

## CONTENT OF PLASTID PIGMENTS IN THE NEEDLES OF *Pinus pallasiana* D. DON IN DIFFERENT FOREST GROWTH CONDITIONS OF ANTI-EROSION PLANTING

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### Abstract

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The influence of different forest growth conditions on the content of plastid pigments in *Pinus pallasiana* D. Don needles in the anti-erosion planting of the steppe zone of Ukraine was investigated. The reduction in the total chlorophyll *a* and *b* concentration in the pine needles in the area with insufficient water supply (xerophilous and mesoxerophilous hygrotopes) was found, compared with more favourable conditions for water supply (mesophilous hygrotopes). The reduction in the content of green pigments in arid conditions is due to chlorophyll *a*. Chlorophyll *b* showed high resistance to moisture deficiency. There is an increase in the concentration of carotenoids in pine needles in forest growth conditions with insufficient water supply (xerophilous and mesoxerophilous), compared with more favourable conditions (mesophilous), which can be explained by the adaptive role of these pigments in the processes of stabilising the functioning of the photosynthetic apparatus in adverse environment, and it is considered as a protective reaction of *P. pallasiana* to the effects of drought.

*Key words:* anti-erosion planting, forest growth conditions, *Pinus pallasiana*, needles, plastid pigments.

### Introduction

Since the second half of the twentieth century, *Pinus nigra* ssp. *pallasiana* (*P. pallasiana*), has been increasingly used in forestry, for planting of cities (Korshikov et al., 1998; Terlyga et al., 2002) and in anti-erosion plantation (Bessonova et al., 2015) in the steppe zone of Ukraine. However, there is a little research on its physiological features and adaptive responses in different growth conditions.

The vital activity of plants depends largely on how effective photosynthetic reactions are, which are affected by many internal and external factors. An important role is played by the pigment chloroplasts. The pigment system of plants is complex and extremely sensitive; it responds dynamically to stress factors of the environment (Bessonova, 2006a; Uspivva, Samko,

2009; Iusypiva, Drotik, 2015). As the energy state of plant organisms depends entirely on the quantitative parameters of the pigment apparatus, their violation leads to a deterioration of the energy supply of plants (Hryhoriukh et al., 2004).

The stability of the functioning of the photosynthetic apparatus is ensured by changes in the content and the redistribution of green and yellow pigments in the light-harvesting complex (LHC) and the reaction centre (RC) photosystem. Increasing or decreasing the concentration of pigments that take part in photosynthesis shows the level of adaptive reactions of the organism to stressful environmental influences (Ivanova, Suvorova, 2015). Moreover, the study on the content of chlorophyll and carotenoids in the leaves provides important information for analysing physiological adaptation and productivity of plant organisms (Andrianova, Tarchevs'kyj, 2000; Titova, 2010; Halil, 2012; Iusypiva, Vegerich, 2014; Bessonova, Ivanchenko, 2016). These indicators are often used in environmental studies as the most important and easily identifiable indicators of the functional state of plants (Ivanova, Suvorova, 2015).

The purpose of this article is to analyse the influence of different forest growth conditions on the content of plastid pigments in the pine needles of *P. pallasiana* in the anti-erosion planting of the steppe zone of Ukraine.

## Material and methods

The study was conducted in *P. pallasiana* trees (*P. pallasiana* D. Don) aged 25–27 years, the artificial stand of which is located on the slope of the southern exposition of wooded ravine Vijs'kovij (coordinates between 48°11'08" N 35°07'45" E and 48°10'41" N 35°10'12" E), situated near Vijs'kove village in Solonyansky district of Dnipropetrovsk region. It belongs to the southern geographical variant of ravine forests of the steppe zone of Ukraine (Belgard, 1971). The total length of the ravine is 3.2 km. The ravine has three forks. It features the areas of natural groves as well as anti-erosion artificial plantations on the slopes (Bessonova et al., 2015). The research area is characterised by low rainfall (420–450 mm) and low humidity factor (0.67). The samples were collected from three sites of artificial planting. The first test area (TA1) is located in a talweg on its flat and slightly raised part 1.5–2 m above the level of the stream, which flows along the bottom of the ravine in a deep streambed. Moistening is groundwater and atmospheric. The mechanical composition of the soil is loam (CL). Forest growth conditions (according to Belgard, 1971) are clay-loam soil (CL<sub>2</sub>) (mesophilic, fresh soil). The second test area (TA2) is laid in the middle, the steepest part of the slope. Moistening is atmospheric transit. Forest growth conditions are CL<sub>1</sub> (mesoxerophilic, somewhat dry, or semi-arid). The third test area (TA3) is located on the top of the slope. Moistening is atmospheric-transit. Forest growth conditions are CL<sub>0-1</sub> (xerophilic, arid).

Two-year-old pine needles were selected at an altitude of 2m from the eastern side of trees growing in identical lighting conditions. The pigments were determined after extraction with 100% acetone on SF-2000. Their concentrations were calculated using the Wettstein formulas (Bessonova, 2006b).

