Rootstock genotype determines phenol content in apple fruits

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ABSTRACT

The effect of weather conditions and eleven apple rootstocks was studied on the phenol content in fruits. Super-dwarf rootstocks P 61 and P 22 determined the highest content of all phenolic compounds tested. Dwarf rootstocks M.9, P 62 and semi-dwarf M.26 determined lower content of all phenolic compounds tested. The content of (+)-catechin, procyanidin B1 and total procyanidins content in apple fruits depended on rootstock genotype and high variation coefficient of these compounds between rootstocks was established. Rootstocks had a lesser effect on the content of quercitrin, (–)-epicatechin, total catechins, phloridzin and chlorogenic acid. No clear differences were found between super-dwarf, dwarf and semi-dwarf rootstock groups. Conditions during the growing season, yield and fruit weight impacted on the content of bioactive compounds. Less than 10% difference in total phenols between the growing seasons was recorded for M.26 and P 22. The greatest differences were recorded in the fruits from trees grown on M.9, Pure 1 and P 66 rootstocks. Rootstock B.396 determined the most stable content of all compounds analysed, except for procyanidin B1 and B2.

Keywords: polyphenols; *Malus* × *domestica*; phenylpropanoid metabolism; antioxidant compounds

Apples in human diet are an important source of different biological active natural plant products, which can contribute positively to prevention of several diseases. Compared to other fruits, apples contain the highest concentration of free phenolics (Sun et al. 2002). Apple studies in the USA indicated that twenty-two percent of the phenolics consumed from fruits are from apples (Vinson et al. 2001).

Various abiotic and or biotic factors can affect apple fruit composition of such defence substances. The biggest differences were found among apple cultivars, where high variability of phenolic content was recorded (Van der Sluis et al. 2001). Growing technologies can stimulate or reduce accumulation of phenolic compounds in apple fruits. Fruit position on a tree and sun exposure have an impact on phenol content (Awad et al. 2001). Weather conditions during the growing season may significantly affect the content of total polyphenols (Mainla et al. 2011).

Various studies demonstrated rootstock effect on tree vegetative and generative development, fruit quality (Kviklys et al. 2012, 2013), uptake and transport of water and minerals (Lochard and Schneider 1981). Therefore a presumption of possible rootstock effect on different pattern of accumulation of bioactive substances in apple fruits could be made.

Impact of rootstocks on fruit phenolics has not been widely tested. Fruit tree studies revealed great differences between rootstocks in accumulation of phenols in lemons (Gil-Izquierdo et al. 2004), peaches and apricots (Scalzo et al. 2005), cherries

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(Usenik and Štampar 2002). Three apple rootstocks were compared in the trial performed in Estonia (Mainla et al. 2011). Rootstock studies performed in the USA demonstrated significant differences in leaf phenolic content among rootstocks (Garcia et al. 2004).

The objective of the present study was to evaluate apple rootstock effect on the content and composition of phenolic compounds in apple fruits, to ascertain rootstock impact on the stability of biological active substances during the growing seasons, to highlight dependence of accumulation and variability of phenolic compounds due to rootstock induced tree vigour.

MATERIAL AND METHODS

The trial was conducted in the experimental orchard of the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry in 2011–2012. Rootstocks for this trial were chosen based on their popularity worldwide (M.9, M.26, partly B.396 and P 22) and suitability for intensive apple growing. According to induced tree vigour rootstocks fall within the following groups: super-dwarf rootstocks P 22, P 59, P 61 and PB.4, dwarf rootstocks M.9, P 62, P 66, P 67, B.396 and Pure 1, semi-dwarf rootstock M.26.

Rootstocks were grafted with cv. Ligol and planted in randomized block design, with four replicates and 3 trees per plot. Twenty randomly selected fruits from each replication were taken for biochemical analysis and carefully mixed. Twenty five randomly selected fruits were chosen from the bulk and analysed in three replicates. The data on the main traits were subjected to the analysis of variance. Significance of differences between rootstocks means was evaluated using *LSD* test at $P < 0.05$. Hierarchical clustering analysis and resulting dendrograms representing similarities between rootstocks were developed with SPSS software (Somers, USA).

