Characteristics of the photosynthetic apparatus of *Pinus sylvestris* L. and *Larix sibirica* Ledeb. from different locations of the Irkutsk region (Eastern Siberia)

M V Oskorbina¹, O V Kalugina¹, L V Afanasyeva² and T M Kharpukhaeva²

¹ Siberian Institute of Plant Physiology and Biochemistry SB RAS, Irkutsk, 664033 Russia
² Institute of General and Experimental Biology SB RAS, Ulan-Ude, 670047 Russia

E-mail: omaria-84@yandex.ru

Abstract. The characteristics of the photosynthetic apparatus of coniferous growing in various forest types and climatic conditions of the Irkutsk region (Taishet, Bratsk, Shelekhov, Irkutsk districts) have been explored. The content of photosynthetic pigments, total, free, and bound water in the needles of *P. sylvestris* and *L. sibirica* were studied. Using a pulse fluorimeter PAM 2500 (Waltz, Germany), the chlorophyll fluorescence in needles was measured based on such induction curve indices as $F_v/F_m$, $Y(II)$, NPQ, and ETR. According to the chlorophyll fluorescence induction curve parameters of Scots pine and Siberian larch needles growing in different forest growth conditions, the most effective potential of primary photochemical processes in PSII was observed in Scots pine. While studying the pigment fund of Scots pine and Siberian larch needles, it was found that the highest values of the content of chlorophyll (Chl) $a$, $b$, and carotenoids were observed in *P. sylvestris* and *L. sibirica* growing in the climatic and forest growth conditions of the Shelekhov district. It is assumed that the revealed characteristics of the parameters reflect the processes of adaptation of the photosynthetic apparatus to various environmental conditions during vegetation development, as well as its high plasticity in different climatic growth conditions.

1. Introduction

The interaction analysis between the structural and functional characteristics of individual organs and tissues of plants is the main point in the study of plant adaptation mechanisms [1]. Nowadays, in the context of increasing planetary climate change, preserving the biosphere role of the vegetation cover is of particular importance. In this regard, it is quite relevant to study photosynthesis, which is the main process ensuring the productivity of forest ecosystems and their adaptation to extreme habitat conditions, in the dominant forest species of the Baikal region (*Pinus sylvestris*, *Larix sibirica*). Their prevalence is based on the undoubtedly high adaptive potential of photosynthesis, as the main physiological process, and the plasticity of the physiological and biochemical parameters of the photosynthetic apparatus of woody plants. Therefore, it seems relevant to carry out a complex of physiological and biochemical studies that will reveal the nature of the photosynthetic apparatus plasticity in the ecological conditions of the Baikal region and determine the adaptive characteristics and productivity of the main woody species.
The plasticity of any organism is widely known to depend on environmental factors [2]. Researchers have shown the high plasticity of the photosynthetic function in natural environments and its ability to modify depending on the combination of climatic conditions [3]. It has been found that the assimilation activity tends to achieve the highest rates under optimal natural conditions [4]. Plant plasticity to unfavourable growing conditions is most often associated with changes in the morphometric parameters of individual plant parts [5], which are based on adaptive changes in physiological and biochemical processes [6, 7].

Quantitative data on the content of photosynthetic pigments can be considered as a component of ecological and physiological characteristics. Together with the other parameters of the functional activity, the characteristics allow identifying the adaptation features that ensure the successful existence of plants in extreme environmental conditions [8, 9].

Photosystem (PS) II is known to play a leading role in the generation and regulation of electron transport in chloroplasts. Therefore, characteristics reflecting the efficiency of PSII are an important indicator of the photosynthetic apparatus activity [10]. An important characteristic of the PSII state during the chlorophyll fluorescence induction is the photochemical quenching coefficient, which characterizes the number of “open” photosystems. The non-photochemical quenching coefficient characterizes the thermal dissipation of the absorbed light quanta energy, depends on various processes, and includes several components differing in the dark relaxation rate [11].

