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INFLUENCE OF META-CHLORO-BENZHYDRYL UREA ON PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF *Saussurea orgaadayi* V. Khan. and Krasnob. CELL CULTURE

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Abstract

Meta-chloro-benzhydryl urea (m-CBU) is an inducer of the human monooxygenase system, its key enzymes belong to the cytochrome P-450 superfamily (CYP). Currently, there is no information about the role of m-CBU in the plant vital activity regulation; however, the participation of CYP in the metabolism of secondary metabolites, for example, flavonoids (Fl), and most phytohormones have been shown. *Saussurea orgaadayi* V. Khan. and Krasnob. is a poorly studied plant species. Its cell culture, in accordance with our data, doubles the total amount of endogenous Fl during the transition from exponential growth to the stationary phase. The present study, for the first time shows statistically significant ($p < 0.05$) differences in the growth responses of the callus culture of the *S. orgaadayi* to m-CBU in different concentrations. It was revealed that the change in the growth index in terms of fresh and dry weight is related to a change in the volume and shape of cells, as well as the occurrence frequency of different groups of cells. Here, for the first time, the dynamics of the content of Fl, accompanying changes in the growth of culture under the influence of m-CBU was assessed. The aim of this work was to determine the role of meta-chloro-benzhydryl urea in the accumulation of flavonoids and the variability of cytomorphological characteristics of *S. orgaadayi* callus culture (cell linear dimensions, volume and shape, the frequency of cells of different sizes, growth index for the fresh and dry biomass). A callus culture derived from cotyledon explants of sterile seedling was repeatedly passaged on a modified Murashige-Skoog (MS) agar nutrient medium supplemented with sucrose, vitamins and growth regulators 0.8 mg/l 2,4-D and 0.5 mg/l 6-BAP. The culture was grown in the dark at a temperature of 22-24 °C in MS medium added with growth regulators and 0.01, 0.1, 1, 10, or 100 μM m-CBU (Sintegal, LLC, Russia). In the control, m-CBU was not added. After 30 days of subculture, $^2/_3$ of the material was used to determine the wet and dry biomass followed by the isolation of flavonoids, and $^1/_3$ of the material was fixed in Clark's solution for 2 days. To prepare micropreparations, the cell culture was macerated in a 3 N hydrochloric acid solution with constant shaking until a homogeneous cell suspension was obtained. Cytophotometric analysis was performed using light microscopy (video camera Moticam 2300, Motic, Spain) with software. The sizes of 100 cells were measured for each variant, their shape was estimated, and the volume was calculated. To calculate the growth index (GI), the initial (beginning of subcultivation, M_0) and the final fresh or dry weight of calli (on day 30 of subculture, M_{30}) were determined and expressed as a percentage of the control: $GI = (M_{30} - M_0)/M_0$. The total amount of Fl in the callus culture was quantified based on the colored Fl complexation with aluminum chloride followed by measurement of the optical density (a UV-1650 spectrophotometer, Shimadzu Corp., Japan). As a result of the studies, a dose-dependent effect of m-CBU on cell growth was established due to their division (0.1 μM) and stretching (1-100 μM), which was accompanied by a 2.1-3.5-fold and 1.5-2.9-fold increase in the GI_f and GI_d of callus culture, respectively ($p < 0.05$). At a concentration of 0.1 μM, the number of small meristematic cells increased by 16 % compared to the control. At the same time, the average volume of large cells decreased by 31 % as compared to the control, which indicates inhibition of cell elongation processes. With the increase in m-CBU concentration, the frequency of cells of two groups increased, by 55 and 30 % for

