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Influence of four different dwarfing rootstocks on phenolic acids and anthocyanin composition of sweet cherry (*Prunus avium* L.) cvs. 'Kordia' and 'Regina'

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Summary

Influence of four different dwarfing rootstocks: 'Gisela 5', 'Gisela 6', 'PHL-C' and 'PiKU1' on the amount of polyphenols (total phenols, total and individual anthocyanins and individual hydroxycinnamic acids) of sweet cherry cultivars 'Kordia' and 'Regina' was researched as well as correlations between polyphenols and colorimetrically measured fruit skin colour. Total phenols (TP) and total anthocyanins (TA) were determined by spectroscopic methods, while individual anthocyanins and hydroxycinnamic were quantified and identified by high performance liquid chromatography (HPLC). 'Gisela 6' and 'PiKU1' had the strongest influence on the TP content of both cultivars. The main hydroxycinnamic acid identified was *p*-coumaric acid (p-CA) which content was influenced by cultivar, vegetation year and interaction of cultivar*rootstock*year. Content of p-CA varied the most on rootstock 'Gisela 5' for both cultivars, with its lowest amount obtained in 2013 and the highest in 2014 in comparison to other rootstocks. The largest difference in TA content in cultivar*rootstock interaction was identified in 'Gisela 6'. Substantial difference in correlation pattern between cvs. 'Kordia' and 'Regina' lead to the conclusion that values *L** and *b** are better TA indicators rather than values *a** and Hue.

Introduction

Due to exceptional organoleptic qualities, market demand for cherries is growing worldwide, which has resulted with significant improvements in production and marketing (Sansavini and Lugli, 2008). Similar to apple, modern sweet cherry production cannot be conceived without the use of dwarfing rootstocks. In selection of suitable sweet cherry rootstocks, the emphasis is given to their compatibility with majority of cultivars in use and adaptability to different agro-ecological conditions (Miljković et al., 2002). The use of dwarfing rootstocks can be limited due to incompatibility issues connected with grafting of scion of different genetic origin (Usenik et al., 2010). Incompatibility is a complex, anatomical, physiological and biochemical process, not yet fully explained (Guclu and Koyuncu, 2012). With respect to grafted fruit trees incompatibility is defined as a phenomenon of premature senescence caused by physiological and biochemical processes (Feucht and Treutter, 1991). Usenik and Štampar (2000) found that low compatibility resulted in a pronounced accumulation of polyphenols, namely *p*-coumaric acid above the graft union of cv. 'Lapins' grafted on different rootstocks (F 12/1, Gisela 5, Weiroot 158), as a stress response to grafting. Higher content of *p*-coumaric acid above the graft point were found in apricot cultivars grafted on heterospecific rootstocks which led to an early graft incompatibility (Usenik et al., 2006). Mng'omba et al. (2008) have found that phenols, especially *p*-coumaric acids and anthocyanins caused poor callus formation at the union, and hence are implicated in graft incompatibility of wild loquat (*Uapaca kirkiana* Müell Arg.).

Fruit quality depends mainly on the scion genotype, but could be influenced by the rootstock (Scalzo et al., 2005; Tavarini et al., 2011) and climatic conditions as well. Different studies with *Prunus* sp. have indicated that rootstock affects vegetative growth, fruit quality and yield efficiency of grafted cultivar (Betrán et al., 1997; Čmelik and Družić-Orlić, 2008). Combined with fruit sweetness (soluble solids content), weight and firmness, fruit skin colour affects significantly consumer acceptance of sweet cherry cultivars as well (Spinardi et al., 2005; Fazzari et al., 2008). Development of red colour in sweet cherry is often used as a ripeness indicator and is directly related with the content and concentration of plant pigments - anthocyanins (Usenik et al., 2015). Major anthocyanins identified in sweet cherries responsible for their red colour are cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside, while peonidin-3-O-rutinoside and pelargonidin-3-O-rutinoside are present in smaller amounts (Kelebek and Selli, 2011). Previous studies on anthocyanins and colour change in sweet cherries revealed that chromatic parameters *L**, *a**, *b** and Hue angle correlated negatively with total anthocyanin levels in sweet cherry (Mozetič et al., 2004; Gonçalves et al., 2007) and weakly correlated in plums (Usenik et al., 2009). Colorimetric ripeness estimation of sweet cherries is considered to be effective since strong correlation coefficient between *a** and cyanidin-3-rutinoside was established (Goncalves et al., 2007). Research done by Pedisić et al. (2009) on sour cherries indicated that correlation between fruit skin colour parameters and total anthocyanins are cultivar dependant as lower *L** values were measured on cultivars with darker colored fruits.

Identification and quantification of polyphenols in a bark bellow and above graft point in different rootostock/cultivar combination is expensive and long procedure (Usenik and Štampar, 2000; Usenik et al., 2006). It is assumed that higher content of polyphenols involved in incompatibility proces could be identified in fruits as well. Higher content of hidroxycinnamic acids, namely neochlorogenic and chlorogenic acids, *p*-coumaric derivatives was determined in fruits of cv. 'Lapins' grafted on 'PiKU 1' roostock than on 'Gisela 5' (Jakobek et al., 2009).

The objectives of this research was to determine the influence of four different dwarfing rootstocks: 'Gisela 5', 'Gisela 6', 'PHL-C' and 'PiKU 1' on the accumulation of polyphenols (total and individual hydroxycinnamic acids, total and individual anthocyanins) in fruits of sweet cherry cultivars 'Kordia' and 'Regina', and to find correlations between polyphenols and colorimetrically measured fruit skin colour.

Material and methods

Plant material

The research was conducted in experimental orchard of the Institute of Pomology of the Croatian Centre for Agriculture, Food and Rural Affairs in Donja Zelina, near Zagreb. Experimental orchard is located on 180 m above mean sea level (AMSL), 45° 55´ north

latitude, 16° 14´ east longitude. The soil in orchard is described as albic stagnosol. The area is characterized by average annual temperature of 10.7 °C, and 855.1 mm of total rainfall. Significant deviation was not recorded in average daily temperature during 2013, while total rainfall was slightely higher than average (970.6 mm). Slightly higher average daily temperatures were recorded in April (13.3 °C), June (20.7 °C) and in July 2013 (24.0 °C). Siginificantly higher precipitation than average were recorded in 2014 (up to 85%), or precisely in February (279 mm) and June (136 mm). In the first quarter of 2014, significantly higher than average temperature were recorded as well (5.6 °C in January; 5.0 °C in February and 10.8 °C in March). The orchard is drip-irrigated with installed black anti-hail nets. Conventional orchard management practices are implemented (pruning, fertilization, pest control).

