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PLANT PHYSIOLOGY

The Elicitor Effect of Yeast Extract on the Accumulation of Phenolic Compounds in *Linum grandiflorum* Cells Cultured In Vitro and Their Antiradical Activity

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Abstract—This paper examines the elicitor effect of yeast extract (YE) in various concentrations (200–1000 mg/L) on the accumulation of phenolic compounds (PC) in flowering flax (*Linum grandiflorum* Desf.) cells cultured in vitro and their antiradical activity. It is established that the total PC content and the content of phenylpropanoids increase in the cell culture, especially at high YE concentrations in the medium (500 and 1000 mg/L). The antiradical activity of flax culture extracts remains in most cases at the control level. Therefore, the elicitation of flowering flax in vitro cultures by YE activates the PC biosynthesis resulting in the accumulation of these secondary metabolites, while the antiradical activity of cell culture extracts does not decrease compared to the control level.

Keywords: flowering flax, *Linum grandiflorum*, callus culture, elicitation, yeast extract, phenolic compounds, antiradical activity

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INTRODUCTION

Effects exercised by various elicitors on plants (in vitro cell and tissue cultures) present a promising research direction in modern biology. Elicitors make it possible to regulate the genetic and production potential of plants, including the accumulation of specialized (secondary) metabolites with pharmacologically valuable properties (Tarchevsky, 2002; Naik and Al-Khayri, 2016; Jamiołkowska, 2020). The elicitation mechanism is quite complex; it involves signal transduction into plant cells implemented with the participation of small messenger molecules: hydrogen peroxide, nitric oxide, oligosaccharins, and various low molecular weight compounds (Tarchevsky, 2002; Ramirez-Estrada et al., 2016). The major factors determining the effectiveness of this process are the elicitor type, its concentration, exposure duration, and application method (Narayani and Srivastava, 2017; Naskar et al., 2021).

In modern studies, elicitors are divided into the biotic, abiotic, endogenous, exogenous, general, and specific types (Jamiołkowska, 2020). Biotic elicitors have a number of advantages over abiotic ones and are normally used to optimize the phytochemical composition of plants (Kannojia et al., 2019). One such biotic elicitor is yeast extract (YE): a set of compounds that have a beneficial effect on plant growth, productivity, microelement composition, and the content of phytohormones and other plant metabolites (Naik and Al-Khayri, 2016; Halder et al., 2019).

One of the earliest "responses" of plant cells to elicitation is the generation of reactive oxygen species (ROS), which is considered their universal nonspecific response (Hasanuzzaman et al., 2020). The antioxidant system represented by high and low molecular weight compounds plays the key role in the ROS content regulation. Low molecular weight antioxidants are considered the second line of cell protection against ROS; they are crucial when the content of high molecular weight antioxidants is low (Erb and Kliebenstein, 2020).

Low molecular weight antioxidants include phenolic compounds (PC): secondary plant metabolites that are diverse in structure and chemical properties (Cheynier et al., 2013; Wagay et al., 2020). Their interaction with ROS prevents/reduces free-radical oxidation processes, thus protecting plant cells from their toxic effects (Kumar and Pandey, 2013). The antioxi-

dant properties of plant PCs-are preserved when they enter the human body; thereby, they are successfully used in pharmacology to treat various diseases (Tungmunnithum et al., 2018).

It must be noted that raw plant materials are becoming less and less accessible with each passing year due to environmental and geopolitical reasons (Nosov, 2012).

Biotechnological approaches, including in vitro cell and tissue cultures, represent an alternative to traditional agronomic farming or the collection of pharmacologically valuable plants for the industrial production of secondary metabolites (Naik and Al-Khayri, 2016; Hidalgo et al., 2018). Their application makes it possible to optimize the growth and productivity of plant cells and automate these processes. It is also important that in vitro cultures are more "metabolically viable" than plants (Nosov, 2012). Developments in this field involve the selection of highly productive cell lines, cell immobilization and permeabilization, bioreactor and metabolic engineering, and elicitation (Hidalgo et al., 2018; Salem et al., 2020). The latter technique represents a promising strategy that can be used to increase the content of secondary metabolites in plant cells in situations when other methods are laborious and ineffective (Naik and Al-Khayri, 2016; Halder et al., 2019).

