

## MORPHOLOGICAL CHARACTERISTICS AND DETERMINATION OF VOLATILE ORGANIC COMPOUNDS OF *DIOSPYROS VIRGINIANA* L. GENOTYPES FRUITS

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### ABSTRACT

The aim of this study was to determine morphometric differences of fruits and seeds between 19 selected American persimmon (*Diospyros virginiana* L.) genotypes. The genotypes of American persimmon have been growing more than 15 years in Forest-Steppe of Ukraine in the M.M. Gryshko National Botanical Garden of NAS of Ukraine. They are well adapted to the climatic and soil conditions. The fruits were collected at the period of their full maturity (October). The population differs in a weight, shape, size and color of fruits and seeds. Their morphometric parameters were following: fruit weight from 2.30 to 81.30 g, fruit length from 8.84 to 49.73 mm, fruit width from 12.84 to 55.34 mm, seed weight from 0.1 to 1.0 g, seed length from 8.30 to 20.88 mm, fruit width from 7.04 to 14.88 mm, seed thickness from 1.98 to 7.09 mm and number of seeds in the fruit from 1 to 8. Partenocarpic fruits are found in some genotypes. The shape indexes of fruits and seeds were found ranging from 0.74 to 1.10 and from 1.01 to 1.77, respectively. During the analysis of qualitative composition and quantitative content of volatiles of fruits from the 4 perspective genotypes of *Diospyros virginiana* 106 compounds were detected. From them 83 compounds were identified. The identified compounds belong to alcohols, saturated and unsaturated aldehydes, ketons, fatty acids, esters, terpenoids etc. The fruits are rich in fat acids. The outcome of the research points to the fact that the genepool of Ukrainian *Diospyros virginiana* is a rich source of genetic diversity and might be used in selection for creation a new genotypes and cultivars.

**Keywords:** American persimmon; fruits; seeds; morphometric characteristics; volatile compounds; fatty acids

### INTRODUCTION

Species of the genus *Diospyros* spp. belong to the family Ebenaceae Gürke (Wallnöfer, 2001). In Europe, the considerable interest of national economy to constitute such kinds of as: *Diospyros kaki* L. (Japanese persimmon) and *Diospyros virginiana* L. (American persimmon), fruit plants as food, *Diospyros lotus* L. (Date plum) – as rootstock, *Diospyros virginiana* – as a rootstock and as a source of high winter hardiness at the hybridization (Bellini, 2002; Grygorieva et al., 2009). The natural range of *Diospyros virginiana* includes the eastern part of North America from Connecticut to Iowa and from Kansas to Florida (Fletcher, 1915; Skallerup, 1953; Holdeman, 1998). Today more than 200 cultivars of *Diospyros virginiana* exist and their fruits have differences in fruits shape, size, color and ripening (Hague, 1911; Spongberg, 1979; Goodell, 1982; Zohary, 2004).

Archaeological and historical records provide evidence of the extensive use and management of American persimmons by Native Americans historically, yet the species is not viewed as a rare, weedy, wild fruit tree that is known primarily by hobbyists and wild harvesters (Ross

et al., 2014). The *Diospyros virginiana* is of great practical interest for fruit growing. In addition, the American persimmon is a valuable decorative and medicinal plant. For the last years was derived good cultivars of *Diospyros virginiana* and some of them are superior the best cultivars of *Diospyros kaki* (Spongberg, 1979; Vitkovsky, 2003; Grygorieva et al., 2011). *Diospyros virginiana* since ancient times are used in the folk medicine (Hamel and Chiltoskey, 1975; Mallavadhani et al., 1998; Foster and Duke, 1999; Briand, 2005). The fruit has been used medicinally as antiseptic and for the treatment of burns, diphtheria, dropsy, diarrhea, gonorrhoea, candidiasis, dysentery, fevers, thrush, fungal and bacterial infections, gastrointestinal bleeding, sore throats (Briand, 2005). Fruits exhibit the antimicrobial and antifungal activities (Rashed et al., 2014).

Also, the persimmon fruit can be considered as a highly nutritional product because of its strong antioxidant capacity induced by a high content of flavonoids, vitamin C, beta-carotene (Oz and Kefalas, 2010; Priya and Nethaji, 2014a).

Biologically active compounds are not only in fruits but in different parts of the plant: bark, wood, leaves, roots. The bark has an antiseptic properties (Briand, 2005) and hepatoprotective and antipyretic activity (Priya and Nethaji, 2015b; 2015c), the leaves showed antimycobacterial effect (Charley et al., 1999; Isfahani et al., 2014) and hepatoprotective and antipyretic activity (Priya and Nethaji, 2015b; 2015c), the roots of American persimmon showed antifungal effect (Wang et al., 2011). Powder of dry leaves has long been used in folk medicine. Shukla et al. (1989) in the leaves of *Diospyros virginiana* found lupeol, betulin, betulinic acid – components that are famous with antitumor properties.

nWegl et al. (2016) published, that some natural compounds could have positive antibacterial effect in pig nutrition. Naphthoquinones from the leaves exhibited fungicidal activity against *Saccharomyces vini*, *Candida mycoderma*, *Hansenula anomala*. Bactericidal activity was found against lactobacilli *Lactobacterium plantarum*, *L. breve*, *L. gracile* and relatively against to the acetic acid bacteria *Acetobacter aceti*, *A. xylinum*, *A. ascendens*, *A. rancens*. It was established by that the antimicrobial activity it exceeds the sulfuric anhydride which is widely used as antiseptic and preservative (Richter, 2001).

