

The high red/far-red ratio supports the acclimation of fern *Platycerium bifurcatum* to high light

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DOI: [10.1556/019.70.2019.23](https://doi.org/10.1556/019.70.2019.23)

Original Article

Cite this article: Oliwa J, and Skoczowski A. 2019. The high red/far-red ratio supports the acclimation of fern *Platycerium bifurcatum* to high light. *Biol. Fut.* 70, 185–197.

Received: 3 January 2019

Accepted: 16 May 2019

Keywords:

chlorophyll *a* fluorescence, reflectance, high-light stress, epiphyte

Background and aims: High light causes disturbances in photosynthetic phosphorylation or damage to the photosystem II (PSII) structure or even assimilation tissues. The value of the red/far-red ratio (R/FR) provides the plant with information on the environmental light conditions, regulating, among others, photosynthetic activity and pigment composition of the plant. The response of the photosynthetic apparatus of the sporotrophophylls and nest leaves of *Platycerium bifurcatum*, grown for 6 months at the low or high R/FR ratio, were studied. Later, the plants were transferred to high light ($1,200 \mu\text{mol quantum} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). **Methods:** Changes in PSII photochemical activity were determined based on non-destructive methods of chlorophyll *a* fluorescence kinetics analysis. The measurement of radiation reflectance from the leaves allowed to determine the content of selected pigments related to the photosynthesis process and to assess changes in the Photochemical Reflectance Index. The calculation of reflectance difference and sensitivity analysis was used to identify so-called “stress-sensitive wavelengths”. **Results and discussion:** Plant growth at high R/FR ratio prepares photosynthetic apparatus of ferns to high light and enables more efficient conversion of absorbed photons. The increase in the amount of photoprotective compounds allows the protection against photoinhibition in the sporotrophophyll leaves that play key roles in plant nutrition and reproduction.

INTRODUCTION

Inside the canopy of tropical forests, the tree’s crown regulates lighting, temperature, and humidity conditions, which results in the creation of a specific microclimate (Nadkarni et al., 2004). However, despite the relatively stable environmental conditions, epiphytes are exposed to abiotic stress, limiting their development. The main role is played by water shortage but also light stress must be taken into account (Oliwa & Skoczowski, 2019; Zotz & Hietz, 2001). Changes in light conditions may result in natural causes (death of trees) or cutting out fragments of the forest. In the second case, in the large area adjacent to the fragment cut, the intensity of light increases significantly in a short time. In plants growing earlier in an environment with limited light availability and changed spectral composition, this results in the lack of the possibility of using excess light energy to create an assimilation force and leads to a decrease in quantum efficiency and sometimes damage to the photosystem II (PSII) structure (Bertamini et al., 2004). For this reason, plants often adopt a strategy to avoid excess light or increase tolerance, as in the tropical climate epiphytes, which grow in the canopy. Understanding the photoprotective strategies of spermatophytes has been the subject of many works, also recently (Demmig-Adams & Adams, 2018; Gould et al., 2018; Malnoë, 2018). However, there is still a lack of information on the response of cryptogams to light stress. In ferns, photoprotective mechanisms are mainly associated with the dissipation of excess energy in the xanthophyll cycle (Tausz et al., 2001). Similarly to other vascular plants and mosses, an important role in the regulation of energy balance during exposure to strong light is also played by the PsbS protein, participating in the remodeling of the PSII-LHCII supercomplex (Dong et al., 2015; Gerotto et al., 2011). The ability of fast regeneration of damage within the photosynthetic apparatus after stress is also very important. The time of restitution after photoinhibition depends on the time of plant exposure to strong radiation, but it is not always possible to completely restore the lost functions.

Energy balance disturbances are characteristic of plants in a state of strong or weaker but prolonged stress and can be registered long before the appearance of its visible symptoms, using fluorescent methods. A well-developed methodology for measuring Chlorophyll *a* (Chl*a*) fluorescence and rich literature allows for an extensive

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assessment of the impact of environmental factors on the photochemical efficiency of PSII (for review, see Kalaji et al., 2014). In turn, the analysis of the OJIP curves provides information on changes occurring at subsequent stages of energy distribution in the light phase of photosynthesis (Kalaji et al., 2014; Strasser et al., 2000).

Reflectance of solar radiation from the leaf blade in the 400–700 nm range is associated with the degree of energy absorption mainly by chlorophylls, as well as carotenoids and anthocyanins (Carter & Knapp, 2001). Leaf reflection measurements allow an effective assessment of the content of photosynthetic and protective pigments, without the necessity of tissue destruction, because the values obtained in this way show a very high correlation with the results of spectrophotometric measurements (Gitelson & Solovchenko, 2018). In addition, leaf reflectance at 970 nm was used to calculate the Water Band Index (WBI), which describes the degree of tissue hydration (Peñuelas et al., 1993a, 1997).

Platyserium bifurcatum is an epiphytic fern occurring naturally in tropical forests of Australia, New Guinea, and in some areas of Central America. Mature sporophyte of *P. bifurcatum* has two types of leaves (Fig. 1). Assimilation functions are performed by sporotrophophylls with a characteristic dichotomic ending, where sporangia are located on the underside of the leaf blade. Oval, quickly browning nest leaves form a conch and attach the plant on the trunk of the tree and collect minerals and water. Both types of leaves differ during the ontogenesis process and in physiological terms (C3 metabolism in sporotrophophylls and probably crassulacean acid metabolism in nest leaves – Oliwa & Skoczowski, 2019).

Previous studies on the physiology of epiphytic ferns, including *P. bifurcatum*, were primarily associated with the cultivation of ornamental plants and micropropagation methods (Aspiras, 2010; Liao & Wu, 2011). However, the increasing anthropopressure in the regions of tropical forests is an important reason to conduct ecophysiological research and determine the acclimation capacity of valuable plant species in high-light conditions. *P. bifurcatum* is one of the most cultivated decorative ferns. For this reason, determining the acclimatization capacity of *P. bifurcatum* to high light and increasing the tolerance to light stress are also of practical importance for plant grower and collectors.

The R/FR ratio provides the plant with information on the intensity of lighting. In natural conditions, there is a

strong relationship between light intensity and spectral composition, especially in places with clearly marked storied plant structure. In the shade, the dominant component of the spectrum of light is the far red, and the red light is very limited due to filtration by canopy (low R/FR ratio – LR). In turn, in sunny places, the red component exceeds the far-red (high R/FR ratio – HR). Changes in the R/FR ratio in the environment are registered by plants through phytochrome mechanisms. This is possible due to the occurrence of two photoreversible phytochrome conformations, i.e., a form absorbing red light (Pr) and far-red (Pfr). The value of R/FR ratio directly modifies the phytochrome properties, activating or inhibiting the shadow avoidance reaction (Franklin & Quail, 2010). Phytochrome also plays an important role in modulating signaling pathways during plant reactions to various abiotic stresses, including light stress (Mani & Guruprasad, 2015). Phytochrome-dependent photoprotective mechanisms are associated with the change in the structure and size of the LHCII pool. PHYA and PHYB play a key role in regulating the level of LHCB gene expression encoding LHCII proteins (Wang & Deng, 2003). This is done by three types of reactions run depending on the intensity of red or far-red light and allows acclimatization of plants to light conditions.

