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VARIATION OF FRUITS MORPHOMETRIC PARAMETERS AND BIOACTIVE COMPOUNDS OF *ASIMINA TRILOBA* (L.) DUNAL GERMPLASM COLLECTION

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ABSTRACT

The objective of this study was to evaluate the morphological parameters and bioactive compounds (antioxidant activity, total polyphenol, flavonoid, and phenolic acid content) of 6 genotypes of dry *Asimina triloba* (L.) fruit from Slovak University of Agriculture in Nitra (Slovakia). Genotypes were obtained from the seeds that were sown in the year 2000. Their morphometric parameters were following: fruit weight from 59.00 to 241.19 g, fruit length from 50.14 to 140.11 mm, fruit diameter from 37.55 to 64.67 mm, number of fruits per cluster from 2 to 8, seed weight from 0.06 to 1.80 g, seed length from 16.33 to 29.11 mm, seed width from 9.56 to 18.33 mm, seed thickness from 4.98 to 9.75 mm, number of seeds in fruit from 4 to 16. The shape indexes of fruits were found ranging from 1.53 to 2.16. The variability of important is the average seeds weight from 7.40 to 35.61%, fruit weight from 14.84 to 32.95%, number of fruits per cluster from 18.21 to 32.54% and a number of seeds in fruit from 13.49 to 27.72%. The other characteristics are more or less stable. Total polyphenol content ranged from 22.13 to 37.36 mg GAE per g, total flavonoid content from 15.10 to 32.03 mg QE per g and phenolic acids content from 0.23 to 0.76 mg CAE per g. All tested samples exhibited DPPH radical scavenging activities with values from 2.84 to 7.04 mg TEAC per g. Antioxidant activity by molybdenum reducing antioxidant power method ranged from 97.25 to 275.41 mg TEAC per g of dry matter. Differences between the genotypes were significant in all observed parameters. This species is potential for propagation and practice used in the Slovak Republic.

Keywords: *Asimina triloba*; Dunal; fruits; seeds; variability; bioactive compounds

INTRODUCTION

The searching of new plant species, which are the valuable source of biologically active compounds, is an actual branch of modern biological science in the last time. The introduction and selection of these plants, cultivation technology and extraction ways of biologically active compounds complex and creation on its basis the new generation of nutritional supplements one of the most important scientific directions **(Brindza et al., 2007; Grygorieva et al., 2014; Monka et al., 2014; Ivanišová et al., 2017; Vinogradova et al., 2017; Horčinová Sedláčková et al., 2018)**. *Asimina triloba* (L.) Dunal. relates to plants that are a source of polysaccharides, free amino acids, mineral compounds, flavonoids, etc.

Species of the genus *Asimina* Adans. belong to the family *Annonaceae* Juss. *Asimina triloba* (pawpaw, paw paw, paw-paw, common pawpaw) is deciduous tree native to eastern North America and Canada **(Layne, 1996)**.

Asimina triloba fruits are rich in nutritive components such as vitamins and minerals **(Templeton et al., 2003; Pomper and Layne 2005)**, a good source of potassium (3000 – 3800 mg.kg^{-1}) and several essential amino acids (mean value: 40 mg.kg^{-1} of protein), and they contain significant amounts of riboflavin (0.06 – 0.15 mg.kg^{-1}), niacin (10 – 12 mg.kg^{-1}), calcium (500 – 800 mg.kg^{-1}), phosphorus (400 – 500 mg.kg^{-1}) and zinc (10 – 12 mg.kg^{-1}) **(Galli et al., 2007)**, to have a high polyphenolic content **(Harris and Brannan, 2009; Brannan et al., 2014; Brannan, 2016)**.

Pawpaws can be used as an alternative to bananas fruits in most recipes **(Jones and Layne, 1995)**. Fruits of pawpaw are very fragrant and resemble a combination of aromas of banana and mango, and may be used commercially in cosmetics and skin products **(Layne, 1996; Brannan and Holben, 2012)**.

