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Soil drought stress and high-temperature effects on photosystem II in different juvenile spruce provenances

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Abstract
With prolonged periods of drought and temperature extremes becoming more frequent, the stress on Norway spruce has increased, as its ecological optimum is in colder and more humid environments. However, it is a tree species with a good adaptability, due to its great geographic and intraspecific variability. In this study, we tested the sensitivity of chlorophyll *a* fluorescence tools for the early detection of drought and heat stress, as well as their combined effect, in four Slovak Carpathian provenances, under controlled laboratory conditions. Responses in photosynthetic parameters of fast and slow (light response curves) kinetics of chlorophyll *a* fluorescence were analysed in spruce seedlings under four treatments: control, drought, heat, and combined drought + heat. Heat and drought + heat stresses led to the decrease of photosynthetic efficiency to a greater extent than in the case of only individual drought stress. The less sensitive parameter impacted by the stresses was the maximum quantum yield of PSII photochemistry (Fv/Fm), while the more sensitive parameters were: the performance index based on absorption of light energy (PI_{ABS}), the effective quantum yield of the PSII (Φ_{PSII}), and the coefficients of non-photochemical fluorescence quenching (NPQ, q_N). The significant variability in responses of individual provenances was also observed. Seedlings from two studied provenances responded better to these abiotic stresses (Pa-680 and Pa-1500); however, Pa-1500 provenance from the highest altitude showed the best ability of resistance to soil drought stress, as well as to high temperature effect. This rapid screening of photosystems II efficiency showed the importance of the selection of more resistant populations to concurrent stresses as one of the measures to mitigate climate change impacts.

Key words: drought; heat stress; *Picea abies*; photosystem II; provenances

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1. Introduction

The preservation of spruce forests in Central Europe is currently becoming a significant challenge, as these stands increasingly suffer from the combined impacts of environmental and climatic changes. These factors can lead to physiological weakening, mortality, and even the degradation of entire tree populations in a specific area (Lindner et al. 2014; Lambers & Oliveira 2019). Understanding the drivers, patterns, and severity of these changes is crucial for implementing mitigation strategies, with which to reduce their negative impact. Over the last decades, wind disturbances and subsequent bark beetle outbreaks, coupled with the increasing frequency of summer droughts and heatwaves, have been major contributors to the mortality of Norway spruce (Eriksson et al. 2007; Hentschel et al. 2014). The interplay of factors such as decreased precipitation, irregular rainfall patterns, reduced air humidity, and rising average temperatures creates a synergistic effect, heightening the vulnerability of common spruce to secondary harmful agents, such as bark beetles. These conditions contribute to an increased number of beetle generations within a single year (Hlásny & Turčáni 2013).

In the context of adaptive forest management, provenance trials play a fundamental role in studying adaptive processes in forest trees. These trials have shown that tree populations from different regions exhibit varying responses to environmental conditions and levels of phenotypic plasticity (Rehfeldt et al. 2002; Aitken et al. 2008; Gömöry et al. 2012, 2015; Bussotti et al. 2015; Robson et al. 2018). Such research aids in identifying resilient

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tree ecotypes that are capable of optimal responses to altered environmental conditions, without compromising physiological processes. Given the importance of photosynthesis in plants, the present study utilized photosynthetic PSII fluorescence techniques as unique and reliable non-invasive tools for screening the effects of drought and high temperatures among different spruce provenances. While these methods have been widely applied in crop plants (Zivcak et al. 2017; Végh et al. 2018; Maier et al. 2022; Noor et al. 2022), they are also applicable for forestry purposes (Bigras et al. 2000; Pšidová et al. 2018; Konôpková et al. 2020; Vastag et al. 2020; Zavadilová et al. 2023).

Both slow and fast chlorophyll *a* fluorescence (Chl *a* fluorescence) kinetics provide parameters that are sensitive to drought stress (Goltsev et al. 2012; Bussotti et al. 2020). However, on the one hand, the utility of these parameters, and protocols for evaluating drought, depends on the severity and duration of the stress (Kalaji et al. 2017; Suresh et al. 2020). On the other hand, photosynthesis is highly sensitive to high temperatures and can be inhibited before other stress symptoms manifest. The photosystem II (PSII) is particularly heat-sensitive, and exceeding critical temperature thresholds can lead to permanent impairment of PSII function, severely limiting photosynthetic capacity (Berry & Björkman 1980; Mathur et al. 2014; Kalaji et al. 2016; Münchinger et al. 2023).

The primary objective of this work was to test the sensitivity of chlorophyll *a* fluorescence tools, which could be used for the rapid analyses of responses to drought and heat stress in *Picea abies* seedlings. The study also aims to comparatively analyse responses to the combined stresses of heat and drought, which are common occurrences that mutually influence each other in natural environments as well as identification of more and/or less sensitive spruce provenances. We suppose that the combined stress effect (drought + heat) will have the biggest impact on photochemical efficiency of PSII photosystems, and these will be less negatively influenced in "marginal" provenances.

2. Material and methods

2.1. Experimental setup

The experimental material consists of 40 Norway spruce seedlings at the age of 5 years. The seedlings originate from 4 different regions across Slovakia from 650 to 1,500 m a.s.l. (see Table 1) of the Western Carpathians (Gene Bank of Forest Trees of Slovakia, OZ Semenoles Liptovský Hrádok). Five individuals per provenance and treatment (C – control group, D – drought treatment, H – heat treatment, D + H – drought and heat treatment) were chosen in 4 weeks of the laboratory experiment, in order to assess their physiological performance under different treatment regimes.