The aim of this study was to determine whether the low and HR ratio values, simulating shade or intense sun exposure, respectively, modify the plant's response to high-light stress, i.e., enable preparing the plant for light shock. Despite many data on the change of radiation reflectance from the leaves of spermatophytes in conditions of various stresses, there is still no information during this process in ferns. Therefore, this work searched for a spectral range, in which the reflectance intensity would change the most in response to light stress. The experiment included both sporotrophophylls and nest leaves due to the structural and functional differences between them.

MATERIALS AND METHODS

Plant material

In the experiment, the 2-year-old sporophytes of *P. bifurcatum* Cav. (C. Chr.) obtained from the Pedagogical

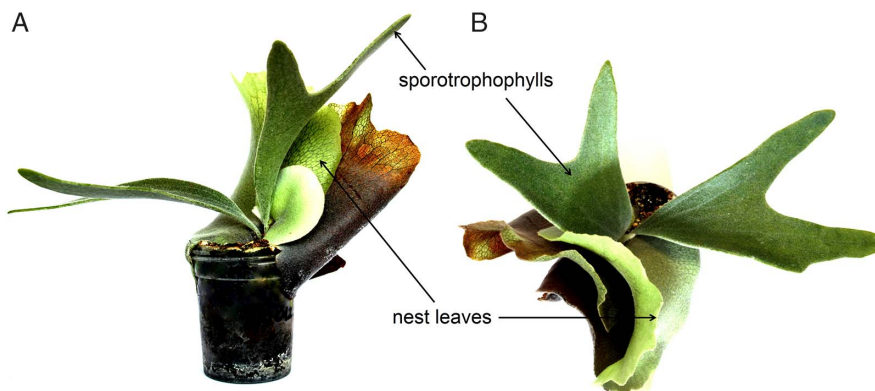


Fig. 1. Sporophyte of *Platyserium bifurcatum*: side view (A) and top view (B). Two types of leaves (sporotrophophylls and nest leaves) are marked with arrows

University of Cracow were used. The measurements were carried out on sporotrophophyll and nest leaves in the second stage of development, according to the classification proposed by Oliwa et al. (2017). Only non-destructive methods were used to determine physiological changes occurring in the same leaves, after different HL exposure times.

Initial growth conditions

The plants were placed for 6 months in the Snijders Scientific Flowstar (Netherlands) climate chamber with LED matrices of light color: blue, red, and far-red (Fig. 2A and B). Half of the plants grew at a HR ratio = 1.9 (Fig. 2A), typical for sunny places. The second part of the plants grew at a LR = 0.25 (Fig. 2B), characteristic of shady places. Differences in the R/FR ratio were obtained by obscuring the far-red light sources. The photosynthetic photon flux density (PPFD) in both cases was $200 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, RH = 60%, temperature $25 \text{ }^\circ\text{C}/15 \text{ }^\circ\text{C}$ (day/night), photoperiod 16/8 h (day/night).

High-light stress

After 6 months, plants from both groups were placed under sodium lamps for 1, 3, and 7 days, at PPFD = $1,200 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, at $25 \text{ }^\circ\text{C}$, photoperiod 16/8 h. The light spectral composition is shown in Fig. 2C.

Measurements of Chla fluorescence kinetics

Measurements of Chla fluorescence kinetics were carried out after 1, 3, and 7 days from the transfer of plants to the elevated light intensity. Parameters of PSII photochemical efficiency were measured according to the method described by Strasser et al. (2000) using a Handy-PEA fluorometer (Hansatech Instruments, UK). Fragments of the leaf blade were acclimated to darkness for 20 min using a clip. Chla fluorescence was induced by radiation of $3,500 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (intensity wavelength at peak 650 nm, spectral line half-width 22 nm). Measurement results were compiled in the PEA Plus program (Hansatech, UK) and MS Excel 2010 and selected parameters were analyzed: F_V/F_M , F_V/F_0 , Area, performance index (PI), and RC/ABS (Oukarroum et al., 2007; Strasser et al., 2004) and fluorescence induction curves (OJIP). The OJIP curves were normalized to steps O and P. The differential curves of Chla fluorescence kinetics (ΔVt) were calculated by subtracting the values of normalized OJIP curves in plants growing for 1, 3, or 7 days at high light, from control curves (Oukarroum et al., 2007). Such presentation of data makes the differences between treatments more visible.

Measurements of radiation reflectance from leaves

Leaf reflectance was measured at $22 \text{ }^\circ\text{C}$ using a CID Bio-Science CI-710 miniature spectrometer (USA) on the upper surface of the leaf blade. Reflectance spectra in the 400–1,000 nm range were recorded using the SpectraSnap software (CID Bio-Science, Camas, WC, USA), and then selected reflectance coefficients were calculated:

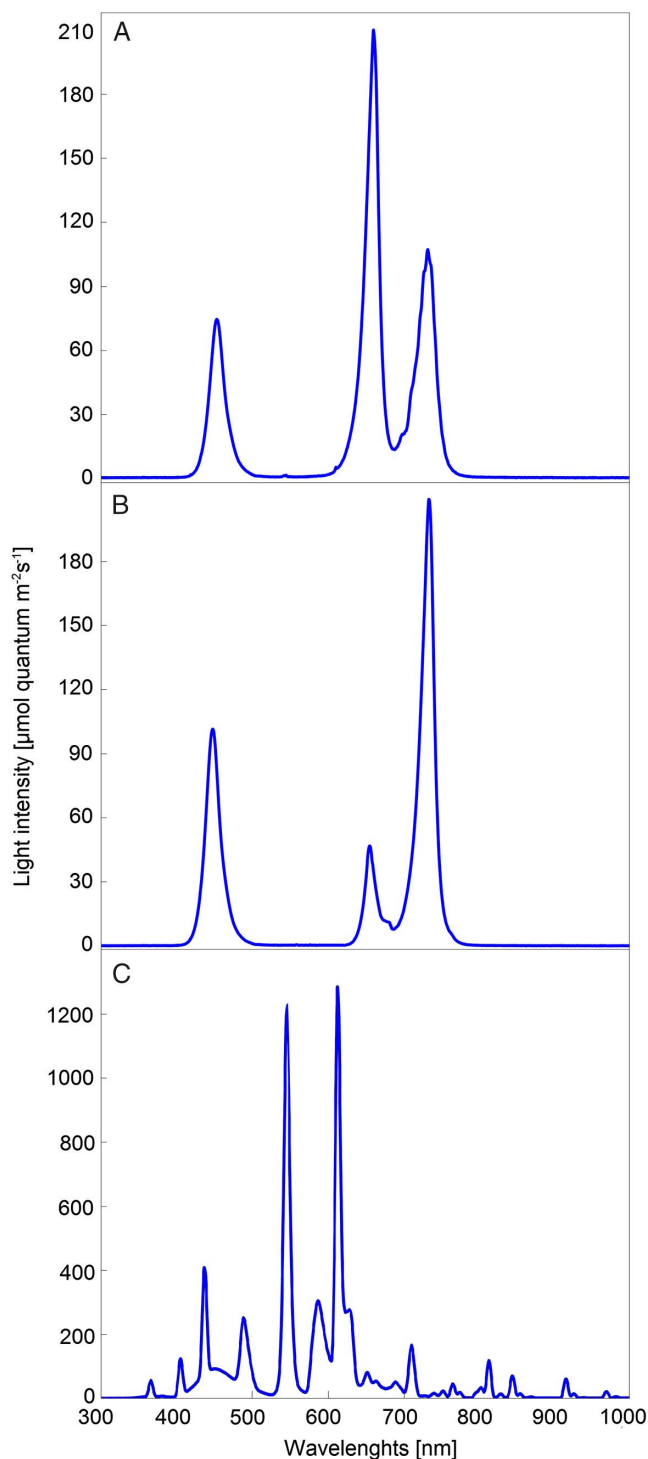


Fig. 2. Spectral composition and intensity of light in the climate chamber with LED matrices in conditions of high (HR – A) and low (LR – B) value of R/FR ratio. Spectral composition and intensity of a sodium lamp light used to cause high-light stress (C)

