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Role of apple clonal rootstocks on yield, fruit size, nutritional value and antioxidant activity of 'Red Chief® Camspur' cultivar

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ABSTRACT

From 2007 to 2015 we investigated impact of three clonal rootstocks for apple (M.9 T337, M.4 and MM.106) on productivity, fruit physical and chemical properties of 'Red Chief® Camspur' cultivar under pedo-climatic conditions of Cacak (western Serbia). Results showed that tree vigour, yield per tree and per unit area, cumulative yield (CY) and yield efficiency (YE) significantly varied among rootstock. These parameters, with exception of YE were lower on M.9T337, and higher on M.4 and/or MM.106. YE was the lowest in M.4. Regarding fruit physical properties, we observed that all evaluated, except L/D ratio, significantly changed through rootstocks. Fruit weight and fruit linear dimensions (length and diameter), of above cultivar were higher and similar on M.4 and MM.106 then on M.9 T337. The MM.106 induced firmer fruits as compared with other both rootstocks. Soluble solids content (SSC) was the highest in whole fruits (pulp + peel) on M.4, and the lowest on M.9T337. In contrast, M.9 T337 induced higher titratable acidity (TA), total phenolic (TPC) and total flavonoid contents (TFC) and antioxidant power (TAC) in both pulp and peel than M.4 and MM.106 rootstocks, respectively. Ripening index (RI) was the highest on MM.106, and the lowest on M.9 T337. Otherwise, peel of this cultivar was better source of above compounds than pulp. Beside malic acid, in both pulp and peel, with exception of *p*-hidroxybenzoic and chlorogenic acids in peel, we detected other organic acids such as gallic, protocatechuic, *p*-hydroxybenzoic, caffeic and chlorogenic as a major and/or minor component.

1. Introduction

Apple is one of the most widely cultivated fruit trees with more than 7,500 cultivars (Dobrzański et al., 2006). This fruit species is a very old because has been cultivated worldwide since 4000 BC. Minor and central Asia, Himalayan regions of India, Pakistan including western China is believed as center of origin of apple (Muzher et al., 2007). World apple production is 89,329,179 tons from 5,293,340 ha with increasing tendency from the past few decades (FAOSTAT, 2017). China produced almost half of this total, followed by USA, Turkey, Poland, India, Italy, etc.

Serbia, the small country on the Balkan Peninsula, produced 240,320 t in 2006 to 328,369 t in 2016, i.e. 252,438 t in average (FAOSTAT, 2017) with also increasing production tendency. In this country, apple is a major fruit crop after European plum and grown in different parts, including Cacak region (western Serbia). The predominant commercial cultivar is 'Idared' (over 55% of all grown cultivars), followed by 'Golden Delicious' (clones B and 'Reinders'), 'Granny Smith', 'Melrose', 'Gala', 'Fuji' and 'Jonagold' with clones, old and new clones of 'Red Delicious', sporadically 'Gloster', 'Mutsu', 'Čadel' and 'Modi®' (Milošević 1997; Milošević et al., 2014a). Recently, newly-bred spur-type of 'Red Delicious' named 'Red Chief® Camspur' is some of the most widely planted apple cultivars in Serbia which characterized with elongated fruit with well-developed calyx lobes.

In past few decades, Serbian apple production is characterized by intensive growing technology with over 2,500 trees per hectare, high yield per tree and/or unit area, introduction of new cultivars and dwarfing rootstocks, satisfactory fruit quality etc. (Milošević et al., 2014a). However, apples have also some technological demands. Namely, they belong to the most difficult fruit species to cultivation. Their difficulty is above all in hand fruit picking and their use for fresh market, but also in the severe technological growth system. The toilful hand picking can be compensated only by cultivation of low stems, which can be achieved by the use of weak growing rootstocks such as M.9 with clones, M.26, and partially MM.106 for spur types of apple cultivars.

In Serbia, since 1960's semi-dwarfing vegetative rootstocks such as M.2, M.7, especially M.4 were suppressed seedlings of wild apple also

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called "forest apple" [*Malus sylvestris* (L.) Mill.] from orchards and importantly contributed to the intensification of its production. So, they have only historical importance (Milošević, 1997). Nowadays, most common rootstock used for apple cultivars is dwarfing clonal M.9 due to their impact to lime induced iron chlorosis, easy propagation and well graft-compatible with apple cultivars. In addition, M.9 induces precocity, high yield per unit area, yield efficiency, large fruits and excellent fruit quality in comparison with other rootstocks (Borbála, 2001). Although has several weaknesses, M.9 was adopted as a dominant rootstock worldwide (Marini et al., 2014).

Currently, in modern and highly intensive orchards in Europe, the MM.106 rootstock used for grafting of spur apple types such as 'Starking', 'Starkrimson', 'Red Chief', 'Scarlet Spur' etc. Under Serbian conditions, 'Idared' since that has a more dwarf tree, often was grafted on MM.106 rootstock in orchards (Milošević, 1997). In addition, although semi-dwarf MM.106 rootstock causes high yield efficiency, fruit size can be smaller than M.9 (Webster and Wertheim, 2003).

Generally, rootstocks can significantly influence a great number of important growing properties of fruit trees and in some fruit species, their influence makes about 50% of economic results (Mezey and Leško, 2014). So, they can influence a tree growth, precocity, productivity, nutritional status of apple tree, (Fallahi et al., 2002; Milošević and Milošević, 2015) alternate bearing (Kviklys et al., 2016; Reig et al. 2018), tolerance to spring frost, winter cold (Robinson, 2004) and drought (Tworkoski et al., 2016), anchorage of a tree, depth of the root system, resistance and tolerance to pests a diseases (Beers et al., 2006), time of budding, fruit sensorial and physical traits and chemical composition (Milošević et al., 2014b; Kviklys et al., 2014, 2017; Lv, 2016).