Some authors pointed out that the leaves extracts can be a potential source for new antimalarial agents (Trigg and Kondrachine, 1998; Ozbilgin et al., 2016).

The results of Priya and Nethaji (2014d) have showed that the ethanolic extracts of leaves and bark of *Diospyros virginiana* normalize the serum level of markers enzymes AST, ALT, GGT and ALP, bilirubin level in CC<sub>4</sub> induced hepatotoxicity.

The leaves of *Diospyros virginiana* by the biochemical composition have the highest content of ascorbic acid and mineral compounds comparing with other species (Richter, 2001; Grygorieva et al., 2012).

The fruits of persimmon are an excellent dietary product, they are used in fresh condition and from them are prepared pastes, jams, syrups, marinades. The fruits were also used to make wine, brandy, white wine vinegar and beer (Bartram, 1772; Briand, 2005). Additionally, the by products from processing of persimmon fruits or leaves can be used in animal nutrition as a source of bioactive compounds (Herkeľ et al., 2016; Gálik et al., 2016) and thereby improve the performance of farm animals.

The aim of this study was to distinguish the best genotypes from our collections of *Diospyros virginiana*, which could be successfully grown on plantations, and to investigate their qualitative and quantitative content of volatile organic compounds of fruits.

## MATERIAL AND METHODOLOGY

### Locating trees and data collection

The objects of the research were 15-year-old plants of *Diospyros virginiana*, which are growing in the Forest-Steppe of Ukraine in M.M. Gryshko National Botanical Garden of NAS of Ukraine (NBG). Observations on the collection's forms of *Diospyros virginiana* in the period

2015 – 2016 were performed during mass fruiting. We have described 19 genotypes of *Diospyros virginiana*.

### Morphometric characteristics

Pomological characteristics were conducted with four replications on a total 30 fruits per genotypes. In the study only one plant (tree) used for per genotype.

The following measurements were taken: fruit weight (FM), in g, fruit length (FL), in mm, fruit width (FW), in mm and seed weight (SM), in g, seed length (SL), in mm, seed width (SW), in mm, seed thickness (ST), in mm, number of seeds in fruit (NS). Data, we are working with, were tested for normal distribution.

### Volatile compounds analysis

The investigation of the volatiles was conducted at the National Institute of Viticulture and Wine "Magarach" under the direction (leadership) of B.O. Vinogradov by the method of Chernohorod and Vinogradov (2006).

The distillation of volatiles of the fruits was carried out by method of Chernohorod and Vinogradov (2006). The volatiles were investigated by the method of chromatography-mass spectrometry using the chromatograph Agilent Technologies 6890 N with the mass spectrometric detector 5973 N (USA) and a capillary column DB-5 length is 30 mm and an internal diameter is 0.25 mm. The carrier gas velocity (Helium) was 1.2 mL.min<sup>-1</sup>. The injector heater temperature was 250 °C. The temperature of thermostat was programmed from 50 °C to 320 °C at the speed 4 °C. The mass spectra library NIST 05 WILEY 2007 with 470 000 spectra and AMDIS, NIST programs were used to identify the investigated compounds. The identification was conducted by comparing obtained mass spectra to mass spectra of standards. The method of internal standard used to determine the quantitative content of compounds.

### Statistical analyses

Basic statistical analyses were performed using SAS System v. 9.2 (SAS 2009); a log-rank test was used for cyclic comparisons and a Student's t test for the cut-through analysis;  $p < 0.05$  was considered to be statistically significant. The DISTRIBUTION analysis (verification of normal distribution of input data), the TREE procedure in SAS 9.2. for further detailed analysis were used. Variability of all these parameters was evaluated using descriptive statistics. Level of variability determined by Stehlíková (1998).

## RESULTS AND DISCUSSION

The weight of the whole fruit is one of significant production characteristics of plant species. Further important features of the fruit and seed are shape, size and color. These parameters of the *Diospyros virginiana* fruit varied significantly. The images of *Diospyros virginiana* fruits of various genotypes are shown on Figure 1, 2. High variability of the size, shape and color of these fruits are evident.



Figure 1 Variability in the shape of *Diospyros virginiana* L. fruits.



Figure 2 Variability in the shape of *Diospyros virginiana* L. seeds.

Table 1 The variability of some morphometric parameters of fruits and seeds for the whole collection of *Diospyros virginiana* L. genotypes from Kyiv.

Characteristics	Unit	<i>n</i>	<i>min</i>	<i>max</i>	<i>mean</i>	<i>CV%</i>
Fruit weight	g	570	2.30	81.30	33.65	68.87
Fruit length	mm	570	8.84	49.73	31.83	32.10
Fruit width	mm	570	12.84	55.34	35.87	29.84
Seed weight	g	510	0.1	1.00	0.57	30.01
Seed length	mm	510	8.30	20.88	15.01	11.55
Seed width	mm	510	7.04	14.88	11.41	15.82
Seed thickness	mm	510	1.98	7.09	4.26	25.39
Number of seeds in fruit		2161	0	8.00	4.05	41.79

Legend: *n* – number of measurements; *min*, *max* – minimal and maximal measured values; *mean* – arithmetic mean; *CV* – coefficient of variation (%).

