Physical Parameters, Total Phenolics, Flavonoids and Vitamin C Content of Nine Sweet Cherry Cultivars

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Sweet cherry tree (Prunus avium L., Rosaceae) is a species with high economic efficiency in production and in the last twenty years the scientific interest increased in the North-East region of Romania. In the area of Iaºi in 2014 the combined influence of the fruits position on tree level in different cultivars was recorded in terms of morphological and biochemical characteristics for nine different cultivars ex situ collected. Fruits collected from two different height-levels areas of tree were used for analysis in each cultivar. Fruit pulp and kernel were investigated in terms of biometry, total solids content, vitamin C, total flavonoids (flavones), and polyphenols. Fruit mass and diameter showed higher values in samples collected from the upper side of the tree on Catalina, Scorospelka, Van and Bucium'cultivars, ranging between 5.8 g / fruit to 9.5 g / fruit and 20.6 to 26.3 mm / fruit respectively. Kernel mass was higher on upper side of the tree on Catalina and Marina cultivars, while on Scorospelka, Van, Bucium and Stefan the values were greater on down half of the tree but without statistical signification. Total solids content of the fruit recorded values between 13.7 to 21.15%, the highest values being recorded in fruits sampled from the upper half of the tree on Catalina, Rivan', Bucium, Stefan, Galata and Marina cultivars. Total flavonoids content recorded values ranging from 2.45 to 4.84 mg catechin g^{-1} DW, with increased values for samples collected from the upper part of the tree. Vitamin C content shows values ranging from 1.98 to 8.62 ascorbic acid 100 g^{-1} DW, the greater value being recorded for Stefan'cultivar from the upper side of the tree.

Keywords: Prunus avium L., fruit size, stone, vitamin C, flavones

The sweet cherry (*Prunus avium* L.) belongs to the group of the earliest fruit crops in the temperate regions. They are also one of the most appreciated fruits in the world due to unique taste and other sensory properties [1].There is a raised interest in their study, due to many nutrients and bioactive compounds that may significantly contribute to a healthy diet [2-4].

It has been shown that fruits, legumes, and cereals have been extensively explored because these products are *basic foods* in the human diet [5]. Fruits and vegetables are a rich source of phytochemicals, such as carotenoids, flavonoids and other phenolic compounds [6]. Studies have indicated that these phytochemicals, especially polyphenols, have high free-radical scavenging activity, which helps to reduce the risk of chronic diseases, such as cardiovascular disease, cancer, and age-related neuronal degeneration [7, 8].

The free radicals are generated in the human body through aerobic respiration and exist in different forms, including superoxide, hydroxyl, hydroperoxyl, peroxyl and alkoxyl radicals. Generally, natural antioxidant enzymes in healthy individuals remove these free radicals [9]. Nevertheless, dietary antioxidants are helpful in assisting the body to neutralise free radicals. Therefore, it is important to consume a diet high in antioxidants, such as fruits and vegetables, to reduce the harmful effects of oxidative stress [6].

In Romania, Sweet cherry (*Prunus avium* L.) is a wild, semi-wild or cultivated species spread on 7,761 ha as fruit growing species [10]. Fruits are very appreciated by consumers for good taste and nutritional values. There are relatively abundant studies conducted about morphological and biochemical properties of the sweet cherry fruits. The sweet cherry fruit size depends on cultivar [11-13], on the climate of the area and year of harvest [14-17] and the applied technology [18, 19]. Jakopic et al. (2015) [20] observed at the apple the influence of the fruit's position within on fruitlets in the cluster. The biochemical properties of sweet cherry fruits are very important subject for research, due especially to the great variety of existing cultivars. The sweet cherry fruits have a high level of antioxidant content [21-23].

The objective of this study was to evaluate nine sweet cherry cultivars with different ripening time and different position of fruits on the crown of the tree, in order to establish the differences of some morphological and biochemical characteristics.

