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Influence of Water Deficit on Contents of Carbohydrates and Nitrogenous Compounds in *Pinus sylvestris* L. and *Larix sibirica* Ledeb. Tissues

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Abstract

In order to study the physiological responses of Siberian conifers to water stress, the contents of total nitrogen protein, free amino acid, starch and content of low-molecular carbohydrates in needles, xylem and inner bark of stems and rough roots and the cellulose and lignin contents in stem and root xylem samples obtained from *Pinus sylvestris* L. (pine) and *Larix sibirica* Ledeb (larch). Trees in three habitats in Eastern Siberia with different amounts of annual precipitation were determined. Pine and larch trees originated from taiga populations had high growth rate of diameter and height increment by 30-40% compared with those from forest-steppe populations. Drought induced an accumulation of sugar due to an increase in monosaccharide concentration in tree tissues. Simultaneously, the content of non-protein forms of nitrogen rose. The tissues of current year shoots were used for identification of biochemical indices of water stress in pine and larch. The increase in the ratio of concentrations of low-molecular carbohydrates to protein nitrogen or the increase in ratio of total concentrations of arginine to the sum of concentrations of glutamic acid, proline and 4-aminobutyric acid in these tissues could be used as a marker of water stress. Water deficit does not have a significant effect on the contents of the main components of xylem - cellulose and lignin.

Key words: carbohydrates, free amino acids, nitrogen, Siberian conifers, stress markers, water deficit.

Introduction

Productivity of forest stands in Russia varies widely, reaching a maximum value only in the most favorable climatic conditions. In Siberia, where about half of all wood stocks of the Russian Federation is concentrated, the preservation of productivity, determined by the genotype of the main forest species, world not be mate realized on a greater part of the forests because of the limiting effects of environmental factors.

Among various environmental factors, water deficit usually limits the growth of woody plants in Siberia, while developing on southern boundary of forest regions. Evergreen conifers, during the early spring, are most often subjected to water stress when transpiration begins to rise in response to increase in air temperature, but the roots are unable to absorb water from the frozen or very cold soil.

The plants react to stress by developing resistance, with implies biochemical, physiological and morphological changes, required for elimination or weakening of various environmental stresses. It is considered that there are three main methods of resistance; 1) a plant bears the impact of stress without undergoing essential changes, 2) a plant resists stress by protective mechanisms, and 3) a plant eliminates the consequence of stress by repairing damage (Mohr and Shopfer 1995). Woody plants subjected to stresses use usually all of these three methods. Common method of plants for avoiding stresses is to develop the stress metabolism.

In this study, we studied charges in the metabolisms of *Pinus sylvestris* (pine) and *Larix sibirica* (larch) as an element of resistance of conifers to water stress in Siberian permafrost region.

Materials and Methods

The trees studied were *Pinus sylvestris* L. (pine) and *Larix sibirica* Ledeb. (larch) trees in three habitats in Eastern Siberia with different amounts of annual precipitation (460 mm in Krasnoyarsk and 350-360 mm in Minusinsk and Chyornoe Ozero).

The total nitrogen and protein contents, free amino acid contents, starch contents and low-molecular carbohydrate content were determined in needles of current year shoots (needles I) and needles of brachyblasts for larch or one-year-old needles for pine (needles II), current year shoots, cambium, xylem and inner bark of the stem and rough roots. Cellulose and lignin were determined in the stem

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xylem. The samples were collected from 10 trees of each species. The inner bark samples were separated into two layers: the external layer, in which parenchyma and resiniferous elements are predominant (bark I), and the inner layer, in which sieve tubes are predominant (bark II). The current year shoots developed needles and xylem.

The contents of total nitrogen and protein were determined using Nessler's reagent, and the content of free amino acids was determined using an amino acid analyzer AAA-339. The content of starch was determined in perchloric acid extracts (Humphreys and Kelly 1961), and the concentration of low-molecular carbohydrate was determined by Bertrand's method with some modifications (Voznesensky et al. 1962). The content of cellulose was determined by Kurschner's method, and of lignin was estimated by the method according to Klasson, as slightly modified by Komarov (Obolenskaya et al. 1965). Soil moisture and wilting moisture were measured by routine methods and are presented as percentages of maximum water holding capacity. Each experiment was performed with a minimum of three replicates for each extracted sample. Data are presented as means and standard errors of the mean. Linear regression analysis was used to evaluate the relationship between content of amino acids in current year shoots and soil moisture.

Results and Discussion

All biometric parameters of sample trees of pine and larch from a taiga population (Krasnoyarsk) were superior to those of pine and larch trees in forest-steppe habitats (Minusinsk and Chyornoe Ozero). The main reason for the delay in tree growth in the southern populations is more or less, the prolonged periods of water deficiency during summer drought, resulting in decreases in annual diameter increment by 25-30% and height increment by 30-40% (Table 1). Sampling was preceded by a droughty period, especially in Minusinsk was long for pine habitats where soil moisture content fell down below wilting point in upper 40-cm soil layer. In Krasnoyarsk, at the same time of drought period, pine and larch trees had some amount of available water (Sudachkova et al. 1996).

Under droughty conditions, carbohydrate accumulation as a result of an increase in the concentration of low-molecular sugars, especially in root tissues and in the cambial zone of pine, was observed this effect was less evident in larch trees because of the lower water deficit (Fig. 1).

At the same conditions, a decrease in protein nitrogen contents and an increase in non-protein nitrogen contents in inner bark storage tissues and in the cambial zone of pine and larch trees were noticed (Fig. 2). We therefore speculated that the quantitative ratio of these two groups of nitrogen compounds, demonstrating opposite tendency to the change occurred under drought, could be used as a marker of water stress. In fact, the ratio of low-molecular carbohydrates and protein nitrogen contents increased in all pine and larch tissues under a drought condition, and drastic differences were found in the cambial zone (Fig. 3).