### Morphometric characteristics

The weight of *Diospyros virginiana* fruits of present study was in the range from 2.30 (DV-17) to 81.30 (DV-09) g (Table 1). Coefficient of variation was 68.87%, which shows a very high degree of variability of fruit

weight. The fruit weight was determined in range from 9.0 to 14 g by Akhund-Zade (1957), from 9.0 to 20.20 g by Chentsova (2008), from 9.0 to 40.0 g by Surkhayev (2006). According to Grygorieva (2011) the fruit weight of American selection cultivars such as Weber, Meader, John Rick was determined as 19.71, 24.10, 26.47 g respectively.

There are genotypes, which reached minimum and maximum values in these characteristic, in Table 2.

The fruit length in our analyses was determined in the range from 8.84 (DV-15) to 49.73 (DV-09) mm. The value of the coefficient of variation was 32.10%, which shows a very high degree of variability of fruit weight. The fruit length was determined in range from 3.18 to 23.00 mm (Chentsova, 2008), in cultivars – from 27.03 to 29.55 mm (Grygorieva, 2011).

In our experiments the fruit width was determined in the range from 12.84 (DV-15) to 55.34 (DV-01) mm (Table 1). The variation coefficient (29.84%) confirmed a very high of variability within the collection. The fruit width was determined in range from 40.0 to 75.0 mm (Spongberg, 1979), from 19.0 to 51.0 mm (Halls, 1990), from 23.00 to 35.00 mm (Chentsova, 2008), in cultivars – from 33.30 to 37.71 mm (Grygorieva, 2011). There are genotypes, which reached minimum and maximum values in these characteristic, in Table 2.

The seed weight in our analyses was determined in the range from 0.1 (DV-15) to 1.0 (DV-13) g. The value of the coefficient of variation was 30.01%, which shows a very high degree of variability of fruit weight. Investigations of Grygorieva (2011) established the range of seed weight of cultivars from 0.40 to 0.57 g.

Seed length was identified in range from 8.30 (DV-08) mm to 20.88 (DV-13) mm (Table 1). The variation coefficient characterizes average degree of variability within the tested collection; genotypes reaching extreme values are listed in Table 2. Grygorieva (2011) determined the average length of the seed in the range from 12.57 to 14.75 mm. Spongberg (1979) determined the average length of the seed in the range from 11.0 to 17.0 mm.

Seed width was identified in range from 7.04 (DV-19) to 14.88 (DV-04) mm. The value of the coefficient of variation fixed the average degree of variability of this characteristic.

Seed thickness was identified in range from 1.98 (DV-06) to 7.09 (DV-04) mm. The variation coefficient (25.39%) confirmed a very high of variability within the collection.

Number of seeds in fruit was identified in range from 1 (DV-05, DV-16, DV-19) to 8 (DV-06). The partenocarpic fruits were found in both – genotypes DV-12 and DV-17. These trees were similar to other trees, that producing seeds, by morphological properties but their fruits had strong difference in shape and taste. Coefficient of variation was 41.79%, which shows a very high degree of variability.

**Table 2** The fruits and seeds variability of *Diospyros virginiana* L. genotypes from the collection.

Genotypes	n	Mean	SD	CV%	Genotypes	n	Mean	SD	CV%
	Lowest values					Highest values			
<b>Fruit weight (g)</b>									
DV-17	30	3.47	0.70	20.24	DV-03	30	62.11	6.99	11.27
DV-12	30	3.83	1.30	32.88	DV-09	30	65.16	9.14	14.02
DV-15	30	3.98	0.65	18.29	DV-18	30	68.77	7.11	10.34
<b>Fruit length (mm)</b>									
DV-12	30	15.15	1.45	9.57	DV-03	30	44.57	2.95	6.63
DV-15	30	15.16	2.93	19.34	DV-18	30	44.58	2.37	5.33
DV-17	30	15.47	1.43	9.26	DV-09	30	45.53	2.39	5.25
<b>Fruit width (mm)</b>									
DV-17	30	17.85	1.44	8.08	DV-18	30	47.15	2.40	5.10
DV-12	30	18.23	1.21	6.64	DV-03	30	47.52	2.29	4.82
DV-15	30	18.59	2.72	14.65	DV-19	30	47.89	2.20	4.60
<b>Seed weight (g)</b>									
DV-15	30	0.14	0.02	16.48	DV-04	30	0.72	0.08	11.18
DV-07	30	0.45	0.07	16.10	DV-03	30	0.73	0.18	25.47
DV-05	30	0.50	0.11	21.94	DV-13	30	0.78	0.10	14.04
<b>Seed length (mm)</b>									
DV-16	30	12.63	0.69	5.49	DV-10	30	16.34	0.87	5.34
DV-06	30	13.35	1.03	7.78	DV-11	30	16.46	0.78	4.74
DV-07	30	14.34	0.85	5.96	DV-13	30	19.00	1.03	5.47
<b>Seed width (mm)</b>									
DV-15	30	8.62	0.77	8.98	DV-01	30	12.70	1.43	11.30
DV-19	30	8.89	0.65	7.39	DV-06	30	13.18	1.57	11.94
DV-14	30	8.911	0.42	4.76	DV-04	30	13.41	0.62	4.69
<b>Seed thickness (mm)</b>									
DV-15	30	2.75	0.19	6.97	DV-13	30	5.17	0.59	11.58
DV-06	30	2.81	0.38	13.56	DV-16	30	5.33	0.52	9.76
DV-14	30	2.90	0.17	6.03	DV-04	30	5.54	0.81	14.68
<b>Number of seeds in fruit</b>									
DV-16	30	2.73	1.50	55.13	DV-01	30	4.86	1.04	21.40
DV-18	30	3.26	0.82	25.33	DV-07	30	5.40	0.81	15.06
DV-10	30	3.36	0.49	14.55	DV-06	30	6.33	1.39	22.07