The trial was conducted on two sweet cherry cultivars 'Kordia' and 'Regina' grafted on four dwarfing rootstock: 'Gisela 5', 'Gisela 6', PHL-C' and 'PiKU1'. Genetic origin of tested rootstocks is shown in the Tab. 1. Trees were planted in a random block design: two trees in 3 repetitions (for each rootstock and cultivar combination). The two-year old trees were planted in 2006, at 4 × 2.5 m distance and trained as a spindle bush.

Tab. 1: Tested rootstocks and their origin

Rootstock	Origin
Gisela 5, Gisela 6	*P. cerasus* L. × *P. canescens*, Germany
PiKU 1	*P. avium* L. × (*Prunus canescens* × *Prunus tomentosa*), Germany
PHL-C	*P. avium* L. × *P. cerasus* L., Czech Republic

At optimal picking maturity (determined by colour and organoleptically), samples were harvested from each tree in the trial. Content of soluble solids in 2013 was in average for cv. Kordia 16.17 °Brix and for cv. Regina 18.63 °Brix, whereas in 2014 cv. Kordia obtained 16.95 °Brix and cv. Regina 17.12 °Brix. Each sample for cultivar and rootstock combination was consisted of 360 fruits (60 fruits taken from each tree in the trial). Fruits were taken from the middle part of the tree crown. Harvesting of fruit samples for cv. 'Kordia' in 2013 started on June 28, and in 2014 on June 16, and for cv. 'Regina' on July 2 in 2013 and on June 23 in 2014. Colour and polyphenol analysis o sweet cherry fruit samples was performed immediatelly after the harvest.

Analysis of total phenols and total anthocyanins

Phenolics were extracted from homogenized pitted fresh sweet cherry fruits. Exactly 5 g of samples were weighed out and extracted using 20 mL of 80% (by volume) aqueous methanol. The mixture was extracted for 20 min in ultrasonic bath at 50 °C, filtered through Whatman No. 40 filter paper (Whatman International Ltd, Kent, UK) using a Büchner funnel. The filtrates were adjusted to 25 mL in a volumetric flask with 80% aqueous methanol. The obtained extract was used for determination of total phenols (TP), total (TA) and individual anthocyanins and individual hydroxycinnamic acids (HCA).

For the determination of total phenols (TP), the adjusted method (Ough and Amerine, 1988; Singleton and Rossi, 1965) with Folin-Ciocalteu reagent was used. The content of TP was measured as follows: 0.25 mL of sample, 15 mL distilled water (dH_2O), 1.25 mL Folin-Ciocalteu reagent (diluted with distilled water in 1:2 ratio) were added to a 25-mL volumetric flask containing and shaken. To the mixture 3.75 mL of saturated Na_2CO_3 solution (m/V) were added with mixing and the solution was immediately filled up to 25 mL with ddH_2O. After incubation at 50 °C for 20 min, the absorbance of the solution was measured by the spectrophotometer Unicam Helios b (Spectronic Unicam, Cambridge, UK) at 765 nm. The results were calculated according to the calibration curve for gallic acid (y = 0.0009x, y = absorbance at 765 nm, x = concentration of gallic acid in mg/L, R2 = 0.9986). The content of TP was expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh mass (FW) of edible part of fruits.

The monomeric anthocyanin pigment content of the aqueous extracts was determined using the pH-differential method (Giusti and Wrolstad, 2001). A spectrophotometer Unicam Helios b (Spectronic Unicam, Cambridge, UK) was used for spectral measurements at 520 and 700 nm. Pigment content was calculated as milligrams cyanidin-3-glucoside/100 g FW using an extinction coefficient of 26900 L/cm/mol and molecular weight of 449.2 g/mol.

HPLC analysis of anthocyanins and hydroxycinnamic acids

HPLC analysis of anthocyanins and hydroxycinnamic was performed using Agilent 1260 quaternary LC Infinity system (Agilent Technologies, Santa Clara, CA, USA) equipped with UV/VIS diode array detector (DAD), an automatic injector and ChemStation software. The separation of phenolic compounds (anthocyanins and hydroxycinnamic acid) was performed on a Nucleosil 100-5C_{18}, 5 µm (250 × 4.6 mm i.d.) column (Macherey-Nagel). The mobile phase composition and the used gradient conditions were described previously by Mitić et al. (2012) and modified by Zorić et al. (2014), instead of 5% solvents contained 3% of formic acid.

For gradient elution, mobile phase A contained 3% of formic acid in water, mobile phase B contained 3% of formic acid in 80% acetonitrile. The elution was as follows: from 0 to 28 min, 0% B, from 28 to 35 min, 25% B, from 35 to 40 min, 50% B, from 40 to 45 min, 80% B and finally for the last 10 min again 0% B. Operating conditions were: constant flow rate 0.8 mL/min, column temperature 22 °C, injection volume 5 µL and equilibration time 2 minutes.

Detection was performed with UV/VIS-DAD by scanning from 220 to 570 nm. Identification of anthocyanins and hydroxycinnamic acids was carried out by comparing retention times and spectral data with those of the authentic standards (anthocyanins were identified at 520 nm, and hydroxycinnamic acids at 280 nm).

The quantifications of anthocyanins, and hydroxycinnamic acids were made by the external standard method. Anthocyanin standards, cyanidin-3-glucoside (Cy-3-G), cyanidin-3-rutinoside (Cy-3-R), were prepared as stock solutions in acidified methanol (1% of formic acid (v/v) in methanol) at a concentration of 100 mg/L. Standards solutions of anthocyanins were prepared by diluting the stock solution to yield five concentrations in a range from 16.67 to 100 mg/L. Quantitative determination was carried out using the calibration curves of the standards Cy-3-G: y = 21.952x, R2 = 0.99; Cy-3-R: y = 14.969x, R2 = 0.99.

