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LOW MOLECULAR WEIGHT COMPOUNDS

Free Amino Acids in Vegetative Organs of *Picea obovata* L. and *Pinus sylvestris* L.

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Abstract—It was shown that the changes in the phenological stages of tree development, associated with the loss of frost resistance and the onset of the growing season, were accompanied by significant changes in free amino acid composition in the meristematic tissues of *Picea obovata* L. and *Pinus sylvestris* L. buds. In winter, in both species, the level of nonproteingeneous amino acids was doubled in comparison with spring. At the same time, *P. obovata* and *P. sylvestris* showed significant species-specific differences in the total content of nonproteaginous amino acids. At *P. sylvestris* their share in the composition of free amino acids reached 40%, which was twice as high as that of *P. obovata*. In the free amino acids in *P. obovata* and *P. sylvestris*, the nitrogen reserve was mainly in the form of glycine corresponding to 13 and 9%, arginine, 12 and 8%, and ornithine, 12 and 15%, respectively. In addition, in *P. sylvestris* an important role in the nitrogen storage was played by γ-aminobutyric acid, about 19%, and valine, about 6%; in *P. obovata* this role was played by lysine and glutamic acid, about 10%. At the same time, the content of proline (an amino acid, which, generally, coordinates the high–low-temperature resistance of plants) in low-resistance coniferous species was low at about 0.04–0.34%. In spring, with bud swelling in both species, the share of arginine and proline increased, and the share of ornithine and γ-aminobutyric acid sharply decreased. In addition to these amino acids, the lysine content in *P. obovata* was doubled. In spring, for *P. sylvestris* a high content of aspartic acid and asparagine of about 19% was characteristic, for *P. obovata* the content of amino acids with a short carbon chain (the total amount of serine + glycine) rose to 22%. As a reliable stress metabolite in both species, one may consider ornithine, whose content in bud meristems in winter was 3–5 times higher than in the meristems of swollen buds in spring.

Keywords: *Picea obovata*, *Pinus sylvestris*, buds, meristems, nonproteingeneous amino acids

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INTRODUCTION

It is known that in the cold-resistant coniferous species of Siberia, in living tissues of wintering buds, protein metabolism has characteristic features associated with the level of water-soluble proteins [1]. For example, Siberian larch, Siberian spruce, and Siberian fir, may accumulate up to 17–30% of water-soluble proteins, and Scots pine and Siberian cedar pine may accumulate no more than 2.5–3.0%. Such a significant difference in protein metabolism, of course, should also affect the exchange of free amino acids, which represent low-molecular weight protein precursors. It should be noted that seasonal changes in free amino acid metabolism in various organs and tissues of conifers have been studied by many authors [2, 3], but information about the free amino acids of living bud tissues has not been found in the scientific literature so far, so this was the goal of our work.

As the objects of our study, *Picea obovata* L. and *Pinus sylvestris* L. were chosen. They were characterized, respectively, by the highest and lowest content of water-soluble proteins in the wintering buds. Comparison of free amino acids composition in meristems of wintering and swollen buds will allow us to reveal species-specificity or similarity in this feature and to estimate seasonal variability and the role of individual amino acids as compounds linking the excess nitrogen entering the plant and not used for the synthesis of stress proteins. Equally important is the evaluation of free amino acids accumulation associated with low-temperature stability of buds in winter.

MATERIALS AND METHODS

We used two representatives of the Pinaceae family, such as Siberian spruce (*Picea obovata* L.) and Scots pine (*Pinus sylvestris* L.). Permanent plots are located in the Central Siberian subtaiga forest-steppe areas in the Mininsk forestry (stands mixed with similar forestry and taxation data on gray medium-podzolic heavy loam soils).

