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Sporulation modifies the photosynthetic activity of sporotrophophyll leaves of *Platycerium bifurcatum*

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Abstract

Platycerium bifurcatum is an epiphytic fern, occurring mainly in the forests of Australia and New Guinea. The location and spatial structure of its sporangia makes this fern a unique research model for physiological experiments. Our research aimed to determine changes in photosynthesis in the sporophilic and trophophilic parts of *P. bifurcatum* leaves during spore tying and maturation. For this purpose, the JIP-test and gas-exchange measurements of leaves were performed. In the study, we showed that changes typical of plant responses to stress factors also happened at critical periods in plant ontogenesis (*e.g*., in the generative phase) not related to environmental stress. Measurements of chlorophyll *a* fluorescence kinetics, the intensities of net/gross photosynthetic rate, and the respiration of sporotrophophilic leaves indicated that the intensities of these processes were related to the location of spores and to the stage of sporulation. The results are the first to describe the photosynthesis process and dark respiration of leaves during sporulation in ferns.

Additional key words: elkhorn fern; epiphyte; generative development; OJIP curve; tropical plants.

Introduction

Analyzing OJIP fluorescence transients, with the JIP-test, can provide a lot of important information about plant bioenergetic state, energy distribution in the light phase of photosynthesis, and communication between individual PSII units (Strasser *et al*. 2000, 2010). A well-developed methodology for chlorophyll (Chl) *a* fluorescence measurements and the extensive literature on the topic allow a comprehensive assessment of the impact of environmental factors on the photochemical efficiency of PSII (Kalaji *et al*. 2014, 2018). In addition, thanks to the nondestructive nature of the measurements, one can observe *in vivo* physiological changes occurring in the same parts of the leaf blade. Used together, fluorescence and gas-exchange measurements provide complete information on the photosynthesis process and on the state of internal balance between energy supply and consumption in the dark phase of photosynthesis.

Many ecophysiological, environmental, and agricultural studies have used Chl *a* fluorescence analysis and the JIP-test to study plant responses to abiotic and biotic environmental stresses (Živčák *et al*. 2008, Kuckenberg *et al*. 2009, Kalaji *et al*. 2014). However, very little attention was given to cryptogams (including ferns), as well as possibility of using the JIP-test in analysis of processes connected with their generative development. Fern growth (like that of many other plant species, for that matter), and in particular, the sporulation phase (generative development), requires energy from photosynthesis. Therefore, the JIP-test, which allows one to assess PSII photochemical efficiency, and gas-exchange analysis can be useful in the study of ontogenesis in ferns.

Platycerium bifurcatum is an epiphyte that occurs naturally mainly in the forests of Australia and New Guinea. It is also one of the most commonly grown ornamental fern species in the world (Kreier and Schneider 2006). Sporophyte of *P. bifurcatum* is a trophically independent

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Abbreviations: ABS/RC – apparent antenna size of active RC PSII; A_M – surface area above the OJIP curve; Chl – chlorophyll; DI_0/RC – total energy dissipation not trapped by the PSII reaction center; ET_0/RC – rate of electron transfer by the active PSII reaction center; F_0 – minimum fluorescence; FL – fluorescence; F_M – maximum fluorescence; F_v/F_0 – indicator of structural damage of thylakoids; F_v/F_M – maximum quantum yield of PS II; OEC – oxygen-evolving-complex; JIP-test – fluorescence transient analysis; P_G – gross photosynthetic rate; PI_{ABS} – performance index of PSII based to absorption; PI_{total} – performance of electron flux to the final PSI electron acceptors; P_N – net photosynthetic rate; PQ – plastoquinone pool; *R* – respiration; RC – reaction center; R_D – dark respiration; RE_0/RC – quantum yield of electron transport from Q_A to the PSI end electron acceptors; S_M – normalized total area over the JIP curve; TR_0/RC – energy trapping of one active reaction center; V_j – the number of closed RCs in relation to the total number of RCs; V_t – normalized fluorescence intensity; φ_{E0} – quantum yield for electron transport from Q_A^- to plastoquinone; φ_{P0} – maximum quantum yield of primary PSII photochemistry; φ_{R0} – quantum yield for reduction of end electron acceptors at the PSI acceptor side; ψ_{E0} – probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-.
†These authors contributed equally to this work.
This work is dedicated to Professor Reto J. Strasser on his 76th birthday.

generation, well adapted to changing water supply conditions (Kluge *et al*. 1989, Rut *et al*. 2008, Rut 2014). Mature sporophytes have two types of leaves: sporotrophophylls and nest leaves. Sporotrophophyll leaves perform assimilation functions (the trophophilic part) and are responsible for reproduction (the sporophilic part). In turn, nest leaves perform mainly mechanical functions, since they fasten the plant onto the tree and accumulate water and mineral substances. Thus, the two types of leaves serve different physiological functions and undergo different types of metabolism (Oliwa *et al*. 2016, 2017; Oliwa and Skoczowski 2019a).