Yeast extract is an elicitor frequently applied to plant cells cultured in vitro (Ramirez-Estrada et al., 2016; Narayani and Srivastava, 2017). Its effects significantly increase the PC content in *Zingiber officinale* callus cultures (Ali et al., 2018), in *Polygonum multiflorum* beard root cultures (Ho et al., 2018), and in callus and suspension cultures initiated from other plants (El-Nabarawy et al., 2015; Bhaskar et al., 2022).

The high biological and antioxidant activity of most plant PCs necessitates expansion of the species diversity of cultures able to accumulate them in vitro. The list of plants promising in this respect includes representatives of the genus *Linum,* which consists of more than 200 species distributed throughout the world (Cullis, 2019). These plants are distinguished by rapid ontogenetic development and are used as commercially important oilseed, fiber, and medicinal crops (Gorshkova, 2007; Zuk et al., 2015). One of their representatives, the flowering flax (*Linum grandiflorum* Desf.), is a valuable ornamental and medicinal plant (Asad et al., 2021). Its extracts feature a wide range of antiviral and antibacterial activities; they inhibit enzymes belonging to the class of bacterial cell hydrolases (tyrosine kinase and phosphodiesterase) and HIV transcriptase (reverse transcriptase of the immunodeficiency virus) (Mohammed et al., 2010). Flowering flax synthesizes various PC, including flavones and their glycosides (luteolin, glucoluteolin, and vicenin) with hypotensive and anti-inflammatory properties (Karuppusamy, 2009; Alamgir, 2017). Valuable biologically active compounds produced by *L. grandiflorum* include lignans (podophyllotoxin and its derivatives: deoxypodophyllotoxin and methoxypodophyllotoxin) that feature high anticarcinogenic, cytotoxic, and antiviral activities and are difficult to produce using other cultures (Asad et al., 2021).

Taking the small number of works examining the elicitation of flowering flax callus cultures with biotic substances, the purpose of this study was to analyze mechanisms regulating the PC accumulation in *L. grandiflorum* in vitro cultures elicited by yeast extract and to assess the antiradical activity of its ethanol extracts.

MATERIALS AND METHODS

The object of this study was a flowering flax (*L. grandiflorum* Desf.) callus culture initiated from hypocotyls of young seedlings. It was cultivated at 24°C with a 16-h photoperiod (5000 lx) in the phytotron chamber of the Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, on the Gamborg medium containing 2,4-dichlorophenoxyacetic acid (2 mg/L). The passage duration was 30 days.

In the course of the experiments, 15-day-old calli (in the middle of the linear growth phase) were transferred to nutrient media containing, in addition to the main components, YE in various concentrations (200, 500, or 1000 mg/L). The basic nutrient medium was used as the control. Callus cultures were analyzed behind 1 and 7 days after the beginning of elicitation. Prior to biochemical tests, they were fixed with liquid nitrogen and stored at −70°C.

The water content in calli was measured after drying them to a constant weight at 70°C (Rogozhin and Rogozhina, 2013).

The lipid peroxidation (LPO) level was determined based on the content of malondialdehyde (MDA) formed in the reaction with thiobarbituric acid (TBA) (Radyukina et al., 2011). A quantity of frozen plant material was homogenized in 0.1 M Tris-HCl buffer (pH 7.5) containing 0.35 M NaCl; then 1 mL of 0.5% TBA solution in a 20% aqueous solution of trichloroacetic acid was added. The reaction mixture was incubated in a water bath (100°C) for 30 min. The optical density of the solution was measured with a spectrophotometer at 532 nm. To compute the MDA content (μmol/g fresh weight), the molar extinction coefficient (1.56×10^{-5} cm^{-1} M^{-1}) was used.

PC were extracted with 96% ethanol for 45 min at 45°C from plant material previously frozen in liquid nitrogen and crushed. The extracts were centrifuged (13 500 *g*, 10 min), and the supernatant liquid was used for the spectrophotometric determination of the total PC content (Folin–Denis reagent) and the content of phenylpropanoids (direct spectrophotometry of the solution) (Olenichenko and Zagoskina, 2005; Olennikov and Tankhaeva 2011). The total PC content was expressed in mg-equiv rutin/g dry weight; the content

of phenylpropanoids, in mg-equiv caffeic acid/g dry weight.

