Ecophysiological reactions of the Siberian stone pine leaf apparatus to climate changes

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Changes in structure and physiological features of different ecotypes of the Siberian stone pine (*Pinus sibirica* Du Tour.) growing in common climate conditions in the south of Tomsk Region are discussed. The grafts of mother trees taken from natural northern, western and eastern marginal populations have been investigated. It is shown that northern ecotypes have smaller size needles, smaller areas of cross section, mesophyll, and conducting bundle, lower green and yellow pigment content as well as decreased functional activity of chloroplasts in comparison with local variants. In the longitudinal direction, differences between the ecotypes are less pronounced, however the local ecotype has higher values of the parameters controlled.

Introduction

Expected global changes of the Earth's atmosphere and climate require that different approaches to environmental monitoring of the vegetation are to be developed. The study of ecological and physiological features of species, types of ecological strategies, and reaction norms of plants to climate changes would allow one to forecast the plant populations redistribution.[1,2] In studying this problem it is reasonable to use edificator-species occupying transcontinental areas and having a wide range of adaptive abilities at all the life levels. The Siberian stone pine (*Pinus sibirica* Du Tour) occupies vast areas from the Urals to the Aldan head river in Eastern Siberia and extends northward along the Yenisei river to the polar circle and southward to the north of Mongolia.[3] Climate peculiarities of the Siberian stone pine area produce response reactions of the whole plant body, especially its leaf apparatus, which result in specific structure and functional features.

Plant response to climate changes can be simulated when growing different ecotypes of the Siberian stone pine in geographical plantations.

This work was aimed at revealing of structure and functional features of the needles of different ecotypes of the Siberian stone pine (*Pinus sibirica* Du Tour) when grown in the south of Tomsk Region in common climate conditions.

Experiment

The results were obtained at the geographical graft plantation founded 30-km southward of Tomsk. Southeast of the West Siberian Plain is an optimal place for growing the Siberian stone pine. Objects of the study were grafts of mother trees taken from natural Siberian stone pine plantations. Four ecotypes were selected for the study; three of them were from boundary points of the growing area: the northern one – from Urengoi (65°50′N 78°10′E), western – from Neviyansk (57°15′N 60°01′E), and the eastern one – from Severobaikalsk (55°40′N 109°25′E), and the fourth one was the local Tomsk ecotype (57°45′N 89°56′E) growing in optimal conditions and serving as a reference. Growing conditions of mother trees are described in Table 1. Temperature factors, humidity, and duration of the growing season evidently changed for plants of different geographical origin.

Table 1. Climate parameters of growing sites and duration of the growing season for mother populations of the Siberian stone pine[4]

Parameter		Ecotype			
		Local	Northern	Western	Eastern
Air temperature, °C	annual mean	–0.6	–7.8	1.5	–3.1
	average minimum in winter	–19.2	–22.0	–20.0	–23.0
	average maximum in summer	18.1	14.0	17.0–19.0	15.0
Precipitation, mm/year		517	524	500–600	350
Humidification factor		1.0	2.25	1.5	0.67
Duration of the growing season, days		114	60–90	130	60–90

Mixed needle samples from 3–4 model trees of each ecotype were used for morpho-anatomical and physiological analysis. For anatomical investigations, the needle was fixed in 70% solution of alcohol.[5] The 30-μm thick microscopic sections were taken from middle parts of needles on a freezing microtome and placed in glycerin. Anatomical parameters were examined using an MPI-5 microscope and temporary preparations using a scale eyepiece and a micrometer eyepiece. The measurements of morphological and anatomical needle parameters were repeated 30–40 times.

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Photosynthetic pigments were determined spectrophotometrically from the optical density at 665, 649, and 440.5 nm wavelengths.[6] For this, a fresh plant sample was fixed in 96% ethanol and then a Shimadzu UV-1601PC spectrophotometer was used. Chloroplasts were isolated from needle with the technique modified for conifers.[7] Functional activity of the chloroplasts was determined spectrophotometrically using potassium ferricyanide.[8] The reaction mixture contained 100 μmole of tris, pH 7.4, 10 μmole of NaCl, 10 μmole of $MgCl_2$, 3 μmole of $K_3Fe(CN)_6$, 4 μmole of ADP, and 10 μmole of KH_2PO_4. The reaction rate in a cell was measured at white-light of 150 $W \cdot m^{-2}$. The intensity of dark respiration was determined from the emission of carbon dioxide.[8]

The data were processed with Excel and Statistica computer programs.