In *P. bifurcatum*, sporangia concentrate in acrostichoids, on the sporophilic part at the dichotomous ends of the leaf blade. They have a single-layer wall and are placed on stalks on the lower surface of the leaves (Raghavan 1989), which helps protect sporangia thanks to less exposure to environmental factors (Holtum and Winter 1999). Sporangia's location and spatial structure make *P. bifurcatum* a unique research model in physiological experiments. Since they are located on a relatively large area separated from the rest of the leaf, it is possible to determine metabolic changes accompanying sporulation both at the site of spore production and in the nonsporulated part. In most cryptogam species, including ferns, such measurements are impossible due to the location of sporangia in numerous, small sori.

The sporulation process begins with the differentiation of an archespore, from which the stem cells of the spores are formed, followed by the tetraspora. *P. bifurcatum* spores have an ellipsoidal shape, flattened on one side; initially green, they become light brown later. Sporoderm is composed of an outer layer (exosporium) and an inner layer (endospore). Exosporium is hard and resistant to chemical substances, but it is permeable to water (Soltis and Soltis 1992, Makgomol 2006). Its spore surface is characterized by diffuse papillary folds and the presence of globular structures.

Earlier research on *P. bifurcatum* have concerned mostly micropropagation and the *in vitro* vegetative propagation (Camloh and Gogala 1992, Ambrožič-Dolinšek and Camloh 1997, Aspiras *et al*. 2010, Liao and Wu 2011). Motivated by growing anthropopressure in tropical forests, ecophysiological studies – some of which used the Chl *a* fluorescence analysis – have been conducted in recent years. They mainly concerned *P. bifurcatum*'s response to drought stress, light stress, and high ozone concentration (Rut *et al*. 2003, Sanusi *et al*. 2011, Oliwa and Skoczowski 2019a,b) and its ontogenetic development in different light conditions (Oliwa *et al*. 2016, 2017). However, according to the authors' knowledge, the scientific literature lacks information on energy distribution and assimilation activity of leaves during the sporulation process not only in *P. bifurcatum*, but in ferns in general. Thus, the following questions seem reasonable: (*1*) Do the sporulating and nonsporulating parts of the leaf differ in photosynthetic activity? (*2*) Does photosynthesis in tissues immediately adjacent to the area of spore formation resemble that which takes place in other parts of the leaf blade?

This work aims to determine changes in photosynthesis in the sporophilic and trophophilic parts of *P. bifurcatum* leaves during spore tying and maturation. The experiment designed to study these processes used the JIP-test and selected parameters of Chl *a* fluorescence to analyze kinetics capture, transport, and energy dissipation in the photosynthetic light phase. The analyses included also the relationship between the stage of sporulation and changes in the intensity of gas exchange measured in the sporophilic and trophophilic part of the leaves.

Materials and methods

Plant material: The experiment used 10-year-old sporophytes of *Platycerium bifurcatum* (Cav.) C. Chr. from the collection of the Institute of Biology of the Pedagogical University of Krakow, Poland. The plants grew under greenhouse conditions with a natural photoperiod, at 22°C and 40–60% relative humidity.

