Quantitative changes in aminoacid proline and chlorophyll in the needles of *Picea abies* Karst. (L.) during stress and adaptation

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In the paper the reactions of stress and adaptation as a response of *Picea abies* Karst. (L.) to the changes in lighting intensity at the level of the physiological functions are characterized. The onset and duration of the reactions have been determined by quantitative variations in photosynthetizing pigments (chlorophyll a, b, a+b) and aminoacid proline. A possible interaction between the two metabolites has been assessed in the needles under stress and in the period of adaptation.

Key words: stress, adaptation, chlorophyll, aminoacid proline, metabolism

INTRODUCTION

Due to formation of stands, natural thinning or thinning induced by elemental factors the intensity of lighting constantly changes. During stress to which Norway spruce is subjected at the initial stage of coenosis formation and as a young stand, due to changes in the intensity of lighting K^+ion transport via cellular membranes increases by 280–435% [1, 2]. During adaptation of the individuals to a new intensity of lighting energetic disbalance of K^+ions disappears. The chain of metabolism is sensitive to changes in the intensity of lighting and regulates the productivity, because there is a direct dependence between light, the quantity of photosynthetizing pigments, photosynthesis and the process of biomass accumulation in the crown. With the aid of the experiments it has been determined [3] that in the phases of stress and initial adaptation the quantity of metabolites taking part in this process increases.

While investigating grass vegetation it has been ascertained that under stress variations occur in the homeostatics of the protoplasm, osmotic potential and electrotransport system in cellular membranes. Also, ATF synthesis and reduction vary. When the organism is subjected to a stress, energy consumption augments, while the energetic pool of a live cell decreases. Such a process of metabolism in a cell of an organism exposed to a stress diminishes the extent of complement and weakens the homeostasis of the system.

The mechanism regulating the adaptation and preservation of plants is rather important and sophisticated. In the experiments it has been ascertained that owing to the activity of the synthesis of proline toxic ammonium is bound in the cell, which forms as soon as an organism gets in the conditions of drought and thus, protects the protoplasm of a cell from hydration. However, in green vegetation the most important property of this aminoacid is its close relationship with chlorophyll in the chain of metabolism. Aminoacid proline is introduced in the synthesis of chlorophyll through the Krebs cycle during the synthesis and reduction of glutamic and keto-glutamic acids [4]. Therefore, the significance of aminoacid proline during stress is understandable, when external factors cause changes in the functions of the synthesis and breathing and in quantitative parameters of metabolites of these functions. An assumption is possible that during adaptation aminoacid proline taking part in the total metabolism favours the restoration of chlorophyll synthesis.

The available data on the activity of proline synthesis under stress and the findings that might characterize quantitative variations in proline during adaptation are not adequate. Therefore, the objective of this work was to characterize the reactions of stress and adaptation as a response elicited by Norway spruce to changes in the intensity of lighting at the level of physiological functions. Also it is necessary to determine the onset and duration of the reactions by quantitative variations in chlorophyll a, b of photosynthesizing pigments and aminoacid proline as well as to assess a possible interaction between the two metabolites in the needles of Norway spruce exposed to stress and during adaptation.

MATERIAL AND METHODS

A scheme of the experiments was described in [3]. *The method of proline analysis.* Aminoacid proline was identified according to the Bates method [5].

The method of analysing chlorophyll a, b. The quantities of pigments in the green needle mass (g.n.m.) have been calculated by the Lichtenthaler formula adapted for the SLT computer programme by Volker Beer [6].

RESULTS AND DISCUSSION

Quantity dynamics of chlorophyll. The data showed that during the experiment the quantity of chlorophyll in the needles of trees noticeably varied, which grew in the part of thinned (25.0 thous. trees/ha) plantations. It must be noted that such a response to the higher intensity of lighting was already distinct during the first hours after thinning. After 5 hours from plantation thinning in the needles of the 3rd verticil the content of chlorophyll a+b was found to be by 130.3% and in the 5th verticil by 146.2% more than in the control. Such a significant increase in chlorophyll in the needles over 24 hours after plantation thinning may be assessed as a stress reaction at the level of chlorophyll synthesis and reduction and a response to a sudden change in the intensity of lighting as a stressor. The stress reaction was most distinct in the plantations where the initial density was highest. The needles of the experimental trees growing in the plantations with the initial density 12.5 thousand trees per ha reacted weaker, because the effect of an increase in the intensity of lighting was less. After 5 hours from plantation thinning chlorophyll a+b in the needles of the 5th and 3rd verticels was only by 121% larger as compared to the control. In the plantations with the initial density 6.2 thous. trees/ha nearly no response of trees to a higher intensity of lighting was observed. In all experimental parts of crowns, the differences between chlorophyll constituted only 5.4 to 10.6%.