$\text{ARI}_1 = (R_{550}^{-1} - R_{700}^{-1})R_{800}$ (Gitelson et al., 2001) – changes in anthocyanin content (Anthocyanin Reflectance Index);

$\text{CRI}_1 = (R_{520}^{-1} - R_{550}^{-1})R_{800}$ (Gitelson et al., 2002) – changes in carotenoids content (Carotenoid Reflectance Index);

SIPI = $(R_{800} - R_{445})(R_{800} + R_{680})^{-1}$ (Peñuelas et al., 1995) – carotenoids to Chla ratio (Structure-Insensitive Pigment Index);

FRI₁ = $(R_{410}^{-1} - R_{460}^{-1})R_{800}$ (Merzlyak et al., 2005) – flavonoids contents of epidermal subcuticular cells (Flavonol Reflectance Index);

PRI = $(R_{531} - R_{570})(R_{531} + R_{570})^{-1}$ (Gamon et al., 1997) – an alternative form of assessment of photosynthesis quantum yield and PSII efficiency (Photochemical Reflectance Index);

WBI = $R_{900}(R_{970})^{-1}$ (Peñuelas et al., 1993a) – degree of hydration of the leaf tissue (Water Band Index).

R_x – in equations means reflectance intensity at a specific x wavelength.

Reflectance difference (RD) and sensitivity analysis allow to identify the wavelength at which the numerical value of reflectance changes the most as a result of stress (Carter, 1993). 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 growing for 6 months under LR or HR.

Stress-sensitive wavelengths (sensitivity) are calculated according to Carter (1993) dividing RD values by 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 in the Statistica 12 program (Statsoft, Poland) using the multifactorial analysis of variance. The significance of differences between means was estimated using Duncan's test at a significance level of $p \leq .05$.

RESULTS

OJIP curves analysis

The OJIP curves for sporotrophophylls and nest leaves growing at LR and HR are shown in Fig. 3A–D. The analysis of curves indicates disturbances caused by light stress only in plants with LR. In both types of leaves growing at LR in high light ($1,200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), there was an increase in the Chla intensity fluorescence between steps O and I (Fig. 3A and B). In sporotrophophylls, a significant increase compared to control took place after just 1 day of high light, and in nest leaves after 3 days. In addition, in the nest leaves, a strong flattening in J–I phase is visible (there is no clear step I – Fig. 2B). The OJIP curves of plants from HR did not differ significantly from the control curves (Fig. 3C and D). Even after 7 days of light

stress, the fluorescence intensity in the O–I phase increased slightly (both in sporotrophophylls and nest leaves). The fluorescence intensity of control plants from HR was higher than those from LR (Fig. 3A–D).

The main effects of light stress in leaves from LR are visible in the O–I phase on differential curves as a positive deviation from the control (Fig. 3E and F). In sporotrophophylls, the greatest deviation from the control was observed after 1 day of high light (Fig. 3E – red line). On days 3 and 7, the deviation decreased. In nest leaves, the trend was reversed (Fig. 3E and F). In sporotrophophylls from HR, the only electron transport disturbances were visible in the O–J phase after 7 days of stress (Fig. 3G). In the nest, disturbances in leaves appear after 1 and 3 days at HR as good visible bands in the phase J–I (Fig. 3H – blue and red lines). However, after 7 days at high light, the differential curve is below the control level (Fig. 3H – green line).

The differential curves $\Delta V_{t_{O-J}}$, $\Delta V_{t_{J-I}}$, and $\Delta V_{t_{I-P}}$ were calculated analogously to ΔV_t , and the values were normalized to the O–J, J–I, and I–P steps, respectively. Characteristic for stress reaction K-band is visible on the O–J differential curve of sporotrophophylls from LR after just 1 day of high light, in leaves from HR after 7 days (Fig. 4A and B, respectively). Stress bands were not observed on the other analyzed curves J–I and I–P (Fig. 4C–F).

In nest leaves from LR, the positive values of K-bands are much lower than in sporotrophophylls (Fig. 4G), and in leaves growing at HR does not occur (Fig. 4H). On the differential curve J–I, the nest leaves from LR show a positive deviation from the control (Fig. 4I). In turn, in the I–P phase, G-band (Fig. 4K – negative values) is observed, which is typical for the abiotic stress (Tsimilli-Michael & Strasser, 2013a). On the differential curves for nest leaves from HR, no stress bands are visible (Fig. 4H, J, and L).

Chla fluorescence parameters

The maximum photochemical efficiency of PSII expressed as the F_v/F_M parameter in sporotrophophylls growing at both LR and HR decreased after exposure to high light, reaching a minimum after 3 days (Table 1). Then, F_v/F_M values for sporotrophophylls growing under HR returned to the control level, while the increase in F_v/F_M value in leaves from LR was significantly lower. In the nest leaves growing initially at LR, the decrease in F_v/F_M after 3 days was smaller than in sporotrophophylls and there was no subsequent return to the control values. In the nest leaves from HR, the F_v/F_M value decreased only after 7 days. An analogous trend as in F_v/F_M was observed in the values of the F_v/F_0 parameter (Table 1).

Area above the Chla fluorescence induction curve represents the size of the reduced plastoquinone pool. The Area value in both types of leaves growing at LR declined already on the first day of high light and remained at a similar level (Table 1). However, in sporotrophophylls from HR, the Area value increased significantly after 7 days at high light.