Seedlings were exposed to 4 weeks of simulated drought conditions. The content of soil water was monitored permanently as soil water potential (SWP (MPa)). It was recorded from 0.0 up to –1.5 MPa using the gypsum blocks (Delmhorst Inc., USA) in each pot with seedlings, and was measured automatically at hourly intervals. Data were stored in data loggers MicroLog SP3 (EMS Brno, Czech Republic). In the laboratory, the mean air temperature during the day was set to 24 °C, relative air humidity was set to 70% in the control group, and the mean air temperature during the day was set to 28 °C, and relative humidity was 50% in the drought-treated group. Daylight was simulated for 14 h using white halogen lamps with a radiation intensity of 400 µmol (photon) m^{-2} s^{-1}. Both groups: The control and drought-treated groups were exposed to heat stress at the end of the experiment. Heat stress (48 °C) was simulated using the water bath (Memmert WNE 22, Germany) and then chlorophyll fluorescence parameters were measured using two different methods (fast and slow kinetics of chlorophyll *a* fluorescence).

Data relating to the long-term averages (period of 1970–2000) of climatic variables for the origin of each provenance were obtained from the WorldClim model (Fick et al. 2017). The forest aridity index (FAI) was calculated using the following equation according to (Führer et al. 2011):

$$FAI = 100\,[T_{VII–VIII} / (P_{V–VIII} + P_{VII–VIII})] \quad [1]$$

where:
$T_{VII–VIII}$ – the mean air temperature during July and August,
$P_{V–VIII}$ – the sum of precipitation from May to July,
$P_{VII–VIII}$ – the sum of precipitation during July and August.

This index is derived from the mean air temperatures in July and August, the sum of the precipitation during the main growth period from May to July, and the sum of precipitation for July and August (Petrík et al. 2020).

2.2. Fast kinetics of chlorophyll fluorescence

Fast kinetics of Chl *a* fluorescence was measured using the Handy PEA (Hansatech Instruments Ltd., United Kingdom) fluorimeter. Samples were shaded using leaf clips for 0.5 h, and then the abaxial part of the clipped needles was subjected to light for 1 second via a saturation light with intensity of 3,500 µmol m^{-2} s^{-1}. Subsequently, Chl *a* fluorescence parameters derived from the OJIP curve were detected. Fundamental parameters of Chl *a* fluorescence were assessed: Fv/Fm – the maximum quantum yield of PSII photochemistry calculated as the ratio of variable Chl *a* fluorescence (Fv) to the maxi-

Table 1. Origin of the Norway spruce provenances with geographical data and mean values of climate characteristics with calculated Forest Aridity Index (FAI).

PV	Altitude (m a.s.l.)	Longitude	Latitude	Locality	Annual Precipitation (1970–2000) (mm)	Annual air temperature (1970–2000) (°C)	Forest aridity index (FAI)
Pa-650	650	48°46′	19°24′	Pohronský Bukovec	808	6.88	3.55
Pa-870	870	49°09′	19°25′	Prosečné	951	6.60	2.65
Pa-1060	1,060	48°59′	19°48′	Tajch	1,011	4.58	2.18
Pa-1500	1,500	48°57′	19°27′	pod Chabencom	1,155	3.42	1.83

mum Chl *a* fluorescence (Fm); RC/ABS – the number of active reaction centres per antenna complex based on the absorption; the index of photochemical activity – performance index based on absorption of light energy – PI_{ABS} and parameter $(1 – V_J) / V_J$ – the measure of forward electron transport, J – step represents an important point of inflection occurring at 2.0 ms at the fluorescence OJIP curve.

2.3. Rapid light curve measurements

The parameters associated with slow kinetics of Chl *a* fluorescence were ascertained using a Mini-PAM (Heinz Walz GmbH, Germany). These parameters were measured on juvenile needles obtained from five Norway spruce seedlings per provenance and treatment. The RLCs were automatically measured under the protocol of Mini-PAM. The actinic light was applied in eight steps, with different PAR intensities from 38 to 616 µmol m^{-2} s^{-1}, each lasting 10 seconds. Illumination periods were interspersed with a 0.8-second saturating pulse from a white halogen lamp (PAR of 2,000–3,000 µmol m^{-2} s^{-1}). The analysed parameters were derived from RLC: Φ_{PSII} – the effective quantum yield of the PSII; q_P – coefficients of photochemical quenching (Zivcak et al. 2013) and q_N – coefficient of non-photochemical quenching; NPQ – non-photochemical quenching. These both these parameters (q_N and NPQ) represent indicators of excess energy in the light or heat-dissipated excitation energy within the antenna complexes.

2.4. Statistical analysis

The data obtained were processed using Statistica 7.1 (StatSoft Tulsa, USA). Variation among treatments and provenances was assessed using a two-way analysis of variance (ANOVA). The size of the differences among the groups (C, D, H, D + H) in individual provenances, as well as among the provenances in individual group/treatment, were tested using Tukey's HSD post hoc test. Prior to the ANOVA, the normality of the data distribution was tested using the Shapiro-Wilk test.

3. Results

According to the soil water potential (Fig. 1), the experiment was completed when the SWP values in most of the spruce provenances decreased below the detectable value –1.5 MPa. A significant decrease was observed especially after day 21, during which SWP dropped below –0.8 MPa. However, the SWP decrease was not uniform for all provenances, with the steepest decline at provenance Pa-1060, and the most gentle decline at provenance Pa-1500.

