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COMPARATIVE CHARACTERISTICS OF WATER-SOLUBLE PROTEINS FROM *Larix sibirica*, *Picea obovata*, AND *Abies sibirica* BUD MERISTEM

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The amount of water-soluble proteins in bud meristem of Larix sibirica *L.,* Picea obovata *L., and* Abies sibirica *L. was shown to increase by 2-3 times in autumn during development of low-temperature resistance. The fractional composition of water-soluble proteins of the studied species and the amino-acid composition of groups of water-soluble proteins with different molecular weight (MW) were similar. Nitrogen accumulated as aspartic acid, glycine, and alanine in high- and medium-molecular-weight proteins. The peptides (MW < 5 kDa) typically had a high content of hydrophobic proline and hydrophilic tyrosine.*

Keywords: *Larix sibirica* L., *Picea obovata* L., *Abies sibirica* L., buds, meristematic tissue, water-soluble protein, fractions, amino-acid composition.

The metabolism of plant cells is directed toward the synthesis of cryoprotector substances under hypothermic conditions [1]. It is thought that the change of content of these compounds in cells is one of the main reactions to the effects of low temperatures. We showed previously [2, 3] that water-soluble proteins of living bud tissues of certain conifers exhibit anti-freezing properties that makes the intracellular water capable of extreme supercooling as the environmental temperature decreases into the low-temperature range.

Herein we continue the study of bud meristem metabolism in endigenous forest conifer species of Central Siberia, in particular, water-soluble cytoplasmic proteins. The functional role of proteins is determined largely by not only their physicochemical properties but also their content in plant tissues. Therefore, the study of changes in the contnet of water-soluble proteins in bud meristem during the yearly cycle of tree development stages provides additional information for assessing the contribution of this very important class of biomolecules to the adaptation of living plant tissues to the lowering of the environmental temperature during winter.

The dynamics of the water-soluble protein content in bud meristem of Siberian larch (*Larix sibirica* L.), Siberian spruce (*Picea obovata* L.), and Siberian fir (*Abies sibirica* L.) were followed from the time when bud scales could be mechanically separated from the meristem (middle of August) until the buds opened and young needles emerged (Fig. 1). Figure 1 shows that the water-soluble protein content in August in meristem of developing buds was comparatively low for all species. However, it began to increase quickly with the onset of autumn and reached a maximum in larch and spruce toward the end of October; in fir, toward the middle of November. A slight reduction in protein content in meristem, which confirmed that metabolic processes occur in living cells even at air temperatures below freezing, was observed even at the end of February. Toward the second half of March (beginning of bud swelling), the content was lower than in winter by ~20% in spruce, 30% in fir, and 40% in larch. Then the level of water-soluble proteins increased sharply in all species, reaching a maximum in the second half of April.

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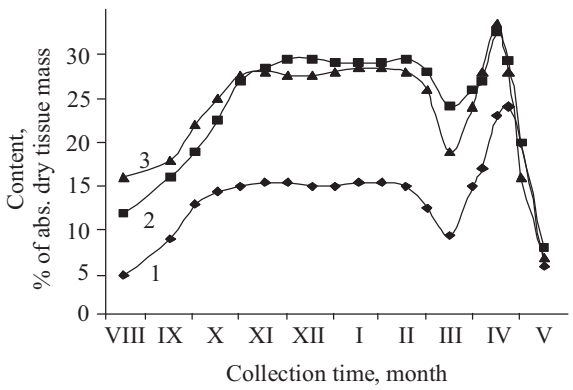

Fig. 1

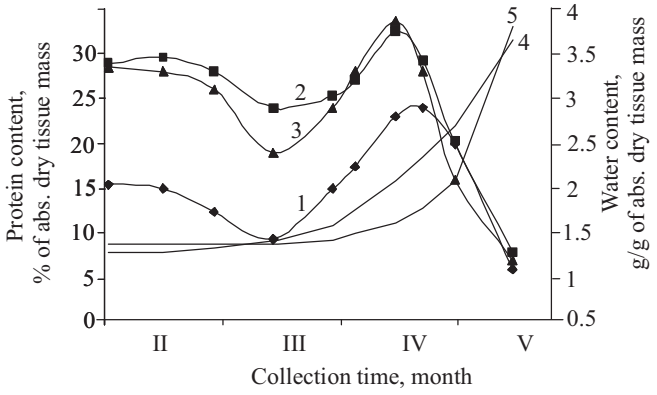

Fig. 2

Fig. 1. Seasonal dynamics of water-soluble protein content in conifer bud meristem: larch (1), spruce (2), fir (3).
Fig. 2. Change of water-soluble protein content in larch (1), spruce (2), and fir (3) meristem during bud swelling; larch water content (4); spruce and fir water content (5).