Note: n – number of measurements; mean – arithmetic mean; SD – standard deviation; CV – coefficient of variation (%).

The number of seeds in fruit was determined in range from 4 to 8 by **Akhund-Zade (1957)**, from 1 to 9 by **Surkhayev (2006)**, from 1 to 5 by **Chentsova (2008)**.

The shape of each object can be characterized by the shape index, i.e. the length to width ratio. Figure 3 represents the shape indexes of fruits and seeds. The shape index of the fruits was found in the range from 0.74 (DV-06) to 1.10 (DV-11), so the genotype's collection demonstrates significant variability in the shape of the fruit, as seen in Figure 1. The shape index of the seed was found in the range from 1.01 (DV-06) to 1.77 (DV-11). These parameters can be used for the identification of the genotypes.

The analysis of coefficient of variation showed the difference of variability of morphological signs between *Diospyros virginiana* samples. Data showed that the most variability of important selection signs are the number of seeds in fruit – from 8.97 (DV-15) to 55.13 (DV-16) %, fruit weight – from 9.00 (DV-14) to 48.18 (DV-01) %, seed weight – from 9.34 (DV-16) to 25.47 (DV-03) % and fruit width – from 3.60 (DV-04) to 22.40 (DV-01) %. These results indicate the promise of breeding in this way of investigations. The stable signs are seed length – from 4.51 (DV-01) to 10.60 (DV-08) % and seed width – from 3.12 (DV-05) to 11.94 (DV-06) % (Figure 4).

The results indicated high correlations between the fruit weight and the fruit length ( $r = 0.962$ ), fruit width ( $r = 0.948$ ), seed width ( $r = 0.584$ ) (Table 3). Slight correlation was found between the fruit weight and the

number of seeds in fruit ( $r = 0.353$ ), seed length and the seed thickness ( $r = 0.283$ ). It was noticed no correlation between the seed width and the seed thickness ( $r = 0.027$ ), fruit weight and the seed thickness ( $r = 0.079$ ). There was a negative correlation between the seed length and the seed width ( $r = -0.242$ ) and fruit weight and the seed length ( $r = -0.271$ ). The results document that between specific characteristics is positive relationship which is very important in *Diospyros virginiana* breeding.

The genetic relationship among the 19 genotypes of *Diospyros virginiana* (Figure 5) was examined by cluster analysis. Dendrogram has showed 2 main group in cluster A and cluster B. Three of 19 genotypes were included in cluster A group no. 1 and 6 genotypes in group no. 2. Seventh of 19 genotypes were included in cluster B group no. 1 and 3 genotypes in group no. 2. The group no. 2 of cluster A and group no.1 of cluster B had the highest mean for fruit morphometric characteristics (weight, length, width), that were significantly different. The results of this assessment related to group 1 of cluster A and group 2 of cluster B had the lowest mean of fruit morphological parameters.

The genetic relationship among 17 genotypes of *Diospyros virginiana* was examined by cluster analysis (Figure 6). Dendrogram has showed two main groups. Tenth of the 17 genotypes were included in cluster group A and seventh were included in cluster group B. The group A had the highest mean than the group B for seed morphometric characteristics (weight, length, width, thickness). So, group A was significantly different with

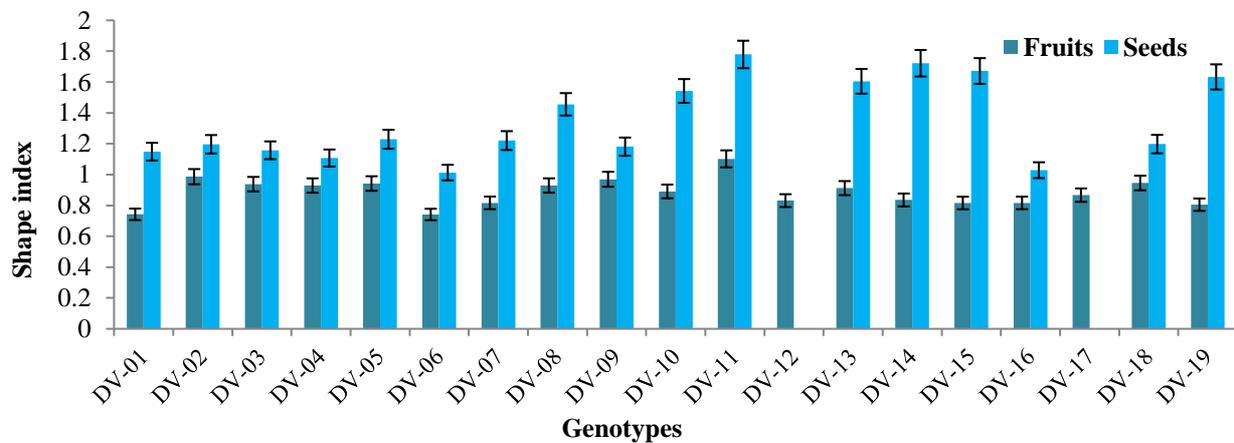


Figure 3 Comparison of the tested *Diospyros virginiana* L. genotypes in the shape index of fruit and seeds.

