The Effect of Light Stressor on Metabolites in the Needles of *Picea abies* (L.) Karst.

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In the paper the reactions of stress and adaptation as a response of *Picea abies* (L.) Karst. to the changes in light intensity (as a stressor) are characterized. The response and duration of the reactions have been determined by amount variations in metabolites - photosynthetizing pigments (chlorophyll a, b, a+b) and aminoacid proline. Possible interaction between the aminoacid proline and chlorophyll has been assessed in the needles of *Picea abies* (L.) Karst. under stress.

Key words: stress, adaptation, light, needles, plantations, chlorophyll, aminoacid proline, metabolism.

Introduction

Picea abies (L.) Karst. is known to be rather tolerant to lack of light. This biological property favours maximal utilization of light by spruce and makes it as a competitor to other productive tree species. However, sudden changes in light may occur in stand-while thinning stands or due to selfthinning caused by environmental factors, so Norway spruce is submitted to stress. Consequently, changes occur at the level of physiological functions. It was established that during stress when young stands are thinned substance transport via cellular membranes in the needles increases up to 280 % (Skuodiene 1987). It depends upon the initial density of plantation and the extent of its thinning. Norway spruce is submitted to stress during mutual competition caused by light and crown closure. It has been determined that the potential of substance transport is affected more in the first years of coenosis forming. As shown by the results of the earlier investigations, in 2-3-year-old experimental plantations of different initial density K^+ ion transport via cellular membranes of needles augmented even 320-435%, as compared with free growing trees. An increase in energy consumption during stress weakens substance assimilation, complementary state of metabolites within a cell and homeostasis of the system in post-stress period and during adaptation, which in all cases is many times longer, as compared to the duration of stress (Skuodienė 1999).

The relationship exists among light, the quantity of pigments of photosynthesis and the process of biomass accumulation in the crown. In the preliminary results of experiments it has been shown that in the phases of stress and initial adaptation the quantity of these metabolites taking part in this process increases (Skuodienė 2000).

The data in the literature (Илкун 1978; Bates et al. 1973; Судачкова et al 1996; Удовенко 1976, 1979) have demonstrated that augmentation of the quantity of metabolites is associated with the stress reaction, which maximally reveals itself in the points of strained metabolism. The processes occurring in generative and vegetative organs in a certain period may be attributed to the conditions provoking stress. The investigations conducted on the analysis of aminoacid proline in conifers have shown that during stress provoked by chemical pollution the aminoacid proline concentration in needles of Norway spruce is elevated four to five times, as compared to the state of conditional adaptation in the conditions of background pollution (Skuodiene 1997).

The mechanisms regulating adaptation and preservation of plants are rather important and sophisticated. There are few data which characterize the post-stress period by quantitative variations in metabolites of trees. A certain role in the process of adaptation is assigned to proline as an aminioacid fulfilling the protective function. In the experiments it has been established (Проценко et al. 1968) that due to the activity of the synthesis of proline toxic ammonium is bonded in the green grass cell. It forms as soon as an organism is drought stressed and thus, protects the protoplasm of a cell from hydration. It may be one of the protective functions of proline when an organism is submitted to stress of drought (Бритиков 1975). Britikov also points out that large quantities of proline in the organism are not harmful as in contrast to other aminoacids. However, for the green vegetation the most important property of this aminoacid is its close relationship to chlorophyll in the chain of metabolism. Aminoacid proline is introduced in the synthesis of chlorophyl through the Krebs cycle during the synthesis and reduction of glutamic and keto-glutamic acids (Бритиков 1975). Therefore, the significance of aminoacid proline during stress is great. Due to external factors the changes occur in the chlorophyll synthesis and in quantitative parameters of this metabolites. Adaptation of plants through the changes in metabolites is a continuating phase of post-stress reaction. Theoretically, it seems that during adaptation aminoacid proline takes part in the total metabolism and favours the restoring of the synthesis of chlorophyll.

Therefore, the objective of this study is to characterize the quantitative variations in aminoacid proline and chlorophyll during stress and adaptation, as a response to light stressor in Norway spruce needles. Also the aim is to determine the response and duration of the reactions by quantitative variations in chlorophyll ***a, b*** and aminoacid proline as well as to assess possible interaction between this metabolites in the needles of Norway spruce submitted to stress and during its adaptation.