The vernal maximum, in contrast with that of winter, lasted less than a week. However, it was quantitatively greater than the mid-winter maximum by ~5–10%. This period typically showed a significant increase in the meristem water content (Fig. 2) and, therefore, an increased probability of intracellular crystallization (frost in April is a normal occurrence for Krasnoyarsk) and cell death. Therefore, the cells required additional protection that was obtained through the synthesis of cryoprotective compounds. Obviously the increased cellular content of water-soluble proteins with antifreezing properties during this period was related to the need for the cells to undergo extreme supercooling because of the rapid increase of water content during bud swelling. Then, their level decreased and was less than 6–8% of the absolute dry tissue mass in all species already by the middle of May (before needle emergence).

Because amino acids are mainly responsible for the physicochemical properties and biological role of proteins, the next phase of the work was to study the amino-acid composition of cytoplasmic meristem water-soluble proteins in dormant conifer buds.

It was found that high- and low-molecular-weight water-soluble proteins had significantly different physicochemical properties [3]. Therefore, it seemed interesting to study the amino-acid composition of proteins of different molecular weight (MW). Preparative column gel chromatography of water-soluble proteins isolated five groups: MW > 100 kDa; 100 > MW > 50; 50 > MW > 17; 17 > MW > 5; and MW < 5 kDa. The molecular weights of the eluted groups of proteins were measured by electrophoresis.

Table 1 presents the results for the amino-acid composition of the water-soluble proteins. A total of 16 amino acids was observed in the proteins. Because asparagine and glutamine in the proteins hydrolyzed to give the corresponding aspartic acid and glutamic acid [4], Table 1 lists the sums of aspartic acid and asparagine and of glutamic acid and glutamine.

The amino-acid compositions of the five studied groups from larch, spruce, and fir had much in common. The amino-acid composition of the peptides (MW < 5 kDa) showed a striking difference from that of the high- and medium-molecular-weight proteins.

Four amino acids dominated in all fractions with MW > 100 kDa; 100 > MW > 50; 50 > MW > 17; and 17 > MW > 5. These were aspartic acid, glutamic acid, glycine, and alanine. This is characteristic of conifers. It can be assumed that the structure of proteins synthesized in autumn in living bud tissues will include primarily amino acids originating from the initial photosynthesis products. Amino acids formed from photosynthesis products (mainly serine, glycine, alanine) were demonstrated experimentally in corn [5] and pea [6] leaves in addition to needles of young Siberian cedar and larch runners [7] to have high metabolic activity. In our instance, the sum of alanine, glycine, and serine in spruce and fir proteins varied in the range 25–30%; in larch, 32–37%. The higher content in larch was probably related to the efflux of amino acids formed in needles during photosynthesis into developed buds before the yearly shedding of needles. Alanine, glycine, and serine were distributed rather evenly in high- and medium-molecular-weight proteins. The quantitative content of serine was about 2-2.5 times less than that of alanine and glycine.

TABLE 1. Amino-Acid Composition of Water-Soluble Protein Groups from Dormant Conifer Bud Meristem, % of Total Amino Acids

