

Different response of photosynthetic apparatus to high-light stress in sporotrophophyll and nest leaves of *Platyserium bifurcatum*

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Abstract

Tropical forest deforestation leads to sudden changes in light conditions, which is a problem for the development of epiphytes, such as *Platyserium bifurcatum*. High light (HL) results in disturbances of the light phase of photosynthesis and changes in leaf pigment composition. The current study determined differences in fast chlorophyll fluorescence transients and changes in radiation reflectance in sporotrophophylls and nest leaves after one and seven days of HL. In addition, a spectral range was identified with the largest change in reflectance induced by HL. Sporotrophophylls exhibited a stronger response of the photosynthetic apparatus to photoinhibition than that of the nest leaves. Moreover, in sporotrophophylls, disorders of electron transport were visible in the whole O-P phase, and in the nest leaves mainly in the O-J phase. Higher accumulation of photoprotective compounds in sporotrophophylls underlined a different reaction to HL.

Additional key words: epiphyte; fern; leaf reflectance; OIJP test.

Introduction

Plants growing in the natural environment are exposed to the simultaneous impact of many abiotic and biotic stress factors, referred to as multistress. It results in the need for developing defense mechanisms at the structural and functional level both on the basis of adaptation inherited from generation to generation, as well as acclimation, which is a direct response to changes in the environment. One of the components of the multistress is high light, often associated with water deficit (Vanhee *et al.* 2011). Practically, every stress factor has a direct or indirect effect on photosynthesis and can cause damage to photosynthetic apparatus (Lichtenthaler and Burkart 1999).

Light is a key factor in the process of photosynthesis. Paradoxically, often the amount of light reaching plants is higher than the plant needs. In such situations, excess light becomes a stress factor that can damage the photosynthetic apparatus. Therefore, the influence of light stress on the photosynthetic apparatus is extensively studied and has been widely described in many scientific papers (for review see Li *et al.* 2009). Imbalance between quantity of light energy and low supply of ADP and NADP⁺ plays an important role and it can lead to disturbances in the

functioning of PSII (Force 2003, Weng *et al.* 2005). Long-term effects of mild to moderate light stress may induce changes in the spatial arrangement of chloroplasts, cause a decrease in chlorophyll (Chl) content or a reduction in the number of active LHCII. Plasticity in the functioning of chloroplasts, *i.e.*, changes in photosystem stoichiometry and increasing plant tolerance to HL (Chernev *et al.* 2006). However, the rapid increase in the light intensity often induces reduction in the photosynthetic capacity of plants, so-called photoinhibition (Bertamini *et al.* 2004). For this reason, the plants have developed various photoprotective strategies, which are the subject of many scientific works. They rely, for example, on the detoxication of reactive oxygen species, which arise after absorption of excess light energy, or on the dissipation of excess energy as heat (Logan *et al.* 2014, Brestic *et al.* 2015, Foyer 2018, Malnoë 2018). In thermal dissipation of excess energy, protective pigments such as anthocyanins play an important role (Cooney *et al.* 2018, Demmig-Adams 2018, Gould *et al.* 2018). In addition, some plants (including ferns, for example *Polystichum acrostichoides*) limit the absorption of light by changing the leaf angle (Forget *et al.* 2018). The plant's response to excess light is also dependent on its growth rate (Demmig-Adams *et al.* 2017). The shade plants may exhibit high susceptibility to photoinhibition.

Received 4 June 2018, accepted 12 September 2018.

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Abbreviations: ABS/RC – apparent antenna size of active PSII RC; Area – surface area; ARI – anthocyanin reflectance index; Chl *a* – chlorophyll *a*; CRI – carotenoid reflectance index; DI₀/RC – total energy dissipation not trapped by the PSII RC; ET₀/RC – rate of electron transfer by the active PSII RC; F₀ – minimum fluorescence; F_v/F₀ – indicator of inhibition of electron donation by OEC; FL – fluorescence; FRI – flavonol reflectance index; F_v/F₀ – indicator of structural damage of thylakoids; F_v/F_M – maximum quantum yield of PSII; HL – high light; M₀ – rate of closure of reaction centers; OEC – oxygen-evolving complex; PI – PSII vitality index; PQ – plastoquinone pool; PRI – photochemical reflectance index; RE₀/RC – quantum yield of electron transport from Q_A⁻ to the PSI end electron acceptors; RC – reaction center; RD – reflectance difference; SIPI – structure-insensitive pigment index; S_M – pool size of the electron acceptors Q_A on the reducing side of PSII; TR₀/RC – energy trapping of one active reaction center; WBI – water band index; φ_{E0} – quantum yield for electron transport from Q_A⁻ to plastoquinone.

However, in this case, the inactivated PSII cores can act as photoprotectors (Malnoë 2018) and provide protection of the photosynthetic apparatus from damage (Bertamini *et al.* 2004, Demmig-Adams 2018).

The sudden change in light conditions is an increasingly serious problem for valuable species of epiphytic ferns growing in tropical forests like *Platycterium bifurcatum* (Barthlott *et al.* 2001, Adibah and Ainuddin 2011). Changes in light conditions in forests may result from natural causes (death of trees) or from anthropogenic pressure (cutting out large fragments of forest). In the latter case, the intensity of light increases on a large area adjacent to the fragment cut. In addition, anthropogenic pressure in tropical forests contributes to the imbalance of the environment and irreversible destruction of the microclimate, which may result in the loss of species diversity.

Currently, it is believed that drought stress is the most important among the factors limiting the development of epiphytes; other factors (including photoinhibition) are of minor importance (Zotz and Hietz 2001). However, according to current knowledge, such an approach does not allow a proper understanding physiology and ecology of epiphytes, hence there is a need to conduct research on the physiological response of these plants to abiotic stresses. In spite of the ecophysiological research, still little is known about the physiological mechanisms that allow epiphytes to cope with light excess. So far it has been demonstrated that biochemical mechanisms based on energy dissipation *via* xanthophyll, similar to those in spermatophytes, are also present in ferns (Eickmeier *et al.* 1993, Tausz *et al.* 2001).

The previous research on *P. bifurcatum* physiology was primarily associated with ensuring proper growing conditions and micropropagation methods of these ornamental plants (Aspiras *et al.* 2010, Liao and Wu 2011). In addition, drought stress response was investigated by Rut *et al.* (2003), and the subject of intense light stress was studied by Sanusi *et al.* (2011). However, there is still a lack of information on the response of the photosynthetic apparatus to the sudden increase in light intensity, *e.g.*, after tree logging in a large forest area. The hitherto rating of light-shock reaction does not fully correspond to the changes of the light intensity in the natural environment (often PPFD $<1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$). The continuous intensification of human activities in tropical climate forests is therefore an important reason for more extended research.

Platycterium bifurcatum sporophyte, as some other species of epiphytic ferns, is characterized by heterophylly (Fig. 1). Long, ribbon-like sporotrophophylls have an assimilatory role, while nest leaves, which support the plant, show different physiological features and even a different type of metabolism (C_3 in sporotrophophylls, CAM in nest leaves – Rut *et al.* 2008, Oliwa *et al.* 2017). Heterophylly is very rarely considered in ecophysiological studies. However, for an assessment of physiological state of the whole plant, conditions of nest leaves should be taken into account, due to possible translocation of metabolites from nest leaves to sporotrophophylls during their terminal stage (Oliwa *et al.* 2017).