During the year, conifers have shown to have significant changes in the PSII maximum quantum yield ($F_{v}/F_{m}$), ($F_v$ – maximum variable fluorescence; $F_m$ – maximum fluorescence intensity at which all reaction centres of PSII are closed) [12]. The maximum $F_v/F_m$ values occurred in the second half of summer and early autumn. That evidently reflected the restoration of the structural and functional integrity of PSII components under favourable temperature conditions [13]. Despite the sufficient knowledge of the biochemical and physiological characteristics of the plant photosynthetic apparatus, comprehensive studies on this issue in certain environmental conditions have not been sufficiently carried out. The purpose of this work is to assess the influence of various forest-plant conditions of the Irkutsk region on the physiological and biochemical characteristics of the photosynthetic apparatus of conifers.

2. Objects and Methods
The forest survey was performed on sample areas in different parts of the Irkutsk region (northern and southern parts) which differ in natural characteristics.

1. Taishet district (northern part): the Biryusa river valley, village Patrikha (abandoned), 373 m a.s.l., 55°17’29.4’’ N, 97°53’44.9’’ E. Average temperature in January −19.4°C, July +18.5°C, annual precipitation −394 mm, annual mean temperature −0.5°C, frost-free season – 96 days. Soil types – soddy forest, forest type: herb-rich pine forest. Microrelief: stony and hummocky. Humidification: atmospheric. Forest stand: three-tiered, average tree height is 20 m.

2. Bratsk district: Bratsk area, 15 km well short of Kezhemskaya village, 463 m a.s.l., 56°26’37.2’’ N, 102°28’54.1’’ E. Average temperature in January −22.6°C, July +18.2°C, annual precipitation −406 mm, annual mean temperature −2.2°C, frost-free season – 94 days. Soil types – soddy podzolic, forest type: sedgy and herb-rich pine forests. Microrelief: even. Humidification: atmospheric, underground. Forest stand: two-tiered, average tree height is 18 m.

3. Shelekhov district: Moty village, the Irkut river valley, 408 m a.s.l., 52°04’26.7’’ N, 103°53’01.2’’ E. Average temperature in January −16.2°C, July +20.2°C, annual precipitation −475 mm, annual mean temperature −1.9°C, frost-free season – 118 days. Soil types – forest grey, forest type: gramineous and green moss pine forest. Microrelief: even. Humidification: atmospheric, underground. Forest stand: two-tiered, average tree height is 22 m.

4. Irkutsk district: southwestern forest part of the city of Irkutsk, 427 m a.s.l., 52°14’21” N, 104°16’07” E. Average temperature in January −5.3°C, July +17.9°C, annual precipitation −480 mm, annual mean temperature −2.1°C, frost-free season – 121 days. Soil types – forest grey, forest type: gramineous and green moss pine forest. Microrelief: even. Humidification: atmospheric, underground. Forest stand: two-tiered, average tree height is 20 m.
The objects of the study were the stands of *L. sibirica* and *P. sylvestris*. Field surveys of forest stands were carried out on sample plots (SP). The SPs were laid in tree stands of similar age and quality according to generally accepted methods described in detail in the manuals [14, 15]. The area of each SP was about 0.01 km². On each SP 3–5 test sites (TS) were chosen for needles samples collection; the size of one TS was approximately 100 m². Needle samples were taken on the south and southwest sides from the middle part of the crowns of 40-60 year old 5–6 trees in the middle of the growing season from July 20 to July 25. Five-seven branches were cut with the delimber. Second-year needles of *P. sylvestris* and all needles of *L. sibirica* were separated from the shoots and mixed thoroughly to form averaged samples for each TS. Next, the needles were frozen in liquid nitrogen to determine pigments.