Preparation of samples. Each apple was cut into slices, frozen and lyophilized with a ZIRBUS sublimator 3 × 4 × 5/20 (ZIRBUS technology, Bad Grund, Germany). The lyophilized slices were ground to a fine powder using a Retsch 200 mill (Haan, Germany).

Extraction. 2.5 g of lyophilized apple powder mixed in 30 mL of ethanol (70%, v/v), and extracted in a Sonorex Digital 10 P ultrasonic bath (Bandelin Electronic, Berlin, Germany) for 20 min at 40°C. Type of extraction, duration, temperature, solvent and its concentration were chosen considering the results of extraction optimization.

Instrumentation and chromatographic conditions. A Waters 2695 chromatograph equipped with a Waters 2998 photodiode array detector was used for HPLC analysis, which was controlled with the Empower® v.2.0. software (Waters, Milford, USA). Chromatographic separations were carried out by using a YMC-Pack ODS-A (5 µm, C_{18}, 250 × 4.6 mm i.d.) column equipped with a YMC-Triart (3 µm, C_{18}, 10 × 3.0 mm i.d.) precolumn (YMC Europe GmbH). The volume of the extract being investigated was 10 µL. The flow rate was 1 mL/min, and gradient elution was used. The mobile phase consisted of 2% (v/v) acetic acid in water (solvent A) and 100% (v/v) acetonitrile (solvent B). The following conditions of elution were applied: 0–30 min, 3–15% B; 30–45 min, 15–25% B; 45–50 min, 25–50% B; and 50–55 min, 50–95% B.

Detection was simultaneously performed at 3 wavelengths: 280 nm (dihydrochalcones, catechins, procyanidins), 320 nm (phenolic acids), and 360 nm (quercetin glycosides). All the phenolic compounds were identified by comparing their retention times and spectra (from 200 to 600 nm) with those of the standard compounds.

Growing conditions. Higher temperatures were recorded in 2011 when the average monthly temperatures were higher than long term average (Figure 1). The summer of 2011 was distinguished by more rainfall in July and especially in August when double the normal monthly rainfall was recorded (Figure 2).

