DOI: 10.32615/ps.2020.011 PHOTOSYNTHETICA 58 (SI): 549-559, 2020

Special issue in honour of Prof. Reto J. Strasser

Origin rather than mild drought stress influenced chlorophyll *a* fluorescence in contrasting silver fir (*Abies alba* Mill.) provenances

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Abstract

The performance of PSII photochemistry in five Central European provenances of silver fir differing in the altitude of origin (250–1,300 m a.s.l.) were studied using the chlorophyll *a* fluorescence parameters derived from the JIP-test. Measurements were performed on two dates in 2016: at the start and at the peak of growing season. Within this period, mild water stress was recorded, allowing us to monitor changes in photochemistry under drought conditions. Our results confirmed that silver fir is generally resistant to water deficit at the PSII level. However, the differences in photochemistry between provenances originating from different climatic conditions were observed. The high-altitude provenances (from wetter and colder localities) achieved higher PSII efficiency, which was also supported by clear trends along the climatic and geographic gradients of the provenance origins. After the drought, comparable PSII efficiency for low-altitude provenances and even higher for these originating in higher altitudes was observed.

Additional key words: altitudinal gradient; climatic gradient; photochemical performance.

Introduction

The merits of studying the physiological limits of silver fir (*Abies alba* Mill.) are based on the very meaning of this species. This species is generally considered an important ecological and functional balancer of European forests and a fundamental species for maintaining high biodiversity in forest ecosystems; it is relatively resistant to wind, snow, and ice storms, which in turn lowers the susceptibility of forest stands to natural disturbances. Moreover, the analysis of tree-rings support the paleoecological evidence, demonstrating that silver fir is more resistant and resilient to drought than is Norway spruce, European beech, and European larch, since sulphur dioxide emissions were substantially reduced (Bošeľa *et al*. 2014, Zang *et al*. 2014, Vitasse *et al*. 2019). For that reason, silver fir could be a particularly suitable replacement for the Norway spruce, which was extensively planted also in lower vegetation

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Received 28 August 2019, *accepted* 22 January 2020.

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Abbreviations: DI_0/RC – total dissipated energy flux per active reaction centres at t = 0; ea – saturated vapour pressure at a given air temperature; es – free-flowing air vapour pressure; ET_0/RC – electron transport flux per reaction centres at t = 0; F_0 – basal fluorescence yield of dark-adapted leaf; F_M – maximum of fluorescence yield of dark-adapted leaf; FNR – ferredoxin-NADP$^+$-reductase; F_t – fluorescence yield at time t after lightening of actinic light; F_V – maximum of variable fluorescence of dark-adapted leaf; M_0 – approximated initial slope of the fluorescence transient; PI_{ABS} – performance index on absorption basis; PQH_2 – plastoquinol, protonated second quinone acceptor; RC/ABS – number of active reaction centres per antenna; TR_0/RC – energy trapping by PSII at t = 0; VPD – vapour pressure deficit; V_t – relative variable fluorescence at time t; ΔV_t – the difference in relative variable fluorescence between stressed and control individuals; δ_{RE0} – probability with which a PSII trapped electron is transferred from PSII electron acceptor side to PSI acceptor side; Φ_{PSII} – maximum quantum yield of PSII photochemistry; Ψ_{ET0} – probability with which a PSII trapped electron is transferred from PSII beyond reduced Q_A, $\Psi_{ET0} = 1 - V_J$; Ψ_{RE0} – probability with which a PSII trapped electron is transferred from reduced Q_A beyond PSI, $\Psi_{RE0} = 1 - V_I$; Ψ_W – soil water potential.

Acknowledgements: The study was financially supported by the grants of the Slovak Agency for Research and Development no. APVV-16-0306 and no. APVV-18-0390. The international cooperation was established within the framework of the COST Action CA15226 CLIMO: Climate-Smart Forestry in Mountains Regions.

belts since the beginning of the 19th century or even earlier, and gradually replaced a substantial part of natural mixed fir-beech forests (Spiecker *et al*. 2004). Currently, the future of spruce stands is at stake and the importance of fir is therefore increasing, especially at higher elevations, which is also associated with a relatively low susceptibility to damage by other disturbance factors, such as bark beetles typically connected with dry and hot summers (Vitali *et al*. 2017). However, much of the studies of silver fir are related to the different races (genetic lineages) as well to the different forest types in which it is present (Liepelt *et al*. 2009, Bošeľa *et al*. 2016, Dobrowolska *et al*. 2017) and there is still lack of studies of physiological responses in silver fir populations. Knowledge about the intraspecific variation in adaptive traits resulting from genetic differentiation due to adaptation to specific environmental conditions is essential for the selection of suitable reproductive material (eventually not original) for future adaptive forest management.

Plant stress responses are always accompanied by changes in structural and functional characteristics of the photosynthetic apparatus. Fluorescence parameters can, in principle, be used as selection tools in plant breeding programs, for rapid screening of wide range of stress effects, and for analysing genotype–environment interaction (Baker and Rosenqvist 2004, Kalaji and Pietkiewicz 2004, Peguero-Pina *et al*. 2007, Niemczyk 2009, Goltsev *et al*. 2016, Matías *et al*. 2016). Even though, PSII has been considered relatively resistant to water deficit (Aziz and Larher 1998), several studies have shown that PSII is injured under drought, resulting in serious changes in the fluorescence curve shape and the appearance of additional bands (Brestič and Živčák 2013, Meng *et al*. 2016, Falqueto *et al*. 2017, Kalaji *et al*. 2018). The O-L-K-J-I-P fluorescence transients and their analysis using JIP-test might be used as indicators of stress tolerance and physiological disturbances before the appearance of visible signs of stress (Kalaji *et al*. 2016). Therefore, there is considerable potential for Chl *a* fluorescence techniques to expand and to provide new insights into the fundamental process of photosynthesis also for forestry purposes (Kalaji *et al*. 2014, Bussotti *et al*. 2020).