The antiradical activity of ethanol extracts of flax callus cultures was determined using the spectrophotometric method based on the ability of PC to inhibit superoxide and $OH^{\cdot}$ previously generated in vitro under model conditions (Volkova et al., 2011). For this purpose, a reaction mixture was used; it consisted of methionine, riboflavin, nitro blue tetrazolium, and the tested extract (in experimental variants) or extractant (in the control variant). The formazan content in the reaction mixture was determined at 560 nm. Antiradical activity expressed as the superoxide inhibition proportion (%) was computed using the formula

$$\text{Inhibition } \% = 100\left(1 - A2/A1\right),$$

where $A1$ is the formazan optical density at a wavelength of 560 nm without the addition of the extract and $A2$ is the formazan optical density at a wavelength of 560 nm with the addition of the tested extract.

Absorption spectra of ethanol extracts of flax callus cultures were measured with a Specord 40 spectrophotometer (Germany) within the wavelength range of 250–350 nm.

The presence of 6-methoxypodophyllotoxin in ethanol extracts of flax callus cultures was analyzed using high performance liquid chromatography (HPLC). An Agilent Technologies system (Model 1100) was used (Kubyshkin et al., 2018). The chromatograph is equipped with a G1379A flow vacuum degasifier, a G13111A 4-channel low-pressure gradient pump, a G1313A automatic injector, a G13116A column thermostat, a G1316A diode array detector, and a G1315B fluorescent detector.

PC were separated using a ZORBAX SB-C18 chromatographic column (2.1 × 150 mm) filled with silica gel with a grafted octadecylsilyl phase with a particle size of 3.5 μm. Eluent composition: methanol as solution A and 0.6% aqueous solution of trifluoroacetic acid as solution B. Chromatography was performed in the gradient mode. The eluent composition changed (by the content of component B) according to the following scheme: 0 min, 8%; 0–8 min, 8–38%; 8–24 min, 38–100%; and 24–30 min, 100%. Eluent flow rate: 0.25 mL/min. Volume of the injected sample: 1 μL. Chromatograms were recorded at 280 nm. To identify 6-methoxypodophyllotoxin, its spectral characteristics and retention time were compared with the respective characteristics of the standard.

Statistical data processing was performed in MS Excel 2007 using generally accepted methods. Biological experiments were conducted with threefold replication; analytical tests, with threefold–fourfold replication. The diagrams show the arithmetic mean values and standard errors of the computed parameters.

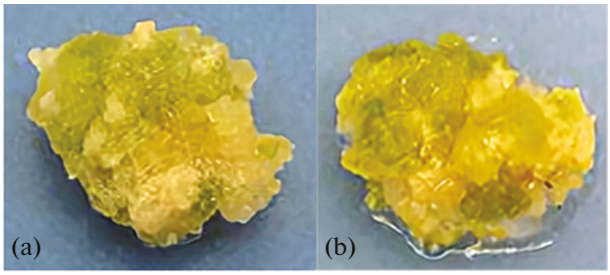


Fig. 1. External appearance of *Linum grandiflorum* callus cultures cultivated (a) on the basic nutrient medium and (b) on the medium containing yeast extract (500 mg/L). Both calli are 22 days old.

RESULTS AND DISCUSSION

Morphophysiological Parameters of Flowering Flax Callus Cultures

Flowering flax callus cultures in the control and experimental variants were of medium density, yellow–green in color, and consisted of coarsely aggregated cell conglomerates (Fig. 1). The green color indicated the formation of chlorophyll-containing cells on the calli surfaces. All cultures feature a low growth rate: the average increase in biomass by the end of the passage is 220 ± 20%.

Water content is an important physiological parameter of in vitro cultures. After one-day elicitation, it remained at the control level in the variant with the lowest YE concentration in the medium (200 mg/L) and increased equally in variants with higher YE concentrations in the medium (500 and 1000 mg/L) (Fig. 2). After a longer elicitation period (seven days), it was similar to that in the control in all experimental variants. Apparently, stretching and vacuolization processes are initiated in flax cells in the initial period of the YE effect, and these processes are concentration-dependent, which was also noted by other researchers (Farjaminezhad and Garoosi, 2021).