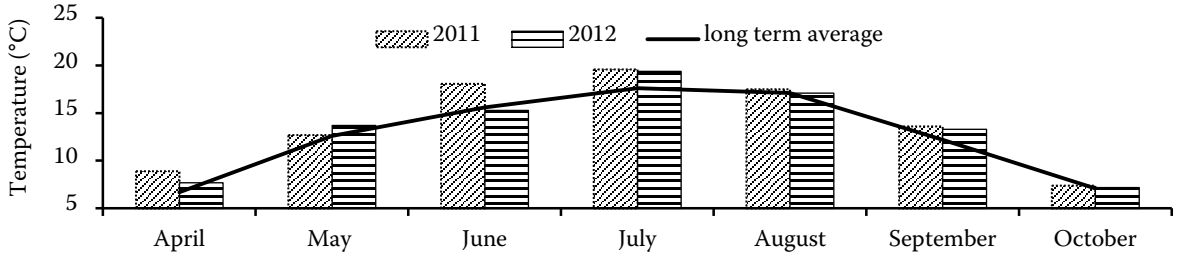

Figure 1. Temperature changes during vegetation periods

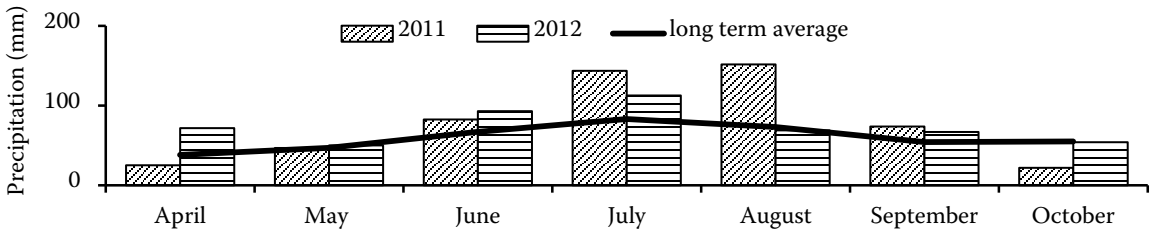

Figure 2. Precipitation during vegetation periods

RESULTS AND DISCUSSIONS

The content of phenolic compounds depends on fruit tissue analysed. Several times more phenolic compounds are found in the peel than in apple flesh (Wolfe et al. 2003, Drogoudi et al. 2008, Leccese et al. 2009). High content of phenols was detected in apple juices as well (Lachman et al. 2006). In our trial, we examined a whole fruit.

Studies of apple and cherry rootstock impact on accumulation of phenolic compounds in fruits and plant tissues revealed benefits of dwarfing rootstocks (Usenik and Štampar 2002, Mainla et al. 2011). That was not so evident in our trial where we tested rootstocks from three different vigour classes.

Hagen et al. (2007) found increased levels of quercetin, epicatechin, procyanidins and phloridzin and Awad et al. (2000) indicated an increase of quercetin in apple peel due to better sun-exposure. However, in our trial we did not identify higher levels of tested compounds in fruits from trees on the super-dwarfing rootstocks, though they resulted in development of slender canopy with better light conditions. Accumulation of certain compounds depended on rootstock genotype but did not depend on rootstock-induced tree vigour. On average of both years, dwarf rootstocks M.9,

Table 1. Effect of semi-dwarf and super-dwarf rootstocks on average content of phenolic compounds in apple fruits (μg/g dry weight) from years 2011 and 2012 and their deviation (%)

Compound	P 22		P 59		P 61		PB.4		M.26	
	(μg/g)	(%)	(μg/g)	(%)	(μg/g)	(%)	(μg/g)	(%)	(μg/g)	(%)
Chlorogenic acid	1047	21	818	43	1020	40	962	46	743	19
Phloridzin	113	35	101	27	112	49	112	58	98.8	42
Procyanidin B1	81.5	182	66.3	151	82.0	56	33.5	49	46.0	219
Procyanidin B2	920	29	711	45	906	23	704	8	706	76
Σ Procyanidins	1001	37	777	51	988	26	737	5	752	82
(+)-Catechin	71.1	22	37.1	24	77.3	23	43.0	59	53.2	6
(–)-Epicatechin	316	21	217	34	329	14	273	24	246	8
Σ Catechins	387	22	254	27	406	16	316	16	299	7
Hyperoside	124	41	135	26	147	24	125	6	102	13
Isoquercitrin	15.9	39	20.5	24	23.3	8	20.1	16	16.9	7
Rutin	20.5	60	18.7	29	21.6	49	21.3	26	15.3	39
Avicularin	84.2	36	75.4	5	94.0	25	78.1	20	69.3	21
Quercitrin	116	31	107	6	130	19	105	24	94.0	11
Σ Quercetin glycosides	361	38	357	11	416	24	350	17	298	15
Σ Phenolic compounds	2909	8	2307	11	2943	17	2476	26	2191	8

Difference between years in absolute values calculated according to formula: 100 – [(year2011 × 100)/year2012]

Table 2. Effect of dwarf rootstocks on average content of phenolic compounds in apple fruits (μg/g dry weight) from years 2011 and 2012 and their deviation (%)

