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Economic valuable traits of promising breeding samples and 'Chornolysta' variety of *Mentha piperita* L. after in vitro sanitation and micropropagation

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Purpose. To study the impact of clonal micropropagation and sanitation *in vitro* by viricide Ribavirin on *ex vitro* plant productivity, quantitative content and qualitative composition of peppermint essential oil components obtained from four breeding samples of peppermint plants (*Mentha piperita* L.) and the 'Chornolysta' variety. **Methods.** The study used methods of field agrotechnical one-factor experiment, essential oil distillation with water vapor according to the Ginzberg method, capillary gas chromatography and statistical analysis. **Results.** Due to the sanitation process *in vitro*, the yield of air-dried leaves of breeding samples increased by 2.9–7.1%, the 'Chornolysta' variety by 51.4% and rhizomes by 2.2–3.8% and 28.5% respectively, which amounted 0.35–2.74 t/ha. A significant increase of essential oil yield of breeding samples from 4.0 to 9.9 kg/ha was shown, and of the 'Chornolysta' variety – up to 28.6 kg/ha. After *in vitro* sanitation and clonal micropropagation of the breeding sample M 01-12, the content of essential oil was more than 4%. The following components of peppermint essential oil were identified: limonene, cineole, menton, mentofuran, isomenton, menthyl acetate, β-caryophyllene, isomenthol, menthol, pulegon, piperitone and carvone. A clear tendency to a decrease in the amount of menton and isomenthol with isomenton and menthol increase in plants, sanitated and propagated *in vitro*, was revealed. **Conclusions**. The use of tissue and organ culture methods and *in vitro* sanitation improves the qualitative composition of terpenoids by increasing the amount of menthol and menton reducing. The data obtained on the composition of terpenoids should be considered in peppermint selection as one of the integral criteria, which should be included in the list of economically valuable characteristics of peppermint plants, such as foliage, biomass of air-dried leaves, plant rhizomes and the amount of peppermint essential oil. Six indicators of the essential oil of the breeding sample M 01-02, namely citric acid, cineole, mentofuran, isomentone, pulegon, carvone, as well as the cineole / limonene ratio, meet the criteria of the European Pharmacopoeia, so it can be considered as promising for cultivation among the studied samples.

Keywords: *peppermint; Mentha piperita L., in vitro; micropropagation; productivity; essential oil, capillary gas chromatography.*

Introduction

Peppermint (*Mentha piperita* L.) is a valuable medicinal essential oil culture that is widely used in medicine, chemical, pharmaceutical, perfumery, cosmetics, food and other industries. Plants are the source for obtaining pharmaceutical leaves, essential oil and biologically active substances (BAS) [1]. Herbal medicinal raw materials of peppermint are leaves, essential oil and its components. Oil (*Menthae piperitae oleum*) and its main component menthol are the part of phyto-collections and about 40 combined medicines of domestic and foreign origin. A herb (*Herba Menthae piperitae*), leaves (*Folia Menthae piperitae*) and peppermint essential oil are used in medicines manufacturing. Peppermint herb contains about 3% of essential oil (inflorescences – up to 6%, stems – 0.4%, leaves – up to 4.8%) [2]. A peculiarity of peppermint, as an essential oil plant, is the presence of secretory structures located on the leaves, shoots and rhizomes of

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the plant, which secrete essential oil, the main components of which are monoterpenes. According to the State Register of Medicines of Ukraine, about 70 pharmaceuticals contain menthol [3]. According to the available data, about 100 biologically active substances (BAS) have been identified in the mint essential oil, among which menthol is predominant [1]. According to the requirements of the European Pharmacopoeia (EP), peppermint essential oil should contain: limonene – 1.0–5.0%, cineole – 3.5–14.0%, menthone – 14.0–32.0%, mentofuran – 1.0–9.0%, isomentone – 1.5–10.0%, menthyl acetate – 2.8–10.0%, menthol – 30.0–55.0%, pulegon – 4.0%, carvone – 1.0% and isopulegon – 0.2% [4].

The content of the essential oil depends on the varietal characteristics, time of harvesting [5], age of the plant [6], conditions of agricultural technology [7], climatic and environmental factors [8]. In addition, the quantitative and qualitative composition of the oil fluctuates significantly along the growing season [9].

Peppermint plants are affected by fungi, bacteria and viruses, which causes a decrease in yield, the amount of BAS and the quality of medicinal raw materials, often causing the death of crops of valuable genotypes [10]. The peppermint is of hybrid origin and its varieties are recommended for vegetative propagation only, since the seed does not reproduce the parent form, a decrease in its productivity is observed during prolonged cultivation. Even in conditions of well-maintained nursery management, the accumulation and transfer of pathogens with planting material occurs.

