MOBILIZATION AND CONSERVATION OF THE GENETIC DIVERSITY OF CULTIVATED PLANTS AND THEIR WILD RELATIVES

Original article
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Anatomy, ploidy level, and essential oil composition of *Hyssopus officinalis* 'Nikitskiy Beliy' *in vitro* and *ex situ*

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Background. Clonal micropropagation is a biotechnological method for plant multiplication. The existing data on the structure of organs *in vitro*, genetic stability, and essential oil composition are limited for *Hyssopus officinalis* L., so this study was aimead at investigating these aspects under a short period of *in vitro* culturing.
Materials and methods. Plants of *Hyssopus officinalis* 'Nikitskiy Beliy' cultivated *ex situ*, *in vitro* and *ex vitro* were analyzed. Conventional methods were applied to study plant anatomy, ploidy level, and relative DNA content, as well as to extract and analyze essential oil. Statistical analysis was performed using the Past 4.03 software.
Results. According to the results obtained, with 6-BAP introduced into MS nutrient medium in optimal concentrations (0.3–0.5 mg/L), the general *in vitro* structure of leaf blades in the developed microshoots was similar to those in *ex situ* plants, while the qualitative and quantitative changes observed were induced by the effect of specific culturing conditions and plant rejuvenation. The analysis of the ploidy level and relative DNA content in the nuclei isolated from the leaf tissue cells of the microshoots *ex vitro* after adaptation revealed no changes compared to the *ex situ* leaf parameters. The mass fraction of essential oil and its component composition in the mother plants and *ex vitro* regenerants were similar.
Conclusion. Cultivation of *Hyssopus officinalis* 'Nikitskiy Beliy' microshoots on MS nutrient medium with 6-BAP optimal concentrations promotes morphogenesis without significant deviations in the ploidy level, relative DNA content, essential oil yield, or its component composition. The developed protocol for clonal micropropagation of *Hyssopus officinalis* 'Nikitskiy Beliy' provides clones identical to the *ex situ* plants.

Keywords: clonal micropropagation, adaptation, leaf blade, light microscopy, flow cytometry, chromatography

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МОБИЛИЗАЦИЯ И СОХРАНЕНИЕ КУЛЬТУРНЫХ РАСТЕНИЙ И ИХ ДИКИХ РОДИЧЕЙ

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Анатомия, уровень плоидности и компонентный состав эфирного масла *Hyssopus officinalis* 'Никитский Белый' *in vitro* и *ex situ*

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Актуальность. Получение идентичных растений при использовании клонального микроразмножения является первостепенной задачей, особенно среди лекарственных культур. Поскольку для *Hyssopus officinalis* L. сведения о структуре органов *in vitro*, генетической стабильности и компонентном составе эфирного масла ограничены, целью нашего исследования было изучение данных аспектов при непродолжительном сроке культивирования *in vitro*.
Материалы и методы. В качестве материала использовали *Hyssopus officinalis* 'Никитский Белый', культивируемый *ex situ*, *in vitro* и *ex vitro*. Исследование анатомии, плоидности, относительного содержания ДНК, извлечение и анализ эфирного масла осуществляли согласно общепринятым методам. Статистический анализ проводили с использованием программного обеспечения Past версии 4.03.
Результаты. Согласно полученным результатам, при введении в состав питательной среды МС 6-БАП в оптимальных концентрациях (0,3–0,5 мг/л) общая структура листовых пластинок микропобегов, в сравнении с таковыми *ex situ*, сохранялась, при этом наблюдали качественные и количественные изменения, связанные с условиями культивирования и омоложением материала. Анализ уровня плоидности и относительного содержания ДНК ядер, выделенных из клеток тканей листьев микропобегов *ex vitro* после адаптации, выявил отсутствие изменений. Массовая доля эфирного масла и его компонетный состав у материнских растений и регенерантов *ex vitro* были подобными.
Заключение. Культивирование микропобегов *Hyssopus officinalis* 'Никитский Белый' *in vitro* на питательной среде МС при введении оптимальных концентраций 6-БАП способствует их нормальному развитию, сохранению уровня плоидности, относительного содержания ДНК, а также не влияет на выход эфирного масла и его компонентный состав. Разработанный протокол для клонального микроразмножения *Hyssopus officinalis* 'Никитский Белый' обеспечивает получение клонов, идентичных растениям *ex situ*.