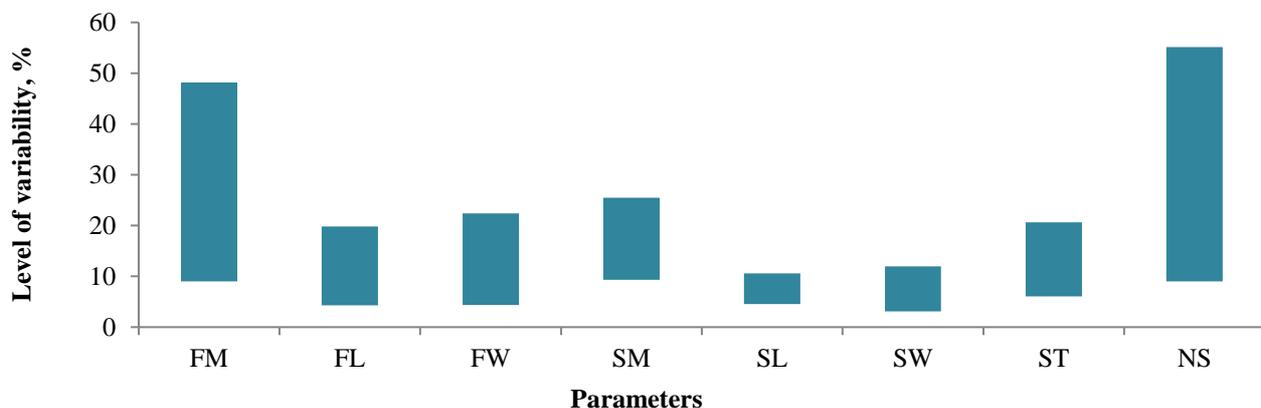


Figure 4 Level of variability of morphological characters of fruits and seeds *Diospyros virginiana* L. (%).

**Table 3** The linear relationship between of the morphometric characteristics of evaluated genotypes of *Diospyros virginiana* L.

Characteristic	r	sr	Confidence Interval r <sub>95%</sub>	r <sup>2</sup>	t	p
F. weight / F. length	0.962	2.803	0.9027 ≤ r ≤ 0.9857	0.926	14.598	0.000
F. weight / F. width	0.948	3.400	0.8681 ≤ r ≤ 0.9803	0.899	12.330	0.000
F. weight / Number of seeds in fruit	0.353	0.506	-0.1197 ≤ r ≤ 0.6961	0.125	1.559	0.137
F. weight / S. length	-0.271	1.324	-0.6459 ≤ r ≤ 0.2087	0.073	1.161	0.261
F. weight / S. width	0.584	1.276	0.1774 ≤ r ≤ 0.8208	0.341	2.970	0.008
F. weight / S. thickness	0.079	0.890	-0.3889 ≤ r ≤ 0.5150	0.006	0.328	0.746
S. length / S. width	-0.242	1.526	-0.6278 ≤ r ≤ 0.2376	0.059	1.032	0.316
S. length / S. thickness	0.283	0.856	-0.1961 ≤ r ≤ 0.6535	0.080	1.218	0.239
S. width / S. thickness	0.027	0.893	-0.4322 ≤ r ≤ 0.4757	0.000	0.112	0.911

Legend: r – Pearson’s correlation coefficient, sr – standard error of the coefficient, min/max – 95% confidence interval for r, r<sup>2</sup> – coefficient of determination, p – significance level.

other group.

The figures clearly identified significant differences between tested *Diospyros virginiana* genotypes. Figures confirm the results from the evaluated variability of morphometric characteristics (Table 1).

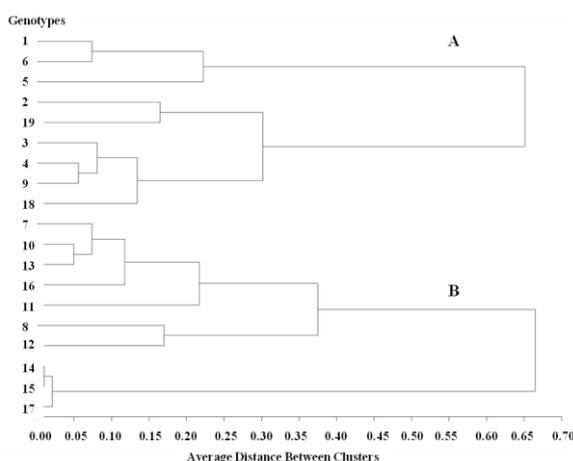
**Volatile organic compounds**

The chromatogram of volatiles of fruits of selected genotypes of persimmon is represented on Figures 1 – 4.