medium-sized cells at 1 and 10 μM, respectively, and by 50 and 57 % for large-sized cells at 1 and 100 μM, respectively. The volume of cells also increased compared to the control, by 61 % at 10 μM for large cells and by 18 % at 100 μM of small cells. m-CBU reduced the total amount of endogenous flavonoids by 80-95 % ($p < 0.05$) upon activation of growth processes in *S. orgaadayi* cells in vitro. The content of the total Fl decreased maximally at 0.01 and 0.1 μM m-CBU and did not differ significantly from the control at 100 μM m-CBU. The maximum 3.5-fold increase in GI_f in the medium with 1 μM m-CBU occurred simultaneously with an 83 % decrease in the amount of Fl. m-CBU can be used in plant biotechnology as a cell growth modulator in callus cultures to reduce the content of growth-inhibiting metabolites. To activate cell division, the most preferable dose is 0.1 μM m-CBU, while to change the content of Fl, which doubles the biomass of the culture, 100 μM m-CBU should be used.

Keywords: *Saussurea orgaadayi*, cell culture, meta-chloro-benzhydryl urea, morphogenesis, flavonoids

Plants, unlike animals and humans, synthesize secondary metabolites, including flavonoids (Fls), the plant specialized metabolites of which more than 6900 compounds have been described [1]. These metabolites are synthesized from p-coumaroyl-CoA by the sequential action of various enzymes, which are believed to form ordered macromolecular multi-enzyme complexes of flavonoid enzymes weakly-bound to membranes (for example, to the endoplasmic reticulum), called flavonoid metabolons [1, 2]. Localization and duration of interactions between specific proteins play an important role in the synthesis of specific FLs during plant growth and response to stressors [1, 3]. FLs protect the plants against leaf-eating insects, pathogenic microorganisms, ultraviolet radiation and high-intensity light, attract pollinating insects, inhibit the formation of reactive oxygen species (ROS), participate in pollen germination, provide biological communication in the rhizosphere during nodulation, increase the efficiency of use nutrients during the aging of the plant and its organs [3-6]. They are capable of metal chelation, which may serve as an in vivo mechanism to reduce toxicity [3]. In addition, FLs act as developmental regulators involved in changes in the transport of the phytohormone indoleyl-3-acetic acid (IAA) and its metabolism [7].

Fls, not synthesized by animals and humans, are essential nutrients. Natural FLs are low toxic and serve for the prevention and treatment of various pathologies [8]. Dietary FLs with antioxidant activity reduce the incidence of atherosclerosis, cardiovascular diseases, diabetes, thrombosis, inflammation in arthritis, neurodegenerative diseases (Alzheimer's and Parkinson's diseases), obesity, hyperlipidemia, and hypertension [4]. Quercetin exerts an antiproliferative effect on cancer cell lines [9]. FLs are a raw material for the industrial production of pharmacological and cosmetic substances, which raises the question of controlling the content of FLs in plants.

Not only exogenous adverse biotic and abiotic factors but also endogenous factors, e.g., a hormonal balance associated with the age of the plant, its organs and cell cultures, can induce the FL biosynthesis [5, 10-12]. Cytochrome-P-450-dependent monooxygenases (CYP), the components of flavonoid metabolons are involved in the FL modification [1, 2, 13]. Meta-chlorobenzhydryl urea (m-CBU) is an inducer of the human cytochrome P-450-dependent monooxygenase system [14] but there is no information on the role of m-CBU in the regulation of plant vital activity.

Multiplicity of metabolic processes and structural elements in plants (at the cellular, tissue, and organ levels) hinders the study of molecular mechanisms. Thereof, heterotrophic cell cultures that are simpler in structure and do not exert photosynthetic activity can serve as the most successful models.

This paper shows for the first time statistically significant ($p \leq 0.05$) differences in the growth responses of the *Saussurea orgaadayi* V. Khan. and Krasnob. callus culture to various concentrations of m-CBU. It was found that the

growth rate (by fresh and dry weight) depends on the volume and shape of the cells and the proportion of different groups of cells. This is the first data on the dynamics of flavonoids as related to in vitro culture growth under the influence of m-CBU.

The work aimed to determine the role of meta-chlorobenzhydryl urea in the accumulation of flavonoids and the variability of cytomorphological parameters (linear dimensions, volume and shape of cells, the rate of different cell groups, the growth index for wet and dry callus biomass) of *S. orgaadayi* callus culture.

