environmental sciences proceedings

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Proceedings

Seasonal acclimation of PSII thermostability via pigments ratio adjustment of Norway spruce (*Picea abies*) †

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† Presented at the 2nd International Electronic Conference on Forests, 2021.

Abstract: Mountainous Norway spruce populations are facing increasing biotic and abiotic pressure at the lower range of their natural distribution due to global climate change. In this study we analysed if Norway spruce is able to acclimate to higher temperatures during the summer and improve the thermostability of its photosynthetic apparatus. We utilized short-term heat stress simulation with water baths followed by fast and slow kinetics of chlorophyll *a* fluorescence. We found that Norway spruce is able to improve its PSII thermostability during summer with maximal performance after short-term heat stress occurring in July and August. This acclimation response was positively correlated with chlorophylls to carotenoids ratio which also significantly differed between the observed months. Moreover, there were no significant changes of assimilation rate between the observed five months. Our results suggest that healthy trees of Norway spruce at lower range of distribution can acclimate to higher temperature during summer.

Keywords: quantum yield, k-step, heat stress, chlorophyll, carotenoids, Carpathians

Citation: Petrík, P; Fleischer, P; Petek, A; Konôpková, A; Kurjak, D. Seasonal acclimation of PSII thermostability via pigments ratio adjustment of Norway spruce (*Picea abies*). *Proceedings* **2021**, *68*, x. https://doi.org/10.3390/xxxxx

Published: date

1. Introduction

We can expect that precipitation and temperature extremes will be more frequent and more severe due to global climate change [1,2]. The health status of spruce stands has already considerably worsened in the Central European region due to drought and heat stress [2,3]. Photosynthesis has been recognized as one of the most thermally sensitive metabolic processes and can be used as a benchmark of trees performance under heat stress [4]. Non-invasive chlorophyll *a* fluorescence and gas-exchange techniques can thus provide new insights into the fundamental process of photosynthesis for forestry purposes [5,6]. Trees might acclimate their photosynthetic performance via pigments adjustment under heat stress [7,8]. Our main hypotheses were that under high temperatures we will observe significant seasonal changes of (I) chlorophylls to carotenoids ratio, (II) PSII thermostability and (III) assimilation rate. Furthermore, we expected to find (IV) significant correlations between pigments to carotenoids ratio and photosynthetic traits which would reflect co-dependence of photosynthetic heat acclimation with pigments content adjustment.

2. Materials and methods

2.1. Site and sampling description

Site is located within Tatra National Park at 1100 m a.s.l. with Southern exposition. The altitude and hydric conditions of the site represent optimal conditions for Norway spruce within the Carpathian Mountain range. Measurements were conducted on 9 individuals of Norway spruce (*Picea abies*) which were around 20 years old with average height of 4m and average diameter at breast height of 11cm. Experiment was conducted in the summer of 2018 where the gas-exchange and fluorescence measurements accompanied with sample collection for pigment concentration analysis were conducted once a month from May till September. All measurements were conducted on branches fully exposed to the sun, at 1m height.

2.2. Gas-exchange measurements

The open gasometric system Li-6400XT equipped with the coniferous leaf chamber fitted with 6400–02B LED light source (LI-COR Biosciences) was used for recording the CO_2 assimilation rate (A, mmol m^{-2} s^{-1}). The conditions in the chamber were stable with CO_2 concentration of 400 mmol mol^{-1}, the saturating PAR of 1000 mmol m^{-2} s^{-1}, the average block temperature of either 30°C or 46 °C and the average RH of 60%. The adaptation inside of the chamber lasted from 1 to 3 min depending on the speed of stabilisation. The leaf area of branches was measured at the end of the experiment in ImageJ software (National Institute of Health, USA) for normalization of A values.

2.3. Pigment content analysis

A homogenized sample mixture (1 g) of spruce needles from each of the 10 individual trees per studied site were analysed as 80% acetone extracts. The chlorophyll contents (Chl a, Chl b, and Chl a+b) and total carotenoids (Car x+c) were determined by spectrophotometry (Cintra, GBS Australia) at 470, 646, and 663 nm and were calculated according to Lichtenthaler [9]. The pigment contents were related to the dry mass unit (mg.g^{-1}).

2.4. Chlorophyll a fluorescence measurements

The needles were dark-adapted for 30min using the leaf clips, and then chlorophyll *a* fluorescence was excited by a saturation pulse with an intensity of 3,500μmolm^{-2}.s^{-1} for 1 s. A plant efficiency analyser, namely, the Handy PEA (Hansatech Ltd., UK), was used for the OJIP transient measurements, which were analysed based on the JIP test (Biolyzer 5 software, Laboratory of Bioenergetics, University of Geneva, Switzerland). We determined the basic fluorescence parameters: The basal fluorescence (F_0), measured 50 μs after illumination and the maximal quantum yield of PSII F_v/F_m, calculated as the ratio between the variable fluorescence ($F_v = F_m - F_0$) and the maximal fluorescence (F_m). Moreover, the photosynthetic performance index on an absorption basis (PI), the density of active reaction centres (RC/ABS) and K-step (Wk) [10].