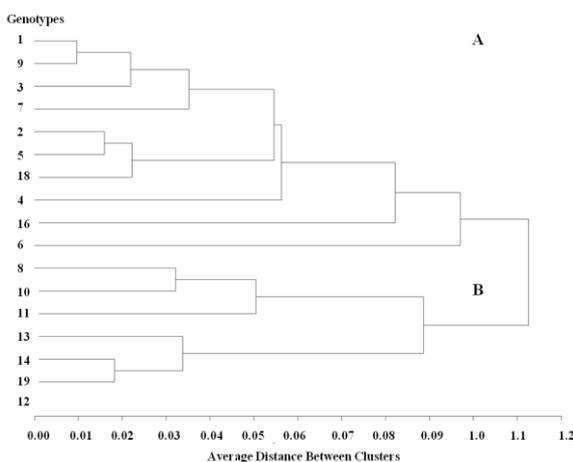
Qualitative composition and quantitative content of identified substances of investigated objects are

represented in Table 4.

It was established that the fruit of DV-03 genotype contained 48 substances, among which were identified 42 substances, genotypes DV-09 – 63 and 52, genotypes DV-18 – 55 and 43, genotypes DV-19 – 46 and 43, respectively. The identified components belong to different chemical classes, including hydrocarbons, alcohols, aldehydes and phenylaldehydes, terpenes, esters and fatty acids.



**Figure 5** Dendrogram of 19 genotypes of *Diospyros virginiana* L. based on morphometric characteristics of fruits.



**Figure 6** Dendrogram of 17 genotypes of *Diospyros virginiana* L. based on morphometric characteristics of seeds.

Table 4 The volatiles of fruits of selected genotypes of *Diospyros virginiana* L.

Component	DV-03		DV-09		DV-18		DV-19	
	1	2	1	2	1	2	1	2
Isoamyl alcohol	0.8	2.45		–		–		–
Acetoin	1.2	2.75		–		–		–
Isobutyl isobutyrate	1.2	3.4		–		–		–
*		–	0.4	3.74		–		–
*		–	0.6	3.97		–		–
Isobutyric acid		–	0.8	4.07		–		–
Butyric acid		–	0.4	4.72		–		–
2-methyltetrahydrofuranone-3		–		–		–	1.0	4.62
Furfural	1.0	5.56	1.4	5.6	1.5	5.61	5.9	5.57
*		–	0.4	6.47		–		–
Heptanol-4	0.7	5.79		–		–		–
Ethyl 3-hydroxybutyrate	2.7	6.88	1.5	6.9	3.9	6.92	0.9	6.88
Caproic acid	0.7	7.24	6.4	7.57	2.8	7.49	1.4	7.33
Furyl methyl ketone		–		–	0.5	7.58	0.5	7.55
Benzaldehyde		–		–		–	0.7	9.2
3- (methylthio) propanal		–		–	0.3	7.83		–
5-Methylfurfurol		–	0.1	9.49	0.5	9.5	0.4	9.50
Octanol	0.8	9.58	0.8	9.6		–	0.2	9.61
Cis-linaloolene oxide		–	1.8	9.82	3.2	9.84	1.4	9.82
Heptanoic acid		–	0.9	10.23	0.9	10.22		–
Trans-linalool oxide	1.3	9.81		–		–		–
Linalool	18.1	10.65	10.0	10.63	7.3	10.63	11.5	10.64
Ethyl 2-ethyl-3-hydroxybutyrate	1.1	10.87		–		–		–
2-ethyl-propanoic acid		–	0.6	11.02		–		–
Nonanal	1.7	11.01	1.8	11.20		–	0.9	11.02
Ho-trienol		–	0.4	11.35	0.4	11.35	0.3	11.36
Benzyl alcohol		–	0.6	11.61	1.2	11.62	0.6	11.62
Phenylacetaldehyde	5.3	12.26	9.1	12.27	13.3	12.29	15.9	12.31
Iodo-2-methylundecane		–	0.8	12.6		–	0.5	12.59
6-methyl-3,5-heptadien-2-one		–		–		–	0.2	12.97
2-nonen-4-one	0.8	12.49		–		–		–
Caprylic acid	2.5	13.42		–	4.1	13.52	3.4	13.50
Terpinene-4-ol		–		–	0.6	13.96		–
Decanal	0.5	14.2	1.3	14.26		–	0.8	14.25
B-phenylethyl alcohol		–	1.5	14.33	3.9	14.3		–
Epoxylinolene		–	1.3	14.38	1.9	14.35	2.3	14.37
Cis-epoxyalinalool	0.6	14.35		–		–		–
$\alpha$ -terpineol	1.9	14.75	2.4	14.8	3.0	14.78	1.3	14.78
Nerol	0.6	15.55		–	0.9	15.58		–
*		–	0.8	15.04		–		–
Nonanoic acid	3.4	16.54	3.1	16.56		–	2.1	16.46
Geraniol	1.6	16.62	1.4	16.63	1.6	16.64	0.8	16.63
*		–		–	3.0	18.59		–
Tetradecane		–		–		–	1.6	16.74
Tetradecene-7		–	1.0	17.08		–	0.3	17.08
Trans-2,4-decadiene		–		–		–	0.1	19.05
Benzonitrile	1.4	16.69		–		–		–
Capric acid	10.2	19.5	5.6	19.52	5.1	18.59	4.7	19.36
*		–		–	2.3	19.86		–
Tetradecanal		–		–		–	0.3	20.04
$\alpha$ -ethylidene phenylacetaldehyde		–		–		–	0.2	20.11
Geranylacetone		–		–		–	0.6	21.78
Eugenol		–	0.5	21.16	1.0	21.17		–
Hexadecene-1		–	0.4	21.24		–		–
Hexadecane		–	0.2	21.60	2.2	21.62		–
*		–	1.3	21.79	1.0	21.79		–
*		–		–	8.2	22.81		–
Hexadecene-8		–	1.0	21.83		–		–
Tetradecanal		–	0.4	22.07		–		–
B-ionone		–	0.5	22.88		–		–

Table 4 (continue) The volatiles of fruits of selected genotypes of *Diospyros virginiana* L.