The primary objective of the present study was to investigate the performance of PSII photochemistry in 16-year-old trees of five contrasting silver fir (*Abies alba* Mill.) provenances during natural mild water stress. The selected provenances cover the altitudinal range of this species in Central Europe (250–1,300 m a.s.l.) representing different microclimatic conditions, which can be reflected in their different gene pools. Considering the assumption that the provenances from contrasting localities have developed different adaptive responses to environmental conditions, which are still recognizable after being transferred and planted to new sites, our questions were as follows: (*1*) How do provenances from various climate conditions differ in their photochemical performance? (*2*) Do geographical and climatic patterns of PSII photochemistry exist across their range? (*3*) To what extent does mild drought stress affect PSII photochemistry of different silver fir provenances?

Materials and methods

Description of experimental plot and plant material: The experiment was conducted at the Hertník silver fir provenance plot located near the town of Bardejov in eastern Slovakia (49.217°N, 21.271°E; 390 m a.s.l.). The experimental plot is a part of the IUFRO 2005 experiment and comprises 17 provenances of silver fir. Initially, each provenance was represented by 105 seedlings planted in three completely randomized blocks (35 seedlings per block) with a 2 × 2 m spacing.

The performance of the PSII photochemistry of five Central European provenances differing in the altitude of origin (250–1,300 m a.s.l.; Fig. 1) were studied. The basic geographic and climatic characteristics of the provenance origin sites and Hertník trial plot (longitude, latitude, altitude, T_{mean} – mean annual temperature, T_{59} – mean temperature during the growing season, P_{year} – annual precipitation, P_{59} – precipitation of the growing season) are shown in text table. Measurements were conducted on two dates in 2016: (*1*) At the start of the season on 24 May, before the trees were exposed to natural stress, and (*2*) at the peak of the growing season on 12 July. Within the tested period, natural drought was recorded, allowing us to monitor changes in photochemistry efficiency in response to water deficit. The measurements were performed under field conditions. Three individuals in two blocks (six individuals) per provenance were selected and measured in three repetitions. To ensure as much homogeneity of the samples as possible, one-year-old shoots that were shaded and both insect- and disease-free from the breast height in the northern part of the crown were used. During the experiment, the age of measured trees was 16 years.

Meteorological data: The meteorological conditions of the experimental site were continuously monitored in an open area in the centre of the provenance plot. The air temperature [°C], relative humidity [%], and incoming global solar radiation [W m^{-2}] (*Minikin*, *EMS*, Brno, Czech Republic) were measured every 2 min and stored as 20-min averages. Precipitation was measured 1 m above the ground [mm] (*MetOne 370R*, USA). The measurements of soil water potential (Ψ_W [MPa]) were carried out on 10 micro-plots at 15, 30, and 50-cm depths using gypsum blocks (*Delmhorst Inc*., USA) and a *MicroLog SP3* datalogger (*EMS*, Brno, Czech Republic) and on two micro-plots in the middle, near the meteorological measurements, at 1-h intervals. Overall, 36 gypsum blocks were used, 12 for each soil depth. We used the mean values of all of the blocks representing the whole site for the analyses to avoid mosaic heterogeneity between and inside the micro-plots. The vapour pressure deficit (VPD [kPa]) was calculated as VPD = es − ea, where es is the saturated vapour pressure at a given air temperature and ea is the vapour pressure of the free-flowing air.

	Trial plot	Tested provenances				
Label	Hertník	PL41	SK01	PL21	SK02	AT
Name	Hertník	Kadlubiska	Staré Hory	Berest-1	Bardejov	Koetschach Valey
Country	Slovakia	Poland	Slovakia	Poland	Slovakia	Austria
Longitude [°]	49.217	50.300	48.750	49.550	49.417	47.100
Latitude [°]	21.271	22.167	19.750	20.950	21.250	13.183
Altitude [m a.s.l.]	390	250	500	690	900	1,300
T_{mean} [°C]	7.2	7.6	6.6	6.2	5.6	1.8
T_{59} [°C]	15	15.9	14.2	13.9	13.2	8.3
P_{year} [mm]	737	591	781	836	849	1,327
P_{59} [mm]	424	354	447	476	482	692

