UDC 634.11:542.943'78
615.279.015
Original research paper
doi: 10.5937/AASer1948097M
Acta Agriculturae Serbica, Vol. XXIV, 48(2019); 97-106

Acta Agriculturae Serbica
FACULTY OF AGRONOMY • ČAČAK •

Role of rootstock and apple fruit tissue in antioxidant activity

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Abstract: During two consecutive years (2018 and 2019) we investigated the effect of two clonal rootstocks on fruit weight, contents of some non-nutrients and total antioxidant capacity (TAC) in the flesh and peel of *cv*. 'Hapke' apple. Results showed that fruit weight was significantly higher on M.9 T337 than on M.26 rootstock and similar in both 2018 and 2019. M.26 rootstock significantly improved titratable acidity (TA), vitamin C content, total phenolic content (TPC), total flavonoid content (TFC) and TAC in comparison with M.9 T337. As regards fruit tissue, the peel was a significantly better source of acids, vitamin C and phenolic compounds, and had higher antioxidant capacity than the flesh. However, the rootstock × fruit tissue interaction for the content of phenolic compounds indicated the complex nature of accumulation and distribution of acidity, vitamin C and phenolic compounds in apples.

Keywords: apple fruit tissue, phenolic compounds, total antioxidant capacity, vitamin C.

Received 30 October 2019 Accepted 10 December 2019

Introduction

Apple (*Malus* × *domestica* Borkh.) is a very popular fruit crop worldwide, with an annual production of more than 83 million tonnes in 2017 (FAOSTAT, 2019), ranking fourth after grapes, citrus and bananas. Its beautiful appearance, crispy flesh, pleasant flavour and sweet taste attract consumers and fetch a high price (Asif Ali *et al*., 2004).

Fruit quality is determined by external and internal (morphophysical, biochemical and organoleptic or sensorial) factors. Therefore, fruit physical and chemical properties play an important role for both producers and consumers. Among them, fruit size, contents of organic matter (sugars, acids, pectins, tannins, starch, cellulose, vitamins, enzymes, phytohormones, phenolic compounds etc.) and biogenic macro- and microelements (N, P, K, Ca, Mg, S and Fe) are the most important for consumers (Nour *et al*., 2010; Milošević *et al*., 2014, 2018). However, in recent years, this nutritional doctrine has changed. Namely, consumers prefer fruits and vegetables not only rich in primary metabolites, but also rich in phenolic compounds with high antioxidant activity i.e. health benefits for the human body (Milošević *et al*., 2018). As known, the chemical composition of apple fruit is very complex since it depends on fruit cultivar, ripeness, physiological condition of the tree as well as on soil and weather conditions (Markuszewski and Kopytowski, 2008) and fruit tissue (Wolfe et al., 2003; Chinnici et al., 2004; Leccese et al., 2009; Milošević *et al*., 2018).

Among cultivars grown in Serbian apple orchards, old and newly selected clones of the 'Red Delicious' group are widespread. It is well known that the characteristic shape of 'Red Delicious' and its clones is the elongated fruit with well-developed calyx lobes (Koukourikou-Petridou *et al*., 2014). 'Hapke' is a clone of the 'Red Delicious' family distinguished by these traits (Milošević *et al*., 2014; Tabakov *et al*., 2016). For the planting of modern commercial apple orchards with 3,000–5,000 trees per hectare, rootstocks such as M.9 T337 with numerous clones (especially NAKB T333) are used in Serbia and worldwide, and also M.26 in some cases (Paunović *et al*., 2015; Milošević *et al*., 2018). As previously noted, apple cultivar and fruit tissue may have a paramount influence on fruit chemical properties, especially phenolic content and total antioxidant activity (Drogoudi *et al*., 2008). The effect of rootstock on these properties in apples is less known because it has not been widely tested. Some sporadic results were obtained by Mainla *et al*. (2011) in Estonia, Kviklys *et al*. (2014) in Lithuania and Milošević *et al*. (2018) in Serbia. For these reasons, the main goal of the present work was to describe variability in the fruit weight and content of some bioactive compounds in the peel and flesh tissue of 'Hapke' apple grafted on two dwarf clonal rootstocks (M.9 T337 and M.26) grown under western Serbian conditions.

Material and method

The privately owned apple orchard located in Prislonica village (43°57ʾ N, 20°26ʾ E) near the town of Čačak (western Serbia) used in this research was established in 2006 in a randomised block design with 5 trees of each rootstock-cultivar combination in four replicates (n = 20). Standard cultural practices were applied, with the exception of irrigation. Fruits from apple cv. 'Hapke' grafted on M.9 T337 and M.26 rootstocks were harvested at commercial maturity in 2018 and 2019. For fruit weight determination, 25 fruits in four replicates (n = 100) were measured with a MAULsteel 5000 G digital balance (Jakob Maul GmbH, Bad König, Germany) in both years.