Component	DV-03		DV-09		DV-18		DV-19	
	1	2	1	2	1	2	1	2
Ethyl laurate	0.8	23.27	1.1	23.25	1.6	23.27	1.3	23.25
Lauric acid	38.6	23.57	37.8	23.52	49.7	23.61	11.6	23.37
*	–	–	–	–	7.0	23.63	–	–
*	–	–	–	–	1.8	24.11	–	–
*	1.0	24.31	–	–	1.0	21.79	–	–
6-Phenyl-dodecane	–	–	–	–	2.8	25.3	–	–
5-Phenyl-dodecane	–	–	–	–	3.1	25.41	–	–
4-Phenyldodecane	–	–	–	–	2.0	25.58	–	–
*	–	–	–	–	4.5	25.84	–	–
3-Phenyldodecane	–	–	–	–	2.6	25.94	–	–
Hexadecanal	–	–	0.7	23.80	–	–	0.6	23.80
*	–	–	0.8	24.32	–	–	–	–
Tridecanoic acid	–	–	0.6	24.77	–	–	–	–
Octadecanal	–	–	1.3	25.32	–	–	1.2	25.32
Methyl myristate	0.6	25.33	–	–	–	–	–	–
Ethylmyristate	3.6	26.21	2.3	26.21	8.5	26.23	1.0	26.21
Myristic acid	101.0	26.61	153.3	26.63	234.6	26.77	89.7	26.54
4-Phenyltridecane	–	–	–	–	5.2	26.98	–	–
3-phenyltridecane	–	–	–	–	1.5	27.33	–	–
Hexahydrofarnesylacetone	0.7	26.79	–	–	–	–	–	–
*	0.6	26.95	–	–	–	–	–	–
Pentadecanoic acid	1.7	27.58	0.9	27.61	5.6	27.69	2.1	27.56
*	–	–	–	–	–	–	1.6	28.04
*	0.6	27.78	3.5	27.79	4.1	27.81	–	–
*	1.0	28.03	4.6	28.05	3.5	27.93	–	–
*	0.6	28.33	3.8	28.35	4.6	28.05	–	–
*	–	–	–	–	3.3	28.35	–	–
Ethyl palmitate	0.9	28.73	2.8	28.74	2.2	28.76	0.9	28.73
Ethyl palmitoleate	3.3	28.79	3.2	28.80	7.1	28.82	1.6	28.79
Palmitic acid	43.3	29.02	115.2	29.19	125.7	29.21	93.9	29.1
Palmitoleic acid	17.0	29.09	35.0	29.20	64.6	29.27	24.0	29.16
*	–	–	–	–	–	–	1.6	29.21
*	–	–	–	–	3.9	29.52	1.8	29.25
11-hexadecenoic acid	3.1	29.14	6.0	29.27	–	–	22.1	29.55
*	–	–	5.1	29.48	–	–	–	–
Methyl 7,10,13-hexadecatrioate	3.4	29.25	–	–	–	–	–	–
7,10,13-hexadecatrienic acid	18.6	29.51	33.4	29.64	33.4	29.68	–	–
Stearic acid	–	–	4.9	31.15	–	–	–	–
Oleic acid	2.2	31.12	2.7	31.21	1.2	31.2	–	–
Linoleic acid	1.4	31.32	2.2	31.36	–	–	–	–
Ethylene-indolate	1.5	31.5	1.0	31.51	2.5	31.52	–	–
Linolenic acid	6.4	31.68	16.5	31.73	10.6	31.72	3.9	31.66
*	0.6	36.11	–	–	–	–	–	–
Pentacosane	–	–	1.5	33.06	–	–	–	–
Heptakosan	–	–	0.9	34.96	–	–	–	–
*	–	–	1.2	37.29	–	–	–	–
Squalene	2.1	37.4	2.9	37.41	3.7	37.44	3.1	37.41

Among the fatty acids of genotype DV-03 fruits were found 32% of myristic acid, 14% of palmitic acid, and 12% of lauric acid; genotype DV-09 – 30, 23, 7%, respectively, genotype DV-18 contained 35% of myristic acid, 19% of palmitic acid, and 10% palmitoleic acid; genotype DV-19 contained 29% of palmitic acid, 28% of myristic acid, and 7% of palmitoleic acid. Palmitic acid was identified as minor constituents by Horvat et al. (1991).