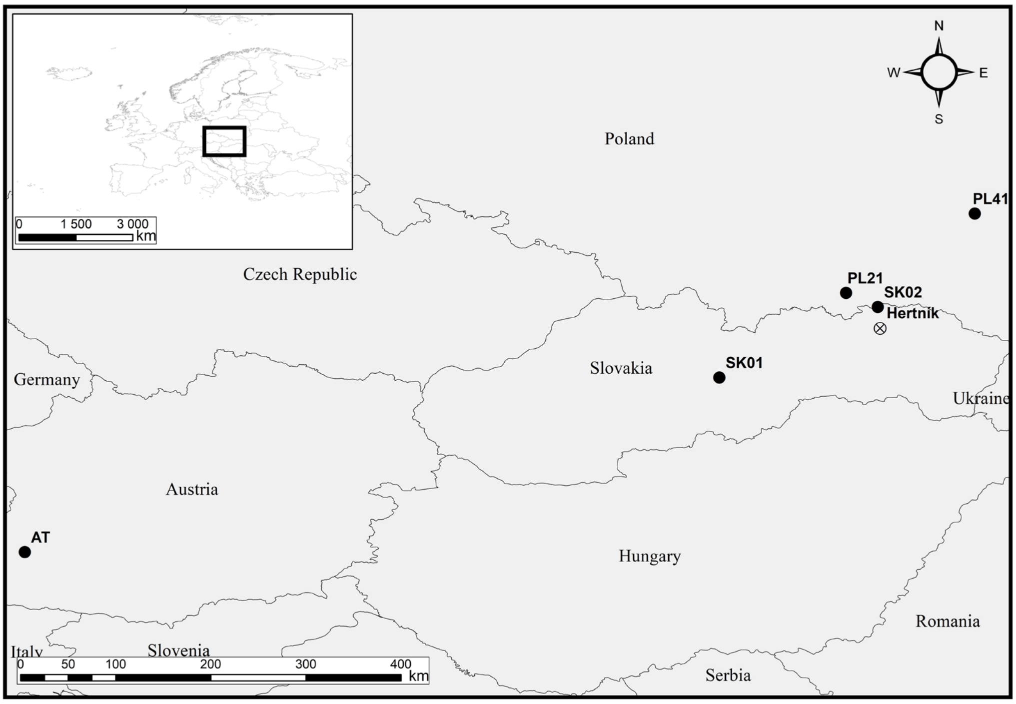

Fig. 1. Localization of the tested provenances and Hertník trial plot.

Chl *a* fluorescence: The performance of PSII photochemistry was studied *via* parameters derived from the fast kinetics of Chl *a* fluorescence measured using a plant efficiency analyzer *HandyPEA* (*Hansatech Instruments Ltd*., UK). The needles were first adapted to the dark for 30 min, and leaf clips that were 4 mm in diameter were used. The samples were then illuminated by a saturation pulse with an intensity of 3,500 μmol(photon) m^{-2} s^{-1} and a duration of 1 s. The transient of Chl *a* fluorescence was analysed based on the JIP-test using *Biolyzer 3.06* software (Laboratory of Bioenergetics, University of Geneva, Switzerland). Since mild drought was observed within days of measurement, we focused on Chl *a* fluorescence parameters for which negative effects of drought have been already described (Redillas *et al*. 2011, Kalaji *et al*. 2016, Falqueto *et al*. 2017, Pšidová *et al*. 2018). The evaluated parameters derived from the JIP-test with the corresponding equations (Strasser *et al*. 2000, Strasser *et al*. 2004) are described in Table 1S (*supplement*). Moreover, for the visualization of additional bands on the OJIP curve, the difference in relative variable fluorescence between stressed individuals and control plants was calculated: $\Delta V_t = V_{t(drought)} - V_{t(control)}$.

Statistical analyses were performed using *R 3.1.2* (*R Core Team*, Austria). Prior to the statistical tests, the data were tested for normality. For several parameters, where the distribution deviated from the normal distribution, data transformation was performed. Differences between provenances were tested using two-way analysis of variance (*ANOVA*) by the GLM procedure using type-III sums of squares, with provenance and conditions as fixed factors. The model $y_{ij} = cond_i + prov_j + cond_i \times prov_j + \varepsilon_{ij}$ was used, where y_{ij} is the measured value of phenotype, $cond_i$ is the effect of the i^{th} condition during the measurements (control and drought), $prov_j$ is the effect of the j^{th} provenance, $cond_i \times prov_j$ is the interaction between the condition and provenance and ε_{ij} is the experimental error. As the insignificant effect of blocks was found in a preliminary study, the data from both blocks were merged. The geographical and climatic trends were assessed using linear regression models; the provenance averages were fitted to the geographical and climate variables of provenance origin. This analysis was performed separately for two dates of measurements: for measurements conducted at the start of the growing season under the optimal growing conditions and at the peak of the growing season after the natural mild drought. The data concerning the long-term averages of climatic variables for trial plot and provenance origins were derived from the WorldClim model with a 30 arc-second resolution (Hijmans *et al*. 2005).

Results

Meteorological conditions: The mean air temperature during the growing season (April–September) in 2016 was 1.9°C above the long-term mean according to WorldClim (Hijmans *et al*. 2005), with a 101 mm precipitation deficit, which represents 80% of the long-term amount during the growing season.

The temperature during all months of the studied period was above the long-term mean temperature, with July being the warmest month (18.5°C, 1.5°C above the long-term mean; Fig. 2*A*,*B*). Precipitation deficits occurred during May (–35% of the long-term amount) and June (−72% of the long-term amount), which represent 43% of the cumulative May–June amount (deficit of 105 mm).

At a broader regional scale, several warm periods and heat waves occurred, during which the daily mean and maximum temperatures were well above average (Fig. 2*C*), and some periods were accompanied by a high VPD and precipitation deficits (Fig. 2*D*). The synergistic effects of these conditions led to a decrease in the soil water potential (Ψ_W) throughout the last decade of June, which was interrupted by rainfall on 1 and 3 July, leading to an increase in Ψ_W around 5 July. This was followed by the second peak of Ψ_W decline, which towards the seasonal minimum of −1.12 MPa on 13 July.

Physiological measurements were performed on 24 May and on 12 July (Fig. 1*C*,*D*). The average daily temperature (17.2°C) and VPD (17.9 kPa) on 24 May were the highest since the beginning of the season. One of the hottest days during the season was 12 July, the average daily temperature reached 23.5°C, with a daily maximum of 34.7°C. Additionally, the previous day was very hot, with a daily maximum of 33.7°C. Higher precipitation occurred