The functional activity of the photosynthetic apparatus of the studied coniferous tree species was assessed by the chlorophyll fluorescence induction indices according to the Genty method [16] using the PAM-2500 (Walz, Germany) device. To record the minimum fluorescence level (*F₀*), branches with needles were darkened for 20 min and then illuminated with low-intensity light signals modulated at low frequencies. Chlorophyll fluorescence intensity (*Fₘ*) was measured after high-intensity light pulse treatment (20,000 μmol·m⁻²·s⁻¹). The maximum quantum yield of photochemical reactions of PSII (*Fᵥ/Fₘ*) was calculated by the formula: $Fᵥ/Fₘ = (Fₘ − F₀)/Fₘ$ [17]; the maximum electron transport rate of PSII (*ETR* – Electron Transport Rate), the quantum yield of photochemical energy conversion in PSII – *Y*(II), the Non-photochemical chlorophyll fluorescence quenching (*NPQ*), and the photochemical quenching with active light (*qP*) were calculated as previously described [17]. The changes in chlorophyll fluorescence indices were recorded under the action of 0.1 μmol·m⁻²·s⁻¹ actinic (acting) light, which supported photosynthesis.

The content of Chl *a* and *b* in the needles was determined using the SF-56 spectrophotometer (LOMO, Russia) in acetone extract [18] during the studied growing seasons. The proportion of chlorophylls in light-harvesting complex (LHC) of the total amount of green pigments was calculated taking into account that all Chl *b* was in LHC, and the Chl *a/b* ratio in LHC was 1.2 [19]. The mean of the pigment content was calculated from 9 samples obtained during all the years of observation. The standard deviation calculated using MS Excel 7.0 varied within ± 0.01-0.08.

To determine the water content in the needles, the generally accepted refractometric method based on the ability of water to leave plant tissues when immersed into a hypertonic sucrose solution was applied, according to [20]. The content of total, free (or weakly bound) and bound (physiologically active) water was calculated. The total water content was determined as the weight difference between raw and dry weighed portions of needles. The water extracted from plant tissues (needles) using a 30% sucrose solution was considered free. That part of the water that did not move into the sucrose solution was considered bound and was defined as the difference between the total water content and its free fraction. All water fractions are determined in wt% of the wet weight of the needles.

To assess the significance of differences, the nonparametric Mann-Whitney test was used (calculated with the STATISTICA 8 software), *n*=3-5.

3. Results and discussion

According to the parameters of the chlorophyll fluorescence induction curve of pine and larch needles (figures 1, 2) obtained in different forest-growing conditions, it is possible to assume that the most effective potential of primary photochemical processes in PSII was observed in *P. sylvestris*. The *P. sylvestris* *Fᵥ/Fₘ* ratio was the same in all of the studied sites and exceeded the *L. sibirica* *Fᵥ/Fₘ* values by 10-30%. *ETR* (light curve parameter characterizing the maximum speed of electrons along the electron transport chain) was maximum in *P. sylvestris* in the forest growing in climatic conditions of the Shelekhov district and exceeded *ETR* values of *L. sibirica* and *P. sylvestris* growing in the other regions by 10-40%. *Y*(II) (quantum yield of photosystem II) was maximal in *P. sylvestris* growing in the Shelekhov district, and in *L. sibirica* growing in the Bratsk district. The minimum *Y*(II) values for both conifers were recorded in the forest growing in climatic conditions of the Taishet district. *NPQ* (non-photochemical quenching indicator) was minimal in *P. sylvestris* growing in the Irkutsk district, and in *L. sibirica* growing in the Taishet and Irkutsk districts.
Figure 1. Parameters of the chlorophyll fluorescence induction curve of *Pinus sylvestris* and *Larix sibirica* needles in different forest sites: 1. Shelekhov district, 2. Taishet district, 3. Bratsk district, 4. Irkutsk district. The asterisks above the columns mean that the differences between *Pinus sylvestris* and *Larix sibirica* are significant at $p<0.05$.

Figure 2. Parameters of chlorophyll fluorescence of *Pinus sylvestris* and *Larix sibirica* needles in different forest zones. 1. Shelekhov district, 2. Taishet district, 3. Bratsk district, 4. Irkutsk district. The asterisks above the columns mean that the differences between *Pinus sylvestris* and *Larix sibirica* are significant at $p<0.05$.