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Table 1. Free amino acids of meristems in *Picea obovata* L. and *Pinus sylvestris* L., % of the total amino acids amount

Amino acids	*Picea obovata*		*Pinus sylvestris*	
	winter buds*	swollen buds**	winter buds*	swollen buds**
Aspartic acid	0.28 ± 0.01	0.29 ± 0.01	0.15 ± 0.01	0.96 ± 0.05
Threonine	0.24 ± 0.01	0.24 ± 0.01	0.08 ± 0.01	0.13 ± 0.01
Serine	0.76 ± 0.03	0.75 ± 0.02	0.37 ± 0.02	0.99 ± 0.05
Asparagine	0.06 ± 0.01	–	–	0.98 ± 0.05
Glutamic acid	0.96 ± 0.03	0.58 ± 0.02	0.44 ± 0.02	0.71 ± 0.04
Glutamine	0.14 ± 0.01	–	0.04 ± 0.01	–
Proline	0.34 ± 0.02	0.53 ± 0.02	0.04 ± 0.01	0.22 ± 0.01
Glycine	1.28 ± 0.05	0.78 ± 0.03	0.66 ± 0.02	1.02 ± 0.05
Alanine	0.66 ± 0.02	0.37 ± 0.02	0.40 ± 0.02	0.56 ± 0.03
Citrulline	–	0.24 ± 0.01	–	–
α-Aminobutyric acid	0.06 ± 0.01	–	–	–
Valine	0.06 ± 0.01	–	0.45 ± 0.02	–
Cystine	0.24 ± 0.01	0.23 ± 0.01	0.09 ± 0.01	0.35 ± 0.02
Methionine	–	–	0.08 ± 0.01	–
Cystathionine	0.06 ± 0.01	–	0.04 ± 0.01	–
Isoleucine	0.14 ± 0.01	0.20 ± 0.01	0.05 ± 0.01	0.12 ± 0.01
Leucine	0.21 ± 0.01	0.39 ± 0.02	0.18 ± 0,01	0.23 ± 0.01
Tyrosine	0.12 ± 0.01	0.12 ± 0.01	0.04 ± 0.01	0.08 ± 0.01
Phenylalanine	0.09 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.10 ± 0.01
β-Alanine	0.05 ± 0.01	–	0.05 ± 0.01	Traces
β-Aminobutyric acid	0.05 ± 0.01	–	0.04 ± 0.01	Traces
γ-Aminobutyric acid	0.61 ± 0.03	0.21 ± 0.01	1.35 ± 0.07	0.71 ± 0.03
Ornithine	1.17 ± 0.05	0.23 ± 0.01	1.06 ± 0.04	0.36 ± 0.01
Lysine	1.04 ± 0.03	0.45 ± 0.02	0.54 ± 0.03	0.50 ± 0.03
Histidine	0.21 ± 0.01	0.17 ± 0.01	0.14 ± 0.01	0.17 ± 0.01
Arginine	1.16 ± 0.05	1.11 ± 0.06	0.59 ± 0.03	2.29 ± 0.96

* End of January.
** End of April.

The collection of meristem samples was carried out as described earlier [4], in winter buds in the state of low-temperature tissue stability and in spring buds with loss of low-temperature stability.

Free amino acids were isolated from water-soluble substances by ion-exchange chromatography on a column with cation exchanger KU-2 [5] and analyzed by an automatic analyzer of amino acids AAA-339M.

The paper presents average arithmetic values of 3–5 analytical repetitions of experiments. The significance of the differences was estimated by comparing the mean values by the Student's test ($P = 0.95$).

RESULTS AND DISCUSSION

In the meristems of wintering and swollen buds of Siberian spruce and Scots pine trees in different periods of our study, more than 35 ninhydrin-positive compounds were found, among which 23 amino acids and two amides were identified reliably (Table 1).

An essential part of the free amino acid pool of both species was nonproteingeneous amino acids. It was about 20% in spruce buds and about 40% in pine buds, relative to the total amount of amino acids. In both spruce and pine buds, the proportion of nonproteingeneous amino acids in winter was twice as high as in spring, confirming the function of these low-molecular weight compounds as stress metabolites, that eliminate excess ammonia nitrogen in tissues [6]. Another explanation for the increased level of nonproteingeneous amino acids is the high content of the total protein accumulating proteaginous amino acids in winter buds. The level of nonproteingeneous amino acids in pine buds was almost twice as high as in spruce

