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RESEARCH PAPER

Diversity of Nuclear DNA Content of Stone Fruits and Their Interspecific Hybrids

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Abstract

The information about genome size is implied in the studies of genetic diversity, the genome size evolution and can be used in breeding of new interesting cultivars. A total of 52 apricot cultivars, including 17 new perspective apricot hybrids planted in South Moravia region were analysed by flow cytometry. In addition, nuclear DNA content and ploidy level of 10 representative cultivars *P. salicina, P. domestica, P. cerasifera* and 22 interspecific hybrids of stone fruit were determined. Only the CV values lower than 5% were considered in analysis. All analysed apricot cultivars were diploid with average value of relative genome size 0.617 ± 0.014 pg/2C. The ploidy and DNA content of plums differ in botanical species. The obtained results were compared according to the geographical origin of cultivars and the theory about genome size evolution was considered. The values of relative genome size of original botanical species related to *P. armeniaca* (*P. mume, P. sibirica, P. ansu*) reached the lowest value in average compared with other cultivars in this study (0.603 pg/2C in average). Two pentaploids and one tetraploid were found in interspecific hybrids, which could be a part of interest of future studies.

INTRODUCTION

Stone fruit belong to the genus *Prunus* in the Rosaceae family and include peach, plums, apricot, cherries and almonds. Apricots and plums grown in temperate regions between 60°C and 35°C north latitudes, as well as south latitudes, are of high importance (Ebert, 2009). Nowadays, many studies are focused on breeding of new stone fruit cultivars for improving the quality, good appearance, storage, yield or nutritional substances of fruit. A great goal is also increasing the resistance to abiotic and biotic stresses, including the resistance to many types of diseases (Szabó, *et al.*, 2010), what can be reached by higher ploidy production (Shi *et al.,* 2015). New species and cultivars and their taxonomic relationships can be assessed upon the ploidy and relative genome size utilization (Šmarda *et al.*, 2006; Olšavská *et al.*, 2012). Previous studies described differences in genome size of cultivars in relation to the climatic conditions, also the correlation of the genome size and altitude, geographical situation, temperature etc. were found (Kolano *et al.*, 2012). Nevertheless, the trend differs for different plant species (Li *et al.*, 2020). Apricots (*Prunus armeniaca*) are diploid with the average genome size of 0.6 pg/2C, what corresponds to 293.4 Mbp (Arumuganathan and Earle, 1991; Li *et al.*, 2020). Plums differs in ploidy, when *P. cerasifera* and *P. salicina* are diploid and *P. domestica* is hexaploid. Exceptionally, the formation of natural polyploidy of the genus *Prunus* may occur (Ramsey and Schemske, 1998).

MATERIALS and METHODS

35 apricot cultivars, 17 apricot hybrids, 3 representatives of *P. cerasifera*, 3 representatives of *P. salicina* and 6 representatives of *P. domestica*, 22 interspecific hybrids of stone fruit (Table 1), three botanical species related to *Prunus armeniaca, P. mume* (the Japanese apricot), *P. ansu* (the Korean apricot), *P. sibirica* (the Siberian apricot) growing in the experimental orchard of the Faculty of Horticulture in Lednice Mendel University in Brno were analysed in the study. The apricot cultivars were divided into five different geographical groups based on the geographical origin: American, Central Asian, East Asian, European and Turkish.