Ключевые слова: клональное микроразмножение, адаптация, листовая пластинка, световая микроскопия, проточная цитометрия, хроматография

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Introduction

The production of medicinal plants using *in vitro* regeneration techniques became popular due to consumer demand, large-scale production in a considerable period within confined space, stable supply of disease-free elite genotypes, and conservation of plants (Manokari et al., 2021). Common hyssop (*Hyssopus officinalis* L.) is widely used as an aromatic and medicinal plant. It is mainly utilized for the production of essential oil applied in the pharmaceutical (as an antioxidant, expectorant, antiseptic, antibacterial and antifungal agent) and perfume industries, as well as in aromatherapy, where its main components, such as *trans*-, and *cis*-pinocamphone, exert a muscle relaxant effect (Plugatar et al., 2023). In the Nikita Botanical Gardens, the work on the development of new hyssop cultivars has been carried out. Plants (*H. officinalis* 'Nikitskiy Beliy') with a number of economically valuable traits (drought resistance, late maturation, raw material yield of 11.39 t/ha, essential oil mass fraction of 0.45% wet weight, and essential oil yield of 51.3 kg/ha) were selected. Research on the qualitative and quantitative composition of bioactive compounds (volatile compounds, phenolics, vitamins, etc.) in the aqueous ethanol extract confirmed the prospects of using the released cultivar to develop products with high biological value (Grebennikova et al., 2017).

Traditionally, hyssop plants are reproduced by seeds; however, this method has some limitations for promising forms and cultivars. To preserve economically valuable traits, plant material obtained through selection breeding needs to be propagated using different vegetative technologies that are of interest for production (Kalinichenko et al., 2013). Currently, biotechnological methods are important for mass multiplication, investigation, and conservation of valuable forms obtained during selection (Bulavin et al., 2021). By now, there are published data regarding different stages of clonal micropropagation of *H. officinalis* (Plugatar et al., 2023). The relevance of such studies has been emphasized for further optimization of the process (Maslova et al., 2021).

Anatomical characteristics mostly reflect the environmental conditions under which the plants have developed and are the result of a complex process that reveals the phenotypical plasticity of these organisms (Rodrigues et al., 2014). The environmental conditions during *in vitro* culturing are very specific due to the use of sealed containers. In addition, high relative humidity, reduced gas exchange, artificial temperature, illumination conditions as well as high variations in CO_2 and ethylene accumulation may occur inside such containers. So, *in vitro* plants can demonstrate anatomical and physiological disorders caused by the effect of micro-environmental conditions (Rodrigues et al., 2014; Martins et al., 2019). Moreover, different concentrations of plant growth regulators also influence the plant structure *in vitro*. Therefore, the anatomy of the *in vitro* material is studied to identify anomalies, optimize the cultivation conditions, and assess the possibility of plant adaptation to *ex vitro* conditions (Plugatar et al., 2023).

During the period of culturing, variable responses of plants to an *in vitro* environment may be accompanied by genetic variations (Ai et al., 2023). Generally, multiplication techniques via buds (existing meristems), shoot tips, nodes, internodes, leaves, and petioles are most frequently chosen to maintain genetic stability (Sliwinska, Thiem, 2007; Rohela et al., 2022). However, newly developed microplants grown from the meristems of the *in vitro* propagated shoots may show genetic variability (Sliwinska, Thiem, 2007). So, the plants grown *in vitro* require assessment of their genetic stability using the ploidy level and/or DNA content analysis (Sliwinska, Thiem, 2007; Fritsche et al., 2022b; Catalano et al., 2023), molecular markers (Çetin, 2018; Bulavin et al., 2021), or both methods (Abdolinejad et al., 2020; Raji, Farajpour, 2021).

Currently, there are data available on essential oil components of *in vitro* microshoots/plants (Guedes et al., 2003; Allahverdi-Mamaghani et al., 2022) and field-acclimated regenerants (Kuźma et al., 2009; Shelepova et al., 2021), but for hyssop such information is very limited. It emphasizes the importance of attaining the unchanged composition of these secondary metabolites after micropropagation for medicinal plants as a source of bioactive metabolites (Sliwinska, Thiem, 2007).