Materials and methods. Meta-chlorobenzhydryl urea (Galodif, CAS: 124057-07-4) was synthesized at the Tomsk Polytechnic University [15], the copyright holders of the drug are Syntegal LLC and Science, Technology, Medicine LLC (Russia). For 0.01, 0.1, 1, 10, and 100 μM m-CBU, a stock m-CBU alcohol solution (100 mM) was diluted.

Callus cultures induced from cotyledon explants of *S. orgaadayi* sterile seedlings were repeatedly subcultured on a modified agar Murashige-Skoog nutrient medium (MS) added with sucrose, vitamins and growth regulators 0.8 mg/l 2,4-D (2,4-dichlorophenoxyacetic acid) and 0.5 mg/l 6-BAP (6-benzynaminopurine) [16]. Calluses were grown in the dark at 22-24 °C on MS medium + growth regulators added with m-CBU (control was not added with m-CBU).

After 30-day subculture, $^2/_3$ of each callus was weighed to determine wet and dry mass and used for flavonoid isolation; $^1/_3$ portions were fixed in Clark's solution (96% ethyl alcohol:glacial acetic acid, 3:1) for 2 days, washed with 96% ethanol (3 times for 30 min) until the smell of acetic acid disappeared, and stored in 70% ethanol at 4 °C [17]. For micropreparations, the calli were macerated in 3 N hydrochloric acid solution with shaking until a homogeneous cell suspension is obtained.

Cytophotometric analysis was performed using light microscopy (Moticam 2300 video camera with software, Motic, Spain). The dimensions (L — length, D — width) of 100 cells were measured. Cell shape was assessed by the L/D ratio, the $1.0 \le L/D \le 1.14$ for round, $1.15 \le L/D \le 1.94$ for oval, and $L/D \ge 1.95$ for elongated cells. The cell volume was calculated according to the Tselniker's formula [18] with the correction factor (k) calculated by the author, which depends on L/D. At $L/D > 2.5$, to determine the cell volume (V, μm^3), the formula for the volume of a cylinder was used: $V = \pi(D/2)2Lk$. At $L/D \le 2.5$, the formula for a rotated ellipsoid was applied: $V = 4/3\pi L/2(D/2)^2$. To calculate the growth index (GI), the initial (at the beginning of subculture, M_0) and final wet or dry weight of calli on day 30 (M_{30}) were determined and expressed as a percentage of the control: $GI = (M_{30} - M_0)/M_0$.

Total FLs of *S. orgaadayi* callus culture were quantified by spectrophotometric assay based on a colored aluminium complex formation (a UV-1650 spectrophotometer, Shimadzu Corp., Japan) [19]. A portion of dry biomass (1 g) was extracted three times (for 60 min each) with 70% ethanol in a boiling water bath, and the extracts were combined. An aliquot of the bulk sample was added with aluminum chloride and acetic acid and allowed for 40 mi (a reference solution without aluminum chloride was prepared for each sample). The optical density of the test solution and the standard rutin solution was measured at $\lambda = 415$ nm. Total Fl content was expressed as rutin equivalents per absolute dry raw material (X_{Fl}): $X_{Fl} = OD_x \cdot K_x \cdot m_{rut} \cdot 100 \cdot 100 \cdot OD_{rut}^{-1} \cdot K_{rut}^{-1} \cdot m_x^{-1} \cdot (100 - W)^{-1}$, where OD_x is the optical density of the test solution; OD_{rut} is the optical density of the rutin solution; m_x is raw material mass, g; m_{rut} is the mass of rutin, g; K_x is the dilution factor of the test solution (1250); K_{rut} is dilution factor of a solution of rutin (2500); W is the weight loss from drying, %.