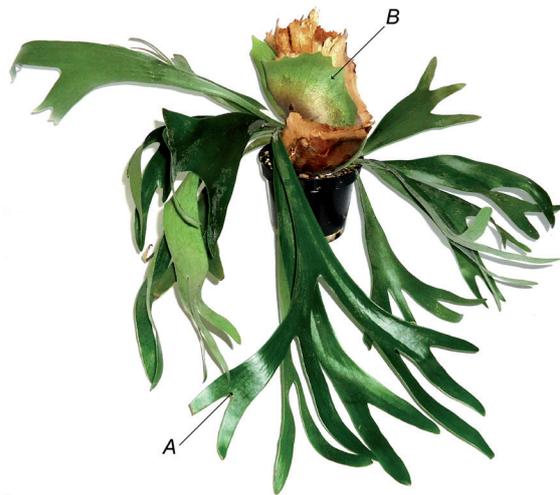


Fig. 1. Epiphytic fern *Platycterium bifurcatum*. The figure shows two types of leaves: sporotrophophylls (A) and nest leaves (B).

The use of nondestructive research methods based on Chl *a* fluorescence (FL) allowed in this study to determine changes at the physiological level occurring in the same leaves after different HL exposure times. Description of the bioenergetic state of the plant using the analysis of Chl *a* fluorescence induction curve (OJIP test) reflects electron transport through subsequent elements of the oxidation-reduction chain and communication between individual PSII units (Kalaji and Guo 2008). The OJIP test and Chl *a* FL parameters are often used for rapid analysis of plant viability and PSII performance estimation under environmental stress conditions (Oukarroum *et al.* 2007, Mehta *et al.* 2010, Kalaji *et al.* 2012, Gururani *et al.* 2015). In turn, reflectance analysis allows to determine changes in leaf chemical composition (including pigment system restructuring) and the degree of light energy utilization (Solovchenko 2010). It also allows to identify the radiation range, in which PAR reflectance spectrum from leaves changes most dramatically in response to light stress (Carter 1993).

HL stress very quickly induces disturbances in the light phase of photosynthesis. Therefore, the aim of this study was to determine differences in the course of the light phase in sporotrophophyll and nest leaves of *P. bifurcatum* after 1 and 7 d from the sudden increase in light intensity. In addition, we investigated whether the HL stress response is accompanied by changes in leaf pigment composition and tissue hydration. An attempt was also made to identify the radiation range, in which the greatest change in reflectance caused by HL occurred (PPFD $\leq 800 \mu\text{mol m}^{-2} \text{s}^{-1}$).

Materials and methods

Plant material and growth conditions: Sporophytes of *Platycterium bifurcatum* grew for six months in an *Angelantoni EKOCH 700* (Massa Martana, Italy) growth chamber. The PPFD was $150 \pm 20 \mu\text{mol m}^{-2} \text{s}^{-1}$, photoperiod 16/8-h (day/night), temperature 25/15°C (day/night), relative humidity of 60%. The analyses were carried out on

sporotrophophyll and nest leaves at their second stage of development (according to the classification of Oliwa *et al.* 2017). In order to elicit an intense light response, the plants were placed for 7 d under sodium lamps (*Philips SON-T AGRO 400W*, The Netherlands), and PPFD was increased to a value of $\leq 800 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Chl *a* fluorescence kinetics: Parameters of PSII photochemical efficiency were measured according to the method described by Strasser *et al.* (2000) using a *Handy-PEA* fluorometer (*Hansatech Instruments*, King's Lynn, UK). The part of intact leaf blade was acclimated to darkness for 20 min using a clip equipped with an iris. Chl *a* fluorescence was induced by PPFD of $3 \text{ mmol m}^{-2} \text{s}^{-1}$. The following fluorescence intensity measurement points were adopted for the OJIP test: O – 20 μs , J – 2 ms, I – 30 ms, P – 300 ms. The differential curves of Chl *a* FL kinetics (ΔVt) were calculated by subtracting the normalized (to O and P points) FL values in plants growing for 1 or 7 d under HL from the normalized values obtained for control. This way of counting makes the differences between treatments more visible. The measurement results were compiled in the *PEA Plus* program (*Hansatech*, UK) and *MS Excel 2010* and selected parameters of the Chl *a* fluorescence kinetics were analyzed: F_V/F_M , F_V/F_0 , Area, PI, S_M , M_0 , ϕE_0 (Strasser *et al.* 2004, Stirbet and Govindjee 2011), F_K/F_J (Srivastava and Strasser 1995) and the following energy flux parameters by RC: TR_0/RC , ET_0/RC , RE_0/RC , DI_0/RC , ABS/RC (Strasser *et al.* 2004, Stirbet and Govindjee 2011). The calculation equations of the used parameters are shown in the following table:

Summary of measured and calculated Chl *a* fluorescence parameters, References: (1) Strasser *et al.* 2004, (2) Yusuf *et al.* 2010, (3) Stirbet and Govindjee 2011.

Parameter	Formula	Reference
F_0	$F_{0.05 \text{ ms}}$	(1)
F_K	$F_{0.3 \text{ ms}}$	(2)
F_J	$F_{2 \text{ ms}}$	(1)
F_I	$F_{30 \text{ ms}}$	(1)
F_M	$F_{300 \text{ ms}}$	(1)
F_V	$F_V = F_M - F_0$	(1)
M_0	$M_0 = 4 (F_K - F_0)/(F_M - F_0)$	(1)
S_M	$S_M = \text{Area}/(F_M - F_0)$	(1)
V_J	$V_J = (F_J - F_0)/(F_M - F_0)$	(1)
V_I	$V_I = (F_I - F_0)/(F_M - F_0)$	(1)
ϕE_0	$\phi E_0 = [1 - (F_0/F_M)] (1 - V_J)$	(1)
ψE_0	$\psi E_0 = (1 - V_J)$	(1)
ABS/RC	$ABS/RC = M_0 (1/V_J) [1 - (F_0/F_M)]$	(1)
TR_0/RC	$TR_0/RC = M_0 (1/V_J)$	(1)
ET_0/RC	$ET_0/RC = M_0 (1/V_J) \psi E_0$	(1)
RE_0/RC	$RE_0/RC = (M_0/V_J) (1 - V_I)$	(3)
DI_0/RC	$DI_0/RC = (ABS/RC) - (TR_0/RC)$	(1)

Radiation reflectance from leaves: Leaf reflectance was measured using a *CID Bio-Science CI-710* spectrometer

(*CID Bio-Science*, Camas, USA) on the upper surface of the leaf. Reflectance spectra in the 400–1,000 nm range were recorded using *SpectraSnap* software. Then the values of content indices of anthocyanins [$ARI_1 = (R_{550}^{-1} - R_{700}^{-1}) R_{800}$, Gitelson *et al.* 2001], carotenoids (Car) [$CRI_1 = (R_{520}^{-1} - R_{550}^{-1}) R_{800}$, Gitelson *et al.* 2002], flavonoids [$FRI = (R_{410}^{-1} - R_{460}^{-1}) R_{800}$, Merzlyak *et al.* 2005] were calculated. In addition, the Car to Chl *a* ratio [$SIPI = (R_{800} - R_{445}) (R_{800} + R_{680})^{-1}$, Peñuelas *et al.* 1995] and tissue hydration index [$WBI = R_{900} (R_{970})^{-1}$, Peñuelas *et al.* 1993] were determined.

