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VARIABILITY OF MORPHOLOGICAL PARAMETERS AND DETERMINATION OF VOLATILE ORGANIC COMPOUNDS OF SWEET CHESTNUT (*CASTANEA SATIVA* MILL.) GENOTYPES FRUITS

Objective *— to select the best genotypes of sweet chestnut (Castanea sativa Mill.) by morphological properties and to investigate of the qualitative and quantitative content of volatile organic compounds in fruits of the collection of M.M. Gryshko National Botanical Garden of the NAS of Ukraine.*

Material and methods. *There was investigated the 28 genotypes of 30-year-old plants that were introduced by seeds from Czech Republic, Carpathians, Kyrgyzstan. Morphometric measurments were conducted: fruit weight, fruit length, fruit width, fruit thickness, hilum length, hilum width. The investigation of the volatiles was conducted by the method of Chernohorod and Vinohradov (2006) using cromatography-mass spectrometry. Basic statistical analyses were performed using PAST 2.17. Hierarchical cluster analyses of similarity between genotypes were computed on the basis of the Bray-Curtis similarity index. Variability of all these parameters was evaluated using descriptive statistics. Level of variability determined by Stehlíková (1998). Correlation between traits was determined using the Pearson correlation coefficient.*

Results. *Morphometric parameters were following: weight of fruits from 1.70 to 18.60 g, length — from 8.07 to 33.39 mm, width — from 16.34 to 40.95 mm, thickness — from 9.02 to 28.70 mm and hilum length — from 6.62 to 31.30 mm, hilum width — from 6.50 to 19.99 mm. Index of fruit shape and hilum was determined in range from 0.81 to 0.98 and from 1.48 to 2.03 respectively. During the analysis of qualitative composition and quantitative content of volatiles of fruits from the 2 selected genotypes 74 compounds were detected, and 27 compounds among them were identified. Identified compounds belong to hydrocarbons, aldehydes, carboxylic acids and their ethers, monoterpenes, sesquiterpenes, triterpenes.*

Conclusions. *Genotypes of seed origin were quite variable. They differ by weight, shape, size and color of fruits. The high level of variation was refered to important selection feature such as mass of fruits, which says about promising of process of selection on this direction. The most genotypes have a small mass of fruits, but certain genotypes characterized by higher sign of fruit mass. The hilum has a various shape and size that can be a diagnostic feature of cultivar characteristic.*

The outcome of the research points to the fact that the collection of Castanea sativa is a rich source of genetic diversity and might be used in selection for creation of new genotypes and cultivars. Investigated plants are promising raw for future pharmacognostic researches.

Key words: sweet chestnuts, Forest-Steppe of Ukraine, fruits, morphometric parameters, variability, volatile compounds.

Introduction

Less common fruit plants can bring significant benefits to improve nutrition and health, creating of conditions for the development of a regional economy, creation of opportunities for the development of small and medium-sized agricultural and processing enterprises, distribution and cultivation of new plant species and for the reproduction of natural resources, for practical use in bioenergetic, pharmaceutical, cosmetic and other purposes. Species that belong to these important plants are *Cornus mas* L. [25, 41], *Cydonia oblonga* Mill., *Pseudocydonia sinensis* Schneid. [26], *Zizyphus jujuba* Mill. [19, 29], *Morus nigra* L. [8], *Diospyros* spp. [24].

Chestnut (*Castanea* Mill.) has been placed in the Fagaceae family. In total, 13 Castanea species are recognized and are native to the temperate zone of the Northern Hemisphere; five in East Asia, seven in North America and one in Europe [12]. The most important of them are: *Castanea sativa* Mill. (Europe, Asia Minor, North Africa), *C. dentata* (Marsh.) Borkh. (USA), *Castanea mollissima* Blume and *C. crenata* Sieb. et Zucc. (Eastern Asia). *Castanea sativa* (sweet chestnuts) is the