Since the information on structural and genetic stability as well as essential oil composition of *Hyssopus officinalis* under *in vitro* conditions is limited, the aim of this study was to investigate these aspects during short-term cultivation.

Materials and methods

The objects of this study included plants of *Hyssopus officinalis* 'Nikitskiy Beliy' grown *ex situ* at the collection plots of the Nikita Botanical Gardens (NBG) located at an altitude of 200 m above sea level under the subtropical climate of the Mediterranean type, and *in vitro* microshoots cultivated on a modified Murashige and Skoog culture medium (MS) with 0.3–0.9 mg/L 6-BAP (Bulavin et al., 2021). The material in culture vessels was kept in the phytochambers of the Unique Scientific Installation "PHYTOBIOGEN" of the NBG or the MLR-352-PE growth chamber (Panasonic, Japan) at a temperature of 24 ± 1°C, 16-h photoperiod, and 37.5 μmol m^{-2} s^{-1} light intensity under basic cool daylight lamps (Ledvance, Smolensk, Russia).

For adaptation, rooted plantlets were transferred into plastic pots with a mixture of soil and perlite (1 : 1), covered with insulators, and grown in a plant growth climate chamber (Conviron, Canada) at 22 ± 1°C, a light intensity of 37.5 μmol m^{-2} s^{-1}, a photoperiod of 16 h, and air humidity of 70–80%. Gradual acclimatization was performed *ex vitro* in a plant growth chamber for two months with subsequent cultivation under greenhouse conditions.

For plant anatomy studies, leaves of the *ex situ* shoots and *in vitro* microshoots were cut and immediately fixed in a solution of formalin, alcohol, acetic acid, and water (1 : 5 : 0.5 : 3.5), dehydrated in alcohols and embedded into paraffin. Sections (8–10 μm) were made on a Rotmic 2A microtome (Orion Medic, Russia), stained with a 0.005% solution of methylene blue, mounted into 60% sucrose, and investigated under the CX-41 light microscope (Olympus, Japan) with a SC 50 digital camera (Olympus, Germany) and the CellSens image processing software version 1.17. In total, 30 cells from ten *ex situ* and *in vitro* leaves were analyzed for each parameter.

The ploidy level and relative DNA content were studied on fresh material. Leaf blades (about 0.5 cm^2) of six *ex situ* and *ex vitro* plants were immersed in a modified WPB buffer (Loureiro et al., 2007) with 2% polyvinylpyrrolidone (K10) supplemented with propidium iodide (50 μg/mL), RNase (50 μg/mL), and *β*-mercaptoethanol (0.3%), grinded up with a safety razor blade. The obtained samples were passed through a 30 mμ CellTrics® filter (Partec, Germany). The analysis was performed using a CyFlow® Ploidy Analyzer (Sysmex, Partec, Germany). *Ficus benjamina* L. plants were used as an external control to determine the relative DNA content (Skaptsov et al., 2016). The measurements were carried out on the same analyzer settings with at least 10.000 nuclei.

In the environments of the South Coast of Crimea, the highest productivity of *H. officinalis* raw materials (yield, and essential oil yield) is observed from the third year of the plants' growing season. Our experiment used five-year-old *ex situ* plants and three-year-old regenerants cultivated in open ground under similar conditions. The biochemical studies of essential oil were carried out on the equipment of the Common Use Center "Physiological and Biochemical studies of Plant Objects" of the NBG (Yalta, Russia). The mass fraction of essential oil was measured in fresh raw materials by hydrodistillation on Ginsberg devices (Shevchuk et al., 2022). The component composition of essential oils was analyzed using a hardware and software complex based on a Chromatek-Crystal 5000.2 chromatograph (Chromatek, Russia) equipped with a mass spectrometric detector (capillary column: CR–5 ms, length: 30 m, inner diameter: 0.25 mm; phase: 5% phenyl 95% polysilphenylenesiloxane, film thickness: 0.25 microns). The temperature of the thermostat was programmed from 75°C to 240°C at a speed of 4°C/min. The evaporator temperature was 250°C. The carrier gas was helium; the flow rate was 1 mL/min. The temperature of the transition line was 250°C. The temperature of the ion source was 200°C. The electronic ionization was 70 eV. The scanning range was 20–450. The scan duration was 0.2. Identification was based on a comparison of the obtained mass spectra with the data from the NIST 14 library (National Institute of Standards and Technology, Gaithersburg, MD, USA) using the NIST MS Search software v. 2.2 (Gaithersburg, USA). Retention indices were obtained by logarithmic interpolation of the reduced retention times using the analytical standard of a mixture of reference n-alkanes (Sigma-Aldrich, Switzerland) and analytical standards (Supelco, USA). The mass fraction of the components in a sample was measured by the percentage normalization method (Adams, 2007; Tkachev, 2008).