Biologically active compounds are not only in fruits but in different parts of the plant: roots, bark, twigs, leaves, flowers, and seeds **(Hui et al., 1989; Zhao et al., 1992, 1993, 1994; Alali et al., 1999; Goodrich et al., 2006; Cuendet et al., 2008; Farag, 2009; Pande and Akoh, 2010)**. The roots, twigs, flowers, and seeds of pawpaw contain acetogenins, which are strong inhibitors of cancer cells **(Ratnayake et al., 1992; McLaughlin and Hui, 1993; Woo et al., 1995; Ko et al., 2011; Sica et al., 2016)**. Pawpaw leaf essential oil has strong activity against cancer cell lines **(Alali et al., 1999; Farag, 2009)**.

Asimina triloba fruit, leaf, bark, and twig extract may be an effective insect feeding deterrent **(Rupprecht et al., 1986; Ratnayake et al., 1992; Zhao et al., 1994; Gu et al., 1999; Sedlacek et al., 2010)**.

Scientific hypothesis

Evaluating of fruit quality formation by their qualitative parameters in the adaptation process of *Asimina triloba* at the agroecological conditions of the experimental base in Nitra.

MATERIAL AND METHODOLOGY

Biological material

The observations of the collection genotypes of *Asimina triloba* in the period 2017 were performed during mass fruiting. We have described 6 genotypes (referred as AzT-01 to AzT-06) of *Asimina triloba*.

Morphometric characteristics

The ripened fruits were picked from trees in maturity stage. Pomological characteristics were conducted with four replications on a total of 30 fruits per genotypes. In the study only one plant was used per genotype. The following measurements were taken: fruit weight, in g, fruit length, in mm, fruit diameter, in mm, and seed weight, in g, seed length, in mm, seed diameter, in mm, number of fruits per cluster, and a number of seeds in fruit.

Chemicals

All the chemicals used were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) and CentralChem (Slovakia).

Preparation of sample extracts

The dry *Asimina triloba* pulp was used for detection of total phenolic content and total flavonoid content. An amount of 0.25 g of each sample was extracted with 20 mL of 80% ethanol for 24 h. Then, the sample in 80% ethanol was centrifuged at 4000 rpm (Rotofix 32 A, Hettich, Germany) for 10 min and the supernatant was used for measurement with the DPPH and molybdenum reducing antioxidant power methods.

Determination of antioxidant activity

Free radical scavenging activity

Free radical scavenging activity of samples was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) **(Sanches-Moreno et al., 1998)**. An amount of 0.4mL of the sample was mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL methanol). The absorbance of the reaction mixture was determined with the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10 – 100 $mg.L^{-1}$; $R^2 = 0.989$) was used as the standard and the results were expressed in $mg.g^{-1}$ Trolox equivalents.

Molybdenum reducing antioxidant power

Molybdenum reducing antioxidant power of samples was determined by the method of **Prieto et al. (1999)** with slight modifications. The mixture of the sample (1 mL), monopotassium phosphate (2.8 mL, 0.1 M), sulfuric acid (6 mL, 1 M), ammonium heptamolybdate (0.4 mL, 0.1 M) and distilled water (0.8 mL) was incubated at 90°C for 120 min, then rapidly cooled. The absorbance at 700 nm was detected with the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10 – 1000 $mg.L^{-1}$; $R^2 = 0.998$) was used as the standard and the results were expressed in $mg.g^{-1}$ Trolox equivalents.

Determination of total polyphenol content

The total polyphenol content was measured by the method of **Singleton and Rossi (1965)** using the Folin-Ciocalteu reagent. A quantity of 0.1 mL of each sample was mixed with 0.1 mL of the Folin-Ciocalteu reagent, 1 mL of 20% (w/v) sodium carbonate and 8.8 mL of distilled water. After 30 min in darkness, the absorbance at 700 nm was measured with the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25 – 300 $mg.L^{-1}$; $R^2 = 0.998$) was used as the standard. The results were expressed in $mg.g^{-1}$ gallic acid equivalents.

Determination of total flavonoid content

The total flavonoid content was determined by the modified method described by **Shafii et al. (2017)**. An aliquot of 0.5 mL of the sample was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminum chloride, 0.1 mL of 1 M potassium acetate and 4.3 mL of distilled water. After 30 min in darkness, the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (1 – 400 $mg.L^{-1}$; $R^2 = 0.9977$) was used as the standard. The results were expressed in $mg.g^{-1}$ quercetin equivalents.