PSII vitality estimated using the PI parameter provided useful information on plant condition, combining information on the number of active reaction centers (RCs) per chlorophyll and initial reactions of the light phase with data on electron flux through RC (Oukarroum et al., 2007). The PI value for the

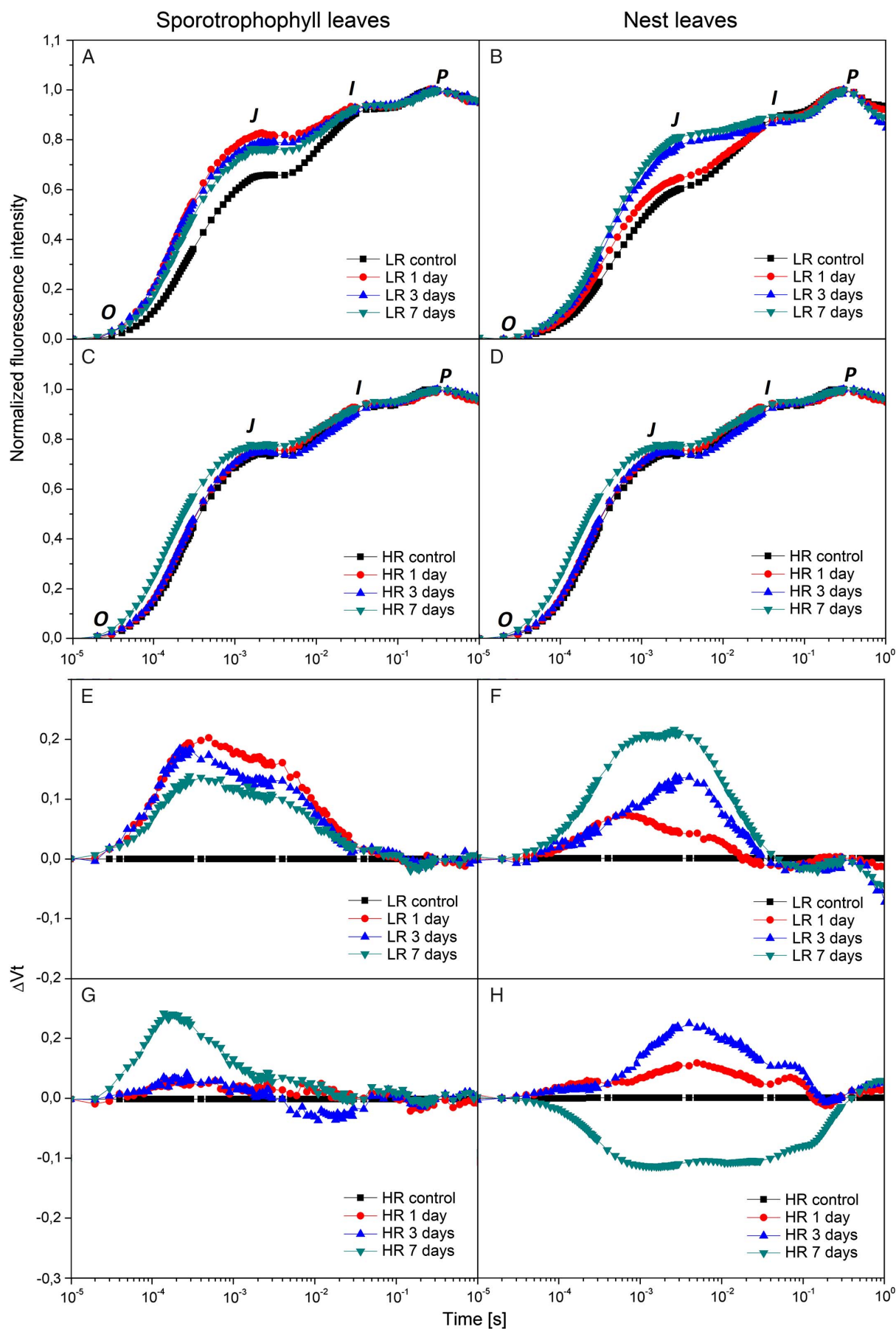


Fig. 3. Induction transients of chlorophyll *a* fluorescence OJIP (A–D) and differential curves ΔV_t (E–H) in sporotrophophylls and nest leaves of *Platycerium bifurcatum* growing at low (LR) or high (HR) value of R/FR ratio (control), then treated with high light ($1,200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) for 7 days, $n = 7$

Sporotrophophyll leaves

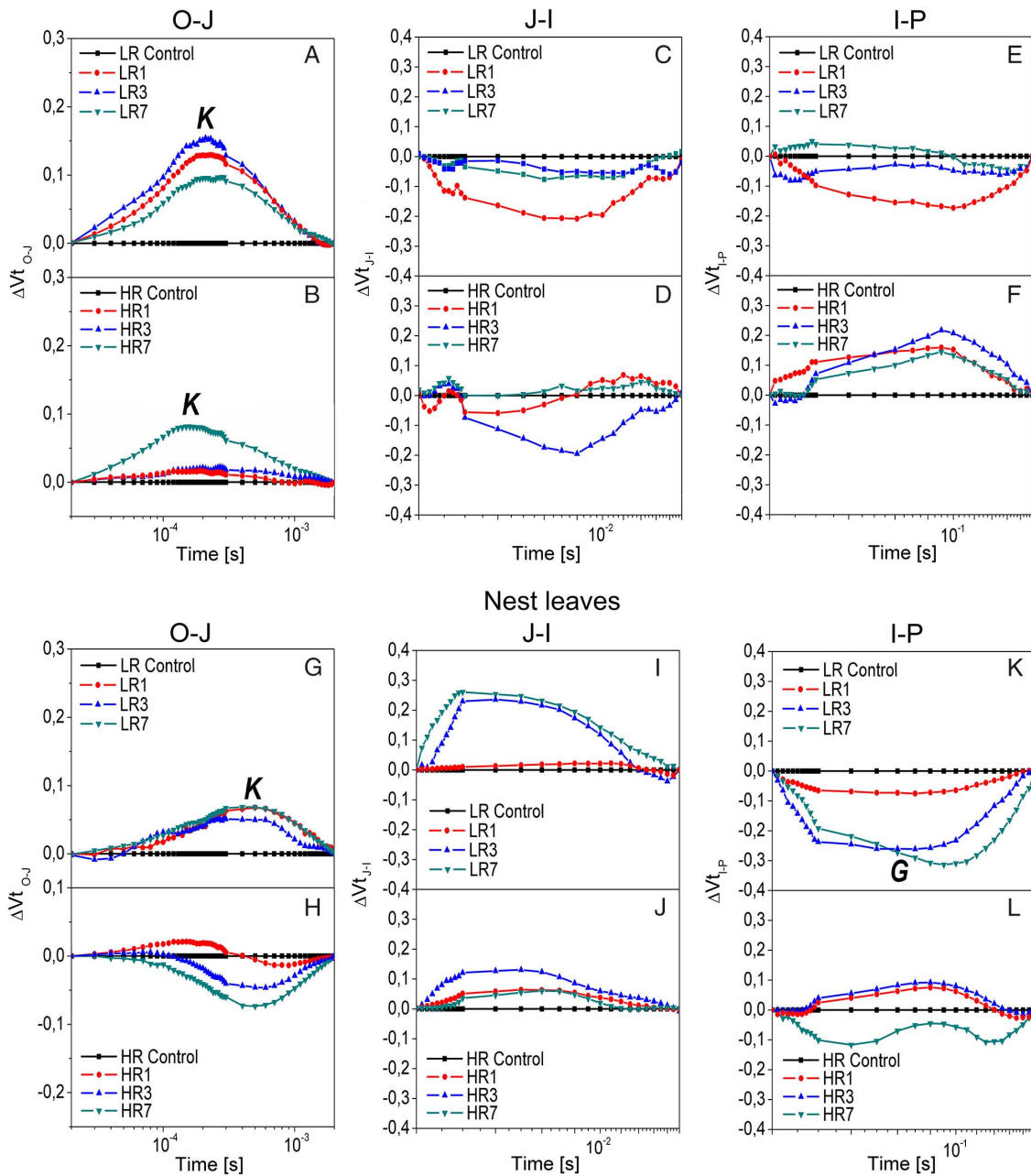


Fig. 4. Differential curves ΔVt for the O-J, J-I, and I-P phases in sporotrophophylls (A-F) and nest leaves (G-L) of *Platycerium bifurcatum* growing at low (LR) or high (HR) value of R/FR ratio (control), then treated with high light ($1,200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) for 7 days, $n = 7$

sporotrophophylls and nest leaves growing at LR declined already on the first day at high light and remained at a similar level till day 7 (Table 1). In sporotrophophylls from HR after 7 days, the PI value increased significantly compared to the control. In turn, in nest leaves growing at HR, no statistically significant changes were observed.