Statistical analysis was carried out using the Past 4.03 software (Hammer et al., 2001). Samples were checked for normality of distribution, and either the *t*-test or the U-criterion was used ($p \leq 0.05$).

Results and discussion

It is well known that the morphogenesis of *in vitro* plants depends on the genotype of the plant material, type of the explant, hormonal composition of the nutrient medium, etc. (Bulavin et al., 2020; Mitrofanova et al., 2022a). The optimal concentration of growth regulators in a nutrient medium supports normal organ development, while their high content provokes structural rearrangements in plant organs *in vitro* (Plugatar et al., 2023). For the *in vitro* introduction of *Hyssopus officinalis* 'Nikitskiy Beliy', shoot segments with a lateral bud were used. Induction of shoot formation was carried out on a modified MS nutrient medium supplemented with 0.5–1.0 mg/L 6-BAP and 0.1 mg/L IBA. Normally formed microshoots were observed after 21 days. They were separated and subcultured on an MS medium supplemented with 0.3–0.9 mg/L 6-BAP. To assess the regenerative potential of hyssop microshoots under *in vitro* culture, their number per explant was analyzed. The optimal concentration of 6-BAP was set at 0.5 mg/L, and 3.3 ± 0.16 normally formed microshoots per explant were obtained. The presence of 0.7–0.9 mg/L 6-BAP in the medium contributed to an increase in the number of microshoots to 4.5 ± 0.18 pieces per explant, while among normally formed organs and their structural parts, the presence of various morphological deviations, such as deformation, thickening, fusion of microshoots, and the formation of single disproportionate leaves were observed. The presence of 0.3–0.4 mg/L 6-BAP in the medium promoted the growth and elongation of microshoots and their spontaneous rooting, as well as a decrease in shoot formation intensity.

Since the leaf is the main photosynthetic organ of *in vitro* and *ex situ* plants, its anatomy was studied. According to the data obtained, epidermis with a cuticle, several subepidermal layers of the collenchyma in the upper and lower parts, oval vascular bundle consisting of the xylem and phloem, and parenchyma were differentiated on the leaf cross-sections along the midrib. In the side part of the leaf, epidermis with the a cuticle, and palisade and spongy mesophyll with lateral veins were distinguished (Fig. 1, A, B). The leaves of *in vitro* microshoots had a similar structure (Fig. 1, C, D). However, among the observed qualitative changes, the cuticular layer, collenchyma, was less developed in the midrib.

Among the quantitative changes, a significant decrease in the leaf height in the midrib and side parts was found *in vitro* compared to *ex situ* (Table 1). Besides, a decrease in the size of the midrib and xylem element number was observed. Under *in vitro* culturing, a reduction in the size of epidermal cells and mesophyll occurred, affecting the general linear parameters.

According to published sources, specific culturing conditions can affect the organ structure of the *in vitro* material. The ecological and anatomical variability analysis of *in vitro* plants revealed mainly quantitative changes (Apóstolo et al., 2005), which is consistent with our data. Photosynthetic tissue structure analyses performed on *in vitro* leaves of ornamental, essential-oil, and fruit crop plants demonstrated the presence of a differentiated mesophyll. One- or two-row palisade cells and two to four rows of spongy mesophyll were found. In some cultivars of *Ficus carica* L., *Lavandula angustifolia* Mill., *Lavandula × intermedia* Emeric ex Loisel. the formation of an isopalisade mesophyll was observed (Mitrofanova et al., 2022b). In this study, the presence of a differentiated mesophyll in *Hyssopus officinalis* 'Nikitskiy Beliy' *in vitro* leaves was identified. Similar data were obtained for *H. officinalis* f. *cyaneus* leaf blades *in vitro*. Besides, some changes in the structure of the organs, namely an increase in the number of spongy mesophyll layers and cell sizes with increased 6-BAP concentrations, were shown (Plugatar et al., 2023).