The Malondialdehyde Content in Flowering Flax Callus Cultures

The redox homeostasis of plant cells determined by the balance between ROS-formation and destruction is of great importance for their vital activity, including situations when cells are affected by various factors (Hasanuzzaman et al., 2020). The lipid peroxidation (LPO) level estimated based on the MDA content is an important criterion of this process (Kolupaev et al., 2019). After one-day elicitation, in flax calli cultivated on media with YE concentrations of 200 and 500 mg/L, it was equal to and 30% lower than the control value, respectively (Fig. 3). A different trend was noted at the highest YE concentration (1000 mg/L): the LPO level exceeded that in the control variant by 25%. Therefore, a short-term exposure of flax cells cultured in vitro to the elicitor causes changes in the

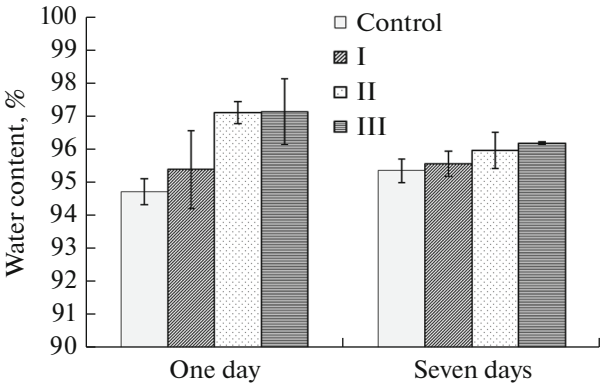


Fig. 2. Water content in *Linum grandiflorum* callus cultures after short-term (one day) and long-term (seven days) elicitation with yeast extract in various concentrations: (I) 200 mg/L, (II) 500 mg/L, and (III) 1000 mg/L. In the control variant, the medium does not contain the elicitor.

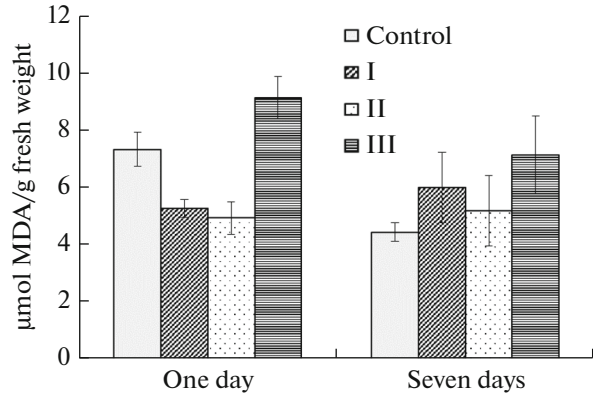


Fig. 3. The malondialdehyde (MDA) content in *Linum grandiflorum* callus cultures after short-term (one day) and long-term (seven days) elicitation with yeast extract in various concentrations: (I) 200 mg/L, (II) 500 mg/L, and (III) 1000 mg/L. In the control variant, the medium does not contain the elicitor.

functioning of their antioxidant system. In the experimental variants, this effect is manifested both in the reduction of the LPO level in the calli (low YE concentrations) and in its increase (the highest YE concentration). Other authors also note that elicitation conditions affect the ROS content in plant cells (Ali et al., 2018; Hasanuzzaman et al., 2020).

Lower (by 30%) LPO levels in flax calli in the control and one of the experimental variants (YE 1000 mg/L) at the end of the experiment (day 7) compared to its beginning (day 1) are of special interest. Apparently, this is because the ROS content in these calli decreases and their pro/antioxidant activity balance is normalized (Naik and Al-Khayri, 2016; Kolupaev et al., 2019). Overall, after a prolonged (7 days) exposure to YE, the MDA content in all experimental variants exceeds that in the control, although statistically significant (by 40%) differences are typical only for the highest YE concentration in the medium (1000 mg/L).

It is known that the plant cell elicitation process depends both on the concentration of the substance and the exposure duration (Tarchevsky, 2002; Makowski et al., 2020). Based on our data, it can be assumed that a short-term (one day) exposure to a high (1000 mg/L) YE concentration presumably activates ROS formation in flax cells cultured in vitro. By contrast, lower YE concentrations (200 and 500 mg/L) reduce the LPO level in flax cells due to changes in the pro/antioxidant balance (Naskar et al., 2021). If the elicitation period is longer (seven days), then the MDA content in flax calli increases relative to the control; apparently, this is due to the excessive ROI formation and disturbances in the redox balance. This is consistent with data reported by other authors: the response of plant cells depends on the exposure duration and the concentration of elicitors (Makowski et al., 2020; Farjaminezhad and Garoosi, 2021).