The two-way ANOVA revealed that the measured parameters of slow- and fast kinetics of chlorophyll *a* fluorescence reacted to the treatments across all provenances. Significant inter-provenance variation indicates differences among provenance responses; however, treatment-by-provenance interactions were not significant, except for q_N (Table 2).

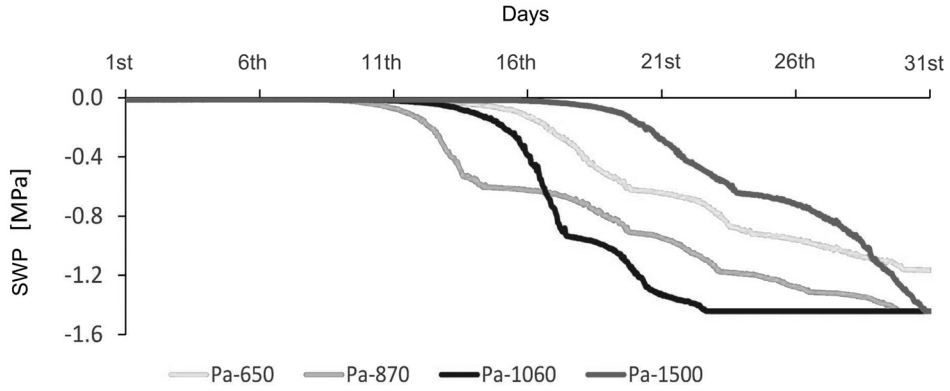


Fig. 1. Course of soil water potential (SWP (MPa)) recorded at one-hour intervals for the individual Norway spruce provenances during one month of soil drought stress.

Table 2. Two-way analysis of variance of chlorophyll *a* fluorescence parameters (SS – sum of squares, MS – mean square, F –statistics, and p – values).

Parameter	Treatment				Provenance				Treatment × Provenance			
	SS	MS	F	p	SS	MS	F	p	SS	MS	F	p
Fv/Fm	1.7	0.6	33.1	<0.0001	0.4	0.1	7.7	0.0001	0.2	0.03	1.6	0.1448
RC/ABC	9.0	3.0	49.7	<0.0001	0.8	0.3	4.2	0.0086	1.0	0.1	1.8	0.0963
PI_{ABS}	103.5	34.5	107.9	<0.0001	3.1	1.03	3.2	0.0280	2.6	0.3	0.9	0.5232
$(1–V_j)/V_j$	1.1	0.4	39.8	<0.0001	0.2	0.08	8.3	0.0001	0.1	0.01	1.5	0.1662
Φ_{PSII}	2.4	0.8	72.7	<0.0001	0.5	0.2	15.2	<0.0001	0.2	0.02	2.0	0.0520
NPQ	40.8	13.6	50.6	<0.0001	13.3	4.4	16.5	<0.0001	3.6	0.4	1.5	0.1703
q_P	0.6	0.2	36.9	<0.0001	0.03	0.01	2.3	0.0908	0.09	0.01	2.0	0.0512
q_N	1.2	0.4	33.6	<0.0001	0.6	0.2	15.9	<0.0001	0.4	0.04	3.7	0.0009

The RC/ABS, $(1–V_j)/V_j$, and PI parameters of fast kinetics of chlorophyll *a* fluorescence were influenced by individual stresses similarly, with no statistical differences among D, H, and D + H treatments in most of the provenances (Fig. 2B–D). The parameter $(1 – V_J)/V_J$ was the last parameter to be influenced by drought stress in Pa-1500 provenance from the highest altitude, and also in Pa-650 provenance from the lowest altitude. In comparison to heat stress, drought did not cause such a significant decrease in the maximum quantum yield of PSII (Fv/Fm) in most of the spruce provenances, except for the Pa-1060 provenance (Fig. 2A). In provenance Pa-1500, the values of fast kinetics parameters did not significantly decrease during drought. Only heat and combined drought + heat stresses affected these measured parameters significantly, except for the parameter $(1 – V_J)/V_J$.

It is possible to derive more detailed information about the treatment's effects on photosynthesis from the analysis of RLC – slow kinetics of Chl *a* fluorescence (Fig. 3, Fig. 4). The actual efficiency of photochemistry (Φ_{PSII}) decreased significantly in D treatment for all provenances except P-1500 (Fig. 3D). The decrease caused by the heat stress was more pronounced in all provenances in comparison with drought stress. Based on the areas below the curves (Fig. 3D), as well as the values of the first RLC points (Table 3), we can conclude that provenance Pa-1500 was able to maintain the highest Φ_{PSII} during the influence of drought (0.771 on average), heat stress (0.482 on average) and combined effect (0.544 on average) of these factors. Regarding NPQ, we observed the highest values in Pa-1500 (above 1.0 in D + H treatment). In drought treatment, Pa-650 and Pa-1500 provenances were able to maintain relatively high levels of thermal dissipation (1.8–2.4) (Table 3, Fig. 3A, Fig. 3D).