To perform statistical analysis (IBM SPSS Statistics for Windows, IBM Corporation, USA), the parametric Student's *t*-test and the nonparametric Mann-Whitney U-test for pairwise comparison of group parameters were used. The

figures show the arithmetic mean values (M) for growth (n = 100) and biochemical (n = 5) parameters with two-sided confidence intervals (M±1.96 SEM). Differences between values marked with different letters are statistically significant at $p < 0.05$.

Results. In our experiments, we studied morphogenesis and Fl accumulation in cotyledon-derived slowly growing callus culture of *S. orgaadayi* depending on the m-CBU concentration in the nutrient medium (Fig. 1). The choice of the crop was based on our preliminary studies [20] which showed a 3-fold excess of the Fl content (0.026±0.006% of dry weight) on day 25 in a slow-growing in vitro culture compared to actively growing culture from *S. orgaadayi* seedling hypocotyls.

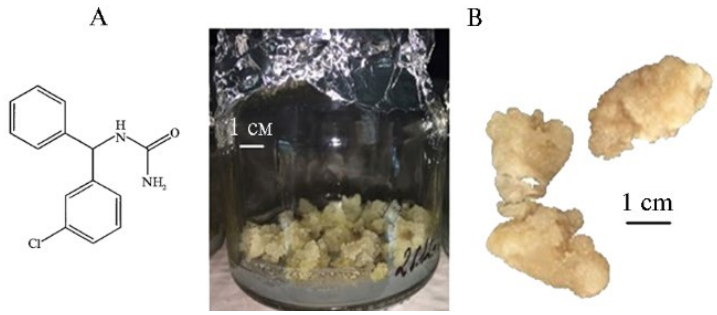

Fig. 1. Meta-chlorobenzhydryl urea (A) and callus cell culture derived from cotyledon explants of *Saussurea orgaadayi* V. Khan. and Krasnob. seedlings (B).

The time of callus subculturing followed from the pattern of an increase in wet weight of the control culture. The callus culture had an S-shaped growth curve showing several specific phases: a 10-day lag phase of low growth, a 15-day logarithmic phase, a 5-day deceleration phase, and a stationary phase starting from day 30 (data not shown). On day 25 of subculturing, the GI_{wet} of the callus in the control was 2.32±0.70. The experiment continued up to day 30 to reach the stationary phase and to increase the yield of secondary metabolites Fls, which is consistent with the data of other authors [12].

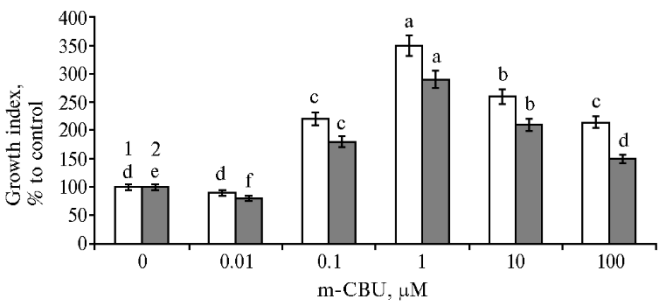

Fig. 2. Relative gain of raw (1) **and dry** (2) **weight of *Saussurea orgaadayi* V. Khan. and Krasnob. callus culture depending on the concentration of meta-chlorobenzhydryl urea** (m-CBU) **in the growth medium.** Differences between each indicator marked with different letters are statistically significant at $p < 0.05$ (M±1.96 SEM).

At 0.1-100 μM, m-CBU stimulated callus growth with a 2.1-3.5-fold increase in GI_{wet} and a 1.5-2.9-fold increase in GI_{dry} values ($p < 0.05$) as compared to the control (Fig. 2). At 0.01 μM, m-CBU did not change GI_{wet} but reduced GI_{dry} by 20% ($p < 0.05$). The GI_{wet} and GI_{dry} values were the highest at 1 μM m-CBU. The m-CBU-treated calli differed from the control in a lighter color and a looser consistency.

