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ARTICLES

Fatty Acid Composition of Total Lipids from Needles and Cultured Calluses of Conifers *Pinus sylvestris* L., *Picea pungens* Engelm., *Pinus koraiensis* Siebold & Zucc., and *Larix sibirica* Ledeb.

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Abstract—A comparative analysis of the fatty acid (FA) composition of lipids in the calluses and needles of Scots pine (*Pinus sylvestris*), white spruce (*Picea pungens*), Korean pine (*Pinus koraiensis*), and Siberian larch (*Larix sibirica*) has been carried out using the method of gas-liquid chromatography–mass spectrometry. In all cases, the total content of monounsaturated FA in the lipids of callus was higher than in the lipids of needles. Oleic acid (C18:1) represented a significant part of the composition of these acids. It was established that the lipids of callus of the studied species are characterized by a lower relative content and a smaller variety of Δ5-acids than the lipids of needles of the same species. It was shown that species-specificity of FA composition of the lipids of needles remains the same in the plant tissues grown in culture in vitro and does not depend on the material used to initiate the callus. Qualitative differences in the FA composition of the callus in culture and the needles of whole plant were detected. It was shown that the embryogenetic ability of the callus is associated with high content of oleic acid.

Keywords: conifers, *Pinus sylvestris*, *Picea pungens*, *Pinus koraiensis*, *Larix sibirica*, callus, needles, lipids, fatty acids

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INTRODUCTION

The processes in Nature that lead to a reduction of the area of coniferous are aggravated by ever-increasing anthropogenic impact [1]. For the preservation and restoration of coniferous forests in Russia it is necessary to combine the methods of traditional breeding with modern biotechnological techniques, such as the development and use of cell cultures in vitro, which is an important tool in physiological and biochemical studies [2]. One of the main features of a plant material, which is necessary for obtaining the culture, is the ability of the cells forming it to divide and grow rapidly. It is known that the most intensive process of callusogenesis, as well as morpho- and embryogenesis in vitro, develops when juvenile tissues or plant organs, i.e., tissues of embryos, seeds and seedlings, are used as a starting material [3, 4]. However, to obtain the callus from plus coniferous trees, it is necessary to use tissues of adult plants, in which it is much more difficult to initiate the callusogenesis and morphogenetic processes [5, 6]. This makes a comparative study of biochemical changes during growth and development of tissues both in vitro and in the plant particularly topical. During embryogenesis, carbohydrates are accumulated first, and then lipids and proteins are stored up [7, 8]. The protein and carbohydrate content in the callus of conifers was studied in sufficient detail earlier [9–11]. However, despite the large attention paid currently to the study of lipids in plants, many issues concerning the lipid metabolism in the callus, including the callus of conifers, were studied insufficiently. In particular, a comparative analysis of the composition of fatty acids (FA) of the total lipids of the callus of two larch species – *Larix sibirica* and *L. gmelinii* was performed; a high degree of unsaturation of FA was detected, and the presence of Δ5-acids was revealed, which is characteristic of conifers [12]. An increase in the content of total lipids and changes in the content of FA in the process of maturation of somatic embryos of Norway spruce were shown, which may indicate an important role of lipids, including FA, in embryonic development [13, 14]. It was concluded that the amount and composition of total lipids required for embryonic development of the callus can help to assess the competence of somatic embryos to complete the subsequent stages of development. It should be noted that the analysis of fatty acid composition of total lipids of plant tissues is of considerable interest because of the extremely important role played by long-chain FA both in the structural-functional organization of cell membranes and in processes of cellular metabolism. Besides the fact that FA are components

of lipids, they serve as intermediaries in the transmission of signals, including the processes of cell differentiation, thereby participating in morphogenesis [15]. Therefore, to understand the specifics of lipid metabolism in the coniferous during callusogenesis and embryogenesis and the possible involvement of FA in these processes, it seems important to analyze the differences in the FA composition of lipids in culture in vitro and in the tissues of plants growing under natural conditions. In this regard, the aim of the present work was a comparative analysis of the FA composition of the lipids of needles of *Pinus sylvestris* L., *Picea pungens* Engelm., *P. koraiensis* Siebold & Zucc., and *L. sibirica* Ledeb. and of the lipids of callus derived from different organs and tissues of these species.

MATERIALS AND METHODS

The non-embryogenic callus lines derived from the needles of *P. sylvestris*, the apical parts of shoots of *P. pungens* branches, the buds with the apical part of the shoots of *P. koraiensis* branches and the embryogenic and non-embryogenic callus lines of *L. sibirica* derived from zygotic embryos at the stage of formation of the cotyledons were used as the research objects. For initiation of the callus cultures of *P. sylvestris, P. koraiensis* and *P. pungens*, a modified Murashige and Skoog medium was used [16]. 2,4-dichlorophenoxyacetic acid (2,4-D, 2 mg/L) and 6-benzylaminopurine (BAP, 0.5 mg/L) were used as growth regulators. The explants and callus derived from them were cultured in the dark at a constant temperature of 24 ± 1°C. The explants were obtained from the apical sections of last year's branches of 10–15 cm in size, with buds and needles from the middle or lower third of the crowns of the trees. For the induction of somatic embryogenesis, isolated zygotic embryos were used at the stage of initiation of cotyledons from seeds collected from the trees of Siberian larch at the age of 40–60 years, growing in the arboretum of the Sukachev Institute of Forest of the Siberian Branch of the RAS (Krasnoyarsk) and in Khakassia. As the objects of comparison of the FA composition of lipids, the needles of mature trees of *P. sylvestris, L. sibirica, P. pungens*, and *P. koraiensis* collected at the experimental plot of SIPPB SB RAS (Irkutsk) were used.

