

Fruit antioxidant capacity and self-incompatibility genotype of Ukrainian sweet cherry (*Prunus avium* L.) cultivars highlight their breeding prospects

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Abstract Breeding for higher levels of health-promoting components are among the priorities for many fruit crops. Such an approach requires breeding material displaying great variations in the trait to be improved. Commercial sweet cherry cultivars demonstrate only limited variability in fruit antioxidant parameters and hence this study was carried out to characterize some Ukrainian sweet cherry cultivars for their antioxidant and antiradical capacity and total phenolic and anthocyanin contents. Ukrainian cultivars had significantly higher ferric reducing antioxidant power and total phenolic contents compared with commercial cultivars. Levels of all four parameters varied considerably in the Ukrainian germplasm, with some cultivars having outstanding values. A total of eight different self-incompatibility genotypes were assigned to the 14 accessions tested. Assigning

cultivars to the appropriate incompatibility groups allowed the design of parental combinations which may result in new genotypes with enhanced functional properties and other valuable fruit traits. Based on the *S*-genotypes, parental combinations were proposed to ensure 50 or 100 % self-compatible seedlings in the offspring population. The most perspective Ukrainian cultivars like ‘Dagestanka’ and ‘Kodrinkaya’ also had reasonable fruit weight (over 8 g), high flesh to pit ratio (approx. 18) and soluble solid contents (over 16 %). Our results demonstrated that adequate variation is available in Ukrainian germplasm for improving fruit health benefits in sweet cherry through directed hybridizations.

Keywords Antioxidant · Functional breeding program · Polyphenolics · Sweet cherry · Self-incompatibility · *S*-genotyping

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Introduction

Higher intake of plant-based foods has been associated with lower risks of several degenerative diseases including heart disease, stroke, hypertension, inflammation and others (Boeing et al. 2012; Crowe et al. 2013; Lock et al. 2005; Root et al. 2012). However, consumption of fruit and vegetables seems to be lower in many European countries than the 600 g population average consumption recommended by the World Cancer Research Fund/American Institute for Cancer

Research as a minimum (WCRF/AICR 2007). Promotional campaigns have been initiated in a range of countries to increase fruit consumption, most without major consequences (Serdula et al. 2004; Stables et al. 2002).

An alternative approach might be to provide fruits with enhanced levels of health-promoting compounds (polyphenolics, vitamins, fibre etc.), most of which have antioxidant or free radical scavenging properties (Cevallos-Casals et al. 2006; Hegedűs et al. 2008; Liu 2003; Olsson et al. 2004). Such crops might be termed as “superfruits” and offered to the consumers as a value-added product. Since the accumulation of beneficial health compounds in fruit is primarily determined by genetic factors (Cevallos-Casals et al. 2006; Hegedűs et al. 2010; Scalzo et al. 2005), breeding programs might benefit from the identification of genotypes with fruits of outstanding antioxidant properties. First, genetic diversity in fruit antioxidant activity, phenolic content and anthocyanin content was characterized among blueberry (*Vaccinium* L. sp.) species and their hybrids (Connor et al. 2002). Heritability estimates in 10 offspring from each of 20 random crosses suggested that increasing antioxidant activity in blueberry through breeding is feasible. Later, similar perspectives were described for raspberry breeding (Connor et al. 2005). Significant differences were also found among 15 peach and nectarine [*Prunus persica* (L.) Batsch] progenies in the fruit antioxidant profile, corroborated by the phenotypic variance of each phytochemical trait analyzed (Cantín et al. 2009).

Designing specific combinations in breeding programs of a self-incompatible species like sweet cherry will require knowledge on the self-incompatible (*S*-) genotype of the parental accessions. Identical *S*-genotypes will prevent cross-fertilization between any perspective parents (Schuster 2012). In sweet cherry, self- and cross-incompatibility is determined by the *S-RNase* gene expressed in the pistil and *SFB* gene responsible for the pollen function (Sonneveld et al. 2005). Both genes have many allelic variants allowing high chances of mating within a population. Until now, 19 *S*-alleles and 49 cross-incompatibility groups (CIG) have been described among cultivated sweet cherries (Schuster 2012; Szikriszt et al. 2013).

The main breeding objectives for sweet cherry include superior fruit quality (e.g. size, visual

appearance, firmness, taste), reduced juvenile phase, large and consistent yields, reduced susceptibility to fruit cracking, self-compatibility, and improved resistance or tolerance to major diseases (Dirlewanger et al. 2009). Compact tree growth and environmental adaptation are also traits of interest in many breeding programs.

Sweet cherry is indigenous to Ukraine, where naturally occurring wild forests were intensively exploited in breeding programs. Hence, Ukrainian sweet cherry cultivars represent great diversity for a range of economically important characteristics (Lukicheva 2010). Some of those cultivars are popular and widely grown in Ukraine (e.g. ‘Krupnoplodnaya’ or ‘Krymskaya noch’), while others are used in breeding programs as source genotypes for several favorable traits (Lukicheva 2010; Turovtsev 1970). For example, breeders use ‘Junsкая rannaya’ and ‘Kutuzovka’ for their high and consistent yields; ‘Dagestanka’, ‘Junsкая rannaya’, ‘Krupnoplodnaya’ and ‘Transportabelnaya’ are used as cultivars amenable to mechanical harvest because their stems detach easily from fruits without juice loss. ‘Krupnoplodnaya’, ‘Transportabelnaya’ and ‘Kutuzovka’ are characterized by firm fruit flesh. Besides the above-mentioned characters, cultivar ‘Kutuzovka’ is also used because of its good-flavored fruits and having considerable tolerance to both winter frost and fruit cracking (Lukicheva 2010). However, as far as we know, the antioxidant capacity of fruits as well as sexual incompatibility has not yet been studied in this germplasm.