Determination of phenolic acids

Total phenolic acids content was determined using the method of Farmakopea Polska (1999). A 0.5 mL of sample extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL Arnova reagent (10% $NaNO_2$ + 10% Na_2MoO_4), 0.5 mL of 1 M sodium hydroxide (w/v) and 0.5 mL of water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1 – 200 $mg.L^{-1}$, $R^2 = 0.999$) was used as a standard and the results were expressed in $mg.g^{-1}$ caffeic acid equivalents.

Statistic analysis

Basic statistical analyses were performed using PAST 2.17. Data were analysed with ANOVA test and differences between means compared through the Tukey-Kramer test ($\alpha = 0.05$). Variability of all these parameters was evaluated using descriptive statistics. Level of variability determined by **Stehlíková (1998)**.

RESULTS AND DISCUSSION

Morphometric measurements of fruits and seeds of selected plants are very important studying parameters because of some reasons such as origin and conditions of growth. It is also necessary indicators at the new conditions of introduction in a new area.

Figure 1 Fruits of *Asimina triloba* (L.) Dunal.

Table 1 The variability of some morphometric parameters of fruits of *Asimina triloba* (L.) Dunal genotypes.

Genotypes	min	max	mean	V%	Genotypes	min	max	mean	V%
Fruit weight (g)									
AzT-01	60.80	211.90	130.83	28.73	**AzY-04**	80.88	241.19	154.63	32.95
AzT-02	67.56	120.05	92.17	15.16	**AzY-05**	59.00	190.45	114.27	23.57
AzT-03	111.23	184.21	149.31	14.84	**AzY-06**	77.19	106.67	106.67	17.81
Fruit length (mm)									
AzT-01	50.14	105.02	84.16	17.59	**AzY-04**	72.19	140.11	108.83	17.11
AzT-02	67.80	91.14	80.77	9.44	**AzY-05**	76.50	112.01	100.15	10.03
AzT-03	90.55	114.68	103.07	7.47	**AzY-06**	64.18	89.96	78.16	10.65
Fruit diameter (mm)									
AzT-01	41.78	64.67	53.03	10.09	**AzY-04**	41.66	59.12	50.33	10.57
AzT-02	37.55	49.69	44.80	6.86	**AzY-05**	41.00	55.06	47.82	7.76
AzT-03	48.82	60.04	55.06	6.00	**AzY-06**	40.69	61.15	50.84	12.77
Number of fruits per cluster									
AzT-01	3	5	3.68	18.21	**AzY-04**	3	6	4.07	29,12
AzT-02	3	5	3.60	22.79	**AzY-05**	3	5	4.00	23.57
AzT-03	2	6	4.07	26.32	**AzY-06**	3	8	4.61	32.54
Seed weight (g)									
AzT-01	0.10	1.11	0.81	35.71	**AzY-04**	0. 60	1.68	1.13	20.57
AzT-02	0.60	1.98	1.21	20.03	**AzY-05**	0.70	1.40	1.15	14.98
AzT-03	1.20	1.60	1.35	7.40	**AzY-06**	0.60	1.50	1.08	24.58
Seed length (mm)									
AzT-01	21.34	29.05	25.73	8.82	**AzY-04**	18,11	29.00	25.52	9.76
AzT-02	18.61	27.11	23.58	9.99	**AzY-05**	16.33	23.81	20.43	10.24
AzT-03	21.10	29.11	26.03	9.94	**AzY-06**	18.25	28.77	23.89	14.46
Seed width (mm)									
AzT-01	9.75	12.78	11.60	7.06	**AzY-04**	10.15	14.90	13.05	7.67
AzT-02	10.29	14.88	12.97	7.51	**AzY-05**	10.78	16.00	13.76	7.98
AzT-03	11.89	18.33	14.86	9.81	**AzY-06**	9.56	12.00	10.88	8.12
Seed thickness (mm)									
AzT-01	5.56	7.34	6.52	7.57	**AzY-04**	5.12	9.39	6.76	12.71
AzT-02	4.98	8.11	6.72	9.91	**AzY-05**	6.16	8.98	7.71	9.02
AzT-03	5.22	9.75	6.96	16.49	**AzY-06**	5.40	8.06	6.26	14.01
Number of seeds in the fruit									
AzT-01	**7**	10	8.50	15.92	**AzY-04**	12	16	13.36	13.49
AzT-02	8	12	9.81	13.52	**AzY-05**	4	12	8.53	27.72
AzT-03	8	14	10.91	17.66	**AzY-06**	7	12	9.90	16.80

Note: n – the number of measurements; min, max – minimal and maximal measured values; mean – the arithmetic mean; V – coefficient of variation (%).

