. Koncentracija mikrohraniva u listu sedam kultivara šipka

	Fe	Zn	Cu	Mn
Cultivar	mg kg^{-1}			
Barski slatki	49.7 ab	8.19 ab	10.2	23.1
Ciparski rani	44.4 ab	7.09 b	11.0	27.0
Glavaš	55.2 ab	8.51 ab	10.2	28.2
Konjski zub	50.5 ab	7.45 ab	10.4	31.4
Sladun	58.8 a	12.9 a	16.7	41.9
Hicaznar	38.9 b	8.10 ab	9.65	24.4
Wonderful	44.1 ab	8.41 ab	11.4	26.3

Different letters within columns indicate significant differences by Tukey HSD test at $P \leq 0.05$.
Različita slova unutar stupaca označavaju značajne razlike s Tukey HSD testom pri $p \leq 0.05$

Cultivar Sladun had significantly higher leaf P concentration than all other cultivars that do not differ among each other. Cultivars (except Sladun) leaf P varied between 0.11 to 0.19% DM what is in sufficiency range for pomegranate (0.1 – 0.2% DM) (Agehara, 2017). Although soil P availability was low, as shown by soil analysis, it seems that pomegranates do not have limitation in P acquisition in calcareous soils.

No significant differences between cultivars were found for leaf K concentrations. In all cultivars there were lower than found in many studies with different cultivars grown on contrasting soils were values were noted to be 0.8 to 1.2% DM (Hasani et al., 2012; Davarpanah et al., 2016.). Although soil K availability was high and no visual symptoms of K deficiency were recorded, K sufficiency ranges should be investigated in future programs for used cultivars. Foliar application of KNO_3 was found to positively affect pomegranate plant K status (Chater and Garner, 2018).

Regarding Ca and Mg leaf concentrations, for both nutrients significant highest values were noted for cultivar Sladun, for Ca not different than Glavaš, Konjski zub and Wonderful and for Mg different to all other cultivars. Leaf Ca was in normal ranges as proposed for Florida pomegranates (0.7 – 1.5%) (Agehara, 2017), but in the other way much lower than was found for cv. Wonderful in Florida (3.5 – 4.7%) (Chater and Garner, 2018) or cv. Ardestani in Florida (2.3 – 2.5%) (Davarpanah et al., 2016). All cultivars had Mg concentrations lower than in any of the mentioned studies although no visual symptoms of Mg deficiency were noted. The chlorosis as a symptom of Mg deficiency is late expressed, what underestimate the importance of latent Mg deficiency without a symptom that also lower plant productivity and affect plant metabolism influencing quality characteristics in agricultural products (Gerendás & Führs, 2013). Similar was found for olives grown on the similar soil (Paskovic et al., 2013).

Leaf Fe concentration significantly differed between cultivars and was highest (58.8 mg/kg Dm) in cv. Sladun and lowest (38.9 mg/kg DM) in cv. Hicaznar (Table 2). All cultivars had Fe concentrations lower than data reported from above cited authors and lower than sufficiency range (60 – 120 mg/kg DM) proposed by Zekri (2017). Fe availability under alkaline and calcareous soils is often far below that required to satisfy plant demand which favour the occurrence of Fe chlorosis (Tagliavini and Rombola, 2001). It seems that pomegranate hardly show typical Fe chlorosis even when grown in Fe-free nutrient solution (Marathe et al., 2017).

Zn leaf concentrations was highest in cv. Sladun and significantly differ only with cv. Ciparski rani. Determined values in all cultivars were much lower than proposed values of 40-70 mg/kg DW or found in other cultivars and agroclimatic conditions (Hasani et al., 2012; Mir et al., 2015). Studied cultivars had sufficient leaf P concentrations what can be possible result of Zn deficiency as it enhances both the uptake rate of P by the roots and the translocation to the shoots. This effect is specific for Zn deficiency and is not observed when other micronutrients are deficient (Marschner, 1995).

Leaf Zn can be significantly increased by foliar application of $ZnSO_4$ what can result in improving marketable yield and reducing fruit split incidence although differences between cultivars was found (Davarpanah et al., 2016; Chater and Garner, 2018).

Leaf Cu and Mn concentrations did not differ between cultivars and values were above sufficiency threshold (20 – 70 mg/kg leaf DW) for Mn and on lower end of sufficiency range (10 – 20 mg/kg DW) for Cu as proposed by Zekri (2017). These values for Mn were much lower than found in even unfertilized plants (Mir et al., 2015; Hasani et al., 2012).

CONCLUSION

The present study is the first report of leaf nutrient concentrations in Croatian pomegranate cultivars grown on calcareous soil. Cultivar differences have been found for P, Ca, Mg, Fe and Zn. Cultivar Sladun had highest concentrations of all nutrients and could be possible option for low-input production.

Our study showed low K and Mg concentration in all pomegranate cultivars, although visual symptoms of deficiency was not detected. Same was found for Fe and Zn. These deficiencies in pomegranates are common alleviated with soil and foliar fertilization so in future its effects should be investigated on studied cultivars

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