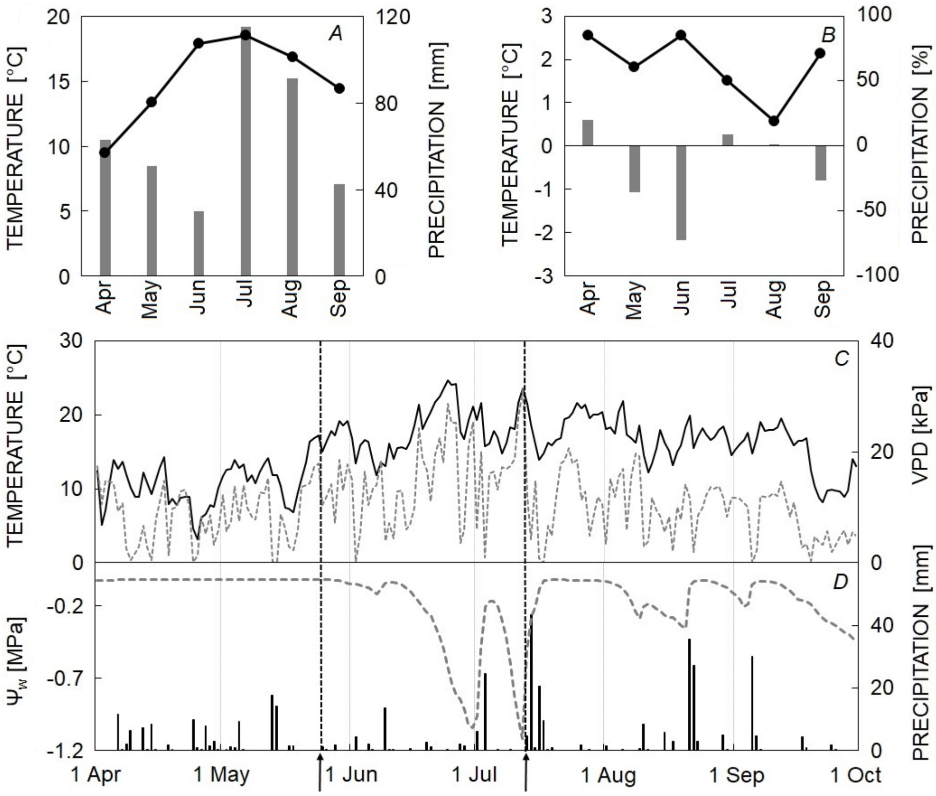

Fig. 2. (*A*) Average monthly temperatures (*black line with circles*) and precipitation (*grey bars*) during the vegetation period in 2016. (*B*) Monthly temperatures (*black line*) and precipitation (*black bars*) anomalies relative to the long-term average. (*C*) Average daily temperatures (*black line*) and vapour pressure deficit (VPD, *dashed grey line*) during the vegetation period (*black arrows* and *black dashed lines* mark 24 May and 12 July, respectively). (*D*) Average daily soil water potential (Ψ_W, *dashed grey line*) and daily precipitation amounts (*black bars*) during the vegetation period (*black arrows* and *black dashed lines* mark 24 May and 12 July, respectively).

for 10 d preceding 24 May (32 mm between 13 and 14 May) and for 9 d preceding 12 July (25 mm on 3 July). As a result of seasonal weather conditions, both days differed significantly in terms of Ψ_W (–0.02 MPa on 24 May and –0.99 MPa on 12 July).

The mostly sunny (partly cloudy in the afternoon), warm day was 24 May during a period with very good water availability (further referred to as 'control condition'). The sunny and extremely hot day was 12 July, with the highest VPD throughout the season and experiencing mild water stress (further referred to as 'drought condition').

Intraspecific variation in photochemical performance along the geographic and climatic gradients: The course of OJIP transients (Fig. 3*A*,*B*) showed a relatively high similarity between the tested provenances within the individual days of measurements. The basic parameters of Chl *a* fluorescence, minimal fluorescence (F_0) and maximal fluorescence (F_M), did not significantly differ between the tested provenances under control conditions or after drought occurrence.

Due to the similarity in courses of OJIP transients, we focused on more complex parameters derived from the JIP-test, where we recorded the differences between provenances in all parameters for both measuring dates (Fig. 4; Table 2S, *supplement*). The performance index (PI_{ABS}), the number of active reaction centres per antenna (RC/ABS), the maximal quantum yield of PSII (Φ_{PSII}), the probability with which a PSII trapped electron is transferred from PSII beyond reduced Q_A (Ψ_{ET0}), from reduced Q_A beyond PSI (Ψ_{RE0}) and from PSII electron acceptor side to PSI acceptor side (δ_{RE0}) reached the highest or at least above-average values in the high-altitude provenances AT and SK02 before and after the mild water deficit (Fig. 4). These results were also confirmed by the altitudinal trends (Fig. 5, Table 1); PI_{ABS}, RC/ABS, Ψ_{ET0}, and Ψ_{RE0} showed significant or at least marginally significant increases along the altitudinal gradient of the provenance origins on both measurement days, thus under the control conditions and after the natural mild drought. Since increasing altitude is closely correlated with rising precipitation and decreasing average temperatures in Central Europe, these climatic variables also showed relationships with PI_{ABS}, RC/ABS, Ψ_{ET0}, and Ψ_{RE0}. The other tested parameters (F_0, Φ_{PSII}, ABS/RC, δ_{RE0}) exhibited climatic trends after drought exposure. Similar to what occurred in the previous case, the high-altitude provenances originating from the relatively wet and cool sites had higher Φ_{PSII} and δ_{RE0} values after