As is shown by the analysis of chlorophyll a+b, at the end of the experiment the situation has obviously changed. In comparison to the control, 21 days following an increase in the intensity of lighting in the thickest plantation (25.0 thous. trees/ha) chlorophyll a+b content in the needles of the 3rd and 5th verticils was only by 2.8% less. In the plantations where the density (12.5 th. trees/ha) was less, chlorophyll content on average was found to be by 18% less than in the control, independent of the place of needles in the crown. In the needles of the plantations with the least density (6.2 thous. trees/ha) and with a higher intensity of lighting the content of chlorophyll was equal to that of control (100%). In accordance with the previous scheme of experiments, a repeated analysis of the chlorophyll content was conducted one (1998) and two (1999) years following an increase in the intensity of lighting. In all cases the content of chlorophyll in the needles of trees growing in the conditions of a higher intensity of lighting was close to that of control (99.7–100%).

While analysing the character of responses of different experimental trees to an increased intensity of lighting by the content of chlorophyll a and b in the needles, the response to stress is exceptionally well visible in the interval of 1–4 days. Additionally, this reaction repeated in the 3rd and 5th verticils. In these verticils of trees exposed to stress the content of chlorophyll a on average increased from 137% to 143% and from 140% to 160%, respectively, as compared to the control. At the same time the content of chlorophyll b increased more intensively (by 270% to 325%).

The post-stress period which lasted 3–5 to 21 days was associated with a weakening of chlorophyll synthesis. It was noted between days 15 and 21. In the response to stress and adaptation, chlorophyll b quantitatively exceeded chlorophyll a. In assessing the state-of-art of plants in terms of adaptation, the ratio of chlorophyll a to b is used as the coefficient of complete adaptation to new conditions.

In accordance with this index, needle adaptation at the level of the synthesis of chlorophyll ends not in all plantations of above densities on day 21 following an increase in the intensity of lighting. As shown by the results of the investigations, a certain exception in transition to adaptation is visible after one (1998) and two (1999) years in the needles of trees growing in plantations with the initial density 12.5 thous. trees/ha and with an increased intensity of lighting. In accordance with the ratio of chlorophyll a to b, needles of trees in plantations of this density and with an increased intensity of lighting had the best characteristics of adaptation after two years. After 21 days, one and two years following an increase in the intensity of lighting the coefficient of the ratio of chlorophyll a to b was close to the characteristic of adaptation during the whole experiment in the plantation with the least initial density (6.2 thous. trees/ha), where the effect of increased intensity of lighting was slightest.

Quantity dynamics of aminoacid proline. Experimental data on the quantity of proline in the needles after 5 hours, one and 21 days, one (1998) and two (1999) years from a thinning-induced increase in lighting intensity, as well as their comparison with chlorophyll dynamics at the same time have demonstrated that, in contrast to chlorophyll, the synthesis of proline was not strong during the first hours and the day when the lighting intensity in the plantations was increased. It had been found that the first day in all density variants the proline content was only by 2.7 to 13.5% higher or even by 13.1% less than in control.

In assessing the results obtained in the plantations of different density it was determined that the response reaction began first in the plantations of the average initial density (12.5 th. trees/ha). On day 21 after an increase in lighting intensity, in needles of the 3rd and 5th verticils proline content was found to be by 147.0% and 156.8%, respectively, higher than in control. The quantity of proline in the needles of trees growing in the thickest and thinned density plantations at that time was equal to or lower than the control and only after one year proline content was found to be by 145.1% to 203.6%, up to 151% higher as compared to the control.

A comparison of the quantity of the aminoacid proline with that of chlorophyll in a period of 21 days showed that the peak of aminoacid synthesis coincides with the minimum activity of chlorophyll synthesis. At the level of the functions of chlorophyll and proline, the response reaction started in the irritation phase and had the character of stress during the first hours and days after an increase in lighting intensity. However, in the period of adaptation a quantitative expression of the two metabolites became essentially different. In assessing the state-of-art of the experimental plantations according to the dynamics of a variation in both metabolites it is possible to make an assumption that there is a close relationship between the quantity of chlorophyll and aminoacid both under stress and in the period of adaptation. Proline takes part in metabolic variations occurring at the onset of a response. As the quantitative variations in this metabolite indicate, it also takes part in adaptation. While investigating a response of Norway spruce to changes in lighting intensity at the level of the functions of chlorophyll and proline, it has been determined that the obtained data support a theoretical assumption that proline as a metabolite plays a regulating (protective) role in restoring the synthesis of chlorophyll in a cell in the post-stress period. The data of chlorophyll analysis have shown that the variations occurring in lighting conditions affect not only the chain of receptors, but also that of adaptation at the level of chlorophyll synthesis in the needles of Norway spruce, and in case other factors are favourable they can influence photosynthesis process and crown productivity.

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KIEKYBINIAI AMINORŪGŠTIES PROLINO IR CHLOROFILO POKYČIAI PICEA ABIES KARST. (L.) SPYGLIUOSE, VEIKIANT STRESUI IR ADAPTACIJAI

S a n t r a u k a

Straipsnyje apibūdinama *Picea abies* (L.) Karst. reakcija į šviesos intensyvumo pokyčius įvairaus tankio želdiniuose. Chlorofilo (a, b, a+b) ir aminorūgšties prolino kiekybiniais pokyčiais spygliuose nustatyta streso ir adaptacijos reakcijų pradžia ir trukmė. Fiziologinių funkcijų lygmenyje įvertinta šių dviejų metabolitų tarpusavio galima sąveika ląstelėje, streso ir adaptacijos metu *Picea abies* (L.) Karst.