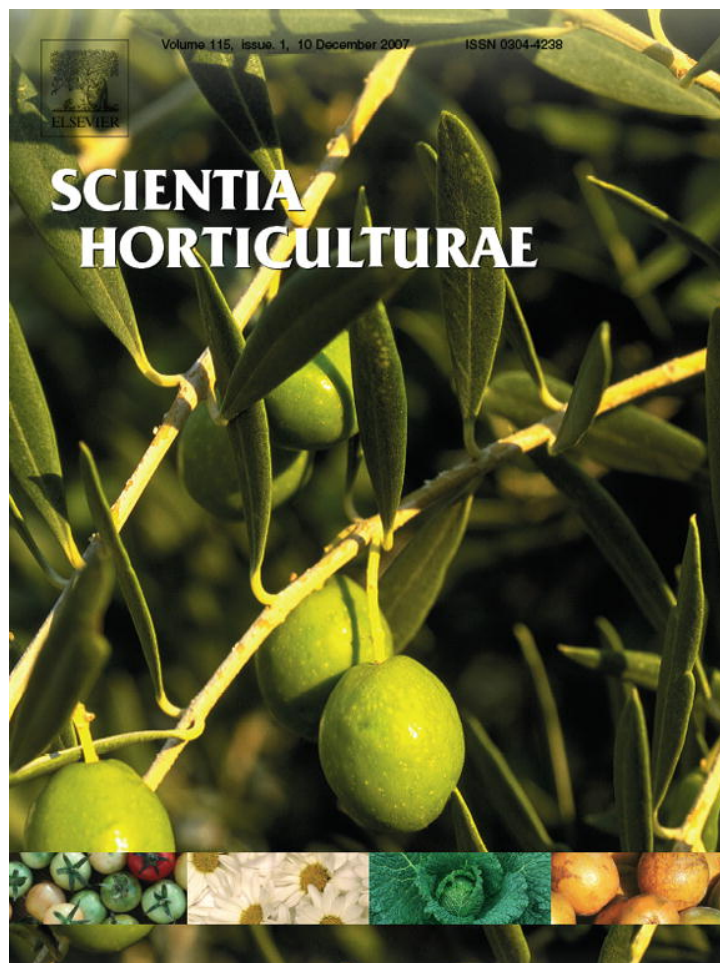


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Effects of cultivar and rootstock on the antioxidant content and physical characters of clingstone peaches

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Abstract

A study was conducted to investigate the variability in the fruit antioxidant content and physical characters of six clingstone cultivars and three breeding selections of peach grafted on three rootstocks. The parameters measured were fruit weight, fruit and stone dimensions, flesh color using CIELAB color variables, total antioxidant activity using the radical DPPH, total phenolics, ascorbic acid, soluble solids and total acid content. Fruit from cultivar PI-E45 had the highest total antioxidant activity ($10.7 \text{ mg g}^{-1} \text{ DW}$) and total phenolic ($6.9 \text{ mg g}^{-1} \text{ DW}$) content, which were up to 6.3- and 5.3-fold greater, respectively, compared with the rest studied cultivars. The highest ascorbic acid content was found in Fortuna ($7.3 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$) and was up to 1.4-fold greater compared with the rest studied cultivars. A high correlation between AEAC and the phenolic content was found, but not between AEAC and the ascorbic acid content. The largest fruit was harvested in cultivar Andross followed with a descending order by PI-E45, PI-IB42, PI-A37 (seedlings of Andross), Fortuna and Loadel \gg Everts and Catherina \gg Romea. Changes in the fruit weight were usually according to changes in stone width. The fruit and stone shape differed among the cultivars but not among the rootstocks studied. Effects of rootstock on the fruit antioxidant contents were not pronounced. Nevertheless rootstocks altered the fruit weight since in all cultivars, apart from Romea and Catherina, when grafted on GF 677 produced the largest fruit (mean 186 g) followed by PR204 (mean 176 g) and even smaller by KID1 (mean 161 g). Results from correlation analyses showed that flesh brightness (measured in frozen fruit) may suggest for more nutritional flesh and small sized fruit may contain a redder and less bright colored flesh.

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Keywords: Ascorbic acid; Flesh color; Fruit and stone dimensions; Fruit weight; Total phenolics

1. Introduction

Due to the short postharvest life of peach, it is essential to cultivate many cultivars with different maturation time in order to extend the harvesting period and supply the industry and market with fruit for longer. Good agronomical attributes of a clingstone peach cultivar are fruit characteristics such as the yellow flesh color, small stone size, absence of pit fragments and red coloration near the stone, high flesh firmness as well as high yield and resistance against frost, insects and diseases. In recent years due to increased public awareness, the nutritional value of fruit is also a parameter ascribing fruit quality.