In order to meet the needs of the pharmaceutical market in high quality raw materials, it is important to maintain at optimum level qualitative and quantitative indicators of cultivated varieties, namely yield, total leaves density, the amount of essential oil and its major components, in particular menthol. Now, one of the most effective ways for obtaining quality raw materials is to improve varietal material by the technique of apical meristem and chemotherapy, based on explants *in vitro* cultivation on nutrient media with antivirals and growth regulators [11]. The effect of growth regulators on the concentration, yield and components of mint essential oil is known [12–14].

In the State register of plant varieties valuable for cultivation in Ukraine for 2019, 5 varieties of peppermint were included [15]. With the exception of the 'Lada' variety, the rest of them were created 10 years or more ago. Anthropogenic and environmental factors of the environment cause the extinction and "degeneration" of cultivated peppermint varieties. Considering the polymorphism of morphological features and component composition of the essential oil and the accumulation of pathogens during vegetative propagation, the method of clonal micropropagation for peppermint culture is promising for rapid reproduction of genetically valuable varieties, breeding specimens and plant sanitation after pathogens influence.

The relevance of this work lies in the systematic study of the effect of plant sanitation in the conditions of clonal micropropagation *in vitro* on the industrial indicators and composition of terpenoids in the essential oil of perspective peppermint samples.

The purpose of the research is to study the effect of clonal micropropagation and sanitation of peppermint (*Mentha piperita* L.) plants *in vitro* by Ribavirin virocide on the productivity of *ex vitro* plants, the quantitative content and qualitative composition of the components of mint essential oil obtained from four breeding specimens and the 'Chornolysta' variety.

Materials and methods

Four breeding samples of peppermint plants and the 'Chornolysta' variety, provided by the Experimental Station of Medicinal Plants of the Institute of Agroecology and Environment of the NAAS of Ukraine, which were tested at the final stage of the breeding process and obtained samples of their essential oil were selected as objects of study. The 'Chornolysta' variety in the pharmaceutical industry is recognized as a medical standard [16].

The methods of *in vitro* culture of isolated tissues and organs and chemotherapy were used for peppermint plants sanitation. For introduction to the culture and micropropagation of plant explants modified Murashige and Skoog (MS) nutrient media with growth regulators (0.75 mg/l 6-benzylaminopurine, 0.1 mg/l of adenine, 0.05 mg/l of indolyl-3-acetic acid, 0.5 mg/l of gibberellic acid) and for one passage of Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, Sigma-Aldrich, USA) at a concentration of 10 mg/l [17] were used.

Along 2015–2017 annually in the field, five experiments were planted in the seedling method in four repetitions in vil. Beresotocha in the Lubenskyi district of Poltava region at the territory of the Experimental station of medicinal plants of the Institute of Agro-ecology and Environment of the National Academy of Agrarian Sciences of Ukraine, in which they tested the variety of peppermint 'Chornolysta' and four promising breeding samples. Vegetatively propagated and not sanitized plants

served as a reference for sanitized by clonal micropropagation and *in vitro* chemotherapy experimental planting material. The mint seedlings used to set up the experiments corresponded to the standards of the normative document [18]. Evaluation was carried out during the period of technical suitability for planting in branching phase (second decade of May).

The plots were of five-rows. The length of one row was 10 m, the total area of the plot was 23 m^2. The distance between the plants was 20 cm (250 plants per plot). Protective strips were laid with the same selection sample or variety that was tested in the experiment. The lateral protective strips were 0.9 m (2 rows), the length of the protective stripe before and after the experiment was 2 m. Plants were selected on each plot using the linear meter method to determine leaves number, yield and chemical components of the essential oil [19]. The plant raw materials for the production of essential oil were taken in compliance with the deadlines determined by the technological regulations for peppermint in the mass flowering phase [18]. Leaves density was calculated as the ratio of the mass of the leaves to the total mint mass. The yield of rhizomes was evaluated in the autumn after the end of the growing season in that part of the area where the aboveground mass was not harvested, since its cutting worsens the quality indicators of rhizomes due to the outflow of nutrients as a result of the after-grass growth. Dug out rhizomes were separated from the mother plant, weighed and recalculated per hectare (t/ha).

Essential oil from peppermint herb was obtained by distillation with water vapor according to the method of Ginsberg [20]. The obtained mint oil was stored in the refrigerator at 4 °C. The analysis of the essential oil components was performed by capillary gas chromatography on an Agilent 7890A chromatograph with a flame ionization detector with automatic sample entry. Column: DB-WAX (Agilent) 60 m × 0.25 mm, fixed phase macrogol 20000 (0.25 μm). Carrier gas: helium 1.5 ml/min, flow separation 1:50. For chromatography, 25 μl of the essential oil was dissolved in 1.5 ml of n-hexane, injection volume: 1.0 μl. Chromatography program: the column temperature was maintained at 70 °C for 15 min, then the temperature was raised to 240 °C for 85 min and maintained for 5 min at 240 °C; sample input temperature – 250 °C, detector temperature – 270 °C.