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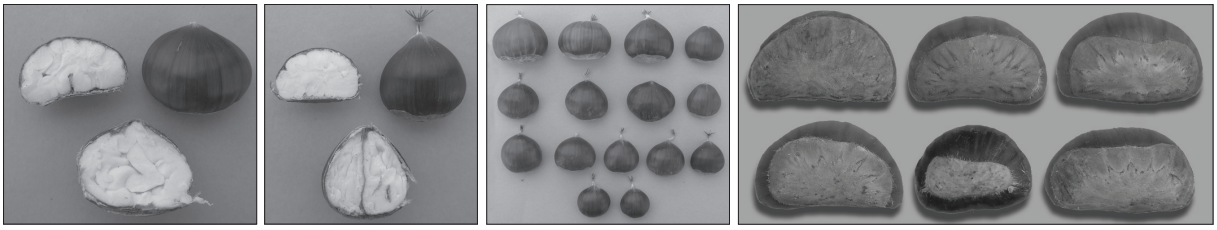

Fig. 1. Variability in the shape of sweet chestnut (*Castanea sativa* Mill.) fruits

most consumed [14]. *Castanea sativa* fruits are eaten in fresh, baked, boild, dried and smoked. Various dishes include fruits of this plant as an ingredient. Also, fruits processed into the flour. It has been used for cooking of cakes, ice-crème, sweets and other confectionery. Sweet chestnuts posses many characteristics that are used by human for different purposes, not only as a part of the food. One of them is the utilization of the sweet chesnut pollen for its pharmacological benefits [23].

Fruits *Castanea sativa* rich in carbohydrates and is a good source of essential fatty acids [26], minerals contents [8, 21], vitamins C and E [20, 46] organic acids [36], polyphenols [33]. From *Castanea sativa* fruits identified a new pyrrole alkaloid, methyl-(5-formyl-1H-pyrrole-2-yl)-4-hydroxybutyrate [1]. Cooked chestnuts are a good source of organic acids and phenolics and have low fat contents, properties that are associated with positive health benefits [27].

There are many authors who have been researching phenotypic diversity among various local populations of sweet chestnut in Italy [5, 30], France [11], Portugal [18], Spain [4, 38], Greece [2], Turkey [15, 37], Romania [9], Slovenia [42], Slovak Republic [39], Czech Republic [17], Bosnia and Herzegovina [32], Iran [7] and India [45]. This researches form basis for the selection of the best types from natural populations of sweet chestnut [10]. Most of the chestnut cultivars, used in commercial production, were obtained with selection studies from natural chestnut populations [15, 45].

The aim of this study was to select the best genotypes of *Castanea sativa* by morphological properties and to investigate of the qualitative and quantitative content of volatile organic compounds in fruits of the collection of M.M. Gryshko National Botanical Garden of the NAS of Ukraine.

Material and methods

Locating trees and data collection

The objects of the research were 30-year-old plants of sweet chestnut from seed origin, which are growing in Forest-Steppe of Ukraine in M.M. Gryshko National Botanical Garden of the NAS of Ukraine (NBG). Seeds were brought from Czech, Carpathians, Kyrgyzstan [31]. They are well adapted to the climatic and soil conditions. Observations on the collection's forms of sweet chestnut in the period of 2013—2015 were performed during mass fruiting. We have described 28 genotypes (referred as CS-01 to CS-28) of sweet chestnut. In autumn, when the nuts began to fall, a sample of 1 kg with burrs was collected from the marked trees. The harvest time was recorded.

Morphometric characteristics

Pomological characteristics were conducted with four replications on a total 30 nuts per genotypes. In the study only one plant (tree) used for per genotype. The following measurements were taken: fruit length (FL), in mm, fruit weight (FS), in g, fruit thickness (FT), in mm, fruit width (FW), in mm and hilum length (HL), in mm, hilum width (HW), in mm. The measurements were made in each nut element as shown in Fig. 1. 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" by the method of Chernogorod

and Vinohradov (2006) [22]. 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 lenght is 30 mm and an internal diameter is 0.25 mm. The carrier gas velocity (Helium) was 1.2 ml/min. The ingector heater temperature was 250 °C. The temperature of termostate was programed from 50 °C to 320 °C at the speed 4 °C. The mas spectra library NIST 05 WILEY 2007 with 470,000 spectra and AMDIS, NIST programs were used to identificate the investigated compounds. The identification was conducted by comparing obtained mas spectra to mas spectra of standarts. The method of internal standart used to determine the quantitative content of compounds.