Breeding for higher levels of health promoting components are among the priorities for many fruit crops including strawberry, peach, sweet cherry and apple (Byrne 2002; Capocasa et al. 2008; Davey and Keulemans 2004; Díaz-Mula et al. 2009). A first step of such a program is screening the available germplasm to find outstanding genotypes that might be used in crosses. For sweet cherries sampled to date, genetic diversity of fruit antioxidant properties seemed to be somewhat restricted (Kelebek and Selli 2011; Usenik et al. 2008). Therefore, the aim of the present study was to screen yet unsampled Ukrainian sweet cherry germplasm for fruit antioxidant parameters. Parental combinations were also proposed for such a breeding program based on the determination of self-incompatibility genotypes.

Materials and methods

Plant material

Twelve Ukrainian sweet cherry cultivars and one hybrid (2115) were used for the experiments (Table 1). Some commercial cultivars ('Alex', 'Celeste', 'Chelan', 'Ferrovia', 'Firm Red', 'Germersdorfi 1', 'Germersdorfi 3', 'Giant Red', 'Katalin', 'Linda', 'Regina', 'Santina', 'Sunburst' and 'Van') were also used in the analysis to compare their antioxidant parameters with the Ukrainian cultivars. Fruit and buds of each accession were harvested from the sweet cherry germplasm collection of Corvinus University of Budapest, Department of Genetics and Plant Breeding (Szigetcsép, Central Hungary, 47°N latitude, 18°E longitude and 95 m altitude).

Fruit quality indices

Fruit and pit weight (g) of 20 randomly selected fruits of each genotype were measured using a 0.01 g sensitive balance. Soluble solid content (SSC), expressed as percentage (%), was determined in the juice of each sample using a PAL-1 handheld digital refractometer (ATAGO Corporation, Tokyo, Japan).

Fruit extraction for antioxidant and antiradical assays

Fruits were picked at the stage of consumption maturity when fruits were fully colored and soft. In the laboratory, cherries were divided into three batches of approximately 100 g fresh weight. Fruits were halved, pitted then homogenized using a 350 W house blender (Bosch MMR0800, Stuttgart, Germany) and centrifuged (Hettich Mikro 22 R; Tuttlingen, Germany) (4 °C, 35 min, 18,750 g). The supernatants were used for the assays. Samples for further analyses were kept at −80 °C until use. For all measurements, a Nicolet Evolution 300 BB spectrophotometer (Thermo Electron Corporation, Cambridge, England) was used.

Antioxidant activity according to the ferric reducing antioxidant power (FRAP) assay

Antioxidant capacity was determined using the FRAP assay (Benzie and Strain 1996). Absorbance was measured at 593 nm at 0 and 6 min. Ascorbic acid was used as a control to obtain the standard curve and FRAP value was calculated relevant to the activity of

Table 1 Pedigree, fruit weight, flesh to pit ratio and fruit soluble solid content of Ukrainian sweet cherry cultivars analyzed

	Cultivar	Pedigree	Fruit weight (g)	Flesh to pit ratio	Soluble solid content (%)
1	Buynayskaya	–	6.28 ± 0.74	17.1 ± 1.5	17.4 ± 1.0
2	Dagestanka	Aprelka Chernaya × Drogana Zeltaya	8.33 ± 1.04	18.1 ± 2.31	16.0 ± 2.4
3	Juns kaya rannaya	–	6.81 ± 1.18	15.5 ± 3.68	16.7 ± 0.8
4	Kodrinskaya	–	8.30 ± 0.61	17.9 ± 1.86	17.3 ± 1.9
5	Krymskaya noch	Bigarreau Goshe × Chernyi orel	7.05 ± 0.66	14.4 ± 1.14	14.9 ± 1.0
6	Krupnoplodnaya	Napoleon bel. × (Valeryi Chkalov + Elton + Zhab)	8.50 ± 0.90	16.1 ± 1.74	14.8 ± 1.2
7	Kutuzovka	Bigarreau Goshe × Chernaya rannaya Naita	5.44 ± 0.60	15.3 ± 1.43	17.7 ± 1.9
8	Melitopolskaya krapchastaya	Frantsis × Vilgelmina Kleindinst	7.40 ± 0.57	19.2 ± 3.03	12.5 ± 1.6
9	Perspektivnaya	Chernaya Daibera × (Krasnaya pozdnaya biutnera + Chernyi orel)	5.99 ± 0.73	17.6 ± 2.83	17.5 ± 1.0
10	Podbelskaya	–	5.10 ± 0.68	16.4 ± 2.55	14.9 ± 1.4
11	Transportabelnaya	Frantsis × Vilgelmina Kleindinst	6.31 ± 0.84	14.4 ± 3.45	17.6 ± 0.8
12	Truzhenitsa stepi	Open pollination from Vinklera Krasnaya Pozdnaya	7.55 ± 0.64	15.4 ± 4.08	17.5 ± 0.9
13	Van	Open pollination from Empress Eugenie	6.13 ± 0.33	16.0 ± 1.3	18.0 ± 1.7
14	Hybrid 2115	–	5.93 ± 0.95	13.6 ± 3.39	16.4 ± 2.1

ascorbic acid and expressed as ascorbic acid (AA) equivalents.

2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity assay

The scavenging of the ABTS was followed by spectrophotometer at 734 nm according to Miller et al. (1993). Trolox was used as a standard and values were expressed as trolox equivalent antiradical capacity (TEAC).