For extraction of total lipids (the fraction of membrane lipids of the callus was on average of 70% of the total amount of lipids, in needles it was of 50%), a sample of the plant material (0.5 g) was fixed in liquid nitrogen, 0.001% ionol was added and the sample was ground to obtain a homogeneous mass [17]. Then 10 mL of a chloroform : methanol mixture (1 : 2 vol/vol) was added, thoroughly mixed and left to stand for 30 min. Chloroform (research grade, stabilized with 0.005% wt of amylene) was removed from the lipid extract under vacuum with a rotary evaporator RVO-64 (Czech Republic). To check the effectiveness of the extraction of lipids (%), nonadecanoic acid (C19:0) was used. FA methyl esters (FAME) were obtained from the extract according to [18]. Additional purification of FAME was carried out by TLC on glass plates with silica gel KSK (Russia) in the chamber with benzene as a mobile phase (Rf = 0.71–0.73). FAME were analyzed by gas-liquid chromatography using a chromatography-mass spectrometer 5973/6890N MSD/DS AgilentTechnologies (USA). Detector was a quadrupole mass spectrometer, method of ionization was electron impact, ionization energy 70 eV. For the analysis, the mode of registration of the total ion current was used. For separation, a capillary column HP-INNOWAX (30 m × 250 μm × 0.50 μm) with stationary phase of polyethylene glycol was used. Helium was used as the carrier gas, flow rate 1 mL/min. Temperature of the evaporator was 250°C; of the ion source, 230°C, and of the detector, 150°C; the temperature of the AUX line was 280°C. The scanning range was 41–450 a.m.u. The volume of the injected sample was 1 μL, the flow divider 5 : 1. FAME mixture was separated under isothermal conditions at 200°C. FA were identified using the library of mass spectra NIST 08, the archive of FAME mass spectra of Christy [19]. The relative content of FA was determined by the method of internal normalization in the weight percentages (wt %) of their total content in the sample, taking into account the coefficient of FA response.

Statistical processing. The table shows means ± standard deviations of four to six biological replicates. Experimental data were analyzed using the statistical package of Microsoft Office Excel 2010. Statistical significance of differences between the compared values was assessed using *t*-test ($p < 0.05$).

RESULTS AND DISCUSSION

FA composition of lipids of the needles and long-living callus cultures derived from various tissues of coniferous branches (in the case of Siberian larch, from zygotic embryos), determined by gas-liquid chromatography-mass spectrometry is presented in Table 1. Significant differences have been found in the content of some FA of total lipids in the callus and needles. Figure 1 shows a typical chromatogram of FA of lipids of the needles and callus of *Picea pungens*. In the callus of this species, the FA spectrum is presented by 25 acids with the number of carbon atoms in the range C14–C22, while the FA spectrum of the spruce needles consists of 24 acids. Palmitic and stearic acids are the main saturated acids of both callus, and pine needles, and the margaric acid is the main one in callus. In the FA spectrum of both the pine needles and callus, unsaturated FA (oleic acid in calli and linolenic acids in needles) dominate. Figure 2 shows typical mass spectra of oleic (Fig. 2a), pinalenic (Fig. 2b), and uniperonic (Fig. 2c) acids. In the mass spectrum of oleic acid, a molecular ion of $m/z = 296$ and a characteristic ion of $m/z = 264$ are presented. The molecular ions of pinolenic and uniperonic acids have $m/z = 292$

and 275, respectively. In the mass spectrum of pinolenic acids, ions of $m/z = 141$, $m/z = 109$ and $m/z = 243$ ($[M\text{-}49]^+$) are presented, which are characteristic of 5,9-dienes. In the lipids of needles of all the studied species, the content of saturated FA was about the same and amounted to 29.6–38.0 wt % of the sum of acids. The content of saturated FA in lipids of the callus of Siberian larch and Korean pine was not significantly different from their content in the needles. In the lipids of the callus of *P. sylvestris*, the content of saturated FA was 1.5 times higher (45.7 wt %), than in the lipids of the needles, and in *Picea pungens* it was 2 times lower than that in the needles and amounted to 16.8 wt %. The lipids of the needles of all species contained lauric acid (C12:0), the highest relative content (12.2 wt %) of which was noticed in the needles of *P. koraiensis*. In Scotch pine and spruce, the contents of this acid was about 2–3 times lower (3.5 and 5.8 wt % respectively). In the callus, lauric acid was found only in the tissues of Siberian larch. Relatively high content of myristic acid (C14:0) is characteristic for the needles of *Picea pungens* (3.7 wt %), while in the rest of the species, the content of this acid was 2 times lower (1.6–1.7 wt %). The tissues of the callus had lower content of myristic acid than the pine needles (0.3–0.6 wt %). Virtually all tissues were found to have minor (less than 1.0 wt %) saturated FA with a carbon chain length of C14–C18. In all species, in the composition of the saturated FA of lipids of the needles, the palmitic (C16:0) acid was dominating, the content of which was the highest of 23.1 wt % in *L. sibirica*, and the lowest of 14.0 wt % in the lipids of the needles of *P. koraiensis*. A high relative content of C16:0 was detected in the lipids of callus of *P. sylvestris* (32.6 wt %); in the total lipids of the callus of *Picea pungens*, its content was the lowest (7.6 wt %). The content of stearic (C18:0) acid in the needles and callus was in the range 1.5–3.7 wt %. The relative content of arachidic acid (C20:0) was slightly higher in the lipids of the needles and callus of *P. sylvestris* than in other species. The content of the other very long chain FA, behenic (C22:0)acid, was the highest in the lipids of the needles of *P. koraiensis* (3.2 wt %).