Fig. 1 shows four stages of sporulation specified for the experiment. Chl *a* FL kinetics and gas exchange were measured in sporophilic parts of leaves: (*1*) with juvenile sporangia at the S_I stage, and with maturing sporangia at (*2*) the S_{II} stage, and (*3*) the S_{III} stage. At the same time, measurements were taken in the trophophilic parts of the leaves (stages T_I, T_{II}, and T_{III}, respectively). Sporotrophophyll leaves without developed sporangia in the sporophilic part constituted the control treatment. Since leaves in the S_{IV} and T_{IV} stages had already lost

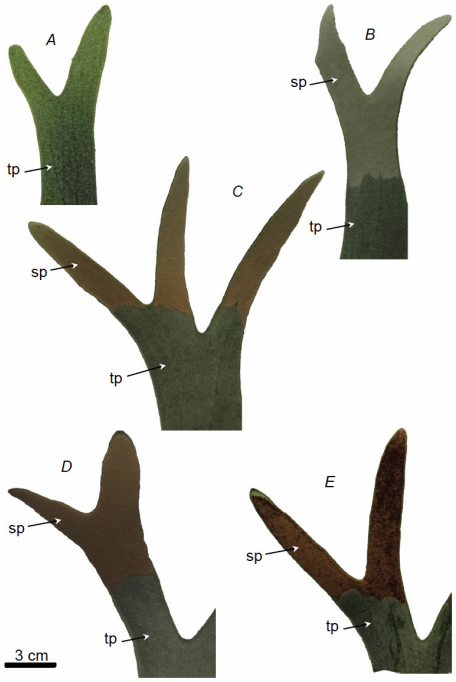

Fig. 1. Different stages of development of the sporangium part on the lower side of *Platycerium bifurcatum* leaf. Nonsporulating leaf (control) (*A*), stage I (S_I) (*B*) – leaf with young sporangium; stage II (S_{II}) (*C*), and stage III (S_{III}) (*D*) – leaves with immature sporangium; stage IV (S_{IV}) (*D*) – leaf with mature sporangium. This classification was proposed by the authors. tp – trophophilic part, sp – sporophilic part.

all of their chloroplasts and thus they did not serve any photosynthetic functions, they were not analyzed.

Chl *a* fluorescence kinetics: According to the method of Strasser *et al.* (2000), the parameters of Chl *a* FL kinetics were determined in the sporophilic and trophophilic parts on the upper and lower sides of the leaves, in successive stages S_I–S_{III} and T_I–T_{III} and in control leaves. The measurements were made with a *Handy-PEA* fluorimeter (*Hansatech Instruments*, UK). Before measurement, leaf blade fragments were acclimated to dark for 30 min. The intensity of the excitation light was 1,000 µmol(photon) m^{-2} s^{-1}, and the measurement time was 1 s. To read the results, the *PEA Plus* program was used (*Hansatech*, UK).

The JIP-test was carried out in the following steps: O – 20 µs, J – 2 ms, I – 30 ms, and P – 300 ms. The JIP curves were normalized to points O and P (V_t). The differential curves (ΔV_t) were calculated by subtracting the values of the normalized JIP curves (V_t) in stages S_I–S_{III} and T_I–T_{III} from control curves (Oukarroum *et al*. 2007, Dąbrowski *et al*. 2016). Similarly, the curves for ΔVt for O–K, O–J, J–I, and I–P phases were drawn.

Net photosynthesis (P_N) and dark respiration (R_D): The P_N and R_D intensities of sporotrophophyll leaves were determined using a *CIRAS-2* infrared gas analyzer (*PP Systems*, Hitchin, Herts, UK). For measurements, a *PLC 4* board chamber (*PP Systems*, Hitchin, Herts, UK) with a measuring surface of 2.5 cm^2 was used. The P_N and R values were determined in a closed system containing 21% of O_2. The CO_2 concentration was 300–400 µmol mol^{-1}. During photosynthesis measurements, PFD was 100 µmol(photon) m^{-2} s^{-1}, while the temperature was 25°C.

Gross photosynthetic rate (P_G) and respiration: The P_G and R intensities of sporotrophophylls were determined using a Clark electrode (*Hansatech Instruments Ltd*., Norfolk, UK), located in a *LD/2* chamber (5-ml volume) at 25°C and connected to a *CB1D* data reading device. To read and analyze the data, the *Acquire* software was used (*Hansatech Group*, Norfolk, UK). The P_G and R values were determined in a closed system containing 21% of O_2. The CO_2 concentration was 300–400 µmol mol^{-1}, PFD was 100 µmol(photon) m^{-2} s^{-1}, and the temperature was 25°C. The rates of photosynthesis and leaf respiration were expressed in µmol(CO_2 production) or (O_2 taken) m^{-2} s^{-1}.

Statistical analysis: To analyze the data, one-way analysis of variance (*ANOVA*) was applied in *Statistica 10.0* (*StatSoft*, Poland). Whenever the *F*-test showed significant differences between the groups, *Tukey*'s HSD test was used to test the pairwise differences between the means, at $p \leq 0.05$. The results shown are the means of 4–10 independent biological replicates.