Standards of hydroxycinnamic acids were prepared as stock solutions in 80% methanol (v/v) at following concentrations: chlorogenic (ChA); *p*-coumaric acid (p-CA) 48 mg/L. Standard solutions were prepared by diluting the stock solution to yield five concentrations in the range from 10.4 to 52 mg/L for ChA and p-CA, from 9.6 to 48 mg/L for p-CA. Quantitative determination was carried out using the calibration curves of the standards (ChA: y = 14.571x, R2 = 0.99; p-CA: y = 10.729x, R2 = 0.99;). For neochlorogenic acid (NChA) lacking reference standard, identification was done according to ChA, and p-Coumaroylquinic acid according to p-CA.

Determination of colour parameters

Fruit skin colour measurements were carried out with portable Konica Minolta Spectrophotometer CM-700d by using *CIE L*a*b** system. Value *L** represents lightness i.e. illumination from 0 (total

dark or black) to 100 (total transparency or white). Value *a** measures redness/greenness (+*a** represents the red, while -*a** represents green). Value *b** measures yellowness/blueness (+*b** represents yellow, while -*b** represents blue) (HUTCHINGS, 1994). Hue angle (°), *Hue = arctg (b*/a*),* represents the hue of the colour (VOSS, 1992) which values are defined as follows: red - pink: 0°, yellow: 90°, bluish - green: 180° and blue: 270° (MCGUIRE, 1992). Chroma was calculated using the following formula: *C = (a*2 + b*2)1/2*, and it is a measure for chromaticity (*C**), and represents the purity or colour saturation (VOSS, 1992).

Statistical analysis

Repeated measures analysis of variance (ANOVA) in two replicates for cultivar * rootstock * year using the statistical software Statistica 10.0 (Stat Soft, Inc., USA) provided estimates of varietal, roostock and annual differences. Standard error was determined using Fisher LSD test with a 0.01 level of significance. Correlation between fruit skin colour parameters and total and individual phenolic was determined by Pearson's *r* coefficient correlation.

Tab. 2: Effect of rootstocks on *Trans Cross-Sectional Area* (TCSA), *Yield Efficiency Index* (YIE) and fruit weight of cvs 'Kordia' and 'Regina'

cultivar	rootstock	TCSA (cm^2)	YEI (%)	Fruit weight (g)
Kordia	Gisela 5	57,75 ad	0,20 ab	9,13 a
	Gisela 6	66,4 ab	0,21 ab	8,45 c
	PHL-C	89,61 c	0,15 abc	9,72 b
	PiKU1	70,77 ab	0,24 b	7,84 d
Regina	Gisela 5	52,68 d	0,16 abc	8,42 c
	Gisela 6	88,11 c	0,13 ac	9,48 ab
	PHL-C	96,40 c	0,02 d	10,31 e
	PiKU1	72,49 b	0,09 cd	9,50 ab
	p	0,021	0,000	0,000

For each cultivar, means having the same letter in each column are not significantly different at P ≤ 0.01 by Fisher LSD Test

Results and discussion

Fruit weight, trans cross-sectional area, yield efficiency index and Phenolic content

Influence of rootstocks on the fruit quality of sweet cherry was studied intensively in past years due to large number of dwarfing rootstocks that can be found on the market. Research was mainly done on one cultivar being grafted on several rootstocks (USENIK et al., 2000; JAKOBEK et al., 2009), or two or more cultivars grafted on one rootstock (SPINARDI et al., 2005). Sweet cherries in our research were grafted on four different rootstocks which in our growing conditions influenced the vigour (measured as cross section of a tree trunk), yield efficiency and fruit weight of cvs. 'Kordia' and 'Regina' (Tab. 2). Both cultivars developed the smallest trees on Gisela 5 rootstock, medium sized on PiKU1 and the highest on PHL-C. Rootstock influence on cultivars can be noticed at Gisela 6, on which cv. 'Kordia' produced trees of similar size as Gisela 5 whereas cv. 'Regina' developed trees of similar size as PHL-C. Difference in maturity timing between rootstocks was not observed for neither of cultivars. Fruit weight was the highest on PHL-C for both cultivars but varied in other rootstocks. For cv. 'Kordia' it decreased as follows: Gisela 5 > Gisela 6 > PiKU1, and for cv. 'Regina' as follows: PiKU1 > Gisela 6 > Gisela 5 (Tab. 2). Special attention was made to harvest samples in optimal ripening stage for each cultivar to accurately evaluate influence of different rootstock on fruit quality.

Content of total phenols (TP) was influenced by rootstock, vegetation year and their mutual interaction as well as their interaction with

Tab. 3: Phenolic content (mg/100 g FW) of sweet cherry cvs 'Kordia' and 'Regina' grafted on 4 different rootstocks*, average of 2 years

cultivar	rootstock	ChA	NChA	p-CA	Cy-3-G	Cy-3-R	TA	TP
Kordia	Gisela 5	1.90 ± 0.65	9.13 ± 1.46	10.53 ± 0.89	3.45 ± 1.04	24.80 ± 16.87	34.41 ± 6.07	175.61 ± 21.69
	Gisela 6	1.66 ± 0.27	9.49 ± 4,44	12.35 ± 5.65	3.89 ± 1.11	32.19 ± 5.38	36.78 ± 6.68	198.48 ± 47.67
	PHL-C	1.56 ± 0.29	8.88 ± 3.58	11.59 ± 4.78	3.46 ± 1.17	27.82 ± 7.49	39.85 ± 13.03	183.37 ± 41.14
	PiKU1	1.76 ± 0.25	9.60 ± 4.65	10.67 ± 4.66	2.34 ± 0,.53	26.34 ± 3.63	33.18 ± 4.51	156.55 ± 25.13
Regina	Gisela 5	1.48 ± 0.19	9.73 ± 3.16	10.21 ± 5.12	2.28 ± 1.51	23.33 ± 7.18	26.19 ± 12.98	149.45 ± 18.99
	Gisela 6	1.17 ± 0.52	8.18 ± 2.43	8.72 ± 2.63	1.17 ± 1.40	15.55 ± 6.21	17.53 ± 8.53	137.40 ± 35.92
	PHL-C	1.44 ± 0.39	9.77 ± 2,52	10.27 ± 3.27	1.76 ± 0.79	19.90 ± 4,.26	23.14 ± 9.66	198.54 ± 87.33
	PiKU1	1.02 ± 0.57	7.71 ± 2,86	8.02 ± 1.82	1.42 ± 1.64	15.86 ± 7.50	20.74 ± 13.55	233.46 ± 156.73
		p value						
cultivar		0.001	ns	0.006	0.000	0.000	0.000	ns
rootstock		ns	ns	ns	0.072	0.005	0.000	0.000
year		ns	0.000	0.000	0.000	0.020	0.000	0.000
cultivar*rootstock		ns	0.036	ns	ns	ns	0.000	0.000
cultivar*year		0.012	ns	ns	0.001	0.000	0.000	0.000
rootstock*year		0.015	ns	ns	ns	ns	0.000	0.000
cultivar*rootstock*year		ns	0.024	0.009	ns	0.030	0.000	0.000