In addition to the above parameters, photochemical reflectance index (PRI), constituting an alternative form of assessing photosynthesis quantum yield and PSII functional efficiency, was also determined [$PRI = (R_{531} - R_{570}) (R_{531} + R_{570})^{-1}$, Gamon *et al.* 1992]; it was analyzed separately based on changes in relative values. In the equations, R_x means reflectance intensity at a specific x wavelength.

A reflectance difference curve and a curve allowing identification of the wavelength, at which the numerical reflectance value changes the most due to stress action (sensitivity analysis) were determined according to the method of Carter (1993). Reflectance difference (RD) was calculated according to the formula: $RD = R_{SP} - R_{CP}$, where: R_{SP} – reflectance intensity (%) in the range of 400–1,000 nm in plants subjected to intense light stress, R_{CP} – reflectance intensity (%) in the range of 400–1,000 nm in control plants.

Stress-sensitive wavelengths (sensitivity) was calculated by dividing the RD values by the control values, according to the formula: $\text{Sensitivity} = RD (R_{CP})^{-1}$

Statistical analysis: The results obtained from seven independent replicates for each of the experimental groups were analyzed by the *Statistica 10.0* program (*Statsoft*, Kraków, Poland) using the analysis of variance (ANOVA) test. The significance of differences between means was estimated using *Duncan's* test at a significance level of $p \leq 0.05$.

Results

Analysis of OJIP curves: A strong stress reaction of the photosynthetic apparatus was observed in sporotrophophylls on the first day of growth under HL. There was a significant increase in FL in the O–J phase of the Chl *a* FL induction curve, a decrease in the FL value in the I–P phase, and a complete lack of I step compared with control (Fig. 2A – black and blue lines, respectively). A further increase in FL intensity on the O–I phase was observed after 7 d under HL. There was an increase in the FL in the J step compared with control, indicating a marked reduction in the efficiency of electron transport. At the same time, the deviation from control on the I–P phase was reduced (Fig. 2A – red line). Nest leaves showed a significantly weaker response to HL stress than that of sporotrophophylls (Fig. 3A). The direction of changes was similar, however, alterations in FL intensity in all phases were much smaller than that in sporotrophophylls, and the shape of the OJIP curve did not differ significantly from

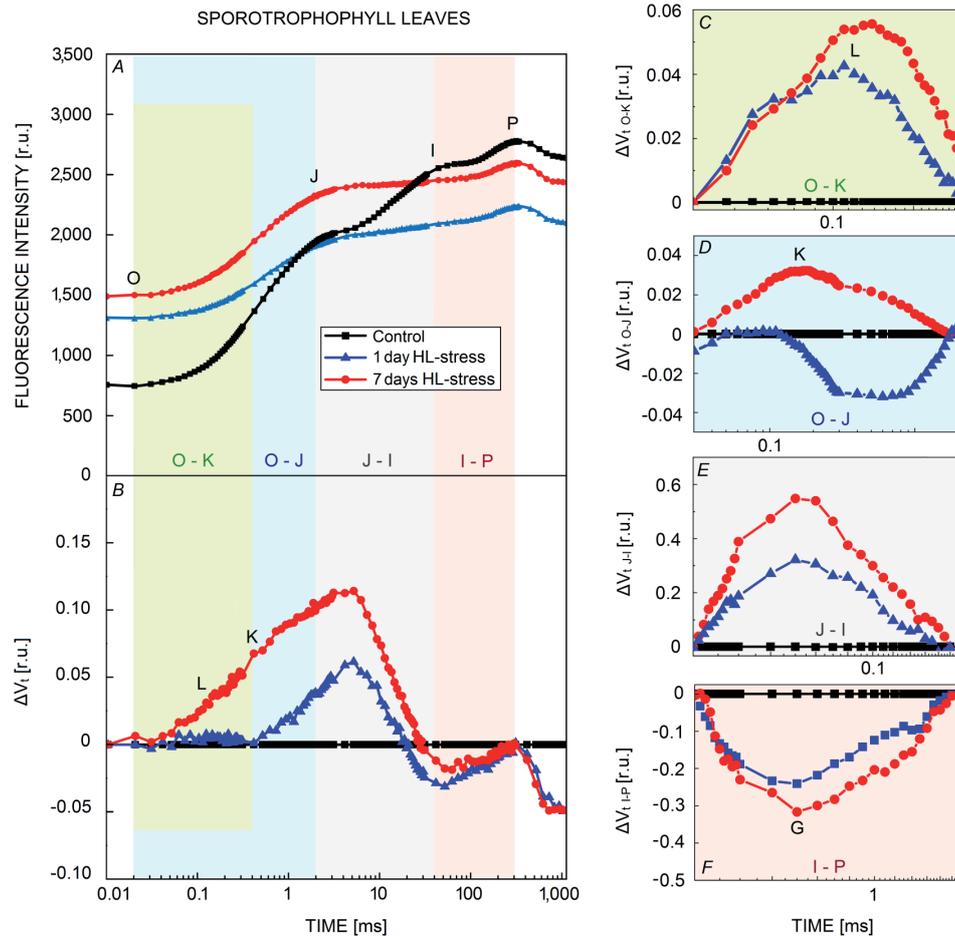


Fig. 2. Induction transients of chlorophyll *a* fluorescence (OJIP) in the sporotrophophylls of *Platycerium bifurcatum* after 1- and 7-d growth under high light and in control conditions (A). Differential curves ΔV_t for the ranges of O–P (B), O–K (C), O–J (D), J–I (E), I–P (F) were obtained by subtracting normalized values of chlorophyll fluorescence intensity of stressed plants from control (see Materials and methods), $n = 7$.

the control. Noteworthy is the lack of significant changes between the 1st and 7th day of growth under HL. It should also be noted that particular steps on OJIP control curves in nest leaves were less marked than in sporotrophophylls (Figs. 2A, 3A).

The differential curves of Chl *a* FL kinetic increase changes (ΔV_t) in sporotrophophylls indicated that significant control deviations occurred over the entire O–P phase (Fig. 2B). However, these differences were significantly lower after one day at HL (especially in the O–I phase) than that after 7 d. Disturbances of the initial stages of the light phase in sporotrophophylls were observed only after a week under HL, which was manifested by an increase in FL at the O–K phase. The main effects of light stress in the nest leaves occurred during the O–J and J–I phases, and the differences between the 1st and 7th day of growth under HL were visible between K–I points (Fig. 3B).