Reflectance analysis

The obtained reflectance spectra showed a typical broad band in the yellow-green range with a maximum at about 550 nm (associated with the presence of chlorophyll) and a significant increase in reflectance in the far-red and infrared

spectrum (Figs 5A, B and 6A, B). Sporotrophophylls growing for 6 months at LR (control) showed initially a lower reflectance percentage in the entire analyzed spectral range than their counterparts growing at HR (Fig. 5A and B – blue lines). The reflectance in plants from the LR increased after 7 days of exposure to high light, whereas it decreased in plants from HR (Fig. 5A and B – red lines). An inverse dependence of reflectance intensity changes was observed in the nest leaves compared to sporotrophophylls (Fig. 6A and B). The leaves of the control plants growing at LR showed higher reflectance values than those growing at HR (Fig. 6A and B – blue lines). However, at high light, the reflectance intensity dramatically decreased in the leaves

Table 1. Values of chlorophyll *a* fluorescence parameters of the sporotrophophyll and nest leaves of *Platycerium bifurcatum* growing at the low (LR) and high (HR) R/FR ratio on consecutive days of exposure (0–7) to high light (1,200 $\mu\text{mol} \cdot \text{photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)

Leaf type	R/FR ratio	Days of high light	Chlorophyll <i>a</i> fluorescence parameters				
			F_v/F_M	F_v/F_0	Area	PI	RC/ABS
Sporotrophophyll	LR	0	0.76 ^a	3.25 ^a	45,880 ^{ab}	1.19 ^b	0.80 ^b
		1	0.68 ^b	2.47 ^{ab}	22,302 ^{cd}	0.53 ^{cd}	0.53 ^{cd}
		3	0.47 ^d	0.95 ^c	14,338 ^d	0.09 ^d	0.36 ^d
		7	0.63 ^b	1.74 ^{bc}	24,705 ^{cd}	0.15 ^d	0.41 ^d
	HR	0	0.75 ^a	3.10 ^a	33,707 ^{bc}	0.87 ^{bc}	0.69 ^c
		1	0.60 ^{bc}	1.69 ^{bc}	25,610 ^{cd}	0.36 ^{cd}	0.50 ^{cd}
		3	0.51 ^{cd}	1.09 ^c	25,764 ^{cd}	0.17 ^{cd}	0.45 ^d
		7	0.75 ^a	3.01 ^a	60,432 ^a	3.85 ^a	1.88 ^a
Nest leaf	LR	0	0.81 ^a	4.25 ^a	22,169 ^a	0.75 ^a	0.51 ^a
		1	0.75 ^{ab}	3.15 ^b	6,477 ^b	0.21 ^b	0.34 ^{bc}
		3	0.69 ^b	2.28 ^{cd}	8,500 ^b	0.16 ^b	0.31 ^{bc}
		7	0.74 ^{ab}	2.84 ^{bc}	11,543 ^b	0.23 ^b	0.37 ^{bc}
	HR	0	0.75 ^{ab}	2.97 ^{bc}	6,559 ^b	0.30 ^b	0.41 ^b
		1	0.73 ^{ab}	2.85 ^{bc}	6,961 ^b	0.24 ^b	0.37 ^{bc}
		3	0.75 ^{ab}	2.68 ^{bc}	12,106 ^{ab}	0.25 ^b	0.35 ^{bc}
		7	0.63 ^c	1.76 ^d	13,783 ^{ab}	0.11 ^b	0.26 ^c

Note. Values for the same leaf type marked with the same letters in the column do not differ significantly at $p \leq .05$ according to the Duncan's test, $n = 7$. R/FR ratio: red/far-red ratio.

growing earlier at LR, and the shape of the reflectance curve was similar to this observed for non-photosynthetic tissues (Fig. 6A – red line). In leaves growing earlier at HR, high light resulted in a strong reflectance increase throughout the analyzed spectral range (Fig. 6B).

The reflectance value at 970 nm, which is an indicator of tissue hydration, was similar in all lighting conditions in the nest leaves. There was a decrease in reflectance at this wavelength in the sporotrophophylls of plants exposed to high light, in particular those growing at HR.

Figs 5C–F and 6C–F show the RD and sensitivity analysis, determining the wavelength at which the reaction of the plant to stress, measured by the change in the intensity of reflection, is particularly evident. A broadband with two extremes at 653 and 690 nm was visible in sporotrophophylls growing at LR (Fig. 5C and D). High light caused the highest increase in reflectance intensity at a wavelength of 653 nm. It can therefore be said that the reflectance at 653 nm illustrates in the present case the sensitivity of *P. bifurcatum* leaves to high-light stress. All RD and sensitivity values were negative (Fig. 5E and F) for sporotrophophylls from the HR. It is possible to determine the maximum difference in reflectance at 698 nm, assuming the most negative value. However, there was no distinct, single peak, for the sensitivity; the maximum sensitivity (the most negative value) was visible in the yellow–green range at 515 nm.

The greatest differences in reflectance in the nest leaves of plants from HR (Fig. 6E and F) were visible in the ranges of approx. 500–650 and 700–900 nm. Two broad stress response bands, one of which is partly in the infrared range, did not have a clearly defined extremum. In this case, the highest RD and sensitivity values were visible at 735 nm. In turn, the highest RD value in the leaves from LR was visible at 735 and 540 nm, which coincides with the sensitivity value (Fig. 6C and D).

The values of reflectance coefficients, such as ARI1, CRI1, SIPI, FRI, and WBI, are shown in Table 2. The

content of anthocyanins (estimated based on the ARI1 coefficient) increased at high light in the both types of leaves only in plants growing earlier at HR. The high intensity of light had no effect on the anthocyanin content in plants from LR. The quantity of carotenoids was significantly higher in the leaves of control plants growing under LR than in those from HR. After 7 days of growth in high light, the amount of carotenoids decreased significantly in plants from LR, whereas it increased in plants from HR (Table 2).

Changes in the carotenoid content to chlorophyll content ratio can be estimated based on the SIPI factor. Nest leaves of the control plants growing at LR showed higher SIPI values than those growing at HR. However, SIPI values after 7 days of high-light exposure decreased in the leaves from LR, whereas they did not change in the leaves from HR (Table 2). SIPI values in sporotrophophylls from LR decreased (similarly to nest leaves) and significantly increased in plants from HR due to light stress (Table 2).

The amount of flavonoid compounds in the leaves is positively correlated with the FRI index value. The increase in light intensity resulted in a significant higher FRI value, with the exception of sporotrophophyll and nest leaves growing earlier under LR.

The photosynthesis quantum yield, described by the PRI parameter, decreased in all analyzed leaves regardless of the R/FR ratio value (Table 2). Sporotrophophylls from HR, which did not respond with a statistically significant decrease in the PRI value to the increase in light intensity, were the exception.