*Values are expressed as the mean ± standard deviation; significant differences at p ≤ 0.05; ns = not significant (p > 0.05)

ChA – Chlorogenic acid; NChA – Neochlorogenic acid; p – Coumaroylquinic acid; Cy-3-G – Cyanidin 3-glucoside; Cy-3-R – Cyanidin 3-rutinoside; TA – Total Anthocyanins, TP – Total Phenols

cultivar (Tab. 3) which is line with the results obtained by JAKOBEK et al. (2009). Also, previous studies reported that TP content in sweet cherries can range from 99.00 - 109.8 mg/100 gFW (KIM et al., 2005; FERRETI et al., 2010). Our research resulted in higher TP content for both cultivars. Rootstocks Gisela 6 and PiKU1 had the strongest influence on the TP content of both cultivars, followed by Gisela 5, and these differences where statistically significant ($p \leq 0.01$). Difference in TP content of both cultivars on PHL-C was not significantly different. JAKOBEK et al. (2009) also identified that cv. 'Lapins' had higher total phenol content grafted on heterogenic rootstocks, with PiKU1 having the highest content of TP in comparison to Weiroot 13, Weiroot 158, Gisela 5 and MaxMa 14. In the study conducted by GONÇALVES et al. (2005), TP content of cvs. 'Burlat', 'Summit' and 'Van' was higher on vigorous rootstocks (MaxMa 14, CAB 11E, *Prunus avium*) as opposite to dwarfing and semi-dwarfing rootstocks (Gisela 5, Edabriz). In our research cv. 'Kordia' shows less variation in TP content in combination with individual rootstocks (21% difference between the lowest and the highest value), while this variation is much higher in cv. 'Regina' (41.15%). Results indicate that this variation in TP content was not connected with either vigour, yield efficiency or fruit weight. Further analysis of vegetation year influence on TP content shows significant variation during 2013 which could be attributed to higher temperatures during final stages of ripening (Fig. 1). This is particularly visible at cv. 'Regina' on PHL-C and especially on PiKU1 rootstocks which in 2013 had the highest and in 2014 the lowest content of TP in comparison to all cultivars and rootstocks in trial, and this difference is statistically significant ($p \leq 0.01$). During 2014, content of TP for both cultivars was significantly lower, however it followed the same pattern as in 2013 for cv. 'Kordia'. For cv. 'Regina', the content of TP between years 2013 and 2014 varied the least in rootstock Gisela 5 (21%) and for cv. 'Kordia' on Gisela 5 (20%) and PiKU1 (22%) (Fig. 1).

As all four rootstocks in our research are of heterogenic origin (Tab. 1), the level of variation in TP content has to be observed not only through genetic origin of rootstock but of both cultivars as well: cv. 'Kordia' being a chance seedling and cv. 'Regina' being a cross between cvs. 'Schneiders Späte Knorpelkirsche' and 'Rube'. Higher content of TP in sweet cherries grafted on heterogenic combinations can be explained by adjustment of genetically different metabolisms of rootstock and cultivar, which causes high stress levels (USENIK et al., 2005). TP content in our research varied significantly less in cv. 'Kordia' between two vegetation years which can lead to a conclusion that this cultivar has formed a more stable graft unions with all four rootstocks unlike cv. 'Regina'. Hence, less variation in the inner quality parameters in sweet cherry fruits makes this cultivar more robust in struggling with adverse climatic conditions and therefore more suitable for intensive fruit production.

Hydroxycinnamic acids content

Variation in the content of hydroxycinnamic acids (HCA) in fruit could be qualitative (presence or absence of certain HCA) or quantitative (amount of present HCA and their ratio), which is affected by numerous factors such as physiological stage of a plant, diseases and pests, and to a lesser degree by agro-ecological conditions (CLIFFORD, 1999). HCA profile and the ratio between the major HCAs, chlorogenic (ChA), neochlorogenic (NChA) and p-coumaric acid (p-CA) are not maturation dependent, except in the very early stages (MOZETIČ et al., 2004). Out of researched individual HCA, p-CA was the most abundant one followed by NChA and ChA (Tab. 3). However, this was not the case for cv 'Regina' grafted on PiKU1 rootstock in 2013, and on Gisela 5 in 2014 when higher content of NChA were identified in fruits (Fig. 2 and 3). USENIK et al. (2015) have identified higher NChA (62.9 - 64.8%) and lower p-CA concentration (32.3 - 34.0%) for cv. Kordia/Gisela 5 combination as well. SERRADILLA et al. (2011) have obtained higher p-CA (62.0%) to NChA (35.0%) ratio in cv. 'Pico Negro' as well as in cv. 'Van' (81.4%; 18.3%) respectively. Hence, it can be concluded that genetic origin of cultivars can have a significant influence on the content and composition of individual HCA. p-CA was influenced by cultivar, vegetation year and interaction of cultivar*rootstock*year. NChA was influenced by vegetation year and interaction of cultivar*rootstock and cultivar *rootstock*year. ChA was influenced by cultivar and cultivar*year and rootstock*year interaction. These differences were statistically significant ($p \leq 0.01$). Rootstock did not have significant influence on the content of either of researched individual HCA (Tab. 3).