The differential curves for the O–K, O–J, J–I, and I–P phases were calculated analogously to ΔV_t (Figs. 2D–F, 3D–F). The L and K bands, characteristic of stress reaction, were evident in both types of leaves treated with HL on O–K and O–J differential curves, respectively. The L band values in sporotrophophylls were significantly

higher after 7 d than after one day at HL (Fig. 2C). There was no such relationship in the nest leaves (Fig. 3C). However, FL intensity in these leaves was higher than that in sporotrophophylls after the first day at HL (Figs. 2C, 3C). The K band is clearly marked only in the nest leaves (Fig. 3D). The K band in sporotrophophylls occurred only after 7 d at HL (Fig. 2D) and had significantly lower values than that in the nest leaves.

Changes in the course of the J–I phase, compared with the control, were visible especially after a longer HL action. The G band, similarly a characteristic of a strong stress (I–P phase), had higher negative values after 7 d under HL (Fig. 2F). In contrast, the nest leaves growing under stress conditions did not show any significant FL increase at the J–I phase in relation to control (Fig. 3E). There were also no typical G bands, both after 1 and 7 d of HL (Fig. 3F).

Chl *a* FL parameters: Changes in PSII photochemical efficiency parameters (F_M , F_V/F_M , F_V/F_0 , Area, PI, S_M , M_0 , F_K/F_I) in plants growing under HL are shown in Fig. 4 as a percentage deviation from the control values. Disturbances in the functioning of the photosynthetic

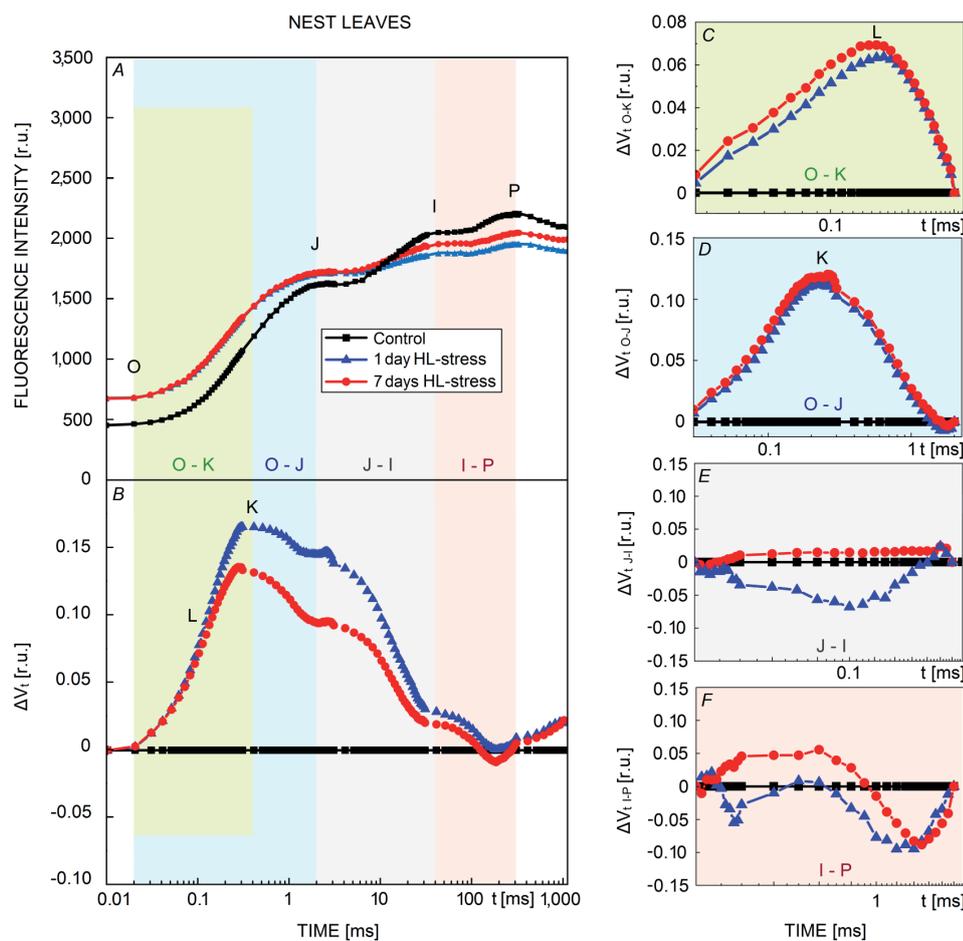


Fig. 3. Induction transients of chlorophyll *a* fluorescence (OJIP) in the nest leaves of *Platycerium bifurcatum* after 1- and 7-d growth under high light and in control conditions (A). Differential curves ΔV_i for the ranges of O–P (B), O–K (C), O–J (D), J–I (E), I–P (F) were obtained by subtracting normalized values of chlorophyll fluorescence intensity of stressed plants from control (see Materials and methods), $n = 7$.

apparatus of sporotrophophylls occurred after the first day of light stress. After this time, a significant decrease in the value F_V/F_0 and Area by at least 50%, and increase in the values of parameters F_K/F_J and M_0 was observed (Fig. 4A). The highest, almost ten-fold decrease was recorded for PI. After 7-d HL, compared to the first day, an increase in the F_M , Area, and M_0 parameters was observed.

A decrease in F_V/F_M , F_V/F_0 , PI, Area, and ϕ_{E0} was lower in the nest leaves compared to sporotrophophylls. Differences between the 1st and 7th day of stress were visible in the PI, Area, F_V/F_0 , S_M , and ϕ_{E0} parameters (Fig. 4B). For the other parameters, no differences were observed between day 1 and 7 of growth under HL. Changes in specific flux parameters in one active reaction center are illustrated in Table 1. An increase in the energy capture and flow through reaction center (RC) (TR_0/RC , ET_0/RC) was recorded after the first day of stress in response to HL in sporotrophophylls. A decrease in RE_0/RC occurred only after 7 d. In the nest leaves, the changes in TR_0/RC and ET_0/RC had a similar trend to that of sporotrophophylls, however, between 1st and 7th day of stress, there was no change in ET_0/RC . The size of the active RC PSII antenna did not change (ABS/RC) under HL conditions. The nest

leaves (both in control and stressed plants) showed greater dissipation of energy than that of sporotrophophylls (DI_0/RC).

Reflectance analysis: In sporotrophophylls, after 7 d of HL stress, the reflectance intensity only slightly increased in the green spectrum (with a peak around 550 nm) whereas in the 700–1,000 nm range was significantly higher as compared with control (Fig. 5A). In nest leaves, an increase in the reflectance intensity was observed in the range of 550–650 nm, while in the far-red part of the spectrum, reflectance decreased under stress conditions (Fig. 5B).

The largest differences in response to stress compared to the control occurred in sporotrophophylls at 535 nm (Fig. 5C). The reflectance difference (RD) curve of the nest leaves was characterized by a completely different shape (Fig. 5D). The minimum difference in RD occurred in the green range at 550 nm, and was the highest at 650 and 690 nm (Fig. 5D). RD in the far-red and near-infrared range had positive values in sporotrophophylls, while negative values in the nest leaves (Fig. 5C,D).