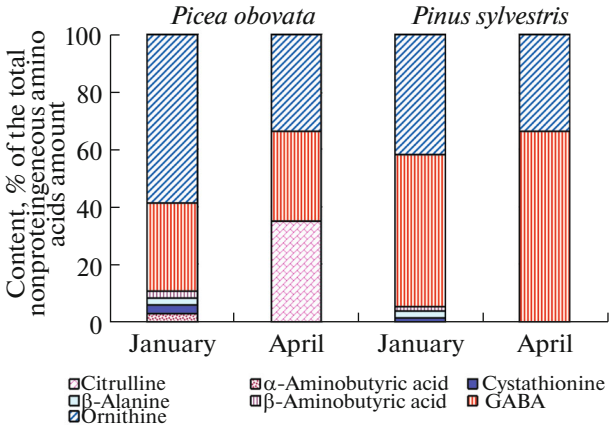

Fig. 1. Changes in the composition of nonproteingeneous amino acids.

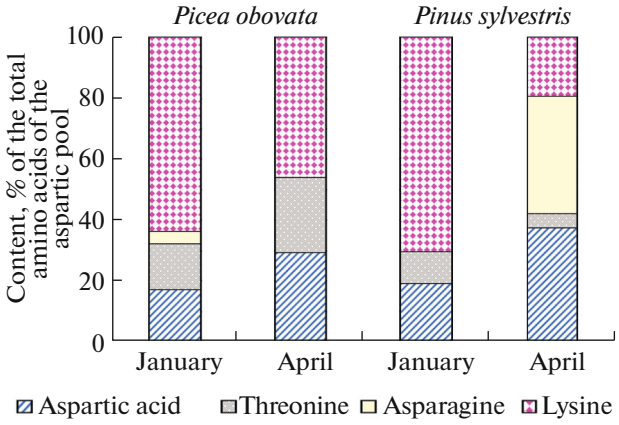

Fig. 2. Changes in the composition of amino acids of the aspartic pool.

ones [7]; i.e., in winter, the significant part of the amine nitrogen in the species with a low water-soluble protein content was stored in the form of nonproteingeneous amino acids.

The composition of nonproteingeneous amino acids in dormant buds was more diverse (Fig. 1). There were six individual components in the spruce buds. Also, there was an increased content of GABA (30%). Five nonproteingeneous amino acids were identified in pine, among which γ-aminobutyric acid (GABA) was predominant and made up about 50%, and the share of ornithine was 39%. When passing from dormancy to the vegetation stage, cystothionine, α-aminobutyric acid, β-aminobutyric acid, and β-alanine, which were present in small amounts in both species in winter, completely disappeared; the content of ornithine and GABA in meristems significantly decreased (Table 1). Simultaneously, the share of GABA in the composition of nonproteingeneous amino acids in pine slightly increased, and in the composition of ornithine it decreased.

In spruce, the share of GABA remained at the same level, and the share of ornithine decreased by almost half. As a result, the level of their content in meristems became equivalent, moreover, the same amount of citrulline in meristems appeared. The appearance of citrulline in the composition of amino acids in spruce in April demonstrated the activation of the ornithine cycle [8] in meristems and was associated with the participation of citrulline in arginine biosynthesis, which later (in May) was accumulated in water-soluble proteins in this species [9]. At the same time, in pine, a significant increase in the arginine content in spring is also evidence of the functioning of the ornithine cycle. Then, the absence of citrulline, the intermediate product of proteaginous arginine formation from nonproteingeneous ornithine, may be explained by the higher activity of the arginine succinate synthetase enzyme than the ornithine carbamoyl transferase. In this case, citrulline will not accumulate, but immediately will turn into arginine succinate and then into arginine.

In the general composition of free amino acids, only for several amino acids the increased contents (more than 9%) were allocated, the share of the others was significantly lower. Spruce buds included among the first ones serine, glutamic acid, glycine, ornithine, lysine, and arginine, and pine buds included aspartic acid, serine, asparagine, glycine, GABA, ornithine and arginine. The total content of these amino acids was 56–79%. Separately, it is necessary to note a significant share of amino acids from photosynthetic products (serine + glycine + alanine), which was equal to 20–27%, and a relatively low share of amino acids derived from pyruvate (leucine + isoleucine + valine). For spruce their share was 4–8%; for pine it was 3–9%. In an insignificant amount (no more than 1–2%), in both species aromatic amino acids—phenylalanine and tyrosine—were present in winter and spring buds.