Cytological analysis revealed morphological heterogeneity of the *S. orgaadayi* cell population (Fig. 3, 4). We identified groups of cells that differed in shape (round, oval and elongated) and size (small, medium and large). We regarded small cells (9.0-35.7 thousand μm^3) as dividing meristematic cells, medium-sized cells (35.75-75.94 thousand μm^3) as growing, and large cells (76.0-323.2 thousand μm^3) as completed growth.

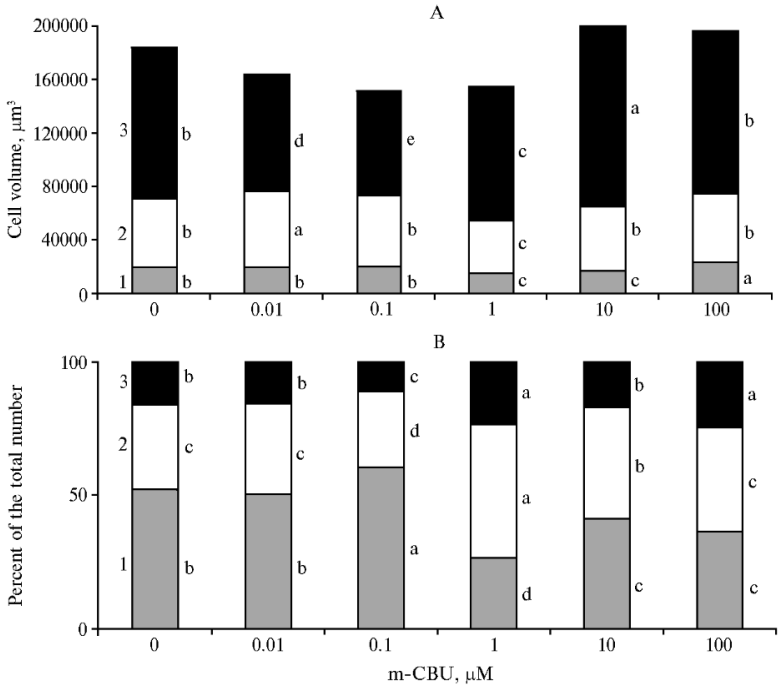

Fig. 3. Cell volume (A) **and the rate of small** (1)**, medium-sized** (2)**, and large** (3) **cells** (B) **in the *Saussurea orgaadayi* V. Khan. and Krasnob. callus culture depending on the concentration of meta-chlorobenzhydryl urea** (m-CBU) **in the growth medium**. Differences between each indicator marked with different letters are statistically significant at $p < 0.05$ ($M \pm 1.96$ SEM).

