Pawpaw Hybrid Genotypes (*Asimina triloba* (L.) Dunal) Cultivated in the Bucharest Area

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Abstract

Pawpaw or Northern banana (*Asimina triloba* (L.) Dunal) is the only representative of the *Annonaceae* family that can be cultivated in temperate areas. The increasing demands from the consumers for this nearly unknown fruit with high nutraceutical potential require specific researches. The aim of this study is to present the fruits pomological characteristics for 23 genotypes analyzed, cultivated in the Bucharest area. For each genotype yield, fruit size, average number of seeds per fruit, average weight of seeds and seeds size were measured. Fruit biochemical analyses as dry matter, total soluble solids, ascorbic acid content, acidity, were done for several genotypes. Fruit quality evaluation through sensorial analyses was conducted for 13 genotypes. The most valuable genotypes from the Experimental field according to the analyses performed and the consumer's preferences are taken in consideration for further research.

Keywords: acidity, ascorbic acid, dry matter, Northern banana, total soluble solids

Introduction

Pawpaw, Northern banana or asimina (*Asimina triloba* (L.) Dunal), *Annonaceae* family, has the origin in North America, having the largest edible fruit from the native fruit trees in this continent (Kral, 1960; Willson and Schemske, 1980; Layne, 1996; Pomper *et al.*, 2003). It has a well-proportioned habitus, 3-7 m height, abundant foliage with shiny dark green colour. The fruit is a berry of different shape and size: elongated or reniform, rarely spherical, green-yellowish and weighting between 50 and 500 grams. The flesh in the initial stages is yellow to yellow–orange, very sweet. The fruits ripen in stages, are climacteric (Koslanund *et al.*, 2000) and perishable (Ghena *et al.*, 2004; Stănică, 2012).

Asimina triloba (L.) Dunal is a species with high frost resistance, surviving at -25 to -30°C. It is also well adapted to different soil types, preferring loose, well-drained soils rich in organic matter with a neutral or slightly acidic pH. One another important quality it is the resistance to diseases and pests, being easy to grow in the organic system (Stănică, 2012).

In Romania it is known since 1926, when in Pianu Nou, Alba County, Ioan Suciu family obtained plants from some seeds brought from Ohio State, some of them until recently viable (Cepoiu *et al.*, 2004; Stănică, 2012). Other *Asimina triloba* (L.) Dunal genotypes are currently found in Romania in the 'Dimitrie Brândză' Botanical Garden of the University of Bucharest and other sites of the city, Geoagiu (Hunedoara county), Simeria, Baia Mare (Cepoiu *et al.*, 2004).

The plant is known and appreciated for its highly nutraceutical fruits (Nam *et al.*, 2018), natural compounds (annonaceous, acetogenins) that can be extracted from leaves, bark and twig tissue

with anticancerogenic and insecticidal properties (Johnson *et al.*, 1996; Ratnayake *et al.*, 1993; McLaughlin *et al.*, 1986; Huang *et al.*, 2003; Avula *et al.*, 2018). Its habitus recommend it as an ornamental plant for landscapes, having special properties in air de-pollution (Szilagyi and Marian, 2011).

In the last 25 years, extended researches were conducted at the Faculty of Horticulture within USAMV Bucharest regarding the potential of this fruit tree and its adaptation in this area (Stănică and Cepoiu, 2003; Cepoiu *et al.*, 2004; Stănică *et al.*, 2004; Cotruț *et al.*, 2005; Stănică *et al.*, 2008; Stănică, 2012). Detailed researches were made in Baia Mare also, including the plant ornamental value (Szilagyi and Marian, 2011; Szilagyi *et al.*, 2016a,b; Szilagyi *et al.*, 2017).

The increasing demands from the consumers for this nearly unknown fruit with high nutraceutical potential require specific researches. The aim of this study is to present the fruits pomological characteristics for more genotypes analyzed, cultivated in the Bucharest area.

Materials and methods

Description of the study site and experimental design

The experience was placed in the Experimental field of the Faculty of Horticulture in Bucharest, located in the North part of the town.

Biological material

Researches were conducted on 23 genotypes planted on two rows (Fig. 1) at 4.0 m × 2.0 m distances between plants. The genotypes were noted R1P1 - R1P11, R2P1 - R2P11 and respectively RP. An integrated fruit growing technology was applied.

The most known genotypes from the asimina collection are: 'Allegheny', 'Potomac', 'Simina' (Fig. 2) (a new Romanian selection under registration) (Stănică, 2012; Ştefan *et al.*, 2018).

For morphological and biochemical fruit characterization, the fruits were noted after fruit set in spring and numbered before the harvesting period, determined the yield. Harvesting period was noted for each genotype, beginning with the first fruits harvested (at the moment when they dropped from tree) to the finals. Fruit weight and size were determined for each fruit/genotype, cumulated data being presented for three years (2016-2018). Seed number and weight were calculated for the fruits harvested in 2018. The method for fruit tree assessment was according to Hoza (2014).

Biochemical analyses

Total soluble solids were determined from flesh juice for each genotype (2018 harvest), with a refractive device Kruss DR301-95 (% Brix) (Yoon, 2005; Saei, 2011; Oltenacu and Lascăr, 2015).

Dry matter and water content of the samples were determined by oven drying for 24 hours at 105°C using a UN110 Memmert oven, method used also by Moura *et al.*, (2005), Skupień, (2006), Delian *et al.*, (2011), Corollaro *et al.*, (2014), Mureşan *et al.*, (2014), Ticha *et al.*, (2015), Bezdadea Cătuneanu *et al.*, (2017).