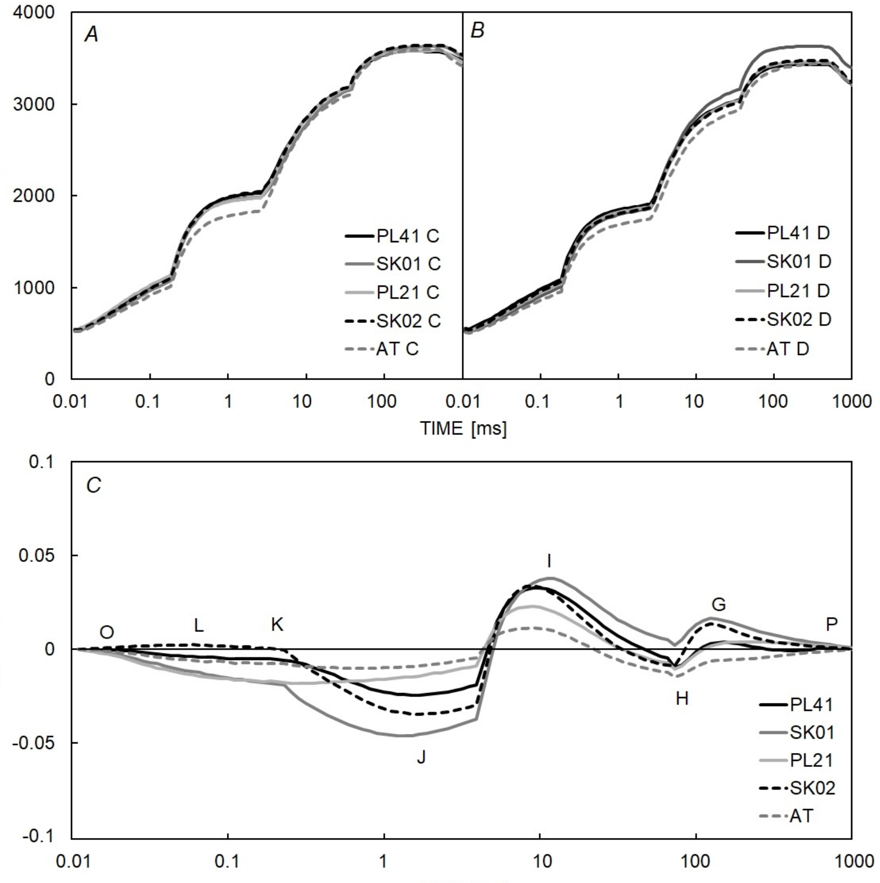

Fig. 3. Transients of mean fluorescence curves with basic steps (O-J-I-P) for the silver fir provenances before (*A*) and after natural mild drought (*B*). Difference of transients between the drought-stressed and control trees with full sequences of bands (O-L-K-J-I-H-G-P) (*C*). The transients were described according to Strasser *et al*. (2007).

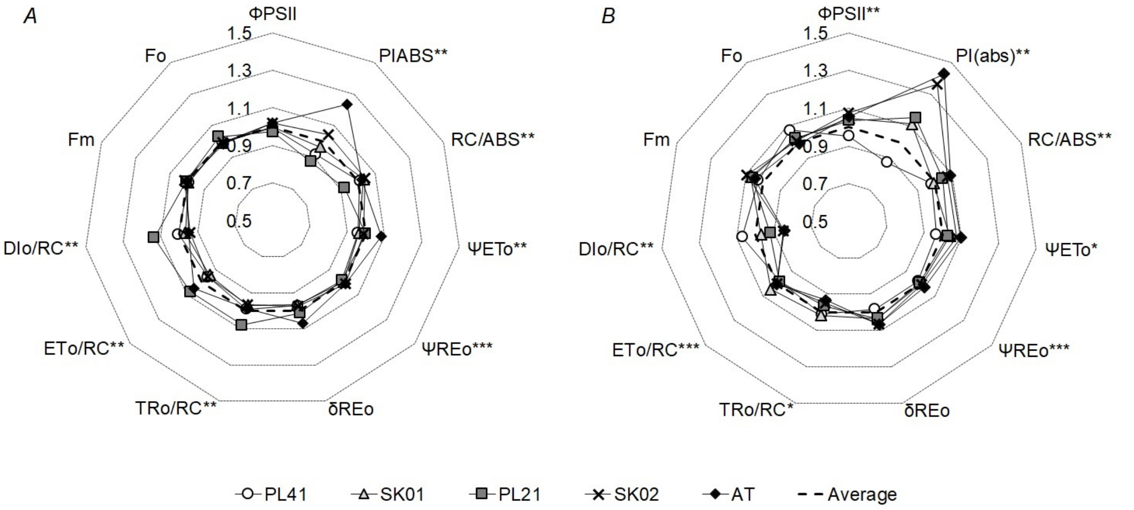

Fig. 4. Radar plots of Chl *a* fluorescence parameters for the silver fir provenances under control conditions (*A*) and after natural mild drought (*B*). The mean values of tested parameters (normalized with the average curve as standard) are displayed. *The asterisks* represent the parameters significantly differing between provenances; *** $P<0.001$, ** $P<0.01$, * $P<0.05$. Φ_{PSII} – maximum quantum yield of PSII photochemistry, PI_{ABS} – performance index on absorption basis, RC/ABS – number of active reaction centres per antenna, Ψ_{ET0} – probability with which a PSII trapped electron is transferred from PSII beyond reduced Q_A, Ψ_{RE0} – probability with which a PSII trapped electron is transferred from reduced Q_A beyond PSI, δ_{RE0} – probability with which a PSII trapped electron is transferred from PSII electron acceptor side to PSI acceptor side, TR_0/RC – energy trapping by PSII at t = 0, ET_0/RC – electron transport flux per reaction centres at t = 0, DI_0/RC – total dissipated energy flux per active reaction centres at t = 0, F_M – maximum of fluorescence yield of dark-adapted leaf, F_0 – basal fluorescence yield of dark-adapted leaf.