Identification of the biochemical components was performed by comparing the chromatograms with a typical mint oil chromatogram, which was in accordance with EF standards [4]. The percentages of the components were calculated by internal normalization, the composition of the essential oil was analyzed, and the results were compared with the corresponding intact plants.

The text and tables show the arithmetic mean values (n = 10) and their standard errors (x ± SE). The results were processed statistically using the program Statistica 10.0. One-way variance analysis was used; differences between the mean values were calculated by the ANOVA method.

Results

The key features of the economic value of breeding samples and zoned peppermint varieties are the yield of above ground mass and rhizomes and the content of essential oil. It is these two components that determine the yield of essential oil per unit area and is the basis for the formation of the quantity and quality of the obtained products. An important feature on which the process of propagation and cultivation of a variety in production depends is the yield of rhizomes. In the experiments, the sanitation effect of the peppermint samples on the biochemical parameters was evaluated by the method of *in vitro* isolated tissues and organs cultivation (Table 1).

In the experimental variants, the yield of air-dry leaves after sanitation and micropropagation *in vitro* increased from 2.9 to 51.4%. However, it should be noted that promising breeding samples, even without *in vitro* culture, were characterized by high yields of air-dried leaves, which exceeded the 'Chornolysta' variety (reference) by 92.1–110%. Due to the improvement, the plant yields of the breeding samples exceeded Chornolista by 30.7–48.6%. The sanitation did not significantly affect the number of air-dried leaves obtained from breeding specimens, as their productivity increased by only 2.9–7.1%.

The yield of rhizomes in sanitised peppermint plants was higher by 2.2–28.5%. The maximum increase of this index was determined in the variety 'Chornolista' ($P \leq 0.01$), in the breeding samples M 01-02 and M 01-12 the yield of rhizomes significantly increased ($P \leq 0.05$), and in the sample M 01-04 the increase was minimal – 2.2%. The rhizome yield increased from 0.35 t to 2.74 t/ha.

The growth of yield indicators after the sanitation and micropropagation *in vitro* caused the accumulation of the amount of essential oil obtained in breeding samples from 4.0 to 9.9 kg/ha, and in the variety 'Chornolysta' – up to 28.6 kg/ha, that amounted 4.1–

Table 1

Indicators of economically valuable traits of peppermint samples under conditions of using clonal micropropagation and *in vitro* sanitation (2015–2017)

Sample, variety	Variant	Yield, t/ha		Leaves density, %	Essential oil content, %	Essential oil yield kg/ha
		Air-dry leaves	Rhisomes			
M 01-02	reference group	2.69±0.06	20.4±0.23	57	3.68±0.14	99.0
	in vitro	2.77±0.07**	21.1±0.26*	60	3.73±0.13	103.3
M 01-03	reference group	2.74±0.07	24.8±0.31	55	3.61±0.11	98.9
	in vitro	2,80±0,06*	25.4±0.37	57	3.65±0.12	102.9
M 01-04	reference group	2,87±0,06	13.9±0.17	55	3.82±0.18	109.6
	in vitro	2.99±0.08**	14.2±0.20	57	3.89±0.14	116.3
M 01-12	reference group	2.94±0.06	13.1±0.19	59	3.97±0.18	116.7
	in vitro	3.15±0.07**	13.6±0.20*	62	4.02±0.19	126.6
'Chornolysta'	reference group	1.41±0.03	9.6±0.16	33	3.76±0.15	52.6
	in vitro	2.12±0.04**	12.3±0.18**	49	3.83±0.16	81.2

Note. Reference group – vegetatively propagated plants; *in vitro* – plants were sanitized and propagated *in vitro* (* $P \leq 0.05$; ** $P \leq 0.01$)

8.5% for breeding specimens and 54.4% for the 'Chornolysta' variety.

The leaves density of vegetatively propagated breeding samples was in the range of 55–59%, after sanitation and clonal reproduction increased by 2–3%. Particular note is the breeding specimen M 01-12, which after *in vitro* sanitation, made up to 62% of leaves density. In the crop structure of the aboveground part of vegetatively propagated plants of the 'Chornolista' variety, the leaves make up only 33%, and after the sanitation *in vitro*, this indicator increased significantly by 16%. Among the studied samples of peppermint for the content of essential oil, a high index of 4.02% was detected in the selection sample M 01-12 after *in vitro* culture (Table 1).

Within the composition of peppermint essential oil limonene, cineole, menthone, mentofuran, isomentone, menthyl acetate, β-caryophyllene, isomenthol, menthol, pulegon, piperitone and carvone were identified by capillary gas chromatography (Table 2).