Measurement of total phenolic content (TPC)

Total phenolic content (TPC) was measured using Folin-Ciocalteu's reagent according to the method of Singleton and Rossi (1965). Absorbance was monitored at 760 nm and the content of soluble phenols was calculated from a standard curve based on gallic acid concentrations.

Determination of total monomeric anthocyanins

The pH differential method was applied to quantify the total monomeric anthocyanin content (TMAC) as described by Lee et al. (2005). The anthocyanin content was expressed as mg cyanidin-3-glycoside/L ($\varepsilon = 26,900$).

DNA extraction

Genomic DNA was extracted from vegetative buds using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). DNA concentrations and purification parameters were measured using a Nanodrop ND-1000 Spectrophotometer (Bio-Science, Budapest, Hungary).

Consensus and allele-specific PCR analysis

PCR was conducted using cherry consensus primer pairs of the PaConsI-F (fluorescently labeled with 6-FAM) and PaConsI-R amplifying the first intron; and PaConsII-F and PaConsII-R for amplification of the second intron according to the protocol described for the primers (Sonneveld et al. 2003).

Approximately 20–80 ng of genomic DNA was used for PCR amplification in a 25 μ l reaction volume, containing 10 $\times$ DreamTaqTM Green buffer

(Fermentas, Szeged, Hungary) which contains KCl and $(\text{NH}_4)_2\text{SO}_4$ at a ratio optimized for robust performance of DreamTaqTM DNA Polymerase in PCR with final concentrations of 4.5 mM MgCl_2 , 0.2 mM of dNTPs, 0.2 μ M of the adequate primers and 0.75 U of DreamTaqTM DNA polymerase (Fermentas). PCR was carried out in a PTC 200 thermocycler (MJ Research, Budapest, Hungary) according to Sonneveld et al. (2003).

The second intron PCR products were separated by electrophoresis in 1.2 % TAE agarose gels for 2 h at 100 V and DNA bands were visualized by ethidium bromide staining. Fragment lengths were estimated by comparison with the 1-kb DNA ladder (Promega, Madison, USA). To determine the exact size of the *S-RNase* first intron region fragments under 500 bp, the fluorescently labeled products were run on an automated sequencer ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Budapest, Hungary). For determination of size (genotyping), GENOTYP-ER 3.7 software and the GS500 LIZ size standard (Applied Biosystems) were used.

Allele-specific PCR analysis was carried out to confirm allele assignment based on consensus PCR. The selective amplifications of the S_1 – S_7 , S_9 – S_{10} , S_{12} – S_{14} and S_{16} *S-RNase* alleles and the *phenylalanine ammonia-lyase* (PAL) gene (used as internal control) were performed according to the protocol described for the primers (Sonneveld et al. 2001, 2003). Allele-specific PCR was carried out with DreamTaqTM DNA polymerase (Fermentas) using a program for 3 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 50–63 °C (according to the protocol described for the allele-specific primers by Sonneveld et al. 2001, 2003), 1 min and 30 s at 72 °C, followed by 5 min at 72 °C.

Statistical analyses

Data presented for each cultivar represent the mean values determined from three independent homogenates. After testing for normal distribution and equality of variances, one-way analysis of variance (ANOVA) and Duncan's multiple range test with $P < 0.05$ or Welch-test was carried out to determine significant differences. Coefficients of antioxidant parameters were compared by examining Pearson correlations. Statistical analyses were carried out and boxplot diagrams were constructed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Physico-chemical parameters of Ukrainian sweet cherries

Average fruit size of the tested Ukrainian cultivars ranged from 5.1 g ('Podbelskaya') to 8.5 g ('Krupnoplodnaya') (Table 1). Fruit to pit ratio varied from 13.6 (hybrid 2115) to 19.2 'Melitopolskaya krapchastaya'. The highest soluble solid content (18.0 %) was observed in 'Van' but several Ukrainian cultivars also had similar values including 'Kutuzovka', 'Transportabelnaya', 'Truzhenitsa stepi', 'Buynayskaya' and 'Kodrinskaya'. The lowest value (12.5 %) was measured in fruits of 'Melitopolskaya krapchastaya'. The variation exhibited by the Ukrainian cultivars for these fruit quality parameters demonstrates the usefulness they could serve when considering parental combinations in a breeding program.

The antioxidant capacity and total polyphenolic contents of all tested Ukrainian cultivars were compared to those of selected commercial cultivars (specified in materials and methods). The number of cultivars in both groups were identical ($n = 13$) and data are plotted on parallel boxplots (Fig. 1). The length of the boxes indicates that both FRAP and TPC data were more variable among the Ukrainian cultivars compared to those of the commercial cultivars.

The median of the data pool was higher in the Ukrainian cultivars, both for FRAP and TPC. The distribution is reasonably symmetric for FRAP values in both groups and for TPC data in the commercial cultivars. Skewed distribution of the TPC data in the Ukrainian group indicates that several cultivars accumulate high quantities of polyphenolics compared to the median.

A Welch test indicated means of FRAP and TPC of the Ukrainian cultivars were significantly higher ($P < 0.001$) than the respective average values of the selected commercial cultivars. Interestingly, four outliers (values between one and a half and three box lengths from the upper end of the box) were registered among the FRAP values of commercial cultivars. Those values were higher compared to the rest of the data and belonged to the cultivars 'Germersdorfi 3' and 'Ferrovia'.