Compound	B.396		M.9		P 62		P 66		P 67		Pure 1	
	(μg/g)	(%)	(μg/g)	(%)	(μg/g)	(%)	(μg/g)	(%)	(μg/g)	(%)	(μg/g)	(%)
Chlorogenic acid	821	9	729	36	781	17	891	53	817	26	935	45
Phloridzin	122	45	85.8	32	98.7	49	107	50	83.7	37	111	51
Procyanidin B1	37.6	79	48.2	43	53.4	300	38.8	12	49.4	13	78.6	47
Procyanidin B2	815	101	577	9	504	9	735	10	737	15	707	35
∑ Procyanidins	852	99	626	6	558	21	773	9	787	15	786	30
(+)-Catechin	57.8	1	49.7	43	35.6	68	52.9	48	38.1	23	77.6	31
(–)-Epicatechin	268	9	222	45	221	12	263	38	294	36	320	30
∑ Catechins	326	7	272	45	257	4	316	40	332	31	397	30
Hyperoside	118	5	87.0	45	100	58	124	17	116	64	127	33
Isoquercitrin	19.8	4	15.4	16	16.5	54	20.1	6	18.2	51	21.3	27
Rutin	19.2	29	15.1	60	17.8	68	20.1	27	20.4	72	21.4	52
Avicularin	79.5	8	59.9	39	65.0	49	81.6	21	71.7	49	86.2	24
Quercitrin	104	6	86.1	25	87.1	40	114	16	94.8	28	115	14
∑ Quercetin glycosides	340	4	264	37	286	52	359	17	321	51	371	26
∑ Phenolic compounds	2462	17	1976	29	1980	15	2447	34	2340	20	2600	36

Difference between years in absolute values calculated according to formula: 100 – [(year2011 × 100)/year2012]

P 62 and semi-dwarf M.26 rootstock were among the 4 rootstocks that determined lower content of all phenolic compounds tested (Tables 1, 2, Figure 3). Analysing separate compounds, super-dwarf P 59 together with the above-mentioned rootstocks determined low total quercetin glycosides content, super-dwarf PB.4 – low total procyanidin content and dwarf rootstock P 67 – low phloridzin. Super-dwarf rootstocks P 61 and P 22 were among the top 4 rootstocks that determined the highest content of phenolic compounds. Besides these two rootstocks, B.396 rootstock was distinguished by high total procyanidins and phloridzin, PB.4 – by chlorogenic acid, Pure 1 – by total catechins and total phenols.

Some researchers stress the relationship between phenolics accumulation in apple fruits and year conditions (Mainla et al. 2011). Despite the variable climatic conditions during the trial period, the difference between years in the total phenolic compounds accumulated in apple fruits was not high and averaged 17% for all rootstocks. The effect of individual rootstocks differed: less than 10% difference of total phenolic compounds was recorded for M.26 and P 22, but around 30% for M.9, Pure 1 and P 66 rootstocks (Tables 1 and 2).

Rather low average differences (< 20%) were recorded for the content of isoquercitrin, quercitrin, catechin and procyanidin B2. Difference between years of procyanidin B1 content was the highest – 76%. Accumulation of rutin and phloridzin was also greatly affected by year fruiting and growing conditions. The analysis of individual rootstocks revealed two – three fold differences of procyanidin B1 content in fruits from trees on P 62, P 22 and M.26 rootstocks. On the other hand, only 12–13% difference was recorded for P 66 and P 67 rootstocks.

The most stable content of all compounds analysed, except for procyanidin B1 and B2, was in fruits from trees on B.396 rootstock.

In spite of small difference of total phenolic compounds, particular compounds varied considerably in fruits from trees on P 22 rootstock almost in all cases exceeding trial averages in absolute values. The same trend was recorded for P 62 rootstock.

There were some rootstocks (M.26, P 59 and P 66) that gave the same yield and similar fruit