Morphological parameters of *Asimina triloba* fruits and seeds

In our study, the weight of *Asimina triloba* fruits (Figure 1) was in the range from 92.17 (AzT-02) to 149.31 g (AzT-03) (Table 1). **Crabtree (2004)** determined the fruit weight from 88.10 to 141.80 g**, Donno et al. (2014)** determined the fruit weight from 39.40 to 238.70 g. Investigations of **Klymenko et al. (2017)** established the range of fruits weight of variety from 39.40 to 238.70 g. In our experiments, the fruit length and diameter were determined in the range from 78.16 (AzT-06) to 108.83 mm (AzT-04) and from 44.80 (AzT-02) to 55.06 mm (AzT-03), respectively. The length and diameter of fruits was determined in the range from 61.98 - 91.16 mm and from 41.27 – 49.65 mm, respectively, by **Donno et al. (2014)**, from 14.0 to 46.0 mm and from 7.0 to 20.0 mm by **Szilagyi et al. (2016)**, from 49.25 to 135.00 mm and from 37.55 to 63.80 mm, respectively, by **Klymenko et al. (2017).** A number of fruits per cluster were identified in the range from 2 (AzT-03) to 8 (AzT-06).

Crabtree (2004) determined the average number of fruits from 1.8 to 3.0, **Klymenko et al. (2017)** determined the number of fruits per cluster from 2 to 7.

Morphological parameters of ranged seeds weight from 0.81 (AzT-01) to 1.35 g (AzT-03), seed length from 20.43 (AzT-05) to 26.03 mm (AzT-03), seed width from 10.88 (AzT-06) to 14.86 mm (AzT-03), seed thickness from 6.26 (AzT-06) to 7.71 mm (AzT-05).

Investigations of **Klymenko et al. (2017)** established the range of seeds weight, length, width and thickness of genotypes from 0.60 to 1.80 g, from 17.10 to 28.80 mm, from 10.86 to 16.46 mm, from 5.54 to 9.15 mm, respectively. **Szilagyi et al. (2016)** determined the weight, length, and width of the seeds in the range from 1.30 to 1.65 g, from 2.51 to 2.69 mm, from 1.30 to 1.36 mm, respectively. A number of seeds in fruit were identified in the range from 4 (Az-05) to 16 (Az-04). The number of seeds in the fruit was determined in the range from 5 to 12 by **Klymenko et al. (2017)**.

The analysis of coefficient of variation showed the difference of variability of morphological signs between *Asimina triloba* samples. Data showed that the most variable important selection signs are the seeds weight from 7.40 to 35.71%, fruit weight from 14.84 to 32.95%, a number of fruits per cluster from 18.21 to 32.54% and a number of seeds in fruit 13.49 to 27.72%.

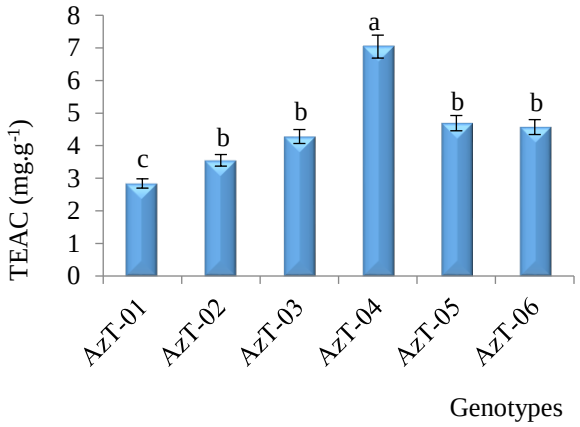

Figure 2 Antioxidant activity of the *Asimina triloba* (L.) Dunal genotypes evaluated by the DPPH method (different superscripts in each column indicate the significant differences in the mean at p <0.05).