In Pa-1060, the ability to dissipate excess energy decreased gradually with the increase of drought, heat stress, and the combined effect of these two factors (Fig. 3C). Interestingly, NPQ significantly decreased to a similar level (below 0.5) in all D, H, and D + H treat-

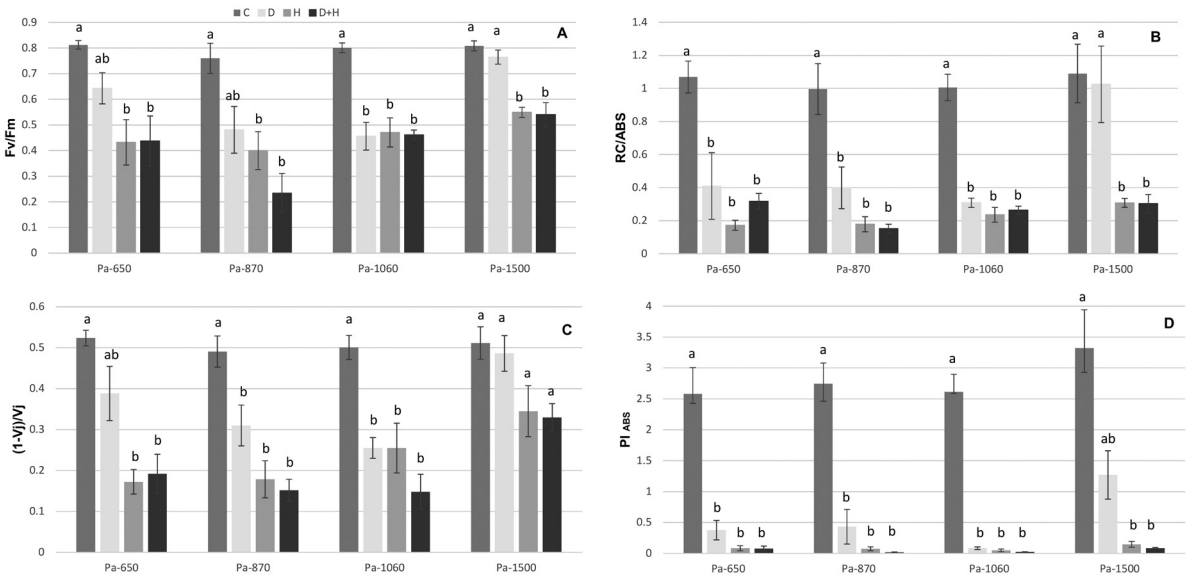


Fig. 2. The changes in the efficiency of photosystem II: A) Fv/Fm – the maximum quantum yield of PSII photochemistry; B) RC/ABS – the number of active reaction centres per antenna complex based on the absorption; C) $(1–V_J)/V_J$ – the measure of forward electron transport; D) PI_{ABS} – performance index of photochemical activity based on the absorption. The graph's bars present means ± SE. Different small letters represent statistically significant differences among the treatments: control (C), drought (D), heat stress (H), drought + heat stress (D + H) at $p < 0.05$ according to post hoc Tukey test.