Material and methods

A scheme of the experiments. For the experiment spruce plantations were chosen, where the initial density was 25.0 thousand trees/ha, 12.5 thousand trees/ha and 6.2 thousand trees/ha. The age of the plantations was 17 years, the site - *Piceetum Hepatico Oxalidosum*. Mean diameter of experimental trees in the plantations with the initial density 6.2 thousand trees/ha was 9.1 cm; 12.5 thousand - 6,6 cm; 25.0 - 5,2 cm, mean heights were 10.6 m , 8.7 m and 7.3 m , respectively. The plantations were divided into two parts. One of them was left for control experiments and in the second part lighting was increased by thinning. After thinning experimental plantations where the density is 25.0 thousand trees/ha in addition give 106 $\mu mol\ s^{-1}\ m^{-2}$ PAR near the 5th whorls from the top of experimental trees. At the density of 12.5 thousand trees/ha in addition trees give 206 $\mu mol\ s^{-1}\ m^{-2}$ PAR and in dense 6.2 thousand trees/ha - give 135 $\mu mol\ s^{-1}\ m^{-2}$ PAR. A total of 5 trees were selected in each part of the plantations. For the analysis of chlorophyll the samples from the 3rd and 5th whorls were taken. The chlorophyll and aminoacid proline were determinated in 500 mg fresh/green mass in 3 replications. The samples were taken after 5 hours, 1, 2, 3, 4, 7, 11, 15, 21 days and 1, 2 years from experimental (after thinning) and control plots.

The method of aminoacid proline analysis. Aminoacid proline has been exstracted and identified according to the scheme of the experiments by the Bates method (Bates at all. 1973). The analyses were conducted by computer according to the SLT biological programme. A comparison of the obtained results with the standard curve of proline was conditioned by high correlation coefficient, K=0,999858. The method of investigation enables very slight quantities of proline to be identified in the material investigated.

The method of analysing chlorophyll. Chlorophyll ***a, b*** were extracted by 96,58% ethanol from the mass of needles fixed and homogenised by liquid nitrogen. Chlorophyll ***a*** was identified at 470 nm (nonometre) and chlorophyll ***b*** at 649 nm. The quantities of pigments in the 500 g green mass (g.m.) have been calculated by the Lichtenhaler formula and adapted for the SLT computer programme by Volker Beer (Lichtenthaler 1987).

Measurement of light. Photosinthetically active radiation (PAR) was measured by the LI-COR Quantum Radiometr/ Photometr (model LI-189). Measurement units - $\mu mol\ s^{-1}\ m^{-2}$.

Results and discussion

The data shows that during the experiment the amount of chlorophyll in the needles of trees in thinned plantations noticeably varied. Five hours after thinning in the initial density 25.0 thousand trees/ha chlorophyll a+b was elevated by 130.3% in the needles of the 3rd whorl and in the 5th whorl by 146.2%, in comparison to the control (Fig. 1). After 24 hours in the 3rd whorl the amount of chlorophyll a+b was 119% and in the 5th whorl - 132.8% larger as compared to the control.

The needles of the experimental trees growing in the plantations with the initial density 12.5 thousand trees per ha responded weaker. At this density the effect of thinning on the amount of chlorophyll was less during the first 5 hours too. Five hours after plantation thinning chlorophyll *a+b* in the needles of the 5th and 3rd whorls was only 121% more, as compared to the control. Twenty four hours after thinning in the needles of the 3rd whorl it was 137.9% larger than in the control. In the needles of the 5th whorl chlorophyll was found to be only 116.8% more than the control.

Such an increase in chlorophyll in the needles 5 and 24 hours after thinning in plantations with the initial density 25.0 and 12.5 thousand trees/ha may be interpreted as a stress reaction and a response to a sudden change in light intensity as stressor. A stress reaction was especially pronounced in the plantations where the initial density was high.

In the plantations with the lowest initial density of 6.2 thousand trees/ha nearly no response of trees to the intensity of light was observed, because there was no essential increase in light. In the experimental parts of crowns the differences between chlorophyll constituted only 5.4 % to 10.6%.