Figure 1. *Asimina triloba* (L.) Dunal genotypes

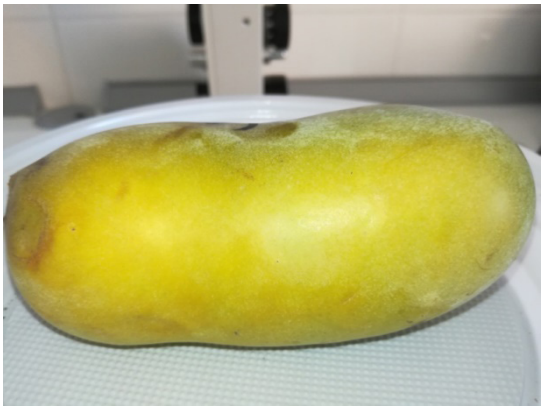

Figure 2. 'Simina' fruit

To determine the content of total titrable acidity, three fruits from the same sample were chosen, removed the skin and the seeds and then crushed the pulp until was obtained a homogeneous paste. 5 g of the sample with 25 ml double distilled water were titrated with 0.1 N NaOH, with the TitroLine easy automatic titrator, up to a pH value of 8.1. The results were calculated according to the following formula and expressed as percentages:

Titratable acidity (%) = (V × N × C ×100) / m;

V = volume of NaOH consumed; N = NaOH; C = equivalent malic acid; m = mass of the sample. C has the values: 0.0067 to express acidity in malic acid (Yoon *et al.*, 2005; Saei *et al.*, 2011; Mureşan *et al.*, 2014; Oltenacu and Lascăr, 2015).

To determine the ascorbic acid content three fruits from the same sample were chosen as well, removed the skin and the seeds and crushed the pulp until a homogeneous paste was obtained. The sample preparation consisted of a conventional trituration extraction of 1g fresh raw material with 10 ml of orthophosphoric acid (2%, v/v) for 1 minute. The obtained extract was homogenized for 15 minutes at 500 rpm and then centrifuged for 5 minutes at 7000 rpm and 4°C. The supernatant obtained by centrifugation was filtered through regenerated cellulose filter (Chanforan *et al.*, 2012; Turmanidze *et al.*, 2017). The chromatographic separation of the compounds was performed using an Agilent Technologies 1200 HPLC equipped with UV-DAD detector through ZORBAX Eclipse XDB-C18 column (4.6 × 50 mm, 1.8 pm id). An isocratic elution with formic acid (0.05% v/v), a flow rate of 0.5 ml/min, at 30°C, and the 2 µl injection volume were used. Curve of calibration was obtained by injecting the ascorbic acid standard at different concentrations. Obtained data were processed using Agilent ChemStation B.04.03 software (Agilent, USA).

Sensorial analyzes were performed in several stages on groups formed by persons with random gender, age and occupation. In the initial stage, tests consisted in the evaluation of size, skin colour, taste and flavour (noted from 1 to 10). In the second stage, tests were detailed, fruit scores being accorded to the following differentiation criteria: fruit size, fruit shape, pulp juiciness, pulp colour scored from 1 to 3; aroma rated from 1 to 4; the attractive appearance and the pulp firmness scored from 1 to 5 and taste scored from 1 to 6.

The analyses were conducted in the Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest.

Results and discussions

Bunch fruits number

One specific element of these species, correlated with the yield, was the number of fruits in a bunch. 'Allegheny' cultivar (Fig. 3 and 4) had the highest number of fruits in a bunch.

Harvesting period

For the harvesting period, data are presented in Figures 5a and 5b. R1P2 genotype was the earliest in terms of fruit ripening and had the longest harvesting period (77 days in 2018). Other early genotypes were: R1P3, R2P9, R1P10, R2P1 and R2P4. Late harvesting period had R1P1, R1P8 and R2P11 genotypes. The results for several genotypes were similar with Pomper *et al.* (2008), their genotypes recorded harvesting period bet-

ween 23-21 days, 18-23 days and respectively 15-25 days in three years analyzed.

Yield and morphological fruits parameters

Several genotypes presented continuity in the annual yield, especially in the last two years. Most of them had alternate bearing especially due to climatic factors and crop management. The results were similar with Pomper *et al.* (2008) experience, regarding discontinuity in production during the analyzed three years (7.0 – 15.7 kg/tree; 3.1 – 7.7 kg/tree; 5.0 – 17.2 kg/tree).

The most productive genotypes in terms of fruit number/tree were R1P6, R2P1 (every year over 100 fruits/tree) and R2P9 (Tab. 1 and 2).

The average fruit weight presented maximum values in 2018, between 182-190 g for R1P3, R1P9, R1P10 and R2P1 genotypes. The results were similar with Brannan *et al.* (2015), genotypes fruits analyzed having weight between 84 and 204 g, fruit length between 7.9 and 11.1 cm and diameter between 4.6 – 6.4 cm. Pomper *et al.* (2008) presented fruit weight variation between 76 – 176 g, 84 – 189 g and respectively 65 – 185 g in the three year experience. The results were also similar with Szilagy *et al.* (2016a) with a six years fruit monitoring experience.

Generally, the fruit shape (height/average diameter) was elongated.