Water deficiency resulted in accumulation of free amino acids, especially in the current year shoots and cambiums of the stem and roots (Fig. 4), but not all amino acids reacted to drought in the same way. The most well-known biochemical indicator of water stress is proline accumulation in plant tissues. In this study, we found that drought induced accumulation of arginine in the current year shoots of both pine and larch (Fig. 5). We therefore suggest that analysis them will be employed as test-materials for revealing biochemical markers of water stress. The maximum concentration of proline was also found in the current year shoots, although it was 3-100-times lower than that of arginine. Further study of the free amino acid contents in the current year shoots in droughty periods showed that water deficit intensification induced an increase in arginine concentration and a decrease in proline concentration (Fig. 6). Analysis of the contents of free amino acids in fluctuating conditions of soil moisture showed that amino acids whose metabolic precursor is glutamic acid were subjected to the greatest changes. A comparison of the correlation coefficients between these amino acid contents and water moisture value showed a correlation with the content of proline, 4-aminobutyric acid (4-ABA) and glutamic acid and a negative way for arginine (Table 2). An increase in the glutamic family proportion of the total free amino acid pool was observed under a drought condition.

Within the pool of these amino acids, a change in the rations of two groups occurred: under the condition of water stress, not only the concentrations of arginine and its biosynthetic precursors, ornithine and citrulline, increased, and the total concentration of glutamic acid, glutamine, proline and 4-aminobutyric acid decreased. The differences became more obvious in comparison to the amount of nitrogen in these amino acid groups (Fig. 7). The contents of different amino acids in needles turning yellow due to drought or due to the approach of autumn might be evidence of specificity of the arginine response to drought (Fig. 8).

To elucidate the characteristics of trophic provision of xylogenesis in water stress conditions, the contents of non-structural carbohydrates and nitrogenous compounds in inner bark per unit of cambium surface were determined. It was found that there was no synchrony in the amounts of these substances in inner bark of the two species under a condition of water deficiency. The accumulation of carbohydrates due to an increase in monosaccharide concentration in the inner bark of pine roots in drought conditions is more remarkable (Table 3).

Clear increase in total nitrogen and especially in protein content in inner bark of the root in comparison to that in the stem was found in larch for wooden part of stem and root of pine there are more carbohydrate compounds and in larch wood – more proteins.

The water deficit did not have a significant effect on the main wood components, cellulose and lignin, only a clear decrease of cellulose content in wooden part of root of both species under drought was noticed (Fig. 9).

Fluctuation in concentration of metabolites in conifer tissues can be a marker of certain types of stress. The use of this kind of marker is grounded for estimation of stress effect and enables identification of species- and stress-specific metabolism fluctuations. Accumulation of low-molecular carbohydrates and decrease in protein content are the responses of some plants to water stress (Levitt 1980, Hulbert 1988), as was confirmed by our study of conifers.

Proline accumulation in plant tissues is a well-known biochemical indicator of water stress in various species of herbaceous and woody plants (Palfi 1969, Britikov 1971, Mori et al. 1971, Stewart and Larher 1980, Shevyakova 1983, Yoshiba et al. 1997). However, observations over a period of several years showed that strong water deficit brought an increase of arginine concentration in pine and larch tissues, but proline concentration increased with decrease of water deficit, whish was opposite tendency against ordinary way. It is known that not all species accumulate proline in response to water or salt stress (Liu and Zhu 1997, Sher and Sen 1987, Schraml and Rennenberg 2000). The maximal concentration of proline is found in current year shoots that are rich in young cells with a high content of cytoplasm, where this amino acid usually accumulates (Heinke and Göring 1982). But even in these tissues, the proline concentration does not exceed 0.1% of tissue mass.

Relative indices are more informative and reliable. As such an index for identification of water stress, the ratio of the concentration of low-molecular carbohydrates to protein nitrogen has been proposed. The ratio of non-structural carbohydrates to total nitrogen in pine and larch tissues increases in response to water stress and may also be used as such a relative index. Moreover, this index differs depending on the species and may indicate the capacity of nitrogen utilization (Sudachkova et al. 2000).

One more relative index that responds to water stress by a drastic rise is the ratio of the total concentration of arginine metabolically connected with ornithine and citrulline to the sum of concentrations of glutamic acid, proline and 4-aminobutyric acid. The change of these amino acids groups demonstrated that degree of redistribution of glutamic acid derivatives pool in the direction of predominate of compounds with high content of nitrogen as a response to water deficit (Fig 10). On the basis of these data, it is thought that accumulation of these amino acids showed the mechanism of binding of ammonia formed after breakdown of protein against drought. Therefore, the ratio of two groups of nitrogen with common compound shows metabolic indicator of water stress. This is confirmed by the correlation between amino acids concentration and moisture content of upper soil layer was negative for the first group; amino acid and was positive for the second group; ammonia.

Table 1. Biometric parameters of Pine (*Pinus sylvestris* L.) and Larch (*Larix sibirica* Ledeb.) trees in natural habitats

Parameter	Pine		Larch	
	Minusinsk	Krasnoyarsk	Chyornoe Ozero	Krasnoyarsk
Age, years	36±2	27±0	30±5	27±0
Height, m	9.2±1.1	12.1±0.5	9.4±1.1	12.1±1.4
Diameter DBHm, cm	11.5±0.7	12.8±1.2	9.6±1.1	12.1±0.3
Height increment, m	0.25±0.03	0.45±0.02	0.31±0.04	0.45±0.05
Wood annual ring width, mm	3.6±0.1	5.2±0.1	3.8±0.2	5.4±0.2

Table 2. Correlation coefficients (r)* between contents of amino acids in content year shoots and soil moisture at a depth of 40 cm

Species	Arginine	Arginine+ citrulline+ ornithine	Proline	4-ABA	Glutamic acid	Whole glutamic acid family
Larch	-0.52	-0.58	-0.69	0.71	0.27	-0.68
Pine	-0.50	-0.52	-0.55	0.46	0.93	-0.55