In peach fruit, phenolic compounds serve as a major source of potential antioxidants (Chang et al., 2000) which are known to enhance the stability of low-density lipoprotein (LDL) to

oxidation and play a significant role in atherosclerosis and coronary heart diseases (Steinberg et al., 1989). Moreover, the phenolic compound chlorogenic acid has been related with high levels of brown rot (*Monilia fructicola*) disease resistance (Gradziel and Wang, 1993; Bostock et al., 1999), and browning reactions in the peach periderm (Cheng and Crisosto, 1995). Measurement of the total antioxidant capacity gives a good measure of the fruit nutritional value (Wang et al., 1996). Although great variation may exist in the peach antioxidant content among different genotypes (free stone peach, Gil et al., 2002; clingstone peach, Chang et al., 2000), information on existing and new peach cultivars is limited.

Rootstock breeding programs aim at producing genotypes that expand the adaptability to different soil conditions, resistance to soil diseases, adapt to new cultivation systems and also provide greater total production and water use efficiency (Caruso et al., 1996; Albás et al., 2004; Yano et al., 2002). The modifications caused by a rootstock on plant development may also change the blooming time, ripening time

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and fruit quality such as the mineral composition and the sugar, organic acid and antioxidant content (Scalzo et al., 2005; Giorgi et al., 2005; Kubota et al., 2001).

The aim of the present study was to determine the variability in the antioxidant content and physical characters in fruit from nine clingstone peach cultivars/genotypes (*Prunus persica* L. Batsch) grafted on three rootstocks. Three of the clingstone peach genotypes and two of the rootstocks studied have recently resulted from a breeding program made at the Pomology Institute and were documented to exhibit good agronomical characteristics (Tsipouridis et al., in press; Tsipouridis and Thomidis, 2005), while the rest cultivars and rootstock are widely grown worldwide.

2. Materials and methods

Fruit was investigated from the clingstone peach [*Prunus persica* (L.) Batsch] cultivars Andross, Catherina, Everts, Fortuna, Loadel, Romea and the breeding selections PI-A37, PI-E45 and PI-IB42, which are selected seedlings of cultivar Andross. The different cultivars were grafted on the peach × almond rootstocks GF 677 and PR204 and KID1 (of the Greek germplasm). Fruit was harvested at commercial maturity during the 2005 growing period from trees maintained in a collection at the Pomology Institute, Naoussa, Greece (40°37'13.40"N, 22°06'59.80"E, at 119 m altitude). The trees were 4 year old, planted in 5 m × 4 m distance in a randomized block design of six trees per genotype/rootstock in two replicate trees per block. Fertilisation and irrigation were conducted at recommended rates to ensure optimum yield and quality. Thinning was applied during the stone hardening period, leaving about 6 cm distances between the remaining fruit.

Twenty fruit per peach genotype/rootstock combination were harvested at the commercial maturity stage, transferred to the laboratory where fruit weight, length and width were measured. The fruit and stone length/width ratio were calculated. Soluble solids content (SSC) and total acidity (TA) were analysed in juice from six fruit using a digital refractometer (model PR-1, Atago, Japan), and TA by titration with 0.1 N NaOH and expressed as malic acid content ($\text{g} \times 100 \text{ mL}^{-1}$). The stone length and width dimensions were measured in 20 fruit after the extractions for the chemical analyses were made.

2.1. Chemical analyses

Eight replicate fruits were rinsed with water, dried using tissue paper and stored at -20°C for chemical analyses. For the antioxidant activity and total phenolics assays flesh samples from five replicate stored fruit were removed using a sharp knife, immediately lyophilized, homogenized using a pestle and mortar and stored at -20°C . For the ascorbic acid assay, samples were readily extracted from the remaining three replicate frozen fruit. All analyses were made in triplicate. Chemicals were purchased from Sigma Chemical Co (St. Louis, MO). Assays were performed using an automated UV/visible spectrophotometer.

2.1.1. Antioxidant activity and total phenolics assay

Freeze-dried samples (400 mg) were homogenized in 10 mL 80% MeOH/H₂O (v/v) in a micro-dismembrator U for 3 min at 2000 rpm. The extract was centrifuged at $5000 \times g$ for 10 min and the supernatant recovered.