At the end of the experiment the situation has obviously changed. After 21 days following an increase in the intensity of light in the densest plantation (25.0 th. trees/ha) chlorophyll $a+b$ in the needles of the 3rd and 5th whorls comprised only 2.8%, in comparison to the control. In the plantations where the density (12.5 th. trees/ha) was lower chlorophyll on average was found to be about 18% less than in the control independing of the place of needles in the crown. In the needles of the plantations with the lowest density (6.2 th. trees/ha) and with increased light intensity, the quantity of chlorophyll was equal to the control (100%).

In accordance with the previous scheme of the experiments a repeated analysis has been conducted in one (1998) and two (1999) years after thinning. In all cases the quantity of chlorophyll in the needles of trees from the thinned plantations was close to the control (99.7%-100%).

Analysing the character of responses of experimental trees to light stressor by amount variations in chlorophyll $a+b$ (Figure 1), and a,b (Figure 2) the reaction of stress may be named in the interval of the first 1-4 days. Moreover, this situation repeated itself in the 3rd and 5th whorls. In these whorls of trees submitted to stress the quantity of chlorophyll ***a*** on average increased up to 137% - 143% and 140% - 160%, respectively (Figure 2), as compared to the control. At the same time the quantity of chlorophyll ***b*** increased more intensively from 270% to 325%. The interval of post-stress period 5-21 days apparently was associated with the decreasing amount of chlorophyll in the needles of experimental trees. In this case the response to light stressor is taking adaptation character and more longer than stress reaction.

In order to assess the plant state in terms of adaptation some authors (Beadle, Jarvis 1977, Young, Britton 1990, Gnojek 1992) use constant ratio of chlorophyll ***a*** to ***b*** as the coefficient of complete adaptation to new conditions. In accordance with this index needle adaptation at the level of the amount of chlorophyll ends not in all plantations of above densities on the 21st day. As established by the results of the investigations (Skuodienė 2001), a certain exception in transiting to adaptation is noted after 1 (1998) and two (1999) years in the needles of trees growing in the plantations with the initial density 12.5 th. trees/ha and with increased light intensity. In accordance with the ratio of chlorophyl ***a*** to ***b*** the needles of trees in the plantations of this density and with increased intensity of light had the best characteristic of adaptation after two years:

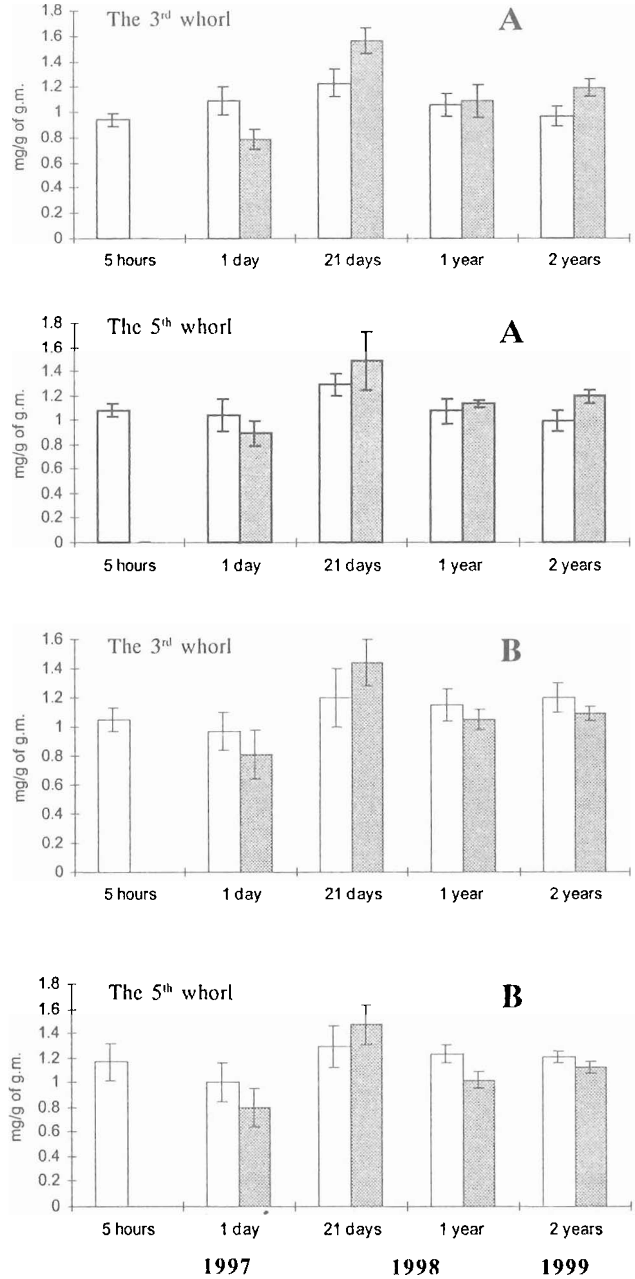

Figure 1. The quantity of chlorophyll ***a+b*** (mg/g of green mass) in the needles of the control (□) and thinned spruce plantations (▒) in 5 hours, 1, 21 days and 1 year (1998), 2 years (1999) after plantation thinning: in density variants 12.5 thousand trees/ha (A); 25.0 thousand tree/ha (B).

Experimental data on the amount of aminoacid proline in the needles after thinning, as well as their comparison with chlorophyll dynamics at the same time have demonstrated that, in contrast to chlorophyll, the

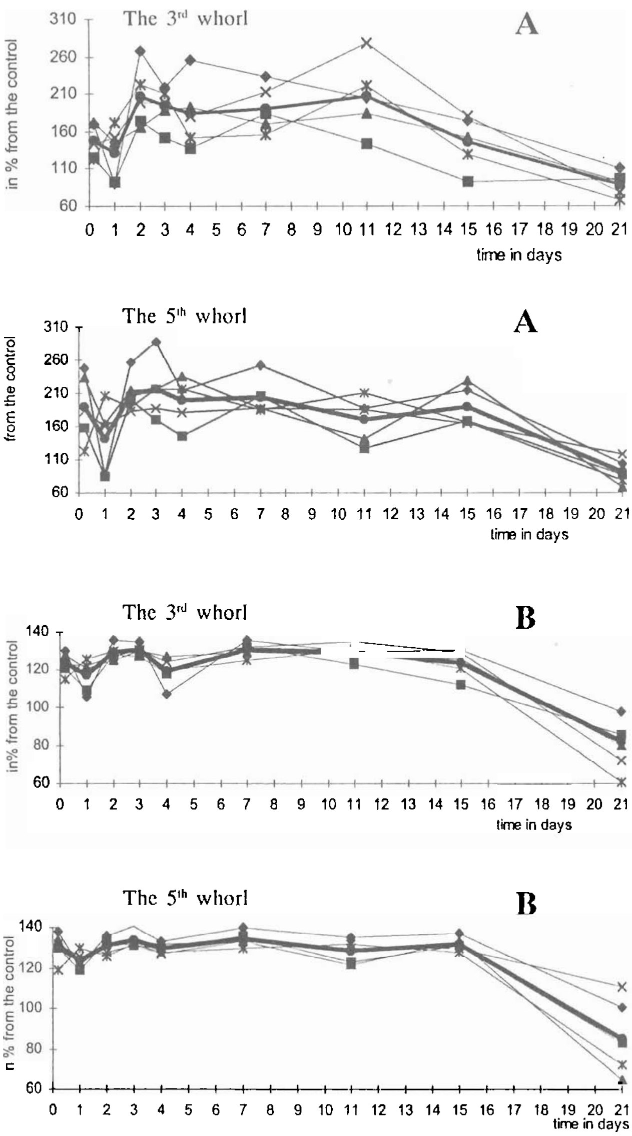

Figure 2. The dynamics of the quantity of chlorophyll *b* (A) and chlorophyll *a* (B) in % from the control, in the needles of thinned spruce plantations of the density 25.0 thousand trees/ha. (Bold line corresponds to mean concentration)