Ferric reducing ability in fruits of the commercial cultivars ranged between 0.84 ('Van') and 3.58 mmol AA/L ('Germersdorfi 3') (Fig. 2a). The Ukrainian sweet cherries had higher FRAP values, ranging from 0.83 ('Junskeya rannaya') to 6.80 mmol AA/L ('Kutuzovka') (Fig. 2b). 'Kodrinskaya', 'Dagestanka' and 'Melitopolskaya krapchastaya' also had high (>4.5 mmol AA/L) FRAP values. The ABTS scavenging capacity of the Ukrainian cherries ranged between 26.3 ('Truzhenitsa stepi') and 57.0 mmol trolox/L

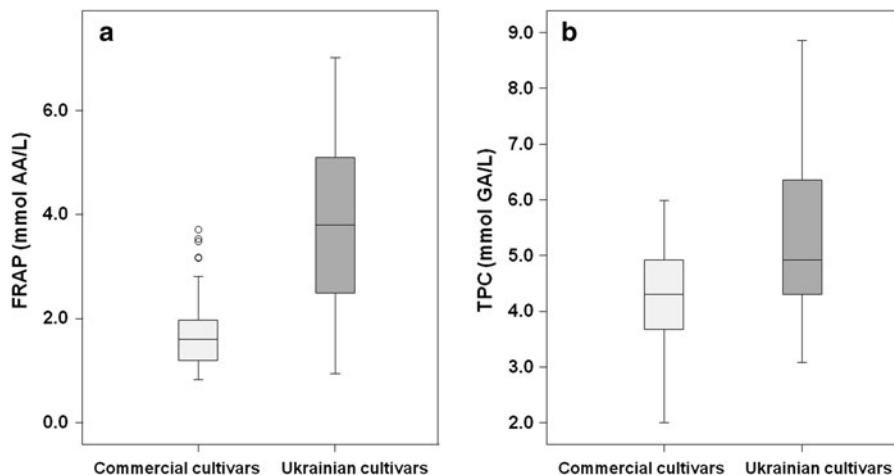


Fig. 1 A boxplot of fruit antioxidant parameters of Ukrainian and commercial sweet cherry cultivars. **a** Ferric reducing antioxidant power (FRAP); **b** total phenolic content (TPC). The dark line in the middle of the box is the median value. Bottoms and tops of boxes indicate the 25th and 75th percentiles,

respectively. Whiskers indicate lower and upper quarters; circles refer to outlier values higher than one and a half box length. Means of the two cultivar groups were statistically significant at $P < 0.001$ according to the Welsch test

(‘Melitopolskaya krapchastaya’). ‘Kodrinskaya’, ‘Dagestanka’, ‘Kutuzovka’ and ‘Perspektivnaya’ were again among the best cultivars (Fig. 3). In this analysis, there were fewer and more overlapping statistically significant groups compared to the FRAP assay. Variations of the TEAC values among cultivars (approx. twofold) were more restricted than variations in FRAP values (over eightfold). The Pearson’s correlation coefficient of FRAP and TEAC values was 0.692.

Two main groups of polyphenolic compounds were also quantified in the fruit extracts of Ukrainian sweet cherries. The lowest and highest total phenolic

contents of commercial cultivars were measured in fruits of ‘Celeste’ (2.02 mmol GA/L) and ‘Katalin’ (5.47 mmol GA/L), respectively (Fig. 4a). TPC values of the assayed Ukrainian cultivars ranged from 3.3 (‘Truzhenitsa stepi’) to 8.1 mmol GA/L (‘Kutuzovka’), presenting a 2.5-fold variation among the samples (Fig. 4b). The anthocyanin contents in fruits of the Ukrainian cultivars ranged between 2.6 (‘Junsкая rannaya’) and 27.1 mg cyanidin-3-glycoside/L (‘Kutuzovka’) (Fig. 5). The antioxidant capacity showed slightly closer correlation with total phenolic contents than anthocyanin contents of fruits

Fig. 2 Ferric reducing antioxidant power (FRAP) in fruits of (a) commercial and (b) Ukrainian sweet cherry cultivars; vertical lines represent SDs ($n = 3$) and different letters indicate significantly different values at $P < 0.05$ according to a Duncan’s multiple range test