Statistical analyses

Basic statistical analyses were performed using PAST 2.17; hierarchical cluster analyses of similarity between phenotypes were computed on the basis of the Bray-Curtis similarity index. The variability of all these parameters was evaluated using descriptive statistics. Level of variability was determined by Stehlikova (1998) [44]. Correlation between traits was determined using the Pearson correlation coefficient.

Results and discussion

The weight of the whole fruit is one of significant production characteristics of plant species. Further important features of the fruit are shape, size and color. These characteristics of the sweet chestnut fruit varied significantly. The images of sweet chestnut fruits of various genotypes are shown on Fig. 1. High variability of the size, shape and color of these fruits are evident.

Table 1. **The variability of some morphometric characteristics of fruits for the whole collection of sweet chestnut (*Castanea sativa* Mill.) genotypes from Kyiv**

Characteristic	n	min	max	$\bar{x}$	V%
Fruit weight, g	840	1.70	20.0	6.85	45.92
Fruit length, mm	840	8.07	33.39	23.74	13.74
Fruit width, mm	840	16.34	40.95	26.52	14.98
Fruit thickness, mm	840	9.02	28.70	16.62	20.57
Hilum length, mm	840	6.62	31.30	21.15	19.58
Hilum width, mm	840	6.50	19.99	12.24	20.66

Note: n — number of measurements; min, max — minimal and maximal measured values; $\bar{x}$ — arithmetic mean; V% — coefficient of variation.

The weight of sweet chestnut fruits of present study was in the range of 1.70 to 20.0 g (Table 1). Coefficient of variation was 45.92 %, which shows a very high degree of variability of fruit weight. Significant differences in fruit weight were reaffirmed a lot of authors from different countries. The fruit weight was determined in range from 2.98 to 6.07 g by Aravanopoulos et al. (2001) [6], from 2.94 to 13.40 g by Bolvansky et al. (2012) [39], from 3.50 to 18.60 g by Solar et al. (2005) [43], from 4.32 to 6.67 g by Mujić et al. (2010) [32], from 4.80 to 10.60 g by Odalovic et al. (2013) [35], from 9.00 to 15.00 g by Pereira-Lorenzo et al. (1996) [38], from 9.41 to 16.60 g by Borghetti et al. (1986) [30], from 10.26 to 39.73 g by Ormeci et al. (2016) [37]. Data comparison shows a high consistency with our results.

The fruit length in our analyses was determined in the range of 8.07 to 33.39 mm (Table 1). The value of the coefficient of variation was 13.74 %, which documented medium degree of variability of the character within the collection. The fruit length was determined in range from 16.41 to 27.75 mm [39], from 19.10 to 24.90 mm [6], from 19.60 to 30.60 mm [35], from 20.00 to 37.00 mm [43], from 20.45 to 24.89 mm [32], from 24.80 to 32.70 mm [38], from 25.80 to 31.40 mm [4], from 30.39 to 34.31 mm [15]. In case of data comparison tested genotypes from Ukraine have low values on this characteristic.

In our experiments the fruit width was determined in the range of 16.34 to 40.95 mm (Table 1). The variation coefficient (14.98 %) confirmed medium degree of variability within the collection. The fruit width was determined in range from 12.00 mm [43] to 42.47 mm [37].