For analysis only intact, young but fully developed leaves of selected cultivars were collected and analysed immediately. All cultivars were analysed in three samples of leaves from different tree. The kit 05-5022 CyStain PI Absolute P® was used for the analysis. According to the protocol of the producer, approximately 0.5 cm^2 of the leaf tissue of a sample and approximately 0.5 cm^2 of the leaf tissue of *Raphanus sativus* cv. Saxa (2C = 1.11 pg DNA) (Doležel *et al*., 1992), which was selected as internal reference standard, were homogenized together in 0.5 mL of Nuclei Extraction Buffer (pH = 1.8) using razor blade. After 60 s, the prepared sample was filtered through a 50 µm membrane (Partec CellTrics®) and supplemented with 2 mL of the solution of Staining Buffer (pH = 7.5), which contained 12 µL of propidium iodide (PI) and 6 µL RNase. The samples were analysed after 30 min of incubation in the dark at room temperature. The samples were analysed in CyFlow Space-3 equipped with a 532 nm laser. The speed of flow was held up to 50 particles per second and at least 5,000 particles were analysed of each sample. The coefficient of variation (CV) values lower than 5% was considered as reliable data. The ploidy was estimated using the same species with known ploidy level as reference and calculated according formula: sample ploidy level = peak position sample/peak position reference × ploidy level reference. 2C nuclear DNA content was calculated according to following formula: 2C (pg) = DNA content of standard × (mean fluorescence value of sample/mean fluorescence value of standard) (Doležel *et al*., 2007). The mean nuclear DNA content was calculated for each cultivar from three replications. The results were also expressed as monoploid genome sizes (1Cx) (i.e., DNA content of the non-replicated base set of chromosomes) according to following formula: 1Cx = 2C genome size/ploidy level × 0.978 × 10^9 bp (Doležel *et al*., 2005). Data were averaged and standard deviation was expressed using STATISTICA 12.

RESULTS and DISCUSSION

All apricot cultivars were diploids in this study and the average relative genome size was 0.617 ± 0.014 pg/2C (301.5 ± 6.9 Mbp). The values ranged from 0.587 pg/2C (Orangered) to 0.644 pg/2C (Bohuticka). The average values for

Table 1. Origin of the analysed interspecific hybrids.

Hybrid	Origin	Hybrid	Origin
LEMY 2017/1	*P. armeniaca* × *P. cerasifera*	Hybrid M 47	*P. armeniaca* × *P. cerasifera*
LEMY 2017/2	*P. armeniaca* × *P. salicina*	Hybrid M 49	Unknown
LEMY 2017/3	*P. armeniaca* × *P. salicina*	Hybrid M 51	Unknown
LEMY 2017/4	Unknown	Hybrid M 56	*P. armeniaca* × *P. cerasifera*
LEMY 2017/5	*P. armeniaca* × *P. cerasifera*	Hybrid M 57	Unknown
LEMY 2017/6	*P. armeniaca* × *P. salicina*	Hybrid M 59	Unknown
LEMY 2017/7	*P. armeniaca* × *P. cerasifera*	Hybrid M 61	Unknown
LEMY 2017/8	*P. armeniaca* × *P. cerasifera*	Flower Queen pluot®	*P. salicina* × *P. armeniaca*
LEMY 2017/9	*P. armeniaca* × *P. cerasifera*	Flower Supreme pluot®	*P. salicina* × *P. armeniaca*
LEMY 2017/10	*P. armeniaca* × *P. cerasifera*	Potesestvenica	*P. salicina* × *P. cerasifera*
LEMY 2017/11	*P. armeniaca* × *P. cerasifera*	Kometa	*P. salicina* × *P. cerasifera*
		Santa Rosa	*P. salicina* × *P. simonii* × *P. americana*

all apricot cultivars with standard deviations are in Table 2. The average values of geographical groups raise in order from American (0.607 pg/2C), Central Asian (0.610 pg/2C), East Asian (617 pg/2C), European (0.621 pg/2C) to Turkish (0.628 pg/2C). The original botanical species

Table 2. Relative genome size of apricot hybrids and cultivars with different geographical origin (1-Turkish, 2-European, 3-East Asian, 4-Central Asian, 5-American, H-Hybrid).