Amino acid	Molecular weight of protein groups, kDa				
	>100	100–50	50–17	17–5	<5
		Larix sibirica L.			
Aspartic acid	16.0	13.9	17.1	14.0	5.7
Threonine	3.9	4.5	5.0	4.3	3.3
Serine	6.8	6.4	5.7	7.5	2.7
Glutamic acid	11.4	16.9	15.7	18.7	12.0
Proline	4.2	3.3	3.7	3.4	31.6
Glycine	13.8	16.3	11.5	11.5	5.3
Alanine	16.5	10.9	14.4	14.2	5.2
Valine	2.6	2.9	2.3	1.7	1.2
Methionine	Tr.	0.7	Tr.	0.2	0.1
Isoleucine	1.5	1.4	1.9	1.5	0.9
Leucine	7.4	8.9	7.9	7.2	2.4
Tyrosine	4.1	5.9	4.6	4.8	19.1
Phenylalanine	3.8	3.2	4.3	3.6	1.3
Histidine	1.4	1.2	1.0	0.6	1.4
Lysine	6.6	3.4	4.4	6.3	5.0
Arginine	Tr.	0.2	0.5	0.5	0.8
Cystine	Tr.	Tr.	Tr.	Tr.	Tr.
Σ hydrophil.	64	69	65	68	58
Σ hydrophob.	36	31	35	32	43
		Picea obovata L.			
Aspartic acid	16.1	19.2	21.9	20.5	5.9
Threonine	4.5	4.5	3.5	2.7	3.0
Serine	5.9	5.0	4.9	4.6	1.0
Glutamic acid	9.2	12.2	10.4	10.4	7.2
Proline	8.2	3.9	3.5	9.0	34.5
Glycine	13.2	13.0	11.9	13.5	8.1
Alanine	10.8	12.8	10.0	10.0	5.1
Valine	2.0	3.2	4.8	3.8	2.1
Methionine	0.8	Tr.	0.9	0.8	1.9
Isoleucine	1.5	1.4	1.2	1.2	0.8
Leucine	5.1	5.6	7.8	4.0	2.2
Tyrosine	3.1	4.0	5.0	4.1	13.8
Phenylalanine	3.0	1.7	1.8	1.5	1.4
Histidine	10.5	6.6	5.9	6.9	5.1
Lysine	3.5	5.2	5.2	5.5	4.6
Arginine	1.6	1.7	1.3	1.5	3.3
Cystine	Tr.	Tr.	Tr.	Tr.	Tr.
Σ hydrophil.	69	71	71	70	52
Σ hydrophob.	31	29	29	30	48
		Abies sibirica L.			
Aspartic acid	18.0	18.9	19.9	21.3	5.8
Threonine	3.8	3.5	4.3	3.0	2.8
Serine	5.5	5.4	4.9	4.7	1.4
Glutamic acid	10.2	11.8	9.9	9.5	7.8
Proline	7.9	3.8	3.9	9.3	33.9
Glycine	12.5	13.3	12.4	12.2	7.5
Alanine	10.9	12.1	10.8	10.4	4.6
Valine	1.8	2.2	4.3	4.1	2.7
Methionine	Tr.	0.4	1.1	0.6	2.1
Isoleucine	1.2	1.5	1.1	1.3	0.5
Leucine	4.9	5.8	8.3	4.7	1.9
Tyrosine	3.9	4.6	5.1	4.3	14.3
Phenylalanine	2.8	1.9	2.0	1.7	1.3
Histidine	11.7	8.3	6.1	6.4	5.5
Lysine	3.4	4.8	4.9	6.0	4.9
Arginine	1.5	1.7	1.0	0.5	3.0
Cystine	Tr.	Tr.	Tr.	Tr.	Tr.
Σ hydrophil.	70	74	68	67	56
Σ hydrophob.	30	26	32	33	44

The total content of glutamine-pool amino-acids, which usually combines those of common origin such as glutamic acid, glutamine, proline, and arginine, varied from 15 to 20% in all species; glutamic acid dominated. Glutamine and glutamic acid (glutamate) are the principal donors of the carbon skeleton during biosynthesis of amino acids incorporated into proteins. Furthermore, they play a key role in metabolic transformations of nitrogen [8]. Therefore, it is fully reasonable that this compound was observed in significant quantities in the water-soluble proteins. The content of arginine, the amino acid richest in nitrogen, was less than 2%. Arginine accumulated in larch preferentially in peptides; in spruce and fir, in high-molecular-weight fractions with MW > 100 kDa and 100 > MW > 50.

The asparagine pool includes aspartic acid, asparagine, threonine, and lysine. Their total content was from 22 to 30%. Aspartic acid dominated and was distributed evenly among the first, second, third, and fourth protein groups. This was characteristic of all species.

The comparatively high content in high- and medium-molecular-weight proteins of aromatic amino acids phenylalanine and tyrosine was interesting. The sum of them was about 8-9% in larch and 6–7% in spruce and fir. Aromatic amino acids are currently thought to be key compounds in the synthesis of nitrogen-free aromatic compounds [9]. Protein synthesis and lignin synthesis compete actively for aromatic amino acids in plant cells. However, the cell wall in meristem is not lignified. Therefore, the amino acids can be freely incorporated into the proteins.

Like most plant proteins, water-soluble proteins of the high- and medium-molecular-weight fractions were depleted in *S*-containing amino acids such as methionine and cystine. The content of the former was ~1%. Cystine was observed only in trace quantities.

The content of amino acids with a branched aliphatic chain (isoleucine, leucine, valine), which have a common biosynthetic pathway, varied in high- and medium-molecular-weight fractions from about 5–9%. Leucine dominated. Similar contents of leucine and isoleucine (about 4–4.5%) were found in the cambial trunk zone of larch and pine and in larch needles [7].