Results

The sporophilic and trophophilic parts of *P. bifurcatum* leaves differed in the PSII photochemical activity, determined by the parameters of Chl *a* FL and JIP curves, and in the amount of CO_2 uptake and O_2 evolved.

Chl *a* fluorescence kinetics: The FL induction curves in both sporophilic and trophophilic parts of the leaves, at all the stages of spore development, had a typical course with clearly marked O-J-I-P steps (Fig. 2). Normalized O-J-I-P curves (V_t in Fig. 2*A–D*) showed that the largest deviations from control occurred in the S_I and T_I phases in the sporophilic part, but also on the upper side in the trophophilic part. In turn, in the sporangium area, the largest differences were observed in the S_{III} stage, that is, during spore maturation (Fig. 2*B*). This relationship was even better visible as positive bands in the double normalized differential curves (ΔV_t) (Fig. 2*E–H*), indicating differences in the energy flux between the sporulating part and the other parts of the sporotrophophyll leaves.

The double normalization of fluorescence curves separately for the O–J phases (Fig. 3) revealed positive K bands. The highest intensity of FL in the K bands was observed in the sporophilic part in stages S_{II} and S_{III} (measured on the lower leaf side) (Fig. 3*B*). This band, however, was visible in the curves of all the analyzed parts of the leaf blades (Fig. 3).

The maturation of sporangia did not adversely affect the maximum photochemical PSII yield in the trophophilic part of the leaves (Table 1). However, the F_v/F_M ratio in the sporophilic part (in the lower part of the leaf blades) decreased by 10–15% in the later stages of sporulation (S_{II} and S_{III}). In turn, the A_M value permanently decreased during spore formation only directly at the sporangium area (S_I–S_{III}, bottom of leaf), with a simultaneous increase in V_j. In the remaining parts of the leaves, the decrease in A_M was temporary and only occurred in stage I. The quantum yields and probability parameters indicate that electron flow outside Q_A decreased in the sporulating part in subsequent stages S_I–S_{III} (ψ_{E0} and φ_{E0}). In the nonsporulating parts of the leaves, ψ_{E0}, φ_{E0}, and φ_{R0} only temporarily decreased.

Parameters representing energy fluxes in one active RC (ABS/RC, TR_0/RC, ET_0/RC, RE_0/RC, and DI_0/RC) increased in the subsequent stages of spore development in both parts of the leaves (Table 1). This trend was not visible only in the ET_0/RC and RE_0/RC values in the trophophilic part.

The performance indexes, PI_{ABS} and PI_{total}, proved to be the most sensitive parameters differentiating PSII activity in individual parts of the sporotrophophylls. During sporulation, PI values significantly decreased, both in trophophilic and sporophilic parts. PI_{total} markedly decreased – down to 70% – on the lower leaf side in the sporophilic part (Table 1).

Gas exchange of leaves: The sporophilic and trophophilic parts significantly differed in P_G values in stages II (S_{II}, T_{II}) and III (S_{III}, T_{III}), and in the P_N values in stage III only (Fig. 4). In both the sporophilic (S_I) and trophophilic (T_I) parts, P_N was significantly lower in stage I than that in the nonsporulating (control) leaves. No such relationship was observed in P_G, however. P_N and P_G intensities in the trophophilic part of leaves in stages T_{II} and T_{III} did not differ significantly from those in the nonsporulating leaves (Fig. 4). In turn, P_N in the sporophilic part was lower than