Cultivar	Group of cultivar	Average 2C (pg DNA)	Average 1Cx (Mbp)
Bohuticka	2	0.644 ± 0.002	314.8 ± 1.1
Marena	4	0.642 ± 0.001	313.9 ± 0.6
Hacihaliloglu	1	0.640 ± 0.011	312.8 ± 5.6
Karola	2	0.637 ± 0.001	311.4 ± 0.4
Sekerpare	1	0.636 ± 0.0005	311.0 ± 0.2
LEM 2017/8	H	0.635 ± 0.000	310.5 ± 0.2
LEM-121	H	0.633 ± 0.001	309.4 ± 0.5
Stark Early Orange	5	0.632 ± 0.001	309.0 ± 0.6
Velkopavlovicka	2	0.631 ± 0.001	308.3 ± 0.6
Hasanbey	1	0.630 ± 0.001	308.1 ± 0.4
LE-3246	H	0.629 ± 0.001	307.4 ± 0.4
LEM-122	H	0.627 ± 0.002	306.7 ± 1.2
Stella	5	0.626 ± 0.001	306.3 ± 0.5
LEM-151	H	0.626 ± 0.001	306.3 ± 0.4
LEM-123	H	0.625 ± 0.002	305.8 ± 1.0
LEM 2017/25	H	0.624 ± 0.004	305.2 ± 1.9
Liaoning	3	0.623 ± 0.001	304.9 ± 0.4
Ananasova	2	0.623 ± 0.004	304.7 ± 1.9
Chuangxing	3	0.623 ± 0.004	304.6 ± 2.1
P. sibirica		0.621 ± 0.001	303.5 ± 0.4
LEM-120	H	0.620 ± 0.001	303.4 ± 0.4
Pozde kvetouci	2	0.618 ± 0.001	302.4 ± 0.5
Salak	1	0.618 ± 0.006	302.4 ± 2.8
Lasgerdi Mashhad	4	0.618 ± 0.002	302.4 ± 1.0
LEM-106	H	0.618 ± 0.0004	302.4 ± 0.2
LEM-139	H	0.616 ± 0.002	301.4 ± 1.0
Kabaasi	1	0.615 ± 0.002	300.8 ± 0.8
Achrori	4	0.615 ± 0.005	300.8 ± 2.4
Pastyrik	2	0.615 ± 0.001	300.7 ± 0.5
LEM 2017/12	H	0.614 ± 0.0002	300.3 ± 0.1
Wondercot	2	0.614 ± 0.001	300.0 ± 0.3
LEM 2017/24	H	0.613 ± 0.003	299.9 ± 1.5
Gvardejskij	4	0.613 ± 0.001	299.6 ± 0.4
Chuang Zhi Hong	3	0.612 ± 0.001	299.1 ± 0.6
Scout	5	0.611 ± 0.001	298.6 ± 0.7
Pozdni chramova	2	0.611 ± 0.005	298.6 ± 2.4
Early Blush	5	0.610 ± 0.001	298.5 ± 0.5
Roxana	4	0.610 ± 0.001	298.3 ± 0.3
H 994	H	0.608 ± 0.002	297.3 ± 1.2
Harcot	5	0.608 ± 0.001	297.3 ± 0.7
P. ansu		0.608 ± 0.001	297.2 ± 0.5
Saimaiti	3	0.607 ± 0.0001	296.6 ± 0.0
LEM 2017/26	H	0.606 ± 0.002	296.4 ± 0.9
Goldrich	5	0.605 ± 0.002	296.0 ± 1.0
LEM 2017/20	H	0.604 ± 0.003	295.5 ± 1.3
LEM 2017/18	H	0.598 ± 0.001	292.5 ± 0.6
Harlayne	5	0.596 ± 0.002	291.4 ± 0.9
Inbeixing	3	0.596 ± 0.001	291.4 ± 0.7
H 1077	H	0.593 ± 0.006	290.0 ± 2.8
Kechpsar	4	0.593 ± 0.002	289.9 ± 0.9
Orangered	5	0.587 ± 0.002	286.9 ± 1.1
P. mume		0.581 ± 0.002	284.2 ± 1.1

related to *P. armeniaca* (*P. mume, P. sibirica, P. ansu*) reached the lowest values in average compared with other cultivars in this study (0.603 pg/2C in average). The lowest 2C-value was determined for *P. mume* (0.581 pg/2C). The relative genome size of apricot hybrids ranged from 0.593 to 0.635 pg/2C with average value 0.617 ± 0.012 pg/2C which was the same value as total average 2C-value of all apricot cultivars.