The analysis of the needle water status showed that the total water content in *P. sylvestris* and *L. sibirica* needles in the areas studied was almost the same (figure 3). However, in the Bratsk district’s climatic and forest-growing conditions, the reduced content of bound (physiologically active) water (10-20%) and the increased content of free water (7-12%) were shown both for *P. sylvestris* and *L. sibirica*.

Figure 3. Water content in *Pinus sylvestris* and *Larix sibirica* needles in different forest sites: 1. Shelekhov district, 2. Taishet district, 3. Bratsk district, 4. Irkutsk district. The asterisks above the columns mean what the differences between contents of the total, free and bound water are significant at $p<0.05$. 
When studying the pigment content of \textit{P. sylvestris} and \textit{L. sibirica} needles, it was found that the highest content values of chlorophyll \textit{a}, \textit{b} and carotenoids were observed in \textit{P. sylvestris} and \textit{L. sibirica} growing in the climatic and forest-growing conditions of the Shelekhov district (table 1). That is probably due to the most optimal moisture conditions of this area, which have the most favourable effect on the photosynthetic apparatus of the studied coniferous species [21]. The minimum values of the chlorophyll content in the LHC of PSII were observed in \textit{P. sylvestris} in the Taishet district (51.6%), and in \textit{L. sibirica} in the Bratsk district (52.4%). The revealed characteristics of the studied parameters reflect the photosynthetic apparatus adaptation and its high plasticity to various environmental conditions of the Baikal region territory.

| Table 1. Content of photosynthetic pigments in \textit{Pinus sylvestris} needles. |
|---------------------------------|----------|----------|----------|----------|
| Chl \textit{a}, mg/g, dry weight | Chl \textit{b}, mg/g, dry weight | Carotenoids, mg/g, dry weight | Chl \textit{a}/\textit{b} LHC, % |
| Shelekhov district               | 3.16±0.22 | 1.07±0.05 | 1.74±0.08 | 2.95 55.63 |
| Taishet district                 | 1.92±0.03 | 0.69±0.01 | 1.07±0.01 | 2.75 58.53 |
| Bratsk district                  | 1.73±0.04 | 0.55±0.02 | 0.95±0.02 | 3.14 53.03 |
| Irkutsk district                 | 2.17±0.06 | 0.61±0.01 | 1.49±0.05 | 3.53 51.50 |

| Table 2. Content of photosynthetic pigments in \textit{Larix sibirica} needles. |
|---------------------------------|----------|----------|----------|----------|
| Chl \textit{a}, mg/g, dry weight | Chl \textit{b}, mg/g, dry weight | Carotenoids, mg/g, dry weight | Chl \textit{a}/\textit{b} LHC, % |
| Shelekhov district               | 5.31±0.17 | 1.89±0.06 | 2.62±0.05 | 2.80 57.80 |
| Taishet district                 | 4.78±0.17 | 1.62±0.09 | 2.59±0.1  | 2.95 55.63 |
| Bratsk district                  | 4.56±0.01 | 1.43±0.01 | 2.41±0.01 | 3.19 52.43 |
| Irkutsk district                 | 5.27±0.27 | 1.32±0.07 | 3.52±0.18 | 3.97 55.78 |

4. Conclusion

According to the results obtained, the important interdependent changes in the structural and functional parameters of the photosynthetic apparatus in different climatic and forest-growing conditions were determined. These changes are a manifestation of the species-specific photosynthetic adaptive strategy of conifers providing high biological productivity and their prosperity in the conditions of the studied region. In a broader sense, the scientific results obtained can serve as the basis for studying the species-specific photosynthetic adaptive strategy of plants while preserving the biological diversity of the vegetation in such unique natural territories as the Baikal region. The characteristics of the structural and functional adaptation of the photosynthetic apparatus of conifers revealed in the study can be of great theoretical and practical importance in ecological and physiological studies characterizing the capacity of forest ecosystems as a valuable sink of greenhouse gases. They also can be important in forest ecosystem monitoring, as well as scientific substantiation and forecasting the dynamics of economic productivity of coniferous stands.

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