Among unsaturated FA of the lipids of needles and callus, mono-, di-, tri-, and tetra-unsaturated FA with *cis*-configuration of hydrocarbon chains were identified. The main unsaturated FA are represented by oleic (C18:1Δ9), linoleic (C18:2Δ9,12) and linolenic (C18:Δ9,12,15) acids. In addition, unusual Δ5-acids were discovered: taxoleic (C18:2Δ5,9), pinolenic (C18:3Δ5,9,12), skiadonic (C20:3Δ5,11,14) and uniperonic (C20:4(Δ5,11,14,17) acids, characteristic of conifers and some other evolutionary ancient taxa [20].

The total content of monounsaturated acids in the lipids of the needles is generally lower than in the callus. The maximum content of 9.7 wt % of these acids in the lipids of the needles were in *Picea pungens*. In the callus, the maximal content of monounsaturated acids was found in the lipids of the embryogenic callus of spruce (44.2 wt %) and the minimal content, in the callus of *L. sibirica* (12.0 wt %). In the composition of monounsaturated fatty acids of the callus, oleic (18:1Δ9) acid was present in a significant proportion, in all cases its content 2–6 times exceeded the content in the needles. Previously we have shown that a high content of monounsaturated oleic acid is typical for the FA composition of the embryogenic callus of larch [21]. It was suggested that the oleic acid can serve as a diagnostic marker of embryogenic potential of the callus of conifers in the selection of promising cell lines at the early stages of embryogenesis. Figure 3 shows the FA composition of the pine needles and callus of Siberian larch with different embryogenic potential. The following cell lines can be highlighted: Cl23, non-embryogenic ones, do not have zones of secondary differentiation; Cl10, embryogenic ones, form a large number of embryos, regenerated plants were not obtained; Cl6, embryogenic, form embryos, regenerated plants were obtained. It follows from Fig. 3 that all these groups differ in the content of oleic acid. For instance, in non-embryogenic line Cl23 oleic acid content was 10.8 wt %; in embryogenic line Cl10 it was 32.5 wt %; in line Cl6, from which the regenerated plants were obtained, the content of acid 18:1Δ9 amounted up to 56.5 wt % of the amount of all acids. Thus, a quantitative relationship has been revealed between the oleic acid content and characteristics of callus lines of larch enabling the assessment of their embryogenic potential. It was also found that the lipids of the callus of white spruce contained up to 41.5 wt % oleic acid; in the lipids of Scots pine callus, the corresponding figure was 25.9 wt % and in Korean pine, 9.0 wt %. Of particular interest is a high relative content of oleic acid in the lipids of the callus of *Picea pungens,* which is 6 times higher than the content of this acid in the lipids of the needles of trees from the natural population (41.5 and 7.4 wt % respectively). As already mentioned, an unusually high content of monounsaturated FA, primarily oleic acid, is associated with high embryogenic potential of tissues, for example, of the callus of Siberian larch [21]. In the studies on the embryogenesis of *Prunus avium, Simmondsia chinensis, Brassica napus* [22–24], the increase of oleic acid content in the process of somatic embryogenesis was reported, too. It was shown that in morphogenic callus of *Triticum aestivum* L. the content of this acid amounted to 17.9 ± 0.9 wt %, while in non-morphogenic callus this value was almost 3 times lower, 7.1 ± 0.5 wt % [25]. It is logical to assume that, as in the callus lines of larch [21], such a high content of oleic acid in the culture of spruce in vitro can be associated with higher morphogenetic potential inherent to this species, compared to other conifers (Siberian pine and larch). In favor of this assumption are the results of an assessment of ability to undergo directional organogenesis and callusogenesis obtained by I.P. Filippova for Siberian spruce (*Picea obovata*), a species closely related to *Picea pungens* [26]. It was shown that the

Table 1. Relative content of fatty acids in the total lipids of the calluses and needles of white spruce, Scotch pine, Korean pine, and Siberian larch