The average relative genome size of plums was higher in average than for apricots. *P. cerasifera* and *P. salicina* were diploid and the values of relative genome size was 0.624 pg/2C and 0.642 pg/2C, respectively. All analysed cultivars of *P. domestica* were hexaploids with average relative genome size 2.046 pg/2C. The values can be compared with determined values of relative genome size of interspecific hybrids (Table 3). The interesting results were found for hybrids 'M 61' (Figure 1a) and 'M 59' (Figure 1b) which were pentaploids and 2C-value was 1.677 pg/2C and 1.604 pg/2C, respectively. Hybrid 'M 57' (Figure 1c) was tetraploid (1.304 pg/2C).

Other interspecific hybrids were diploids in this study and the relative genome size ranged from 0.607 to 0.733 pg/2C. The average value of *P. armeniaca* × *P. salicina* was 0.624 pg/2C, where lowest value was determined for 'LEMY 2017/2' (0.588 pg/2C) and the highest 2C-value was determined for hybrid 'Flower Queen pluot®' (0.652 pg/2C). The average 2C-value of *P. armeniaca* × *P. cerasifera* was 0.615 pg/2C. The lowest value was determined for hybrid 'LEMY 2017/10' (0.584 pg/2C) and the highest value was determined for hybrid 'LEMY 2017/8' (0.641 pg/2C).

The origin of interspecific hybrids 'M 61', 'M 59' and 'M 57' can be determined by the higher ploidy, which could be caused by crossing the diploid *P. armeniaca* or *P. cerasifera* or *P. salicina* with hexaploid *P. domestica*. In addition, the shape of the leaves can be compared (Figure 1). The leaves of hybrids 'M 61' and 'M 59' are similar as leaves of *P. domestica*. In addition, the hybrid 'M 59' has black felt fruit similarly as apricot. Thus, the origin of the hybrid 'M 59' may

Table 3. Ploidy level and relative genome size of interspecific hybrids.

Cultivar	Origin of hybrid	Ploidy Level	Average 2C (pg DNA)	Average 1Cx (Mbp)
Hybrid M 61	Unknown	5×	1.677 ± 0.001	327.9 ± 0.2
Hybrid M 59	Unknown	5×	1.604 ± 0.022	313.8 ± 4.3
Hybrid M 57	Unknown	4×	1.304 ± 0.001	318.7 ± 0.1
Hybrid M 49	Unknown	2×	0.733 ± 0.0003	358.2 ± 0.1
Flower queen pluot®	*P. salicina* × *P. armeniaca*	2×	0.652 ± 0.002	318.7 ± 1.0
LEMY 2017/6	*P. armeniaca* × *P. salicina*	2×	0.643 ± 0.015	314.3 ± 7.4
LEMY 2017/8	*P. armeniaca* × *P. cerasifera*	2×	0.641 ± 0.004	313.3 ± 2.1
LEMY 2017/4	Unknown	2×	0.638 ± 0.003	311.7 ± 1.2
LEMY 2017/1	*P. armeniaca* × *P. cerasifera*	2×	0.636 ± 0.004	311.0 ± 2.0
Flower supreme pluot®	*P. salicina* × *P. armeniaca*	2×	0.632 ± 0.003	309.0 ± 1.4
Hybrid M 56	*P. armeniaca* × *P. cerasifera*	2×	0.631 ± 0.001	308.5 ± 0.5
Hybrid M 51	Unknown	2×	0.625 ± 0.003	305.6 ± 1.2
Potesetvenica	*P. salicina* × *P. cerasifera*	2×	0.624 ± 0.002	305.1 ± 1.1
LEMY 2017/9	*P. armeniaca* × *P. cerasifera*	2×	0.623 ± 0.003	304.5 ± 1.3
Santa Rosa	*P. salicina* × *P. simonii* × *P. americana*	2×	0.619 ± 0.004	302.9 ± 1.8
Hybrid M 47	*P. armeniaca* × *P. cerasifera*	2×	0.611 ± 0.003	298.8 ± 1.4
Kometa	*P. salicina* × *P. cerasifera*	2×	0.607 ± 0.010	296.8 ± 4.8
LEMY 2017/3	*P. armeniaca* × *P. salicina*	2×	0.605 ± 0.001	296.1 ± 0.7
LEMY 2017/7	*P. armeniaca* × *P. cerasifera*	2×	0.605 ± 0.004	295.9 ± 2.1
LEMY 2017/11	*P. armeniaca* × *P. cerasifera*	2×	0.604 ± 0.002	295.2 ± 1.1
LEMY 2017/5	*P. armeniaca* × *P. cerasifera*	2×	0.601 ± 0.001	293.8 ± 0.3
LEMY 2017/2	*P. armeniaca* × *P. salicina*	2×	0.588 ± 0.018	287.5 ± 8.8
LEMY 2017/10	*P. armeniaca* × *P. cerasifera*	2×	0.584 ± 0.004	285.6 ± 1.9