* - $P \leq 0.05$

Table 3. Amounts of carbohydrates and nitrogenous compounds in the inner bark of larch and pine, g.m^{-2} cambium surface

Geographic point	Species	Tree part	Non-structural carbohydrates	Total nitrogen	Amino acids	Proteins
Chyornoe Ozero	Larch	Stem	19.0±0.9	4.7±0.1	0.6±0.0	29.5±0.5
		Roots	26.7±0.8	7.3±0.2	1.6±0.0	46.4±1.1
Krasnoyarsk	Larch	Stem	27.7±1.0	6.2±0.1	1.7±0.0	34.5±0.7
		Roots	42.1±1.3	7.6±0.2	1.9±0.0	40.5±0.8
Minusinsk	Pine	Stem	25.3±0.7	2.3±0.1	2.0±0.0	13.1±0.3
		Roots	55.7±1.4	2.6±0.1	3.4±0.0	17.1±0.3
Krasnoyarsk	Pine	Stem	45.1±1.0	3.2±0.1	2.7±0.0	21.2±0.5
		Roots	31.9±0.9	2.7±0.1	1.6±0.0	18.4±0.4

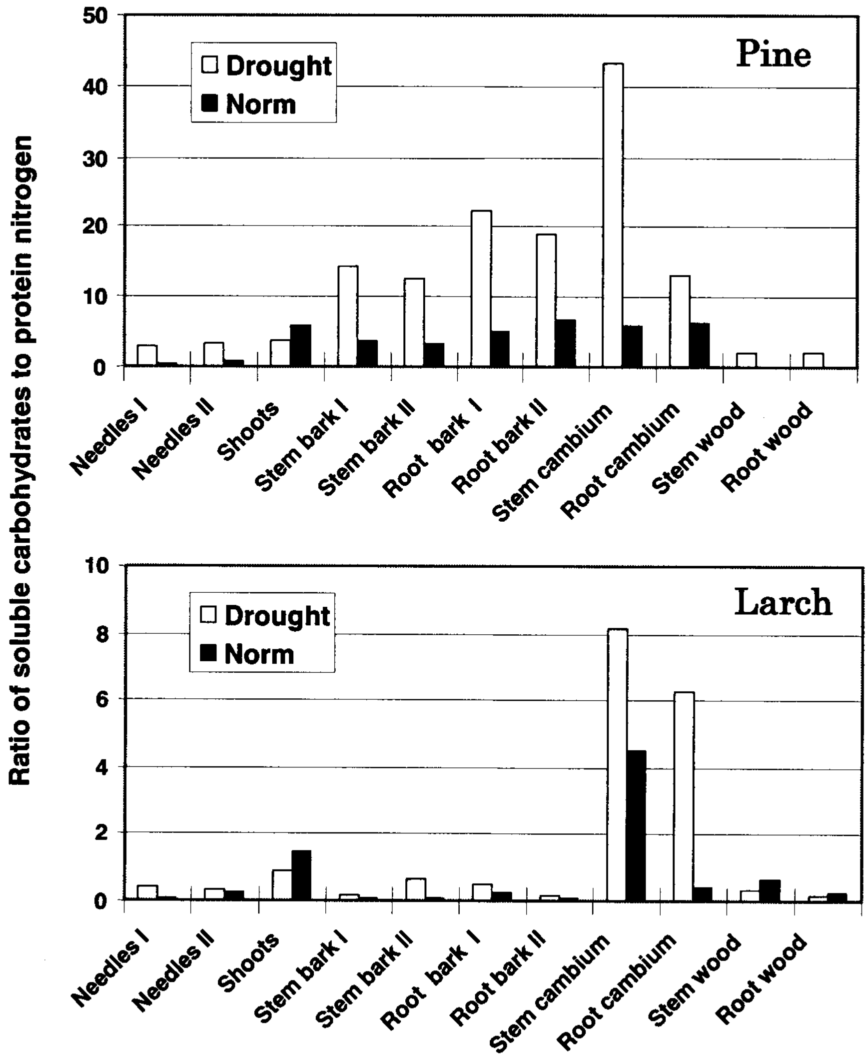

Fig. 1. Effects of drought on contents of low-molecular carbohydrates in pine and larch tissues.

Note: **Needle I**; current year, **Needle II**; one year old, **Bark I**; external layer, **Bark II**; inner bark