Fruit sampling i.e. flesh and peel sampling for chemical analysis was performed using the procedure described earlier in our similar investigation (Milošević *et al*., 2018). Titratable acidity (TA) was determined by titration with 0.1N NaOH solution up to pH 8.1 using a Metrohm 719S titration device (Titrino, Herisau, Switzerland) and was expressed as percent of malic acid. Ascorbic acid (vitamin C) was determined by the 2,6-dichloroindophenol method (Arya et al., 2000). Data are given as milligrams per 100 g fresh weight (mg/100 g fw).

Total phenolic content (TPC) was determined spectrophotometrically using the Folin-Ciocalteu method (Singleton *et al*., 1999). Values were expressed as milligrams of gallic acid equivalent (GAE) per 100 g fw (mg GAE/100 g fw). Total flavonoid content (TFC) was measured by the aluminium chloride colorimetric assay (Zhishen *et al*., 1999) using catechin equivalent (CE) as a standard. TFC was expressed as milligrams of CE per 100 g fw (mg CE/100 g fw). TAC was evaluated by the phosphomolybdenum method (Prieto et al., 1999). Ascorbic acid (AA) was used as standard and TAC was expressed as µg of AA per g fw (µg AA/g fw).

All compounds were estimated by using a UV–VIS spectrophotometer model MA9523-SPEKOL 211 (Iskra, Horjul, Slovenia). Their contents were expressed as means ± SE of triplicate analyses per rootstock and fruit tissue in both years.

All data in the present study were subjected to analysis of variance (two-way ANOVA) and means were separated by LSD test at $P \leq 0.05$.

Results and discussion

Apple fruit weight and fruit size are important factors influencing consumer acceptance and orchard profitability. For these reasons, pomologists want to know which practices and treatments influence mean fruit weight and mean fruit value (Marini *et al*., 2001). Fruit weight as a cultivar-specific property can be improved by cultural practices such as fertilisation, irrigation, pruning, thinning, spraying with foliar nutrients and hormones etc. (Greenhalgh *et al*., 1977; Milošević *et al*., 2014, 2019), crop load, rootstock (Milošević *et al*., 2018) and

choice of environmental conditions suitable for apple growing. In the present study, significantly higher fruit weight was found in 'Hapke' on M.9 T337 in comparison with M.26 (Table 1). These results were expected due to the higher yield produced by M.26 rootstock in both years (data not shown). In our earlier study we found similar fruit weight values for this cultivar (Milošević *et al*., 2014).

Year-by-year variations in fruit weight were not observed, as indicated by the stronger effect of rootstock than season, which is in agreement with the results of other researchers (Tabakov *et al*., 2016). The effect of the rootstock × year interaction on this property was also not significant.

Table 1. Average fruit weight of 'Hapke' apple cultivar grafted on two clonal rootstocks during two consecutive years

Parameter		Fruit weight (g)
Rootstock (A)		
M.9 T337		176.25 ± 0.75 a
M.26		168.12 ± 0.64 b
Year (B)		
2018		171.37 ± 1.02 a
2019		173.00 ± 2.04 a
Interaction A × B		
M.9 T337	2018	174.75 ± 0.75 a
	2019	177.75 ± 0.74 a
M.26	2018	168.00 ± 0.50 a
	2019	168.25 ± 1.75 a
ANOVA		
A		*
B		ns
A × B		ns

The different letter(s) in the column indicate significant differences among means for each rootstock and fruit tissue at $P \leq 0.05$ by LSD test.
The asterisk in the column indicates significant differences at $P \leq 0.05$ by *F* test. ns: not significant.

Data in Table 2 revealed that the content of chemicals significantly varied between rootstocks and also between fruit tissues. In addition, the effect of the rootstock × tissue interaction on all evaluated biochemical compounds was significant. Sasnauskas *et al*. (2007) also reported that the fruit quality and

biochemical composition of apple were significantly influenced by scion (cultivar), rootstock and their interaction.

Titratable acidity can be an important indicator of taste of apple fruits (Harker *et al*., 2002) and their internal quality, since consumers often have distinct preferences for acid or sweet tasting apples (Daillant-Spinnler *et al*., 1996). The predominant acid in apples is malic acid, followed by citric acid etc. (Nour *et al*., 2010).