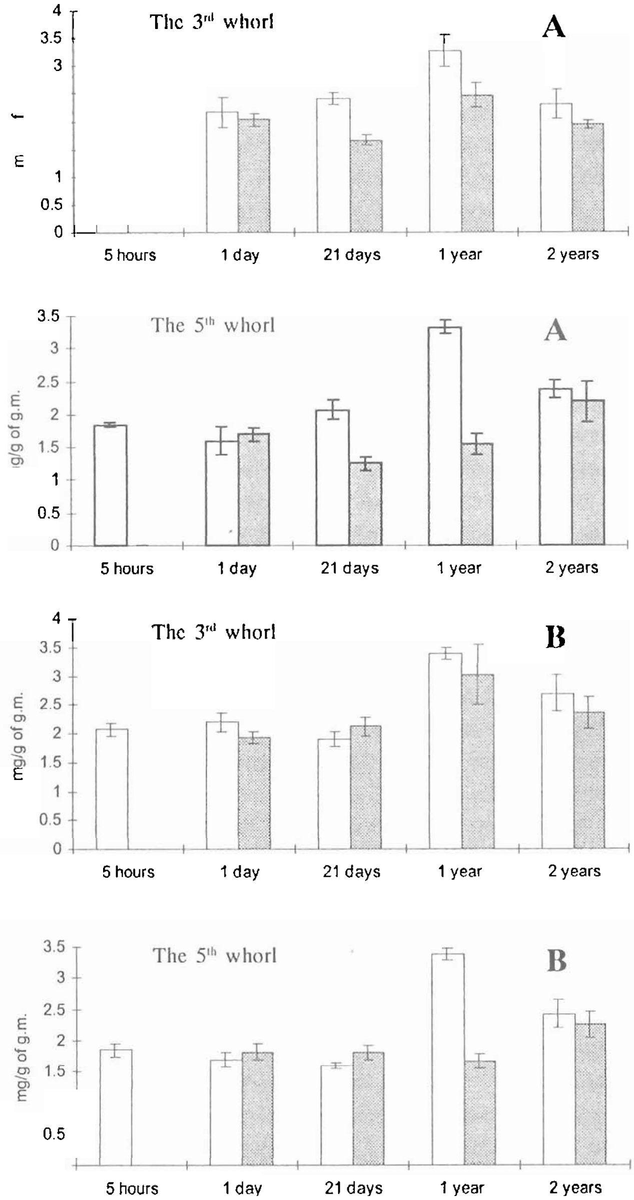

Figure 3. The quantity of aminoacid proline (mg/g of green mass) in the needles of the control (▨) and thinned spruce plantations ( ) in 5 hours, 1-21 days and 1 year (1998), 2 years (1999) after plantation thinning: density 12.5 thousand trees/ha (A); 25thousand trees/ha (B).

accumulation of the aminoacid proline content was not strong during the first days. The quantity of proline in the needles of trees growing in the plantations with increased light intensity was compared with the control. It was found that the first days in all density variants proline comprised only 2.7 to 13.5% more or even up to 13.1% less than the control (Figure 3). The results obtained in the plantations of different density showed that a response began first in the plantations of average initial density (12.5 th. trees/ha). Only on the 21^{rst} day after an increase in light intensity in needles of the 3rd and 5th whorls proline was found to be 147.0% and 156.8%, respectively, more than the control. The quantity of proline in the needles of trees growing in the thickest (25.0 th. trees/ha) and thinned dense plantations at that time was equal or lower than the control (109.6% -115.9%). In these plantations only after one year aminoacid proline was found to be from 145.1% to 203.6% up to 151% more, as compared to the control.