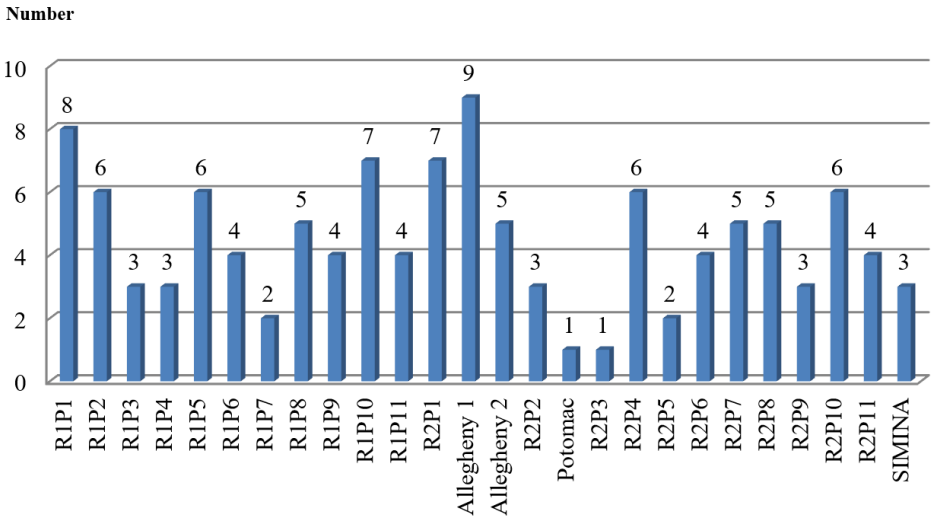

Figure 3. Number of fruits in a bunch/tree

Figure 4. Fruits of 'Allegheny' cultivar

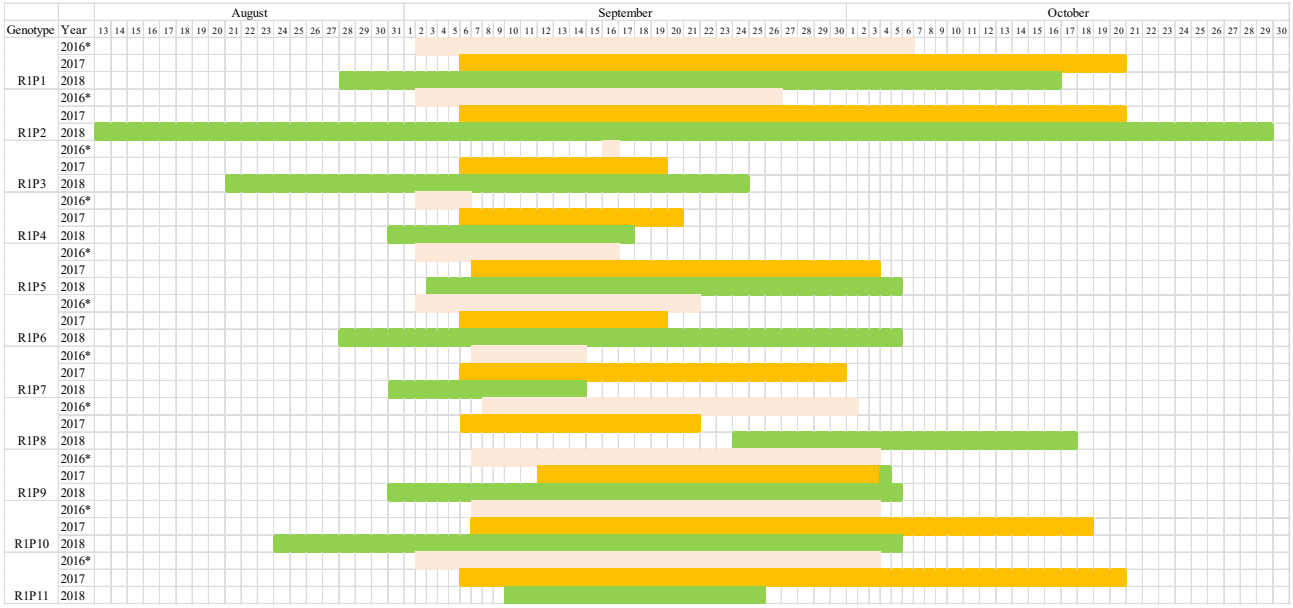

Figure 5a. Establishing harvesting period for genotypes in terms of maturation

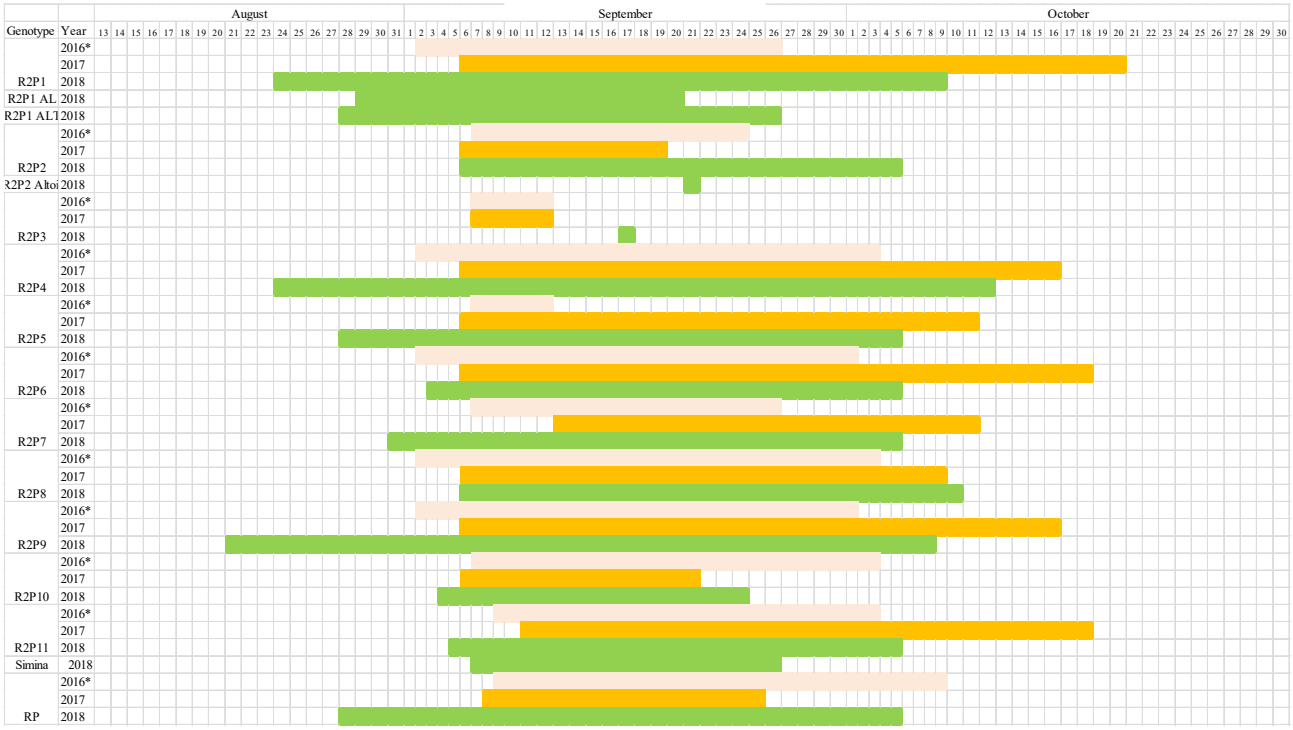

Figure 5b. Establishing harvesting period for genotypes in terms of maturation

Seeds morphological characterization

Following the determinations made for 2018 production, the average number of seeds/fruit varied according the genotype from 2 to 11 seeds (Fig. 6 and 7). The seed mass expressed in g/fruit varied from 3.7 g/fruit to 14.2 g/fruit.

The average seed weight compared to other fruit species such as apple or pear was much higher with values between 0.9 and 1.8 g. The lowest values were obtained at R1P1 and R2P2 genotypes respectively, at 'Potomac' variety (Fig. 8 and 9). Crabtree *et al.* (2014) registered also the rate of the seed weight in the fruit for several

Table 1. Fruit parameters characterization

			R1P1	R1P2	R1P3	R1P4	R1P5	R1P6	R1P7	R1P8	R1P9	R1P10	R1P11	R2P1	Al-1	Al-2	R2P2	R2P3	P-1	R2P4	R2P5	R2P6	R2P7	R2P8	R2P9	R2P10	R1P11	S	RP
Fruits	Height (cm)	2016*																											