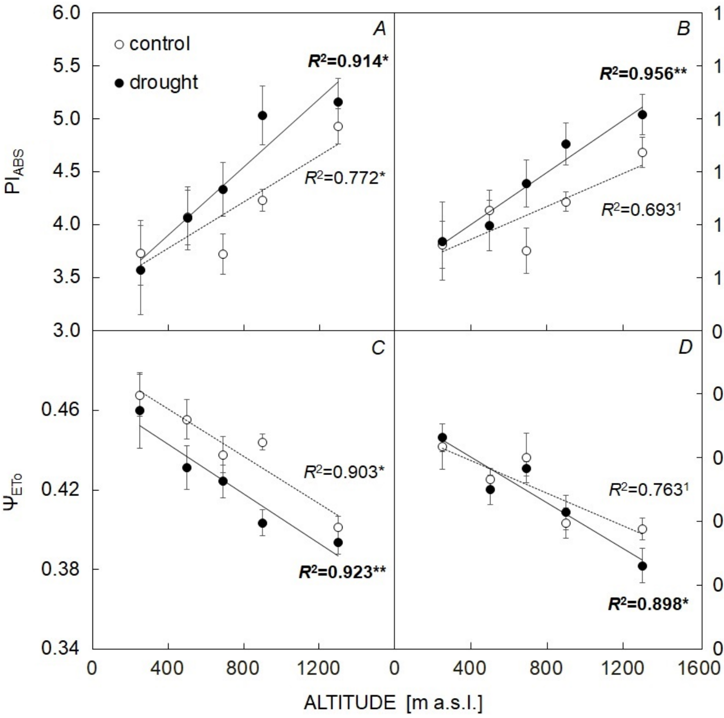

Fig. 5. Linear regressions of the performance index (PI_{ABS}) (*A*), number of active reaction centres per antenna (RC/ABS) (*B*), probability with which an electron trapped by PSII will reach the secondary quinone electron acceptor Q_B (Ψ_{ET0}) (*C*), and probability with which an electron trapped by PSII will reach the PSI acceptor site (Ψ_{RE0}) (*D*) against the altitude of provenance origins under control conditions (*empty circle*, *dashed line*) and after natural mild drought (*solid circle*, *solid line*). The provenance means ± standard error of mean are presented. Significant (*** $P<0.001$, ** $P<0.01$, * $P<0.05$) and marginally significant (^{1} $P<0.1$) trends are shown.

natural drought treatment compared to the low-altitude provenances. The F_0 also exhibited improved values in the high-altitude provenances; this parameter decreased along the altitudinal and precipitation gradients and conversely increased along the temperature gradient.

In terms of the electron transport flux per RC (ET_0/RC) and the energy trapping by PSII (TR_0/RC), we observed the highest values for the high-altitude provenances on May; however, after mild drought, the increase was the most pronounced for the low-altitude provenances. Moreover, the total dissipated energy flux per active RC (DI_0/RC) was the highest for the low-altitude provenances on both dates

Table 1. Linear regressions of Chl *a* fluorescence parameters against climate characteristics at the sites of provenance origins. Significant (*** $P<0.001$, ** $P<0.01$, * $P<0.05$) and marginally significant (^{1} $P<0.1$) models are shown. Φ_{PSII} – maximum quantum yield of PSII photochemistry, PI_{ABS} – performance index on absorption basis, RC/ABS – number of active reaction centres per antenna, Ψ_{ET0} – probability with which a PSII trapped electron is transferred from PSII beyond reduced Q_A, Ψ_{RE0} – probability with which a PSII trapped electron is transferred from reduced Q_A beyond PSI, δ_{RE0} – probability with which a PSII trapped electron is transferred from PSII electron acceptor side to PSI acceptor side, F_0 – basal fluorescence yield of dark-adapted leaf, Alt – altitude, T_{mean} – mean annual temperature, T_{59} – mean temperature during the growing season, P_{year} – annual precipitation, P_{59} – precipitation of the growing season.

OJIP parameter	Climatic variable	Control			Drought		
		R^2	P	slope	R^2	P	slope
PI_{ABS}	Alt	0.772	0.049^{*}	709.2	0.914	0.011^{*}	571.7
	P_{59}	0.906	0.013^{*}	238.1	0.661	0.094^{1}	150.7
	P_{year}	0.907	0.012^{*}	523.3	-	-	-
	T_{59}	0.924	0.009^{**}	–5.6	0.670	0.090^{1}	–3.5
	T_{mean}	0.946	0.005^{**}	–4.4	0.657	0.096^{1}	–2.7
RC/ABS	Alt	0.693	0.080^{1}	4,462.2	0.956	0.004^{**}	3,877.7
	P_{59}	0.850	0.026^{*}	1,532.6	0.695	0.079^{1}	1,025.2
	P_{year}	0.846	0.027^{*}	3,357.0	0.688	0.082^{1}	2,239.0
	T_{59}	0.854	0.025^{*}	–35.5	0.731	0.065^{1}	–24.2
	T_{mean}	0.879	0.018^{*}	–28.0	0.717	0.070^{1}	–18.7
Φ_{PSII}	Alt	-	-	-	0.667	0.092^{1}	45,958.2
F_0	Alt	-	-	-	0.928	0.008^{**}	–26.4
	P_{59}	-	-	-	0.764	0.053^{1}	–7.4
	P_{year}	-	-	-	0.741	0.061^{1}	–16.1
	T_{59}	-	-	-	0.738	0.062^{1}	0.2
	T_{mean}	-	-	-	0.708	0.074^{1}	0.1
Ψ_{ET0}	Alt	0.903	0.013^{*}	15,111.6	0.858	0.024^{*}	14,851.5
	P_{59}	0.891	0.016^{*}	4,655.7	0.740	0.062^{1}	4,197.0
	P_{year}	0.898	0.014^{*}	10,264.9	0.710	0.073^{1}	9,029.1
	T_{59}	0.909	0.012^{*}	–108.6	0.698	0.078^{1}	–94.1
	T_{mean}	0.884	0.017^{*}	–83.248	0.674	0.088^{1}	–71.9
Ψ_{RE0}	Alt	0.763	0.053^{1}	29,727.6	0.875	0.020^{*}	24,680.4
	P_{59}	0.656	0.096^{1}	8,547.9	0.935	0.007^{**}	7,911.0
	P_{year}	-	-	-	0.920	0.010^{**}	17,233.3
	T_{59}	0.663	0.093^{1}	–198.358	0.916	0.011^{*}	–180.831
	T_{mean}	0.676	0.088^{1}	–155.688	0.914	0.011^{*}	–140.469
δ_{RE0}	Alt	-	-	-	0.700	0.077^{1}	22,511.7
	P_{59}	-	-	-	0.907	0.012^{*}	7,943.5
	P_{year}	-	-	-	0.907	0.012^{*}	17,438.7
	T_{59}	-	-	-	0.905	0.013^{*}	–183.284
	T_{mean}	-	-	-	0.925	0.009^{*}	–144.013

of measurements, but no significant trends were observed for these parameters (Table 1).