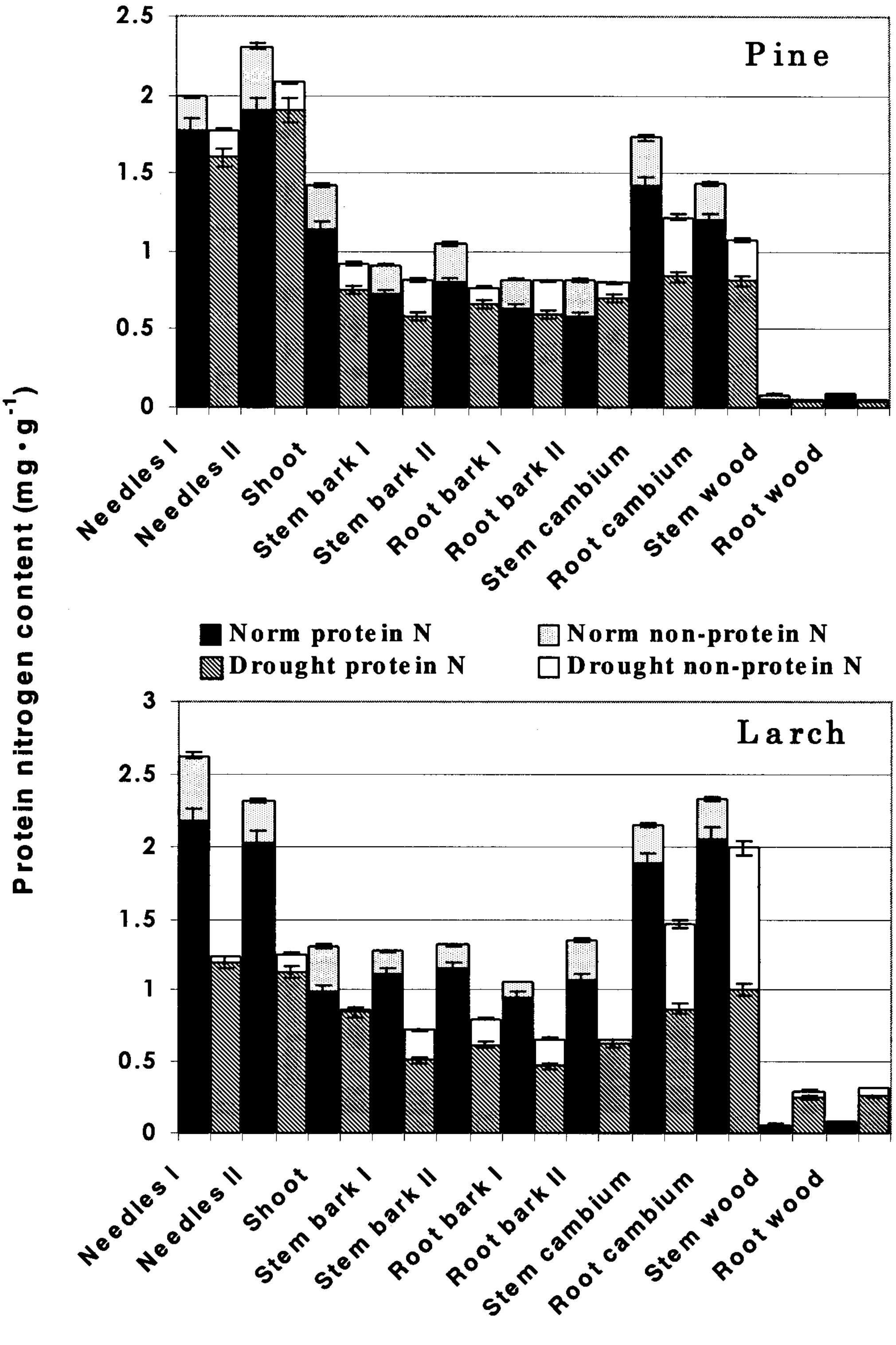

Fig. 2. Effects of drought on protein nitrogen contents in pine and larch tissues.

Note: **Needle I**; current year, **Needle II**; one year old, **Bark I**; external layer, **Bark II**; inner bark

