Obtaining a primary suspension cell culture of *Dracocephalum palmatum* Stephan ex Willd

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Abstract. Dracocephalum palmatum Steph. grows on the southern slopes of the Oymyakon plateau in Yakutia (Northeast of Russian Federation) in conditions of harsh continental climate with continuous permafrost. The aboveground phytomass of the plant contains various complexes of secondary metabolites including polyphenolic compounds. It is a potential source of secondary metabolites needed for practical use in the pharmaceutical industry. The aim of the study is to obtain a primary suspension cell culture of Dracocephalum palmatum, growing in the conditions of the Cold Pole — Oymyakon. The work includes optimization of the nutrient medium for introducing calluses into a suspension culture, analysis of the dynamics of biomass growth of the obtained suspension culture, and morphological characteristics of the cells of the suspension culture. The callus cell cultures of Dracocephalum palmatum, cultivated on Murashige-Skoog (MS) medium with the addition of 0.5 mg/L α-naphthylacetic acid (NAA) and 0.2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), were most successfully transitioned into suspension culture. The maximum biomass growth of cell suspension culture was observed when cultivated in liquid MS medium with the addition of 2,4-D (0.5 mg/L), 6-benzylaminopurine (0.5 mg/L), and NAA (0.5 mg/L). The primary cell suspension culture of Dracocephalum palmatum, cultivated for 22 days, had an increase in wet weight of 9,2084 g, dry weight — 0,34135 g, and contained dedifferentiated aggregates of parenchyma-like cells and single round-shaped cells. Samples of the obtained cell suspension culture of Dracocephalum palmatum will be used for the analysis of secondary metabolites and for the development of optimal cultivation conditions in a bioreactor.

1 Introduction

The genus *Dracocephalum* L. (family *Lamiaceae*) includes more than 74 species of essential oil-bearing perennial plants, common in the northern parts of the world with a moderate climate. Representatives of the genus *Dracocephalum* are known as medicinal herbs and have been part of traditional Tibetan and Uyghur medicines for centuries [1–3]. Dragonheads are sources of secondary metabolites with antioxidant, anti-inflammatory, anticancer, and antidiabetic activities [4–8].

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Dracocephalum palmatum Steph is a promising source of polyphenolic compounds and other groups of secondary metabolites. Extracts obtained from the aerial parts of *Dracocephalum palmatum*, growing in the conditions of a harsh continental climate and permafrost in the territory of the Oymyakon district of Yakutia (Northeast of Russia), were identified to contain flavones, flavonols, flavan-3-ols, flavanones, anthocyanins, condensed tannins, lignans, stilbenes, phenolic acids, many of which were identified for the first time for representatives of the genus *Dracocephalum* L. [9]. Extracts obtained from *Dracocephalum palmatum* showed anti-cancer activity [10, 11].

The aim of this study is to obtain a primary cell suspension culture of the plant *Dracocephalum palmatum* Steph, growing in the conditions of the Cold Pole - Oymyakon. In order to achieve this objective, the following tasks were set: optimize the nutrient medium for introducing calluses into suspension culture; study the growth dynamics of the biomass of the obtained suspension culture; study the morphology of the cells of the suspension culture. Samples of callus culture of *Dracocephalum palmatum* obtained in our earlier works were used to initiate the *in vitro* suspension culture.

2 Methods

2.1 Optimization of the nutrient medium for obtaining a primary cell suspension culture in vitro

The Murashige and Skoog (MS) nutrient medium was employed for cultivation purposes without the use of agar. The prepared MS nutrient medium underwent treatment in a steam sterilizer «BK-75-01» (Tyumen Medical Equipment and Instruments Plant, Russia) at a temperature of 120°C for 2 hours before use. In aseptic conditions, a specific combination of synthetic hormones were added to the nutrient medium: 2,4-dichlorophenoxyacetic acid (2,4-D), α-naphthylacetic acid (NAA), kinetin, and 6-benzylaminopurine (BAP). The medium was poured into 250 ml conical flasks to a volume of 100 ml. A piece of callus was placed in each flask with the nutrient medium using pincettes, after which the flasks were placed on an orbital shaker PSU-20i (Biosan, Latvia) with constant mixing at a speed of 120 rpm.