Antioxidant activity was measured using the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical (Blies, 1958), which has an intense violet color, but turns colorless as unpaired electrons are sequestered by antioxidants. Reaction mixtures containing 0 or 10 μL extract, 2300 μL 106.5 μM DPPH in MeOH and 690 μL H₂O were vortexed, and then held at room temperature for 1 h. The absorbance of the reaction mixtures were measured at 517 nm and ascorbate equivalent antioxidant capacity (AEAC) (mM) was extrapolated from a standard curve prepared using 0–2.7 mM ascorbate.

Total soluble phenolics were determined with the Folin-Ciocalteu reagent and results were expressed as mg gallic acid $\times 100 \text{ g}^{-1}$ DW.

2.1.2. Ascorbic acid assay

Samples (2 g) from the flesh of three frozen peaches stored at -20°C were readily removed using a sharp knife. The samples were extracted in 3 mL of ice-cold 6% (w/v) metaphosphoric acid containing 0.2 mM diethylene triamine pentaacetic acid (DTPA). The extract was centrifuged at $10,000 \times g$ for 4 min at 4°C and the supernatant recovered. Ascorbic acid content was measured using the reflectometer Merck RQflex following the manufacture's instructions.

2.1.3. Color determination

Color determinations were made in the flesh of frozen fruit. Using a sharp knife 1 cm depth of the fruit's cheek was removed and the CIELAB L^* (brightness or lightness; 0 = black, 100 = white), a^* ($-a^*$ = greenness, $+a^*$ = redness) and b^* ($-b^*$ = blueness, $+b^*$ = yellowness) color variables were measured using the chromatometer Minolta (Minolta, Ramsey, NJ).

2.2. Statistical analysis

MANOVA using genotype and rootstocks as variables were performed using SPSS (SPSS Inc., Chicago, Illinois, USA). LSD's were calculated in cases that significant at $P < 0.05$ variance was found. Correlation analyses were also made.

3. Results and discussion

The highest AEAC was found in PI-E45 (10.7 mg g^{-1} DW) followed with a descending order by Everts \gg Andross and Catherina \gg PI-A37, Loadel, PI-IB42, Romea and Fortuna (1.7 mg g^{-1} DW) (Fig. 1 and Table 3). The highest total phenolic content (6.9 mg g^{-1} DW) was also found in the cultivar PI-E45 followed with a descending order by Everts and PI-IB42 \gg Andross and PI-A37 \gg Catherina, Fortuna, Loadel and Romea (1.3 mg g^{-1} DW). There was no significant correlation between the time of harvest and the fruit antioxidant content (data not shown); nevertheless the highest AEAC and

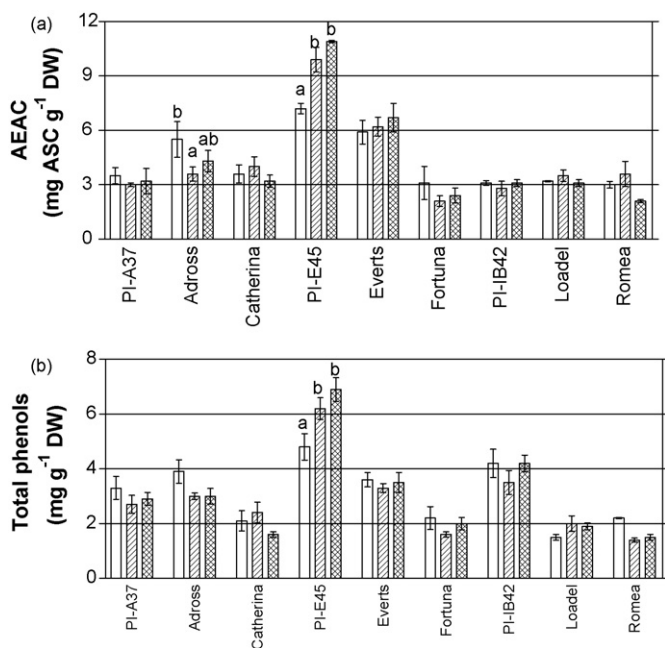


Fig. 1. (a) Ascorbate equivalent antioxidant capacity (AEAC) (mg g⁻¹ DW) and (b) total phenolics (mg g⁻¹ DW) in nine peach genotypes grafted on GF677, KID1 and PR204. Values represent mean ± S.E. Letters denote significant differences (*P* < 0.05). Open bars represent plants grafted on GF677, hatched bars represent plants grafted on KID1 and double hatched bars represent plants grafted on PR204.

total phenolic content were found in PI-E45 and Everts both being the latest harvested cultivars. González et al. (2003) found higher antioxidant content in late compared with early ripening raspberry cultivars.