Sensitivity values in sporotrophophylls were the

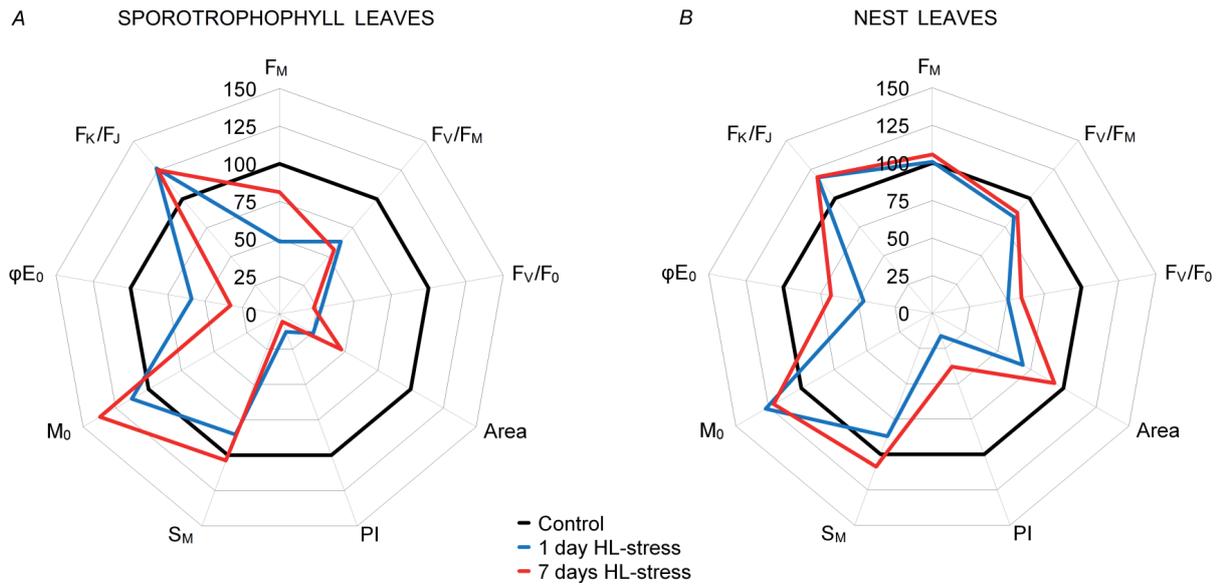


Fig. 4. Values of chlorophyll *a* fluorescence parameters of the sporotrophophyll (A) and nest leaves (B) of *Platycerium bifurcatum* after 1- and 7-d growth in high light (PPFD = 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The values are presented as the percentage of the control, $n = 7$.

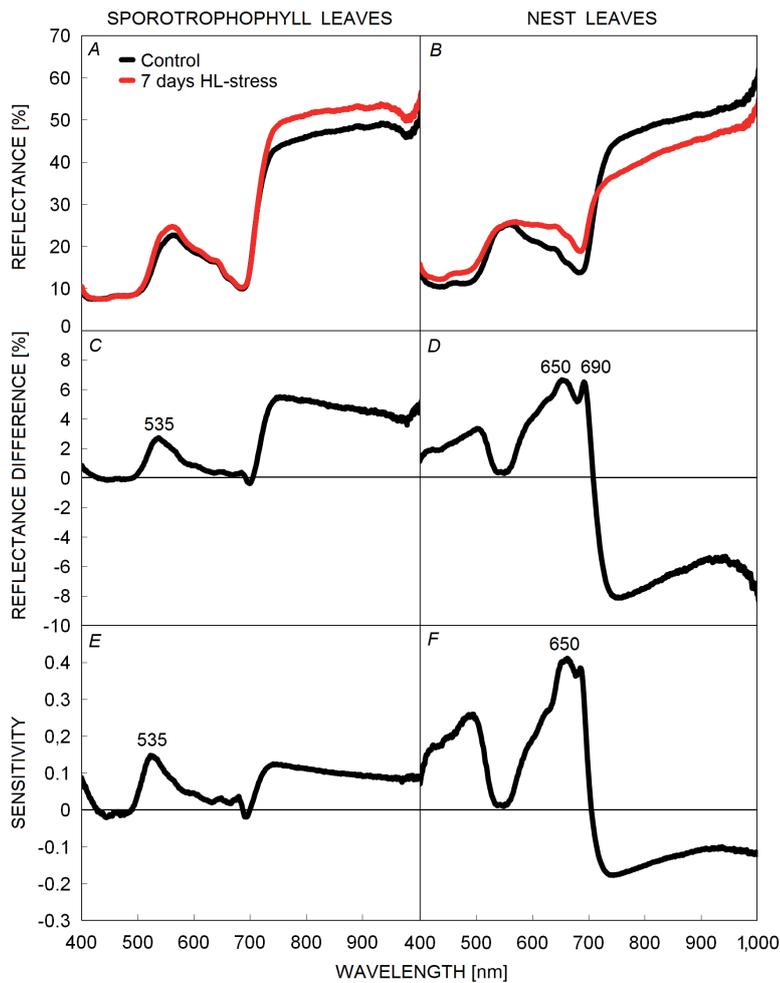


Fig. 5. Intensity of reflectance from sporotrophophylls (A) and nest leaves (B) of *Platycerium bifurcatum* after 7-d growth in high light (PPFD = 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and in control conditions (PPFD = 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Reflectance difference, produced by subtracting the mean values control plants from values of the stressed plants (C,D). Sensitivity (no units), produced by dividing percentage reflectance difference by percentage reflectance values for control plants (E,F; methodology of Carter 1993), $n = 7$.

Table 1. Values of specific energy fluxes expressed per active PSII reaction center (RC) of the sporotrophophyll and nest leaves of *Platycerium bifurcatum* after 1 and 7 days growth in high light (PPFD = 800 $\mu\text{m}^{-2} \text{s}^{-1}$) and in control conditions (PPFD = 150 $\mu\text{m}^{-2} \text{s}^{-1}$). Values for the same leaf type marked with the same letters in the column do not differ significantly at $p \leq 0.05$ according to the Duncan's test, $n = 7$.

Leaf type	Days of irradiation	Chlorophyll <i>a</i> fluorescence parameters				
		TR ₀ /RC	ET ₀ /RC	DI ₀ /RC	RE ₀ /RC	ABS/RC
Sporotrophophyll	0 (control)	1.87 ^b	0.47 ^d	1.41 ^b	0.55 ^a	0.19 ^{abc}
	1	3.24 ^a	1.70 ^b	1.54 ^b	0.58 ^a	0.22 ^a
	7	3.64 ^a	2.13 ^a	1.51 ^b	0.34 ^b	0.20 ^{ac}
Nest leaf	0 (control)	2.48 ^b	0.50 ^d	1.99 ^a	0.58 ^a	0.16 ^{bc}
	1	3.20 ^a	1.04 ^c	2.15 ^a	0.36 ^{bc}	0.15 ^c
	7	3.18 ^a	0.98 ^c	2.19 ^a	0.49 ^{ac}	0.18 ^{abc}