For the passage of the cell suspension culture, one-third of the volume of the primary cell suspension culture was transferred to 100 ml of fresh nutrient medium in 250 ml flasks.

2.2 Method for studying the dynamics of biomass growth of a suspension cell culture in vitro

The overall characterization of biomass growth in an *in vitro* suspension culture was analyzed using the commonly accepted indicator - the growth index [12]. The growth index (I) is calculated based on the following formula:

$$I = \frac{X_{max} - X_o}{X_o},$$

where X_{max} - is a content of cellular biomass at the end of the cultivation cycle, g;

and X_o- is a content of cellular biomass at the beginning of cultivation cycle, g.

Measurements by date were taken by 3 replications (three samples from each flask, n=3). Weighing of the selected samples (wet weight and dry weight) of the cell suspension culture was carried out on high-precision analytical scales Adventurer™ Pro (Ohaus, USA) with a readability of 0.0001 g.

2.3 Method for studying the morphological characteristics of the cells of the suspension culture

The morphological characteristics of suspension culture cells of *D. palmatum* were studied visually. Observation was carried out on pressed temporary slides stained with a freshly prepared 0.1% methylene blue solution, using a Primo Star light microscope (Zeiss, Germany) with an integrated AxioCamErc 5s camera at magnifications of x100 and x400.

3 Results

To introduce *Dracocephalum palmatum* into suspension cell culture, a callus culture line of this plant species that has been derived in the education and science laboratory "Molecular genetics and cell technologies" from November 10, 2018 was used. The Murashige-Skoog (MS) standard medium was utilized as the nutrient medium without agar. The following cultivation options with corresponding concentrations of phytohormones were tested (Table 1).

Table 1. Phytohormonal composition of nutrient media MS for obtaining a suspension culture of *Dracocephalum palmatum* cells.

Nutrient media variant	Phytohormones, mg/L		
	2,4-D	BAP	NAA
1	1	1	1
2	0.2	-	0.5
3	-	0.2	0.5
4	0.5	0.5	0.5

During continuous cultivation on an orbital shaker, variants of suspension biomass of *Dracocephalum palmatum* cells were obtained (Fig. 1).

In all variants of the MS nutrient medium, different degrees of callus transition into suspension and different rates of growth were observed. Cells from the callus variant used showed a high ability to acclimatize in liquid nutrient medium in all tested cultivation variants.

When cultivated in the MS nutrient medium variant No. 1 with the addition of 1 mg/L 2,4-D, BAP, and NAA, good growth was initially observed, however, during subsequent transplants, a noticeable slowdown in biomass growth was apparent (Fig. 1A). When cultivated in the MS nutrient medium variant No. 2 with the addition of 0.2 mg/L 2,4-D and 0.5 mg/L NAA, we observed an intensive transition of callus cells to suspension culture (Fig. 1B).

In cultivation variant No. 3 with the addition of 0.2 mg/L BAP and 0.5 mg/L NAA, the slowest transition of callus cells into suspension was observed, and after two passages, biomass growth ceased (Fig. 1C).

When cultivated in the nutrient medium variant No. 4 with the addition of 0.5 mg/L 2,4-D, BAP, and NAA, the best growth of suspension biomass was observed compared to other variants of the nutrient medium (Fig. 1D).

a b c d

Fig. 1. Cultivation of suspension cell cultures of *Dracocephalum palmatum* in different nutrient medium variants.

3.1 Study of biomass growth dynamics of suspension cell culture of Dracocephalum palmatum

Observation of biomass growth of suspension cell culture of *Dracocephalum palmatum* on MS medium variant No. 1 was carried out from April to September 2023, during this time the growth of cell biomass slowed down after the third passage (Table 2).

Table 2. Growth dynamics of wet and dry biomass of suspension cell culture of *Dracocephalum palmatum* when cultivated on MS medium (variant No. 1)

Planting date	**21.04.2023**		**27.04.2023**		**02.05.2023**	
Cultivation time, days	1	6	1	5	1	6
Wet weight, g	2,7896± 0,0850	5,9903± 0,2033	1,9967± 0,2740	3,2365± 0,1504	1,0927± 0, 3658	1,0987± 0,2303
Dry weight, g	0,1562± 0,0480	0,4675± 0,0530	0,1479± 0,0393	0,2281± 0.0175	0,0851± 0,02125	0,0990± 0,0246

Observation of biomass growth of suspension cell culture of *Dracocephalum palmatum* on MS medium variant No. 2 was carried out from January to March 2024, during this time, the growth cycle equal to 20-22 days was observed (Table 3).