Fatty acid	*Larix sibirica* Ledeb.		*Pinus koraiensis* Siebold & Zucc.		*Pinus sylvestris* L.		*Picea pungens* Engelm.	
	needles	callus	needles	callus	needles	callus	needles	callus
C12:0	0.2 ± 0.1	0.1 ± 0.0	12.2 ± 1.8	–	3.5 ± 0.4	–	5.8 ± 0.4	–
C14:0	1.6 ± 0.1	0.4 ± 0.1	1.7 ± 0.2	0.5 ± 0.0	1.7 ± 0.1	0.6 ± 0.1	3.7 ± 0.0	0.3 ± 0.1
C15:0	0.4 ± 0.1	0.4 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	–	2.1 ± 0.0	0.3 ± 0.0	0.3 ± 0.1
C16:0	23.1 ± 0.6	21.8 ± 1.3	14.0 ± 0.2	24.4 ± 2.0	17.3 ± 0.8	32.6 ± 1.1	19.4 ± 1.9	7.6 ± 0.3
C16:1	2.1 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	1.6 ± 0.3	1.0 ± 0.1	0.6 ± 0.1	1.7 ± 0.1	0.1 ± 0.0
C17:0-a	0.9 ± 0.1	3.0 ± 0.0	1.7 ± 0.2	2.1 ± 0.3	–	1.9 ± 0.1	3.3 ± 0.2	4.6 ± 0.5
C17:0	1.1 ± 0.1	0.7 ± 0.0	0.8 ± 0.1	0.6 ± 0.2	–	1.9 ± 0.1		0.5 ± 0.1
C16:3 (*n*-3)	2.2 ± 0.1	–	0.7 ± 0.1	–	2.1 ± 0.2	–	0.3 ± 0.0	–
C18:0	2.4 ± 0.1	2.6 ± 0.0	2.3 ± 0.2	1.8 ± 0.1	3.7 ± 0.4	3.3 ± 0.1	2.3 ± 0.3	1.5 ± 0.2
C18:1 (n-9)	3.4 ± 0.1	10.8 ± 1.4	4.4 ± 0.2	9.0 ± 1.3	3.2 ± 0.4	25.9 ± 0.1	7.4 ± 0.4	41.5 ± 2.6
C18:1 (*n*-7)	0.4 ± 0.1	0.5 ± 0.0	0.8 ± 0.1	5.6 ± 0.5	0.8 ± 0.1	1.8 ± 0.1	0.6 ± 0.2	1.2 ± 0.2
C18:2 (Δ5,9)	0.7 ± 0.0	1.6 ± 0.1	0.8 ± 0.1	0.2 ± 0.0	–	0.6 ± 0.1	2.0 ± 0.2	4.7 ± 0.3
C18:2 (*n*-6)	7.8 ± 0.2	37.6 ± 1.5	16.3 ± 0.9	34.5 ± 0.6	18.7 ± 0.4	16.4 ± 0.1	17.0 ± 0.3	21.7 ± 2.5
C18:3 (Δ5,9,12)	1.0 ± 0.1	6.4 ± 0.4	3.4 ± 0.4	1.0 ± 0.2	4.1 ± 0.4	0.7 ± 0.1	4.2 ± 0.3	2.3 ± 0.2
C18:3 (*n*-3)	43.9 ± 0.9	6.6 ± 0.2	25.1 ± 2.7	13.0 ± 0.2	27.2 ± 0.5	4.2 ± 0.4	22.1 ± 0.5	4.4 ± 0.8
C18:4 (Δ5,9,12,15)	2.3 ± 0.2	0.7 ± 0.1	1.7 ± 0.2	0.2 ± 0.0	1.4 ± 0.2	–	2.9 ± 0.3	0.4 ± 0.1
C20:0	0.7 ± 0.2	0.7 ± 0.1	1.1 ± 0.1	0.4 ± 0.1	1.6 ± 0.3	1.0 ± 0.0	0.4 ± 0.1	0.4 ± 0.1
C20:2 (*n*-9)	0.4 ± 0.1	–	0.5 ± 0.0	–	1.2 ± 0.2	–	0.1 ± 0.0	–
C20:2 (*n*-6)	–	0.6 ± 0.1	–	0.2 ± 0.0	–	1.0 ± 0.2	–	4.2 ± 0.5
C20:3 (Δ5,11,14)	2.8 ± 0.1	2.7 ± 0.4	5.7 ± 0.4	2.9 ± 0.3	7.8 ± 0.2	2.0 ± 0.2	4.1 ± 0.4	0.8 ± 0.1
C20:4 (Δ5,11,14,17)	1.7 ± 0.1	–	1.0 ± 0.2	–	1.3 ± 0.1	–	0.7 ± 0.1	–
C22:0	1.3 ± 0.2	1.3 ± 0.1	3.2 ± 0.6	0.6 ± 0.1	1.8 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	0.7 ± 0.2
FA < 1%	0.7 ± 0.0	0.9 ± 0.0	1.2 ± 0.1	1.2 ± 0.1	2.6 ± 0.0	2.6 ± 0.3	1.3 ± 0.0	3.2 ± 0.3
Σ_{SFA}	31.7 ± 0.6	31.4 ± 0.5	38.0 ± 2.5	31.3 ± 1.3	29.6 ± 2.2	45.7 ± 0.9	36.6 ± 2.3	16.8 ± 1.2
Σ_{MUFA}	5.8 ± 0.4	12.0 ± 1.4	6.4 ± 0.2	16.9 ± 1.1	7.2 ± 0.8	29.3 ± 0.1	9.7 ± 1.0	44.2 ± 3.1
Σ_{PUFA}	63.7 ± 0.3	56.6 ± 0.9	55.8 ± 2.3	51.9 ± 0.9	63.2 ± 1.5	25.0 ± 1.1	52.8 ± 1.3	39.4 ± 4.2
Weight of FAME, mg/g of dry weight	23.2 ± 1.0	27.4 ± 3.1	65.4 ± 1.9	39.5 ± 0.2	37.5 ± 2.7	63.3 ± 1.1	21.3 ± 1.9	72.4 ± 0.4