The amino-acid composition of the peptides (MW < 5 kDa) deserves special attention. The peptides contained significantly less of the amino acids that usually dominate in conifer proteins. A feature of these proteins was an unusually high content of the hydrophobic amino acid proline, which reached 32–35% of the total amino acids. It is thought that proline accumulates in cytoplasm and, therefore, in living plant tissues [10, 11]. Based on this, the results are completely reasonable. Proline can accumulate in meristematic bud tissue due to its influx from needle cells or chlorophyll-bearing phloem cells.

Researchers have previously noted [12, 13] vigorous synthesis in winter wheat and rape of proteins enriched in proline caused by low temperatures. However, that amount of proline in the water-soluble proteins would significantly reduce their hydrophilicity, i.e., change their solubility. At least a part of the peptides with a high proline content can become insoluble and be adsorbed on the surface of cellular membranes and cell walls if the water content of dormant bud meristem is reduced [14]. Furthermore, the high proline content in the winter water-soluble proteins may be due to its protective functions because the role of proline in plant proteins is reported to be related to its ability to preserve their structure under stressful conditions [15].

Alanine (~5%) must be mentioned in addition to proline as a hydrophobic amino acid present in the peptides. Another observed feature of the peptides is their relatively high content of the hydrophilic aromatic tyrosine. We have already noted the elevated tyrosine content in high- and medium-molecular-weight proteins. Tyrosine, which increases the hydrophilicity of the water-soluble peptides, is probably formed from phenylalanine during biosynthetic processes in the absence of competition during winter by protein and lignin synthesis for it.

Water-retention capability is a very important property of proteins during winter [16]. It depends to a large extent on the presence in them of polar (hydrophilic) amino acids. Certain researchers reported a high hydrophilicity for proteins synthesized under hypothermic conditions [17, 18]. Measurement of the ratio of hydrophilic and hydrophobic (nonpolar) amino acids in water-soluble proteins can to a certain extent reflect the water-retention capability and solubility of proteins in addition to their ability to become insoluble under certain conditions.

Table 1 gives the ratio of hydrophilic and hydrophobic amino acids in the water-soluble cytoplasmic protein groups. Despite the high proline content in the peptides, they remain generally hydrophilic and water-soluble. It was also found (including in model systems) that the cytoplasmic soluble phase became gelatinous and immobile if all groups of soluble cytoplasmic compounds were concentrated both in the initial period of autumnal partial dehydration and during ice formation outside of the organs or cells [3]. For this, the part of the low-molecular-weight water-soluble proteins with a high proline

content can be adsorbed by the surface of cellular membranes. Adsorption of proteins on membranes can provide them additional protection during low-temperature deformations caused by cell dehydration.

Thus, the following conclusions can be made based on the study of water-soluble cytoplasmic bud meristem proteins of Central Siberian frost-resistant forest conifers *L. sibirica*, *P. obovata*, and *A. sibirica* growing under identical conditions: 1) Water-soluble cytoplasmic proteins are synthesized vigorously, increasing their content by 2-3 times, in bud meristem during autumn upon development of low-temperature resistance in the studied conifer species; 2) the composition of water-soluble meristem proteins of dormant buds is heterogeneous; larch contains 28 pure proteins; spruce, 32; fir, 33; electrophoretic patterns of the proteins were very similar; 3) the amino-acid compositions of water-soluble proteins in the studied conifer species had much in common; aspartic and glutamic acids, glycine, and alanine dominated in fractions with MW > 100 kDa; 100 > MW > 50; 50 > MW > 17; and 17 > MW > 5; a signature of the peptides (MW < 5) was an unusually high content of hydrophobic proline and hydrophilic tyrosine.

The observed features of the seasonal dynamics and composition of water-soluble proteins suggested that these biopolymers, which are synthesized in large quantities during autumn in larch, spruce, and fir meristem, fulfill identical functions during low-temperature resistance. Highly hydrophilic high- and medium-molecular-weight proteins provide supercooling capabilities for intracellular solutions. The more hydrophobic peptides provide supercooling and can also participate in formation of a rigid protein framework that protects membranes from dehydration and low-temperature deformations.

EXPERIMENTAL

We summarized results from studies conducted in 1998-2008. We studied meristematic tissues of vegetative buds from Siberian larch (*L. sibirica*), Siberian spruce (*P. obovata*), and Siberian fir (*A. sibirica*).