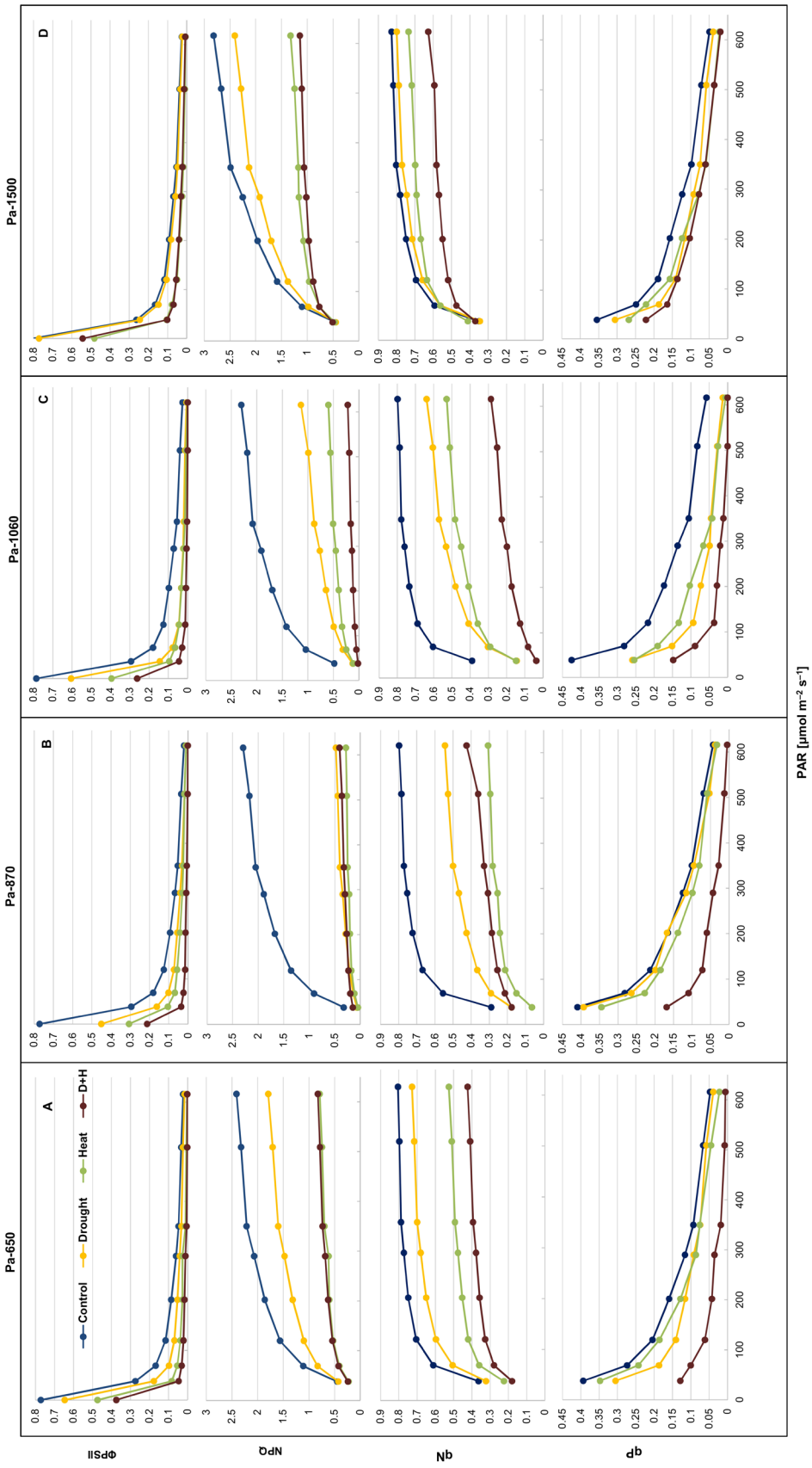


Fig. 3. Rapid Light Curves (RLCs) of fluorescence parameters Φ_{PSII}, NPQ, q_N and q_P per different Norway spruce provenances: A) Pa-650, B) Pa-870, C) Pa-1060 and D) Pa-1500; and per different treatments: control, drought, heat, and combined drought + heat stress (D + H).

Table 3. Variability of the individual parameters of the slow kinetics of chlorophyll a fluorescence (Φ_{PSII}, q_P, q_N, NPQ) among different treatments (C – control, D – drought, H – heat, combined D + H). Different small letters indicate the statistically significant differences among the treatments (ANOVA, post hoc Tukey test). Data represent the average values with their standard errors.

Treatment	Provenances			
	Pa-650	Pa-870	Pa-1060	Pa-1500
		Φ_{PSII}		
C	0.772 ±0.02^{a}	0.772 ±0.02^{a}	0.785 ±0.01^{a}	0.806 ±0.01^{a}
D	0.646 ±0.05^{b}	0.646 ±0.05^{b}	0.605 ±0.06^{b}	0.771 ±0.03^{a}
H	0.474 ±0.05^{bc}	0.474 ±0.05^{bc}	0.396 ±0.01^{c}	0.482 ±0.03^{b}
D + H	0.375 ±0.08^{c}	0.375 ±0.08^{c}	0.262 ±0.03^{c}	0.544 ±0.04^{b}
		q_P		
C	0.393 ±0.02^{a}	0.410 ±0.01^{a}	0.423 ±0.04^{a}	0.355 ±0.03^{a}
D	0.306 ±0.04^{a}	0.393 ±0.03^{a}	0.259 ±0.04^{b}	0.355 ±0.03^{a}
H	0.348 ±0.03^{a}	0.345 ±0.02^{a}	0.255 ±0.02^{b}	0.268 ±0.02^{ab}
D + H	0.129 ±0.05^{b}	0.169 ±0.02^{b}	0.148 ±0.04^{b}	0.222 ±0.05^{b}
		q_N		
C	0.805 ±0.02^{a}	0.798 ±0.01^{a}	0.794 ±0.02^{a}	0.828 ±0.01^{a}
D	0.728 ±0.05^{ab}	0.544 ±0.08^{b}	0.639 ±0.06^{ab}	0.800 ±0.02^{a}
H	0.576 ±0.08^{b}	0.307 ±0.05^{c}	0.529 ±0.04^{b}	0.735 ±0.03^{ab}
D + H	0.564 ±0.11^{b}	0.424 ±0.05^{bc}	0.286 ±0.05^{c}	0.627 ±0.09^{b}
		NPQ		
C	2.410 ±0.23^{a}	2.294 ±0.17^{a}	2.221 ±0.24^{a}	2.816 ±0.18^{a}
D	1.793 ±0.49^{ab}	0.477 ±0.06^{b}	1.136 ±0.22^{b}	2.406 ±0.28^{a}
H	0.789 ±0.26^{b}	0.280 ±0.06^{b}	0.599 ±0.07^{bc}	1.323 ±0.15^{b}
D + H	0.827 ±0.29^{b}	0.404 ±0.24^{b}	0.220 ±0.05^{c}	1.144 ±0.46^{b}

ments. In the case of the coefficients of photochemical- and non-photochemical quenching, these were the most negatively affected in Pa-1060 (Fig. 3C), in comparison with the less negatively affected coefficients in Pa-1500 (Fig. 3D). After the influence of combined stresses, we observed the qN level at about 0.6 in Pa-1500, but only at about 0.3 in Pa-1060 (Table 4). Individual drought, as well as individual heat, caused the highest decrease of q_N in provenance Pa-870 (Fig. 3B), however, the combined stress effect was most pronounced in provenance Pa-1060 (Fig. 3C). Photochemical quenching was significantly influenced only in D + H treatment in Pa-650, Pa-870, and Pa-1500 provenances. For Pa-1060 provenance, Q_p decrease was observed, even under the individual treatments (D, H) (Fig. 3D).