The total phenolic content was positively correlated with AEAC (*r* = 0.842), which suggests that phenolic compounds greatly contribute to the total antioxidant capacity. Similar results were reported for other clingstone (Chang et al., 2000), peach and nectarine cultivars (Gil et al., 2002). Mean values of the total phenolic content were similar when compared with other reports made on a fresh weight basis, considering that drying reduces weight by about ten times (Chang et al., 2000).

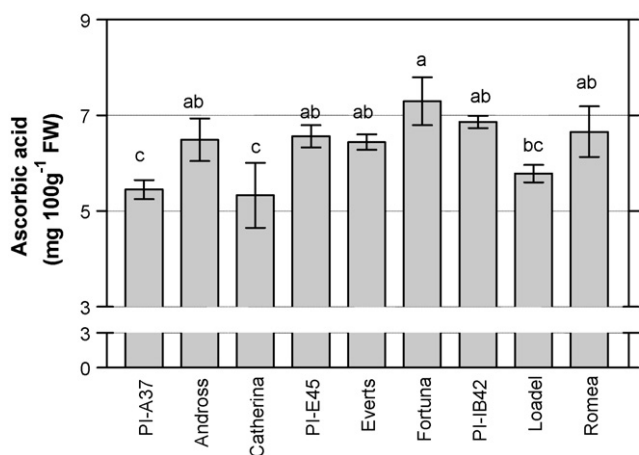


Fig. 2. Mean values (± S.E.) of ascorbic acid content (mg 100 g⁻¹ FW) in nine peach genotypes. Letters denote significant differences (*P* < 0.05).

Table 1
Pearson product moment correlations and (*P* value) between peach physical and chemical characters

	1	2	3	4	5	6	7	8	9	10	11
1. Fruit weight	1										
2. Fruit length/width	0.844 (0.004)	1									
3. Stone length/width	-0.623 (0.073)	-0.127 (0.744)	1								
4. <i>L</i> *	0.570 (0.109)	0.725 (0.027)	-0.339 (0.371)	1							
5. <i>c</i> *	-0.911 (0.001)	-0.745 (0.021)	0.527 (0.145)	-0.725 (0.027)	1						
6. <i>b</i> *	0.190 (0.624)	0.069 (0.861)	0.305 (0.426)	0.323 (0.397)	-0.216 (0.577)	1					
7. SSC	-0.265 (0.491)	-0.482 (0.198)	0.219 (0.571)	-0.093 (0.812)	0.318 (0.404)	0.344 (0.365)	1				
8. Total acids	-0.351 (0.354)	-0.340 (0.371)	-0.043 (0.912)	-0.629 (0.070)	0.401 (0.284)	-0.807 (0.009)	0.070 (0.857)	1			
9. Total phenols	0.461 (0.212)	0.602 (0.086)	-0.232 (0.547)	0.760 (0.018)	-0.549 (0.126)	-0.277 (0.471)	0.026 (0.948)	0.277 (0.471)	1		
10. AEAC	0.160 (0.681)	0.453 (0.220)	-0.048 (0.902)	0.735 (0.024)	-0.304 (0.473)	0.275 (0.473)	0.382 (0.310)	0.275 (0.473)	0.842 (0.004)	1	
11. Ascorbic acid	0.320 (0.401)	-0.194 (0.616)	-0.471 (0.200)	0.317 (0.405)	-0.112 (0.774)	0.140 (0.719)	0.308 (0.420)	0.140 (0.719)	0.263 (0.494)	0.191 (0.622)	1

Regarding the flesh ascorbic acid content, the cultivar Fortuna had the highest (7.3 mg 100 g⁻¹ FW) and PI-A37 and Catherina had the lowest (mean 5.3 mg 100 g⁻¹ FW) content, while the rest cultivars had intermediate values (Fig. 2, Table 3). There was no significant correlation between the ascorbic acid content and AEAC (Table 1); a finding also reported by Gil et al. (2002) for free stone peach and nectarine cultivars and suggests that antioxidants other than ascorbic acid may mostly contribute to the total antioxidant capacity in peach fruit.