In evaluated genotypes we determined the fruit thickness in the range of 9.02 to 28.70 mm (Table 1). The value of the coefficient of variation was 20.57 %, which documents a high degree of variability of the characteristic within the collection. The fruit

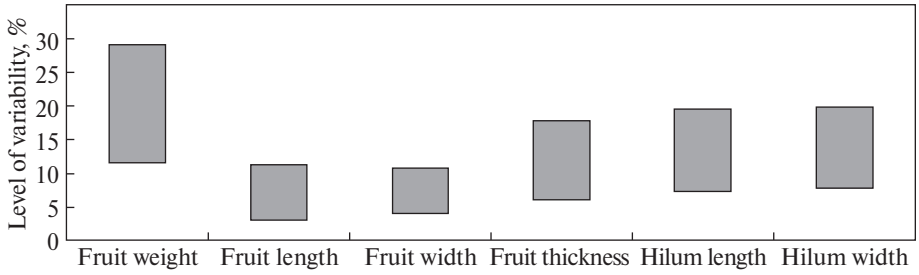

Fig. 2. Variability level according to the minimum and maximum means of a coefficient of variation (CV) depending on morphological character of sweet chestnut (*Castanea sativa* Mill.) fruit

thickness was determined in range from 10.80 mm [6] to 27.29 mm [32].

Hilum length was identified in range from 6.62 mm to 31.30 mm (Table 1). The value of the coefficient of variation documented a high degree of variability of these characteristic. The hilum length was determined in range 12.00—32.00 mm [43], 12.90—14.50 mm [6], 19.00—31.00 mm [35].

Hilum width was identified in range from 6.50—19.99 mm (Table 1). The value of the coefficient of variation documented a high degree of variability of these characteristic. The hilum width was determined in range from 6.00 mm [6] to 16.00 mm [32, 43]. The comparison of previous data shows high consistency with our results. Significant differences in morphometric characteristics were reaffirmed a lot of authors from different countries.

The analysis of coefficient of variation showed the difference of variability of morphological signs between *Castanea sativa* samples. Data showed that the most variability of important selection signs are the fruits weight — from 11.60 to 29.04 %. The other characteristics are more or less stable (Fig. 2).

The shape of each object can be characterized by the shape index, i.e. the length to width ratio. Fig. 3 represents the shape indexes of fruits and hilum. The shape index of the fruits was found in the range from 1.48 (CS-04) to 2.03 (CS-23), so the genotype's collection demonstrates significant variability in the shape of the fruit, as seen in Fig. 1. The shape index of the hilum was found in the range from 0.81 (CS-20) to 0.98 (CS-12). This parameter can be used for the identification of the genotypes.

The results of the analysis are given in Table 2. Obtained data indicated high correlations (r = = 0.63—0.94). Also, results document that between specific characteristics exists positive relationship which is very important in sweet chestnut's breeding.

The genetic relationship among the 28 genotypes was examined by cluster analysis. The figure

Table 2. **The linear relationship between morphometric characteristics of evaluated genotypes of sweet chestnut (*Castanea sativa* Mill.)**

Characteristic	r	sr	Confidence interval $r_{95\%}$	r^2
FW/FL	0.85	1.60	0.70≤r≥0.93	0.73
FW/FS	0.92	1.38	0.83≤r≥0.96	0.85
FW/FT	0.91	1.13	0.83≤r≥0.96	0.84
FW/HL	0.68	2.48	0.42≤r≥0.84	0.47
FW/HW	0.67	1.58	0.39≤r≥0.83	0.45
HL/HW	0.94	0.69	0.88≤r≥0.97	0.89
FL/FS	0.94	1.20	0.87≤r≥0.97	0.88
FL/FT	0.82	1.63	0.64≤r≥0.91	0.67
FL/HL	0.70	2.43	0.44≤r≥0.85	0.49
FL/HW	0.63	1.64	0.34≤r≥0.81	0.40
FS/FT	0.90	1.19	0.80≤r≥0.95	0.82
FS/HL	0.80	2.00	0.62≤r≥0.90	0.65
FS/HW	0.74	1.42	0.51≤r≥0.87	0.55
FT/HL	0.78	2.13	0.57≤r≥0.89	0.61
FT/TP	0.77	1.35	0.55≤r≥0.88	0.59

Note: r — Pearson's correlation coefficient; sr — standard error of the coefficient; r^2 — coefficient of determination.