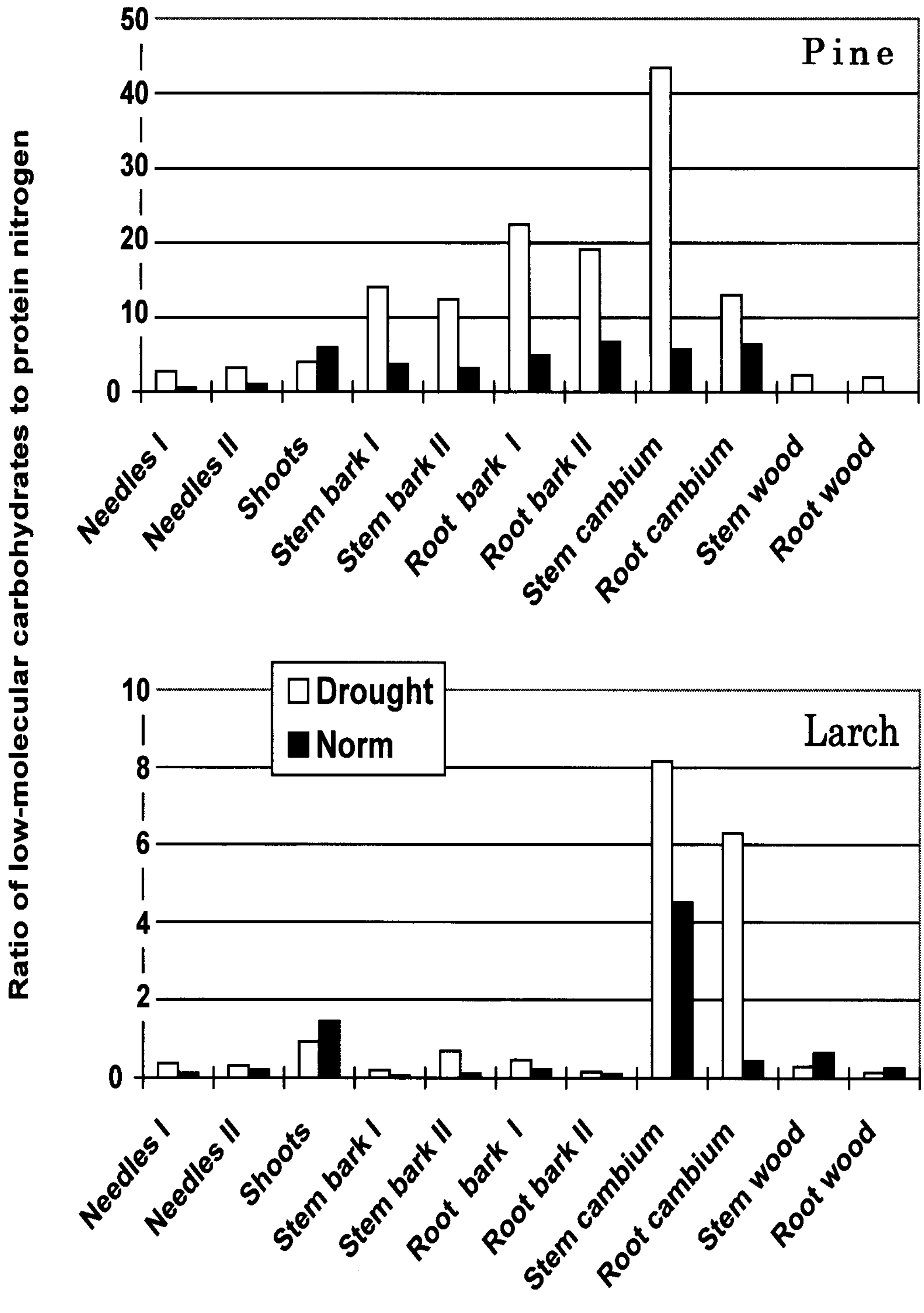

Fig. 3. The ratios of low-molecular carbohydrates to protein nitrogen concentrations in pine and larch tissues under drought conditions.

Note: **Needle I**; current year, **Needle II**; one year old, **Bark I**; external layer, **Bark II**; inner bark

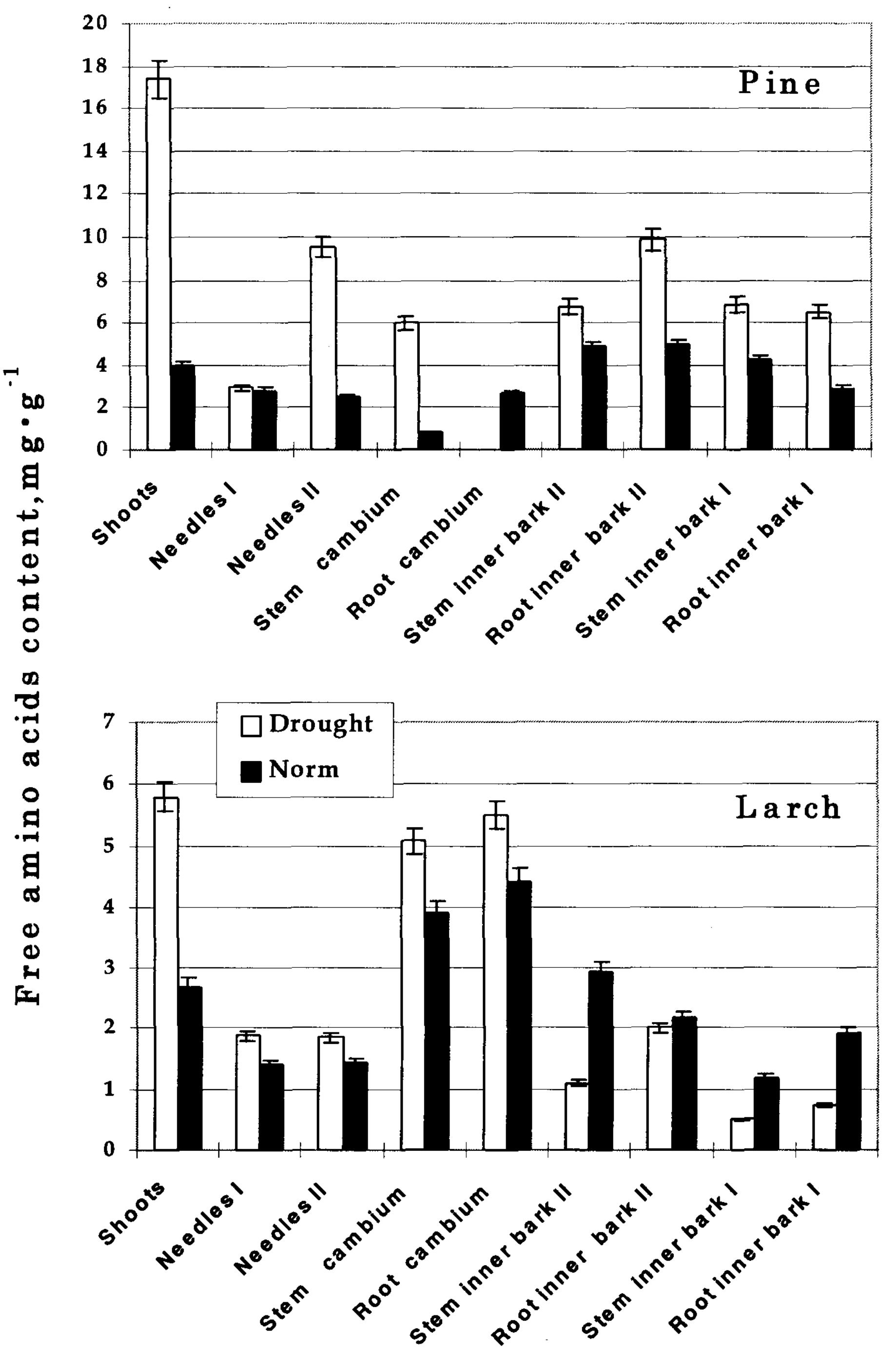

Fig. 4. Contents of free amino acids in pine and larch tissues under drought conditions.