Sign "–" means that the acid was not detected. "FA < 1%" means that the total amount of FA was less than 1% of the sum of acids. Σ_{SFA} is the sum of saturated FA. Σ_{MUFA} is the sum of monounsaturated FA. Σ_{PUFA} is the sum of polyunsaturated FA. The table shows mean values ± SD for three to six biological replicates.

reproduction rate of zygotic embryos in the tissue culture of Siberian spruce was significantly (4–10) times higher than in the culture of Siberian pine and Scots pine [26]. It was found that, under identical conditions of cultivation, in Siberian spruce, 15 adventitious buds on average were formed per an explant, while in the Siberian larch and Scots pine, only four buds [27]. It was also found that the formation of the callus in conifers occurs with different intensity, the maximum induction was observed from terminal and lateral buds of Siberian spruce (formation of the callus was obtained in 53–65% of the explants on average) [27]. A possible reason for the increase of oleic acid content in morphogenic tissues of plants can be, as noticed by a number of researchers, the participation of this acid and its derivatives in the work of the signaling networks, including, the processes of cell differentiation [15, 23, 28].

In the composition of monounsaturated FA, also, palmitoleic, and *cis*-vaccenic (18:1Δ7) acids were identified in relatively small amounts, content of the latter in all cases was higher in the callus.

The main polyunsaturated FA of the lipids of the needles and callus of four species of coniferous were linoleic (18:2Δ9,12) and linolenic (18:3Δ9,12,15) acids. The content of linoleic acid in the lipids of the

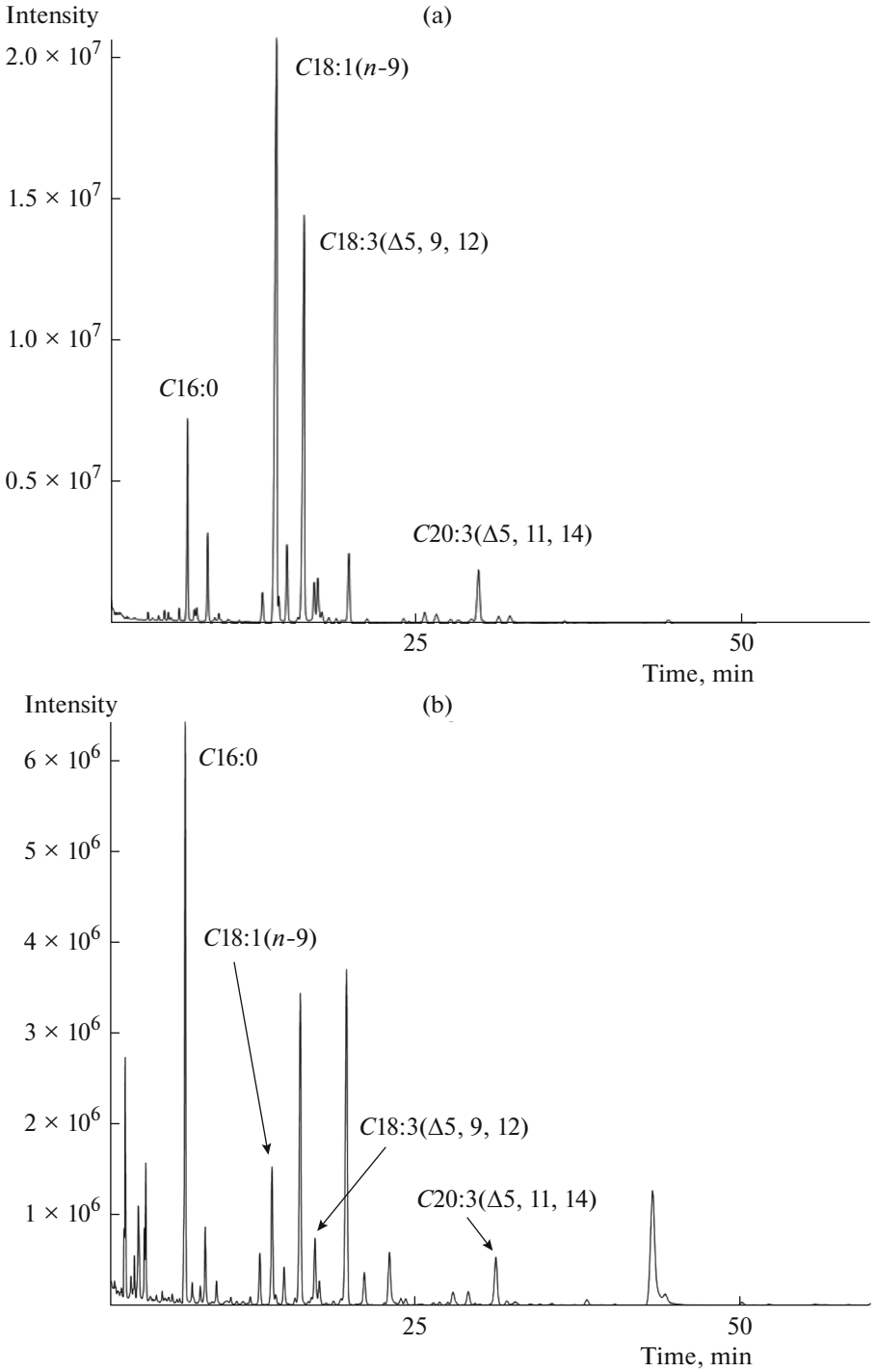

Fig. 1. FA chromatograms of the lipids of the callus (a) and needles (b) of *Picea pungens* Engelm.