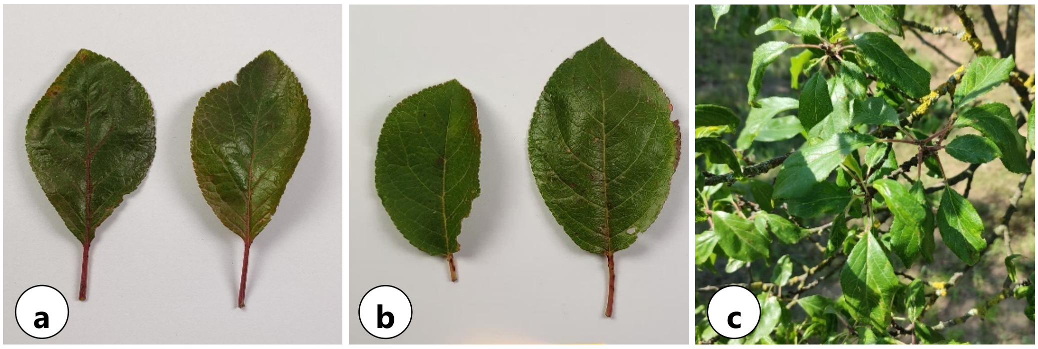

Figure 1. Leaves of polyploid interspecific hybrids with unknown origin. **a)** Hybrid 'M 61', **b)** Hybrid 'M 59', **c)** Hybrid 'M 57'.

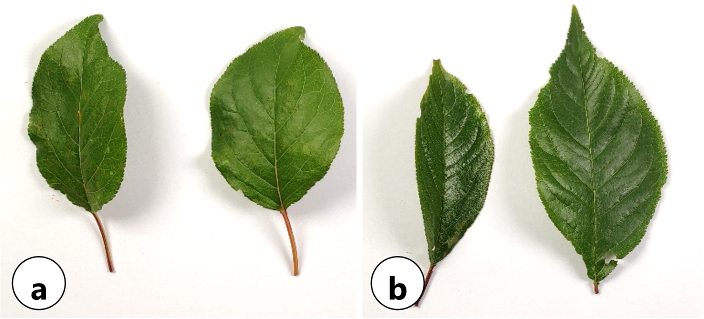

Figure 2. Leaves of diploid interspecific hybrids **a)** Hybrid 'M 47' (*P. armeniaca* × *P. cerasifera*), **b)** 'LEMY 2017/2' (*P. armeniaca* × *P. salicina*).

be *P. domestica* × *P. armeniaca*. The leaves of hybrid 'M 57' are similar to leaves of *P. salicina*. Thus, the hybrid may be originated from *P. domestica* × *P. salicina* crossing. The results need to be confirmed with other methods.

Similarly, the determination of origin of diploid interspecific hybrid needs the using of genetical analysis, because there was no statistically significant difference of the relative genome size values. The examples of the leaves of interspecific hybrids *P. armeniaca* × *P. cerasifera* and *P. armeniaca* × *P. salicina* are on the Figure 2. The hybrid 'M 47' (Figure 2a) has palmate and tender leaves similarly as *P. armeniaca*. Conversely, the leaves of 'LEMY 2017/2' (Figure 2b) are club-shaped and waxy.