Note: **Needle I**; current year, **Needle II**; one year old, **Bark I**; external layer, **Bark II**; inner bark

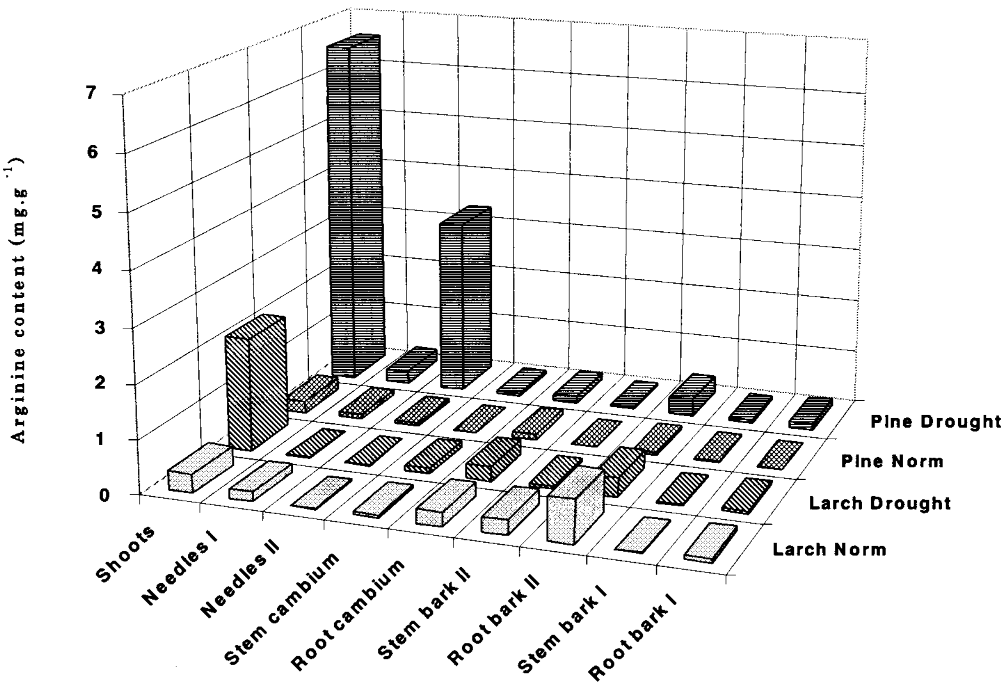

Fig. 5. Arginine contents in pine and larch tissues in normal moisture conditions and in drought conditions.

Note: **Needle I**; current year, **Needle II**; one year old, **Bark I**; external layer, **Bark II**; inner bark

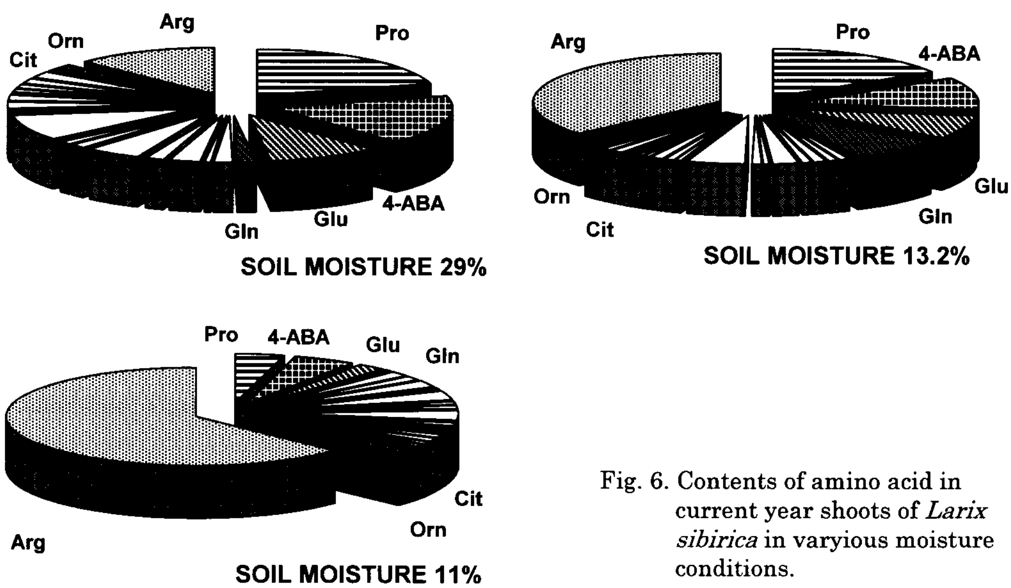

Fig. 6. Contents of amino acid in current year shoots of *Larix sibirica* in varyious moisture conditions.

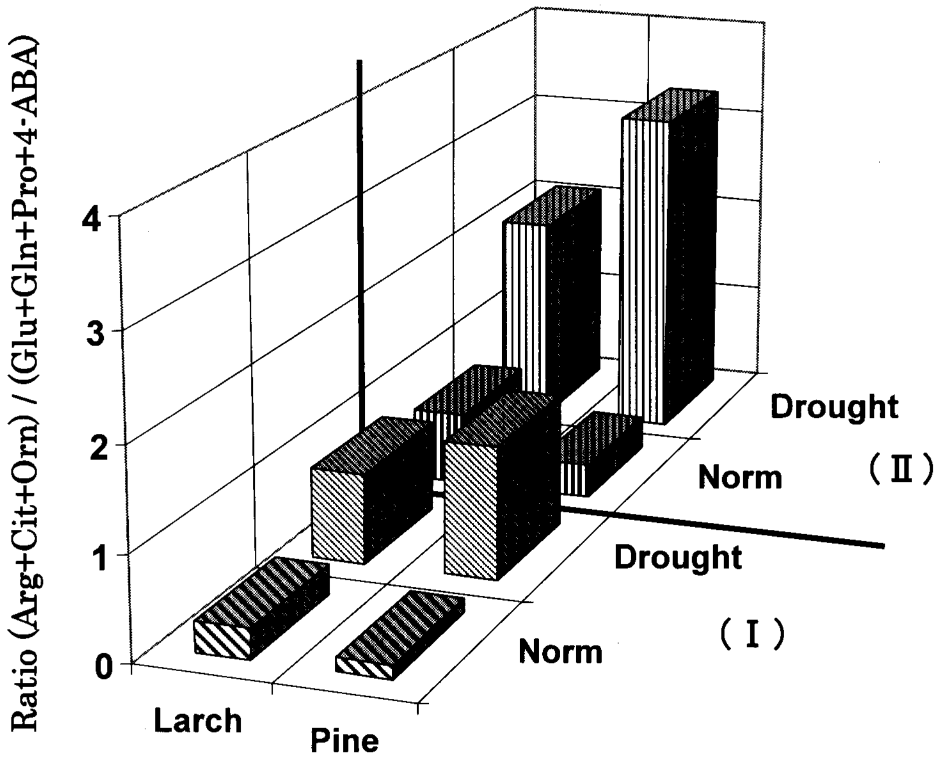

Fig. 7. Mass ratio of two amino acid groups (Arg+Cit+Orn) / (Glu+Gln+ Pro+4-ABA) (I) and incorporated into them agairnst nitrogen (II) in current year shoots of pine and larch under drought conditions.