Table 3. Growth dynamics of wet and dry biomass of suspension cell culture of *Dracocephalum palmatum* when cultivated on MS medium (variant No. 2).

Planting date	**10.01.2024**		**02.02.2024**		**22.02.2024**		**15.03.2024**	
Cultivation time, days	1	22	1	20	1	20	1	22
Wet weight, g	1,0102± 0,4438	5,9367 ± 1,7428	1,1722 ± 0,4382	10,2186 ± 2,3069	5,1093 ± 0,7017	8,586± 1,537	2,862± 0,5156	13,07525 ± 0,99725
Dry weight, g	0,06125 ± 0,0273	0,3377 ± 0,0873	0,0537 ± 0,0336	0,4026± 0,0874	0,2268 ± 0,2527	0,31155 ± 0,05575	0,10385 ± 0,01858	0,5183± 0,0217

Observation of biomass growth of suspension cell culture of *Dracocephalum palmatum* on MS medium variant No. 4 was also carried out from January to March 2024, a growth cycle of 20-22 days was also observed (Table 4, Fig. 2).

Table 4. Growth dynamics of wet and dry biomass of suspension cell culture of *Dracocephalum palmatum* when cultivated on MS medium (variant No. 4).

Planting date	**25.01.2024**		**16.02.2024**		**07.03.2024**	
Cultivation time, days	1	22	1	20	1	20
Wet weight, g	1,0099± 0,2943	10,2186± 0,5772	3,4062± 0,1103	11,7585± 0,3195	3,9195± 0,5322	16,0338± 0,0948
Dry weight, g	0,0722± 0,01295	0,4026± 0,0448	0,1342± 0,0269	0,4628± 0,0672	0,1542± 0,0269	0,5305± 0,1279

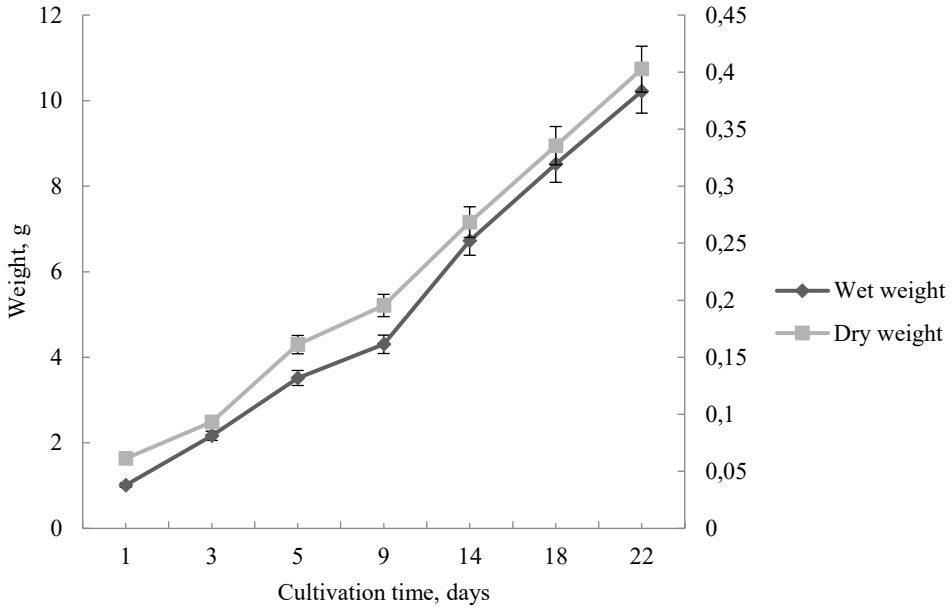
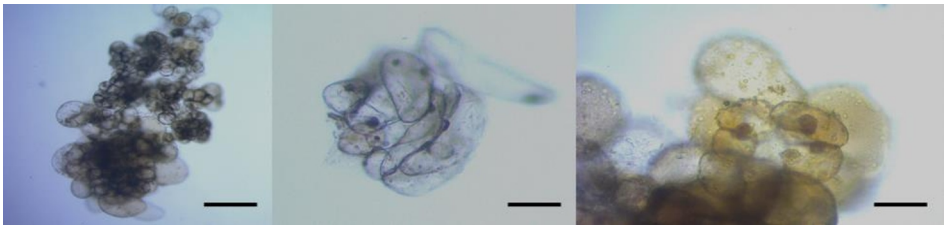