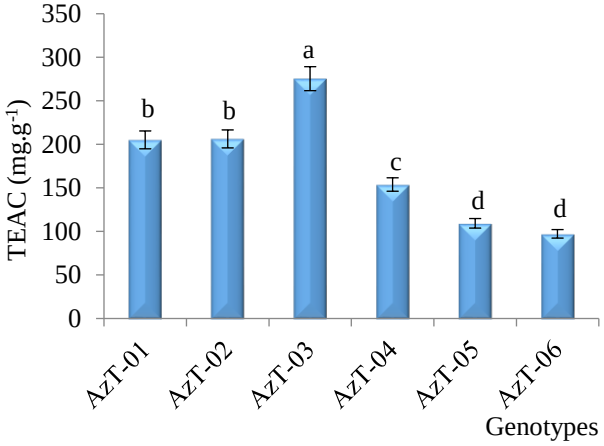

Figure 3 Antioxidant activity of *Asimina triloba* (L.) Dunal genotypes evaluated by the molybdenum reducing antioxidant power (different superscripts in each column indicates the significant differences in the mean at p <0.05).

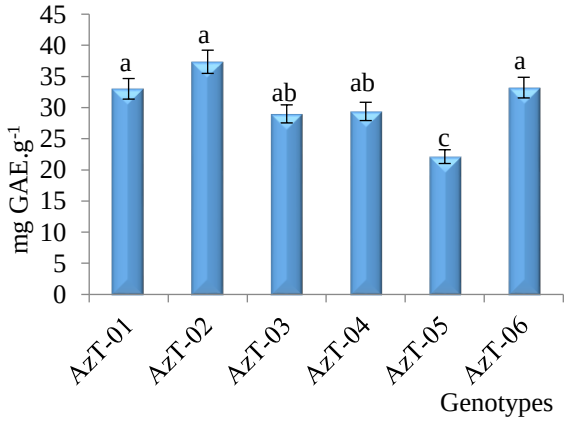

Figure 4 Total polyphenol content in *Asimina triloba* (L.) Dunal genotypes (different superscripts in each column indicate the significant differences in the mean at p <0.05).

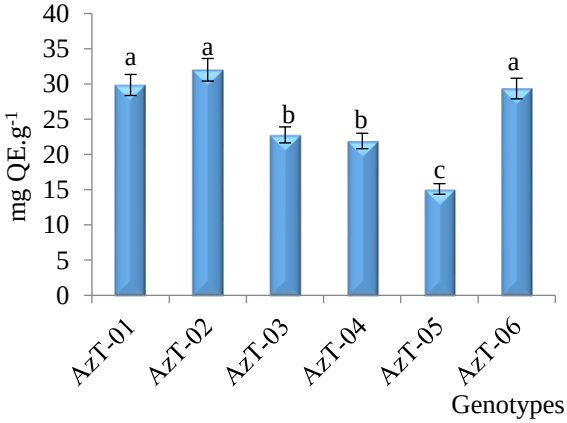

Figure 5 Total flavonoid content in *Asimina triloba* (L.) Dunal genotypes (different superscripts in each column indicate the significant differences in the mean at p <0.05).

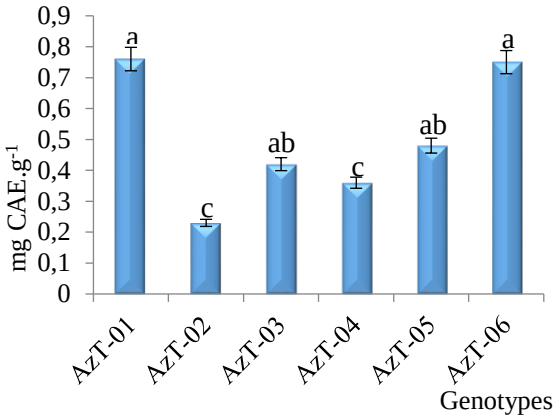

Figure 6 Total phenolic acid content in *Asimina triloba* (L.) Dunal genotypes (different superscripts in each column indicate the significant differences in the mean at p <0.05).