Aspartic acid, asparagine, threonine, and lysine were part of the asparagine pool (Fig. 2).

In winter, lysine dominated in both species, and their share in this group averaged 64–70%. It is known that lysine, possessing cryoprotective action in cell membranes, accumulated during hardening in the vegetative organs (roots and leaves) of winter wheat [10]. In our case, the increased content of free lysine in meristems in winter (twice as much as in spring) was characteristic only of spruce (Table 1). Probably, this species has a significant role in the cryoprotection of cell membranes, since lysine is a part of the dominant amino acids. In the pine, the level of lysine in meristems in January and April was approximately the same and closed to its level in spring spruce buds. It

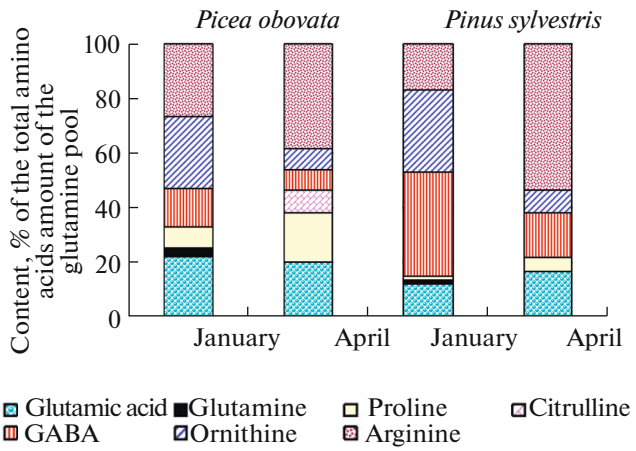

Fig. 3. Changes in the composition of amino acids of the glutamine pool.

should be noted that in spring asparagine appeared in the pine meristems, and the amount of aspartic acid increased almost by 10 times (Table 1). As a result, the ratio of amino acids in the aspartic acid pool in this species changed in favor of the latter. There were no significant changes in the spruce meristems, and the total share of the asparagine pool decreased by approximately 15% from the winter level.

Both in spruce and in pine, amino acids of the glutamine pool prevailed (37–50%), which included glutamic acid, and a number of acids associated with it by its origin—glutamine, GABA, proline, arginine, and its metabolic precursors—citrulline and ornithine (Fig. 3).

When the tree moved from dormancy to vegetation, the most significant changes in the content and ratio of individual components occurred in this group of amino acids. In the smallest amount, the glutamine pool contained glutamine, which was determined in both species exclusively in dormant buds (in winter), and ornithine and arginine were present in spruce and pine buds in the glutamine pool in the largest amount. Ornithine is preferentially accumulated in pine, and in spruce, an approximately equal level of accumulation of both amino acids was observed. In spring, the nature of the quantitative changes in the arginine and ornithine levels in both species was similar: the content of arginine increased, and the content of ornithine decreased.

The storage of amino groups in the composition of arginine and its metabolic precursors (ornithine and citrulline) was associated with the accumulation of ammonium nitrogen, which is not used for the synthesis of stress proteins [11]. In the tissues of conifers, the elevated level of these proteins is considered as an indicator of water stress [12]. Considering that water content of the meristems of the dormant buds of spruce and pine in winter was also significantly lower than that in the vegetative period [13], it is likely that a higher level of accumulated arginine + ornithine is possible in connection with water deficiency. In addition, it is known that under certain conditions arginine may slow the hydrolysis of proteins [10]. Therefore, its relatively high content in January and in April, when frosts are still possible in Siberia, will help to protect protein structures from damage.

The role of GABA and proline is most often discussed in the literature in connection with the features of plant metabolism under action of a low-temperature stress factor. In plant organisms, GABA is formed by decarboxylation of glutamic acid with the enzyme glutamate decarboxylase [8]. This phenomenon with various types of stress, including low-temperature stress, was obtained in many plants [14, 15]. It is assumed that GABA was accumulated in those plants where protein synthesis is slow, i.e., proteaginous glutamic acid is not actively used. Probably, this may explain the increased level of GABA in pine, the species for which in winter the lower total protein content was characteristic, due to its minimum level in the cytosol of meristematic cells. It was shown that, accumulating in cells, GABA exerted the same protective effect on cell membranes as proline did [10].