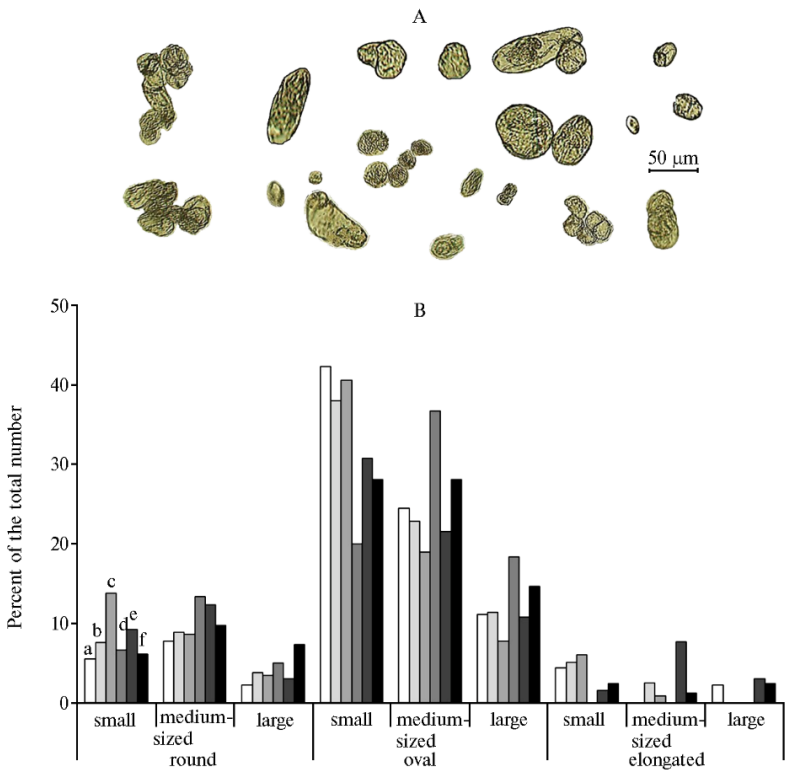

Fig. 4. Shape (A) **and the rate** (B) **of cells of various shapes and sizes in the *Saussurea orgaadayi* V. Khan. and Krasnob. callus culture depending on the concentration of meta-chlorobenzhydryl urea in the growth medium:** a — 0 (control); b — 0.01 μM; c — 0.1 μM; d — 1 μM; e — 10 μM; e — 100 μM.

In the control callus culture on day 30, the rate of small cells was 1.6 and 3.4 times higher than medium and large cells, respectively (Fig. 3, B). The average volume of small cells was 20.2±2.3 thousand μm^3, of medium and large cells 51.5±1.4 and 112.1±2.6 thousand μm^3 (Fig. 3, A).

The m-CBU was shown to affect cell growth in the calli. At 0.1 μM m-CBU, the number of small meristematic cells increased by 16% compared to the control, which could indicate activated cell division (see Fig. 3, B). The average volume of large cells decreased by 31% compared to the control (see Fig. 3, A), which indicated inhibition of cell elongation. With an increase in the m-CBU concentration, there was an increase in the rate of medium-sized cells by 55 and 30% for 1 and 10 μM and large cells by 50 and 57% ($p < 0.05$) for 1 and 100 μM (see Fig. 3, B). In large cells the volume increased by 61% ($p < 0.05$) for 10 μM, in small cells by 18% ($p < 0.05$) for 100 μM compared to the control. The number of small cells decreased by 49, 20, and 30% ($p < 0.05$) for 1, 10, and 100 μM m-CBU, and their volume decreased by 22 and 17% ($p < 0.05$) for 1 and 10 μM (see Fig. 3).

m-CBU increased the proportion of round cells of all sizes (Fig. 4, B). The greatest changes occurred in the rate of small cells (a 2.5-fofld increase, $p < 0.05$, for 0.1 μM m-CBU). The rate of medium-sized and large cells increased 1.7-fold and 2.3-fold, respectively ($p < 0.05$) for 1 μM m-CBU. The highest rate increased by 3.3 times ($p < 0.05$) was recorded for large round cells at 100 μM m-CBU.

A change in growth processes in the cell culture could indicate a change in the levels of endogenous substances that regulate growth. Among the latter, there are Fl modulating the homeostasis of plant hormones auxins [7]. We found out that the total content of Fl in the control subcultured calli at the stationary stage (day 30) was 0.049±0.008% of dry weight, which is 50% ($p < 0.05$) more than on day 25 at the inflection point of the logarithmic section of the growth curve. These data indicate a slowdown in growth with an increase in the of endogenous Fl content.

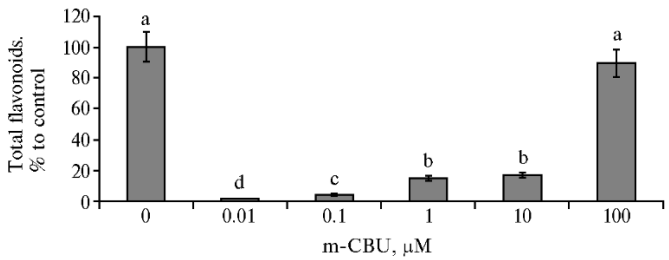

Fig. 5. Total flavonoids in the *Saussurea orgaadayi* V. Khan. and Krasnob. callus culture depending on the concentration of meta-chlorobenzhydryl urea (m-CBU) **in the growth medium.** Differences between each indicator marked with different letters are statistically significant at $p < 0.05$ (M±1.96 SEM).