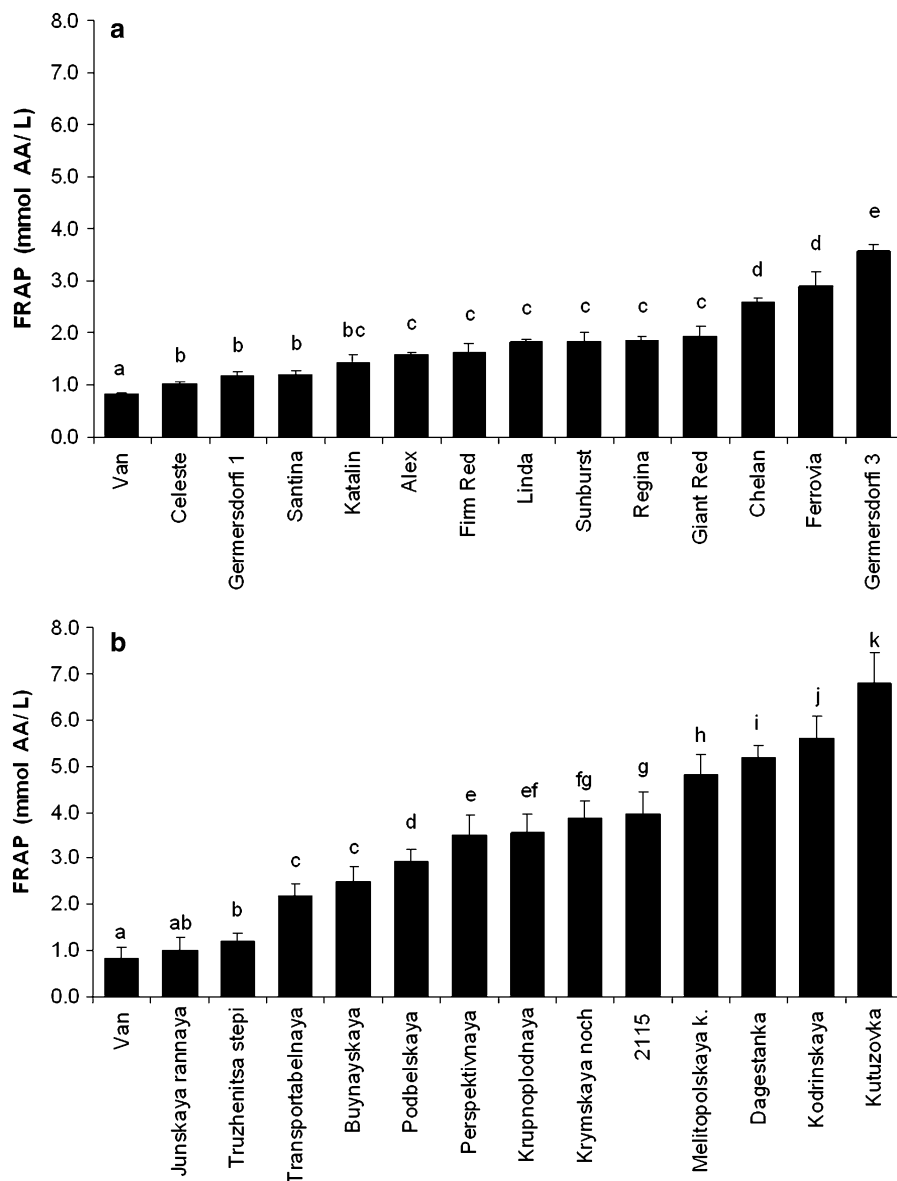
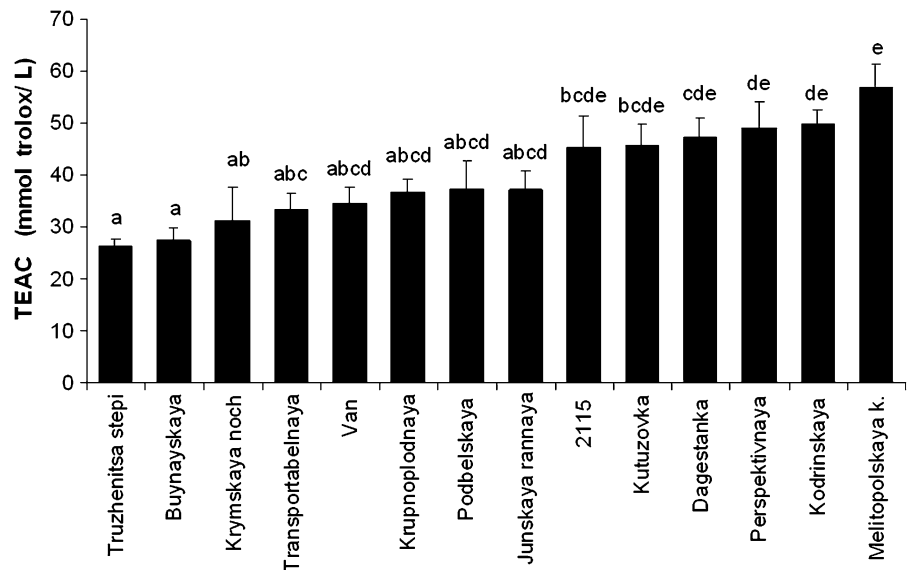


Fig. 3 Trolox equivalent antiradical capacity (TEAC) in fruits of Ukrainian sweet cherry cultivars; vertical lines represent SDs ($n = 3$) and different letters indicate significantly different values at $P < 0.05$ according to a Duncan's multiple range test



both for FRAP ($r_{\text{FRAP/TPC}} = 0.842$ and $r_{\text{FRAP/TMAC}} = 0.761$) and TEAC ($r_{\text{TEAC/TPC}} = 0.877$ and $r_{\text{TEAC/TMAC}} = 0.554$), primarily due to ‘Melitopolskaya krapchastaya’, a cultivar with high polyphenolic but low anthocyanin contents.

S-genotyping of Ukrainian sweet cherries

The *S*-genotypes were determined using consensus and allele-specific PCR. First and second intron regions of the *S-RNase* gene display an allele-specific length variation, which allows the efficient identification of specific *S*-alleles. In addition, doubtful alleles can be verified using allele-specific primers. Sizes of the fragments amplified in the second intron analysis ranged between 400 and 2,000 bp (Table 2). Only one of the two alleles was amplified in the case of ‘Buynayskaya’, ‘Junskaya rannaya’, ‘Kodrinskaya’, ‘Kutuzovka’, ‘Perspektivnaya’ and ‘Truzhenitsa stepi’. Three cultivars (‘Krupnoplodnaya’, ‘Melitopolskaya krapchastaya’ and ‘Truzhenitsa stepi’) amplified a band of approx. 2,000 bp, but the amplification was rather weak. The identity of their bands required further analysis.

To support the *S*-genotype determination, first intron sizes were also determined with fluorescently labeled primers. All cultivars amplified two different sizes of fragments that ranged between 305 and 523 bp. Some alleles show very similar sizes for both first and second introns, e.g. S_2/S_7 or S_9/S_{10} and S_{16}/S_{32} (Sonneveld et al.

2003), and hence reliable discrimination between those alleles requires allele-specific PCR.