Experimental part

Materials and methods

Samples of fruits were collected in 2014 from nine sweet cherry cultivars from experimental plot of Fruit Growing Research Station Iasi-Romania and used for subsequent analysis. Trees are planted at 5×4m distance as palmette crown system, grown on *P. mahaleb* L. seedlings rootstock. Catalina, Rivan and Scorospelka cultivars have early ripening time, Van, Bucium and Stefan cultivars, medium ripening time, while Galata, Hedelfingen and Marina cultivars have late ripening time. From each cultivar, a sample (15 fruits) was collected, each in three replicates from two different areas of the tree. For fruit and stone weight (g), a high precision balance (Radwag, 0.01 sensivity) has been used. For biometric

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determinations (width, D in mm; thickness, d, in mm; length, H in mm) of the fruit pulp and stone, a digital caliper Luumytools was used. Dry weight content of sweet cherry samples was determined using gravimetric method by evaporation at mild temperature (105°C) for three days (until weight stability). Thus, it was determined the quantity of dry matter both in pulp and skin. The results of dry weight were expressed in g 100 g^{-1} [24]. To avoid confusions, we should emphasize that throughout this paper, the term, fruit pulp (or fruit) refers actually to epicarp (skin) and mesocarp (pulp), while the term 'stone' refers to sclerified endocarp. Material extraction was performed with 80% methanol. Fresh sweet cherries fruits samples were homogenized with 80% methanol and then they were stirred for 30 min . After their centrifugation at 3000 rpm for 15 min at 4°C, the supernatants were used for the next determinations. The total polyphenols content was determined by using a modified Folin-Ciocalteu method [25]. The appropriately dilute sample was added Folin-Ciocâlteu reagent and mixed thoroughly. After four minutes, 15% Na_2CO_3 was added. The absorbance of resulting bleu-colored solution was read at 765 nm after two hours, against the blank (distilled water). The amount of the total phenolic content was expressed as mg gallic acid equivalent (mg GAE g^{-1} DW) (R^2=0.99). Three readings were taken for each sample and the result averaged. The flavonoids content was measured following a spectrophotometric method [26]. Briefly, methanol extract were appropriately diluted with distilled water. Initially, 5% $NaNO_2$ solution was added to each test tube; after five min , 10% $AlCl_3$ solution was added and then at six minutes 1.0 M NaOH was added. Finally, water was then added to the test tube and mixed well. Absorbance of resulting pink-colored solution was read at 510 nm against the blank (distilled water). Total flavonoids content was expressed as mg catechin equivalent (mg CE g^{-1} DW) (R^2 =0.98). Three readings were taken for each sample and the result averaged. The ascorbic acid content was estimated with potassium ferrocyanide solution [27]. Results were expressed as mg ascorbic acid 100 g^{-1} DW.

All results were analyzed statistically using the Duncan's Multiple Range test ($p \leq 0.05$) for bifactorial experiences. Factor A was position of fruit on the crown of the tree as follows: a_1- upper part (upper half) of the tree (UP); a_2- lower part (lower half) of the tree (LP). Factor B refers to cultivar, as follow: b_1-Catalina, b_2- Rivan, b_3- Scorospelka, b_4-Van b_5- Bucium, b_6- Stefan, b_7- Galata, b_8- Hedelfingen, b_9 - Marina. The statistical analysis was performed with the XLSTAT software (ProAcademic, 2011, Addinsoft).

Results and discussions

The obtained results show a larger heterogeneity within all investigated cultivars, both when sampled from the upper and lower parts of the trees.

The fruit weight varied between 5.48g (Rivan) and 9.29g (Stefan) in the year 2014, for samples collected from UP. In the upper part of the tree, the fruit weight ranges between 4.57g (Galata) and 9.40g (Bucium). For Catalina, Scorospelka, Van and Bucium fruit weight was lower in the UP than in LP, but for Rivan, Stefan, Galata and Hedelfingen fruit weight values were slightly greater in the LP than UP (table 1). However, relatively significant differences occur only in Galata cultivar: 5.56 g when sampled from upper part of the tree, while in the lower part, the registered value was found to be 4.57 g.

Fruit width also varies in small limits, in cultivars sampled from the two parts of the tree: in the UP the highest value was found in Bucium (26.34 g), and the smallest, in Galata (20.3 g) cultivars; in the LP the highest value was also recorded in Bucium (27.03 g), and the smallest, in Galata (19.03 g). This pattern could be related rather to cultivars features, than other factors affecting fruit formation and growing.