Statistical analysis was carried out using Microsoft Office Excel 2007. The data was analysed as mean ± SEM (standard error of the mean). In order to estimate the significant differences between paired data, Student's t-test method was used at the level of significance  $p \leq 0.05$ .

## Results and discussion

The results of our study indicate that the forest-based conditions affect the content of chlorophyll in *P. pallasiana* needles. The lowest concentration of this pigment in the investigated organs is observed in plants of the upper third of the slope (TA3); the highest concentration is observed in mesophilic fresh soil growth conditions (TA1) (Table 1).

Table 1. Influence of forest growth conditions on the content of chlorophylls (*a* + *b*) in pine needles of *Pinus pallasiana*, mg g<sup>-1</sup> wet weight, M ± m, n = 4.

Test area	20 May	Share of reference data (%)	18 July	Share of reference data (%)	5 September	Share of reference data (%)
TA1	1.17 ± 0.03		1.33 ± 0.04		1.37 ± 0.03	
TA2	1.12 ± 0.04*	95.7	1.23 ± 0.02*	92.5	1.18 ± 0.03	86.1
TA3	1.02 ± 0.02	87.2	1.08 ± 0.03	81.2	1.05 ± 0.04	76.6

Note: \* the value differs insignificantly from TA1 ( $p \leq 0.05$ ).

Table 2. Influence of forest plant conditions on the content of chlorophyll *a* and *b* in the pine needles of *Pinus pallasiana*, mg g<sup>-1</sup> wet weight, M ± m, n = 4.

Test area	20 May	Share of reference data (%)	18 July	Share of reference data (%)	5 September	Share of reference data (%)
Chlorophyll <i>a</i>						
TA1	0.82 ± 0.02		0.90 ± 0.03		0.93 ± 0.04	
TA2	0.76 ± 0.02*	92.7	0.78 ± 0.02	86.7	0.76 ± 0.02	81.7
TA3	0.70 ± 0.01	85.4	0.67 ± 0.03	74.4	0.65 ± 0.02	69.9
Chlorophyll <i>b</i>						
TA1	0.35 ± 0.02		0.43 ± 0.04		0.42 ± 0.02	
TA2	0.36 ± 0.01*	102.9	0.44 ± 0.03*	102.3	0.46 ± 0.03*	109.5
TA3	0.32 ± 0.02*	91.4	0.41 ± 0.04*	95.3	0.40 ± 0.02*	95.2

Note: \* the value differs insignificantly from TA1 ( $p \leq 0.05$ ).

The difference between the content of chlorophyll in the needles of plants in TA2 relative to TA1 is minor (insignificant at  $p \leq 0.05$ ) in May and July. However, it becomes reliable in September and makes 13.9%. The difference in this indicator in trees of TA3 compared to TA1 is reliable in all months and makes 12.8% in May, 18.8% in July and 23.4% in September. This is in line with the data of other researchers who found that deterioration of water supply reduces the accumulation of chlorophyll in assimilation organs of plants (Bessonova et al., 1976; Shmat'ko et al., 1989; Kisljuk et al., 1997; Loggini et al., 1999; Hryhoriukh et al., 2004). In their research paper, Korytova et al. (1976) indicate that the pine tree grown in a fresh subor has a higher total chlorophyll content *a* and *b* than that grown in semi-arid wood conditions.

The effect of insufficient water supply to plants on the content of each of the forms of green pigments (*a* and *b*) is uneven. As can be seen from Table 2, the number of chlorophyll *a* in TA2 decreases significantly in July and September by 13.3% and 18.3%, respectively, when compared with TA1 and – even more – by 25.6 and 30.1%, respectively, when compared with TA3.

It was found that the growth conditions do not affect the concentration of chlorophyll *b* in the pine needles (Table 2). Consequently, this form of green pigment shows greater resistance to insufficient soil moisture.

The obtained data are consistent with the results of a number of researchers (Kushnirenko, Medvedeva, 1969), which indicate that under more arid conditions, the reduction in the concentration of green pigments occurs because of chlorophyll *a*, which is a more labile form. The study of the content of plastid pigments in leaves of *Robinia pseudoacacia*, growing in different growth conditions (fresh and arid), also showed that the amount of chlorophyll *b* is almost the same in both variants of the experiment during the vegetation (Bessonova et al., 1976).

Similar results were obtained in experiments with common pine, which grew in a fresh subor and semi-arid wood conditions (Korytova et al., 1976). The fact that chlorophyll *b* is a less mobile component of the pigment system and its content almost does not change during the vegetation was pointed out by Novikov and Vitkovskaja (1959). Stroganov et al. (1970) explained the stability of this form of chlorophyll by a stronger bond of pigment with membranes of chloroplasts.