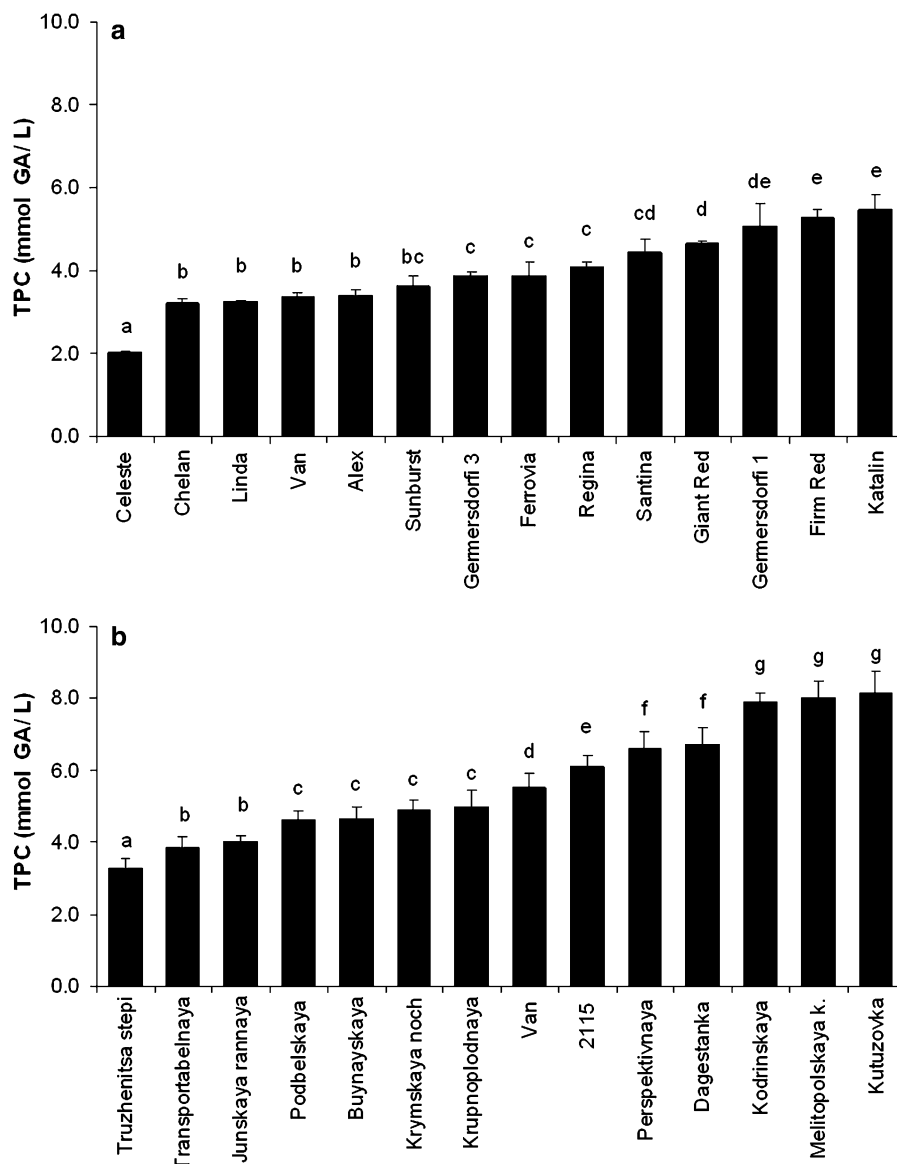
Combining the results provided by two runs of consensus PCRs and allele-specific PCR allowed the identification of the following seven *S-RNase* alleles: S_1 (in five cultivars), S_2 (three cultivars), S_3 (eight cultivars), S_4 (three cultivars), S_5 (six cultivars), S_6 (one hybrid) and S_9 (one cultivar and one hybrid). A total of eight different *S*-genotypes were assigned to the 14 accessions tested. The most frequent allelic combination was S_1S_3 (cross incompatibility group II) with four cultivars, followed by S_4S_5 (CIG V) with three cultivars and S_3S_5 (CIG VII) with two cultivars. One cultivar was added to each of the incompatibility groups I, IV, VIII, X and XVI. Cultivars from the same incompatibility group cannot be used as parents in a breeding program.

Discussion

Some Ukrainian sweet cherries are rich sources of antioxidants

Traditional breeding for the enhanced health-effects of fruit requires broad diversity in fruit antioxidant parameters among breeding materials. Screening Ukrainian sweet cherry cultivars for fruit antioxidant and antiradical capacity as well as total phenolics and total anthocyanin contents demonstrated large variations, with some accessions being characterized by

Fig. 4 Total phenolic content (TPC) in fruits of (a) commercial and (b) Ukrainian sweet cherry cultivars; vertical lines represent SDs ($n = 3$) and different letters indicate significantly different values at $P < 0.05$ according to a Duncan's multiple range test