According to the obtained data, the maximum content of menthol, as the main marker component of essential oil, contain plants of the 'Chornolysta' variety – 30.7–33.3% and the selection sample M 01-02 – 28.6–29.4% (Fig. 1–4).

In the samples of essential oil of vegetatively propagated plants and after *in vitro* sanitation, the amount of menthol was from 15.4 to 33.3%, mentone to 15.1–50.9%. According to the results of the chromatographic analysis, an increase in the total amount of menthone, menthol, isomenton and isomenthol in essential oil of healed peppermint plants in culture was observed.

Limonene is the precursor of the main components and its concentration in the essential

Table 2

The component composition of the essential oil obtained from vegetatively propagated and sanitized breeding samples and the variety of peppermint 'Chornolysta' (%)

Essential oil components	Standard ratios EP, %	Sample/Variety									
		M 01-02		M 01-03		M 01-04		M 01-12		'Chornolysta'	
		VP	*in vitro*	VP	*in vitro*	VP	*in vitro*	VP	*in vitro*	VP	*in vitro*
Limonene	1.0–5.0	1.0	0.8	1.1	1.0	0.9	0.7	0.8	1.2	1.1	1.0
Cineole	3.5–14.0	5.0	4.4	2.1	2.4	3.4	3.0	2.8	2.8	3.8	3.1
Menthone	14.0–32.0	33.9	36.9	15.1	17.0	33.6	50.9	37.4	18.4	23.5	21.8
Mentofuran	1.0–9.0	3.2	3.4	8.4	5.0	7.0	4.8	5.3	6.7	4.8	4.5
Isomentone	1.5–10.0	5.9	7.1	21.6	26.1	5.8	6.8	6.2	18.7	4.0	3.3
Menthyl Acetate	2.8–10.0	2.6	1.6	5.3	3.3	11.2	3.5	8.8	6.2	3.0	2.9
β-caryophyllene	–	0.2	0.2	0.8	1.0	0.6	1.1	0.7	0.8	1.0	1.6
Isomenthol	–	6.5	5.8	2.1	1.6	2.4	1.4	2.1	1.6	6.5	6.4
Menthol	30.0–55.0	28.6	29.4	19.7	22.4	25.7	15.4	21.8	27.2	30.7	33.3
Pulegon	to 4.0	3.1	1.9	11.3	8.6	4.1	6.3	9.2	10.7	11.1	11.9
Piperitone	–	0.1	0.2	0.3	0.4	0.3	0.5	0.4	0.3	0.4	0.4
Carvone	to 1.0	0.2	0.1	0.5	0.5	0.0	0.1	0.0	0.4	0.6	0.9
Other Compounds	–	9.7	8.4	11.7	10.8	4.9	5.6	4.5	5.0	9.5	8.9
Cineole / Limonene	more than 2.0	5.0	5.8	2.0	2.4	3.9	4.3	3.5	2.3	3.5	3.1

Note. VP – vegetatively propagated plants; *in vitro* – sanitized plants.

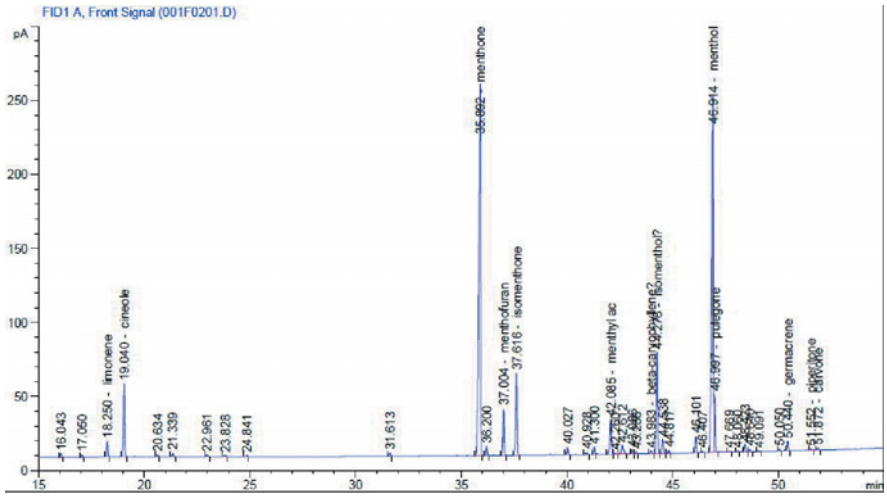

Fig. 1. Chromatogram of peppermint essential oil compounds of breeding sample M 01-02 before *in vitro* sanitation and reproduction