The information about the genomes of specific cultivars of stone fruit are limited so far. In previous study of Arumuganathan and Earle (1991) the relative genome size of apricot was 0.61 pg/2C. Li *et al*. (2020) determined the range of genome size of apricot 0.597–0.673 pg/2C with average value 0.637 pg/2C. In our study the range was 0.587 to 0.644 pg/2C with average value 0.617 of pg/2C and similar to Li *et al.* (2020); all apricot cultivars were diploid in this study.

According to Arumuganathan and Earle (1991) 2C value for *P. domestica* was 1.85 pg. However, Žabka *et al*. (2018) determined higher value as 2.10 pg and in this study it is estimated as 2.046 pg. Tamarzizt *et al.* (2015) determined the range of the relative genome size of Tunisian *P. salicina* 0.457 to 0.971 pg/2C. In our study we analysed relative genome size of three cultivars of *P. salicina* (Ozark Premier, Black Amber, Crimson Glo) and the average value was 0.642 pg/2C. The differences may be caused by different cultivar analysing.

The higher ploidy was determined for all cultivars of *P. domestica* and three interspecific hybrids. In our study hybrids 'M 61' (unknown) a 'M 59' (unknown origin) were pentaploids and 'M 57' (unknown origin) was tetraploid. The formation of a natural polyploidy of the genus *Prunus* is the result of chromosomal pairing and fusion of unreduced gametes (Ramsey and Schemske, 1998). Within *Prunus*, pentaploids are known in case of interspecific hybrids such as 'Herkules', hybrid of hexaploid *P. domestica*

'Ontario' and diploid *P. salicina* 'Formosa'. This hybrid was created by pollination with unreduced pollen (2n) of the variety 'Formosa' (Neumüller, 2011; Guerra and Rodrigo, 2017; Głowacka *et al.*, 2021). Further, it is known as pentaploid hybrid 'Damas GF 1869' (*P. domestica* × *P. spinosa*) (Filiti *et al.*, 1987; Reig *et al.*, 2019) and according to Žabka *et al.* (2018), *P.* × *fechtneri* was estimated as pentaploid too. Well known tetraploids are *P. spinosa* and *P. dasyphylla* (Žabka *et al.*, 2018) and many types of tetraploids ($2n = 4x = 32$) are known among the genera *P. spinosa* and hybrids *P. cerasifera* with some of the Eurasian plum species (Zhebentyayeva *et al.*, 2019). The information of genome size estimation obtained about interspecific hybrids can be used to determine their origin. According to Žabka *et al.* (2018), the 2C value for *P. spinosa* was 1.40 pg and similar value was determined for hybrid 'M 57' (1.304 pg). Thus, *P. spinosa* can be the parent of the hybrid. For the purpose of the origin, the shape of the leaves was also studied. The leaves of hybrids 'M 61' and 'M 59' have similar shape as *P. domestica* and hybrid 'M 57' has similar leaf shapes as *P. salicina*. Thus, the hybrids may be originated from crossing *P. domestica* with diploid *Prunus*. The theory of the origin of interspecific hybrids need to be proved by further insight. So future researches are needed for this topic.

CONCLUSION

In our study, a total of 52 apricots cultivars, including 17 new perspective apricot hybrids planted in South Moravia region were analysed by flow cytometry. In addition, ploidy level and nuclear DNA content of 10 representative cultivars *P. salicina, P. domestica, P. cerasifera* and 22 interspecific hybrids of stone fruit were determined. Ploidy and relative genome size was measurable information used in breeding, because of its utilization in studies of evolution relationship between plant species and their variety. Comparing the nuclear DNA content of different apricot cultivars, there is a difference between different geographical groups. When the highest 2C value was found for Turkish apricots and the lowest value was for American apricots. The difference between the highest value (Bohuticka) and the lowest value (Orangered) was 0.057 pg. Some polyploids were found within the interspecific hybrids. This study can be useful in future research of genetic diversity of apricots and also some interspecific hybrids. Further study is needed for the clear classification within the taxonomic ranks.

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ADDITIONAL INFORMATION

This research was presented at the 1st International Symposium of Biodiversity Studies and was published in the abstract e-book in the proceedings of the Symposium.

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