Content of Phenolic Compounds in Flowering Flax Callus Cultures

PC are effective antioxidants the formation of which is typical for all plant cells and tissues (Karuppusamy, 2009). Their biosynthesis is a distinctive feature of plants belonging to the genus *Linum*, including the flowering flax (Tchoumtchoua et al., 2019; Asad et al., 2021). The total PC content makes it possible to determine the contribution of these low molecular weight antioxidants to the formation of plant cell responses to the effect of the factor studied (Karuppusamy, 2009). Data presented in Fig. 4a indicate that the flax culture exposed to the short-term (one day) effect of YE in a low (200 mg/L) concentration features the lowest PC content equal to that in the control variant. At higher concentrations of the elicitor in the medium (500 and 1000 mg/L), the PC content increases significantly (by two and three times, respectively). Based on these data, it can be concluded that short-term exposures to YE contribute to the PC accumulation in flowering flax callus cultures.

Similar (to a certain extent) trends were observed after a longer (seven days) exposure to the elicitor: in the presence of high YE concentrations (500 and 1000 mg/L) in the medium, the total PC content in cultures increases by almost 200 and 300%, respectively compared to the control. The YE concentration of 200 mg/L also increases the total PC content, but to a much lesser extent (by 35% relative to the control).

Our results are consistent with scarce data collected by other researchers on the stimulatory effect of biotic elicitors on PC accumulation in callus cultures of various plant species (El-Nabarawy et al., 2015; Bhaskar et al., 2022). Correlations between the content of these metabolites elicited in in vitro cultures on the one hand and the elicitor concentration and exposure

duration on the other hand were noted (Ali et al., 2018; Nunes da Silva et al., 2021). There is evidence that some elicitors not only increase the content of PC, including flavonoids, but concurrently reduce the viability of cultures, which slows down cell growth and causes their death (Jesionek et al., 2018). For instance, the strategy aimed to induce the biosynthesis of such an antitumor compound as paclitaxel in yew suspension cultures slows down their growth, which makes it difficult to use elicitation on a large scale (Salem et al., 2020).

Content of Phenylpropanoids in Flowering Flax Callus Cultures

Changes in the accumulation of phenylpropanoids, the simplest specialized phenolic metabolites the formation of which is typical for flax (Volynets, 2013), can be an important factor characterizing the response of plant cells to elicitation. In the initial period of the study (day 1), their content in the control variants of flax callus cultures was 30% higher than at the end of the experiment (day 7) (Fig. 4b). Such a reduction can be determined by changes in the formation of phenylpropanoids in the course of the passage, which was noted by other authors as well (Asad et al., 2021).

Elicitation of flax callus cultures is accompanied by the accumulation of phenylpropanoids in them, and this effect depends on the YE concentration in the nutrient medium and exposure duration. The content of these phenolic metabolites increases after a short-term (1 day) exposure to YE in concentrations of 200, 500, and 1000 mg/L by 20, 50, and 175%, respectively, compared to the control. Therefore, the higher the elicitor concentration in the nutrient medium, the greater the increase in the content of phenylpropanoids in flax calli. This is consistent with data provided by other authors and indicating a direct correlation between the concentration of the active exogenous factor and the response of cells manifested in the PC accumulation in them (Ali et al., 2018; Halder et al., 2019; Makowski et al., 2020).

A comparison of phenylpropanoid concentrations in flax calli after a prolonged (7 days) elicitation also indicates their greater accumulation in the experimental variants compared to the control, which is manifested most clearly at high YE concentrations (500 and 1000 mg/L). Interestingly, the content of phenylpropanoids is equal to that in the previous study period (one-day exposure).