Apple fruits with large size, good coloured peel, shapely and marked aroma have high market value. Fruit contains numerous bioactive primary and secondary compounds with potentially valuable nutritive, health and pharmacological potential (Lv, 2016; Kviklys et al., 2014, 2017). Recent studies have shown that the apple cultivar may substantially influence the fruit chemical properties, especially phenolic content and total antioxidant activity (Drogoudi et al., 2007), reflecting a qualitative genetic control (Sha et al., 2011). Rootstock influence on these properties is less studied, only in Estonia (Mainla et al., 2011) and Lithuania (Kviklys et al., 2014, 2017). From this point, the main goals of this study were: *i*) assess role of different clonal rootstocks on tree vigour, precocity and yield performances, *ii*) evaluate the possibilities of the rootstock to change the fruit physical and chemical properties, and *iii*) assess how the rootstocks perform the distribution of compounds in some parts of the fruits of 'Red Chief® Camspur' grown under western Serbian pedo-climatic conditions.

2. Materials and Methods

2.1. Plant material, field trial and ecological conditions

The apple orchard with *cv*. 'Red Chief® Camspur', belonging to red-delicious apples with dark-red peel colour, grafted on M.9 T337, M.4 and MM.106 rootstocks was established in early spring 2006 with 1-year-old nursery trees with sylleptic shoots at 3.0 m × 1.2 m planting distance; training system was slender spindle. The orchard was located in Prislonica village (43°57′N, 20°26′E) near Cacak city, western Serbia at 320 m a.s.l. and managed following the usual standard procedures under non-irrigated practice. Fruit thinning was not performed in order to maintain the apparent effect of the rootstock. Treatments were distributed using the randomized complete block design with five trees for each rootstock-cultivar combination in four replicates ($n = 20$).

Orchard was established on Vertisol or "Smonitza" soil with the followed chemical properties: clay loamy, pH_{nKCl} – 5.20, organic matter – 1.71%, N total – 0.15%, available P_2O_5 – 73.0 mg kg^{-1} and K_2O – 280.9 mg kg^{-1}, 0.07% – CaO and MgO – 4.7 mg kg^{-1}, respectively. Soil is rich source in available P_2O_5 and K_2O, whereas other macronutrients are in a moderate to low range.

Weather data during the experimental period were in the range of long-term averages; characterized by the average annual temperature of 11.3 °C and total annual rainfall of 690.2 mm. The average air temperature during vegetative cycle was 17.0 °C. There was no severe spring frost damage or other weather disturbances during the experimental period.

2.2. Experimental procedure

2.2.1. Tree growth and yield attributes

Vegetative growth was evaluated by measuring the trunk diameter 20 cm above graft union, and converting to trunk-cross sectional area (TCSA) from 2007 to 2015. Yield per tree (kg) and per hectare (t) and CY per tree (kg) from 2008 to 2015 of each rootstock–cultivar combination were measured from the harvest data using ACS electronic scale (Zhejiang, China). The YE (kg cm^{-2}) was calculated as the ratio of the total cumulative yield (kg) per final TCSA (cm^2). Measurements were performed every year.

2.2.2. Fruit physical properties

Fruit sampling (25 fruits from every tree in four replicates, i.e. total 100 fruits per each rootstock-cultivar combination) was performed in 2014 and 2015 at commercial maturity which was determined using the starch iodine test and picked from the all part of canopy (top, central and basal). The fruits were immediately taken to the laboratory at Faculty of Agronomy in Cacak and their physical properties were determined. Fruit weight (g) was measured with a digital balance MAULsteel 5000 G (Jakob Maul GmbH, Bad König, Germany). Fruit linear dimensions - length (L) and diameter (D) (both in mm) were measured using caliper gauge Sttarret 727 (Athol, MA, USA). Length/diameter ratio (L/D ratio), also called fruit elongation index, was calculated.

Flesh firmness (FF) was determined with an hand penetrometer Bertuzzi FT-327 (Facchini, Alfonsine, Italy) with an 8 mm-diameter plunger, on both cheeks of the fruit after skin removal and was express in N.

2.2.3. Fruit chemical properties

Fruit sampling for chemical analysis, about 50 per each rootstock-cultivar combination, was performed at technological maturity i.e. after five weeks of storage at 2 °C in 2014 and 2015. According to standard laboratory procedures as previously described by AOAC (1995), fruit samples and subsamples were prepared for analytical determinations.

2.2.3.1. Determination of soluble solids content and titratble acidity. The SSC (°Brix) was determined by hand refractometer Carl Zeiss 32-G (Carl Zeiss, Jena, Germany) at 20°C, from juice extracted from the fruits. Titratable acidity (% of malic acid) was analyzed in juices by titration with 0.1 mol L^{-1} NaOH, up to pH 8.1 using a titrimeter Metrohm 719 S (Titrino, Herisau, Switzerland). The ripening index (RI) was calculated based on the SSC/TA ratio. Above evaluations were accomplished in whole fruits.

2.2.3.2. Determination of secondary metabolites. Levels of secondary metabolites evaluated were determined from fruit flesh (pulp) and from peel. The peel and flesh were collected separately. Whole fruits were peeled to a thickness of approximately 1 mm, and the remaining flesh was cut into pieces before all samples were flash-frozen in liquid nitrogen. Afterward, the samples were ground to a fine powder and stored at -80 °C before further analysis. All analyses were performed in triplicate.

2.2.3.2.1. Chemicals and reagents. Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, ascorbic acid, rutin, quercetin, and organic acid standards (gallic acid, protocatechuic acid, p-hidroxybenzoic acid, caffeic acid