Content of p-CA of cv. Kordia in combination with rootstocks was decreasing in following order: Gisela 6 > PHL-C > PiKU1 > Gisela 5, whereas for cv 'Regina' it followed different pattern: PHL-C > Gisela 5 > Gisela 6 > PiKU1. These differences were not statistically significant. The content of NChA varied in both cultivars depending on rootstock, and these difference were statistically significant ($p \leq 0.01$). This is especially visible at cv. 'Kordia' which NChA content decreased in following order: PiKU1 > Gisela 6 > Gisela 5 > PHL-

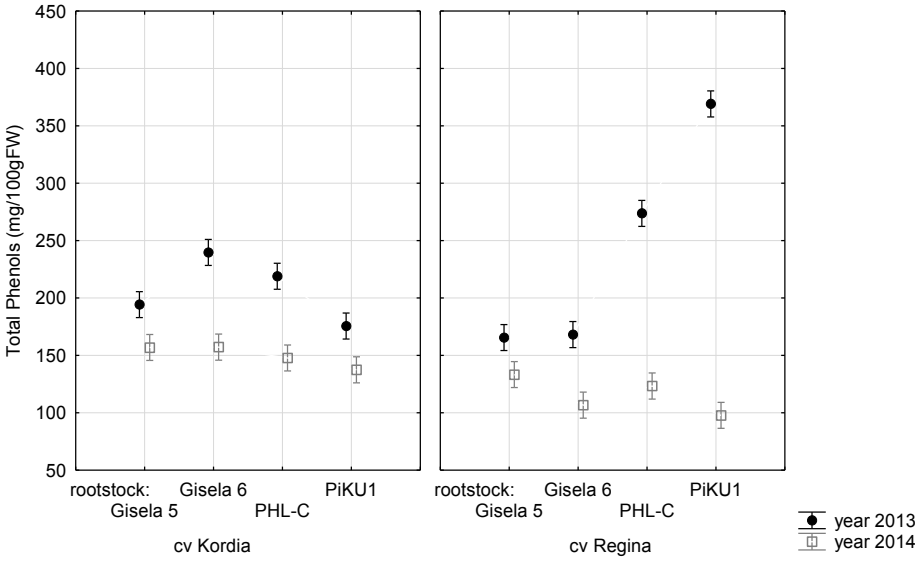

Fig. 1: Total phenol content (mg/100 g FW) of cvs. 'Kordia' and 'Regina' grafted on 4 different rootstocks during 2013 and 2014

C. In cv. 'Regina' content of NChA decreased in same manner as for p-CA (Tab. 3).

These differences in the individual HCA variation in fruits of both sweet cherry cultivars depending on a graft union with individual rootstocks prompted further analysis to investigate the influence of vegetation year. Content of both p-CA and NChA was higher in 2013 for all cultivar and rootstock combinations (Fig. 2 and 3). Cv. 'Kordia' had higher content of both p-CA and NChA on all rootstocks except on Gisela 5 during 2013. Both cultivars had similar content of NChA on PHL-C rootstock in 2013. Cv. 'Kordia' has shown more variation in the ratio of HCA in 2013, which is especially visible in combinations with Gisela 6 and PHL-C rootstocks.

Rootstock PiKU1 and PHL-C had similar influence on the content of p-CA of both cultivars in 2014 (Fig. 3). The most significant influence on the content of p-CA was visible in rootstock Gisela 5, on which cv. 'Regina' obtained the lowest and cv. 'Kordia' the highest amount of mentioned HCA. Weather conditions had influenced the NChA content of both cultivars in 2014. Statistically significant differences in the content of NChA in 2014 were observed only in cv. 'Kordia' in combination with Gisela 5 and cv. 'Regina' in combination with PHL-C.

Cultivar*rootstock interaction in our research was significant only

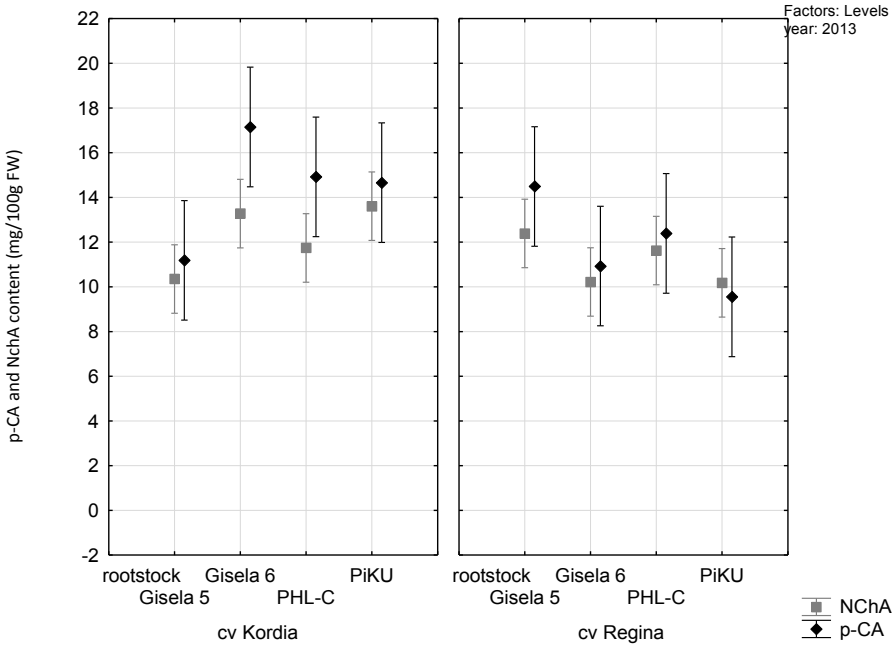

Fig. 2: p-Coumaric acid (p-CA) and neochlorogenic acid (NChA) (mg/100 g FW) of cvs. 'Kordia' and 'Regina' grafted on 4 different rootstocks during 2013