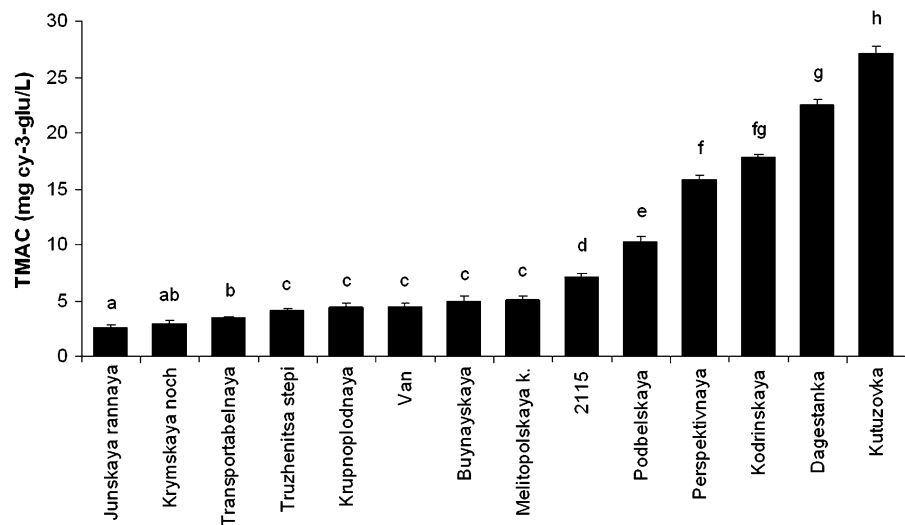
outstanding values. In FRAP values, we detected an approx. eightfold variation, which exceeded the two to fourfold variation obtained for commercial cultivars in this study and others published previously (Kelebek and Selli 2011; Usenik et al. 2008). The range of variations was indeed more restricted in the group of commercial cultivars (Fig. 1). The highest FRAP value of 'Germersdorfi 3' was an outlier in the commercial cultivar group and was nearly identical with the median of the Ukrainian group.

Combining results from each of the four assays, 'Kutuzovka', 'Kodrinskaya', 'Dagestanka', 'Melitopolskaya krapchastaya' and 'Perspektivnaya' can all be

accepted as perspective genotypes in functional breeding programs to enhance fruit antioxidant properties. This approach is gaining in popularity among breeding objectives of several fruit crops, including both berries and stone fruits (Capocasa et al. 2008; Connor et al. 2002; Díaz-Mula et al. 2009).

Most of the high antioxidant capacity fruits were also rich sources of anthocyanins in both flesh and skin. For 'Melitopolskaya krapchastaya', colorless polyphenolics may have contributed sizably to the antioxidant and especially the antiradical potential similarly to other sweet and sour cherry cultivars (Papp et al. 2010; Piccolella et al. 2008; Usenik et al. 2008).

Fig. 5 Total monomeric anthocyanin content (TMAC) in fruits of Ukrainian sweet cherry cultivars; vertical lines represent SDs ($n = 3$) and different letters indicate significantly different values at $P < 0.05$ according to a Duncan's multiple range test



This explains why antioxidant capacity was more closely correlated with total phenolic rather than anthocyanin contents of fruits. In conclusion, anthocyanins contribute a lot to the antioxidant capacity of dark colored fruits but some cultivars with lightly colored fruit may also have high antioxidant capacity, attributable to other polyphenolic compounds, as described similarly for sour cherry (Papp et al. 2010).

Most Ukrainian cultivars had fruit weights higher than cultivar 'Van'. Fruit weight of 'Van' was very similar to the value (6.62 g) determined by Vursavus et al. (2006). Although rootstock and crop load may also influence fruit weight (Goncalves et al. 2004), similar values from the two studies indicate that comparison of the data is feasible. Cultivars with the highest fruit weight ('Krupnoplodnaya' and 'Dagestanka') showed similar values like the top Turkish cultivar, '0-900 Ziraat' (Vursavus et al. 2006) but were smaller than 'Lapins' (Usenik et al. 2008) or 'Sunburst' (Radicevic et al. 2008). Since early ripening cultivars tend to produce fruit characterized by low weight, 'Junsкая rannaya' seems to be a perspective genotype by yielding quite big fruits in the first days of June.

Flesh to pit ratio was highest for 'Melitopolskaya krapchastaya' being slightly lower than that of '0-900 Ziraat' in a previous study (Vursavus et al. 2006). However, cultivars 'Dagestanka', 'Kodrinskaya' and 'Perspektivnaya' also had higher flesh/pit ratios than 'Van'. Soluble solid content of mature sweet cherry was reported to be 14–16 % depending on cultivar (Kader 1999). Except for 'Melitopolskaya

krapchastaya' all cultivars had SSC values within this range or higher. In this study, the highest values approached 18 %, as similarly described for 'Vega' (Radicevic et al. 2008). It was shown that a minimum SSC of 16.0 % satisfies the majority of consumers (Crisosto et al. 2003), supporting the breeding perspectives for such Ukrainian cultivars.

Parental combinations based on self-incompatibility genotypes

Antioxidant characterization of fruits indicated that several Ukrainian cultivars (e.g. 'Kutuzovka', 'Kodrinskaya', 'Dagestanka' and 'Melitopolskaya krapchastaya') might be perspective donor genotypes in a functional cherry breeding program. Parental self-incompatibility genotypes determine successful breeding combinations, and hence, the *S*-genotypes of the assayed cultivars had to be assigned.

The demand for sweet cherry is optimum when 10 g average fruit weight can be achieved (Horvitz et al. 2003); therefore, large fruited cultivars may be considered as perspective parents. 'Sunburst', 'Lapins' and 'Carmen' might be good choices as having average fruit weight over 10 g (Radicevic et al. 2008; Usenik et al. 2008). 'Carmen' is S_4S_5 , and hence, is cross-incompatible with 'Kodrinskaya', but compatible with three of the other Ukrainian cultivars with high antioxidant capacity fruits. 'Sunburst' and 'Lapins' are self-compatible cultivars (Sonneveld et al. 2001) and can be used with any of the Ukrainian cultivars. Self-compatibility is also among the most

Table 2 Contribution of several *S*-allele typing strategies for the determination of *S*-genotypes of Ukrainian sweet cherry cultivars