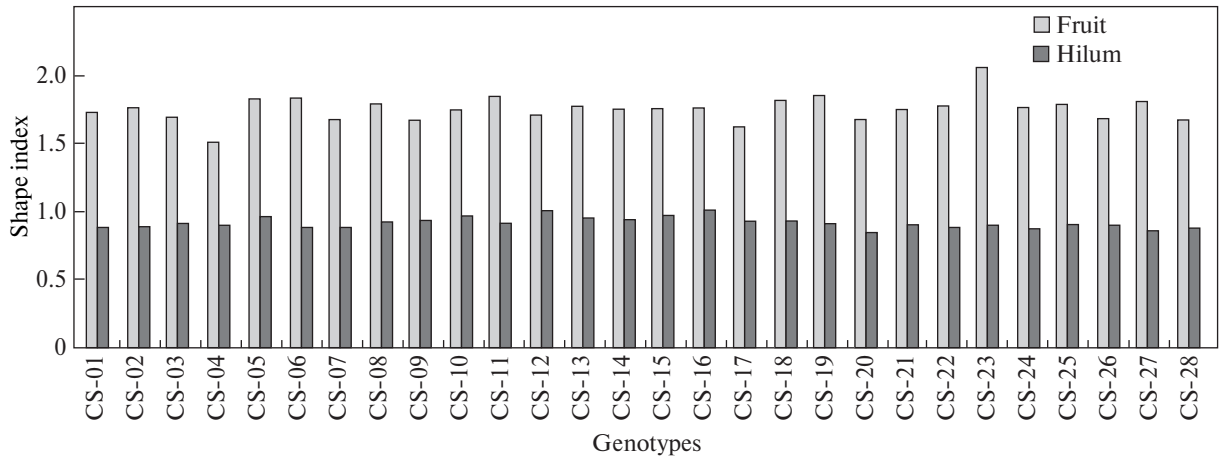

Fig. 3. Comparison of tested sweet chestnut (*Castanea sativa* Mill.) genotypes in the shape index of fruit and hilum