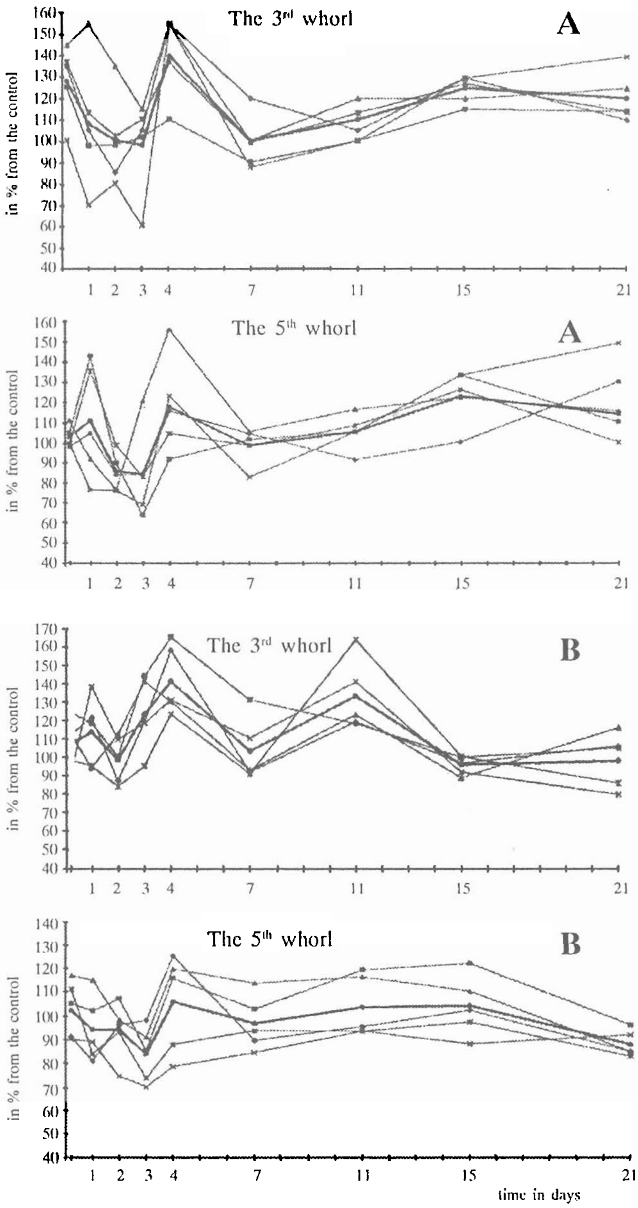

Figure 4. The dynamics of the quantity of aminoacid proline in % from the control in the needles of thinned spruce plantations in density 12.5 thousand trees/ha (A); 25.0 thousand trees/ha (B). (Bold line corresponds to mean concentration)

In general results by comparing quantity dynamics of aminoacid proline with chlorophyll in a period of 21 days it is obvious that the maximum of the activity accumulation of aminoacid proline coincides with the minimum of the activity of chlorophyl accumulation. However, according to the character of the dynamics of the quantity of chlorophyll and aminoacid proline as a response to light stressor started and had the character of stress during the first hours and days (1-4) after thinning. In the period of adaptation a quantitative expression of the two metabolites becomes essentially different. In assessing the state of the experimental plantations according to the dynamics of a variation in both metabolites it is possible to make an assumption that there is close relationship between the quantity of chlorophyll and aminoacid proline both under stress and in the period of adaptation. Aminoacid proline takes part most actively in metabolic variations occurring at the onset of a response. As quantitative variations in this metabolite indicate it also takes part in adaptation.

While investigating a response of Norway spruce to the changes in light intensity as a light stressor at the level of the physiological functions by the analysis of chlorophyll and aminoacid proline quantity it has been determined that the obtained data support the theoretical assumption (Britikov,1975), that proline as a metabolite plays a protective role in the post stress period. The data of our investigations have shown that aminoacid proline plays a radical role in restoring the synthesis of chlorophyl in a cell in the post-stress period. The data of the analysis of chlorophyll have demonstrated that the variations occurring in lighting conditions affect not only the chain of receptors but also that of adaptation at the level of the quaranty of chlorophyll in the needles of Norway spruce. In case there are favourable factors they can influence the photosynthesis process and crown productivity.

Conclusions

It has been determined that due to thinning in spruce plantations of different density the changes occurring in light intensity affect receptor-adaptation chain of chlorophyll and may influence the process of photosynthesis and crown productivity. An assumption is made that in this process the role of aminoacid proline is associated with a change in chlorophyll metabolism, radically with restoring-adaptation function, which occurs during light stress.

It has been ascertained that during the reaction of stress the amount of chlorophyll in needles is very considerable from 137 % to 325%, as compared to the control .The reaction of stress occurs during the first hours 5 hours and 1-4 days after plantation thinning. Depending upon the initial density of plantations the process of adaptation with the weakening of chlorophyll amount of accumulation lose from 21 day till 2 years. It has been determined that aminoacid proline joins metabolic changes at the onset of a response to stress reaction. However, its greatest activity has been observed during adaptation.

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ЭФФЕКТ СВЕТА КАК СТРЕССОРА НА МЕТАБОЛИТЫ В ХВОЕ *PICEA ABIES* (L.) KARST.

Л. Скуодене

Резюме

В статье рассматривается реакция ели обыкновенной на изменение световых условий как стрессора в насаждениях после их прореживания. Стресс-адаптационный период характеризуетсяс по изменению количества хлорофилла *а*, *б* *а+б* и аминокислоты пролина. На уровне физиологических функций дается оценка возможного взаимодействия метаболитов во время стресса и адаптации.

Ключевые слова: стресс, адаптация, свет, хвоя, насаждения, хлорофилл, аминокислота пролин, метаболизм.