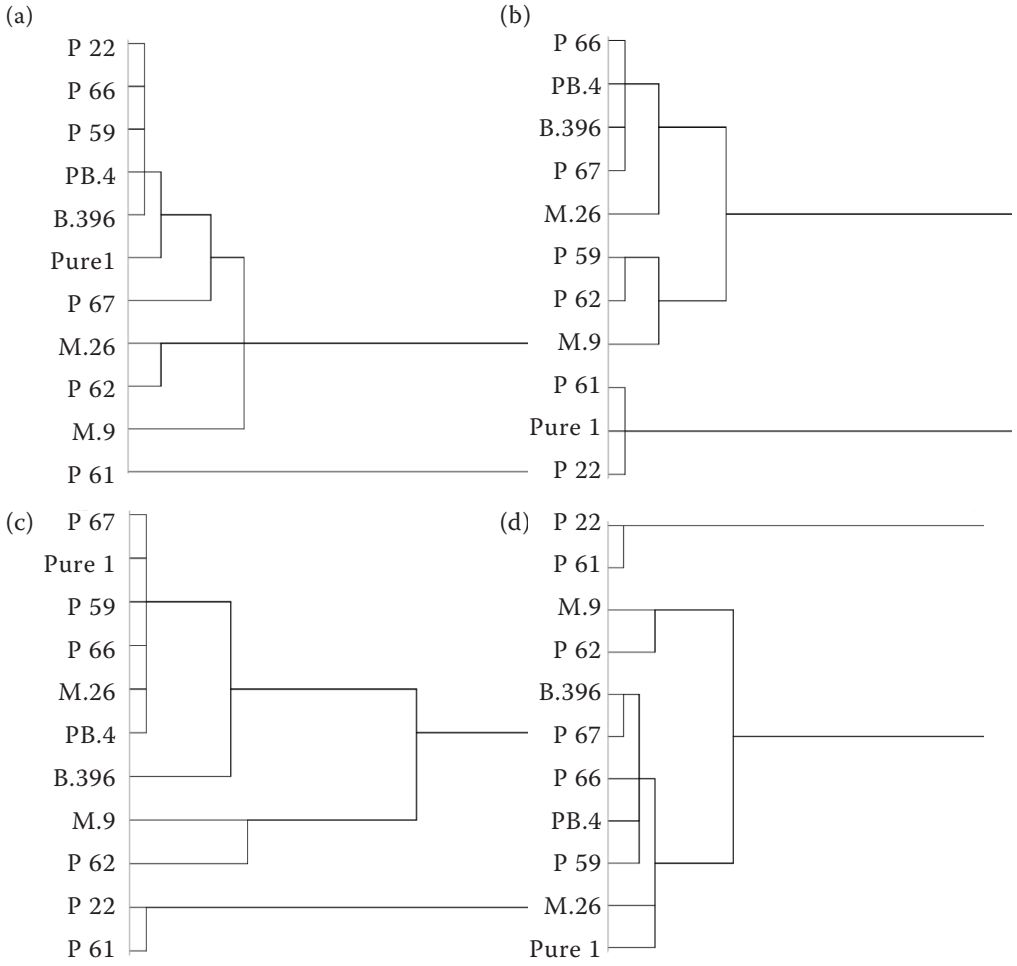

Figure 3. Dendrogram representing apple rootstock similarities between total quercetin glycosides (a); catechins (b); procyanidins (c), and phenolic compounds content (d) in apple fruit

weight in both years (the data is not presented). If the difference of the tested compounds between years was small in fruits from trees grown on M.26 and P 59 rootstocks, one of the biggest differences was detected on P 66. On the other hand, low variation of the tested compounds in fruits from trees on B.396 rootstocks did not depend on threefold yield differences and great variation in fruit weight, showing primary rootstock genotype effect on the stability of total phenolic content