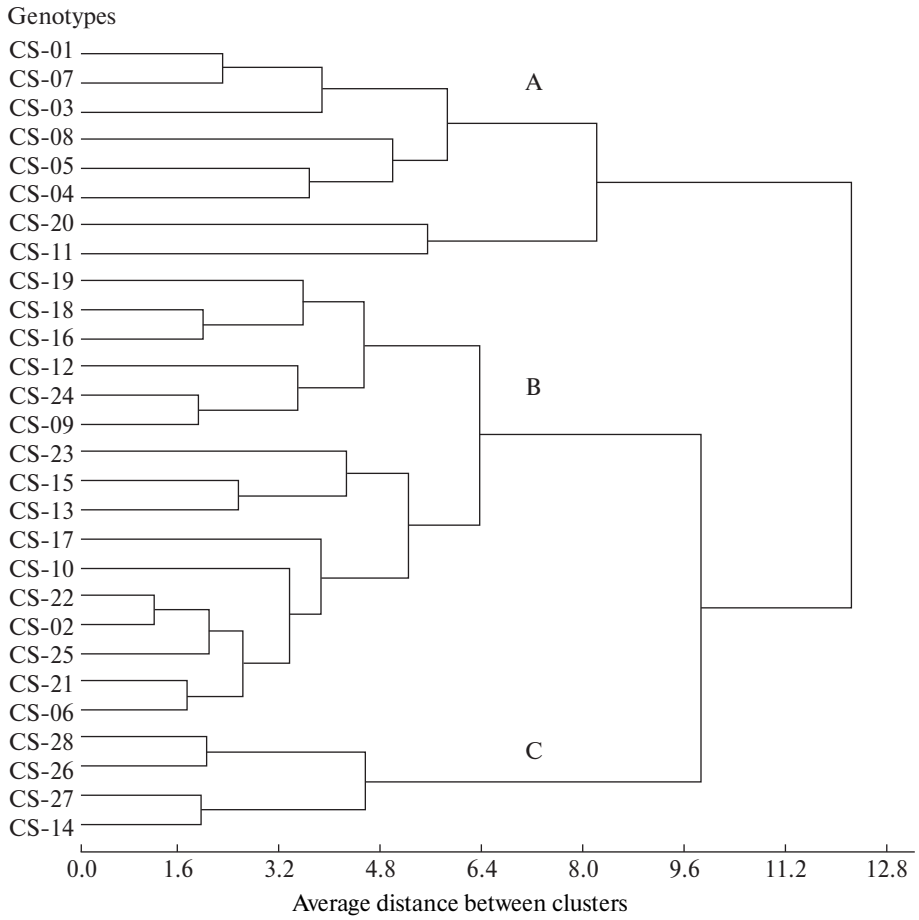

Fig. 4. Dendrogram of 28 genotypes of sweet chestnut (*Castanea sativa* Mill.) based on morphometric characteristics of fruits

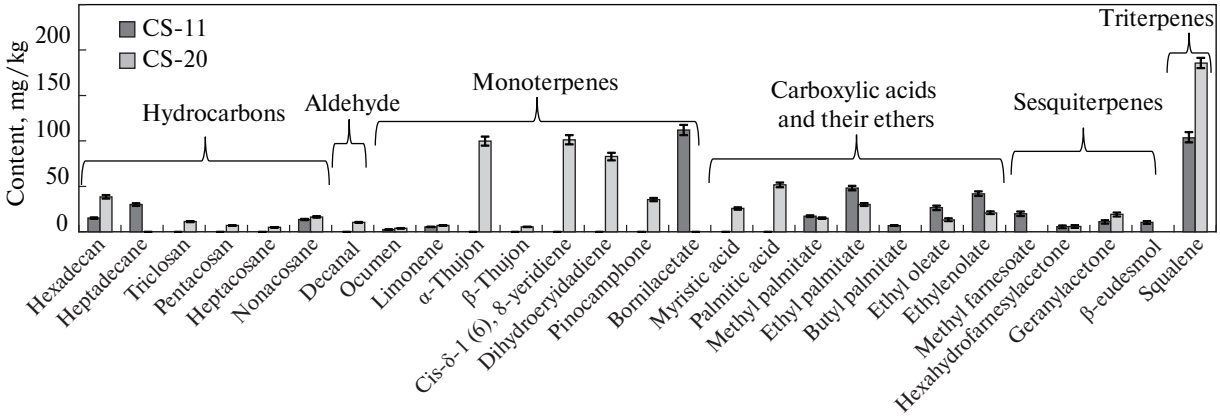

Fig. 5. The volatiles compounds of fruits of selected genotypes of sweet chestnut (*Castanea sativa* Mill.), mg per 1 kg

clearly identified significant differences between tested sweet chestnut genotypes. Dendrogram has showed 3 main groups (Fig. 4).

Eight of 28 genotypes were included in cluster group A, 16 genotypes — in group B, 4 genotypes — in group C. The group B had the highest mean for morphological characteristics (fruit weight, fruit length, fruit width, fruit thickness, hilum length, hilum width), that were significantly different with other groups. The results of this assessment related to group C had the lowest mean of morphological parameters. Figure confirms the results from the evaluated variability of morphometric characteristics (Table 1).

Qualitative composition and quantitative content of volatiles compounds of investigated objects are represented in Fig. 5.

It was established that the fruits of CS-11 genotype contained 19 substances, among which were identified 15 substances, genotypes CS-20 — 28 and 22, respectively. Among them 11 compounds only are common for investigated genotypes of sweet chestnut. The identified components belong to different chemical classes, including hydrocarbons, aldehydes, carboxylic acids and their ethers, monoterpenes, sesquiterpenes, triterpenes. Content of volatile compounds was 469.6 (CS-11) and 791.7 (CS-20) mg per 1 kg. Bornilacetate (112.0 mg per 1 kg) and squalene (103.5 mg per 1 kg) prevailed in fruits of CS-11, in fruits of CS-20 — squalene (185.6 mg per 1 kg). Hexanal (48.30—52.50 %) was the main aromatic composition as reported Silvanini et al. (2014) [3]. Differences in volatile compounds are mainly related to the cultivar [13]. By the data of Gounga et al. (2017) [16], 55 volatile organic major compounds were identified in fresh roasted chestnut. Monoterpenes and derivatives of butane, pentane, hexane, and heptane were identified as important aroma impact compounds by Krist et al. (2004) [47].

