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LIPIDS FROM BUD MERISTEM OF *Larix sibirica*

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The total lipids were isolated from meristematic bud tissues of Larix sibirica *L. and cell membrane complexes. Their group composition was established. The dynamics of seasonal variation were studied. The principal lipids were found to be polar lipids residing in the membranes. Lipids in membranes were largely (up to 75% of the mass) replaced in winter by proteinaceous material. The principal lipids in the membranes were phospholipids.*

Key words: *Larix sibirica* L., meristematic tissues, cell membrane complex, lipids, fractional composition.

The most frost-resistant conifer among those forming Siberian and Far-Eastern old growth forests is the Siberian larch (*Larix sibirica* L.) [1, 2]. It withstands temperatures as low as -60°C during winter under natural conditions. Meristematic tissues of its buds can retain vitality after freezing at -196°C [3].

Structural components of living cells, the biological membranes, were found to be the least resistant to low temperatures. We previously noted that meristematic tissues of vegetative buds of *Larix sibirica* L. become stable during dormancy owing to an increased fraction of protein mass in the cell membranes. However, the liquid phase of the membranes that is necessary for their normal functioning is determined significantly by the condition of the membrane lipid matrix.

We studied seasonal variations of the group composition of larch meristem bud lipids (Table 1) and found that the content of total lipids varies during bud development and depends on the season. The vernal maximum in their content was clearly seen [15.91% of the absolute dry mass (adm) of tissue] and occurred during the period preceding opening of cones, when the maximum content of neutral lipids was also noted (9.5%). Then, their content decreased by almost three times. This agrees with data in the literature [6], in which it was shown that the neutral lipids (NL) in meristematic cells of maple and walnut began to drop precipitously during maximal bud swelling. The minimum content of total lipids in larch meristematic bud tissues was observed in September (9.5%) and coincided in time with their NL content (2.8%). A comparison of our results and those obtained previously for the cambial zone of *Larix sibirica* L. [7] indicates that the yearly dynamics of the total and NL contents in these tissues are similar. It can be hypothesized that a similar exchange of substances in meristematic bud tissues and the cambial zone explains the overall trend in the yearly dynamics of lipid content.

Polar lipids represent a significant part of the lipids in meristematic bud tissues. Their fraction at different times of the year varied from 39.7 to 75.8% of the total cell lipids. The polar lipids included glyco- (GL) and phospholipids (PL). The yearly dynamics of their contents were opposed in nature. The maximum content of PL [~7.5% of the adm of tissue] coincided with the time of minimum content of GL (~1%) and occurred in December. The change of polar lipid content is due primarily to a qualitative restructuring of the cell-membranes.

The main lipid mass of meristematic tissues is located in the membranes. Table 2 and Fig. 1 show the group compositton of cell-membrane complex (CMC) lipids.

The NL content over the studied period did not exceed 0.5% of the adm of tissue (Table 2). However, Fig. 1 shows that their fraction in the cell-membrane complex (CMC) increased by 2-2.5 times during winter and made up >5% of the total membrane lipids.

The GL content varied during the studied period from 0.9 to 4.5% of the adm of tissue (from 2.3 to 21.7% of the adm of the CMC). The dynamics of their content are opposed to those for NL and PL. The GL content increased in spring to >45% of the total lipids of the CMC. The GL content dropped sharply in autumn and remained low during dormancy, <10% of the total membrane lipids.

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TABLE 1. Group Composition of Lipids from Meristematic Tissues of Larch Buds, % of Absolute Dry Mass

Lipid composition	Sample-collection month									
	8	9	10	11	12	1	2	3	4	5
Total	13.5	9.6	11.6	12.7	12.3	11.8	11.7	13.2	15.9	12.5
Neutral	3.5	2.9	3.7	4.2	3.8	3.6	3.6	3.7	9.6	3.0
Glycolipids	4.5	1.7	1.3	1.0	1.0	0.9	0.8	2.3	2.6	4.5
Phospholipids	5.5	5.0	6.6	7.4	7.5	7.3	7.3	7.2	3.8	5.0