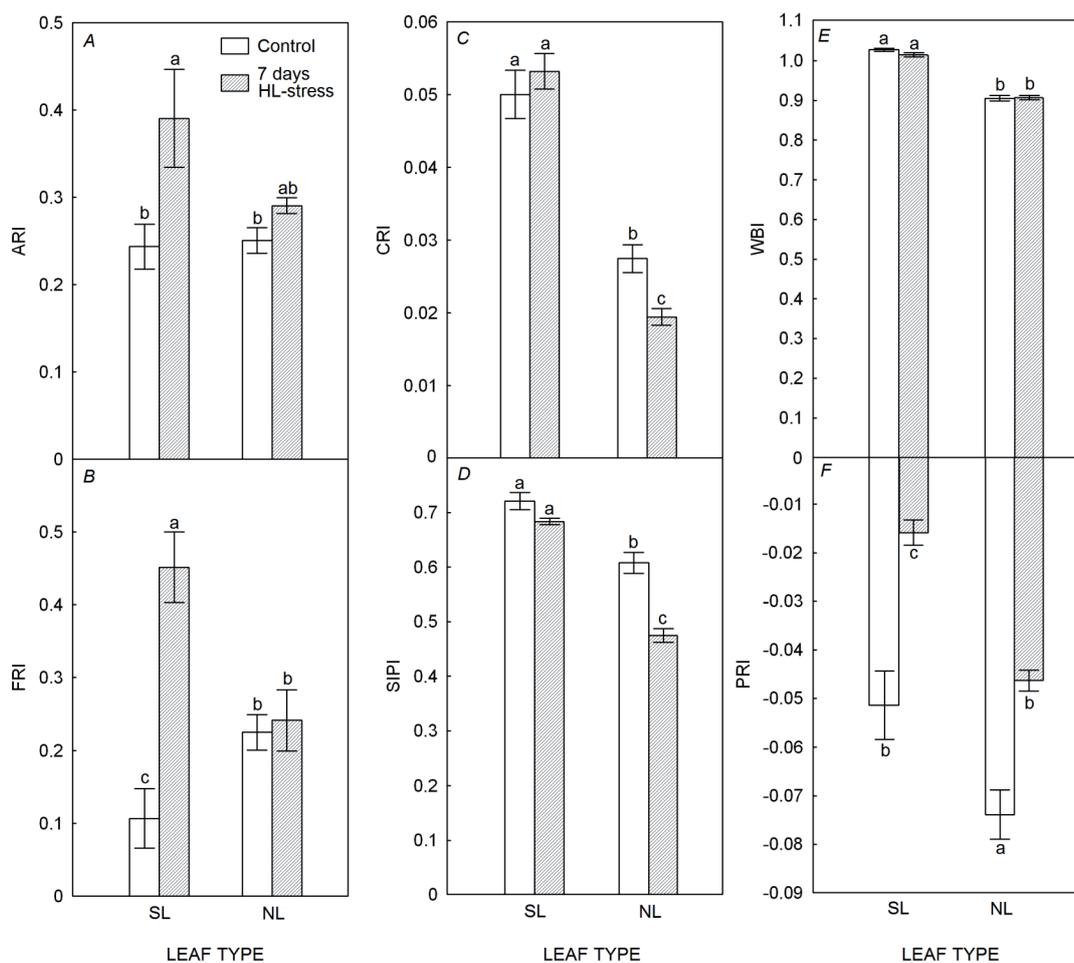


Fig. 6. Changes in the pigment composition and water content in sporotrophophylls (SL) and nest leaves (NL) of *Platycerium bifurcatum* after 7-d growth in high light (PPFD = 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and in control conditions (PPFD = 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$): A – amount of anthocyanins (ARI), B – amount of flavonoids (FRI), C – amount of carotenoids (CRI), D – ratio of carotenoids to chlorophyll content (SIPI), E – amount of water (WBI), F – photochemical reflectance index (PRI), $n = 7$. Bars marked with the same letters do not differ significantly at $p \leq 0.05$ according to the Duncan's test, $n = 7$.

highest in the green and infrared spectrum, with maximum values at the same wavelength as for RD (Fig. 5E). The minimum sensitivity occurred at 695 nm. In turn, the nest leaves showed maximum sensitivity to light stress in the red spectrum (650 nm – Fig. 5F).

Changes in pigment composition and hydration of sporotrophophylls and nest leaves are shown in Fig. 6, using ARI, CRI, SIPI, FRI, and WBI parameters.

There was a large increase in the content of anthocyanin compounds (ARI) and flavonoids (FRI) after 7 d of HL

stress in sporotrophophylls compared with the control (Fig. 6A,B). There were no changes in the content of Car pigments (CRI – Fig. 6C) and the Car/Chl ratio (SIPI – Fig. 6D). In turn, the ARI value did not change, and CRI and SIPI decreased in the nest leaves under HL (Fig. 6A,C,D). The higher flavonoid contents (FRI) in control plants did not change after 7 d of plant growth under HL compared to sporotrophophylls. HL did not change tissue water content in both types of leaves (WBI – Fig. 6E). Differences in hydration between sporotrophophylls and nest leaves remained at the same level as in control plants.

Photosynthesis quantum yield associated with changes in the composition of xanthophyll pigments (PRI) was significantly lower in the nest leaves than that in sporotrophophylls both in control and under HL. However, HL caused a significant decrease in this coefficient in both types of leaves (Fig. 6F).

Discussion

Light stress in the epiphytic ferns: Light stress occurs when the amount of PAR absorbed by the photosynthetic pigments of the antenna complex is too large compared to the plant's capacity to convert it into the energy of chemical bonds. It results in decreased photosynthetic efficiency and photoinhibition, and in extreme cases, irreversible damage to PSII. However, even a short-term impairment of PSII can contribute to the loss of a significant part of the daily gain of assimilates (Raven 1989). Photoinhibition is often associated with the degradation of the D_1 protein, responsible for Q_A binding and deactivation of the repair mechanism, which regulates the balance between active and inactive RCs PSII (Flexas *et al.* 2001, Zhou *et al.* 2007). These inactive RCs, play an important role in PSII restitution by absorbing and dissipating excess energy after stress factor activation (Lee *et al.* 2001).

In epiphytes, light stress and associated photoinhibition rarely results in plant's death, however, when another stress factor is involved, especially drought, it often leads to inhibition of photosynthetic light phase processes (Lovell *et al.* 1994). Then even moderate irradiation can contribute to serious disturbances in ontogenetic development (Durand and Goldstein 2001). Many epiphytes, including ferns, are less susceptible to HL stress than other plant species growing in the shade of tree crowns. Despite the relatively low values of the compensation point, many epiphytes have developed the ability to tolerate intense light (Lüttge *et al.* 1986). Among eight species of tropical epiphytic ferns subjected to several minutes of PAR action, with a photon flux intensity of $\leq 1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$, none of the plants showed signs of photoinhibition (Hietz and Briones 2001). It is worth noting, however, that the evaluation included only the F_v/F_m ratio. However, the long-term effect of HL (hours, days) more strongly differentiates epiphytic ferns in terms of excess light tolerance and resistance to photoinhibition. The degree of tolerance is often related to the distribution of plants within the canopy (Hietz and Briones 2001). Species growing on higher parts of trees and those growing on the edge of the forest usually show

higher energy demand and higher tolerance for intensive radiation than those growing deep in the forest (Hietz and Briones 1998).

Despite different light intensity or diurnal temperature fluctuations, the relatively stable microclimate within the canopy of the tropical forest promotes the development of epiphytes and contributes to their species richness (Parker 1995). Light stress, however, can affect fern species distribution. Durand and Goldstein (2001) showed that some native species of ferns in Hawaii are characterized by lower tolerance for increased light intensity than the invasive species present there.