		2017	5.9	7.2	7.8	8.4	8.0	6.7	6.4	8.2	9.0	7.2	6.7	8.5			6.1	6.0		7.4	6.6	7.1	6.6	7.0	7.4	8.1	8.3		6.1
		2018	7.0	8.1	9.1	6.1	8.6	8.3	7.1	9.8	9.6	10.0	7.6	9.1	8.3	8.3	5.8	8.3	7.7	9.9	7.0	8.8	8.2	7.9	7.7	8.3	10.0	9.8	8.0
	Average		6.4	7.7	8.4	7.3	8.3	7.5	6.8	9.0	9.3	8.6	7.1	8.8	8.3	8.3	5.9	7.2	7.7	8.6	6.8	7.9	7.4	7.4	7.5	8.2	9.1	9.8	7.1
	Diamm. (cm)	2016*																											
		2017	3.9	4.5	4.8	7.9	4.8	3.9	4.3	5.0	5.3	4.3	4.6	5.3			4.2	3.9		3.9	3.8	4.1	4.5	4.4	4.4	4.8	4.7		3.3
		2018	4.3	5.0	6.4	5.5	5.3	5.4	4.6	5.6	5.8	5.9	5.4	5.9	4.9	4.6	3.9	4.7	4.4	4.9	4.2	5.0	5.3	4.8	4.7	5.1	5.3	5.6	4.4
	Average		4.1	4.7	5.6	6.7	5.0	4.6	4.4	5.3	5.6	5.1	5.0	5.6	4.9	4.6	4.0	4.3	4.4	4.4	4.0	4.6	4.9	4.6	4.5	5.0	5.0	5.6	3.9
	Weight (g)	2016*	88.6	60.8	35.0	116.2	70.1	87.4	158.5	92.4	105.6	95.1	74.3	44.2			111.8	137.3		149.3	129.6	85.7	61.6	100.9	101.8	113.4	47.3		75.2
		2017	51.8	83.2	92.9	145.9	110.2	56.2	67.6	107.9	152.4	95.5	80.9	129.7			65.3	51.4		68.5	70.8	66.4	71.1	84.8	93.2	87.3	106.4		41.3
		2018	75.7	116.6	189.5	147.6	120.7	140.4	98.8	167.5	183.9	182.9	122.6	182.2	116.1	109.7	50.0	92.5	78.9	138.6	70.4	122.2	130.7	102.5	92.1	120.1	155.4	158.0	115.3