Conclusions

The results of the experiment, presented in this work, are consistent with the results reported earlier. Evaluating of 28 genotypes of sweet chestnut determined the weight of the fruits in the range from 1.70 g (CS-26) to 18.60 g (CS-20), lenght from 8.07 mm (CS-28) to 33.39 mm (CS-11), width from 16.34 mm (CS-28) to 40.95 mm (CS-11), thickness from 9.02 mm (CS-26) to 28.70 mm (CS-11) and hilum length from 6.62 mm (CS-26) to 31.30 mm (CS-07), hilum width from 6.50 mm (CS-23) to 19.99 mm (CS-07). The results about relationship between specific characteristics were indicated as a high correlation (r = 0.63—0.94). Also, presented results showed significant differences in the evaluated characteristics.

In this study 27 volatile compounds in the fruits of sweet chestnut were detected, which belong to hydrocarbons, aldehydes, carboxylic acids and their ethers, monoterpenes, sesquiterpenes, triterpenes. Bornilacetate and squalene are prevailed compounds in the fruits.

Obtained results are important for breeding new varieties of sweet chestnut as well as their practical use.

This study is significant as first selection work in Ukraine. Researches of adaptation studies will also be required for the selected sweet chestnut genotypes.

The results of the study are helpful for understanding the variability and attempting the selection of superior desirable sweet chestnut accessions for bringing to commercial cultivation.

Investigated plants are promising raw for future pharmacognostic researches.

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ВАРІАБЕЛЬНІСТЬ МОРФОЛОГІЧНИХ ПАРАМЕТРІВ ТА ВИЗНАЧЕННЯ ЛЕТКИХ РЕЧОВИН ПЛОДІВ ГЕНОТИПІВ КАШТАНА ПОСІВНОГО (*CASTANEA SATIVA* MILL.)

Мета — відібрати найкращі генотипи каштана посівного (*Castanea sativa* Mill.) за морфологічними показниками та вивчити якісний склад і кількісний вміст летких речовин у плодах рослин з колекції Національного ботанічного саду імені М.М. Гришка НАН України.

Матеріал і методи. Досліджено 28 генотипів 30-річних рослин каштана посівного насінного походження з Чехії, Карпат, Киргизстану. Вивчено морфометричні показники (маса, довжина, ширина і товщина плоду, довжина та ширина рубчика). Дослідження летких речовин проводили за методикою Черногорода та Виноградова (2006) з використанням хромато-мас-спектрометрії. Статистичний аналіз виконували за допомогою PAST 2.17. Ієрархічний кластерний аналіз подібності генотипів здійснено за індексом подібності Брей-Кертіса. Варіабельність досліджених параметрів оцінено з використанням методів описової статистики. Рівень варіабельності визначали за Stehlikova (1998). Наявність зв'язків між параметрами встановлювали за коефіцієнтом кореляції Пірсона.

Результати. Виявлено варіабельність морфометричних параметрів: маса плоду — від 1,70 до 18,60 г, довжина плоду — від 8,07 до 33,39 мм, ширина плоду — від 16,34 до 40,95 мм, товщина плоду — від 9,02 до 28,70 мм, довжина рубчика — від 6,62 до 31,30 мм, ширина рубчика — від 6,50 до 19,99 мм. Величина індексу форми плодів та рубчика становила від 0,81 до 0,98 та від 1,48 до 2,03 відповідно. При аналізі складу та вмісту летких речовин плодів двох відібраних генотипів виявлено 74 речовини, з них ідентифіковано 27 сполук (вуглеводні, альдегіди, карбонові кислоти та їх ефіри, монотерпени, сесквітерпени, три терпени).