Fig. 2. Growth dynamics of suspension culture *Dracocephalum palmatum* when cultivated on MS medium No. 4 (2,4-D (0.5 mg/L); BAP (0.5 mg/L), NAA (0.5 mg/L); planting date: 25.01.2024). Left Y axis is wet biomass; right Y axis is dry biomass.

3.2 Characteristics of the morphological structure of suspension cell culture of Dracocephalum palmatum

The morphological structure of cells of the obtained variants of *Dracocephalum palmatum* suspension culture was studied on pressed temporary slides stained with freshly prepared solution of 0.1% methylene blue. Observations were conducted using a light microscope at magnifications of x100 and x400.

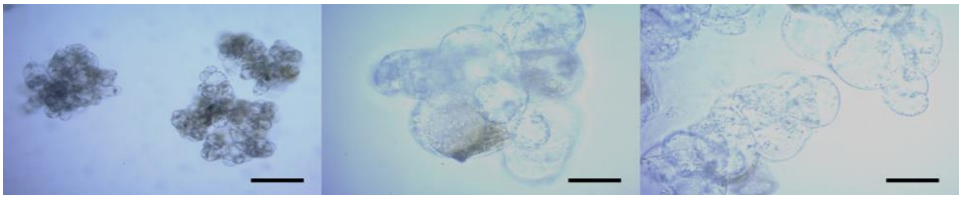
When cultivated on MS medium No. 1, the suspension culture had biomass predominantly non-morphogenetic, non-structured, watery dark brown shade with light beige areas. The biomass mainly consisted of dedifferentiated aggregates of parenchyma-like cells, single cells of round shape with dark cytoplasm and elongated form (Fig. 3).



a b c

Fig. 3. Cells of suspension culture *Dracocephalum palmatum* when cultivated on MS medium No. 1 (2,4-D (1 mg/L), BAP (1 mg/L), NAA (1 mg/L)). a and b - magnification x100, scale bar = 100 µm; c - magnification x400, scale bar = 50 µm.

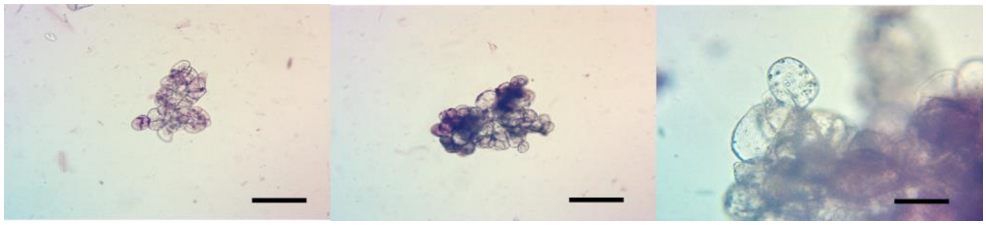
When cultivated on MS medium No. 2, the suspension culture had predominantly non-morphogenic, homogeneous, slightly swollen watered biomass of a light beige shade with rare dark speckles. The biomass consisted of dedifferentiated aggregates of parenchyma-like cells and individual large round cells with transparent cytoplasm (Fig. 4).



a b c

Fig. 4. Cells of suspension culture *Dracocephalum palmatum* when cultivated on MS medium No. 2 (2,4-D (0.2 mg/L), NAA (0.5 mg/L)). a - magnification x100, scale bar = 100 µm; b and c - magnification x400, scale bar = 50 µm.

When cultivated on MS medium No. 4, the suspension culture had predominantly homogeneous biomass of light-gray color with beige speckles. The biomass consisted of dedifferentiated aggregates of parenchyma-like cells and large cells of round and oval shape with semi-transparent cytoplasm (Fig. 5).



a b c

Fig. 5. Cells of suspension culture *Dracocephalum palmatum* when cultivated on MS medium No. 4 (2,4-D (0.5 mg/L); BAP (0.5 mg/L); NAA (0.5 mg/L)). a and b - magnification x100, scale bar = 100 µm; c - magnification x400, scale bar = 50 µm.