* Al-1 = 'Allegheny' 1; Al-2 = 'Allegheny'' 2; P-1 = Potomac'; S= 'Simina'

Table 2. Yield characterization

			R1P1	R1P2	R1P3	R1P4	R1P5	R1P6	R1P7	R1P8	R1P9	R1P10	R1P11	R2P1	Al-1	Al-2	R2P2	R2P3	P-1	R2P4	R2P5	R2P6	R2P7	R2P8	R2P9	R2P10	R1P11	S	RP
Yield	No fruits/tree	2016*	182	40	2	35	8	47	6	10	28	29	25	106			30	2		10	9	36	54	50	36	27	34		50
		2017	94	85	10	21	15	16	30	5	14	21	21	161			17	6		112	16	148	71	33	72	12	49		12
		2018	60	72	9	19	17	143	4	18	15	72	22	114	28	31	15	1	1	71	7	61	17	67	104	28	56	16	9
	Kg/ tree	2016*	16.1	2.4	0.1	4.1	0.6	4.1	1.0	0.9	3.0	2.8	1.9	4.7			3.4	0.3		1.5	1.2	3.1	3.3	5.0	3.7	3.1	1.6		3.8
		2017	4.9	7.1	0.9	3.1	1.7	0.9	2.0	0.5	2.1	2.0	1.7	20.9			1.1	0.3		7.7	1.1	9.8	5.0	2.8	6.7	1.0	5.2		0.5
		2018	4.8	7.9	1.7	2.8	2.0	19.4	0.4	3.0	2.7	12.9	2.7	20.7	3.2	3.3	0.7	0.1	0.1	9.8	0.5	7.4	2.2	6.8	9.2	3.3	8.3	2.5	1.0

* Al-1 = 'Allegheny' 1; Al-2 =' Allegheny' 2; P-1 = 'Potomac'; S= 'Simina'

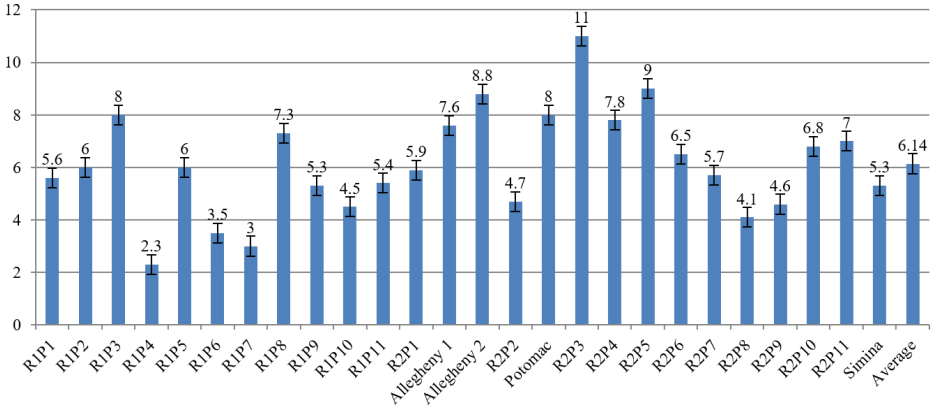

Figure 6. Seed number/fruit

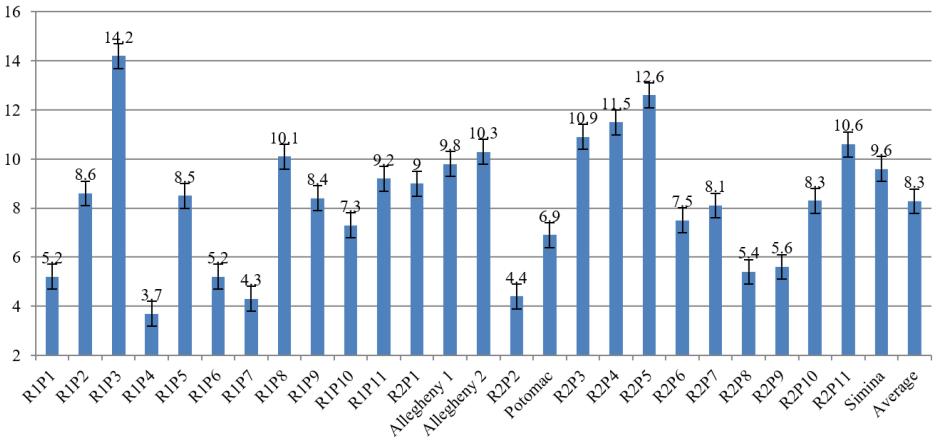

Figure 7. Seed mass (g)/fruit

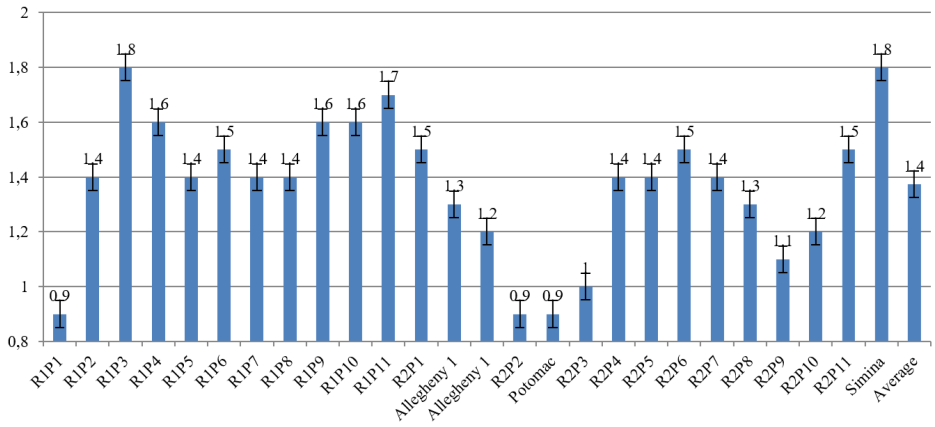

Figure 8. Average seed weight

pawpaw cultivars, highlighting the importance of its low value.