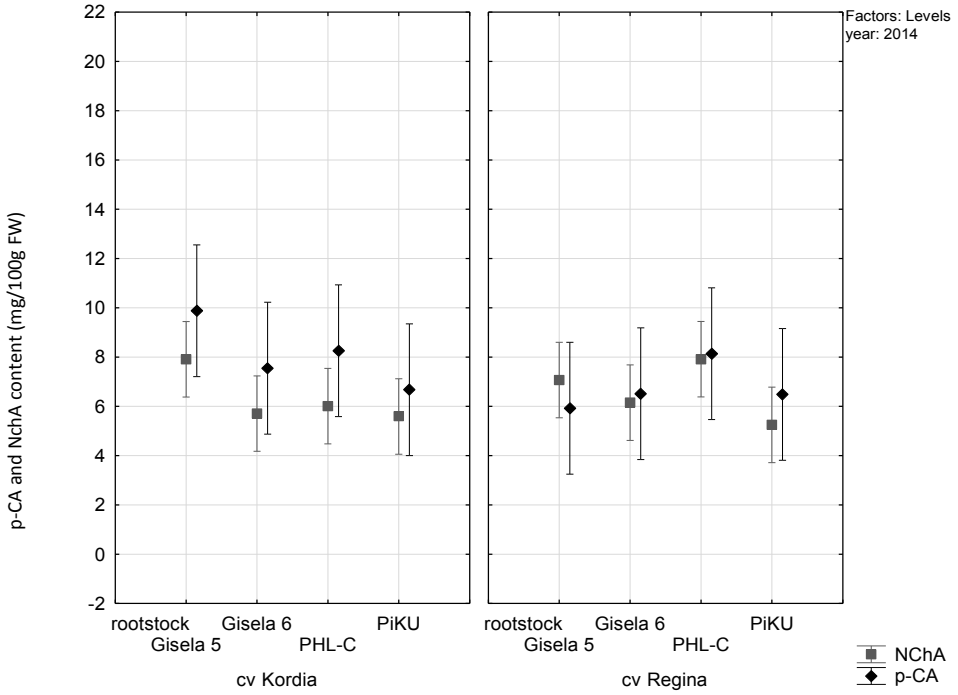

Fig. 3: p-Coumaric acid (p-CA) and neochlorogenic acid (NChA) (mg/100 g FW) of cvs. 'Kordia' and 'Regina' grafted on 4 different rootstocks during 2014

for the content of NChA. The influence of different rootstocks on the content of p-CA and their role in incompatibility process in sweet cherry with heterospecific rootstock combinations having higher p-CA content in phloem above the graft point was previously investigated (USENIK and ŠTAMPAR, 2000). With regard to polyphenol content in specific cultivar and rootstock combinations it can be assumed that heterospecific graft combinations carry a low level of stress due to adjustment between two different genotypes. All of our combinations are of heterospecific genetic origin, therefore the presence of certain level of stress is expected and can be quantified through the content of individual HCA.

Total and individual anthocyanins content in fruit

Content of total anthocyanins (TA) was influenced by cultivar, rootstock, vegetation year and their mutual interaction (Tab. 3). Difference in the TA content of cultivar and rootstock combinations can be better observed in their interaction with the vegetation year. TA was in average lower in 2014 than in 2013 for both cultivars on all rootstocks except for cv. Kordia/Gisela 5 combination which had 24 % higher content of TA in 2014. Also, content of TA in cv. 'Kordia' varied significantly on PHL-C rootstock, and for cv. 'Regina' varied the most on PiKU1 (Fig. 4). Cv. 'Regina' showed similar distribution of TA content in both vegetation years forming in that sense more stable connection with all four rootstocks. Cv. 'Kordia', however reacted differently in combination with individual rootstocks when it comes to TA content in both vegetation years. Distribution of TA content in 2013 and 2014 follows similar pattern as the distribution of p-CA (Fig. 2, 3) except for Kordia/Gisela 5 combination in 2014. This can be explained by HCA being a precursors in flavonoids biosynthesis via the phenylpropanoid pathway.

The content of cyanidin 3-rutinoside (C-y3-R) for rootstock*cultivar combinations in trial followed TA pattern, except for cv. 'Kordia', where the lowest C-y3-R content was determined in Gisela 5, and highest in Gisela 6. The amount of Cy-3-R ranged between 84.75 % (cv. 'Kordia') – 85.21 % (cv. 'Regina'), and Cy-3-G ranged between 7.56 % (cv. 'Regina') – 9.12 % (cv. 'Kordia') which is in accordance with the ratio obtained by USENIK et al. (2008; 2015). Differences between mentioned individual anthocyanins for cultivar/rootstock combinations in trial were not statistically significant (Tab. 3).

Colour parameters

Fruit skin colour is one of the most important fruit quality parameter and directly connected with consumer acceptance and purchase decisions (TIJSKENS et al., 2011). It is renowned that cultivar and agro-ecological conditions affect cherry fruit colour which is linked to the TA content. Our results revealed that not only cultivar and year, but rootstock and interaction of cultivar*year and cultivar*rootstock*year had significant influence on chromatic values *L*, a*, b*,* Hue and Chroma (Tab. 4).

Cultivar * rootstock combinations in our research indicated that high chromatic value *a** corresponds to higher values *b** and Chroma and to the lower Hue value of the sweet cherry fruit skin. In contrast, chromatic value *L** does not follow this pattern, nonetheless it is connected with the content of TA in cv. 'Regina' (Tab. 3, 4), as lower value *L** of fruit skin corresponds to the higher TA content of cv. 'Regina' on different rootstocks. For all cultivar and rootstock combinations, except for Kordia/Gisela 6, it was identified that lower value *L** corresponds to higher TA, Cy-3-G, Cy-3-R and ChA content. Change in values *a*, b*,* Chroma and Hue is not linked with the change in phenolics content.

Fruits of cv. 'Regina' were of lighter colour, with less red and yellow colour than cv. 'Kordia's, hence the higher Hue and Chroma values. Our results are in line with the research done by USENIK et al. (2009) on cv. 'Kordia' measured with chromameter Konica Minolta CR-10 in Slovenia. Several authors have identified the rootstock influence on fruit skin colour, however their results differ depending on chromatic parameter which was affected by this interaction. CANTIN et al. (2010) have reported significant influence of different rootstocks on value *L** of cvs. 'Van' and 'Stark Hardy Giant' sweet cherries. In our research, cultivar and rootstock interaction has affected the value *b**, Hue and Chroma. GADŽE et al. (2010) have also identified significant influence of rootstocks to fruit colour of sweet cherry 'Lapins'. Values *L** and *b** of cv. 'Lapins' were higher and value *a** was lower on Gisela 5 the PiKU1, however these differences were not statistically different.