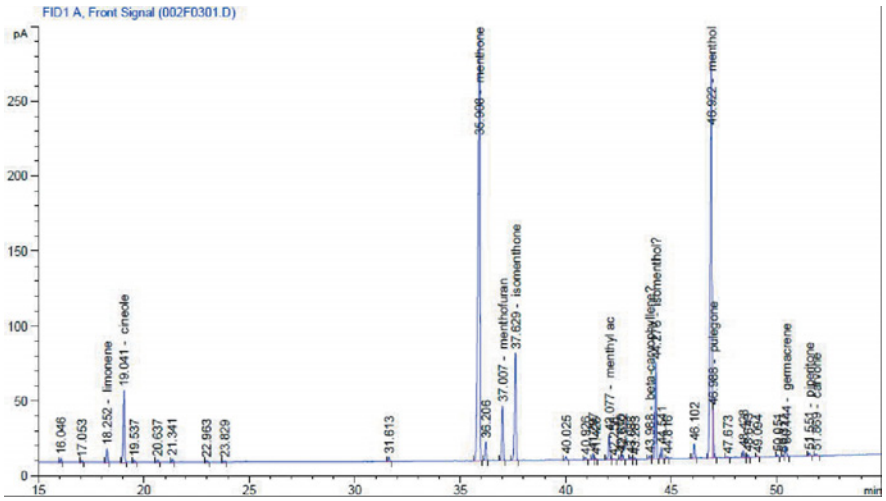

Fig. 2. Chromatogram of peppermint essential oil compounds of breeding sample M 01-02 after *in vitro* sanitation and reproduction

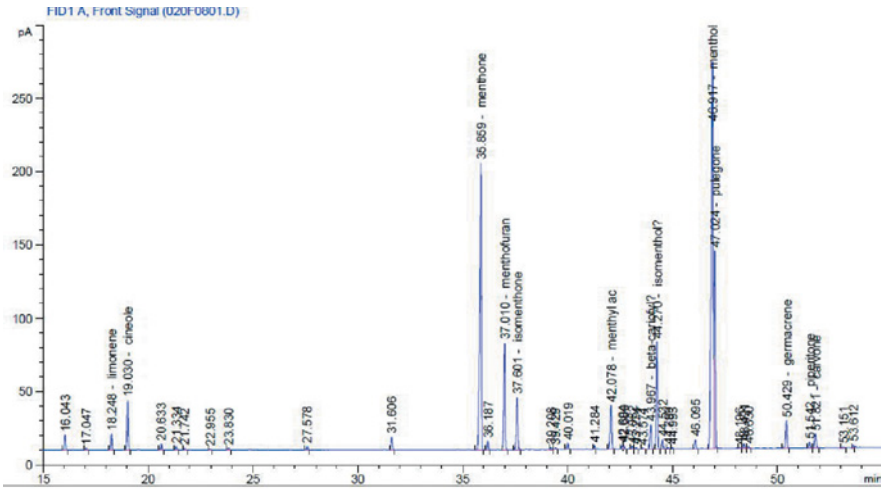

Fig. 3. Chromatogram of essential oil compounds of peppermint 'Chornolysta' variety leaves before *in vitro* sanitation and reproduction

oil samples after plant sanitation and clonal micropropagation decreased by 0.1–0.4%, except for the M 01-12 sample. By the amount of 1.8-cineole, which in its pure form has a camphor smell and a burning taste, the essential oil of vegetatively propagated variety M 01-02 con-

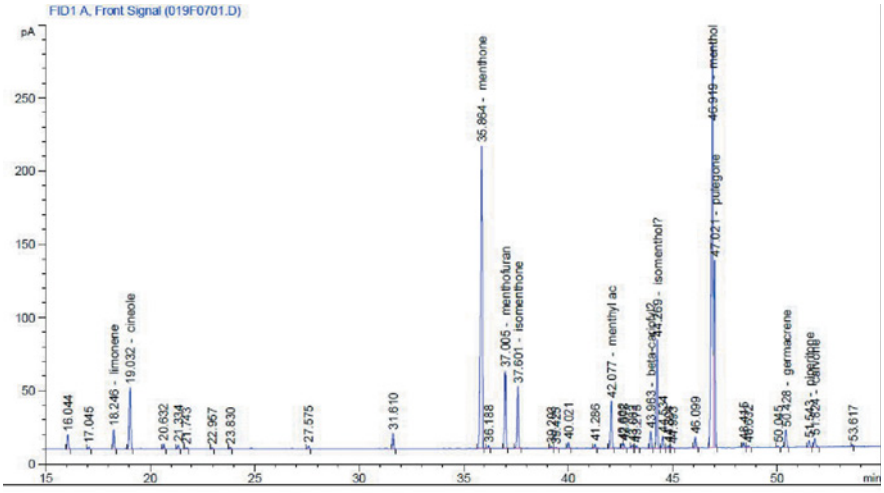

Fig. 4. Chromatogram of essential oil compounds of peppermint 'Chornolysta' variety leaves after *in vitro* sanitation and reproduction

tains its maximum amount – 5.0 and 4.4% – in sanitized plants. Essential oil obtained from vegetatively propagated plants of the 'Chornolysta' variety also meet EP standards. The cineole / citric acid ratio is an integral indicator of the quality of peppermint essential oil. Essential oil samples before and after sanitation and *in vitro* micropropagation meet EP standards (more than 2.0%), which distinguishes them among the previously studied varieties of Ukrainian breeding [21].