Table 2. Phenolic compounds and total antioxidant capacity of apple *cv*. 'Hapke' grafted on two clonal rootstocks

Parameter		Titratable acidity (%)	Vitamin C (mg 100/g fw)	Total phenolic content (mg GAE 100/g fw)	Total flavonoid content (mg CE 100/g fw)	Total antioxidant capacity (µg AA/g fw)
Rootstock (A)						
M.9 T337		0.26 ± 0.00 b	8.25 ± 0.02 b	208.00 ± 0.02 b	93.74 ± 0.01 b	103.37 ± 0.00 b
M.26		0.35 ± 0.01 a	11.35 ± 0.00 a	210.35 ± 0.01 a	99.89 ± 0.02 a	107.87 ± 0.01 a
Tissue (B)						
Flesh		0.13 ± 0.00 b	8.65 ± 0.01 b	201.49 ± 0.02 b	71.18 ± 0.02 b	103.37 ± 0.01 b
Peel		0.48 ± 0.00 a	10.95 ± 0.00 a	216.87 ± 0.01 a	122.44 ± 0.01 a	108.82 ± 0.01 a
Interaction A × B						
M.9 T337	Flesh	0.10 ± 0.00 d	7.05 ± 0.02 d	200.55 ± 0.03 d	67.07 ± 0.01 d	101.28 ± 0.01 d
	Peel	0.41 ± 0.01 b	9.46 ± 0.01 c	215.46 ± 0.01 b	120.41 ± 0.00 b	105.46 ± 0.01 c
M.26	Flesh	0.17 ± 0.00 c	10.25 ± 0.00 b	202.42 ± 0.01 c	75.30 ± 0.02 c	107.40 ± 0.00 b
	Peel	0.54 ± 0.01 a	12.45 ± 0.00 a	218.29 ± 0.01 a	124.48 ± 0.02 a	110.24 ± 0.01 a
ANOVA						
A		*	*	*	*	*
B		*	*	*	*	*
A × B		*	*	*	*	*

The different letter(s) in columns indicate significant differences among means for each rootstock and fruit tissue at $P \leq 0.05$ by LSD test.
The asterisks in columns indicate significant differences at $P \leq 0.05$ by *F* test. ns: not significant.

In the present study, M.26 induced a higher TA content than M.9 T337 in 'Hapke' fruits. However, in our earlier work on apple (Milošević *et al*., 2018), M.9 T337 caused a higher total acid content in 'Red Chief® Camspur' fruits than the semi-dwarf M.4 and MM.106 rootstocks, respectively. In many pomologies worldwide, M.9 T337 and M.26 rootstocks are classified as dwarf rootstocks for apple, with M.26 as somewhat more vigorous. Also, as the canopy of *cv*. 'Hapke'

grafted on M.26 was sparse, the fruits were more exposed to sunlight, which contributed to the increased acid content of the fruit (Avad *et al*., 2000).

Hence, studies on the effect of rootstock on the accumulation of TA in apple fruits revealed benefits of dwarfing rootstocks, as previously reported by Mainla *et al*. (2011). In our study, 3.7-fold more TA were found in the peel than in the apple flesh, which is in agreement with the results of other authors (Drogoudi et al., 2008; Milošević *et al*., 2018). Drogoudi *et al*. (2008) also reported that the green peel of 'Granny Smith' apple had the highest TA content, as opposed to the lowest in the yellowish-green peel of 'Fyriki' apple in comparison with seven apple cultivars, indicating an important role of peel colour in TA accumulation (Nour *et al*., 2010). Also, results from more studies reported that clones (genotypes) of the 'Red Delicious' group had smaller to moderate TA contents (Koukourikou-Petridou et al., 2007; Ahmed et al., 2013; Milošević *et al*., 2018). In general, our range of TA values was within the range determined by other researchers (Sasnauskas *et al*., 2007; Nour *et al*., 2010; Contessa and Botta, 2016).

In our study, higher contents of vitamin C, TPC, TFC and TAC were found in *cv*. 'Hapke' grafted on M.26 than on M.9 T337, and the peel was a better source of these compounds in comparison with the fruit flesh (Table 2). This is in accordance with the previous results obtained for several apple genotypes showing that the peel tissue is the fruit portion with the highest bioactivity (Leccese et al., 2009).

Studies on the influence of apple rootstock (Mainla *et al*., 2011; Kviklys *et al*., 2014) on the accumulation and distribution of phenolic compounds in apple fruit tissues (Wolfe et al., 2003; Chinnici et al., 2004; Drogoudi *et al*., 2008; Leccese et al., 2009) revealed benefits of dwarfing rootstocks (Milošević *et al*., 2018). Awad *et al*. (2000) indicated an increase of some phenolic compounds such as quercetin in apple peel due to better sun-exposure.

As regards the effect of rootstock on the contents of these compounds in this trial, we did not identify higher levels of tested compounds in fruits from trees on the dwarfing M.9 T337 rootstock. Probably, the slender canopy with better light conditions of M.26 may be the major reason for this phenomenon in our study. Similar results were found by Kviklys *et al*. (2014).