Correlation between phenols and colour parameters

Polyphenol content determination is time-consuming and expensive procedure. Aiming to decrease these costs, different methods of fast

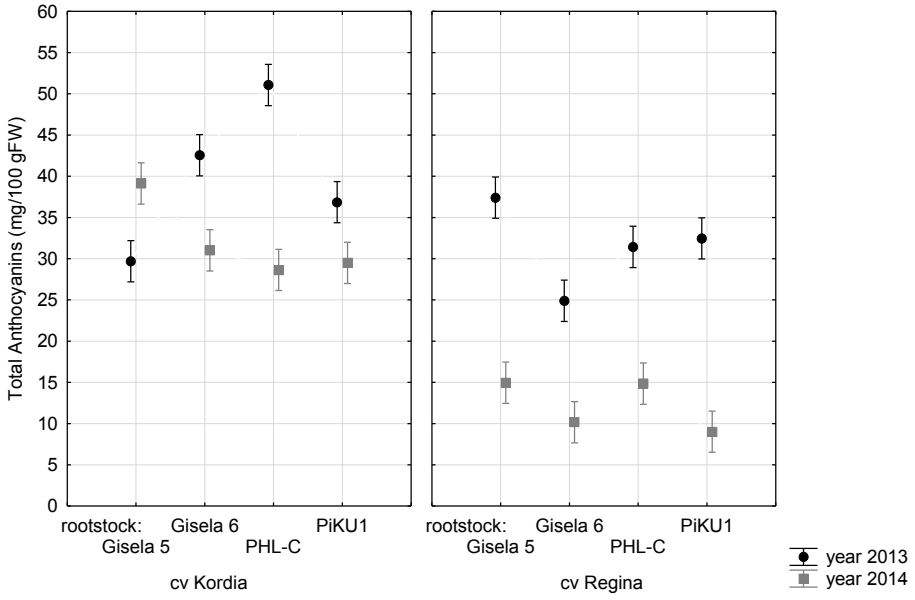

Fig. 4: Total anthocyanin content (mg/100 g FW) of cvs. 'Kordia' and 'Regina' grafted on 4 different rootstocks during 2013 and 2014

Tab. 4: Colour characteristics of sweet cherry cvs 'Kordia' and 'Regina' grafted on 4 different rootstocks, average values for 2013 and 2014*

cultivar	rootstock/year	*L**	*a**	*b**	Hue	Chroma
Kordia	Gisela 5/2013	43.42 ± 0.45	4.52 ± 1.17	2.04 ± 0.39	24.69 ± 2.97	4.96 ± 1.21
	Gisela 5/2014	42,56 ± 0.67	3.70 ± 1.24	2.05 ± 0.39	29.99 ± 4.66	4.24 ± 1.26
	Gisela 6/2013	43.63 ± 0.45	4.33 ± 1.08	2.02 ± 0.36	25.30 ± 2.37	4.78 ± 1.12
	Gisela 6/2014	43.52 ± 0.48	5.78 ± 1.81	2.70 ± 0.57	25.55 ± 2.36	6.39 ± 1.88
	PHL-C/2013	43.34 ± 0.43	4.02 ± 0.84	1.86 ± 0.25	25.14 ± 2.41	4.43 ± 0.85
	PHL-C/2014	42.98 ± 0.59	3.59 ± 0.95	1.97 ± 0.30	29.48 ± 3.99	4.10 ± 0.96
	PiKU1/2013	43.24 ± 0.35	4.35 ± 1.07	1.92 ± 0.38	24.13 ± 2.61	4.76 ± 1.12
	PiKU1/2014	42.99 ± 0.34	4.29 ± 1.03	2.09 ± 0.28	26.53 ± 3.08	4.78 ± 1.04
Regina	Gisela 5/2013	42.03 ± 0.36	3.46 ± 0.71	1.66 ± 0.18	26.07 ± 3.39	3.84 ± 0.70
	Gisela 5/2014	44.62 ± 0.47	6.33 ± 2.16	2.99 ± 0.74	25.91 ± 2.37	7.01 ± 2.26
	Gisela 6/2013	43.26 ± 0.42	3.68 ± 1.11	1.71 ± 0.34	25.46 ± 3.21	4.06 ± 1.13
	Gisela 6/2014	44.20 ± 0.27	4.63 ± 1.16	2.49 ± 0.35	28.84 ± 2.67	5.26 ± 1.18
	PHL-C/2013	42.76 ± 0.46	4.26± 0.96	1.75 ± 0.28	22.68 ± 2.71	4.61 ± 0.98
	PHL-C/2014	44.03 ± 0.31	4.56 ± 1.21	2.43 ± 0.39	28.61 ± 2.78	5.17 ± 1.25
	PiKU1/2013	42.68 ± 0.56	4.06 ± 0.99	1.89 ± 0.30	22.55 ± 2.59	5.22 ± 1.72
	PiKU1/2014	44.11± 0.34	4.85 ± 1.52	2.56 ± 0.53	28.47 ± 2.62	5.49 ± 1.59
		p - values				
cultivar		0.000	ns	ns	ns	ns
rootstock		0.000	ns	0.002	ns	ns
year		0.000	0.000	0.000	0.000	0.000
cultivar*rootstock		ns	0.000	0.000	0.001	0.000
cultivar*year		0.000	0.000	0.000	ns	0.000
rootstock*year		0.006	0.004	ns	0.002	0.001
cultivar*rootstock*year		0.000	0.000	0.000	0.000	0.000

*Values are expressed as the mean ± standard deviation; significant differences at $p \leq 0.01$; ns = not significant ($p > 0.01$)

and non-destructive identification of fruit inner quality parameters have been developed with higher or lesser accuracy. In this research we attempted to connect distribution of total and individual phenols, total and individual anthocyanins and individual hydroxycinnamic acids identified in cherry fruits (inner quality parameters) and colorimetric values of cherry fruit skin (outer fruit quality parameters) in order to determine whether these parameters are cultivar and rootstock dependant. Following the statistical analysis of all above mentioned parameters, we have conducted further analysis concerning the average data for each cultivar and rootstock. Overall, we have identified that colorimetrical values of cherry fruit skin (*L**, *a**, *b**, Chroma and Hue) are negatively correlated with polyphenols identified in this research (Tab. 5 and 6). Mozetič et al. (2004) have also identified strong negative correlation between TA and value L* (r = -0.81), b* (r = -0.86), Hue (r = -0.69) and Chroma (r = -0.96) on sweet cherry cv. 'Petrovka', which is darker coloured sweet cherry. In cv. 'Kordia' we have not observed statistically significant correlation for value Hue with any of the observed inner fruit quality parameter. Correlation of medium strength was determined between value *L** and *a** and ChA, value *b** and NChA and TA. Chroma was in medium negative correlation with both ChA and NChA (Tab. 5).