Discussion

The investigations we have carried out showed the intraspecific variability of structure and functional parameters under study, which is evidence of adaptability of the leaf apparatus and large genetic potential of the Siberian stone pine. The local ecotype grows in the habitat, which is a reference ecological point: to the north of the point, natural growing conditions become worse due to general cooling and increase of the humidification factor, to the east – due to decrease of the moisture content on the territory, and to the west – due to the increase of the humidification factor (see Table 1).

Table 2 presents the measurement data on morpho-anatomical features of the needle. Variations of the structure parameters evidently depend on climate parameters of the graft-mother habitats. First, there are parameters invariable from south to north and from west to east. Second, there are parameters, variability of which is specific for each ecotype. For instance, the northern ecotype has needle length, mesophyll area, mesophyll cell area, and the area of the conducting bundle that are lower than those in the local ecotype by 28, 29, 27, and 16%, while the stomatal density is higher by 16%.

Table 2. Morpho-anatomical parameters of the needle of Siberian stone pine grafts of different geographical origin

Parameter		Ecotype			
		Local	Northern	Western	Eastern
Needle length, mm		110.3	79.1	107.8	101.6
Area of cross section, $\mu m^2 \cdot 10^4$	cross section	45.7	36.1	36.7	35.7
	mesophyll	29.3	20.8	22.7	18.3
	mesophyll cell	0.1	0.09	0.1	0.08
	conducting bundle	12.9	10.8	11.4	13.4
	resin channels	3.6	3.8	3.8	3.9
Stomas	density, pcs/mm^2	41.1	48.9	42.1	41.1
	length, μm	50.4	51.9	57.6	53.6
Cell thickness, μm	epidermis	12.8	14.7	11.3	13.9
	hypodermis	12.1	13.3	12.2	14.3

At the northern boundary of the growing area the temperature is the main factor affecting the growth; here needle of a number of conifers is characterized by quite small length and areas of anatomical structures.[9] High stomatal density of northern ecotypes is typical for xeromorfic leaves; just such needle structure is formed under overmoistened conditions.[10]

Differences between ecotypes were less pronounced in the longitudinal direction (see Table 2). Thus, the western ecotype differed from the local one in transversal section area by 20%, mesophyll area by 22%, and increase of stoma sizes by 22%. The eastern ecotype was characterized by still larger decrease of the cross section area, mesophyll area, and mesophyll cell area. Needles of western ecotypes had definitely less epidermis thickness and larger stoma sizes in comparison with the eastern ecotype. This could well be due to the different humidification factor for mother areas, which is higher in the west than in the east.

The variability of the pool of needle photosynthetic pigments of ecotypes under study was mainly related to different rates of growth and development of needles of model objects. In the end of July, all ecotypes had optimally developed photosynthetic apparatus. Nevertheless, chlorophyll and carotenoid contents as well as functional activity of chloroplasts at the level of the photosystem II differed (see Fig. 1). As follows from the investigations conducted, northern and eastern ecotypes had less developed assimilation apparatus in conditions of the south of Tomsk Region in comparison with the local ecotype, which is evident from the lower content of green and yellow pigments and Hill reaction rate.

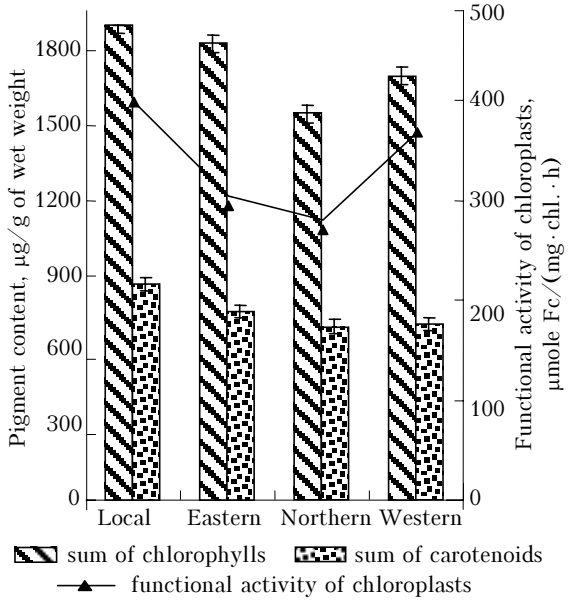

Fig. 1. Content of photosynthetic pigments and functional activity of isolated chloroplasts of one-year needles of different Siberian stone pine ecotypes (end of July); Fc means ferrycyanid, chl. is the sum of chlorophylls "*a*" + "*b*".