The fruit thickness varies in the sweet cherry fruits as follow: from 18.23 mm in Rivan cultivar, to 22.47 mm in Stefan cultivar, when collected from UP. When analyzed from LP, these values ranged from 17.01 mm in Galata to 22.74 mm in Stefan cultivars. There were not found significant differences of samples collected from UP and LP, in terms of fruit thickness, when compared each cultivar; only for Catalina cultivar, this value was slightly increased when sampled from lower part (22.18), comparatively to upper part (20.84).

The maximum fruit length in analyzed samples, collected from UP, was recorded in Hedelfinger cultivar (22.89 mm), while the minimum was observed in Rivan cultivar (18.41 mm). When collected from LP, the fruit length ranged from 18.38 (Rivan) to 23.39 mm (Stefan) cultivars. There were no significant differences in term of fruit length of samples collected from UP and LP, with a slight exception in the case of Catalina and Galata cultivars. However, no pattern has been identified in this case (table 1).

Stone weight ranges from 0.17g (Rivan) to 0.38g (Stefan) cultivars, in the case of samples from UP and from 0.17g (Rivan) to 0.42g (Bucium) for samples from LP. For cultivars Catalina and Marina, stone weight registered greater values in UP, while values for Scorospelka, Van, Bucium, Stefan and Hedelfinger were slightly increased in LP, yet with no significant statistical differences. Stone width recorded values ranging from 7.62 mm (Scorospelka) to 9.54 mm (Catalina) for samples collected from UP. Stone width of Stefan and Marina cultivars was sensitively greater in UP than LP. Stone thickness was slightly greater for samples collected from UP in the case of Scorospelka, Stefan and Marina cultivars, but no significant statistical differences were found. Stone length of Rivan cultivar registered a significantly diference: 8.61 mm in UP and 9.67 mm in LP, the statistical difference being significant. In the case of Stefan, Galata'and Marina cultivars, fruit length was greater for samples collected from UP than LP yet without significant statistical differences, while for Catalina, Scorospelka, Van' Bucium and Hedelfingen cultivars, the stone length values were greater for samples collected from LP than UP but no significant statistical differences registered (table 2).

Total dry matter in studied sweet cherry cultivars range from 13.69% (Scorospelka) to 21.15% (Stefan), both values being registered for samples collected from UP (table 3). However, in the case of Stefan cultivar, relatively large difference occurs between samples from upper part (21.1) and lower part (17.69). For samples from the LP, the values range from 14.49% (Catalina) to 19.44% (Hedelfingen). The values were greater for samples collected from UP for cultivars: Catalina, Rivan, Bucium, Stefan, Galata and Marina, while for Scorospelka, Van'and Hedelfingen, the total dry matter values were slightly greater in LP; no significant statistical differences were registered. The values of humidity of the fruits range between 78.85% (Stefan) and 86.31% (Scorospelka), greater values being recorded in UP for Scorospelka, Van and Hedelfingen cultivars (table 3).

For Catalina, Rivan, Bucium, Stefan, Galata and Marina cultivars, values of humidity of the fruits collected from LP were greater than for UP. No significant statistical differences were established between values of the fruit

Sample[1]	Fruit weight[2] (g)	Fruit width D (mm)	Fruit thickness D (mm)	Fruit length H (mm)
a_1b_1	8.29^{bc}	22.89^{cde}	20.84^{bc}	20.58^{abcd}
a_1b_2	5.48^{de}	22.26^{de}	18.23^{d}	18.41^{ef}
a_1b_3	5.80^{d}	20.62^{ef}	18.73^{d}	19.13^{def}
a_1b_4	8.25^{bc}	25.70^{ab}	21.73^{abc}	21.34^{abc}
a_1b_5	9.2^{ab}	26.34^{ab}	21.94^{abc}	22.29^{ab}
a_1b_6	9.29^{ab}	26.20^{ab}	22.47^{ab}	22.75^{a}
a_1b_7	5.56^{de}	20.31^{ef}	18.27^{d}	20.03^{cdef}
a_1b_8	8.51^{abc}	24.18^{bcd}	21.30^{abc}	22.89^{a}
a_1b_9	7.49^{c}	24.09^{bcd}	20.85^{bc}	22.30^{ab}
a_2b_1	8.69^{ab}	25.79^{ab}	22.18^{abc}	22.72^{a}
a_2b_2	5.05^{de}	20.81^{ef}	17.93^{de}	18.38^{f}
a_2b_3	6.09^{c}	21.80^{def}	18.52^{d}	19.13^{def}
a_2b_4	8.60^{ab}	25.82^{ab}	22.24^{abc}	21.92^{abc}
a_2b_5	9.40^{a}	27.03^{a}	21.98^{abc}	22.20^{ab}
a_2b_6	9.00^{ab}	25.65^{abc}	22.74^{a}	23.29^{a}
a_2b_7	4.57^{e}	19.03^{f}	17.01^{e}	18.91^{def}
a_2b_8	8.34^{abc}	24.13^{bcd}	21.14^{abc}	23.15^{a}
a_2b_9	7.49^{bc}	24.27^{bcd}	20.64^{c}	21.78^{abc}
LSD 5%	0.92	2.71	1.42	1.84