Overall, drought had a lesser effect on fluorescence parameters, in comparison with heat. In the majority of cases, values of the fluorescence parameters did not dif-

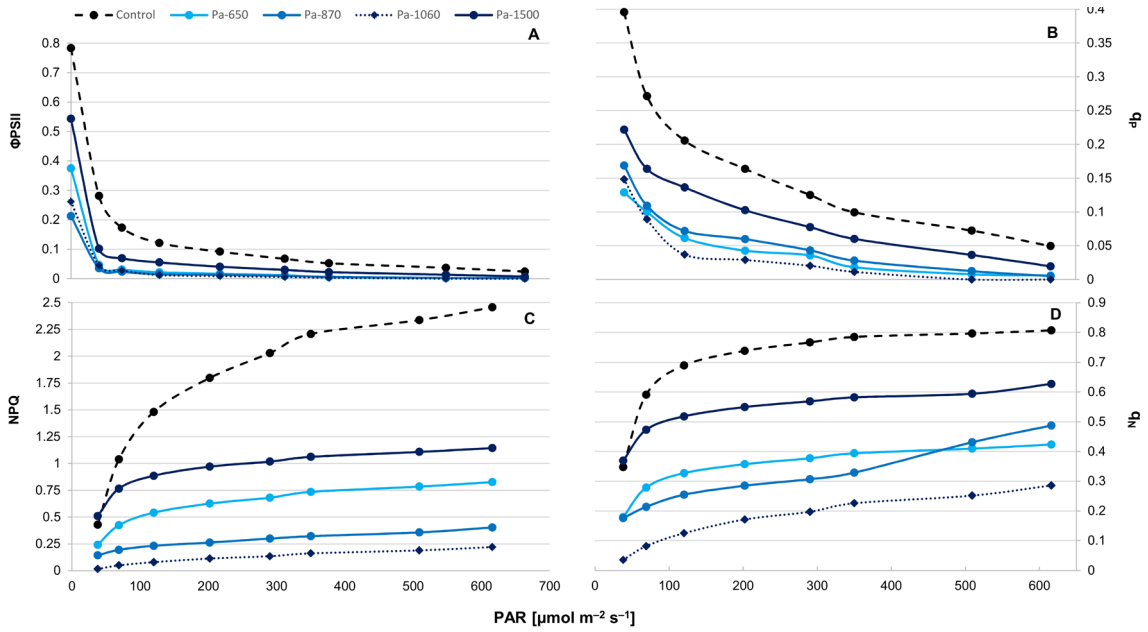


Fig. 4. Variability of Rapid Light Curves (RLCs) of fluorescence parameters: A) Φ_{PSII}, B) q_P, C) NPQ, D) q_N among different Norway spruce provenances under combined drought + heat stress (D + H). Control represents the average value of all controls in individual provenances.

Table 4. Variability of the individual parameters of slow kinetics of chlorophyll *a* fluoresceence (Φ_{PSII}, q_P, q_N, NPQ) among different Norway spruce provenances under combined drought + heat stress (D + H). Control represents the average value of all controls in individual provenances. Different letters indicate the statistically significant differences among provenances (ANOVA, post hoc Tukey test). Data represent the average values with their standard errors.

	D + H Treatment			
Provenances	Φ_{PSII}	q_P	q_N	NPQ
Control	0.784 ±0.02^{a}	0.396 ±0.01^{a}	0.807 ±0.02^{a}	2.460 ±0.15^{a}
Pa-650	0.375 ±0.08^{bc}	0.129 ±0.05^{b}	0.424 ±0.11^{bc}	0.827 ±0.29^{bc}
Pa-870	0.213 ±0.07^{c}	0.169 ±0.02^{b}	0.488 ±0.05^{bc}	0.404 ±0.24^{bc}
Pa-1060	0.262 ±0.03^{c}	0.148 ±0.04^{b}	0.286 ±0.05^{c}	0.220 ±0.10^{c}
Pa-1500	0.544 ±0.04^{b}	0.222 ±0.05^{b}	0.627 ±0.09^{b}	1.144 ±0.46^{b}

fer significantly between H and D + H treatments. However, combined drought and heat stress significantly influenced all parameters of the slow kinetics of Chl *a* fluorescence in all provenances (Table 4, Fig. 4). Moreover, variability among provenances in responses to this type of stress was proved by the exhibition of the highest values of Φ_{PSII} (Fig. 4A), q_N (Fig. 4C) and NPQ (Fig. 4D) in Pa-1500 provenance.

4. Discussion

Forest trees are frequently exposed to abiotic stresses, such as drought, high temperature, and their combined impact. A precipitation deficit and heat periods often intensify the inadequate growth conditions for spruce during the vegetation season, which usually leads to the loss of optimal conditions for survivability and production in Central Europe (Allen et al. 2010; Petrík et al. 2023). As stress tolerance is the major target of much research, the efficient and reliable methods and tools that are useful in the screening of these stress effects are required. In this study, we present several fluorescence techniques that provide information about the leaf photosynthetic efficiency and photosystem II performance in spruce seedlings of 4 provenances under different stresses. The study of their physiological response to heat and drought conditions can help to better understand their reactions, which also differ across regions.

The experiment confirms that drought and thermal stress negatively influences the photosynthetic process in spruce seedlings, but not to the same extent in all cases. Overall, (in most cases) fluorescence parameters were more affected by heat and combined heat + drought stress than by individual drought. The drought effect on chlorophyll fluorescence parameters depends on the degree of water deficit or the length of the stress influence (Kalaji et al. 2016). The combination of high temperature and drought affected the performance of photosynthesis more intensively than the effect of these factors separately, but for many parameters the difference between H and D + H treatments was not statistically significant. It may be caused by the fact that drought, to a certain extent, increases the resistance of the photosystem to high temperatures (Wang et al. 2010). The antagonistic effect of water deficit, and the effect of high temperature, can increase the resistance of PSII to the action of high temperature during dehydration (Lu & Zhang 1999). The increased thermostability of PSII is associated with the accumulation of soluble components that are synthesised during drought stress and contribute to the stabilisation of the thylakoid membrane, which is directly affected by temperature stress (Berry & Björkman 1980).