Effect of mild drought on PSII photochemistry in different silver fir provenances: From the basic fluorescence parameters, the differences between the control and drought conditions were confirmed just in F_M; the mild drought resulted in a decline in F_M of all tested provenances except SK01.

The differences in the JIP-derived parameters between the control and drought conditions were confirmed for the maximal quantum yield of PSII (Φ_{PSII}) and the probability with which the electron trapped by PSII will reach the secondary quinone electron acceptor Q_B (Ψ_{ET0}), however, the reduction of Φ_{PSII} was negligible (from 0.87 to 0.86). The other JIP-derived parameters were not negatively affected by natural mild drought. Actually, a slight increase in most of the parameters after drought was observed, especially in the high-altitude provenances, which further increased the differences between the individual provenances.

The differences in kinetics (ΔV_t) between days of measurements showed only moderate changes in the I-band (ΔV_I) after mild drought; the L- and K-bands absented (Fig. 3*C*). Moreover, the amplitudes of the L- and

K-bands had negative courses; however, the occurrence of two peaks at 20 ms (ΔV_{20ms}) and at 100 ms (V_{100ms}) in the stressed individuals was recorded. The V_{20ms} and V_{100ms} significantly increased (Table 2S) on average from 0.78 to 0.80 and from 0.967 to 0.972, respectively. Moreover, the interprovenance differences in ΔV_{100ms} were significant. The kinetics changes were the most pronounced in the low-altitude provenances (Fig. 3*C*). In contrast, the provenance AT from the highest altitude, thus from the wettest and coolest location, behaved relatively homogeneous and without strong oscillation between the control and drought conditions. Moreover, the high-altitude provenances (SK02 and AT) showed the lowest values of relative variable fluorescence (V_t) within individual transient steps for both measurement dates.

Overall, we can state that the high-altitude provenances had the highest overall photochemical efficiency under optimal conditions and even better performance after mild drought period compared to the low-altitude provenances.

Discussion

The present study explored the PSII photochemistry of silver fir provenances of climatically contrasting origin but growing at the same experimental plot Hertník, which is situated below the fir climatic optimum and fitted with conditions expected for the end of the 21st century in Europe (Vitasse *et al.* 2019). The suboptimal growing condition for fir was in 2016 enhanced by the precipitation deficit during the growing season, thus the intraspecific variation of PSII photochemistry response to natural mild drought could be observed.

Natural mild drought induced changes in the maximal photochemical efficiency of PSII (Φ_{PSII}), which is considered a basic parameter for revealing the degree of PSII damage and photochemistry performance under many stresses (Genty *et al.* 1989, Brestič *et al.* 2013, Dąbrowski *et al.* 2016, Živčák *et al.* 2014). Overall, Φ_{PSII} was slightly reduced in fir provenances. However, Force *et al.* (2003) suggested using more complex parameters (*e.g.*, performance indices, energetic fluxes at the reaction centre of PSII and at excited cross-section levels) to evaluate PSII performance rather than a single parameter, such as the aforementioned Φ_{PSII}. The advantage of these parameters is that they can indicate the effects of stress before the appearance of visible physiological disturbances (Christen *et al.* 2007, Meng *et al.* 2016). However, we observed no significant changes in the absolute performance index (PI_{ABS}), the number of active RCs, or the RC/ABS, and observed just a slight increase in these parameters, especially for high-altitude provenances. The specific energy fluxes (ET_0/RC, DI_0/RC, TR_0/RC) operated relatively effectively in the high-altitude provenances under optimal conditions. However, these fluxes increased after the mild drought treatment in the low-altitude provenances, probably reflecting a negative response due to the greater number of inactivated RCs (Strasser *et al.* 2010).

Aditionally, several drought studies considering Chl *a* fluorescence transients have used relative fluorescence (V_t) data that were normalized and displayed as kinetic differences (ΔV_t), permitting the identification of the appearance of individual bands of fluorescence curve. Severe drought may result in the presence of the L-band at 0.15 ms and the K-band at 30 ms. The positive L-band is an indicator of relatively weak usage of excitation energy or reduced stability of PSII as a result of de-stacking of the thylakoid membranes (Strasser and Stirbet 1998, Strasser *et al.* 2004, Oukarroum *et al.* 2007, Falqueto *et al.* 2017). The K-band indicates irreversible changes in PSII, either by the modification of light-harvesting complexes (LHCs) or by the inhibition of electron transport from pheophytin to the primary quinone acceptor Q_A; moreover, the K-band may be associated with the inactivation of the oxygen-evolving complex (OEC) (Srivastava *et al.* 1997, Strasser 1997, Lazár *et al.* 1999). However, negative amplitudes for the L- and K-bands after mild drought stress were observed for all tested provenances except SK02. The same behaviour was described for maple genotypes (Banks 2018) and drought-tolerant cultivars of barley (Oukarroum *et al.* 2007), where a more hyperbolic shape of the initial fluorescence curve was attributed to an increase in the energetic connectivity among PSII antennae (Strasser 1981).