[1]a_1- up half of the tree (UP); a_2- down half of the tree (LP); b_1-Catalina, b_2- Rivan, b_3- Scorospelka, b_4 -Van b_5 - Bucium, b_6 - Stefan, b_7 - Galata, b_8 - Hedelfingen, b_9 - Marina
[2]Different letters after the numbers within a column corresponds with statistically significant differences for P 5% according to Duncan's Multiple Range test.

Table 1
INFLUENCE OF THE POSITION ON THE TREE (a) AND CULTIVAR (b) on the physical features of sweet cherry fruit (RSFG Iasi, 2014, n=3)

Sample[1]	Stone weight[2] (g)	Stone width D (mm)	Stone thickness D (mm)	Stone length H (mm)
a_1b_1	0.37^{ab}	9.54^{ab}	7.67^{ab}	11.35^{ab}
a_1b_2	0.17^{e}	8.10^{fgh}	7.11^{bcde}	8.61^{f}
a_1b_3	0.23^{de}	7.62^{h}	6.26^{fg}	8.96^{ef}
a_1b_4	0.31^{bc}	9.01^{bcde}	7.23^{bcd}	9.85^{d}
a_1b_5	0.37^{ab}	9.04^{abcde}	7.74^{ab}	10.07^{d}
a_1b_6	0.34 ^{ab}	8.82^{bcdef}	7.50^{abc}	10.28^{cd}
a_1b_7	0.38 ^{ab}	8.67^{cdefg}	6.48^{defg}	12.00^{a}
a_1b_8	0.35 ^{ab}	8.34^{defgh}	7.06^{bcde}	10.93^{bc}
a_1b_9	0.36 ^{ab}	8.43^{cdefgh}	7.66^{ab}	10.26^{cd}
a_2b_1	0.30^{bcd}	9.87^{a}	8.20^{a}	11.65^{ab}
a_2b_2	0.17^{e}	8.94^{bcdef}	7.34^{bc}	9.67^{de}
a_2b_3	0.25^{cd}	7.89^{gh}	5.94^{g}	8.97^{ef}
a_2b_4	0.34 ^{ab}	9.26^{abc}	7.41^{bc}	10.22^{cd}
a_2b_5	0.42^{a}	9.19^{abcd}	7.84^{ab}	10.13 ^{cd}
a_2b_6	0.35^{ab}	8.34^{defgh}	7.10^{bcde}	10.06^{d}
a_2b_7	0.38 ^{ab}	8.88^{bcdef}	6.41^{efg}	11.80^{ab}
a_2b_8	0.36 ^{ab}	8.46^{cdefgh}	6.87^{cdef}	11.16^{ab}
a_2b_9	0.33^{bc}	8.15^{efgh}	7.20^{bcd}	10.05^{d}
LSD 5%	0.07	0.76	0.67	0.829

[1]a_1- up half of the tree (UP); a_2- down half of the tree (LP); b_1-Catalina, b_2- Rivan, b_3- Scorospelka, b_4 - Van, b_5 - Bucium, b_6 - Stefan, b_7 - Galata, b_8 - Hedelfingen, b_9 - Marina.
[2]Different letters after the numbers within a column corresponds with statistically significant differences for P 5% according to Duncan's Multiple Range test.