Chlorophyll *b* is one of the leading factors in the stability of pigment-protein complexes of photosynthetic membranes (Tanakav R., Tanaka A., 2011). As a specific chlorophyll of antenna complexes, chlorophyll *b* increases light gathering in low light, and in bright conditions, it takes part in the dissipation of the excess of absorbed light energy, in the formation of the light energy migration paths within the antenna and from the antenna to the photosystems, and is also a direct component of centres of thermal dissipation (e.g. antenna protein Lhcb 4). The lack of chlorophyll *b* causes changes in the size and composition of the photosynthetic complexes of granal thylakoids, which affects the nature of their stacking in the membrane. This limitation of lateral diffusion of macromolecular protein-pigment complexes complicates the processes of their repair, and the limitation of the diffusion of plastoquinone and plastocyanin can seriously limit the linear transport of electrons (Vojcehovskaja, 2015). According to Bossmann et al. (1999), the lack of chlorophyll *b* destabilises the light-harvesting antenna, first of all, LHC2, because of proteolytic degradation of the Lhcb 1–2 proteins, which leads to a decrease in the intensity of photosynthesis. Consequently, maintaining the content of chlorophyll *b* in *P. pallasiana* needles in conditions of poor water supply (CL<sub>0-1</sub>) at the same level as in the conditions of better water supply (CL<sub>2</sub>) has a significant adaptive value.

Changes in the concentration of green pigments in arid forest growth conditions led to a violation of the ratio of chlorophylls *a* and *b* during the month when the moisture content in the soil in the upper part of the slope was significantly lower than that of talweg. Although the difference in the value of this parameter between the variants of the experiment was not evident in May, it increased and became significant in July and September. As the negative influence of arid conditions of growth affects the content of chlorophyll *a* and almost does not affect the concentration of chlorophyll *b*, then the chlorophyll *a/b* ratio decreases in TA2 and TA3 plants relative to TA1 (Table 3).

The content of carotenoids is higher in the pine needles of *P. pallasiana* in the arid forest growth conditions of the upper (CL<sub>0-1</sub>) and medium (CL<sub>1</sub>) slopes in comparison with

Table 3. Influence of forest growth conditions on Chlorophyll *a/b* Ratio,  $M \pm m$ ,  $n = 4$ .

Test area	20 May	18 July	5 September
TA1	2.34 ± 0.05	2.09 ± 0.10	2.11 ± 0.11
TA2	2.21 ± 0.04*	1.77 ± 0.05	1.65 ± 0.05
TA3	2.18 ± 0.06*	1.63 ± 0.11	1.62 ± 0.07

Note: \* The value differs insignificantly from TA1 ( $p \leq 0.05$ ).

Table 4. Influence of forest growth conditions on carotenoid content in the needles of *Pinus pallasiana*,  $mg\ g^{-1}$  wet weight,  $M \pm m$ ,  $n = 4$ .

Test area	20 May	Share of reference data (%)	18 July	Share of reference data (%)	5 September	Share of reference data (%)
TA1	0.12 ± 0.005		0.19 ± 0.006		0.20 ± 0.010	
TA2	0.14 ± 0.004	116.7	0.24 ± 0.004	126.3	0.25 ± 0.006	125.0
TA3	0.15 ± 0.009	125.0	0.25 ± 0.008	131.6	0.28 ± 0.007	140.0

this indicator in plants of talweg ( $CL_2$ ). In the trees of the upper part of the slope (TA3), in comparison with those in the talweg (TA1), the concentration of yellow pigments increases in May by 25.0%, in July by 31.6% and in September by 40.0%. The conditions in September were the most unfavourable because of the lack of rain during August. In the plants in TA2, the number of carotenoids in the needles also increases compared with this indicator in the area with favourable conditions of water supply (TA1), but to a lesser extent (Table 4). In our study, the growth of the concentration of carotenoids in the tissues of *P. pallasiana* needles under water-deficit conditions can be considered as a protective reaction to the effects of drought. A number of research articles also provide data on the increase in the content of carotenoids in the assimilation bodies of plants under the influence of stress factors of moderate strength, mainly in drought-resistant species (Tuba, 1984; Taran, 1999; Bessonova, Ponomarova, 2017).

Carotenoids play an important role in the adaptive reactions of plant organisms. They expand the spectral range of photosynthetic radiation, take part in energy migration, neutralise active forms of oxygen, protect chlorophyll from photodegradation, regulate the heat exchange of the lamina and stabilise photosynthetic membranes (Adams et al., 1990; Demmig-Adams, 1990; Demmig-Adams, Adams, 2000; Hryhoriukh et al., 2004; Ladygin, Shirshikova, 2006; Shadchyna et al., 2006; Ralituho et al., 2007).

## Conclusion

Thus, chlorophyll *a + b* content in the needles of *P. pallasiana* under conditions of insufficient water supply (xerophilous,  $CL_{1-0}$  and mesoxerophilous,  $CL_1$ ) is lower than that in the trees under fresh growth conditions (mesophilous,  $CL_2$ ). The reduction in the concentration of green pigments in TA2 and TA3 relative to the TA1 indices is due to chlorophyll *a*; chlorophyll *b* showed high resistance to poor water supply. This leads to a decrease in the

chlorophyll *a/b* ratio in the needles of plants in more arid areas compared with this indicator in trees of mesophilic growth conditions.

The quantity of carotenoids in needles under insufficient water supply is higher than that in the trees under fresh forest growth conditions, which can be explained by the adaptive role of these pigments in the processes of stabilising the functioning of the photosynthetic apparatus under adverse growth conditions and considered as a protective reaction of *P. pallasiana* on the effect of drought.

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