Висновки. Генотипи насінного походження з колекції Національного ботанічного саду імені М.М. Гришка НАН України відрізняються за масою, формою, розміром і кольором плодів. Найбільший рівень мінливості виявлено у такої важливої для селекції ознаки, як маса плоду, що свідчить про перспективність селекції за цим показником. У більшості генотипів невелика маса плодів, лише в деяких цей показник є високим. Рубчик має різну форму та розмір, що можна використовувати як діагностичні ознаки для характеристики сорту. Результати дослідження свідчать про те, що колекція каштана їстівного є джерелом генетичної різноманітності та може бути використана для відбору і створення нових генотипів та сортів. Рослини каштана посівного — перспективний матеріал для фармакогностичних досліджень.

Ключові слова: каштан посівний, Лісостеп України, плоди, морфометричні параметри, мінливість.

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ИЗМЕНЧИВОСТЬ МОРФОЛОГИЧЕСКИХ ПАРАМЕТРОВ И ОПРЕДЕЛЕНИЕ ЛЕТУЧИХ ВЕЩЕСТВ ПЛОДОВ ГЕНОТИПОВ КАШТАНА ПОСЕВНОГО (*CASTANEA SATIVA* MILL.)

Цель — отобрать лучшие генотипы каштана посевного (*Castanea sativa* Mill.) по морфологическим показателям, изучить качественный состав и количественное содержание летучих веществ в плодах растений из коллекции Национального ботанического сада имени Н.Н. Гришко НАН Украины.

Материал и методы. Исследованы 28 генотипов 30-летних растений каштана посевного семенного происхождения из Чехии, Карпат, Кыргызстана. Изучены морфометрические показатели (масса, длина, ширина и толщина плода, длина и ширина рубчика). Исследование летучих веществ проводили по методике Черногорода и Виноградова (2006) с использованием хромато-масс-спектрометрии. Статистический анализ выполняли с помощью PAST 2.17. Иерархический кластерный анализ сходства между генотипами осуществлен по индексу сходства Брей-Кертиса. Вариабельность исследованных параметров оценивали с использованием методов описательной статистики. Уровень изменчивости определяли по Stehlikova (1998). Наличие связей между параметрами устанавливали по коэффициенту корреляции Пирсона.

Результаты. Выявлена вареабельность морфометрических параметров: масса плода — от 1,70 до 18,60 г, длина плода — от 8,07 до 33,39 мм, ширина плода — от 16,34 до 40,95 мм, толщина плода — от 9,02 до 28,70 мм, длина рубчика — от 6,62 до 31,30 мм, шири-

на рубчика — от 6,50 до 19,99 мм. Величина индекса формы плодов и рубчика составляла от 0,81 до 0,98 и от 1,48 до 2,03 соответственно. При анализе состава и содержания летучих веществ плодов 2 отобранных генотипов выявлены 74 вещества, из них идентифицированы 27 соединений (углеводороды, альдегиды, карбоновые кислоты и их эфиры, монотерпены, сесквитерпены, тритерпены).

Выводы. Генотипы семенного происхождения из коллекции Национального ботанического сада имени Н.Н. Гришко НАН Украины отличаются по массе, форме, размеру и цвету плодов. Наибольший уровень изменчивости выявлен у такого важного для селекции признака, как масса плода, что свидетельствует о перспективности селекции по этому показателю. У большинства генотипов небольшая масса плодов, лишь у некоторых этот показатель высокий. Рубчик имеет разную форму и размер, что можно использовать как диагностические признаки для характеристики сорта. Результаты исследования свидетельствуют о том, что коллекция каштана съедобного является источником генетического разнообразия и может быть использована для отбора и создания новых генотипов и сортов. Растения каштана посевного — перспективный материал для фармакогностических исследований.

Ключевые слова: каштан посевной, Лесостепь Украины, плоды, морфометрические параметры, изменчивость.