Under the action of m-CBU, the total content of Fl changed compared to the control (Fig. 5). It was shown for the first time that a decrease (by 80-95%) in the total amount of endogenous Fl caused by m-CBU activates cell growth in the *S. orgaadayi* in vitro cell culture. The total Fl content decreased to its minimum at low m-CBU concentrations and remained at the control level at 100 μM m-CBU. The maximum (a 3.5-fold) increase in biomass, noted at 1 μM m-CBU, occurred with an 83% decrease in the total Fl.

Thus, our study showed the effect of m-CBU on the growth of *S. orgaadayi* callus culture, which is probably due to the m-CBU effect on cell elongation and division. Dose-dependent changes in the accumulation of culture biomass (see Fig. 2) occurred together with a change in the rate of cells of various sizes and shapes (see Fig. 3, 4). The action of m-CBU at the lowest concentration (0.01 μM) reduced GI_{dry}, which could be due to a decrease in the volume of large cells by 23% with an equal control of the ratio of cell groups ranked by size. The highest

GI_{wet} and GI_{dry} observed under the action of 1 μM m-CBU were accompanied by an increased frequency of medium-sized and large cells and a decrease in the proportion of small cells (see Fig. 3, B). The concentration of 0.1 μM m-CBU had a lesser effect than 1 μM m-CBU, which manifested itself in an increase in the frequency of small cells and, accordingly, in a decrease in the proportion of medium-sized and large cells.

The highest stimulation of callus growth occurs under the influence of 0.1-10 μM m-CBU, leading to abundant small round meristematic cells. Growth acceleration results in a change of secondary metabolism and 80-95% lower total Fl ($p < 0.05$) compared to the control (see Fig. 5). At a high concentration (100 μM), m-CBU significantly stimulates culture growth without a noticeable change in the Fl level. Dose-dependent patterns of callus growth and Fl content under the influence of m-CBU may probably indicate a change in the functioning of enzymes involved. Goncharuk et al. [21] who studied the effect of exogenous cadmium and glyphosate on the phenolic compounds also indicate modification of FLs in cell culture: the sum of phenolic compounds increased when phenylpropanoids and flavonoids decreased.

FLs which could affect callus growth represent a highly diverse class of polyphenolic secondary metabolites possessing various properties. Along with catechins and quercetin known for their antioxidant activity, some FLs at high doses exert pro-oxidant effect and can damage cells [8].

FLs are involved in cell cycle regulation [22]. Possible mechanisms include the direct interaction of Fls (for example, quercetin) with Raf and MEK protein kinases which determine the transmission of mitotic signals, or their binding to the AhR receptor and the formation of a complex with ARNT which stimulates the transcription of the cell cycle inhibitor CDKNB1 [22, 23].

In addition, an indirect effect of FLs on growth due to changes in the transport of auxins and their metabolism is also possible. It is known that the spatiotemporal distribution of auxins, determined by the mechanisms of polar transport, plays a decisive role in their physiological effects [24]. Using *tt4* mutants as an example, it was shown that Fl deficiency enhances the flow of IAA [25]. Flavonols are thought to directly modulate IAA transport, a process that is reduced in the F39H-defective *tt7* mutant that overproduces kaempferol [25]. Non-glycosylated kaempferol and quercetin compete with the auxin transport inhibitor 1-N-naphthylphthalamic acid for a high-affinity binding site for a complex containing proteins PGP1, PGP2, and MDR1/PGP19 which belong to ATP-binding cassette transporters. FLs also act as regulators of IAA oxidase involved in the IAA degradation. FLs with o-hydroxyls in the B ring (quercetin, myricetin, luteolin) inhibit the enzyme activity while FLs with p-hydroxyls (apigenin, naringenin, naringin) stimulate it [26]. As a result, the first group of Fls are stimulates plant growth while the second group inhibits it.