Samples of essential oil in the amount of mentofuran, which is synthesized from pulegon and contained mainly in peppermint flowers, meet the standards of EP too. Among the samples of essential oil of peppermint plants which have been healed *in vitro*, there is a tendency for mentofuran decreasing by 0.3–3.3%, excepting the breeding sample M 01-02.

Menthyl acetate in all samples of essential oil meets the criteria of EP requirements, excepting varieties of samples M 01-02, which has slightly reduced values – 2.6% in the control variant, and in plants, healed and propagated *in vitro* – 1.6%. According to the amount of pulegon, only the essential oil of the breeding sample M 01-02 meets the standards of EP, and in breeding samples M 01-03, M 01-04, M 01-12 and 'Chornolysta' before and after sanitation and micropropagation *in vitro* exceeds them.

According to the total content of marker compounds of the mint essential oil, namely menton, isomentone, menthol, isomenthol – peppermint plants can be arranged in the following sequence: breeding samples M 01-02 (74.9–79.1%); M 01-04 (67.5–74.5%); M 01-12 (65.9–67.5%); M 01-03 (58.5–67.1%) and 'Chornolysta' variety (64.7–64.8%).

The amount of menthone in breeding specimens M 01-02, M 01-04 and vegetatively propagated M 01-12 exceeds the recommended EP standards. Isomenton in the breeding sample M 01-03 exceeds the norm by 2 times, and after sanitation and cloning – by 2.5 times, and the content of menthol in all studied varieties is less than the EP standards.

As a result of factor analysis of the main components of the breeding peppermint samples essential oil, it was found that the first axis of the main components is 48.25% of the variance in the set of indicators, of which cineole, β-caryophyllene, isomenthol and pulegon were of the greatest meaning (Table 3). A slightly smaller contribution to the dispersion was made by isomentone, mentofuran and menthol.

The second axis of the major components is 28.11% of the total dispersion, which is dominated by the amount of limonene, carvone, piperitone and menthone.

The third axis (PC3) covers 14.41% of the variance. The maximum value in it is the content of menthyl acetate. We consider it necessary to evaluate significant changes in the synthesis of isoprenoids in plants in the process of clonal micropropagation of breeding samples of peppermint. First of all, their important difference is the high content of menthone, excepting the breeding sample M 01-03. Thus, in the variety M 01-12, the menthone after *in vitro* culture decreased by 2 times – from 37.4 to 18.4%, while in the breeding sample M 01-04 it increased – from 33.6 to 50.9%. Menthone is relatively stable in the breeding sample M 01-02 and the 'Chornolysta' variety. Menthone biosynthesis can occur by the restoration of pulegon or piperitone, as well as in the process of menthol oxidation [9]. Obviously,

Table 3

Contribution of features into the main components of peppermint varieties

Essential oil components	F1 (48,25%)	F2 (28,11%)	F3 (14,41%)
Limonene	0.176	0.498*	0.185
Cineole	0.917**	0.025	0.003
Menthone	0.313	0.576*	0.082
Mentofuran	0.541*	0.000	0.280
Isomentone	0.618*	0.322	0.016
Menthyl acetate	0.048	0.205	0.671*
β-caryophyllene	0.786**	0.132	0.053
Isomenthol	0.793**	0.180	0.011
Menthol	0.392*	0.290	0.173
Pulegon	0.795**	0.007	0.038
Piperitone	0.312	0.597*	0.033
Carvone	0.407	0.517*	0.064
Minor compounds	0.176	0.498*	0.185

Note. F1, F2, F3 are the first, second and third axes of the principal components respectively.

with the use of *in vitro* culture method in plants of the variety M 01-12, the path of pulegon metabolism shifts towards the synthesis of isomentone, which, according to Fedchenkova Yu. A. [16], is caused by the decrease in the activity of the enzyme systems associated with the menthone accumulation in the leaves.

In the group of terpenoids studied, the first significant feature is the monocyclic terpene cineole (Table 3) with pronounced bacteriostatic action, which is intensively accumulated in the plants of the breeding sample M 01-02. The second most important marker is the pulegon, which is an important precursor of menthone and menthol in their biosynthesis. These biochemical characteristics in the complex are the most important characteristics of the secondary biosynthesis of metabolites in the leaves of peppermint plants.