The assessment of leaf hydration level using the WBI indicated that high-light stress in plants from the HR spectrum resulted in a higher water accumulation in tissues, whereas no significant differences were observed at LR. Generally, sporotrophophyll leaves were characterized by greater hydration (Table 2).

Sporotrophophylls

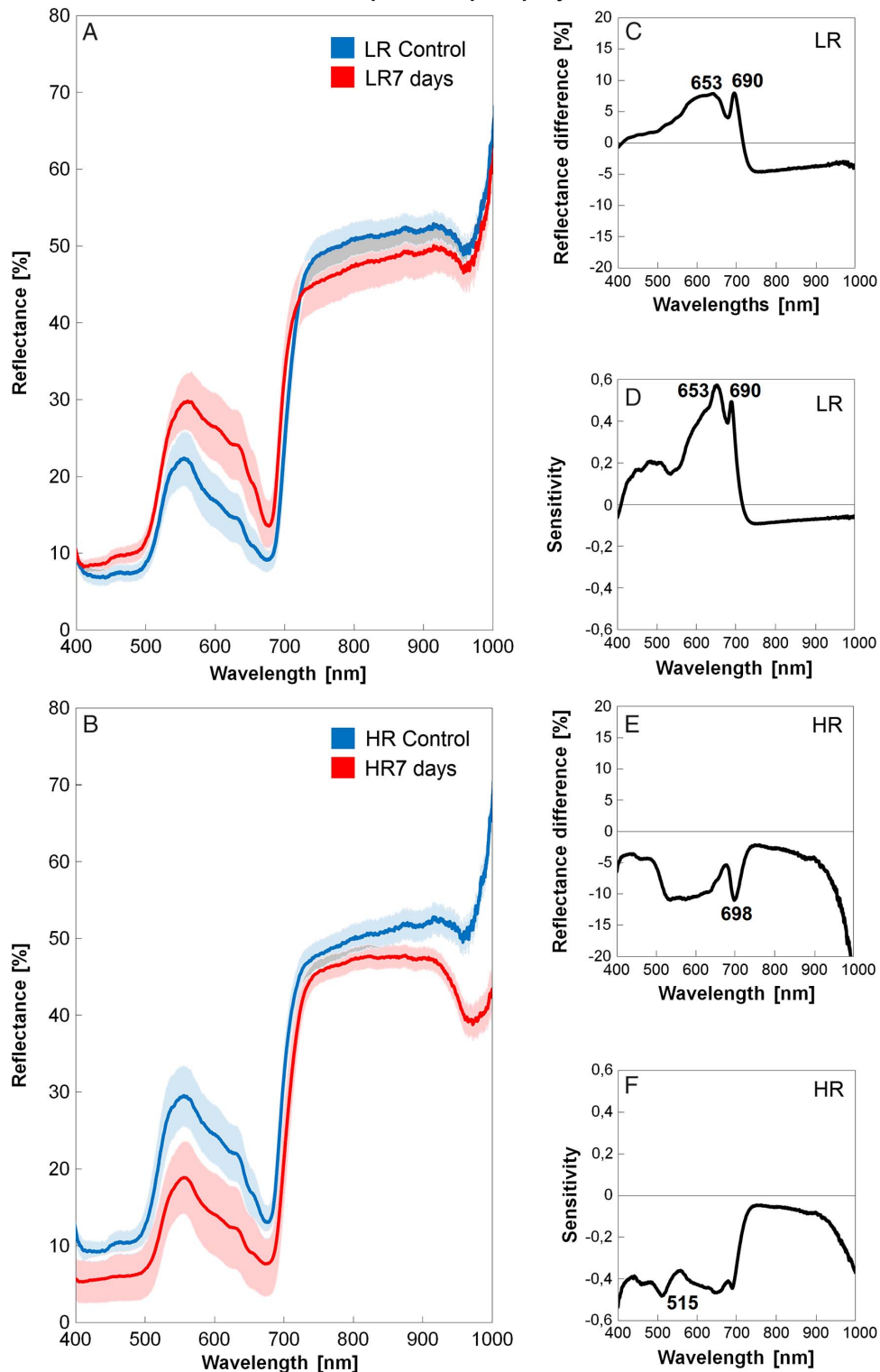


Fig. 5. Reflectance intensity from sporotrophophyll leaves of *Platycerium bifurcatum* growing at (A) – low (LR), (B) – high (HR) R/FR ratio before exposure and 7 days after exposure to high light ($1,200 \mu\text{mol} \cdot \text{quantum} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). Reflectance difference and sensitivity calculated on the basis of reflectance intensity % (see “Materials and Methods” section) for leaves growing at LR (C, D) and HR (E, F), $n = 7$. The shaded area on the curves means $\pm SD$

DISCUSSION

In many epiphyte species, even moderate irradiation combined with other stress can lead to disturbances of ontogenetic development (Durand & Goldstein, 2001). The ability

to absorb light quanta and the reaction rate in the electron transport chain in the light photosynthetic phase is closely correlated with the plant’s demand for assimilates, which decreases under stress conditions (Strasser et al., 2000). When the amount of the light absorbed by the pigments of