As is shown in Refs. 11 and 12, the content of photosynthetic pigments in needles in the north is lower than in south regions. When grown under optimal conditions the ability to form protein-lipidic complexes remains low. The formed adaptation mechanisms allow northern plants to overcome unfavorable conditions. Plants with lower productivity are usually characterized by higher resistance.

Northern and eastern ecotypes differ from local trees in the dark respiration rate most of all (see Fig. 2).

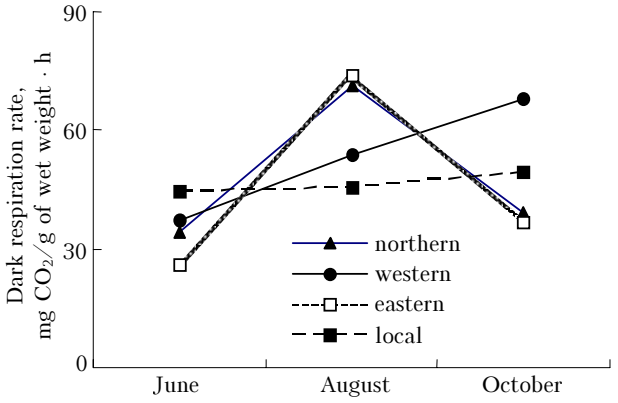

Fig. 2. Respiration rate of one-year needles of different Siberian stone pine ecotypes.

In the beginning of vegetation period the rates of carbon dioxide emission were similar for all the variants under study; with leaf apparatus formation, differences were observed and strongly depended on the temperature of ambient air. The increase of respiration rate for the northern ecotype is seemingly related to the growing conditions of mother populations. Depressing total metabolic activity and growth, stress conditions can intensify the respiration for synthesizing specific metabolites with protective functions.[13]

Conclusion

Thus, morpho-anatomical features, pigment complex, functional activity of chloroplasts, and the dark respiration rate play a certain diagnostic role in estimation of the state of tree bodies and can serve an indicator of the environment–tree interrelation. We have ascertained that quantitative morpho-anatomical and physiological parameters of needles are characteristic for an ecotype and seemingly caused by growing conditions of mother populations. The obtained results have shown the highest conservatism of the northern ecotype in comparison with the western and eastern ones when grown under optimal conditions; its adaptation mainly concerns its resistance, but not the increase of productivity. The experiments conducted allow one to assume that under conditions of warming along with precipitation enhancement, structure and functional changes in every ecotype will concern those anatomical, morphological, and physiological parameters which will promote the most effective adaptation of the Siberian stone pine to new environmental conditions and, hence, its area will expand farther to the north.

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References

1. V.I. Piyankov, L.A. Ivanov, and X. Lanbers, Ecology, No. 4, 243–251 (2001).
2. M. Gratani, P. Meneghini, P. Pesoli, and M.F. Crescente, Trees, No. 17, 515–521 (2003).
3. N.T. Mirov, *The Genus Pinus* (The Roland Press Company, New York, 1967), 602 pp.
4. *Reference Book on the Climate of the USSR* (Gidrometeoizdat, Leningrad, 1990), 302 pp.
5. A.T. Mokronosov, in: *Mesostructure and Functional Activity of a Photosynthetic Apparatus* (Publishing House of the Ural State University, Sverdlovsk, 1978), pp. 5–30.
6. A.A. Shlyk, in: *Biochemical Methods in Physiology* (Nauka, Moscow, 1971), pp. 154–170.
7. A.P. Zotikova, G.M. Ananiev, S.I. Allakhverdiev, I.B. Ganago, and E.I. Simonova, Fiziologiya Rastenii **34**, Is. 3, 271–277 (1987).
8. V.F. Gavrilenko, M.E. Ladygina, and L.M. Khandobina, *Large Laboratory Manual on Plant Physiology* (Vyssh. Shkola, Moscow, 1975), 392 pp.
9. G.M. Kozubov, in: *Problems of Plants Adaptation to Extreme Northern Conditions* (Petrozavodsk, 1975), p. 85–104.
10. S.A. Mamaev, *Forms of Intraspecies Variability of Woody Plants* (Nauka, Moscow, 1972), 284 pp.
11. V.A. Mudrik and G.E. Vilchek, Ecology, No. 4, 267–273 (2001).
12. Yu.E. Novitskaya, *Physiologo-Biochemical Bases for Pine Growth and Adaptation in the North* (Nauka, St. Petersburg, 1985), 186 pp.
13. T.K. Golovko, *Plants Respiration. Physiological Points* (Nauka, St. Petersburg, 1999), 204 pp.