2.5. Rapid Light Curves

RLCs were recorded using a fluorimeter Pam-2500 (Waltz, Germany). The measurements consisted of nine levels of actinic illumination with increasing intensities from 5 to 2,018 μmol m^{-2}.s^{-1} and a duration of 30 s. The illumination periods were separated by a 1-s saturating flash with an intensity of 14,038 μmol m^{-2}.s^{-1}. RLCs for the electron transport rate (*ETR*), non-photochemical quenching (*NPQ*), and the effective quantum yield ($\phi PSII$) were measured. All curves were quantified as the sum of the individual points of the curve.

2.6. PSII sensitivity to heat stress simulation

The measurements were conducted at control temperature (30 °C) and stressing temperature (46 °C). The temperature of 46 °C was chosen as a threshold temperature in which the changes of PSII photochemistry certainly occur [11,12] and it is still lower than 50 °C, which can affect the results because of depigmentation and overall disorganization of PSII [13]. Heat stress was simulated using a WNE22 water bath (Memmert, Germany). The shoot was enclosed in a glass Erlenmeyer flask and exposed to a temperature of 46 °C for 30 min by immersing the flask in a water bath. All measurements were repeated twice per individual and the values were averaged.

2.7. Statistical analysis

The normal distribution and homogeneity of variance between dates were firstly tested by Shapiro-Wil test and Barlett's test. The significance of differences between the dates were tested by analysis of variance. Principal component analysis was used for all traits to visualize temporal dynamics and to find covariance between the traits. All statistical analyses were conducted in R 3.6.3 software (R Core Team, Austria).

3. Results and discussion

The analysis of variance revealed that chlorophyll content (a+b), chlorophylls to carotenoids ratio (ab/xc), quantum yield (F_v/F_m), performance index (PI_{abs}) and K-step (W_k) differed significantly during the vegetation season (Tab 1). We found no significant differences of assimilation rate (A) during the vegetation season (Tab 1).

Table 1. Results of One-Way ANOVA for all tested traits with Month as a fixed factor

Trait	df	SumSQ	MeanSQ	F	p
A	4	0.40	0.13	0.02	0.99
a+b	4	0.84	0.21	6.02	***
ab/xc	4	4.06	1.02	7.68	***
F_v/F_m	4	0.19	0.05	13.94	***
PI_{abs}	4	11767	2942	2.97	*
W_k	4	0.19	0.05	3.26	*

The ab/xc ratio was the lowest in May and peaked during August. Acclimation of thermostability of PSII reflected in higher values of F_v/F_m and PI at 46 °C occurred as we can see significantly higher values of these two traits under short-term heat stress during June, July and August in comparison to May (Figure 1). The A values obtained at 46 °C show no significant changes during the vegetation season which might suggest that Norway spruce was able to maintain high photosynthetic performance. Low values of the ab/xc ratio manifest a weakened photo-protective function of the photosynthetic apparatus [14].

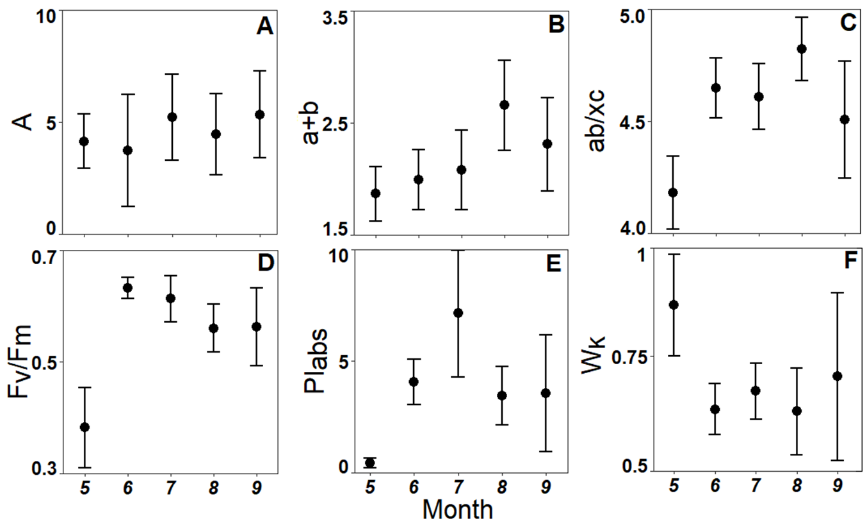

Figure 1. Average values with 95% confidence intervals for assimilation rate (A), chlorophyll content (B), chlorophylls to carotenoids ratio (C), quantum yield (D), performance index (E) and K-step (F).

We found positive significant correlation between Fv/Fm and ab/xc which corresponds to higher PSII performance at 46°C during July and August. Furthermore, Wk negatively correlates with ab/xc ratio which also reflects acclimation of PSII performance under heat stress by adjustment of ab/xc ratio within vegetation season. Acclimation of

PSII thermostability via pigments adjustment with emphasis on xanthophyll-cycle were observed for *Solanum tuberosum* and *Pinus sylvestris* [7,8].