callus of larch and Korean pine was significantly, 2–5 times higher than in the needles of these species. In white spruce, this excess was less pronounced. The content of linoleic acid in the lipids of the Scots pine callus was less than in the lipids of the needles. The content of linolenic acid in the lipids of the needles of larch was 7 times higher than in the callus, and amounted to 43.9 wt %. The content of this acid in the lipids of the needles of the cedar reached 25.1 wt %, while in the callus, the content of linolenic acid was 13.0 wt %, and was the highest for the culture in vitro of the four investigated species. In the callus of spruce and pine, the content of 18:3Δ9,12,15 acid was 4.4 and 4.2 wt %, that was 5–6 times lower than in the tissues of needles (22.1 and 27.2 wt % respectively).

It is known that oleic acid is the substrate for the synthesis of not only linoleic acid but also Δ5- and

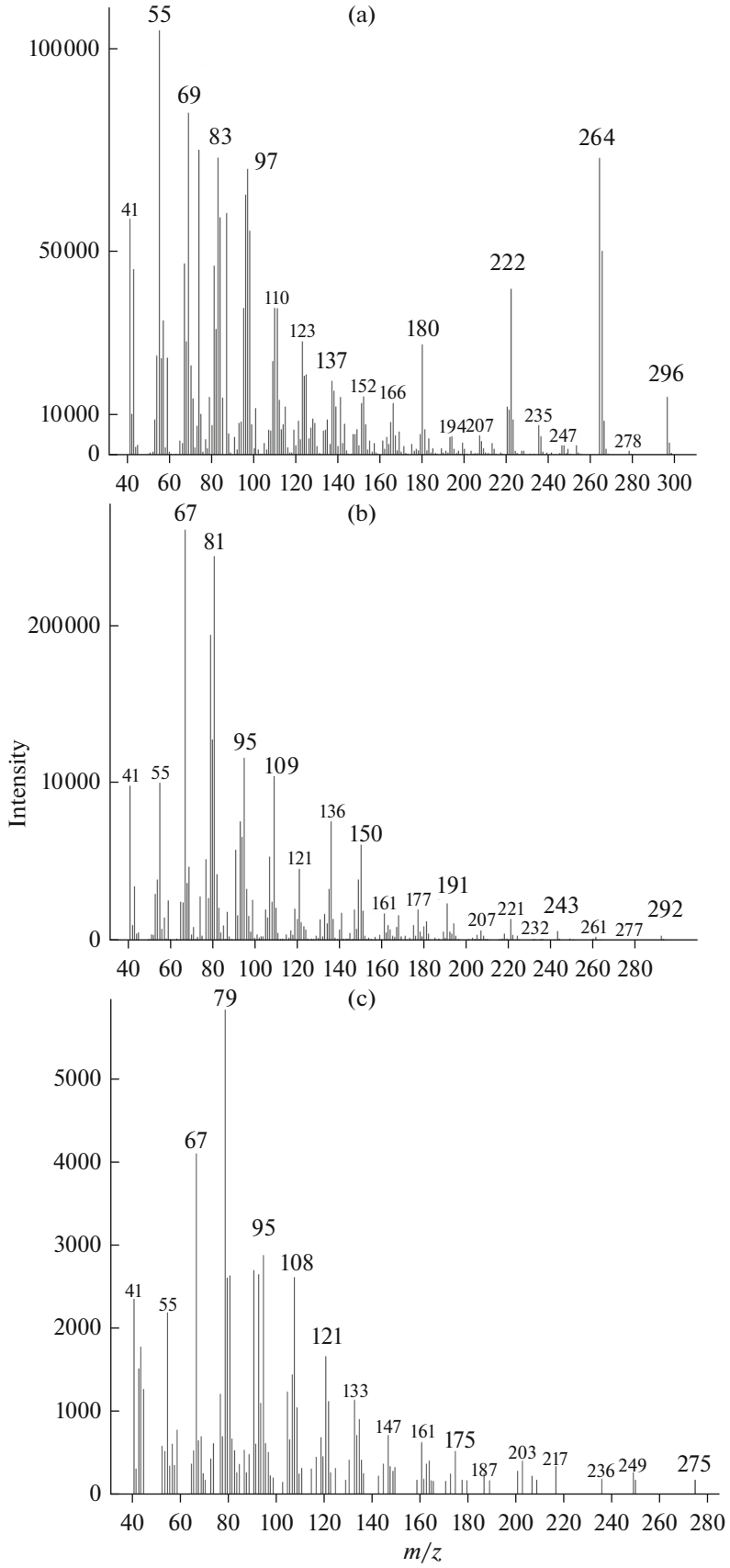

Fig. 2. Mass spectra of fatty acids: oleic (a), pinolenic (b), uniperonic (c).