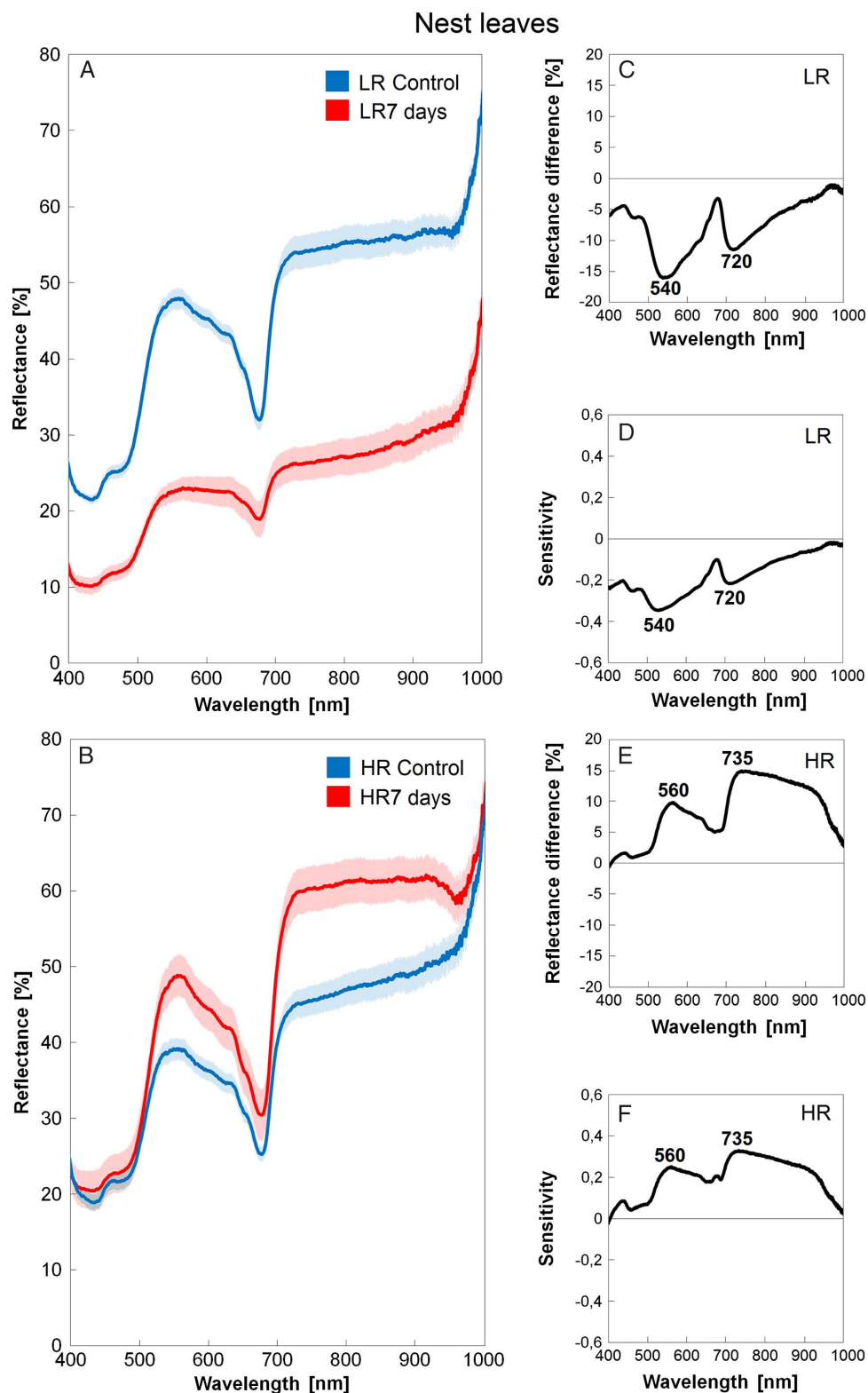


Fig. 6. Reflectance intensity from nest leaves of *Platycerium bifurcatum* growing at (A) – low (LR), (B) – high (HR) R/FR ratio before exposure and 7 days after exposure to high light ($1,200 \mu\text{mol} \cdot \text{quantum} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). Reflectance difference and sensitivity calculated on the basis of reflectance intensity % (see “Materials and Methods” section) for leaves growing at LR (C, D) and HR (E, F), $n = 7$. The shaded area on the curves means $\pm SD$

the antenna complex is too large compared to the possibility of transforming it into the energy of chemical bonds, photoinhibition occurs, and in extreme cases, irreparable damage to PSII (Zhou et al., 2007). This causes changes in the fluorescence induction curve shape and the values of the

Chl a fluorescence kinetics parameters as well as remodeling of the leaf pigment composition.

Changes in fluorescence intensity in the O–J phase of OJIP curves are strongly dependent on light. They describe the absorption capacity of LHCII (light-harvesting complex)

Table 2. Values of leaf reflectance parameters of the sporotrophophyll and nest leaves of *Platycerium bifurcatum* growing at the low (LR) and high (HR) R/FR ratio before exposure and 7 days after exposure to intense light ($1,200 \mu\text{mol} \cdot \text{photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)

Leaf type	R/FR ratio	Days of high light	Reflectance parameters					
			ARI1	CRI1	SIPI	FRI	PRI	WBI
Sporotrophophyll	LR	0	0.004 ^b	0.043 ^b	0.710 ^{ab}	0.903 ^b	-0.029 ^a	1.027 ^b
		7	0.005 ^b	0.034 ^b	0.623 ^c	0.908 ^b	-0.071 ^b	1.014 ^{bc}
	HR	0	0.004 ^b	0.029 ^b	0.639 ^{bc}	0.492 ^c	-0.030 ^a	0.992 ^c
		7	0.008 ^a	0.091 ^a	0.760 ^a	1.197 ^a	-0.047 ^{ab}	1.207 ^a
Nest leaf	LR	0	0.005 ^b	0.022 ^a	0.608 ^a	0.179 ^b	-0.006 ^a	0.906 ^b
		7	0.006 ^b	0.008 ^c	0.393 ^c	0.225 ^b	-0.042 ^c	0.914 ^b
	HR	0	0.007 ^b	0.010 ^c	0.454 ^b	0.145 ^b	-0.025 ^b	0.874 ^c
		7	0.009 ^a	0.015 ^b	0.491 ^b	0.509 ^a	-0.056 ^d	1.000 ^a

Note. 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 test, $n = 7$. R/FR ratio: red/far-red ratio; ARI1: Anthocyanin Reflectance Index; CRI1: Carotenoid Reflectance Index; SIPI: Structure-Insensitive Pigment Index; FRI: Flavonol Reflectance Index; PRI: Photochemical Reflectance Index; WBI: Water Band Index.

and energy transport efficiency between LHCII and the PSII RC (Tsimilli-Michael & Strasser, 2013a). In PPFD = $1200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, only the plant curves from LR were significantly deviated from the control curves (Fig. 3A and B). Thus, in both types of leaves growing at LR, the limitations on electron transport mainly relate to the donor side of PSII.

As suggested by Oukarroum et al. (2007) and other authors, distinct K-band (Fig. 4A, B, G) is the result of an imbalance in PSII between the electrons leaving acceptor RC and the electrons donated by the donor side. This is related to the uncoupling of the Oxygen-Evolving-Complex (OEC; Guisse et al., 1995). In sporotrophophylls from HR, the fluorescence intensity of K-band on the O–J differential curves from the first day was lower than in those of LR (Fig. 4A, B). Also in the nest leaves, the positive K-band was visible only in plants growing at LR (Fig. 4G). Thus, both types of leaves growing at a high R/FR ratio have a higher tolerance of light stress. In turn, flattening of the course of OJIP curve in the I–P phase indicates the disturbances of energy transport from Q_A to Q_B (Fig. 3B). The negative G-band visible on the I–P differential curve (Fig. 4K) shows the course of PSI acceptor side reduction and corresponds to the increasing number of NADPH molecules per active RC (Tsimilli-Michael & Strasser, 2013b). Similar relationships were also observed in the case of other abiotic stresses and during leaves aging (Kalaji et al., 2017). The analysis of the differential J–I and I–P curves (Fig. 4I–4L) confirms the observation that nest leaves acclimated to high light by a high R/FR ratio (HR) show smaller deviations from the control than the leaves growing at LR.

Light stress in sporotrophophylls from LR and HR caused an initial decrease in F_V/F_M and F_V/F_0 values especially after 3 days of high light (Table 1). In the high light, decrease in F_V/F_M values is described in *P. bifurcatum* and in other tropical epiphytic ferns, e.g., *Asplenium cuspidatum* (Hietz & Briones, 2001; Sanusi et al., 2011). However, in sporotrophophylls of *P. bifurcatum* growing at HR, after 7 days of high light, the parameters values returned to the control level. This indicates that the effect of high light did not cause irreparable damage to the photosynthetic apparatus. The rapid increase of light intensity often leads to the degradation of the D_1 protein responsible for Q_A attachment in PSII and RCs deactivation (Maxwell &

Johnson, 2000). However, then the inactive RCs take over and dissipate excess light energy, enabling fast restitution after stress (Lee et al., 2001). In turn, the decrease in the F_V/F_0 value is related to the limitation of acceptor function of the PSII RC and informs about the damage of the thylakoid structure in chloroplasts (Pereira et al., 2000). According to Kalaji et al. (2012) and others, the F_V/F_0 value is also directly proportional to the OEC efficiency on the PSII donor side. Moreover, only in sporotrophophylls from HR, there was no decrease in the PRI parameter value. It illustrates quick, reversible changes in the profile of xanthophyll pigments, induced by stress factors, where higher values mean greater photosynthetic efficiency (Peñuelas et al., 2011). Therefore, both the PRI and F_V/F_M and F_V/F_0 values show that the efficiency of PAR utilization did not only decrease in sporotrophophylls from HR.