TABLE 2. Group Composition of Cell-Membrane Complex (CMC) Lipids

Lipid composition	Sample-collection month									
	8	9	10	11	12	1	2	3	4	5
Total	9.6 / 51.4	6.6 / 32.5	7.9 / 32.0	8.5 / 26.0	8.4 / 25.7	8.1 / 24.8	8.0 / 29.1	9.2 / 36.7	6.0 / 24.2	9.0 / 40.5
Neutral	0.3 / 1.4	0.3 / 1.6	0.5 / 1.8	0.5 / 1.5	0.4 / 1.3	0.4 / 1.3	0.5 / 1.7	0.4 / 1.7	0.2 / 0.8	0.2 / 0.9
Glycolipids	4.0 / 21.7	1.42 / 7.0	1.1 / 4.5	0.7 / 2.4	0.8 / 2.5	0.8 / 2.4	0.7 / 2.6	1.9 / 7.7	2.3 / 9.0	4.2 / 18.7
Phospholipids	5.3 / 28.2	4.8 / 23.9	6.3 / 25.7	7.2 / 22.2	7.2 / 21.8	6.9 / 21.1	6.8 / 24.7	6.9 / 27.4	3.6 / 14.4	4.7 / 20.9

In the numerator, % of absolute dry mass (adm) of tissue; in the denominator; % of adm of CMC.

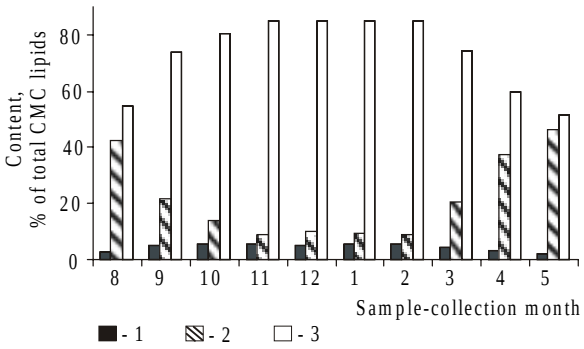

Fig. 1. Group composition of cell-membrane complex (CMC) lipids from meristematic tissues of *Larix sibirica* L. buds: neutral lipids (1), glycolipids (2), phospholipids (3).

The PL dominated the membrane lipids during the whole bud-development period. Compared with green wood and the cambial zone of Siberian larch [8, 9], the PL content in bud meristem was ~7% of the adm of tissue. Their content increased in winter by 1.5 times compared with that in September (Table 2). The PL content calculated per adm of CMC decreased. This confirms the hypothesis about the synthesis of membrane structures during autumn and winter. This is also consistent with a determination of the mass fraction of membranes in meristematic tissue. During spring (April), the PL content decreased to 3.6% of the adm of tissue. This agrees with the idea that the protein—lipid membrane complex is variable and decomposes to form structural material if it is needed for any cell part [10]. Next, the PL content increased before bud swelling to 4.6% due to an increase of cell dimensions. Biosynthesis of PL must accelerate during this period in order to maintain their previous amount.

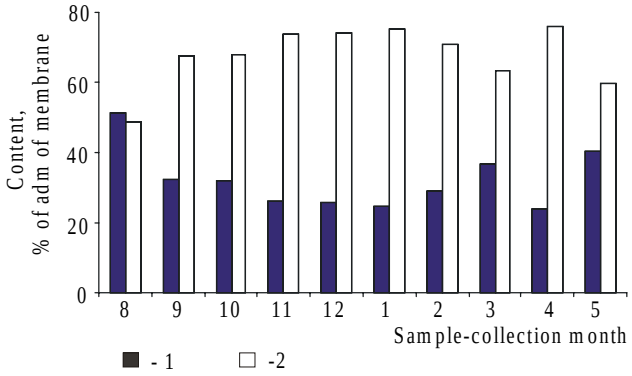

Fig. 2. Seasonal dynamics of lipid(1):protein(2) ratio in cell-membrane complex of *Larix sibirica* L. meristematic bud tissues.