Among other saturated fatty acids were identified butyric, caproic (hexanoic), caprylic (octanoic), nonanoic, capric (decanoic), lauric (dodecanoic), tridecanoic, pentadecanoic ones. Also we identified that some genotypes contain stearic acid  $C_{17}H_{35}COOH$  and its unsaturated derivatives: oleic acid  $C_{17}H_{33}COOH$  (one double bond), linoleic acid  $C_{17}H_{31}COOH$  (two double bonds) and linolenic acid  $C_{17}H_{29}COOH$  (three double bonds). Latter they were identified in all genotypes.

In present study among alcohols isoamyl alcohol and heptanol-4 (DV-03), octanol (DV-03, DV-09, DV-19), benzyl alcohol (DV-09, DV-18, DV-19) were identified. Saturated aliphatic aldehydes nonanal and decanal were identified in the three genotypes DV-03, DV-09 and DV-19. Phenylacetaldehyde was identified in all genotypes. Some genotypes also contained long straight-chain aliphatic saturated aldehydes such as tetradecanal, hexadecanal and octadecanal.

According to **Besada (2013)**, the high accumulation of phenylacetaldehyde and lipid-derived aldehydes are related with loss of astringency of fruits. Regarding to the previously described volatile compounds of the *Diospyros kaki*, **Besada et al. (2013)** benzyl alcohol and some related compounds such as acetaldehyde, hexanol-1, 3-methyl-1-heptanol, 1-undecanol, and aliphatic saturated and unsaturated aldehydes such as hexanal, heptanal, octanal, decanal, (E)-2-octenal, (Z)-2-nonenal, (E)-2-decenal, (E,E)-2,4-heptadienal were identified. **Taira et al. (1995)** identified such volatile compounds of astringent *Diospyros kaki* fruits as n-butanol, hexanol-1, (Z)-3-hexen-1-ol, 2-methyl hexanol, acetoin and acetic acid.

Flavour and aroma are important quality features in persimmon fruits. Flavour is formed by the combination of sweetness and sourness from carbohydrates, organic acids and aroma volatile compounds (**Besada et al., 2013**). In general, fruit volatile compounds refer to aliphatic esters, alcohols, aldehydes, ketones, lactones, terpenoids (monoterpenes, sesquiterpenes) and apocarotenoids. Fatty acids are the major primary precursor substrates of many character-impact aroma compounds in most fruits. Aliphatic alcohols, aldehydes, ketones, organic acids, esters and lactones, ranging from C<sub>1</sub> to C<sub>20</sub>, are all derived from fatty acid precursors through three key biosynthetic processes:  $\alpha$ -oxidation,  $\beta$ -oxidation and the lipoxygenase pathway. Sensor analysis is used for the estimation of ripening stage and storage life of *Diospyros kaki* fruits (**Baietto and Wilson, 2015**). Among the identified volatiles responsible for flavour in this study were linalool,  $\alpha$ -terpineol and geraniol in all the genotypes and nerol in DV-3 and DV-18, terpinene-4-ol in DV-18 genotype. They all belong to terpenoids. Geraniol and nerol have a rose odour, nerol has a weaker odour (**Acree and Arn, 2004**). As was stated by **Martineli et al. (2013)** the volatiles from *Diospyros kaki* were mainly represented by terpenes hydrocarbons, followed by straight-chain esters.

The fact that less part of identified volatile compounds in American persimmon flesh in this study were reported by other scientists could be explained, first of all by the absence of available studies for *Diospyros virginiana*. Therefore, our results were compared with studies performed for *Diospyros kaki*.

## CONCLUSION

The results of the experiment, which presented in this work, are consistent with the results reported earlier. In evaluating of 19 genotypes of American persimmon we determined the weight of the fruits in the range from 2.30 to 81.30 g, fruit length from 8.84 to 49.73 mm, fruit width from 12.84 to 55.34 mm, seed weight from 0.1 to 1.0 g, seed length from 8.30 to 20.88 mm, seed width from 7.04 to 14.88 mm, seed thickness from 1.98 to 7.09 mm and number of seeds in fruit from 1 to 8.

The shape index of the fruits was found in the range from 0.74 to 1.10. The shape index of the seed was found in the range from 1.01 to 1.77. The most variability of important selection signs are the number of seeds in fruit – 8.97 – 55.13%, fruit weight – 9.00 – 48.18%, seed weight – 9.34 – 25.47% and fruit width – 3.60 – 22.40%.

Obtained results are important for breeding new cultivars of *Diospyros virginiana* as well as their practical use. In this study 106 volatile compounds in the fruits of *Diospyros virginiana* were detected. Among them 83 compounds were identified, which belong to alcohols, saturated and unsaturated aldehydes, ketones, fatty acids, esters, terpenoids. The fruits are rich in fat acids. They are considered as precursors of many specific aroma compounds. Aldehydes are thought to be responsible for the loss of astringency by persimmon fruits.

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### Acknowledgments:

The publication was prepared with the active participation of researchers in international network AGROBIONET, as a part of international program "Agricultural biodiversity to improve nutrition, health and quality of life" within the project ITMS 25 110 320 104 Innovation of test methods and procedures for the detection of sources of bioactive substances for the improvement of health and quality of life. Experimental activities were realized in laboratories of Excellent center for the conservation and use of agrobiodiversity at the Faculty of Agrobiolgy and Food Resources, Slovak Agricultural University in Nitra. Natalia Hudz (51600967) is thanking to the International Visegrad Scholarship Fund for the realization of research at Institute of Biodiversity Conservation and Biosafety.

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