Table 2
INFLUENCE OF THE POSITION ON THE TREE (a) AND CULTIVAR (b) ON THE PHYSICAL SWEET CHERRY FRUIT STONE (RSFG IASI, 2014, n=3)

Sample[1]	Total dry matter (%)[2]	Humidity (%)	Flavones mg catechin g^{-1} DW	Vitamin C mg acid ascorbic 100 g^{-1} DW	Polyphenols mg GAE g^{-1} DW
a_1b_1	17.15^{abcd}	82.85^{abcde}	4.39^{ab}	6.73^{abc}	37.84^{bcde}
a_1b_2	15.54^{abcd}	84.46^{abcd}	3.27^{bcd}	8.51^{ab}	36.01^{bcde}
a_1b_3	13.69^{d}	86.31^{a}	4.84^{a}	3.22^{abcd}	44.33^{ab}
a_1b_4	15.65^{abcd}	84.35^{abcd}	3.78^{abc}	4.15^{abcd}	40.05^{abcd}
a_1b_5	19.23^{abc}	80.78^{de}	3.69^{abcd}	2.48^{bcd}	40.46 ^{abcd}
a_1b_6	21.15^{a}	78.85^{e}	3.82^{abc}	8.62^{a}	38.15 ^{bcde}
a_1b_7	18.85 ^{abc}	81.15^{cde}	2.45^{d}	1.9^{d}	30.49^{e}
a_1b_8	19.11 ^{abc}	80.89^{de}	3.48^{bcd}	2.83^{abcd}	33.71^{cde}
a_1b_9	17.99^{abcd}	82.02^{abcde}	3.57^{abcd}	9.2^{cd}	38.46 ^{bcde}
a_2b_1	14.49 ^{d}	85.51^{ab}	4.28^{ab}	4.66^{abcd}	46.96^{a}
a_2b_2	15.24^{cd}	84.76^{abcd}	4.52^{ab}	4.66^{abcd}	36.99 ^{bcde}
a_2b_3	14.63 ^{d}	85.37^{abc}	4.48^{ab}	6.44^{abcd}	42.80 ^{ab}
a_2b_4	15.81^{abcd}	84.19^{abcd}	3.52^{abcd}	3.11^{abcd}	39.16 ^{abcd}
a_2b_5	15.29^{abcd}	84.71^{abcd}	3.19^{bcd}	5.40^{abcd}	37.60 ^{bcde}
a_2b_6	17.69^{abcd}	82.31^{abcde}	4.39^{ab}	4.63^{abcd}	39.23 ^{abcd}
a_2b_7	18.08^{abcd}	81.92^{bcde}	2.70^{cd}	4.2^{d}	30.71^{e}
a_2b_8	19.44^{ab}	80.56^{de}	3.31^{bcd}	8.3^{cd}	32.73^{de}
a_2b_9	15.48^{abcd}	84.52^{abcd}	4.04^{ab}	3.6^{d}	41.75^{abc}
LSD 5%	3.48	3.69	1.15	5.29	7.24

Table 3
INFLUENCE OF THE POSITION ON THE TREE (a) AND CULTIVAR (b) ON THE CHEMICAL FEATURES OF SWEET CHERRY FRUIT
(RSFG Iasi, 2014, n=3)

[1]a_1- up half of the tree (UP); a_2- down half of the tree (LP); b_1-Catalina, b_2- Rivan, b_3- Scorospelka, b_4-Van b_5- Bucium, b_6- Stefan, b_7- Galata, b_8 - Hedelfingen, b_9 - Marina
[2]Different letters after the numbers within a column corresponds with statistically significant differences for P 5% according to Duncan's Multiple Range test.

samples from UP and LP. Total flavonoids content range between 2.45 mg catechin g^{-1}DW (Galata collected from UP) and 4.84 mg catechin g^{-1}DW (Scorospelka from UP). Total flavonoids content was fairly greater for fruits sampled from UP in Catalina, Scorospelka, Van, Bucium and Hedelfingen cultivars (table 3). Vitamin C content largely varies from 1.9 to 8.62 mg ascorbic acid 100 g^{-1} DW, the greater value being recorded for Stefan on UP (table 3); for samples collected from UP vitamin C content range between 1.9 (Galata) and 8.62 mg ascorbic acid 100 g^{-1} DW (Stefan), while for samples collected from LP, vitamin C content range between 3.6 (Marina) and 6.44 mg ascorbic acid 100 g^{-1} DW (Scorospelka) (table 3). Catalina, Rivan, Van, Stefan, Hedelfingen and Marina cultivars registered higher vitamin C content in fruits collected from UP. Polyphenols content varies from 30.49 (Galata from UP) to 46.96 mg GAE g^{-1} DW (Catalina from LP). For Scorospelka, Van, Bucium, and Hedelfingen polyphenols content was greater in the UP, while for cultivars Catalina', Rivan, Stefan, Galata and Marina polyphenols content was greater in the LP (table 3).