4 Discussion

Among the tested cultivation variants, the best growth of the suspension culture of *Dracocephalum palmatum* was achieved on MS medium No. 4 with the addition of 2,4-D (0.5 mg/L), BAP (0.5 mg/L), and NAA (0.5 mg/L). In cultivation variant No. 3 with the

addition of BAP (0.2 mg/L) and NAA (0.5 mg/L), the growth of biomass stopped after two passages. In the MS nutrient media variants No. 1 and No. 2, the growth index values are lower compared to those on MS nutrient medium No. 4 (Table 5).

Table 5. Growth index of wet and dry biomass of suspension culture of *Dracocephalum palmatum* on different variants of MS nutrient media.

Nutrient media variant	Phytogormones, mg/L			Cultivation time, days	Wet biomass growth index	Dry biomass growth index
	2,4-D	BAP	NAA			
1	1.0	1.0	1.0	6	1.1473±0.1356	1,3098±0,6831
2	0.2	-	0.5	21	6.2971±0.7365	5,5606±0,0523
3	-	0.2	0.5	–	–	-
4	0.5	0.5	0.5	21	9.1154±1.058	5,5731±0,6073

Thus, the growth index of the wet biomass of *Dracocephalum palmatum* suspension culture when adding 1 mg/L of phytohormones to the MS medium (variant No. 1) is 9 times lower than in the variant of the nutrient medium with the addition of phytohormones at 0.5 mg/L (variant No. 4). Concurrently, in variant No. 1, the degradation phase occurs almost immediately after the first passage, the culture growth slows down almost twice over the same period of time, which was 6 days. This variant's degradation phase occurs after the 3rd passage, as the suspension mass remained unchanged on the 18th day.

The conducted cytological analysis of the suspension cultures shows that in the variant of the nutrient medium No. 2, the biomass consists of dedifferentiated aggregates of parenchyma-like cells, as well as single round and vermiform cells. The suspension biomass is light beige with rare dark speckles. In variant No. 4 of the nutrient medium, the biomass consists of dedifferentiated aggregates of parenchyma-like cells and single large round cells with transparent cytoplasm, irregular and vermiform cells are absent.

5 Conclusion

Dracocephalum palmatum Steph. is a perennial herbaceous plant of the northern regions. The plant grows on the southern slopes of the Oymyakon Highlands in Yakutia, where a harsh continental climate is typical and the continuous layer of permafrost is near the surface. The study indicates that the aboveground biomass of the plant is rich in secondary metabolites, including polyphenolic compounds. The plant is valuable as a source of secondary metabolites that are sought after for various applications in the pharmaceutical industry.

In this work, a suspension cell culture of *Dracocephalum palmatum* was first obtained based on a previously obtained callus cell line, initiated from leaf explants of sterile seedlings cultivated from wild plant seeds. The work included optimization of the nutrient medium for introducing calluses into a suspension culture, analysis of the growth dynamics of the biomass of the obtained variants of the suspension culture, and study of the morphological characteristics of the cells in the suspension cultures. The best growth of the biomass of the suspension cell culture of *Dracocephalum palmatum* was observed when cultivated in liquid MS nutrient medium with the addition of 2,4-D (0.5 mg/L), BAP (0.5 mg/L), and NAA (0.5 mg/L). After being cultured for 22 days, the primary suspension cell culture of *Dracocephalum palmatum* showed a wet weight increase of 9.2084 g and a dry weight increase of 0,34135 g. The culture contained dedifferentiated aggregates of parenchyma-like cells and individual round cells.

The developed variant of the *Dracocephalum palmatum* suspension culture has been transferred to Russian Collection of Cultivated Cells of Land Plants of the Institute of Plant Physiology of the Russian Academy of Sciences and displayed under the designation NEFU

Dpalm-2 (registry No. 118). Samples from the suspension cell culture of *Dracocephalum palmatum* will be used to analyze secondary metabolites and to establish optimal cultivation conditions in a bioreactor.

The work was carried out with the ongoing funding support of the laboratories of NEFU (RP No. 6, 2021-2024).

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