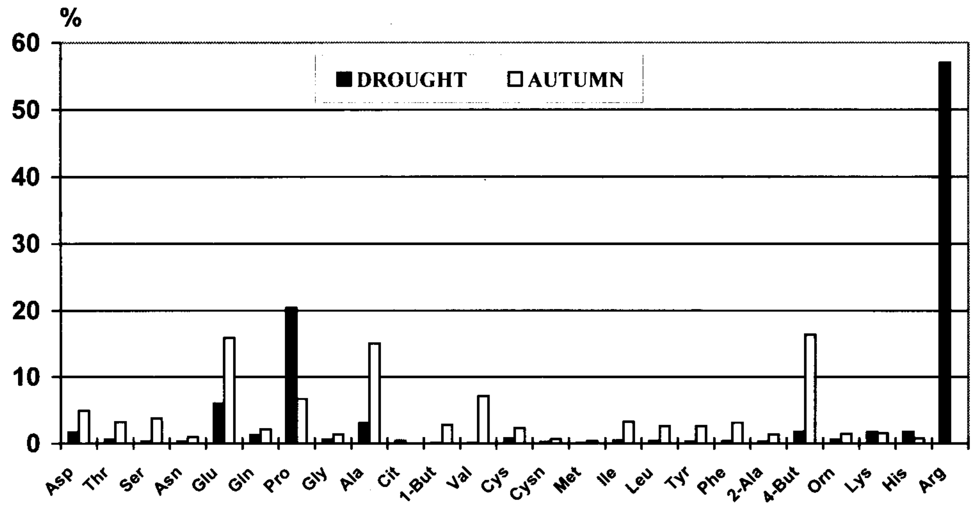

Fig. 8. Contents of free amino acids in needles of *Larix sibirica* yellowing due to drought or due to the approach of autumn, percentage.

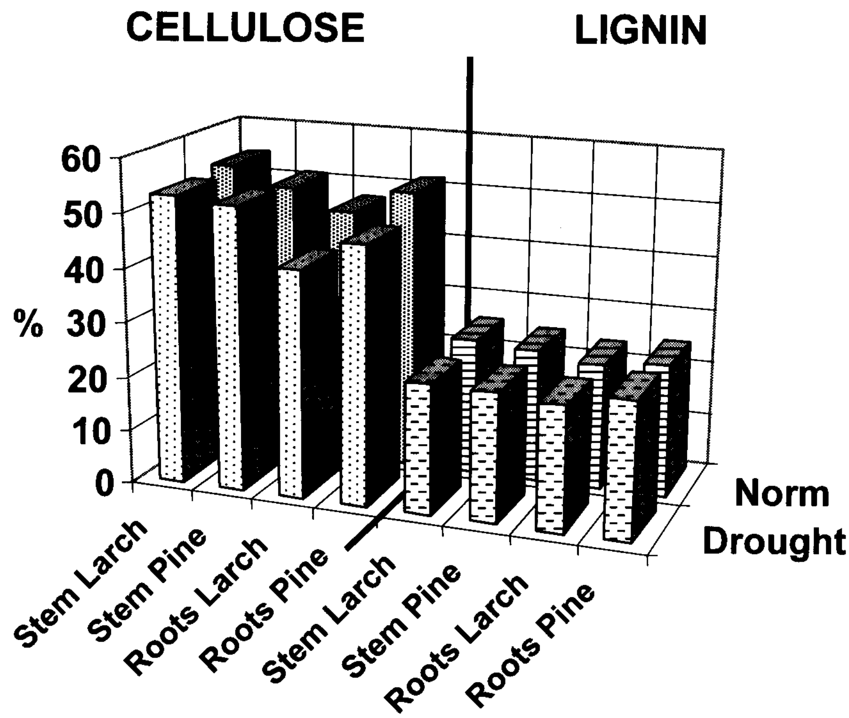

Fig. 9. Cellulose and lignin contents in the stems and roots of pine and larch under drought conditions.