Fruit weight and fruit dimensions as width, thickness and length are very important properties of sweet cherry cultivars being the parameters; they actually confer the appropriate commercial appearance of fruits. Generally, sweet cherry cultivars with large fruits (in both dimensions and weight) are increasingly valued [28]. But these parameters are strongly influenced by climate conditions and cultivation systems. Lichev et al. (2004) [28] show that the fruit size of Van cultivar range between 6.6 to 9.2 g in three different years of harvest. Also, Kurlus (2004) [29] shows that the fruit size of six different sweet cherry cultivars range between 5.8 g to 8.1 g as average of four years. Our research results showed a great variability in the fruit size between sweet cherry cultivars but also from the two considered areas of the tree. Perez-Sanchez et al. (2010) [30] studied 20 sweet cherry cultivars from Spain and found that endocarp width range between 0.86 to 1.08 cm, the thickness endocarp between 0.63 mm and 0.82 cm. Demirsoy and Demirsoy (2004) [31] reported for local Turkish sweet cherry cultivars values of fruit weight ranging from 2.9 to 7.6 g; there were also large variations in terms of fruit thickness (15.0 to 20.3 mm) and fruit length (16.0 to 21.8 mm). Bieniek et al. (2011) [32] studied over the three years the fruit morphology of Lithuanian sweet cherry cultivars, and found that there was even a variability of investigated parameters within the three-year conducted study. As a mean of three years, the weight of fruits in the analyzed cultivars was in the range of 3.78 g to 6.45 g; fruit length was 1.66 to 2.13 g and fruit width, 1.80 g to 2.17 g. In the same study, a consistent variability has been also identified for vitamin C content; this varied in investigated cultivars from one year to other. As a mean of three years, the content of vitamin C was found in the range of 5.55 to 8.15 mg acid ascorbic 100 g^{-1} and dry matter has values from 14.44% to 17.24%. Gündoðdu and Bilge (2012) [33] found for standard Turkish sweet cherry cultivars that vitamin C content ranged between 6.01-11.44 mg/100g. Overall, the heterogeneous content of vitamin C, here recorded, has been also found for other fruit species from *Rosaceae*; for instance, in *Prunus cerasifera* biotypes, the content of vitamin C ranged between 3-7 mg % [34]. Total phenolics ranged in our study between 30.49 and 46.96 mg GAE g^{-1}. However, other studied reported even large variations of polyphenols in investigated sweet cherry cultivars, or in derived products (wines), thus with no possibility to cluster the results or to compare them [35-39]. Total flavonoids are also a variable parameter within our investigated sweet cherry cultivars (2.45-4.84 mg catechin g^{-1}). Mahmood et al. (2013) [29] found values of total flavonoids in sweet cherry fruits in various maturity stages, ranging from 36.61 to 51.80 mg catechin g^{-1}.

Conclusions

Analysis of physical parameters and biochemical properties of nine sweet cherry cultivars revealed very heterogeneous biological profiles. The position of fruits within trees architecture differently influenced fruit size and biochemical content; for some sweet cherry cultivars as Catalina, Scorospelka, Van and Bucium the fruit size and total flavonoids content were greater in the upper part of the trees; contrarily, for others cultivars as Rivan, Stefan, Galata and Hedelfingen the fruit size was greater in the lower part of the trees. Overall, no significant differences in terms of physical parameters, antioxidant indices (total flavonoids, phenolics and vitamin C) in sweet cherry fruits of cultivars were found in samples analyzed from the two parts of the trees. Despite the obtained results are heterogeneous and consistent with those derived from other studies, comparisons with other results and the attempt to group cultivars in different clusters is difficult to be done.

Abbreviations: GAE gallic acid equivalent; CE catechin equivalent; DW dry weight; UP the upper of the half part of the tree; LP the down of the half part of the tree

Acknowledgements: This study has been partially financed by the Ministry of Agriculture and Rural Development, Grant No. ADER 3.1.2. /2015, with title 'Management of in situ and ex situ fruit crop resources.

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Manuscript received: 20.11.2016