Changes in the geometry and structure of conducting bundles are also characteristic of *in vitro* culture. In *Vitex negundo* L., a concave vascular bundle was described, with a few xylem elements and a thin layer of the phloem. Veinlets transversing the mesophyll were represented by inconspicuous collateral bundles surrounded by undistinguished endodermis (Manokari et al., 2021). For *Cynara scolymus* L. leaves *in vitro*, limited development of a conducting bundle consisting of several elements without fibers was shown (Apóstolo et al., 2005).

According to our analysis, the *in vitro* leaf midrib of *Hyssopus officinalis* 'Nikitskiy Beliy' was characterized by the decreasing height and width, and a diminished number of xylem elements, developed to a sufficient extent and observed on the cross-sections. The change in the midrib size was probably associated with the correlative relationships during the lamina development depending on its linear parameters.

In *in vitro* culture, the plant genome can be affected, and somaclonal variation (spontaneous mutagenesis) may occur. It is believed that introduction into *in vitro* culture destabilizes the genetic and epigenetic program of intact plant tissue (Neelakandan, Wang, 2012) and can lead to variations at the level of chromosomes and DNA sequences (Neelakandan, Wang, 2012; Ryabushkina, 2014; Plugatar

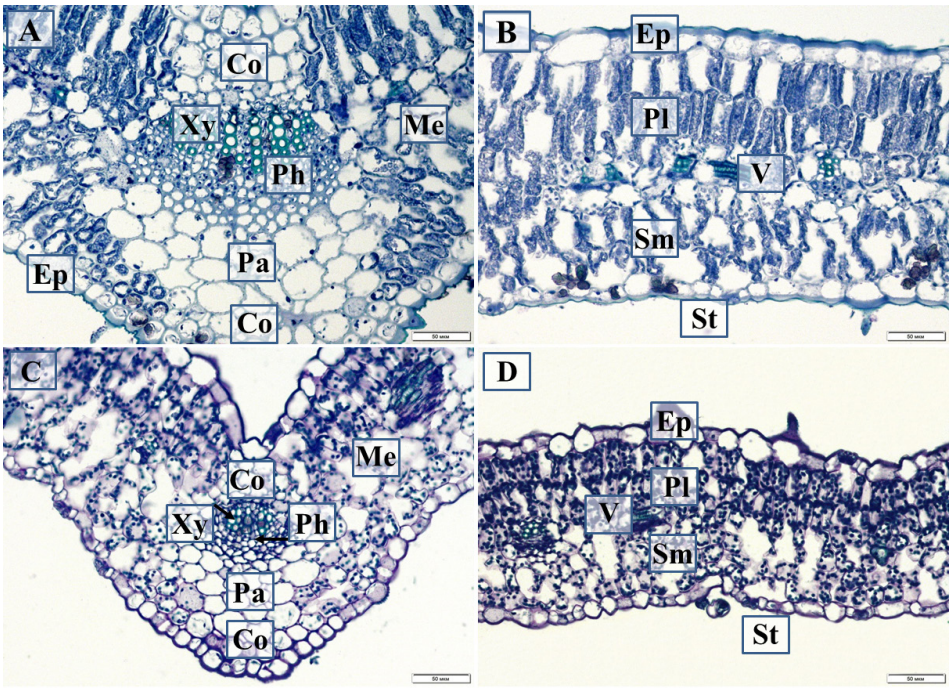

Fig. 1. ***Hyssopus officinalis*** **L. 'Nikitskiy Beliy' cross-sections of the leaf** ***ex situ*** **(A, B) and** ***in vitro*** **(C, D):**
Co – collenchyma; **Ep** – epidermis; **Me** – mesophyll; **Pa** – parenchyma; **Ph** – phloem; **Pl** – palisade; **Sm** – spongy mesophyll; **St** – stomata; **V** – vein; **Xy** – xylem (light microscopy)