According to the progressive decline of the SWP, some of the provenances were able to maintain higher values of SWP for a longer period. The capacity to reduce drought-induced needle dehydration could provide an indication of the relative degree of drought tolerance of the investigated provenances (Schiop et al. 2017). The Pa-1500 provenance showed better photosystem performance, not only under drought treatment, where SWP was maintained at –1.2 MPa, but also under temperature stress. It may indicate that the photosynthetic response of individual spruce provenances to extreme heat cannot be separated from their responses to drought stress, and this may be in connection with their adaptation strategy.

According to Kunert et al. (2020), spruce species mortality is less related to drought stress and more related to low thermal tolerance; hence, Norway spruce has a more negative wilting point than most species. High temperature caused serious damage which is reflected in the parameters of chlorophyll *a* fluorescence in almost all of the tested provenances, except for the Pa-1500, with its origin in the highest elevation in 3 tested parameters (q_P, q_N, $(1–V_J)/V_J$). In the study of the impact of heat stress on 6 tree species, Kunert et al. (2022) observed relatively low thermotolerance of Norway spruce compared to other tested species, despite the fact that drought tolerance in Norway spruce was relatively high compared to other tested tree species. Indeed, the thermotolerance of conifers is usually lower than in broadleaved species (Húdoková et al. 2022; Münchinger et al. 2023). Nevertheless, thermal tolerance of Norway spruce does not depend only on environmental stress conditions, but it is primarily influenced by the inherent ability to cope with high temperatures, which is evolved in trees growing on specific sites across generations (Wang & Ingvarsson 2023). When trees need to withstand high temperatures or dry conditions, they can use transpiration to cool the plant organism, in order to prevent overheating of the assimilation apparatus or close stomata to prevent loss of

water (Zavadilová et al. 2023). Therefore, the trees that are tolerant to drought could develop several adaptations to cope with higher temperatures (Henry et al. 2019). Exposure to heat stress thus exhibits a considerable threat to conifer species in forests of temperate climate systems (Kunert et al. 2022). While drought affects forest ecosystems during longer periods, the impact of heat on all ecosystem levels usually intensifies.

During summer heat waves and dry periods, Norway spruce growing on steep and sun-exposed stands are faced with overheating of their assimilation apparatus; whereby, leaf temperature can be even higher than the air temperature. While in well-watered trees leaf temperature is usually cooler than the air temperature in the range of 3–7 °C, in drought-stressed plants, leaf temperature can rise and become 3–5 °C greater than the temperature of the environment (Cook et al. 2021). Plants are able to adapt to heat stress by temporary reciprocal conversion of PSII heterogeneity up to 40 °C (Mathur et al. 2014). When seedlings are exposed to high temperatures of 42–43 °C, chlorophyll fluorescence parameters indicate the impairment of the acyclic photophosphorylation and donate water to PSII. After exposure to temperatures in the range of 44–46 °C, the first injuries appear in the Calvin cycle when the need to use NADPH and ATP are observed. Bigras et al. (2000) observed visible damage of needles and low photochemical efficiency after heat stress in families with intensive height growth, which were more vulnerable to high temperatures than families with lower height growth after the same treatment. Temperatures close to the range 45–48 °C enhance degradations of light-harvesting complexes, thus causing the occurrence of their disconnection from reaction centres of PSII, and damage of the electron-transport chain, similar to the denaturation of D1 and D2 proteins (Mathur et al. 2014).

The parameters of the fast Chl *a* fluorescence indicated the reduced capacity of the primary photosynthetic processes. Fv/Fm represents an average value of the efficiency for all the PSII units in the measured excited cross-section, and also the units with inactivated reaction centres (Brestic & Zivcak 2013). It is a relatively stable parameter, that is insensitive to stomatal effects, which usually reflects only strong drought stress or prolonged water deficit, e.g. in field conditions. The Fv/Fm measurements during drought stress make sense, as they can draw attention to the effects of co-occurring stresses (heat stress, photoinhibition, etc.), or to the early phases of leaf senescence. On the other hand, parameters PI_{ABS}, RC/ABS, and $(1–V_J)/V_J$ are linked with the efficiencies of electron transfer in PSII, which are more sensitive to stress than the parameter that is related to maximum quantum yield of PSII (Fv/Fm) (Kalaji et al. 2012; Zurek et al. 2014). PI_{ABS} is found to be a very sensitive parameter, reflecting the functionality of both photosystems II and I, and it provides quantitative information on the current state of plant performance under stress conditions (Strasser et al. 2004). Moreover, a very good positive correlation between PI_{ABS} and CO_2 assimilation capacity under drought stress was observed (Van Heerden et al. 2007). Thus, the different sensitivities of these parameters of the fast chlorophyll *a* fluorescence was observed in our study, with a lower sensitivity of Fv/Fm, and the highest sensitivity for the PI_{ABS} parameter. However, strong heat stress significantly influenced a stable parameter such as Fv/Fm, and its values significantly dropped to under the disturbance limit of 0.725 (Critchley 1998).