The presented work assumes that a rapid change in PPFD from 150 to $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ must cause stress reactions in plants growing earlier under a 5-fold lower light intensity. However, it should be noted that in the natural environment plants are subject to fluctuations in the intensity of light, which results, for example, from the movement of leaves within the canopy and changing the position of the sun on the sky during the day. These light fluctuations are known as sunflecks (Chazdon 1988, Zhang *et al.* 2016). Thus, when analyzing the effect of light on plant metabolism under controlled conditions, it must be considered that growth chambers never fully reflect natural conditions. This is because in the environment occurrence of sunflecks may cause even local photoinhibition occurring only in the illuminated leaf fragment.

Analysis of OJIP curves: Abiotic stress factors such as strong light or drought usually result in disturbances in the electron transport chain (Foyer *et al.* 2012, Gururani *et al.* 2015). They are reflected in the shape of the Chl *a* FL curve. Therefore, methods based on the OJIP test are often used in ecophysiological studies to assess the condition of the photosynthetic apparatus, especially PSII (Misalski *et al.* 2016).

The analysis of OJIP curves shows that sporotrophophylls show a stronger response to HL than the nest leaves, especially after 7 d of growth under stressful conditions. Lack of definite differences in FL Chl *a* curves between the first and seventh day in nest leaves indicates a fast but less sudden response of the photosynthetic apparatus and its lower susceptibility to excessive photon density or faster regeneration. It is interesting, because according to Rut *et al.* (2008), *P. bifurcatum* nest leaves utilize CAM metabolism. In turn, Adams and Osmond (1988) suggested that CAM-photosynthesis epiphytes, growing in the shade, exhibited high sensitivity to photoinhibition in HL.

Excitation energy losses during transfer to the PSII reaction center were visible in both types of leaves, although they intensified after a longer period of plant growth under HL only in sporotrophophylls. These disturbances were reported by the FL increase (compared with control) in the O–K phase of the induction curve (Figs. 2A,B; 3A), high F_0 (O – point) value and the appearance of the L–band on the O–K differential curve (characteristic for the effects of abiotic stress). The increase in Chl *a* FL intensity on the first phase of the OJIP curve is usually associated with a decrease in energy transport efficiency between LHCII and the PSII reaction center (Tsimilli-Michael and

Strasser 2013b). It also indicates a weaker connection of antenna complexes, which is related to the abnormal structure of thylakoids. An increase in L band intensity in sporotrophophylls after 7 d under HL (Fig. 2C) is probably associated with progressive degradation of LHCII proteins. It involves FtsH6 protease, constitutionally present in small amounts in the thylakoids and participating in plant acclimation to the conditions of strong radiation (Želisko *et al.* 2005). In turn, the occurrence of the maximum L band shifted in time, observed after 7 d, demonstrated slower energy transfer from the antennas to RC (Kalaji *et al.* 2018). This shift in the nest leaves was observed after just one day under HL and confirmed a faster response to stressful conditions than that in sporotrophophylls (Fig. 3C).

The analysis of the O–J phase of Chl *a* FL induction curves (Figs. 2A, 3A) as well as differential curves ($\Delta V_{t_{O-J}}$ – Figs. 2D, 3D) allow to describe the state of the PSII donor side under stress conditions and illustrate the size and absorption capacity of LHCII (Tsimilli-Michael and Strasser 2013a). The biggest differences between the 1st and 7th day of HL stress were visible in nest leaves in the course of the O–J phase, which is strongly light-dependent (Dąbrowski *et al.* 2016). Differential curves for this phase visualized the presence of a distinct K band (Fig. 3D). High positive K band values (observed already after 1 d of plant growth in HL) prove the rapid oxidation-reduction imbalance between the OEC and the PSII reaction center. They are caused by OEC damage, which is extremely sensitive to changes in environmental conditions (Strasser 1997, Kalaji *et al.* 2018). The lack of the K band in sporotrophophylls, after 1 d under HL and only a low intensity K band after 7 d (Fig. 2D), suggests induction of a mechanism that would compensate for the abnormal functioning of OEC. However, these mechanisms do not sufficiently improve PSII function. This is evidenced by large deviations from the control values in other phases of the OJIP curve (Fig. 2A), as well as decreased values of Chl *a* FL parameters, such as F_v/F_0 , F_v/F_M , PI, F_K/F_1 , especially after 7 d of stress (Fig. 4A). It seems, however, that changes in the functioning of OEC caused by stress are too complicated to be explained only by the OJIP test.

Disturbances of electron transport at the plastoquinone reduction stage, observed in sporotrophophylls (Fig. 2E), increased during the longer exposure to HL, as evidenced by clear bands in the J–I phase. On the other hand, negative G band values in the I–P phase illustrated the course of reduction of the PSI acceptor side. More negative G band values, visible after 7 d, corresponded to the growing number of NADPH molecules per active RC under abiotic stress (Tsimilli-Michael and Strasser 2013a). Similar dependencies were also observed in the case of other abiotic stresses, *e.g.*, micro- and macro-nutrient deficiencies or salt stress, as well as during the aging process (Bąba *et al.* 2016, Dąbrowski *et al.* 2016, Kalaji *et al.* 2018). In contrast to sporotrophophylls, curves obtained for the nest leaves at J–I and I–P phases (Fig. 3E–F) did not indicate disturbances in energy flux between the plastoquinone pool and PSI and proved lower susceptibility of the photosynthetic apparatus to photoinhibition. In

conclusion, the stage of the photosynthetic light phase, at which the main HL effects occur, depends on a leaf type. Disturbances in the initial reactions of the light phase were characteristic for the nest leaves. In sporotrophophylls, subsequent reactions associated with the reduction of PQ and PSI acceptors were primarily impaired. However, negative changes in LHCII were visible only after a longer period of HL exposure.

Chl *a* FL parameters: The effects of photoinhibition and disintegration of the photosynthetic apparatus are reflected in changes in Chl *a* FL parameters (Fig. 4). The decrease in the maximum quantum yield of PSII (F_v/F_M) by 37% in sporotrophophylls and by 13% in nest leaves, compared with control, occurred after 1 d of plant growth in HL. It indicated, especially in sporotrophophylls, strong photoinhibition, which already occurred at PPFD of 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which is almost half lower than this applied by Sanusi *et al.* (2011) in their study on *P. bifurcatum*. A decrease in F_v/F_M after a 2-d HL exposure was also demonstrated for eight other species of epiphytic ferns growing in tropical forests of Mexico (Hietz and Briones 2001). This decrease for *Asplenium cuspidatum*, which grows naturally in the shade inside the canopy, was 37%, whereas the average F_v/F_M value decreased by 10% in *Elaphoglossum petiolatum*, growing in sites with better sunlight exposure (in comparison to control). A strong light intensity disturbed the growth and development, and limited functioning of photoprotective mechanisms in the leaf also in epiphytic orchid species, which was associated with a decrease in the F_v/F_M value (Stancato *et al.* 2002).