Fl oxidation by plant peroxidases or other enzymes is an integral stage of normal plant growth and development [27, 28]. Due to oxidation, FLs possess prooxidant properties and can damage biological structures, which reduces cell viability [28].

The m-CBU can regulate the activity of heme-containing enzymes of the cytochrome P-450 (CYP) family of monooxygenases in humans. Enzymes of this group are found in all kingdoms and catalyze various chemical reactions [13, 25]. Changes in the *S. orgaadayi* cell culture growth and the Fl content influenced by m-CBU can probably also be explained by the regulation of the functioning of CYPs involved in Fl metabolism [2]. It has been shown that *CYP82D* encodes flavone-6-hydroxylase and 7-demethylase and is responsible for the biosynthesis

of Fls in sweet basil, while *CYP75* regulates the expression of flavonoid-3′-hydroxylase and flavonoid-3′,5′-hydroxylase which are involved in the synthesis of most anthocyanins in red grapes [13]. CYP93G1, the closest homologue of CYP93G2 in rice, is flavone synthase II (FNSII) which catalyzes the direct conversion of flavanones to flavones [30].

Plant hormones could act as other endogenous *S. orgaadayi* cell culture growth regulating factors the metabolism of which is also regulated by CYP. For example, CYP79B2/B3 is responsible for the conversion of L-tryptophan (L-Trp) to indole-3-acetaldoxime in an alternative L-Trp-dependent auxin biosynthesis pathway [13], cytokinin hydroxylase CYP735A1 and CYP735A2 catalyze the biosynthesis of trans-zeatins, and CYP72C1 inactivates brassinosteroids in *Arabidopsis thaliana* [13, 25].

Membrane mechanisms of m-CBU action on plant cells are also possible, by analogy with human cells. Such mechanisms may involve the blockade of fast neuronal sodium channels, which limits the propagation of the electrical potential along biological membranes. As a result, m-CBU stabilizes the concentration gradient of ions by regulating the water-electrolyte balance and prevents changes in the membrane permeability and transmembrane potential of the cell. Also, m-CBU enhances microsomal oxidation, showing a detoxifying effect in cells.

In our experiment, high Fls in 30-day subculture caused the completion of *S. orgaadayi* callus active growth in the control, which is consistent with the data on an increase in Fl accumulation at the stationary stage of cell culture [11, 12] and in leaves of *Lactuca sativa* L. plants that have completed their growth [5]. It has also been shown [12, 29] that in long-term suspension culture, the oxidative status (ROS accumulation) increases, and activation of antioxidant enzymes and secondary metabolites occurs. It should be expected that m-SBU increases the biomass of the cell culture, probably due to a lower level of F1 and, consequently, a longer time of active cell growth.

Our findings and other data [2, 12, 25, 28-30] allow us to assume the influence of m-SBU on the activity of enzymes that regulate the content of Fls and plant hormones, as well as enzymatic antioxidants.

Thus, the slow-growing *Saussurea orgaadayi* callus culture derived from the cotyledon explant produces flavonoids. Meta-chlorobenzhydryl urea (m-CBU) added to the growth medium at a concentration of 0.01 to 10 μM decreases the content of secondary metabolites in the callus. A lower level of flavonoids activates the growth in plant cell culture. Callus biomass increases with maximum growth index at 1 μM m-CBU. A further increase in the m-CBU concentration slows down the biomass growth. In the calli, the rate of cells of different types and sizes changes depending on the m-CBU concentration. Under the influence of 0.1 μm m-CBU, the number of small meristematic cells increased, and starting from 1 μM m-CBU, the round and oval cells of medium and large size are abundant while a proportion of small cells decreases. At 10 μM m-CBU, the cells were the largest in size, and at 100 μM m-CBU, the maximum number of large cells appeared. These data indicate acceleration of cell division at low m-CBU concentrations and cell elongation at medium and high m-CBU concentrations. Our findings show a dose-dependent effect of m-CBU on cell growth through a change in the content of flavonoids.

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