Table 2 The correlation coefficients of a linear relationship between the biological activities of tested *Asimina triloba* (L.) Dunal genotypes.

Parameter	Polyphenols	Phenolic acids	Flavonoids	DPPH method
Phenolic acids	-0.013*			
Flavonoids	0.976*	0.184*		
DPPH method	-0.394	-0.326	-0.518	
MRP method	0.286*	-0.329	0.240*	-0.359

Note: Significant according to the *t*-test (p <0.05).

These results indicate the promise of breeding in this way of investigations. The stable signs are seed width from 7.06 to 9.81%.

Antioxidant activity of *Asimina triloba* measured with the DPPH and molybdenum reducing antioxidant power methods

The antioxidant activity of *Asimina triloba* genotypes evaluated by the DPPH method (Figure 2) ranged from 2.84 (AzT-01) to 7.04 mg TEAC.g^{-1} (AzT-04). The degree of mean variability of parameters was confirmed by the variation coefficient (31.77%) in all the genotypes tested.

The antioxidant activity evaluated by the molybdenum reducing antioxidant power (Figure 3) varied from 97.25 (AzT-06) to 275.41 mg TEAC.g^{-1} (AzT-03). The degree of mean variability of parameters was confirmed by the variation coefficient (35.07%) in tested genotypes.

Nam et al. (2017) from methanolic extracts fruits obtained with DPPH method found the antioxidant activity to be 4.18 mg.mL^{-1}. **Donno et al. (2014)** found the total antioxidant activity from 889.6 to 1519.6 mg.kg^{-1}. **Kobayashi et al. (2008)** in ripe fruits from 15.57 to 17.04 mmol TE.g^{-1} FW. Antioxidant activity of *Asimina triloba* fruits could be influenced by the degree of maturity and cultivars **(Kobayashi et al., 2008; Donno et al., 2014)**. Since there are differences in genotypes of *Asimina triloba* cultivars grown under different geographic and agroecological conditions, our results are difficult to compare with the previously reported.

Total polyphenol, flavonoid, and phenolic acid content

The total polyphenol content (Figure 4) in *Asimina triloba* genotypes ranged from 22.13 (AzT-05) to 37.36 mg GAE.g^{-1} (AzT-02). The variation coefficient (16.87%) confirms the high variability of the parameter.

In our mind, the differences between the present and previously conducted studies may be attributable to the plant geographical origin as well as the different methods of extraction.

The total flavonoid content (Figure 5) varied from 15.10 (AzT-05) to 32.02 mg.g^{-1} QE (AzT-02). The variation coefficient (25.39%) supported the observations on high variability of this parameter.

The total phenolic acid content was found to vary significantly among the various *Asimina triloba* genotypes (Figure 6), which may be due to their different botanical and regional origins. The mean total phenolic acid of the studied fruits genotypes was 25.16 mg.g^{-1} CAE, with the highest phenolic acid recorded by genotypes AzT-02 at 32.02 mg.g^{-1} CAE, indicating its superior antioxidant potential. The variation coefficient (25.39%) supported the observations on high variability of this parameter. **Kobayashi et al. (2008)** found in ripe fruits the total phenolic content from 64.11 to 98.42 mg.g^{-1} GAE.

Correlation analysis

Correlation analysis was used to explore the relationships between the individual polyphenols, phenolics, flavonoids compounds and antioxidant capacities (DPPH and MRP methods) measured for all fruit extracts from six *Asimina triloba* genotypes (Table 2). It was observed a strong linear correlation between the polyphenol and flavonoid contents (r = 0.976).

CONCLUSION

Asimina triloba is a new introductive species in the European conditions. Originally it was used for decorative purposes. Nowadays it uses more widespread in many European countries as promising fruit plant. Fruits have specific taste, aroma, energy, therapeutic and pharmaceutics properties and uses. Presented results of this study showed

that *Asimina triloba* was well adapted in the conditions of Nitra in the Slovak Republic. All genotypes produce fruits comparable with foreign cultivars by morphological parameters and also by the content of biologically active compounds. Thus, this species is potential for propagation and practice used in the Slovak Republic.

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