The largest fruit was produced by the cultivar Andross (mean 214 g) followed with a descending order by PI-E45, PI-IB42, PI-A37, Fortuna and Loadel (mean 183 g) \gg Everts and Catherina (mean 160 g) \gg Romea (mean 132 g) (Tables 2 and 3). Cultivar Romea is very early maturing that accounts for its small fruit weight. Changes in the fruit weight were usually according to changes in stone width, as indicated by a significant positive correlation between fruit weight and stone width ($r = 0.674$) (data not shown).

Linear correlations were found between the fruit length and width ($r = 0.764$; $P = 0.014$) and between the stone length and

width ($r = 0.822$; $P = 0.007$) dimensions (data not shown). The shapes of fruit from PI-A37 and Andross were close to sphere (length/width ratio = 0.977), in Romea was more elongated (0.839) and in the rest cultivars had an intermediate shape (0.923) (Table 2). Regarding the stone shapes Romea, Catherina and PI-A37 had the most oval (1.527), followed by PI-E45 (1.426) and the rest cultivars (1.309). There was little relationship between the fruit and stone shape (no significant correlation was found between the fruit and stone length/width ratio); nevertheless in Romea both fruit and stone shapes were the most oval among the studied cultivars. Similar results were reported by Quilot et al. (2004) for seven peach genotypes and suggest that the fruit and stone length and width are symmetrical with the fruit exhibiting a more spherical and the stone a more oval shape.

Cultivar Andross had the most yellow colored flesh (high b^* and low a^* values) whereas Romea had the most red colored (high a^* values) flesh. The brightest colored flesh (high L value) was found in PI-E45, Everts, PI-A37 and Andross; whereas the darkest colored flesh was found in Caterina, Romea, Loadel and PI-IB42.

Table 2

Mean values of fruit weight (g), fruit and stone length/width (L/W), skin color parameters L^* , a^* and b^* , soluble solids content (SSC) (%), titratable acidity (TA) (g 100 mL⁻¹) in nine peach genotypes grafted on three rootstocks

	PI-A37	Andross	Cather	PI-E45	Everts	Fortuna	PI-IB42	Loadel	Romea
Fruit weight; LSD = 23.9									
GF 677	198.0	262.0	159.2	198.5	174.3	197.2	196.4	192.3	138.2
KID1	159.3	171.2	142.7	174.7	146.4	172.3	174.2	167.0	141.7
PR204	184.6	207.6	168.9	187.6	165.8	180.1	192.4	174.3	117.4
Fruit L/W; LSD = 0.05									
GF 677	0.972	0.929	0.911	0.990	0.935	0.936	0.930	0.935	0.885
KID1	0.993	0.918	0.899	0.982	0.907	0.957	0.955	0.930	0.849
PR204	0.976	0.917	0.922	0.947	0.938	0.895	0.892	0.908	0.874
Stone L/W; LSD = 0.11									
GF 677	1.52	1.25	1.56	1.49	1.32	1.32	1.29	1.32	1.49
KID1	1.51	1.33	1.49	1.41	1.34	1.37	1.28	1.28	1.52
PR204	1.46	1.26	1.57	1.38	1.34	1.32	1.29	1.33	1.62
L^* ; LSD = 4.3									
GF 677	100.1	101.6	97.6	99.7	96.5	97.1	96.4	95.1	93.2
KID1	96.4	96.4	92.0	102.0	99.5	97.0	95.6	97.4	95.1
PR204	99.4	97.2	93.9	101.0	101.0	98.5	97.8	96.2	98.1
a^* ; LSD = 2.2									
GF 677	4.2	3.4	10.9	3.7	8.1	7.0	7.9	8.7	11.9
KID1	5.4	4.0	9.6	5.3	9.1	6.6	6.8	8.1	11.9
PR204	5.6	4.4	9.9	6.7	8.0	6.3	6.3	9.1	12.0
b^* ; LSD = 8.9									
GF 677	-18.3	-19.1	-26.8	-17.1	-18.6	-30.1	-42.0	-49.2	-19.6
KID1	-22.2	-10.4	-18.9	-26.6	-19.6	-23.6	-26.6	-51.0	-18.8
PR204	-18.7	-20.1	-16.3	-21.9	-24.0	-17.0	-21.9	-46.0	-21.2
SSC; LSD = 3.66									
GF 677	11.7	14.1	15.6	15.1	14.7	14.7	12.1	12.7	16.0
KID1	12.8	14.7	14.6	14.4	13.6	14.4	13.0	13.9	13.6
PR204	12.3	14.8	14.4	14.9	13.9	12.9	13.6	14.0	14.8
TA; LSD = 0.85									
GF 677	5.2	5.4	7.2	5.6	5.0	6.5	6.2	8.9	5.8
KID1	5.5	6.0	9.1	4.9	5.9	6.2	5.3	10.7	6.0
PR204	4.3	6.2	6.4	5.0	5.0	6.0	6.2	8.9	6.1

LSD at 95% level of significance are presented.