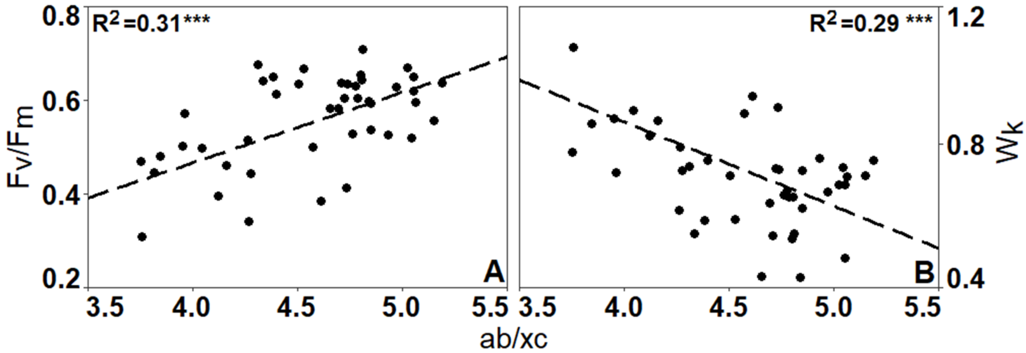

Figure 2. Linear regressions between chlorophylls to carotenoids ratio, quantum yield (A) and K-step (B).

The PCA analysis showed that the performance at control temperature (30 °C) does not change significantly throughout vegetation season as all 95% confidence ellipses overlap. The shift of 95% confidence ellipses of traits measured at 46°C shows that the May measurements significantly differ from all other months (Figure 2). Recent studies are showing that Norway spruce is able to acclimate its photosynthetic apparatus under heat stress at sites with optimal environmental conditions [15,16], but not at sub-optimal sites [17]. We can see that the R^2 for significant linear regressions is around 0.3 and thus other unknown factors are influencing the quantum yield and K-step under short-term heat stress. Also, we have found no significant correlation between PI_{abs} and pigments content. Seasonal changes of PSII thermostability might be affected by phenological age of needles, lipid structure of thylakoid membrane, antioxidants production etc. Further analysis of photosynthetic related traits is needed to assess the adaptive potential of Norway spruce under heat stress.

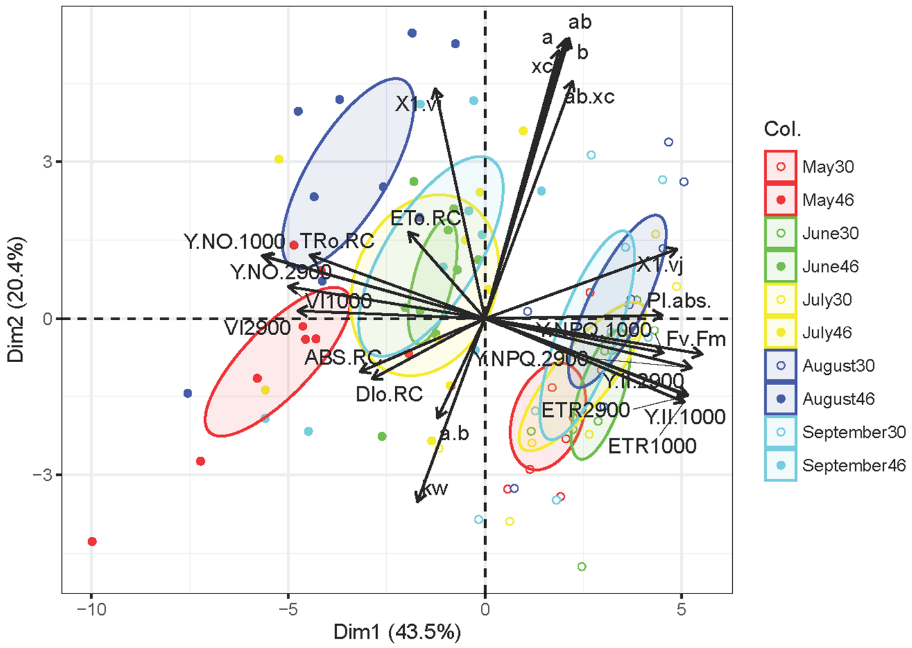

Figure 3. Scatter plot of PCA with 95% confidence ellipses for each measured month at control (30°C) and stressed (46°C) temperature.

4. Conclusion

Our data suggests that Norway spruce (*Picea abies*) trees growing under optimal hydric conditions are able to mitigate the impact of heat stress on photosynthetic performance within the vegetation season. Seasonal acclimation of PSII thermostability correlates with chlorophylls to carotenoids ratio. Nevertheless, other unknown factors are influencing the seasonal acclimation of PSII thermostability and thus further studies of related traits are needed.

Acknowledgments: The study was financially supported by the grant of the Slovak Agency for Research and Development no. APVV-16-0306.

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