Runners of the last year were collected monthly on test plots of the Krasnoyarsk suburbs from trees of growth classes II-III. Meristematic tissues were isolated from vegetative buds and hommogenized with chilled distilled water without protective additives. The homogenate was centrifuged at 22,000 g for 30–40 min. The precipitate was washed twice with icewater and centrifuged under the same conditions. The supernatants containing water-soluble compounds were combined and used to determine the content of total water-soluble cytoplasmic protein (WSCP) by the literature method [19]. Gel-filtration over Sephadex G-150 was used for preparative separation of protein group fractions from meristem of dormant buds (January) for subsequent amino-acid analysis [20, 21]. The amino-acid composition of proteins was determined on an automated amino-acid analyzer AAA 339 M (Mikrotechna, Czech Rep.). Proteins were hydrolyzed and samples for amino-acid analysis were prepared as before [22].

Figures 1 and 2 show the average multi-year results for determination of the dynamics of WSCP content during the yearly cycle and meristem water content during bud swelling. Table 1 presents average results from studies of amino-acid compositions of water-soluble proteins conducted in January 2006, 2007, and 2008 that were calculated from the arithmetic means of three biological and three analytical repetitions each year. The biological repetition in each experiment consisted of runners taken from 10 trees. The significance of the differences was estimated by comparing averages using the Student criterion at significance level $P = 0.05$ [23].

REFERENCES

1. T. I. Trunova, *Plants and Low-Temperature Stress* [in Russian], Nauka, Moscow, 2007.
2. P. V. Mironov, *Izv. Vyssh. Uchebn. Zaved., Lesn. Zh.*, **6**, 24 (1999).
3. P. V. Mironov, E. V. Alaudinova, and S. M. Repyakh, *Low-Temperature Resistance of Living Conifer Tissues* [in Russian], Sib. Gos. Tekh. Univ., Krasnoyarsk, 2001.
4. B. P. Pleshkov, *Practicum on Plant Biochemistry* [in Russian], Agropromizdat, Moscow, 1985.
5. G. V. Udovenko, *Fiziol. Rastit.*, **5**, 932 (1965).
6. T. C. Ta and K. W. Joy, *Planta*, **1**, 117 (1986).
7. N. E. Sudachkova, *Metabolism of Conifers and Wood Formation* [in Russian], Nauka, Novosibirsk, 1977.
8. S. M. Brei, *Nitrogen Exchange in Plants*, Agropromizdat, Moscow, 1986.

9. A. C. Neish, *Phytochemistry*, **1**, 1 (1961).
10. N. I. Shevyakova, *Fiziol. Rastit.*, **4**, 768 (1983).
11. F. Hainke and H. Goring, *Wiss. Beitr. Martin-Luther-Univ.*, **10**, 1286 (1982).
12. O. I. Kolosha, O. V. Petrova, and P. S. Mishustina, *Dokl. Akad. Nauk SSSR, Ser. Biol.*, **10**, 938 (1978).
13. M. Stefl, J. Trcka, and P. Vralny, *Biol. Plant.*, **2**, 119 (1978).
14. E. V. Alaudinova, S. Yu. Simkina, and P. V. Mironov, *Khvoinye Boreal′noi Zony*, **4**, 487 (2007).
15. M. Perras and F. Sarhan, *Plant Physiol.*, **2**, 577 (1989).
16. E. V. Alaudinova and P. V. Mironov, *New Achievements in the Chemistry and Chemical Engineering of Plant Raw Material* [in Russian], Book 2, N. G. Bazarnova and V. I. Markin (eds.), Izd. Alt. Univ., Barnaul, 2009.
17. C. L. Hew, *Eur. J. Biochem.*, **2**, 33 (1992).
18. C. A. Knight, E. Driggers, and A. L. DeVries, *Biophys. J.*, **4**, 252 (1993).
19. G. N. Buzun, K. M. Dzhemukhadze, and F. L. Mileshko, *Fiziol. Rastit.*, **29**, 198 (1982).
20. L. A. Osterman, *Research Methods for Proteins and Nucleic Acids* [in Russian], Nauka, Moscow, 1981.
21. G. A. Kochetov, *Practical Handbook of Enzymology* [in Russian], Nauka, Moscow, 1980.
22. B. P. Pleshkov, *Practicum on Plant Biochemistry* [in Russian], Agropromizdat, Moscow, 1985.
23. A. Bernstein, *Statistical Solutions Guide* [in Russian], Statistika, Moscow, 1968.