In cv. 'Regina', unlike cv. 'Kordia' *a** values and Chroma did not have statistically significant correlation with any of the inner quality parameters (Tab. 6). Furthermore, the intensity of association between values *L**, *b** and Hue and majority of polyphenols was much stronger than in cv. 'Kordia' (Tab. 6).

Substantial difference between cultivars when it comes to correlation coefficient between fruit skin colorimetric values and fruit phenolic content prompted further analysis on the rootstock level. Obtained results showed that individual rootstocks have different correlation pattern. For Gisela 5, value *L** had strong association with p-CA (r = -0.76), Cy-3-R (r = -0.83), Cy-3-G (r = -0.87) and TA (r = -0.96). Strong association was determined between values *a**, *b** and Chroma and mentioned phenolic acids as well on Gisela 5 (data not shown). In PiKU1 strong association was found as well between *L** and Cy-R-G (r = -0.83), Cy-3-R (r = -0.84) and TA (r = -0.80). Value *b** correlated with TA (r = -0.93), Cy-3-G (r = -0.76), Cy-3-R (r = -0.74) and NChA (r = -0.75).Gisela 6 had strong association only between value *L** and TA (-0.74), and between Hue and ChA (r = -0.79). PHL-C was the only rootstock where correlation between anthocyanins and chromatic values was not determined. The only association found for this rootstock was between TP and *L** (r = -0.72) and Hue (r = -0.71).

Mozetič et al. (2004) have determined that the content of anthocyanins rises during ripening of sweet cherries and leads to the development of new colour palette which results in decrease of the intensity of red colour. Change in value *L** and Chroma during the ripening of sweet cherry 'Petrovka' correlated strongly with the change in the TA content. Gonçalves et al. (2005) obtained similar results on sweet cherry cultivars 'Burlat', 'Summit' and 'Van'. Influence of the rootstock on sweet cherry fruit skin colour development and correlation association between outer and inner fruit quality parameters was

Tab. 5: Correlation coefficients (r), *p* values and the probability levels for phenolic content as well as colour parameters of cv. 'Kordia'

		*L**	*a**	*b**	Hue	Chroma
Chlorogenic acid	r	-.5248	-.5101	-.4219	.4491	-.5037
	p	0.037*	0.044*	0.104 ns	0.081 ns	0.047*
Neochlorogenic acid	r	.0414	-.4885	-.5744	.2103	-.5059
	p	0.879 ns	0.055 ns	0.020*	0.434 ns	0.046*
p-Coumaroylquinic a.	r	.1373	-.3456	-.4499	.0712	-.3640
	p	0.612 ns	0.190 ns	0.080 ns	0.793 ns	0.166 ns
Cyanidin 3 glucoside	r	.0352	.1360	.1366	-.1589	.1380
	p	0.897 ns	0.616 ns	0.614 ns	0.557 ns	0.610 ns
Cyanidin 3 rutinoside	r	-.3013	.0271	.0599	-.0308	.0325
	p	0.257 ns	0.921 ns	0.826 ns	0.910 ns	0.905 ns
Total anthocyanins	r	-.0355	-.3765	-.5068	.0867	-.3985
	p	.896 ns	0.151 ns	0.045*	0.749 ns	0,126 ns
Total phenols	r	.2288	-.1616	-.2353	.0136	-.1731
	p	0.394 ns	0.550 ns	0.380 ns	0.960 ns	0.521 ns

**, * = significant at p ≤ 0.01 and 0.05, respectively; ns = not significant (p > 0.05)

Tab. 6: Correlation coefficients (r), p - values and the probability levels for phenolic content and colour parameters of cv. 'Regina' grafted on 4 different rootstocks

		*L**	*a**	*b**	Hue	Chroma
Chlorogenic acid	r	-.3934	-.0823	-.3384	-.6451	-.1179
	p	0.132 ns	0.762 ns	0.200 ns	0.007 **	0.664 ns
Neochlorogenic acid	r	-.7711	-.4688	-.6844	-.5071	-.4749
	p	0.000 **	0.067 ns	0.003 **	0.045 *	0.063 ns
p-Coumaroylquinic a.	r	-.7384	-.4365	-.6053	-.3509	-.4643
	p	0.001 **	0.091 ns	0.013 *	0,.183 ns	0,.070 ns
Cyanidin 3 glucoside	r	-.8341	-.4627	-.6688	-.5229	-.4500
	p	0.000 **	0.071 ns	0.005**	0.038 *	0.080 ns
Cyanidin 3 rutinoside	r	-.7898	-.4237	-.6516	-.5548	-.4314
	p	0.000 **	0.102 ns	0.006 **	0.026 *	0.095 ns
Total anthocyanins	r	-.8683	-.4691	-.6887	-.5666	-.4564
	p	0.000 **	0.067 ns	0.003 **	0.022 *	0.076 ns
Total phenols	r	-.5941	-.2933	-.4684	-.6079	-.2219
	p	0.015 *	0.270 ns	0.067 ns	0.012 *	0.409 ns

**, * = significant at p ≤ 0.01 and 0.05, respectively; ns = not significant (p > 0.05)

not researched to this extent so far. Our results instigate that when deciding on sweet cherry ripeness stage (harvest window), rootstock influence on individual cultivars must be taken into consideration as well.

Conclusions

Rootstock had strong influence on the quality parameters of cvs. 'Kordia' and 'Regina', namely on the content of TP, TA, Cy-3-R and Cy-3-G, as well as on the values *L** and *b**. TA content in fruits of both cultivars was influenced by vegetation year. Cv. 'Regina' showed similar distribution of TA content in both vegetation years forming in that sense more stable connection with all four rootstocks. Cv. 'Kordia', however reacted differently in combination with individual rootstocks when it comes to TA content in both vegetation years. With substantial difference in correlation pattern in our research between cvs. 'Kordia' and 'Regina' it can be concluded that values *L** and *b** are better TA indicators rather than values *a** and Hue as previous studies suggested. Also, with TA content following the same distribution pattern as p-CA content, it can be assumed that mentioned chromatic values could be used as indicators of phenolics content for specific cultivar and rootstock combinations. Having in mind that rootstock had strong influence on inner and outer fruit quality parameters which corresponds to existence of correlation association between researched phenolics and colour parameters, further analysis is needed to investigate influence of the cultivar*rootstock interaction on correlation pattern.

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