A significant decrease in parameters of fast kinetics of Chl *a* fluorescence derived from RLCs was observed mainly in provenances Pa-870 and Pa-1060. The measurement of slow fluorescence kinetics reflected both stomatal and non-stomatal effects (Baker 2008). As for spruce species, abscisic acid (ABA) signaling with consequent rapid stomatal closure plays a key role in response to drought stress. Stomatal conductance appears to play only a minor role in the temperature limitation of photosynthesis. Higher temperatures increase the rate of CO_2 absorption, however, at around 27 °C, the reverse reaction occurs. According to Salvucci & Crafts-Brandner (2004), a decrease in CO_2 assimilation is associated with the inhibition of RuBisCo activation via a direct effect on RuBisCo activase. Instead of further converting CO_2 and water into sugars, sucrose is converted back into CO_2 and water. This even occurs when leaf temperatures rise to 40 °C or higher, as plants burn more carbon dioxide through photorespiration than they take in from the atmosphere. Norway spruce as a C3 plant disposes of higher photorespiration under stressful conditions, which consumes part of the electrons flowing within the linear electron transport chain. Photorespiration can also be understood as a certain form of plant protection in the event of a reduction/absence of CO_2 intake, resulting in the formation of precursors for the synthesis of certain amino acids.

In the case of tree assimilatory organs being unable to efficiently utilise the absorbed energy in photochemical processes, incoming photons can cause severe damage to the reaction centres. This leads to photoinhibition, where the formation of photochemically inactive reaction centres, that are capable of converting excitation energy into heat, can protect the surrounding photosystems from potential damage (Zhang & Xu 2003). Further, alternative paths of electron transport are also used, such as the aforementioned photorespiration, and the Mehler reaction, which release excess energy and protect against oxidative stress. Our study suggests that some of the provenances (Pa-1500 and Pa-650) had used their greater ability for non-photochemical dissipation (NPQ, q_N) of absorbed energy in the form of heat under stressful conditions. A higher non-radiative dissipation capacity is correlated with an increased supply of violaxanthin, antheroxanthin, zeaxanthin, and the content of deepoxanthophylls (Demmig-Adams & Adams 1996). It was

also found that zeaxanthin is able to directly quench the energy of excited chlorophylls. Interestingly, the actual fraction of photochemically active PSII reaction centres (unconnected PSII units, q_p) was significantly declined only under the combined drought and heat stress in most of the provenances, except Pa-1060. In this provenance, also individual stress factors (heat, drought) significantly decreased these photochemically active centres.

Observable intraspecific variations in the response of PSII to drought and heat were revealed across the altitudinal gradient of origins of the tested provenances. Our findings revealed that provenances from the lowest altitude (Pa-650), and the highest altitude (Pa-1500), did not respond to drought by a significant decrease in more assessed parameters, while in the provenances with the optimal conditions for Norway spruce distribution and similar FAI (Pa-870, Pa-1060), mostly a significant decrease of Chl *a* fluorescence parameters were observed. Pa-650 population originating from a more arid region with higher temperatures and lower precipitation during the vegetation season could develop better mechanisms of protection and defense against these abiotic stressors. On the other hand, however, the seedlings from Pa-1500 provenance, originating from the most humid area with the lowest average temperatures, seem to cope with drought stress very well. Under these alpine conditions (1,500 m a.s.l.), the seedlings could develop adaptation mechanisms that are linked to stony soils with low water availability, winter stress, overheating, and high irradiation of assimilative apparatus, which would also help to cope with drought conditions.

Kapeller et al. (2012), in their study of different Norway spruce populations across the eastern Alpines, assume that the impact of climate warming is different for individual population groups. In general, variability in climate response increases with higher temperatures and less precipitation. Moreover, those authors observed that populations originating from currently warm and drought-prone regions appear to be well-suited to their specific climate conditions, and could be viable candidates for expanded utilisation in the future. Zhang et al. (2018) also tested physiological responses to drought and long-term high temperatures in spruce species in northern China. Those authors found that *Picea crassifolia* Kom. from higher elevations exhibited greater photosynthetic acclimation than *Picea wilsonii* Mast., and the differences were mainly related to variations in stomatal conductance and the Fv/Fm.

Several studies claim that tree provenances originating from elevated altitudes face a more demanding setting, that is characterised by lower temperatures, increased elevations, and the possibility of arid conditions. Over successive generations, these provenances have probably evolved physiological mechanisms in order to effectively manage intermittent drought stress (Pšidová et al. 2018; Jamnická et al. 2019; Marešová et al. 2022; Zavadilová et al. 2023).

5. Conclusions

In natural conditions, it is complicated to eliminate the action of any of the abiotic factors of the environment. The mutual relationship between temperature and drought can be manifested by a synergistic, but also antagonistic, effect on the physiological processes of woody plants. Therefore, in this experiment with young spruce seedlings, we aimed the study the effect of individual soil drought stress, individual high-temperature stress, and their combined effect. We used effective fluorescence tools for the rapid screening of stress factors impacting the photosynthesis process. Heat stress and combined stressful conditions (drought and heat), influenced more photosynthesis efficiency and photosystem II performance than individual drought. Our results show that provenances Pa-1060 and Pa-1500 are the most different to each other in terms of greater adaptability of provenance from the highest elevation to the stressful conditions. These differences may be caused by existing adaptations to specific site conditions (humidity, temperature, freezing, etc.) in the place of their origin.

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