Table 3
P values for the effects of cultivar and rootstock and their interaction on fruit physical and chemical characters of peach

	Genotype	Rootstock	Genotype × rootstock
Fruit weight	<0.001	<0.001	<0.001
Fruit length/width	<0.001	0.085	0.238
Stone length/width	<0.001	0.978	0.349
<i>L</i> *	<0.001	0.199	0.051
<i>a</i> *	<0.001	0.735	0.491
<i>b</i> *	<0.001	0.026	0.003
SSC	0.007	0.549	0.554
TA	<0.001	<0.001	<0.001
Total phenolics	<0.001	0.354	0.004
AEAC	<0.001	0.957	0.052
Ascorbic acid	0.001	0.100	0.060

The rootstocks GF 677, PR204 and KID1 were previously found to give better yield, relatively good fruit quality and resistance to frost damages in the table peach cultivar May Crest when compared to other 10 peach rootstocks (Tsiouridis and Thomidis, 2005). In the present study the above rootstocks did not have pronounced effects on the fruit antioxidant content in most cultivars studied (Tables 2 and 3, Fig. 1). In the case of PI-E45 grafted on GF677 the fruit had lower AEAC and total phenolics compared with the rest rootstocks; a response that could be attributed to the greater fruit weight produced in GF677. An opposite trend on the effects of peach rootstocks on the antioxidant content and fruit weight was similarly documented by Giorgi et al. (2005). Significant effects of different rootstocks such as Barrier 1, Citation, GF677, Ishtara and Julior on the peach antioxidant content have been shown (Scalzo et al., 2005; Giorgi et al., 2005; Kubota et al., 2001). In the present study the lack of substantial rootstock effects on the fruit antioxidant content and other chemical characters may be due to their close genetic origin (peach × almond hybrids).

GF 677 produced the largest fruit (mean 186 g) followed by PR204 (mean 176 g) and smaller by KID1 (mean 161 g) (Tables 2 and 3). However, in cultivar Romea fruit weight was similar among the studied rootstocks, which may be attributed to its very short fruit development period that may minimize differences in fruit quality between the graft combinations. Similar results were reported by Caruso et al. (1996) for another very early ripening peach cultivar. The fruit and stone shape were not affected by the rootstocks studied.

Correlation analyses showed that flesh brightness (*L* value) was positively correlated with AEAC ($r = 0.735$) and total phenolic ($r = 0.760$) content, suggesting that flesh brightness may indicate for a more nutritional flesh in clingstone peach. An association between the flesh redness and the antioxidant β -cryptoxanthin content in clingstone peach cultivars has also been documented (Tourjee et al., 1998) while in the pomegranate fruit the anthocyanin content were likely to be greater in small sized or red colored pomegranates (Drogoudi et al., 2005).

Flesh redness (*a** value) was negatively correlated with fruit weight ($r = -0.911$), fruit length ($r = -0.977$) and stone width ($r = -0.691$), while the flesh brightness was positively correlated with fruit length ($r = 0.712$) and stone width

($r = 0.691$) (data not shown). It is suggested that smaller sized clingstone peaches may contain a redder and less bright colored flesh.

4. Conclusions

Results from the present study have allowed the characterisation in the nutritional attributes and other fruit quality characteristics in widely grown and new selections of clingstone peach as well as new rootstock selections. A pronounced cultivar effect on the fruit antioxidant content was found; AEAC and total phenol content varied up to 6.3- and 5.3-fold, respectively. Less pronounced effects of rootstocks on the peach antioxidant content were found and this may be attributed to the close genetic origin of the studied rootstocks; nevertheless changes in the fruit weight were documented. The cultivar Everts and the genotype PI-E45 should be considered as a good source of antioxidants. Moreover, results from correlation analyses showed that flesh brightness may suggest for a more nutritional flesh in clingstone peaches.

The cultivars PI-E45, PI-IB42 and PI-A37 are selected seedlings of Andross and should be suggested for widespread cultivation due to its relative good fruit weight, good fruit quality and distribution in the maturation time; PI-A37 mature 25 days earlier than Andross and the PI-IB42 and PI-E45 mature four and 10 later than Andross, respectively (Tsiouridis et al., in press).

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