Based on the data obtained, it can be assumed that the biosynthesis of phenylpropanoids is activated in flax in vitro cultures mainly at the initial elicitation stages. For instance, after a short-term exposure to the elicitor, the accumulation of these PC depends on its active concentration, reaches the maximum at high doses, and then no further activation of this process is observed. An increase in the content of phenylpropanoids and an increase in the expression of genes of the phenylpropanoid pathway in the course of exogenous treatment of in vitro plant cultures with biotic elicitors represented by polysaccharides (including YE) have been described in the literature. This is typical for some representatives of the families Labiatae and Boraginaceae and for such a valuable medicinal crop as *Hypericum perforatum* (Park et al., 2016; Shakya et al., 2019). All this can indicate the regulatory role of phenylpropanoids that are distinguished by a high radical-binding capability in the maintenance of redox homeostasis in plant cells.

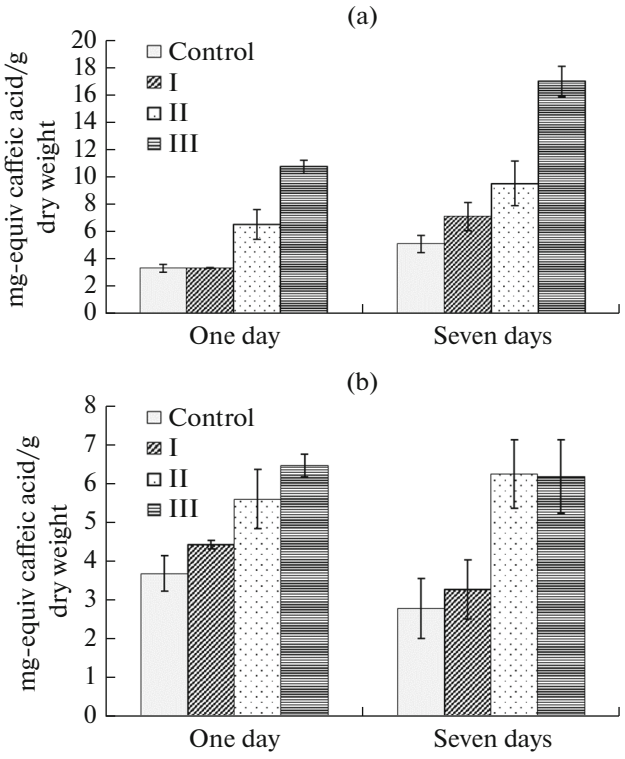


Fig. 4. (a) Total PC content and (b) content of phenylpropanoids in *Linum grandiflorum* callus cultures after short-term (one day) and long-term (seven days) elicitation with yeast extract in various concentrations: (I) 200 mg/L, (II) 500 mg/L, and (III) 1000 mg/L. In the control variant, the medium does not contain the elicitor.

Phenolic Compounds and Lignans in Flowering Flax Callus Cultures

The composition of PC and changes in it caused by exogenous impacts are important indicators of the metabolic activity of plant cells and tissues (Cheynier et al., 2013). The absorption spectra of ethanol extracts of flax callus cultures were examined to establish the presence of certain metabolites in them. The wavelength range from 250 to 350 nm, where the main and additional absorption maxima of most PC are recorded (Volynets, 2013), was used. In all studied ethanol extracts of flax callus cultures, the absorption

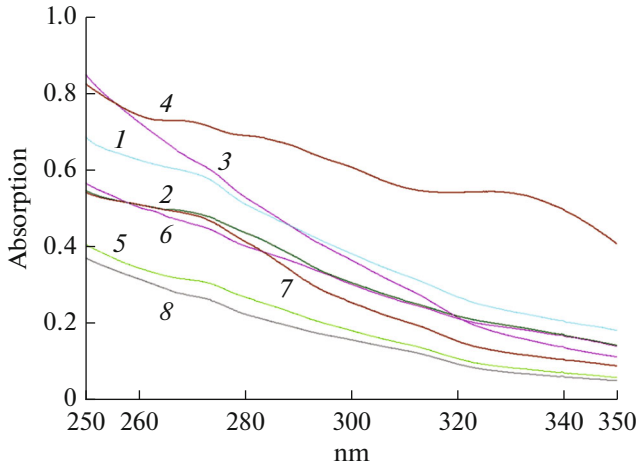


Fig. 5. UV absorption spectra of ethanol extracts of *Linum grandiflorum* callus cultures after short-term (one day; variants *1–4*) and long-term (seven days; variants *5–8*) elicitation with yeast extract in various concentrations: 200 mg/L (variants *2* and *6*), 500 mg/L (variants *3* and *7*), and 1000 mg/L (variants *4* and *8*). In control variants (*1* and *5*), the medium does not contain the elicitor.