The PI combines information on RCs concentration per Chl (RC/ABS), quantum performance of primary PSII photochemistry and efficiency of electron transport outside of Q_A (Oukarroum et al., 2007). Thus, changes in PI values depend on the properties of the antenna, trapping efficiency or electron transport. The logarithmic function of the relative electron transport activity and the logarithmic function of relative PI are linearly related (Oukarroum et al., 2007). The ferns growing in HR are characterized by a slightly lower vitality (PI values) than those from LR. However, the PI value of sporotrophophylls from HR after 7 days of high light was significantly higher than the control value (Table 1). In turn, in sporotrophophylls from LR, the average PI value at that time declined significantly. Increasing the vitality of sporotrophophylls from HR can be interpreted as a result of acclimation to new light conditions and an attempt to compensate for losses caused by light stress. This is confirmed by the values of the Area parameter reflecting the size of the plastoquinone pool (Kalaji et al., 2011). Also in the other ornamental plants, the reduction of the R/FR ratio in the light spectrum by far-red supplementation increased the photosynthesis and positively correlated with yield photon flux (Park & Runkle, 2017).

In nest leaves from HR, no statistically significant changes in the Area and PI values were noted (Table 1). Also in the reflectance analysis, no significant increase in the pigment content was observed. In turn, the reflectance curve

of nest leaves form LR under light stress was similar the curve of the necrotic tissue, characteristic for the third stage of nest leaf development (Fig. 6A; Oliwa et al., 2017).

The sporotrophophylls growing under LR showed at high light, although expected, the low increase in radiation reflectance (Figure 5A). A similar reaction occurred in many species of seed plants subjected to short-term stress (dehydration, increased ozone concentration, and herbicide treatment or nitrogen deficiency; Carter, 1993; Carter & Knapp, 2001). High light reflectance in the PAR range is the result of low pigment contents (Maxwell & Johnson, 2000). Light reflectance from a single leaf is directly correlated with its chemical composition and allows to determine pigment content (Linke et al., 2008). Simultaneously, it is a good alternative to biochemical methods, which are often subject to errors resulting from pigment instability, and also require tissue destruction (Solovchenko, 2010). The described differences in response to the high light stress between two types of leaves are associated with their different functions. Sporotrophophylls are responsible for assimilation. Nest leaves have mainly mechanical functions and in ontogenetic development they quickly lose assimilation functions (Oliwa et al., 2017).

Leaf reflectance coefficients, measured in narrow wavebands, can serve as indicators of plant stress. In *P. bifurcatum*, maximum RD and sensitivity values partly coincide with the range determined by Carter (1993) for eight species of spermatophytes in response to environmental stresses. The first maximum in *P. bifurcatum* fern occurred at 653 nm and the second located in the vicinity of 690 nm, as in the case of spermatophytes (Carter, 1993).

In the fern *P. bifurcatum*, maximum negative RD and sensitivity values do not indicate the stress response, but rather the degree of changes occurring during acclimatization of the plant to other lighting conditions. The sensitivity index, as suggested by Carter (1993), appears to be a more sensitive stress detector in a typical stress reaction that occurred in sporotrophophylls from LR.

ARI1 proved to be a sensitive indicator correlated linearly with anthocyanin contents in the leaves of many plant species (Steele et al., 2009). The increasing content of anthocyanins after 7 days of high light in both leaf types from HR allowed to minimize the effects of light stress by photosystem “shading.” This may explain the increase in Chl a FL parameters related to PSII photochemical efficiency (F_v/F_M , F_v/F_0) and plant vitality (PI). Accumulation of anthocyanins is a very frequent reaction of plants to a high PAR intensity because these compounds protect the photosynthetic apparatus (Merzlyak & Chivkunova, 2000). The analogous to ARI1 increase in the value of carotenoids (CRI1) seems to be justified by the need to protect the photosynthetic apparatus by dissipating excess energy, e.g., in the xanthophyll cycle. Carotenoids absorb mainly blue-range radiation, while Chl a blue and red; therefore, the SIPI parameter based on reflectance in both these ranges can be used as an indicator of the quantity ratio of these compounds (Peñuelas et al., 1993a). Values of carotenoids to Chl ratio increased significantly in both leaf types from the HR spectrum and decreased in plants from LR. The increase in the amount of carotenoids in relation to the amount of Chl is a frequent response of plants to high light.

In addition to anthocyanins and carotenoids, flavonoids also play an important role in photoprotection (Solovchenko, 2010). Their rapid generation under stress conditions inhibits the production of reactive oxygen species (Agati et al., 2012). The FRI values demonstrated a significant increase in the flavonoid content in the reaction to high light – more noticeable in the sporotrophophyll leaves. The rapid synthesis of flavonoids in leaves acclimated to intense light correspond well with our results of Chl a FL analysis. However, it should be remembered that in the process of dissipating excess energy, in addition to the synthesis of photoprotectants, an important role is played by PsbS proteins, the amount of which is regulated by phytochrome signaling (Dong et al., 2015). Thus, the PSII efficiency recovery may be associated with a change in the structure and size of the LHCII pool (Wang & Deng, 2003).

Tissue hydration measured with the WBI value in most of the objects was within the range characteristic for green plants, i.e., 0.8–1.2 IU (Peñuelas et al., 1997). Nevertheless, significant higher WBI values in both types of leaves from HR under high light is worth noting, although the mechanism of this phenomenon is not clear.

CONCLUSION FOR FUTURE BIOLOGY

The sporophytes of *P. bifurcatum* growing at high R/FR ratio have a higher tolerance to high light than plants growing at the low R/FR ratio. Therefore, increasing the R/FR ratio in the light spectrum can acclimated the photosynthetic apparatus of ferns for light stress. An important role in this process is played by photoprotective pigments such as carotenoids, anthocyanins, and flavonoids, which in plants growing at a high R/FR ratio are contained in a larger amount than in plants growing at a low R/FR ratio. A different reaction of sporotrophophylls and nest leaves provides effective protection of the organs responsible for the production of assimilates and reproduction, i.e., sporotrophophylls.

The sensitivity analysis is an effective indicator of the assessment of light stress in epiphytic fern *P. bifurcatum* sporotrophophylls. However, the above methods of reflectance spectra analysis are not suitable for very low amounts of pigments.

Funding Statement: This project was funded by the Pedagogical University in Krakow as part of statutory research BSM162/G/2018.

Data Accessibility: Data were generated at Department of Plant Physiology, Pedagogical University of Cracow. Raw data supporting the findings of this study are available from the corresponding author JO on request.

Competing Interests: The authors declare no competing interests.

Authors' Contributions: AS and JO designed the research and analyzed the data. JO conducted the research and wrote

the paper. Both the authors have read and approved the final version of the manuscript.

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