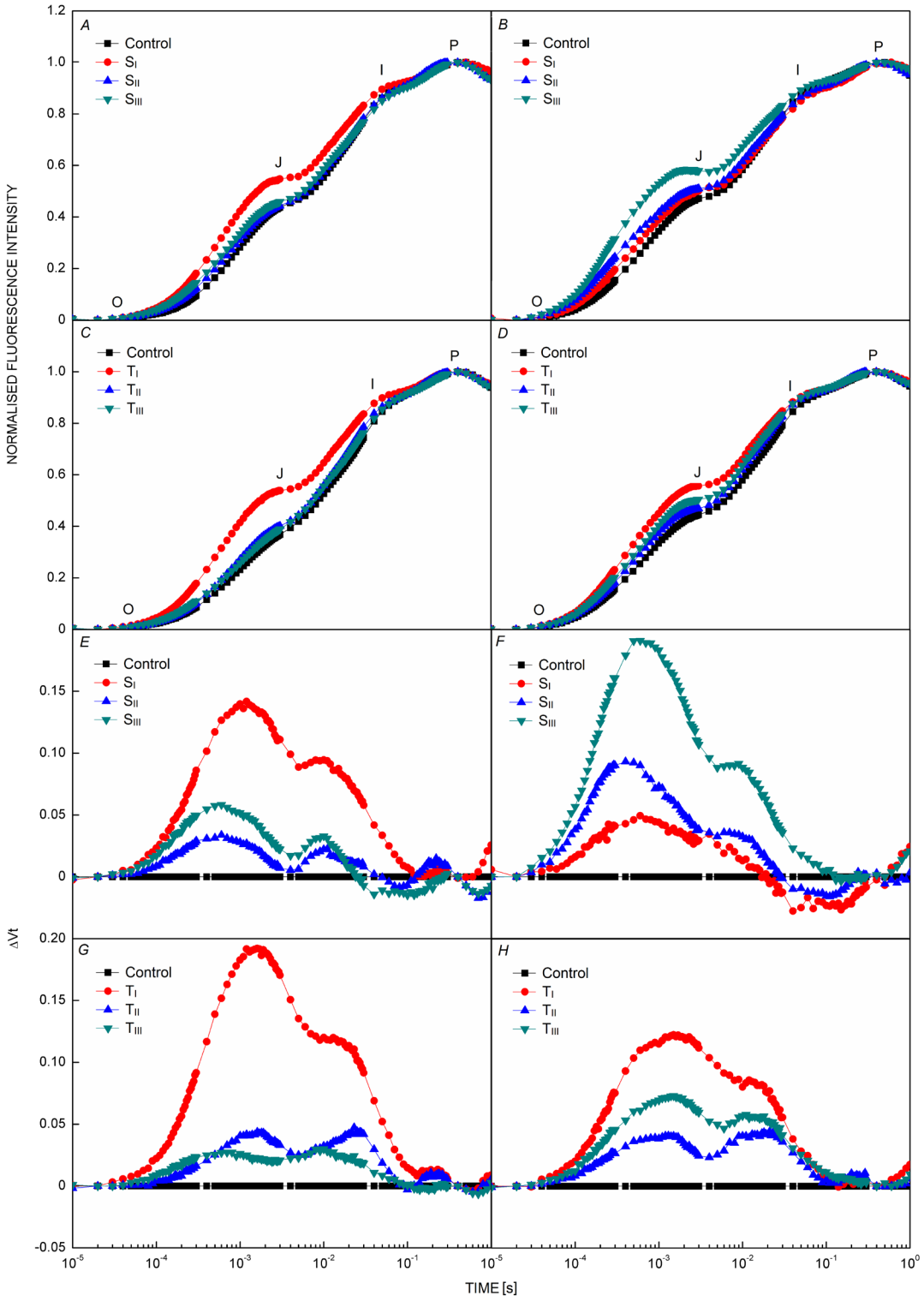

Fig. 2. Normalized OJIP curves (*A–D*) and differential curves ΔVt (*E–H*) in the nonsporulating leaf (control) and in the subsequent stages of sporulation in the sporophilic (S_I–S_{III}) and trophophilic parts (T_I–T_{III}) of *Platycerium bifurcatum* sporotrophophyll leaves. Sporophilic part – upper side of leaf blade (*A*,*E*); sporophilic part – bottom side (*B*,*F*); trophophilic part – upper side (*C*,*G*); trophophilic part – bottom side (*D*,*H*).

that in the control in the first stage of spore maturation [S_I, by 4.7 μmol(CO_2) m^{-2} s^{-1}] and that in stage S_{III}. In the sporulating leaves at the S_I stage, P_G [11.3 μmol(O_2) m^{-2} s^{-1}] was higher than P_N [7.8 μmol(CO_2) m^{-2} s^{-1}]. In the later stages (S_{II}, S_{III}), this discrepancy was smaller (Fig. 4).