According to the data presented in Table 3, the seed height varied between 1.79 and 2.85 cm, the average diameter varied between 1.01 and 1.25 cm, the results of the shape index varied between 1.88 and 2.67 respectively. Following the results obtained, the seed form was elongated. Szilagy *et al.* (2016a) noticed an average of 1.51 g/seed, 2.59 cm height and 1.33 cm diameter.

Figure 9. Seeds of R2P2 genotype

Table 3. Determining the shape of asimina seeds

Genotype	Height (cm)	Diameter (cm)	Shape index	Shape
R1P1	2.13	1.01	2.11	Elongated
R1P6	1.79	1.01	1.77	Elongated
R1P10	2.57	1.15	2.24	Elongated
R1P11	2.85	1.07	2.67	Elongated
R2P1	2.56	1.12	2.28	Elongated
'Allegheny' 1	2.39	1.09	2.21	Elongated
'Allegheny' 2	2.28	1.03	2.22	Elongated
R2P2	2.06	1.05	1.96	Elongated
R2P4	2.39	1.14	2.11	Elongated
R2P6	2.35	1.25	1.88	Elongated
R2P8	2.25	1.10	2.04	Elongated
R2P9	2.15	1.05	2.05	Elongated
R2P10	2.60	1.05	2.46	Elongated
Average	2.34	1.09	2.15	

Total soluble solids and dry matter

Dry matter varied from 17.18% (R2P2) to 31.05% (R2P8), 'Allegheny' 2 cultivar presenting a value of 9.47%. R1P1, R1P4 and R1P9 presented also high values.

Total soluble solids varied from 15.40% (R2P2) to 27.90% (R1P4) (Fig. 10).

The results are similar with Brannan *et al.* (2015), their studied genotypes registering total soluble solids between 19.9% and 28.0% and dry matter between 23.4% and 28.9%.

Ascorbic acid content and titrable acidity in 'Allegheny' cultivar

Ascorbic acid content varied between 18.16 and 19.13 mg/100 g and titrable acidity between 0.08 – 0.12 g malic acid/100 g fresh fruit (Tab. 4).

Sensorial analysis of asimina fruits

The first test of consumer preferences was made in 2017, having five testing parameters. The most appreciated fruits were of R1P6, R2P9 and R2P10 genotypes. The most appreciated for the fruit size were R2P10 fruits while for the skin colour R1P6 genotype fruits (Tab. 5).

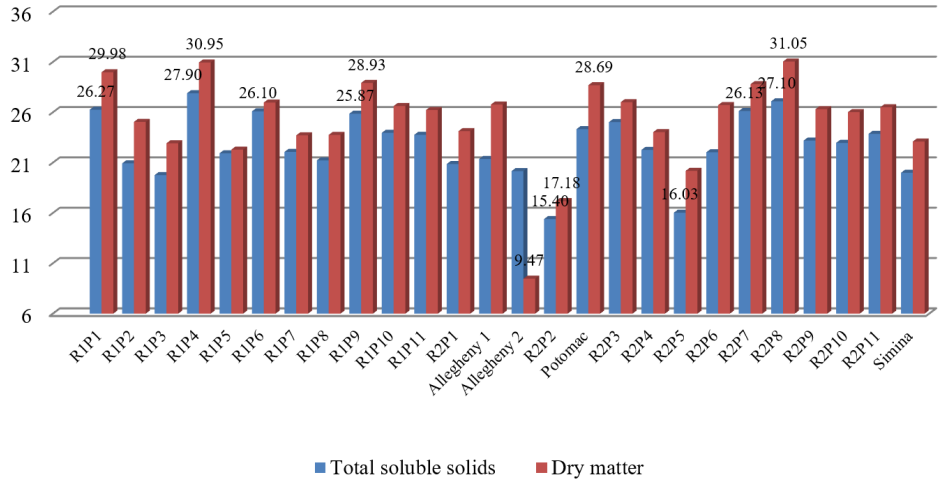

Figure 10. Total soluble solids and dry matter content

Table 4. Titrable acidity and ascorbic acid content in 'Allegheny' cultivar fruits

Cultivar	Titrate acidity (g malic acid/100 g fresh product)	Ascorbic acid mg/100 g
'Alegheny' 1	0.12	18.16
'Alegheny' 2	0.08	19.13
Average	0.10	18.64