Рис. 1. Поперечные срезы листьев ***Hyssopus officinalis*** **L. 'Никитский Белый'** ***ex situ*** **(A, B) и** ***in vitro*** **(C, D):**
Co – колленхима; **Ep** – эпидерма; **Me** – мезофилл; **Pa** – паренхима; **Ph** – флоэма; **Pl** – столбчатый мезофилл; **Sm** – губчатый мезофилл; **St** – устьице; **V** – боковая жилка; **Xy** –ксилема (световая микроскопия)

Table 1. Quantitative anatomical parameters of ***Hyssopus officinalis*** **L. 'Nikitskiy Beliy' leaf blade cross-sections**

Таблица 1. Количественные анатомические показатели поперечных срезов листьев ***Hyssopus officinalis*** **L. 'Никитский Белый'**

Parameter	*Ex situ*	*In vitro*
Leaf cut height, midrib (μm)	403.64 ± 8.87	236.51 ± 10.24*
Midrib height (μm)	97.18 ± 4.61	53.28 ± 2.92*
Midrib width (μm)	157.85 ± 11.17	86.12 ± 2.21*
Vessel number	47.9 ± 4.11	33.6 ± 3.4*
Leaf cut height, side part (μm)	251.16 ± 7.67	171.18 ± 12.46*
Cell height, upper epidermis (μm)	29.75 ± 1.03	24.01 ± 1.11*
Cell width, upper epidermis (μm)	33.66 ± 1.51	30.09 ± 2.25*
Cell height, lower epidermis (μm)	22.29 ± 0.77	14.99 ± 0.67*
Cell width, lower epidermis (μm)	23.44 ± 1.17	17.15 ± 0.84*
Cell height, palisade mesophyll (μm)	41.92 ± 0.83	38.41 ± 1.24*
Cell width, palisade mesophyll (μm)	11.26 ± 0.33	16.97 ± 0.74*
Cell height, spongy mesophyll (μm)	27.69 ± 0.96	17.38 ± 1.06*
Cell width, spongy mesophyll (μm)	12.75 ± 0.64	22.64 ± 1.53*
Number of the palisade mesophyll layer	2.2 ± 0.13	2.0 ± 0.00
Number of the spongy mesophyll layer	3.6 ± 0.16	3.2 ± 0.13
Palisade height (μm)	88.21 ± 5.37	63.43 ± 5.43*
Spongy mesophyll height (μm)	121.03 ± 4.11	61.24 ± 6.07

Note: * – an asterisk indicates a significant difference between the two parameters in a line

Примечание: * – звездочка указывает на существенную разницу между двумя параметрами в строке

et al., 2023). Therefore, the ploidy level of the nuclei isolated from the leaves of *H. officinalis* 'Nikitskiy Beliy' *ex vitro* plants after adaptation was compared with the ploidy of *ex situ* plants. On the histogram comparing the *ex situ* and *ex vitro* leaf nuclei of *H. officinalis*, the main fluorescence peak, corresponding to the G_1/G_0 (2C) phases, and the next small one, conforming to G_2 (4C), were identified (Fig. 2). Since the number of peaks was similar, no changes were detected in the *ex vitro* samples, and their ploidy corresponded to 2x. The relative DNA content analysis did not reveal any changes as well: 2C = 1.02 pg for *ex situ* plants versus 2C = 1.05 for *ex vitro* plants.

The essential oil of *H. officinalis* is the main physiologically active component of the plant. It has antibacterial, antiviral, antifungal, and expectorant effects. Typically, the yield of essential oil obtained by steam hydrodistillation from dried and fresh hyssop plant material is 0.15–0.30% and 0.30–0.80%, respectively. The main components are isomeric pinocamphone, *β*-pinene, pinocarvone, limonene, linalool, *β*-caryophyllene, germacrene D, thujone, and myrtenol (Velikorodov et al., 2015).