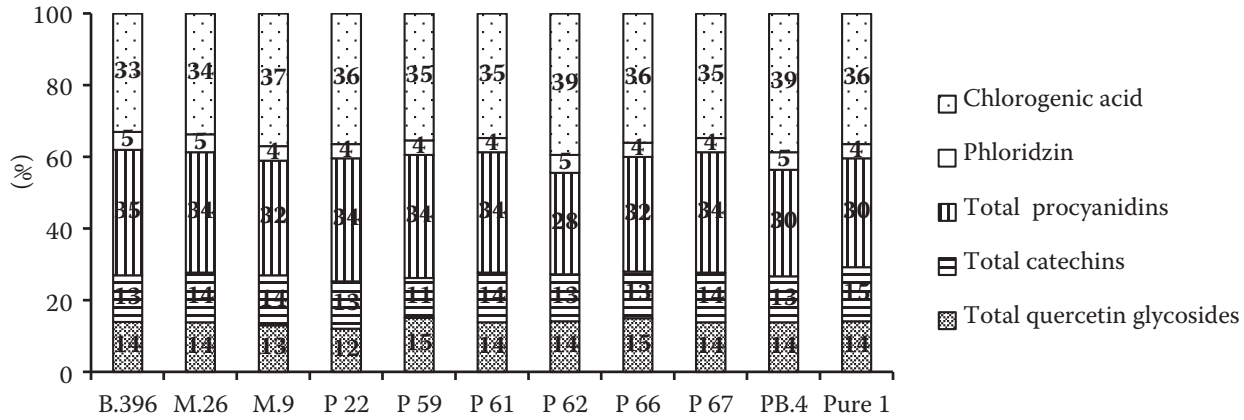

Figure 4. Rootstock effect on the composition of phenolic compounds in apple fruits (average of 2011, 2012)

Table 3. Coefficient of variation of phenolic compounds determined by rootstock genotypes

Year	Hyperoside	Isoquercitrin	Rutin	Avicularin,	Quercitrin	Σ Quercetin glycosides	(+) Catechin	(–) Epicatechin	Σ Catechins	Procyanidin B1	Procyanidin B2	Σ Procyanidins	Phloridzin	Chlorogenic acid	Σ Phenolic compounds
2012	15.1	13.5	15.9	12.2	13.4	12.5	40.6	15.6	17.8	35.5	19.7	20.4	14.9	17.2	15.7
2011	26.2	25.5	26.1	20.1	17.4	21.1	22.1	19.1	19.4	41.7	25.4	24.2	11.5	15.0	16.7

in apple fruits. Usually, investigations confirm the importance of the cultivar as a factor for the quantitative composition of apples (Tsao et al. 2003, Ceymann et al. 2012).

On average, chlorogenic acid was a dominating compound in apple fruits, which agrees with the findings of other researches (Marks et al. 2007, Duda-Chodak et al. 2010). P 62 and PB.4 rootstocks determined the highest share (39%) of it (Figure 4). Similar quantities of total procyanidins were detected as well. The share of total procyanidins was lower in fruits from trees grown on P 62. The B.396 was the only rootstock which determined higher content of procyanidins than chlorogenic acid. Equal amount of both compounds was accumulated in fruits from tress on M.26, P 59, P 61 and P 67 rootstocks. The share of total quercetin glycosides and total catechins varied from 13% to 15% of the total phenolic compounds and was the same for all rootstocks, only P 59 rootstock determined lesser amount of total catechins.

The average apple yield in the trial was 48.6 t/ha in 2012 and only 22.3 t/ha in 2011. The content of the two main apple fruit phenols depended on the yield. In 2012, 'in the year on' chlorogenic acid accounted for 40% of the total phenol content and only 32% in 2011 when apple yield was twice as low. Conversely, apple fruits contained lesser amount of total procyanidins in 2012 (27%) and more in 2011 (39%).

Fruits accumulated less phenols in 2011 and high variation (CV > 20%) between rootstocks was detected for most of the tested compounds (Table 3). In 2012, the variation between rootstocks was medium, except for (+)-catechin and procyanidin B1. Despite the great differences in yield and fruit weight between years, the variation between rootstocks was medium in both years for quercitrin, (–)-epicatechin, total catechins, phloridzin, chlorogenic acid and total phenols.

In conclusion, super-dwarf rootstocks P 61 and P 22 determined the highest content of all phenolic compounds tested. Dwarf rootstocks M.9 and P 62 and semi-dwarf rootstock M.26 resulted in lower content of all phenolic compounds tested. No clear differences were found between super-dwarf, dwarf and semi-dwarf rootstock groups. Rootstock impact on the accumulation of total phenols was different: less than 10% difference between years was recorded for M.26 and P 22, but around 30% for M.9, Pure 1 and P 66 rootstocks. The most stable content of all compounds analysed, except for procyanidin B1 and B2, was in fruits from trees on B.396 rootstock. Environmental conditions, fruit yield and fruit weight had an impact on rootstock – dependent variation of phenolic compounds. On average of two years, the highest variation coefficient was established for (+)-catechin, procyanidin B1 and total procyanidins content. Medium variation coefficient was recorded for quercitrin, (–)-epicatechin, total catechins, phloridzin, chlorogenic acid and total phenols.

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