Until now, the ecophysiological studies on light stress in epiphytes were mainly based on the F_v/F_M ratio analysis, but other parameters of PSII efficiency were not determined (Hietz and Briones 2001, Stancato *et al.* 2002). However, as our research shows, the F_v/F_0 and PI coefficients are more sensitive indicators of environmental stress, better describing different response during the light phase. The decrease in the F_v/F_0 value indicates damage to the thylakoid structure in chloroplasts (Pereira *et al.* 2000). Changes in PI values determine the overall vitality of the plant and its viability, combining information on the number of active RCs on Chl and initial reactions of the light phase with data on electron flux through RC (Kalaji *et al.* 2014). A decrease in sporotrophophyll vitality, to 13% of the control value after 1 d of HL stress, indicated a drastic reduction in the efficiency of energy conversion in PSII (Bąba *et al.* 2016). However, in the nest leaves, despite the initial decrease in PI, a slight improvement in RC energy efficiency was observed after 7 d (Fig. 4). This can be explained by an entirely different ontogenesis compared to sporotrophophylls, *i.e.*, short period of leaf growth and the following fast aging process, combined with the loss of assimilation properties (Oliwa *et al.* 2017). The study of Lovelock *et al.* (1994) demonstrated that a short leaf life span in some tropical fern species was associated with their ability to regenerate faster after photoinhibition. It is possible that this was due to the faster rate of photosynthesis (Reich *et al.* 1997).

The OEC in both types of leaves was damaged under

HL (Fig. 4, F_K/F_J value – Srivastava and Strasser 1995). In addition, an increase of M_0 value was observed. This parameter is related to the rate of closure of reaction centers (Baba *et al.* 2016). In sporotrophophylls, this parameter increased between 1st and 7th day under HL, which was not observed in the nest leaves. This confirms the different reaction to HL stress in the photosynthetic apparatus of sporotrophophylls. Changes in the values of TR_0/RC , ET_0/RC (Table 1) in both types of leaves indicate an increase in the efficiency of trapped and transport of electrons by RC. HL does not affect the amount of energy dissipated in the form of heat (DI_0/RC). However, in the nest leaves, DI_0/RC values were significantly higher than that in sporotrophophylls.

Reflectance analysis: Plant pigments strongly absorb visible radiation in the 400–700 nm range. The decrease in pigment contents in the tissue results in a reduction of the absorption capacity, and thus an increase in the intensity of reflectance. This corresponds to the degree of light energy utilization, which can be treated as an indicator of assimilate-production efficiency (Araus *et al.* 2001). Reflectance analysis, as opposed to classical biochemical methods, allows for fast and nondestructive assessment of leaf pigment composition (Gamon and Surfus 1999, Solovchenko 2010). In turn, measurements performed on individual leaves allow to avoid interpretation problems resulting from the complex spatial structure of the plant (Linke *et al.* 2008).

The increase in reflectance intensity in sporotrophophylls after 7 d of HL is typical for stressful conditions. It is probably associated with changes in the leaf color profile (Solovchenko 2010). An increase in PAR reflectance is usually associated with a decrease in the Chl content and is a general detector of plant stress (Cibula and Miller 1996, Carter and Knapp 2001). Differences in reflectance between sporotrophophylls and nest leaves in the far-red and near-infrared range may result from different morphological and anatomical structure of these leaves.

The analysis of reflectance difference (RD) and determination of stress-sensitive wavelengths are also helpful in the assessment of changes caused by stress in plants (Cibula and Carter 1992). The 535-nm band, visible in sporotrophophylls (Fig. 5C,E), and 690 nm in nest leaves (Fig. 5D,F), correspond to the bands observed in various plant species subjected to stress, such as drought, deficiency of mineral elements, fungal infections, and other (Carter 1993, Moran *et al.* 2000). The reflectance coefficient showed usually the highest sensitivity to stress in the 535–540 nm and 685–700 nm ranges (Carter 1993). RD spectral bands of 535 and 650 nm can therefore be characteristic of the HL-stress response in *Platyserium*, and possibly in other ferns. However, acclimation to intense light by growing plants at an elevated R/FR ratio in the light spectrum can change this picture (Oliwa and Skoczowski – unpublished data). However, the maximum RD and sensitivity values fall within the range of 400–850 nm in all cases.

Changes in pigment composition of sporotrophophylls influenced by HL were observed to a much greater extent

than that in the nest leaves. The pronounced increase in the content of anthocyanins and flavonoids demonstrated the defense response of sporotrophophylls (Fig. 6A,B). Accumulation of photoprotectants, including anthocyanins and flavonoids, is very common in plants exposed to intense radiation in the PAR and UV range and is intended to protect thylakoid membranes (Chalker-Scott 1999, Gitelson *et al.* 2003, 2009, Steele *et al.* 2009). Excess PAR implies, in addition to disturbances in electron transport, the generation of singlet oxygen in the P_{680} triplet, especially dangerous for chloroplast membranes (Baroli and Melis 1998, Burritt and MacKenzie 2003). Rapid production of flavonoid compounds, as a response to HL, supports the inhibition of reactive oxygen species production and stabilizes chloroplast membranes (Agati *et al.* 2012). In turn, the presence of Car pigments of the xanthophyll cycle in thylakoids separates LHCII that receives excess energy from the PSII reaction center. This enables the protection of the most sensitive part of the photosynthetic apparatus (Walters and Horton 1993, Gilmore and Yamamoto 1993). There was no increase in the amount of anthocyanins and flavonoids in cover leaves. In addition, a slight decrease in Car contents and a quantitative ratio of Car to Chl (SIPI), which usually increases during HL exposure, was observed (Young and Britton 1990). This suggests that the main defense mechanisms were aimed to protect assimilation organs (*i.e.*, sporotrophophylls).

The decrease in the amount of water in the tissue results in a lower value of the WBI parameter (Peñuelas *et al.* 1993). WBI values (Fig. 6E) were typical for assimilating tissues in both leaf types (0.8–1.2 i.u. – Peñuelas *et al.* 1997). Lack of significant changes in the water content under HL conditions compared to control may indicate the absence of disturbances in leaf stomatal conductance. On the other hand, lower WBI values in nest leaves were results of their different morphological and anatomical structure (Oliwa *et al.* 2017).

Reduction of the photochemical reflectance index (PRI), which occurred in both types of leaves, describes rapid changes in the pigment profile of the xanthophyll cycle under HL and is associated with deepoxidation of xanthophyll pigments (Filella *et al.* 1996, Sims and Gamon 2002, Peñuelas *et al.* 2011). In addition, it illustrates the intensity of nonphotochemical dissipation (Naumann *et al.* 2008). Lower PRI values in the nest leaves than that in sporotrophophylls in both control and HL conditions indicated better light use by the nest leaves (Gamon *et al.* 1997 – Fig. 6F).

In summary, *P. bifurcatum* sporotrophophylls showed a stronger response to HL stress than the nest leaves. The disturbances of electron transport occurring at various stages of the light phase led to a decrease in the vitality of the photosynthetic apparatus. However, it seems that sporotrophophylls use photoprotective mechanisms associated with the accumulation of flavonoid compounds and anthocyanins more efficiently than the nest leaves. Differences in the response to HL between the two types of leaves may result from the different role they play in the examined fern.

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