The sporulation process resulted in a higher increase in

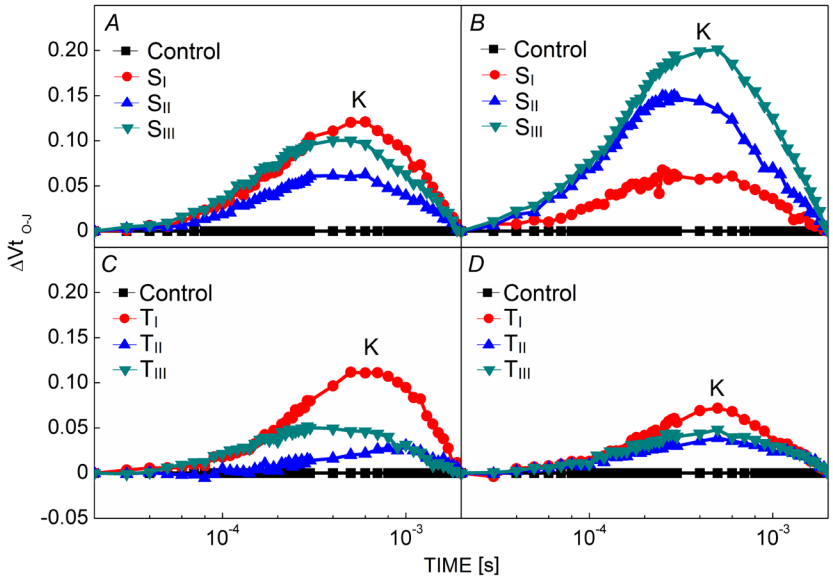

Fig. 3. The O–J phase of differential curves (ΔV_t) in the nonsporulating leaf (control) and in the subsequent stages of sporulation in the sporophilic (S_I–S_{III}) and trophophilic parts (T_I–T_{III}) of *Platycerium bifurcatum* sporotrophophyll leaves. Sporophilic part – upper side of leaf blade (*A*); sporophilic part – bottom side (*B*); trophophilic part – upper side (*C*); trophophilic part – bottom side (*D*).

respiratory intensity in the sporophilic part than that in the trophophilic part, especially in the juvenile stage of spore development (S_I) (Fig. 4). In stages S_{II} and S_{III}, respiration intensity decreased to lower levels than that observed in stage S_I. Despite this, the breathing process was more intense than that in the trophophilic part.

In the trophophilic part, the the R_D and R value did not significantly differ from the control in the subsequent T_I–T_{III} stages. Depending on the measurement method used, the intensity of breathing in the S_I stage increased by 70% to upwards of even 170%, compared to the control.

Discussion

Photosynthesis is the main metabolism process, and its course strongly correlates with the stage of plant development. Photosynthesis phases include light absorption by the antenna system, electron transfer in reaction centers, energy stabilization in secondary processes, and the synthesis and transport of photosynthetic products (Blankenship 2014). Understanding how the photosynthetic apparatus functions in the sporophilic and trophophilic parts during spore maturation may help understand the processes of growth, development, and reproduction – not only of *P. bifurcatum*, but of ferns in general.

Along with the progressive sporulation process in the individual parts of *P. bifurcatum* leaves, a change in photosynthetic activity occurred, a process associated with both PSII functioning and CO_2 assimilation. The differences in the energy flux in the sporophilic part, visible on the OJIP and V_t curves, seem to be a natural consequence of sporangia formation and spore maturation (Fig. 2*B*). Changes in PSII functioning, however, were also visible on the upper side of the sporophilic part of the leaf, and in the trophophilic parts that did not directly participate in the formation of sporangia (Fig. 2). The O–J and J–I phases of the FL induction curves suggest that the fundamental difference between the sporulating part of the leaf and the others manifests in the largest changes in the absorption and distribution of energy at different stages of sporulation. In the area of spore formation, the increase in amplitude of the ΔV_t bands in stages S_I–S_{III} (Fig. 2*F*) suggests a progressive loss of LHCII absorption capacity, as was the case in other plants under stress (Tsimilli-Michael and Strasser 2013). Changes in part O–J during spore development indicate the reorganization of PSII units, which translates into communication between them (Strasser *et al*. 2004, Joliot and Joliot 2005, Stirbet 2013). These values increased in steps J and I, which means that the number of electron carriers on the PSII acceptor side was limited (Lazár 2006), and that the oxygen-evolving complex (OEC) might be dysfunctioning (Kalaji *et al*. 2018). These increases confirm the decrease in the S_M value in the S_I–S_{III} phases in this part of the leaf, suggesting a reduction in the size of the PSII and PSI electron carrier pool (from Q_A to ferredoxin) (Bąba *et al*. 2016). On the other hand, in the remaining parts of the leaf (*i.e*., above the sporulating part and in the trophophilic part), temporary disturbances at this stage of electron transport were visible only in stage I. In the next stages, the leaves returned to a state similar to that of nonsporulating (control) leaves. Quick compensation of excitation energy losses and restoration of effective energy transport in PSII units in the trophophilic part of the leaf suggest that it may play an important role in supplying energy to the sporulating part, in which the presence of spores hinders effective energy absorption.