The identified components of the essential oil of perspective breeding peppermint samples fluctuate within: limonene from 0.7% to 1.2%, cineole – 2.1–5.0%, menthone – 15.1–50.9%, mentofuran – 3,2–8.4%, isomentone – 5.8–26.1%, menthyl acetate – 1.6–11.2%, β-caryophyllene – 0.2–1.1%, isomenthol – 1,4–6.5%, menthol – 15.4–29.4%, pulegon – 1.9–11.3%, piperitone – 0.1–0.5%, carvone – 0.0–0.5% (Table 2).

Thus, after sanitation and micropropagation *in vitro*, the breeding sample of peppermint M 01-02 contains 3.7% of essential oil and 28.6–29.4% of menthol, at the same time the breeding sample of M 01-12 contains the largest amount of essential oil – 4.02 and menthol in it – 27.2%, but the pulegon twice exceeds the EP criteria.

Six indicators of the essential oil of the breeding sample M 01-02, namely: limonene, cineole, mentofuran, isomentone, pulegon and carvone, meet the criteria of the European Pharmacopoeia, so it can be considered promising among the studied breeding samples.

Considering that in the studied breeding samples during the period of mass flowering, the precursors of menthol biosynthesis, namely pulegon, isomentone and menton differed in high content, and menthol, in turn, was low, it is necessary to trace the component composition of essential oil in other phases of mint flowering – at its beginning and ending.

The experiments were conducted along 2014–2017 according to the scientific topic "Biotechnological basis of reproduction of essential oil medicinal plants of Lamiaceae family for obtaining high quality planting material» (state registration number 0116U001994).

Conclusions

As a result of sanitation, the yield of air–dried leaves of perspective breeding specimens increased by 2.9–7.1%, and in the 'Chornolysta' variety – by 51.4%, compared to control. The yield of rhizomes of breeding specimens increased by 2.2–3.8%, of the 'Chornolysta' variety – by 28.5%, which corresponds to increase of biomass yield from 0.35 to 2.74 kg/ha. Clonal micropropagation and sanitation of peppermint plants *in vitro* resulted in an increase in the amount of essential oil obtained in the breeding specimens of 4.0–9.9 kg/ha, and in the 'Chornolysta' variety – 28.6 kg/ha, which is in breeding plant samples 4.0–8.5%, and in the variety 'Chornolysta' – 54.4%. The maximum content of essential oil (more than 4%) was observed in the breeding sample M 01-12 after sanitation *in vitro*.

Thus, the sanitation and clonal micropropagation *in vitro* causes an improvement in the quali-

tative composition of terpenoids. Increasing the amount of menthol in the conditions of simultaneous reduction of menthone is advisable to take into account in the process of mass clonal micropropagation of peppermint plants, which is a necessary condition for selection and provides an increase in leaves density, yield of air-dry leaves, rhizomes and amount of essential oil.

Six indicators of the essential oil of the breeding sample M 01-02, namely: limonene, cineole, mentofuran, isomenton, pulegon, carvone, as well as the ratio of cineole/limonene meet the criteria of the European Pharmacopoeia, therefore, among the studied breeding samples it can be considered promising for cultivation.

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Шкопинська Т. Є.[1*], Коломієць Ю. В.[1], Григорюк І. П.[1], Куценко Н. І.[2] Господарсько-цінні ознаки перспективних селекційних зразків та сорту 'Чорнолиста' *Mentha piperita* L. після їх оздоровлення й мікророзмноження *in vitro*. *Plant Varieties Studying and Protection*. 2019. Т. 15, № 4. С. 424–433. https://doi.org/10.21498/2518-1017.15.4.2019.188722