Table 5. Appreciation of pawpaw fruits according to 2017 sensorial analysis

Genotype	Size 1-10	Skin colour 1-10	Taste 1-10	Flavour 1-10	Total 9-40
RP	5.8	5.3	7.1	7.1	25.3
R2P1	7.5	7.5	7.0	8.0	30.0
R2P2	7.6	7.0	8.0	7.4	30.0
R2P4	5.0	5.0	9.0	9.0	28.0
R2P6	8.3	6.7	8.0	9.3	32.3
R2P7	6.4	5.5	8.0	8.1	28.0
R2P8	5.0	5.3	8.3	8.3	26.8
R2P9	7.5	7.9	9.3	9.6	34.2
R2P10	9.4	7.9	9.0	8.5	34.8
R1P1	5.8	4.9	8.1	7.9	26.7
R1P4	6.7	5.2	7.1	7.2	26.2
R1P6	7.5	8.3	9.3	10.0	35.2
R1P7	7.5	7.5	5.3	6.3	26.5
R1P8	5.0	5.0	9.3	8.7	28.0
R1P10	7.0	5.8	8.9	8.7	30.4
Average	6.8	6.32	8.11	8.27	29.49

Table 6. Sensory analysis of the asimina fruits of certain genotypes (2018)

Genotype	Fruit size* (1-3)	Fruit shape* (1-3)	Attractiveness* (1-5)	Pulp firmness* (1-5)	Pulp juiciness* (1-3)	Pulp colour* (1-3)	Taste* (1-6)	Flavour* (1-4)	Total* (1-32)
R1P1	2.00	2.00	2.33	1.33	2.33	2.33	3.00	2.33	17.67
R1P2	2.33	1.78	2.33	2.00	1.67	2.11	2.22	2.00	16.44
R1P5	1.80	1.80	1.40	1.40	1.20	1.80	1.60	2.00	13.00
R1P8	2.82	2.36	2.55	2.09	1.91	2.18	4.18	2.64	20.73
R1P9	1.88	1.63	1.50	1.75	1.63	1.50	2.13	1.50	13.50
R1P10	2.33	2.33	2.00	2.33	1.67	2.00	5.00	3.33	21.00
R2P4	2.00	2.13	1.88	2.13	1.88	1.75	3.38	2.38	17.50
R2P5	2.00	2.00	2.29	2.43	2.43	2.14	4.29	2.71	20.29
R2P6	2.27	2.27	2.27	2.00	2.18	2.00	3.36	2.45	18.82
R2P8	2.27	2.27	2.45	2.45	2.00	2.00	4.00	2.64	20.09
R2P9	1.89	2.11	1.56	2.00	1.78	2.00	3.78	2.67	17.78
R2P11	2.50	2.30	2.20	2.10	1.80	1.80	4.10	2.90	19.70
RP	2.10	2.10	1.90	2.20	2.00	2.10	3.30	2.20	17.90
Average	2.17	2.08	2.05	2.02	1.88	1.98	3.41	2.44	18.03

* data were statistically processed according to the rate and average calculated for each parameter

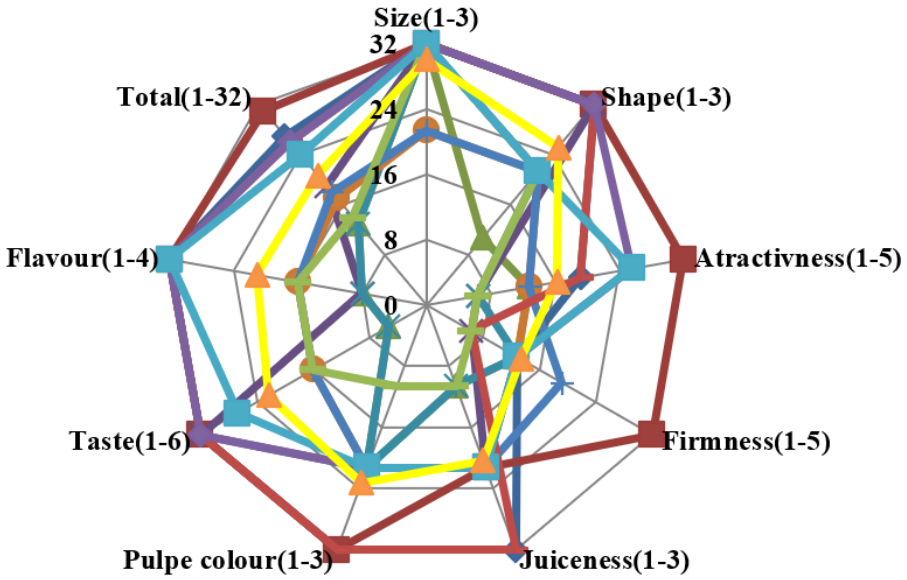

Figure 11. Parameters evaluated by sensory analysis for R1P8 genotype

For the second testing (2018), the most appreciated genotypes were R1P8 that received the best scores in terms of fruit size, fruit shape and attractive appearance; R2P8 obtained the best score from the point of view of the pulp firmness; R1P1 recorded the best score in terms of pulp colour while R1P10 accumulated the highest score in the taste and aroma criteria. The most appreciated genotype, based on the final score, was R1P10 (Tab. 6).