Vitamin C (also called ascorbic acid, ascorbate, AA) is a water soluble organic compound that participates in many biological processes i.e. in maintaining human health and is positively correlated with antioxidant activity. In the present study, the apple peel contained a 1.27 times higher vitamin C content and 1.05 times higher antioxidant activity compared with the flesh. This is in accordance with the previous results obtained in several apple genotypes showing that the peel tissue is the fruit portion with the highest bioactivity (Drogoudi *et al*., 2008; Leccese *et al*., 2009). Drogoudi *et al*. (2008) reported much lower values of vitamin C in the flesh of several apples than those obtained in our study, whereas Planchon *et al*. (2004) and Nour *et al*. (2010) reported

ranges between 2.9 and 12.3 mg 100 g^{-1} fw and 6.18 and 18.70 mg 100 g^{-1} fw, which supported our results. Ahmed *et al.* (2013) reported the values of vitamin C in fruits of 'Red Delicious' on MM.111 between 6.98% ('Red Chief') and 7.83% ('Starking Delicious'). In addition, ancient or old apples are richer in vitamin C and phenolic compounds than newly bred and/or commercial apples, but generally depending on the genetics of cultivar, fruit position in the trees, fruit size, fruit skin colour and maturity stage (Contessa and Botta, 2016). Interestingly, the flesh of 'Hapke' on M.26 had better TAC values than the peel of fruits of this cultivar on M.9 T337.

The comparison of the TPC and TFC values obtained in the present study with those of other studies suggests similar trends although differences in the units used and analytical methods employed make a direct comparison difficult (Drogoudi *et al*., 2008). For example, the edible part of peeled apple fruits contained TPC between 31.42 and 90.12 mg GAE 100 g^{-1} fw (Contessa and Botta, 2016), whereas Kähkönen *et al*. (1999) reported 12 mg GAE 100 g^{-1} dw in the flesh of an unspecified cultivar. However, higher contents of phenolic compounds in the peel than the flesh were also reported by other researchers (Wolfe *et al*., 2003; Chinnici *et al*., 2004; Leccese *et al*., 2009; Milošević *et al*., 2018). For instance, Leccese *et al*. (2009) reported that the content of TPC was higher in the peel (varied between 1.72 and 3.75 mg GAE g^{-1} fw) than in the flesh tissue (varied between 0.30 and 0.84 mg GAE g^{-1} fw). In our earlier research (Milošević *et al*., 2018), the peel of 'Red Chief® Camspur' contained TPC between 203.12 and 340.05 mg GAE 100 g^{-1} dw, and TFC between 48.34 and 77.42 mg RUE 100 g^{-1} dw. In this study, the peel was also a richer source of these compounds than the edible part of the fruits.

The significant interaction between rootstock and fruit tissue indicated that the accumulation and distribution of acids and phenolic compounds and antioxidant activity in apple fruits have a complex nature and can be attributed to the biochemical and physiological processes during their growth, development and maturity.

Conclusion

The obtained results showed that rootstocks significantly modified the fruit weight of 'Hapke' apples, being 1.04 times higher on M.9 T337 rootstock than on M.26. Year alone and the rootstock × year interaction had no effect on this property, indicating a stronger effect of rootstock than the other sources of variation. On the other hand, rootstock, fruit tissue and their interaction significantly changed titratable acidity and the contents of vitamin C and phenolic compounds in the fruits of this cultivar. Higher values were found on M.26 rootstock than on M.9 T337. The peel was a richer source of acidity and phenolic compounds, and had better antioxidant activity than the fruit flesh.

The physicochemical characteristics of 'Hapke' apples should be considered together for the choice of better rootstocks under growing conditions aimed at selecting rootstocks with improved fruit weight, acidity, phenolic compounds and antioxidant capacity.

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ULOGA PODLOGE I TKIVA PLODA JABUKE U ANTIOKSIDATIVNOJ AKTIVNOSTI

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Rezime

Tokom dve uzastopne godine (2018 i 2019) ispitivali smo uticaj dve vegetativne podloge na masu ploda, sadržaj nekih nenutritivnih jedinjenja i ukupni antioksidativni kapacitet u mezokarpu i pokožici ploda jabuke sorte 'Hapke'. Rezultati su pokazali da je masa ploda bila veća na podlozi M.9 T337 u odnosu na M.26, ali slična u obe godine istraživanja. Podloga M.26 je značajno poboljšala sadržaj ukupnih kiselina, vitamina C, ukupnih fenola i ukupnih flavonoida i ukupni antioksidativni kapacitet u poređenju sa M.9 T337.
Što se tiče tkiva ploda, pokožica je bila značajno bolji izvor ukupnih kiselina, vitamina C i fenolnih jedinjenja i imala je veći antioksidativni kapacitet od mezokarpa. Međutim, interakcija podloga × tkivo za pomenuta jedinjenja pokazuje složenu prirodu njihove akumulacije i distribucije u plodu.

Ključne reči: tkivo ploda jabuke, fenolna jedinjenja, ukupni antioksidativni kapacitet, vitamin C.