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Мета. Встановити ефективність впливу клонального мікророзмноження та оздоровлення рослин м'яти перцевої (*Mentha piperita* L.) в культурі *in vitro* віроцидом Ribavirin на продуктивність рослин *ex vitro*, кількісний вміст і якісний склад компонентів м'ятної ефірної олії, отриманої з чотирьох селекційних зразків й сорту 'Чорнолиста'. **Методи.** У дослідженнях використано методи польового агротехнічного однофакторного досліду, перегонки олії ефірної з водяною парою за методикою Гінзберга, капілярної газової хроматографії та статистичного аналізу. **Результати.** Завдяки процесу оздоровлення в культурі *in vitro* врожайність повітряно-сухих листків селекційних зразків збільшилася на 2,9–7,1%, а сорту 'Чорнолиста' – 51,4% та кореневищ на 2,2–3,8% і сорту 'Чорнолиста' – 28,5%, що складає 0,35–2,74 т/га. Показано достовірне зростання виходу ефірної олії у селекційних зразків від 4,0 до 9,9 кг/га, а у сорту 'Чорнолиста' – 28,6 кг/га. Після оздоровлення та клонального мікророзмноження в культурі *in vitro* у селекційного зразка М 01-12 прослідковувалось накопичення вмісту ефірної олії більше 4%. Нами ідентифіковано наступні компоненти ефірної олії м'яти перцевої: лімонен, цинеол, ментон, ментофуран, ізоментон, ментіл ацетат, β-каріофілен, ізоментол, ментол, пулегон, піперитон та карвон. Встановлено чітку тенденцію до зменшення кількості ментону та ізоментолу та одночасне збільшення ізоментону і ментолу у рослин, що оздоровлені та розмножені в умовах культури *in vitro*. **Висновки**. Застосування методів культури тканин і органів й оздоровлення *in vitro* спричиняє поліпшення якісного складу терпеноїдів за рахунок збільшення кількості ментолу та зменшення ментону. Отримані дані щодо складу терпеноїдів необхідно враховувати в селекції м'яти перцевої як одного із інтегральних критеріїв, який необхідно ввести до переліку господарсько-цінних ознак культури м'яти перцевої: облиствіння, біомасу повітряно-сухих листків, кореневищ рослин та кількість виходу м'ятної ефірної олії. Шість показників ефірної олії селекційного зразка М 01-02, а саме лімонен, цинеол, ментофуран, ізоментон, пулегон, карвон, а також співвідношення цинеол/лімонен відповідають критеріям європейської фармакопеї, тому серед досліджуваних селекційних зразків його можна вважати найперспективнішим для культивування.

Ключові слова: *м'ята перцева; Mentha piperita L.; культура in vitro; мікророзмноження; врожайність; ефірна олія; капілярна газова хроматографія.*

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Шкопинская Т. Е.[1*], Коломиец Ю. В.[1], Григорюк И. П.[1], Куценко Н. И.[2] Хозяйственно-ценные признаки перспективных селекционных образцов и сорта 'Чорнолиста' *Mentha piperita* L. после их оздоровления и микроразмножения *in vitro* // Plant Varieties Studying and Protection. 2019. Т. 15, № 4. С. 424–433. https://doi.org/10.21498/2518-1017.15.4.2019.188722

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Цель. Установить эффективность воздействия клонального микроразмножения и оздоровления растений мяты перечной (*Mentha piperita* L.) в культуре *in vitro* вироцидом Ribavirin на продуктивность растений *ex vitro*, количественное содержание и качественный состав компонентов мятного эфирного масла, полученного из четырех селекционных образцов и сорта 'Чорнолиста'. **Методы.** В исследованиях использованы методы полевого агротехнического однофакторного опыта, перегонки эфирного масла с водяным паром по методике Гинзберга, капиллярной газовой хроматографии и статистического анализа. **Результаты.** Благодаря процессу оздоровления в культуре *in vitro* урожайность воздушно-сухих листьев селекционных образцов увеличилась на 2,9–7,1%, а сорта 'Чорнолиста' – 51,4% и корневищ – на 2,2–3,8% и на 28,5% соответственно, что составило 0,35–2,74 т/га. Показано достоверное увеличение выхода эфирного масла у селекционных образцов от 4,0 до 9,9 кг/га, а у сорта 'Чорнолиста' – до 28,6 кг/га. После оздоровления и клонального микроразмножения в культуре *in vitro* у селекционного образца М 01-12 прослеживалось накопление содержания эфирного масла более 4%. Были идентифицированы такие компоненты эфирного масла мяты перечной: лимонен, цинеол, ментон, ментофуран, изоментон, ментилацетат, β-кариофилен, изоментол, ментол, пулегон, пиперитон и карвон. Установлена четкая тенденция к уменьшению количества ментона и изоментола, а также одновременное увеличение изоментона и ментола у растений, оздоровленных и размноженных в условиях культуры *in vitro*. **Выводы**. Применение методов культуры тканей и органов и оздоровления *in vitro* улучшает качественный состав терпеноидов за счет увеличения количества ментола и уменьшения ментона. Полученные данные о составе терпеноидов необходимо учитывать в селекции мяты перечной как одного из интегральных критериев, который необходимо отнести к перечню хозяйственно-ценных признаков культуры мяты перечной: облиственность, биомасса воздушно-сухих листьев, корневищ растений и количество мятного эфирного масла. Шесть показателей эфирного масла селекционного образца М 01-02, а именно: лимонен, цинеол, ментофуран, изоментон, пулегон, карвон, а также соотношение цинеол/лимонен соответствуют критериям европейской фармакопеи, поэтому среди исследуемых селекционных образцов его можно считать перспективным для культивирования.

Ключевые слова: *мята перечная; Mentha piperita L.; культура in vitro; микроразмножение; урожайность; эфирное масло; капиллярная газовая хроматография.*

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