Cultivar	Fragment length (bp)		<i>S</i> -allele	Allele-specific PCR							<i>S</i> -genotype	Incompatibility group
	1st intron PCR	2nd intron PCR		<i>S</i> ₁ 820 bp	<i>S</i> ₂ 640 bp	<i>S</i> ₃ 960 bp	<i>S</i> ₄ 820 bp	<i>S</i> ₅ 300 bp	<i>S</i> ₆ 470 bp	<i>S</i> ₇ 584 bp	<i>S</i> ₉ 495 bp	
1 Buynayskaya	<u>305</u> 419	680 –	<i>S</i> ₃ <i>S</i> ₂		<i>S</i> ₂	<i>S</i> ₃				na	<i>S</i> ₂ <i>S</i> ₃	IV
2 Dagestanka	310 440	680 550	<i>S</i> ₃ <i>S</i> ₉			<i>S</i> ₃					<i>S</i> ₉	XVI
3 Junszkaya ramnaya	<u>462</u> <u>523</u>	– 850	<i>S</i> ₅ <i>S</i> ₄				<i>S</i> ₄	<i>S</i> ₅				V
4 Kodrinskaya	440 500	– 850	<i>S</i> ₅ <i>S</i> ₄				<i>S</i> ₄	<i>S</i> ₅			na	V
5 Krymskaya noch	310 440	680 600	<i>S</i> ₃ <i>S</i> ₁	<i>S</i> ₁		<i>S</i> ₃					na	II
6 Krupnoplodnaya	<u>462</u> <u>523</u>	2,000 850	<i>S</i> ₅ <i>S</i> ₄				<i>S</i> ₄	<i>S</i> ₅			na	V
7 Kutuzovka	310 440	680 –	<i>S</i> ₃ <i>S</i> ₅	na		<i>S</i> ₃		<i>S</i> ₅		na	<i>S</i> ₃ <i>S</i> ₅	VII
8 Melitopolskaya krapch.	310 450	680 2,000	<i>S</i> ₃ <i>S</i> ₅	na		<i>S</i> ₃		<i>S</i> ₅			<i>S</i> ₃ <i>S</i> ₅	VII
9 Perspektivnaya	<u>420</u> <u>455</u>	– 600	<i>S</i> ₂ <i>S</i> ₁	<i>S</i> ₁	<i>S</i> ₂					na	na	I
10 Podbelskaya	<u>305</u> <u>455</u>	680 600	<i>S</i> ₃ <i>S</i> ₁	<i>S</i> ₁		<i>S</i> ₃						II
11 Transportabelnaya	<u>305</u> <u>455</u>	680 600	<i>S</i> ₃ <i>S</i> ₁	<i>S</i> ₁		<i>S</i> ₃	na				<i>S</i> ₇ <i>S</i> ₃	II
12 Truzhenitsa stepi	420 <u>462</u>	– 2,000	<i>S</i> ₂ <i>S</i> ₅		<i>S</i> ₂			<i>S</i> ₅		na	<i>S</i> ₂ <i>S</i> ₅	VIII
13 Van	310 450	680 600	<i>S</i> ₃ <i>S</i> ₁	<i>S</i> ₁		<i>S</i> ₃					<i>S</i> ₇ <i>S</i> ₃	II
14 Hybrid 2115	410 500	550 400	<i>S</i> ₉ <i>S</i> ₆		na	na	na		<i>S</i> ₆		<i>S</i> ₉	X

Underlined numbers indicate sizes obtained on the automated sequencer

na no amplification

important tree characteristics in cultivation and selection for this trait can be performed using molecular markers (Sonneveld et al. 2005; Zhu et al. 2004).

To maximize the ratio of self-compatible seedlings, cultivars ‘Kutuzovka’, ‘Dagestanka’ and ‘Melitopolskaya krapchastaya’, sharing the S_3 -allele, should be crossed with ‘Sunburst’ (S_3S_4) as a pollen parent. In this case, pollen carrying the S_3 -SFB will be recognized and rejected, and 100 % of the offspring population will be SC. Since ‘Kutuzovka’ is characterized by relatively small fruits, it should be crossed with ‘Sunburst’ or other large fruited cultivars. In contrast, a 50 % ratio of SC seedlings can only be achieved by crossing ‘Sunburst’ (S_3S_4) and ‘Kodrinskaya’ (S_4S_5). Díaz-Mula et al. (2009) found ‘Santina’ had the highest total phenolics content among 11 sweet cherry cultivars. The SC cultivar, ‘Santina’, has the S -genotype of S_1S_4 (Wünsch and Hormaz 2004). Among the Ukrainian cultivars, ‘Perspektivnaya’ showed high total phenolic content and radical scavenging capacity. Crossing ‘Perspektivnaya’ (S_1S_2) as a seed parent with ‘Santina’, will result only in SC seedlings. ‘Dagestanka’ cannot be crossed with ‘Burlat’, ‘Chelan’ and other cultivars of the XVI cross-incompatibility group; while ‘Kutuzovka’ and ‘Melitopolskaya krapchastaya’ sharing the S_3S_5 genotype cannot be crossed with each other and ‘Hedelfingen’, ‘Tünde’ or other perspective cultivars from the CIG VII.

The physico-chemical parameters of fruits with high antioxidant capacity indicate ‘Dagestanka’ and ‘Kodrinskaya’ also have relatively large fruit, an advantageous flesh/pit ratio and acceptable soluble solid content. In addition, ‘Kutuzovka’ had high antioxidant and anthocyanin contents coupled with favorable tree and fruit characteristics (Lukicheva 2010). All data confirm that Ukrainian cultivars display great genetic diversity and some of them should be included as effective parents in functional breeding programs. In general, these cultivars have much higher contents of bioactive compounds and display favorable fruit characters similar to or better than the widely grown commercial cultivars.

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