GABA and glutamic acid are easily converted into each other. This allows us to consider GABA as a reserve fund for glutamate. Currently, GABA takes the role of the compound supplying nitrogen to synthetic processes, as it is a donor of amino groups and a carbon skeleton in protein synthesis. Therefore, in species that accumulate water-soluble proteins during formation of cryoprotected tissue, GABA was consumed, as we confirmed by our studies. So, in the wintering spruce buds, GABA content was three times lower than in pine, and glutamic acid, on the contrary, was higher by approximately 35% (Table 1). Simultaneously, in the meristem tissues of the spruce and pine buds, there was a significantly increased GABA content in winter compared with spring (Table 1). It is important to note that when studying dormant buds of various birch species, a close GABA content, specifically 5–10% of the total amino acids, was observed [16].

Cryoprotective free proline functions are associated with its specific physicochemical nature [8]. Today, there are already the proofs of the proline protective role under cold stress [17, 18]. It was shown that proline in cooled water may form strong hydrogen bonds with its molecules, influencing the structure of ice crystals, thus providing a protective effect on the cell membranes. However, in our experiments, an increased level of proline in winter was not found; its content in spruce buds was about 3%, and in pine buds

it was less than 1%. In spring, the level of proline rose (Table 1). This is in good agreement with the proline content decrease in April as one representative of water-soluble proteins [19]. Consequently, in the meristem tissues of the buds of frost-hardy coniferous species, free proline may not be considered as a specific stress metabolite at low-temperatures.

CONCLUSIONS

When the phenological stages of tree development are interchanged due to the loss of low-temperature stability and the onset of the growing season, the content and composition of free amino acids in the meristems of *P. obovata* and *P. sylvestris* buds would be changed as follows:

(1) In the meristems of wintering buds of both species, the level of nonproteingeneous amino acids was doubled in comparison with spring buds. In this case, *P. obovata* and *P. sylvestris* showed significant species-specific differences in the total nonproteingeneous amino acids (in *P. sylvestris* its content was twice higher than in *P. obovata*) and in the quantitative ratio of their individual components.

(2) In winter, the nitrogen supply of free amino acids in *P. obovata* and *P. sylvestris* was mainly in the form of glycine, arginine, and ornithine. In addition, in pine, GABA played a significant role in the storage nitrogen (19.3%), while in spruce this role was fulfilled by lysine and glutamic acid (about 10%).

(3) In winter, *P. obovata* was characterized by a higher level of proteingeneous amino acids (primarily glutamic acid and glycine). For *P. sylvestris* a share of valine was 10 times higher than that for *P. obovata*, and three times higher than the share of nonproteingeneous GABA.

(4) In the cryoprotection of cell membranes in *P. sylvestris* buds an important role belongs to GABA; in *P. obovata* it belongs to lysine. In winter, their content in meristems was 1.0–1.4% of the total amino acids, and in spring buds it was reduced by half. At the same time, in the studied species the proline content (amino acid, in the presence of which, generally, high– low-temperature stability of plant tissues was coordinated) in winter it was low (0.04–0.34%).

(5) With the swelling of buds in both species, the share of arginine and proline increased, and the share of ornithine and GABA sharply decreased. In spring buds of *P. sylvestris*, a high content of aspartic acid + asparagine reached 19%; for *P. obovata* the content of amino acids with a short carbon chain (serine + glycine) increased to about 22%.

Thus, the study and analysis of free amino acids allowed us to identify seasonal and species-specific differences between *P. obovata* and *P. sylvestris* according to the feature studied. Seasonal changes are well coordinated with the information in the literature on the cryoprotective functions of individual free amino acids in plants and the species-specific features of meristem metabolism inherent in frost-resistant conifers with different morphology of their buds. As a reliable stress metabolite in spruce and pine, only ornithine may be considered, the content of which in winter bud meristems was 3–5 times higher than in spring buds.

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