According to the data obtained, the mass fraction of essential oil in mother plants and in *ex vitro* regenerants was 0.33% and 0.30% wet weight, respectively. The dominant

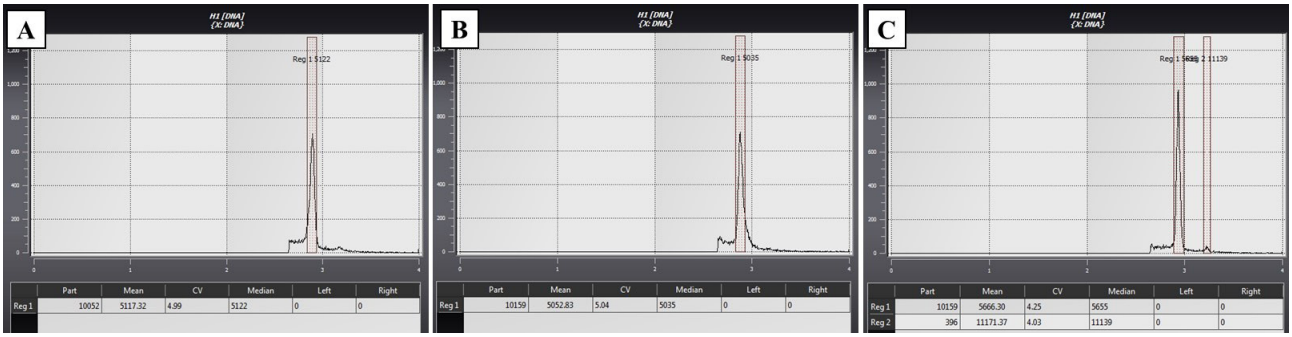

Fig. 2. Ploidy level histograms of the *Hyssopus officinalis* L. 'Nikitskiy Beliy' nuclei isolated from leaves: A – external control of *Ficus benjamina*; **B –** nuclei sample isolated from the leaves of *ex situ* plants; **C –** samples of nuclei obtained from the leaves of *ex vitro* plants after adaptation

Рис. 2. Гистограммы уровня плоидности ядер *Hyssopus officinalis* L. 'Никитский Белый', выделенных из листьев: А – внешний контроль *Ficus benjamina*; **В –** образец с ядрами, выделенными из листьев растений *ex situ*; **С –** образец с ядрами, полученными из листьев побегов растений *ex vitro* после адаптации

Indirect regeneration via callus is considered the main source of somaclonal variation. Thus, for example, FCM-analysis of the nuclei isolated from the shoot segments of *Asparagus officinalis* L. obtained from anther callus revealed that the DNA content in the nuclei had three types of peaks, corresponding to 2x, 4x, and 8x, that represented the diploid, tetraploid and octaploid DNA levels, respectively (Shiga et al., 2009). Meanwhile, no changes in the ploidy level of the callus-derived regenerants of *Eulophia ochreata* Lindl. during micropropagation were shown (Shriram et al., 2014). Cytogenetic studies confirmed that the highest percentage of chromosomal abnormalities were observed, as expected, in callus and protoplast cultures, with a predominance of polyploidy, followed by aneuploidy (Duta-Cornescu, 2023).

In the case of direct regeneration, researchers marked a reduction in the activity of genomic rearrangements, associated with prolonged tissue cultivation in the presence of plant growth regulators (Skaptsov et al., 2016). At the same time, when *Cattleya tigrina* A. Rich. (Orchidaceae) *in vitro* plants were analyzed, regenerants with a doubled ploidy level (26.5%) were identified (Fritsche et al., 2022a). As endopolyploidy is a common feature in orchid tissues, an attempt was made to find evidence of its influence on the cytogenetic stability of regenerated plantlets. Although no direct correlation between the explants' endoploidy and changes in the regenerants' ploidy level was found, the authors emphasized that micropropagation of genetically superior plants can lead to obtaining double ploidy regenerants (Fritsche et al., 2022b). Our data complied with the results earlier obtained on *Hyssopus officinalis* f. *cyaneus* Alef. nuclei isolated from the leaves of *in vitro* microshoots and *ex situ* plants (Plugatar et al., 2023) and attested to the genetic stability of *H. officinalis* 'Nikitskiy Beliy' during short-term *in vitro* culturing.

components in all analyzed samples were bicyclic monoterpene ketones, such as pinocamphone (43.72 and 43.30%) and isopinocamphone (24.97 and 25.88%). Minor components, including mirtenol (5.24 and 5.09%), *β*-pinene (4.20 and 4.70%), elemol (4.57 and 4.40%), and bicyclogermacrene (4.60 and 3.66%), varied slightly (Table 2).