Schansker *et al.* (2005) described that the J-, I-, and P-bands of the fluorescence transient represent the subsequent kinetic bottlenecks of the electron transport chain as well as the accumulation of reduced Q_A^- because of inhibited electron transfer between Q_A^- and Q_B (J-band), the limitation of plastoquinol reoxidation (PQH_2) (I-band), and a transient block on the acceptor side of PSI attributed to inactive ferredoxin-NADP$^+$ reductase (FNR) (P-band). Several researchers noticed a gradual increase in V_J and V_I in response to drought stress (Redillas *et al.* 2011, Kalaji *et al.* 2018, Pšidová *et al.* 2018). As in the previous cases (L- and K-bands), we noticed that the decrease in the J-band (shown by the increase in Ψ_{ET0}) in stressed individuals of all provenances indicates a relatively fast transport of electrons during the initial phases of fluorescence. This phenomenon is probably associated with seasonal changes in photochemical processes caused by increasing ambient air temperatures within the growing season. The gradual increase in photochemical capability, with the maximum at the peak of the growing season, was also observed in other conifers, such as *Picea abies*, *Pinus cembra*, and *Pinus sylvestris*, attributed to the gradual recovery of PSII after winter (Ottander *et al.* 1995, Lundmark *et al.* 1998, Stecher *et al.* 1999). Ottander *et al.* (1995) suggested that conifers are able to downregulate their PSII during the winter by partially reducing the amount of Chl and reorganizing the LHC into large aggregates to prevent the destruction of thylakoids. The most pronounced decreases in V_L, V_K, and V_J were observed in the low-altitude provenances. However, the provenances from relatively high altitudes showed below-average values of V_t for both measuring dates, which was also confirmed by the significant altitudinal and climatic trends. These provenances behaved relatively homogeneous without greater oscillation between the start and the peak of the growing season. Given that the PSII reorganization after winter is strongly temperature dependent (Ottander *et al.*

1995), it may also reflect the intraspecific variability in the onset and duration of recovery processes as a result of local adaptation of individual provenances. The behaviour of the I-band was tracked as the probability with which an electron trapped by PSII will reach the PSI acceptor site (Ψ_{RE0}). The decrease in Ψ_{RE0} was observed after natural drought for the low-altitude provenances, whereas the high-altitude provenances showed a slight increase. Similar results were described for European beech provenances by Pšidová *et al*. (2018), where Ψ_{RE0} was strongly affected by combined natural drought and heat in low-altitude provenances, while electron transport efficiency was shown to be enhanced and was less sensitive to stress occurrence for individuals originating from higher altitudes. The last part of Chl *a* fluorescence transient describing the efficiency of electron transfer from PQH_2 to PSI acceptors (δ_{RE0}) was not significantly affected by mild water stress, and climatic and altitudinal trends were apparently just for the stressed trees. These findings are also supported by those of Yan *et al*. (2013) and Pšidová *et al*. (2018), in which the electron transfer from PQH_2 to PSI is less sensitive to heat and combined heat and drought, respectively, compared to previous steps of fluorescence transient. Moreover, we found that the natural drought resulted in two consistent bands between the I- and P-bands. In a previous study by Tsimilli-Michael *et al*. (1998), the P-band was split into two bands (H- and G-bands), whereas the G-band was considered equivalent to P-band. Later, Strasser *et al*. (2007) described the H- and G-bands as two different peaks between I and P as a consequence of slow-down multiple turnover events within the electron transport chain: the second reduction of Q_B^- to Q_B^{2-} (H-band at 20 ms) and the formation of a second protonated quinone acceptor PQH_2 (G-band at 100 ms).

In summary, 9-d-long natural mild drought did not significantly injure the PSIIs of young fir trees. The downregulation of photochemical processes by moderate water stress was not observed, what is in accordance with results presented by Kalaji *et al*. (2018) for *Tilia cordata*, where the first changes in JIP-derived parameters were recognized after 14 d without watering. However, our results well document the intraspecific variability in the performance of PSII. The fir provenances originating from relatively high altitudes (from wetter and colder localities) achieved and maintained relatively high efficiency of PSII photochemistry, even more they enhanced their efficiency at PSII level after mild water stress. These findings concur with those from provenances of other species, *i.e.*, *Fagus sylvatica* (Kučerová *et al*. 2018, Pšidová *et al*. 2018), *Quercus suber* L. (Aranda *et al*. 2005), *Picea abies* (Oleksyn *et al*. 1998, Jamnická *et al.* 2019), and also *Abies alba* (Peguero-Pina *et al*. 2007, Konôpková *et al*. 2018), reflecting the variation in the adaptation and acclimation abilities of forest tree species under conditions of a changing climate with possible use for forestry purposes.

Conclusion: Our study focused on assessing the response of different silver fir (*Abies alba* Mill.) provenances to naturally occurring mild drought conditions at the PSII level using the parameters derived from JIP-test. Related to the longevity of forest tree species, it is important to focus attention on methods of studying adaptation and acclimation mechanisms. The fast kinetics of Chl *a* fluorescence allows effective, fast, and especially nondestructive assessments of the health status and the performance of PSII *in situ* and thus evaluation of relatively large sets of plant material.

The results of the present study showed that young fir trees were resistant to 9-d long natural mild water stress at the PSII level. The photochemical efficiency parameters were maintained at the same level for both measurement days, even more, individuals from relatively mesic and cool localities slightly enhanced their PSII performance after drought occurrence. However, for low-altitude provenances, the changes on Chl *a* fluorescence transients with appearance of additional bands were observed, especially on the last part of curves, which is probably the first indicator of incipient PSII changes. Therefore, there is still an open question whether and how the different provenances would respond during long-lasting or more severe drought periods.

On the other hand, we proved the intraspecific variation in photochemical efficiency with clear patterns along the climatic and geographic gradients, which are a result of fixed genetically driven responses to conditions of the origin localities. These observed differences in the adaptation and acclimation capability of PSII can provide an important insight for the selection of suitable seed material for future reforestation programmes as a way to mitigate the impact of climate change on forest ecosystems.

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