The lipid:protein ratio in the studied CMC can indicate characteristic changes in the membrane structure. The seasonal dynamics of the lipid:protein ratio in the CMC of meristematic bud tissues are plotted in Fig. 2. Lipids in the membranes were replaced during autumn and winter by structural proteins. The lipid:protein ratio varied from 1:1 in August to 1:3 in November. The formation of resistance to low temperatures that was associated with the onset of the autumn—winter period was accompanied by a reduction of lipid content in the CMC to its minimum value (~25% of CMC mass) and a replacement of lipids by membrane structural proteins. Therefore, it can be assumed that namely the membrane lipids are the most probable site of damage at low winter temperatures. The plant, striving to protect itself, developed a mechanism for reducing the fraction of this component in the membranes to its minimal value. It should be noted that the content of membrane lipids per dry mass of tissue was practically constant. Therefore, the reduction of the lipid fraction in the CMC occurs only because of greater protein synthesis and an increase of membrane mass fraction in the plant cell. These features of lipid content in meristematic tissues of Siberian larch buds have not been previously described.

The results suggest that the formation of a stable meristem state is accompanied, first, by a replacement of part of the membrane lipid matrix by proteinaceous material (Fig. 2). Obviously, this reduces the probability of their damage by the cold. Second, the membranes of wintering tissues typically have a significantly higher PL content (~85% of total CMC lipids). Their content decreased substantially in spring (Fig. 1). This agrees with the literature on the involvement of PL in formation of winter hardiness of conifers [11, 12]. Furthermore, the increased fraction of NL (by 2-2.5 times) in the lipid CMC of wintering cells and the significant reduction (by 5-7 times) of GL content are interesting.

EXPERIMENTAL

Results of observations made from 1998 to 2001 are summarized. We studied meristematic tissues of vegetative buds of Siberian larch (*Larix sibirica* L.). Shoots of the last year (one-year) were cut from 55-60-year-old trees growing near Krasnoyarsk in natural old growth stands. Collections were made at different times of the year that corresponded to different phenological states of the tree. After removing bark together with bud scales, meristematic tissues of vegetative buds were cut at the boundary with the shoot xylem.

The total cell-free homogenate was prepared by grinding with a pestle in a mortar. The destruction and subsequent tissue fractionation were performed at ~0°C in order to minimize possible autolytic changes occurring as a result of mixing enzymes and substrates located in different cell compartments. We used laboratory equipment and materials that were cooled beforehand to this temperature. Protective agents were added to the medium for cell homogenization: protease inhibitor, sulfhydryl-group protector, and a reductant. We added EDTA (acid form) to inactivate lipolytic acylhydrolase and phospholipase D, the activities of which depend on the presence of Ca^{2+} ions.

Meristematic tissues were homogenized in distilled water (0°C) with protective agents: ascorbic acid (0.05 g), NaOH (0.012 g), EDTA (0.01 g), β-mercaptoethanol (0.05 g), phenylmethylsulfonyl fluoride (PMSF, 0.025 g), and distilled water to bring the volume to 10 mL. The homogenization was carried out for 2 min. The mass ratio of meristematic tissues to medium was 1:20. The pH of the medium during homogenization was 7.2-7.5 [13].

The homogenate was centrifuged at 500 g for 3 min. This precipitated nuclei and cell walls of high density and large sizes. The supernatant was carefully decanted. The precipitate was homogenized again with a fresh portion of medium and centrifuged under the same conditions. The supernatants containing the cellular organelles, their fragments, and cell membranes were combined and centrifuged at 22,000 g for 30 min. The precipitate, from here called the CMC, was washed twice with icewater, centrifuged under the same conditions, and used to determine the total lipid content and study their group composition.

Lipids from meristematic bud tissues and cell membranes were isolated by the method of Bligh and Dyer using $CHCl_3$—*i*-PrOH (1:2, v/v) [14].

Nonlipid impurities were removed by washing the extract with distilled water and passing it over a column of Sephadex G-25 [15]. The purified extract of total lipids was evaporated in vacuum at temperatures <38°C and used to separate the NL and polar lipid fractions.

Total lipids were separated over a chromatography column (1200×30 mm) packed with silica gel (KSK, Voskresensk Chemical Combine, 200×325 mesh). The silica gel was activated before use for 8 h at 120°C. The mass ratio of silica gel to lipid extract was 75:1.

The column was eluted successively with $CHCl_3$ (525 mL), $(CH_3)_2CO$ (2000 mL), and *i*-PrOH (525 mL). The flow rate was ~3 mL/min, at which NL, GL, and PL were successively eluted.

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