It is known that *in vitro* culture makes it possible to obtain new compounds that have not previously been found in intact plants (Thiem et al., 2011; Luczkiewicz et al., 2015). There are published evidences that demonstrate differences in the chemical composition between *in vitro* material and greenhouse plants (Guedes et al., 2003; Allahverdi-Mamaghani et al., 2022). Meanwhile, a study on *Hypericum androsaemum* L. emphasized that the composition of essential oils was complex, had a variable number of compounds, and depended on the time of harvest and the origin (*in vivo* versus *in vitro*) of the biomass (Guedes et al., 2003).

Clonal micropropagation of valuable essential-oil cultivars implies the production of clones identical not only morphologically and genetically but also biochemically. The results obtained by Kuźma et al. (2009) demonstrated the similarity of chemical profiles and relative amounts of compounds between *in vitro* plants after acclimatization and cultivation for two years in the field and plants propagated from seeds and grown under the same conditions, which is important in view of the biological activity and commercial application of essential oil. According to our data, short-term *in vitro* culturing (six to seven months) of *H. officinalis* 'Nikitskiy Beliy' microshoots on the modified MS nutrient medium containing 6-BAP in optimal concentrations did not significantly affect the biosynthesis pathways of essential oil, which is confirmed by the data obtained by gas chromatography on the regenerated plants after three years of open-ground cultivation.

Table 2. **Component composition of essential oil in *Hyssopus officinalis* L. 'Nikitskiy Beliy'**
Таблица 2. **Компонентный состав эфирного масла *Hyssopus officinalis* L. 'Никитский Белый'**

Component	Retention time	Plant samples	
		Ex situ	*Ex vitro*
		Component mass fraction (%)	
α-pinene	4.56	0.18	0.20
sabinene	5.29	0.96	0.99
β-pinene	5.44	4.20	4.70
β-myrcene	5.54	0.90	0.87
D-limonene	6.56	0.73	0.72
β-phellandrene	6.61	1.08	0.89
(Z)-ocimene	6.90	0.92	0.79
linalool	8.29	1.24	1.22
α-thujone	8.56	0.59	0.64
trans-**pinocamphone**	10.23	43.72	43.30
isopinocamphone *cis*-pinocamphone	10.77	24.97	25.88
terpinene-4-ol	10.81	–	0.21
α-terpineol	11.19	0.74	0.88
myrtenol	11.32	5.24	5.09
methyl myrtenate	14.27	0.36	0.43
β-bourbonene	17.27	0.36	0.41
methyl eugenol	17.44	0.26	0.35
β-caryophyllene	18.37	0.54	0.60
allo-aromadendrene	19.60	0.76	0.54
germacrene D	20.19	1.97	1.72
bicyclogermacrene	20.65	4.60	3.66
elemol	22.06	4.57	4.40
(+)-spathulenol	22.93	0.41	0.47
ledol	23.76	0.26	0.26
γ-eudesmol	24.48	0.44	0.66
Content, %	monoterpenes	8.97	9.16
	sesquiterpenes	8.23	6.93
	Terpenes in total:	**17.20**	**16.09**
	alcohols	12.90	13.19
	ethers	0.62	0.78
	phenols	–	–
	ketones	69.28	69.82
	Terpenoids in total:	**82.80**	**83.79**
Quantitative content of all identified components, %		100	96.15

Conclusion

Cultivation of *Hyssopus officinalis* 'Nikitskiy Beliy' microshoots on an MS nutrient medium with 0.3–0.5 mg/L 6-BAP promotes morphogenesis without significant deviations. Leaf blades *in vitro* retained the overall structure, while some qualitative and quantitative changes associated with the effect of cultivation conditions and material rejuvenation were observed. *Ex vitro* regenerants demonstrated genome stability, which was confirmed by flow cytometry. After *ex situ* cultivation at the collection plot, essential oil yield and component composition of the regenerated plants was similar to the mother plants. The developed protocol for clonal micropropagation of *Hyssopus officinalis* 'Nikitskiy Beliy' provides clones identical to *ex situ* plants.

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