maximum was recorded at 260 nm (Fig. 5). This corresponds to the main absorption maximum of hydroxybenzoic acids (235–270 nm). At other wavelengths, no clearly manifested maxima were recorded in the UV region, which can be due to low PC concentrations in ethanol extracts of callus cultures (although an additional maximum was sometimes present at 330 nm). This confirms the presence of phenylpropanoid compounds in the extracts represented by conjugates of *p*-hydroxybenzoic, *p*-coumaric, ferulic, and caffeic acids, which was also noted in our earlier studies (Goncharuk et al., 2018). The significant similarity of UV spectra of extracts obtained from all studied variants of flowering flax callus cultures must also be noted; this may indicate that the composition of their phenolic complexes is similar as well. Literature sources also note the absence of differences in the phenolic complex composition between in vitro plant cell cultures, including those exposed to various exogenous factors (Ossipov et al., 2022). The absorption spectra of extracts obtained from different variants of flax callus cultures indicate that the PC concentrations in them are different, which is consistent with the measurement data (Fig. 4).

Lignans are important and pharmacologically valuable metabolites synthesized by flax plants: these dimeric phenylpropanoids are considered primary antioxidants neutralizing free radicals (Mohammed et al., 2010; Alamgir et al., 2017). According to the literature, these compounds are accumulated in photomyxotrophic flax callus cultures and elicitation exercises a stimulating effect on their formation (Karuppusamy, 2009; Naik and Al-Khayri, 2016).

Most literature sources report that flax plants, including flowering flax, produce lignans such as podophyllotoxin, deoxypodophyllotoxin, and 6-methoxypodophyllotoxin (MPTOX) (Mohammed et al., 2010). HPLC chromatography has made it possible to establish the presence of MPTOX in ethanol extracts of flowering flax callus cultures (Fig. 6). Its content was low and fairly similar in all variants. Therefore, the biosynthesis of MPTOX, a derivative of the phenylpropanoid pathway, is typical for flowering flax callus cultures. The ability of cells cultured in vitro to biosynthesize secondary metabolites specific to intact plant tissues has been reported repeatedly in the literature (Nosov, 2012; Halder et al., 2019).

All of the aforesaid indicates that flowering flax cells cultured in vitro retain the ability to accumulate PCs, including lignans, specific to intact plants. The elicitor effect does not change their composition, but increases their content. Therefore, elicitation of flax cells with YE makes it possible to regulate their biosynthetic activity with regard to the accumulation of PC (mainly phenylpropanoids: biologically active metabolites featuring antioxidant activity).

Antiradical Properties of Extracts of Flowering Flax Callus Cultures

The antiradical activity of plant extracts is an important parameter characterizing their suitability for pharmacology (Mohammed et al., 2010; Alamgir, 2017). The antiradical activity of PC is manifested in relation to free radicals formed under the impact of external factors of various nature; therefore, it was important to compare the antiradical activity of plant extracts of flowering flax callus cultures in the control and experimental variants. Data presented in Fig. 7 indicate that after a short-term (one day) exposure to YE, the parameter values in different variants are similar, and the differences between them are statistically insignificant. After seven days of elicitation, the antiradical activity of extracts of callus cultures was equal in the control variant and in the variant with the YE concentration of 200 mg/L; this antiradical activity value was the highest in the experiment; and it was 50% higher than that after a short (one-day) elicitation period. In other variants, the antiradical activity was lower: by 17% in extracts of calli cultivated on the medium with a YE concentration of 500 mg/L and by 4.5 times in extracts of calli cultivated on the medium with a YE concentration of 1000 mg/L. Therefore, the above-described elicitation strategy for flowering flax callus cultures either inhibits the antiradical activity of their ethanol extracts (after seven days of exposure to high YE concentrations) or has no effect on the process studied (short-term (one-day) exposure to YE). This reflects the multifunctionality of the effect exercised by low molecular weight antioxidants and difficulties hindering the evaluation of their contribution to the antiradical activity of plant extracts. Published