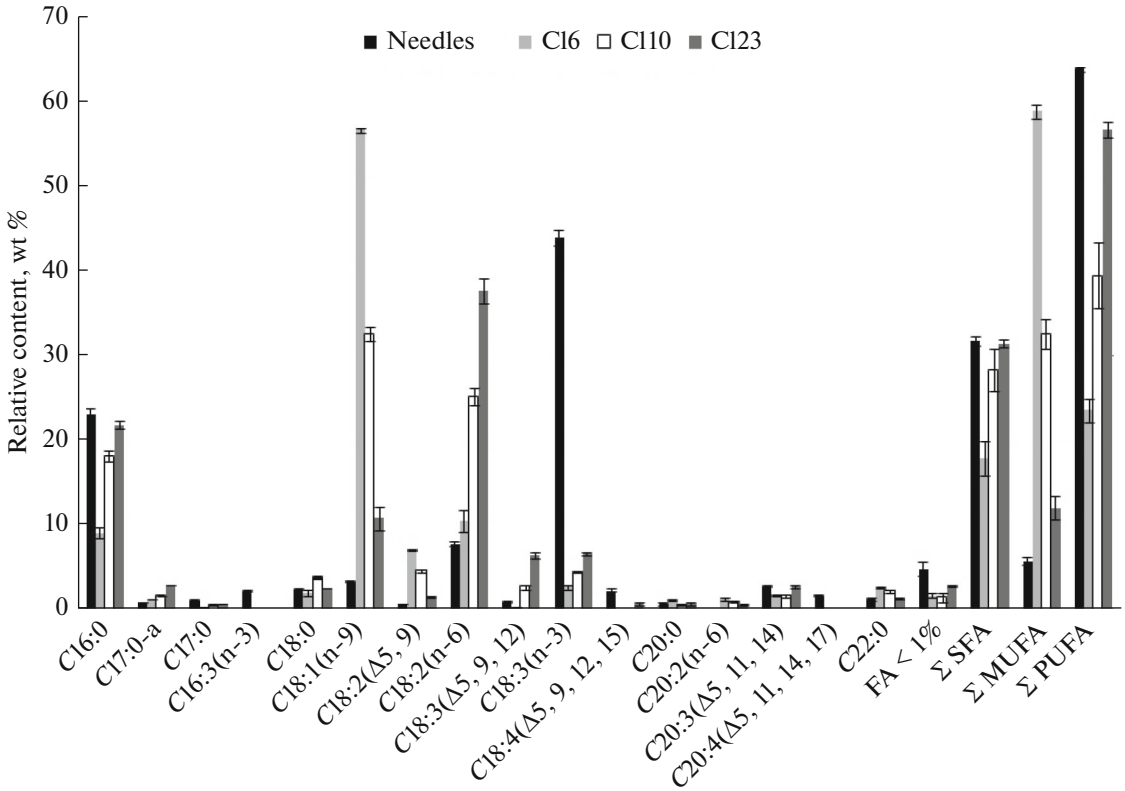

Fig. 3. FA composition (% of total acids) of the total lipids of the needles and calli of embryogenic and non-embryogenic cell lines of Siberian larch. Cl6, cell line 6, embryogenic callus, producing regenerants. Cl10, cell line 10, embryogenic callus, producing no regenerants. Cl23, non-embryogenic callus (does not produce regenerants). "FA < 1%" means that the total amount of FA contained in less than 1% of the sum of acids; Σ_{SFA}, the sum of saturated FA; Σ_{MUFA}, the sum of monounsaturated FA; Σ_{PUFA}, the sum of polyunsaturated FA.

Δ6-polyunsaturated acids in the membranes of plants [29]. Figure 4 shows the results of determination of the relative content of polyunsaturated Δ5-acids in the needles (Fig. 4b) and callus (Fig. 4a) of the studied species. It can be seen that one of these acids, uniperonic acid, is presented only in the lipids of the needles in all four species. The highest content of this acid, 1.7 wt %, was found in the lipids of the needles of larch, and the lowest content was in the callus of spruce needles, 0.7 wt %. The highest among the acids of this group was the content of pinolenic acid (C18:3Δ5,9,12) in callus of both studied types of tissues. The total content of Δ5-acids in all cases was higher in the lipids of the needles, except Siberian larch, in the lipids of the callus of which the corresponding figure was 11.4 ± 0.5 wt % of the sum of FA, and in the lipids of the needles it was 8.5 ± 0.4 wt %. In spruce, the high total content of these FA was detected in the tissues of the callus (8.2 wt %) and needles (13.9 wt %), however, the greatest total content of Δ5-acids was in the needles of *P. sylvestris* (14.6 wt %) and in the callus of *L. sibirica*. In general, the lipids of callus differ from the lipids of needles in smaller relative content of Δ5-acids, and uniperonic acid is absent in the lipids of the callus of the examined conifers.

Of particular interest are the results of the comparative analysis of the FA composition of the needles and callus of Scots pine, as in this case, the FA composition of the needles and callus, obtained directly from them, was determined. The data presented in Table 1 show the characteristic differences between the FA composition of total lipids of the callus obtained from different starting material (see Materials and Methods) and the needles of trees of the natural population. These differences include the higher content of monounsaturated acids in the lipids of callus, the smaller variety and low content of Δ5-acids, no acids with carbon chain length less than 14. It is logical to assume that these differences are typical for all types of the callus of studied conifers, irrespective of the organ or tissue from which they are derived.