Some of the genotypes with the highest scores in 2018 were appreciated also in 2017, but not with the highest values. Brannan *et al.* (2012) noticed in pawpaw pulp sensorial analyses testing besides mango and papaya fruits that identification of pawpaw seemed to increase with increasing age,

Figure 12. Fruits of the highly appreciated R1P8 and R1P10 genotypes

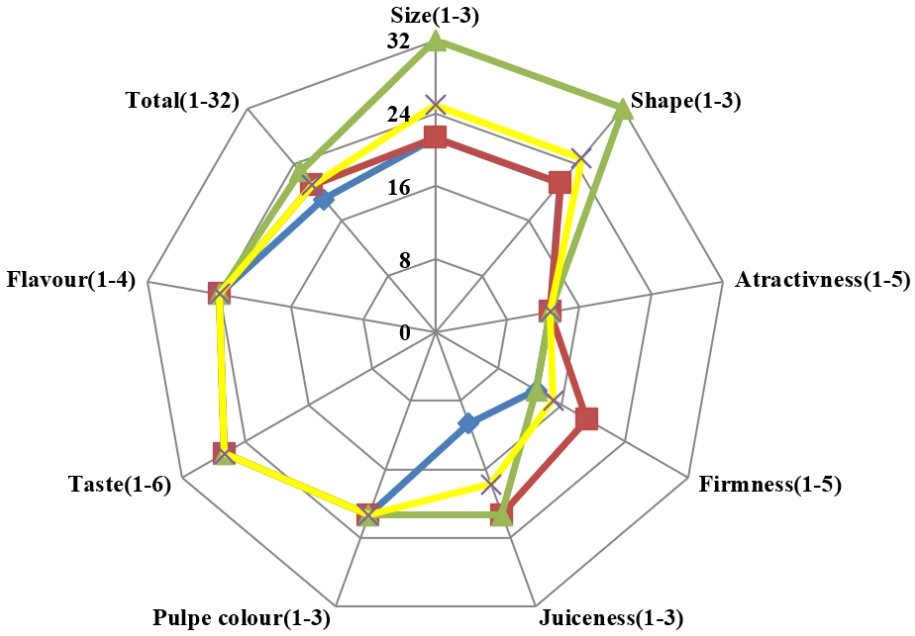

Figure 13. Parameters evaluated by sensory analysis at R1P10 genotype

education and consumption. Further researches are required to observe these characteristics.

The most appreciated criteria for the R1P8 genotype (Fig. 11 and 12) were the taste, the size of the fruit and the flavour while the least was the pulp juiciness.

The most appreciated parameters for R1P10 genotype (Fig. 12 and 13) were the taste, aroma, size and shape of the fruit. The parameter with the lowest accumulated score was the pulp firmness.

The most appreciated criteria for R2P8 genotype (Fig. 14 and 15) were the taste, aroma, attractive appearance and firmness of the pulp. The criteria with the lowest scores were the succulence and the pulp colour.

Conclusions

This study presented a pomological description with several parameters for the genotypes from Bucharest collection, highlighting the most valuable genotypes according to the analyses performed and also to the consumer's preferences. Its qualities recommend the pawpaw as a promising fruit for diversify the present fruit species consumed in Romania and also in Europe. The measurements and the analyses recommend:

Figure 14. Fruit of the R2P8 genotype

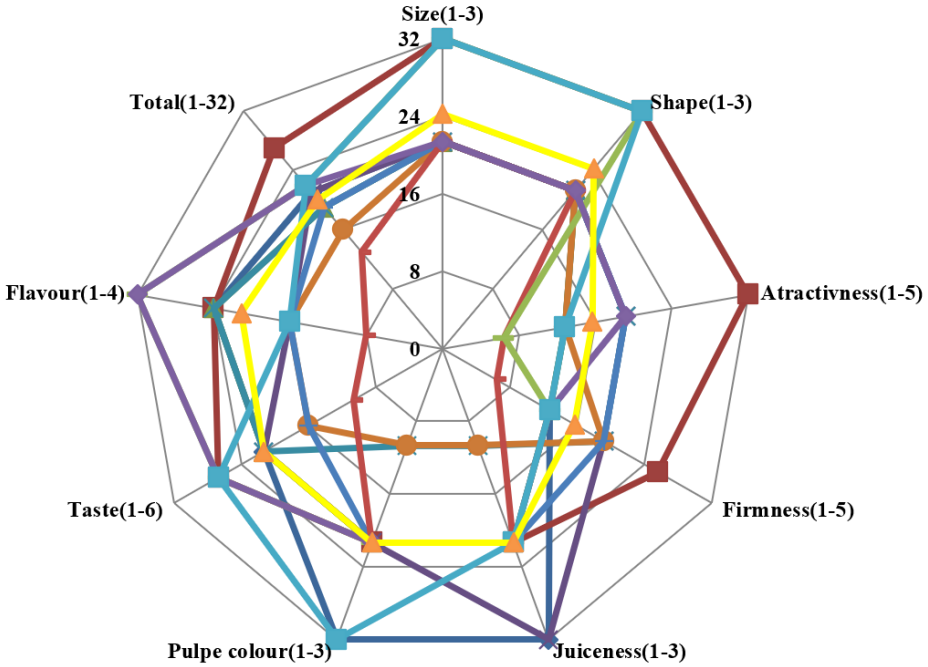

Figure 15. Parameters evaluated by sensory analysis at R2P8 genotype

for earliness and lateness: R1P2 and R1P8; yield/tree: R1P6 and R2P1; fruits sizes: R1P3, R2P1 and R2P4; seeds sizes: R1P4 and R2P2; fruits biochemical parameters: R1P4, R2P8, R2P9 and 'Allegheny'. From the sensory analysis test, promising genotypes were: R1P10, R1P8, R2P5 and R2P8. The most valuable genotypes from the Experimental field according to the analyses performed and the consumer's preferences were taken in consideration for further research.

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