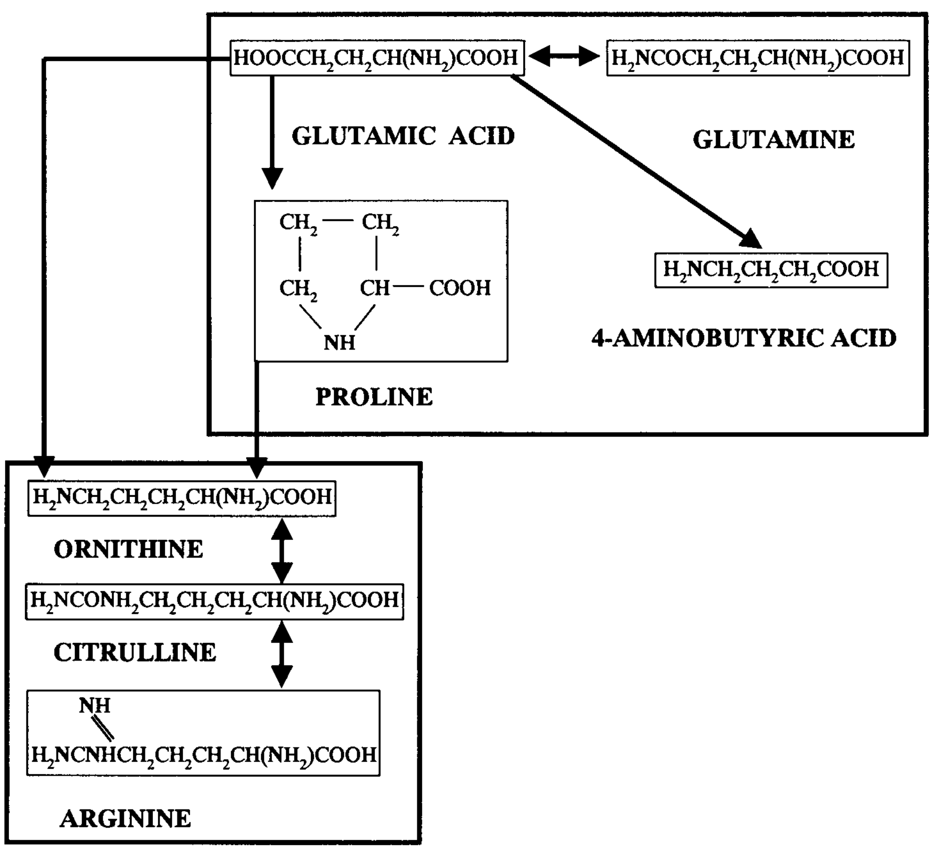

Fig. 10. Metabolic interactions of glutamic acid derivatives.

Conclusions

The tissues of current year shoots are regarded as test-materials for identification of biochemical and physiological indicators of water stress in pine and larch.

Relative biochemical indices are more informative and reliable for identification of water stress than are concentrations of metabolites. The ratio of the concentration of low-molecular carbohydrates to protein nitrogen and the ratio of total concentration of arginine, ornithine, and citrulline to the sum of concentrations of glutamic acid, proline, and 4-aminobutyric acid in tissues are good relative biochemical indices.

The ratio of carbohydrate to total nitrogen differs significantly depending on the species and can be used as an index of capacity of nitrogen utilization.

The water deficit does not have significant effect on either the contents of cellulose and lignin in wooden parts or on labile compounds in the inner bark of pine and larch.

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References

Britikov, E.A. (1975) Biological role of proline. Moscow, Nauka, 88p.

Heinke, F. and Göring, H. (1982) Stressbedingte Prolinakkumulation bei Pflanzen. Wiss. Beitr. M. Luther-Univ. Halle-Wittenberg, 17: 171-172.

Hulbert, C., Funkhouser, E.A., Soltes, E.J. and Newton, R.J. (1988) Inhibition of protein synthesis in loblolly pine hypocotiles by mannitol-induced water stress. Tree Physiology, 4: 15-26.

Humphreys, F.R., and Kelly, J. (1961) A method for determination of starch in wood. Anal. Chem. Acta, 24 (1): 66-70.

Levitt, J. (1980) Responses of plants to environmental stresses. Vol. II. Water, radiation, salt and other stresses. N.Y., L. etc: Acad. Press. 607 p.

Liu, J. and Zhu, J.K. (1997) Proline accumulation and salt-stress induced gene expression in a salt-hypersensitive mutant of Arabidopsis. Plant Physiol., 114: 591-596.

Mohr, H. and Shopfer, P. (1995) Plant Physiology. Berlin, Heidelberg: Springer-Verlag. 629 p.

Mori, T., Sakagami, Y. and Doi, K. (1971) Change in free amino acid content in *Cryptomeria japonica* transplants under various soil moisture conditions. J. Jap. Forest. Soc., 53: 350-354.

Obolenskaya, A.V., Scheglov, V.P., Akim, G.L., Akim, E.L., Kossovich, N.L. and Emelianova, I.Z. (1965) Practical works in wood and cellulose chemistry. Moscow, Lesnaya promishlennost'. 411 p. (in Russian).

Palfi, G. (1969) Das Prolin, die dem Wassermangel der Pflanzen anzeigerde Aminosäure. Acta biol. Szeged., 15: 65-69.

Schraml, C. and Rennenrerg, H. (2000) Senssitivität von Ökotypen der Buche (*Fagus sylvatica* L.) gegenüber Trockenstress. Forstw. Cbl., 119: 51-61.

Sher, M. and Sen, D.N. (1987) Proline accumulation in arid zone plants. J. of Arid Environments, 13 (3): 231-236.

Shevyakova, N.I. (1983) Metabolism and physiological role of proline in plant under water and salt stress. Plant Physiology, 30: 768-783 (in Russian).

Stewart, G.R. and Larher, F. (1980) Accumulation of amino acids and related compounds in relation to environmental stress. Biochemistry of plants. A comprehensive treatise. N.Y., Acad. Press, 5: 609-635.

Sudachkova, N.E., Milyutina, I.L., Kudashova, F.N., Semenova, G.P. and Kozhevnikova, N.N. (1996) Drought influence on free amino acids composition in tissues of *Pinus sylvestris* and *Larix sibirica*. Lesovedenie, 2: 57-67 (in Russin).

Sudachkova, N.E., Milyutina, I.L. and Semenova, G.P. (2000) Interspecific variability of biochemical parameters of Siberian conifers as a strategy of biodiversity maintenance. *In*: Proc. Internat. Conf. "Biodiversity and dynamics of ecosystems in North Eurasia" : Novosibirsk, Russia, pp. 163-164.

Woznesensky, V.L., Gorbacheva, G.I., Shtan'ko, T.P. and Phylippova, L.A. (1962) Sugar determination by Feling liquid discoloration. Plant Physiology, 9: 255-266 (in Russian).

Yoshiba, Y., Kyosue, T., Nakashima, K., Yamaguchi-Shinozaki, K. and Shinozaki, K. (1997) Regulation of levels of proline as an osmolite in plants under water stress. Plant Cell Physiol., 38: 1095-1102.