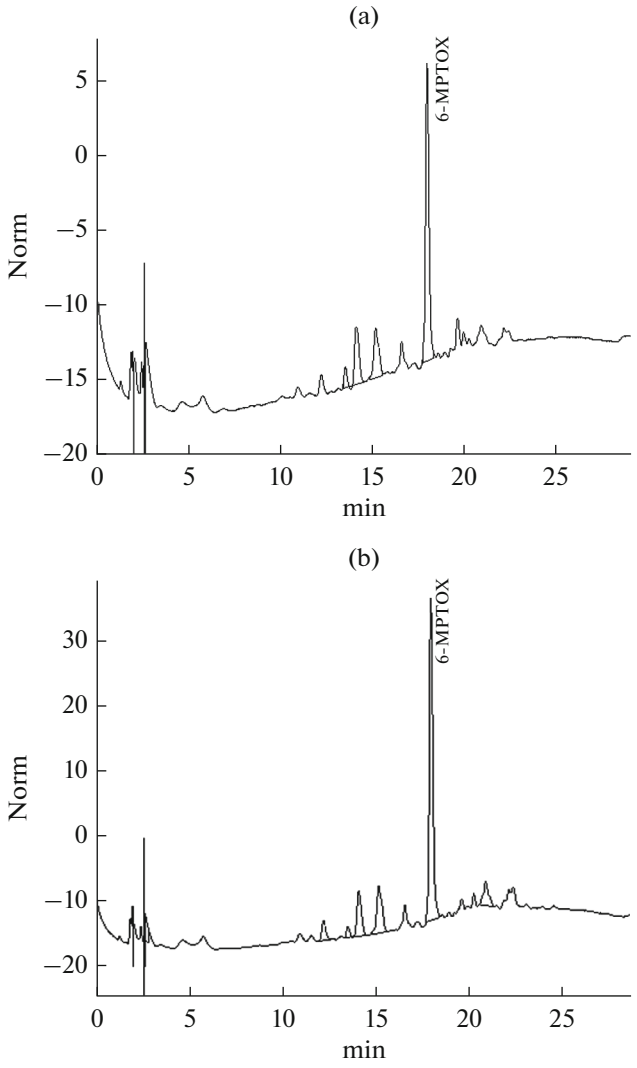


Fig. 6. HPLC chromatograms of ethanol extracts of flowering flax callus cultures recorded at 280 nm: (a) control variant and (b) experimental variant (yeast extract 500 mg/L). The mAU data are indicated on the axis of ordinates; the compound (6-methoxypodophyllotoxin (MPTOX)) retention time (min), on the axis of abscissas.

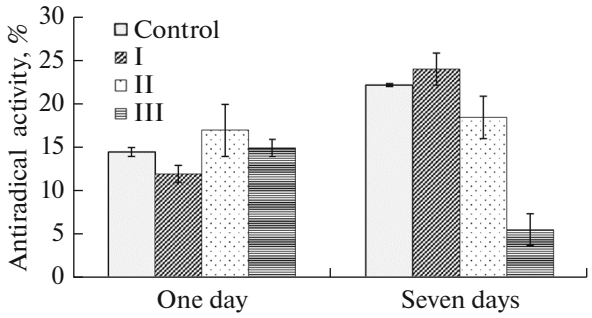


Fig. 7. Antiradical activity of ethanol extracts of *Linum grandiflorum* callus cultures after short-term (one day) and long-term (seven days) elicitation with yeast extract in various concentrations: (I) 200 mg/L, (II) 500 mg/L, and (III) 1000 mg/L. In the control variant, the medium does not contain the elicitor.

sources also note that the content of phenolic secondary metabolites in plant cells and tissues does not always correlate with the antiradical and antioxidant activity of their extracts (Ali et al., 2018).

CONCLUSIONS

All of the aforesaid confirms that the potentiality to extract specialized metabolites, including PC, from in vitro cultures of plant cells and tissues varies depending on their type, origin, elicitation parameters, and external factors. Being an environmentally safe biotic compound, YE stimulates the formation of PC; however, its elicitor effect largely depends on the concentration and exposure duration. Based on the data presented, it can be concluded that further transcriptomic, metabolomic, and proteomic studies are required to optimize the formation of PC in plant cell cultures and to understand the regulation of their biosynthesis under elicitation conditions. Such studies will also make it possible to solve both fundamental and applied issues associated with the production of unique plant metabolites with antioxidant properties.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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