The obtained results indicate significant qualitative and quantitative differences in the content of the individual FA of lipids in the needles and callus. For instance, the lipids of the callus of studied species are characterized by a lower relative content and smaller variety of Δ5-acids in comparison with the needles of the same species. The callus of all species of coniferous trees contain significantly more monounsaturated acids in their lipid composition than the needles do.

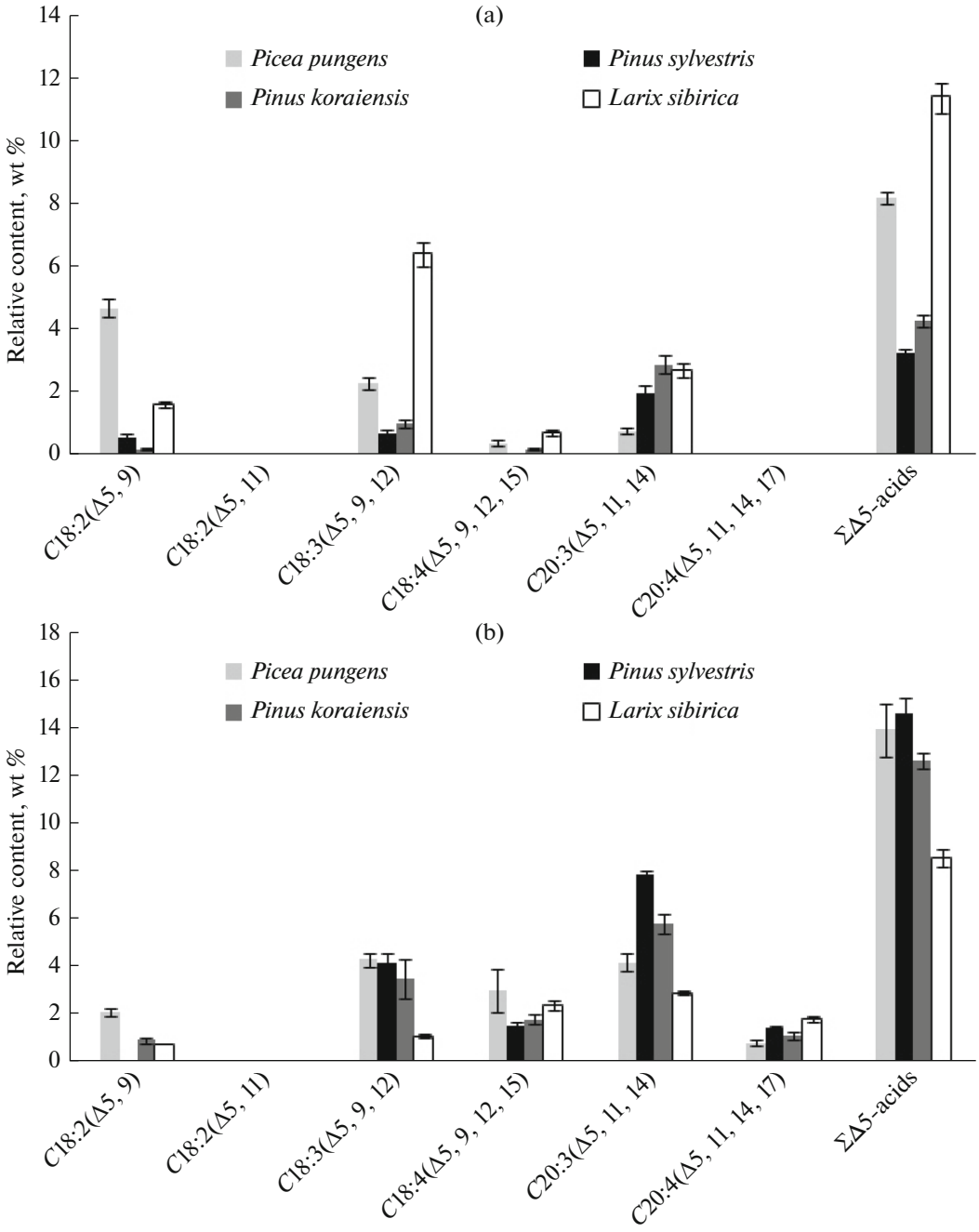

Fig. 4. Relative content of Δ5-acids in the total lipids of the callus (a) and needles (b) of spruce, Scots pine, Korean pine and Siberian larch. ΣΔ5-acids is the sum of Δ5-acids.

According to the obtained and published data, a high content of oleic acid in the tissues of the callus of white spruce may indicate a higher morphogenetic potential of in vitro culture of this species compared to some other species of coniferous and may be a marker of embryogenic activity. The obtained data suggest that the most significant differences in the FA composition of total lipids of the needles and callus do not depend on the material used for initiation of the callus, and are probably related to the peculiarities of the biosynthesis of these compounds in the culture in vitro. Further study of the patterns of changes in lipid and FA composition of the callus of conifers during embryogenesis will allow getting closer to understanding the biological role of rearrangements of lipid metabolism in this process.

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