The O–K and O–J phase differential curves (ΔV_t; Fig. 3) showed that the characteristic L and K stress bands occured not only in the case of a typical response to environmental stress. Therefore, these bands can also be used to describe changes in energy distribution in PSII during propagation and generative development of plants. This shows new possibilities of using Chl *a* fluorescence kinetics analysis in ontogenesis and plant reproduction studies, and in developmental biology in the broad sense. Visible L and K bands in the sporophilic part of the leaf (especially, in stages S_{II} and S_{III}; Fig. 3) indicate an imbalance in

Table 1. Selected parameters of chlorophyll *a* fluorescence kinetics measured on the upper and lower sides of the sporophilic and trophophilic part of *Platycerium bifurcatum* leaves. The control was nonsporulating leaves; S_I–S_{III} – following stages of sporulation measured in the sporophilic part of leaf; T_I–T_{III} – following stages of sporulation measured in the trophophilic part of leaf. Parameter values marked with *different letters* differ significantly in a given part of the leaf, according to *Duncan*'s test at $p \leq 0.05$, $n = 4$–10.

Parameter	Sporophylic part								Trophophilic part							
	Upper leaf side				Bottom leaf side				Upper leaf side				Bottom leaf side			
	Control	S_I	S_{II}	S_{III}	Control	S_I	S_{II}	S_{III}	Control	T_I	T_{II}	T_{III}	Control	T_I	T_{II}	T_{III}
Measured parameters and basic JIP-test parameters																
A_M	32.425^{a}	15.877^{b}	35.334^{a}	36.165^{a}	24.744^{a}	11.208^{b}	13.328^{b}	13.163^{b}	33.411^{a}	18.098^{b}	31.841^{a}	32.982^{a}	25.102^{a}	14.206^{b}	24.649^{a}	23.009^{a}
F_0	195^{c}	178^{c}	264^{b}	306^{a}	157^{ab}	96^{c}	130^{b}	177^{a}	212^{b}	211^{b}	219^{b}	257^{a}	176^{b}	165^{b}	202^{a}	207^{a}
F_M	$1{,}331^{b}$	879^{c}	$1{,}595^{a}$	$1{,}549^{a}$	$1{,}074^{a}$	434^{c}	634^{b}	745^{b}	$1{,}306^{a}$	$1{,}086^{b}$	$1{,}403^{a}$	$1{,}366^{a}$	$1{,}146^{b}$	846^{c}	$1{,}325^{a}$	$1{,}258^{ab}$
F_v/F_M	0.85^{a}	0.80^{a}	0.83^{a}	0.80^{a}	0.85^{a}	0.78^{b}	0.79^{b}	0.76^{b}	0.84^{a}	0.80^{a}	0.84^{a}	0.81^{a}	0.85^{a}	0.80^{a}	0.85^{a}	0.84^{a}
F_v/F_0	5.84^{a}	3.92^{b}	5.07^{a}	4.14^{b}	5.89^{a}	3.51^{b}	3.88^{b}	3.24^{b}	5.18^{a}	4.13^{b}	5.41^{a}	4.40^{b}	5.60^{a}	4.06^{b}	5.58^{a}	5.13^{a}
V_j	0.40^{b}	0.53^{a}	0.41^{b}	0.44^{b}	0.45^{b}	0.49^{b}	0.51^{b}	0.60^{a}	0.33^{b}	0.52^{a}	0.37^{b}	0.35^{b}	0.43^{b}	0.55^{a}	0.46^{ab}	0.50^{ab}
S_M	28.47^{a}	22.91^{b}	26.54^{ab}	29.11^{a}	27.00^{b}	33.44^{b}	26.46^{b}	23.05^{c}	30.59^{a}	21.01^{c}	26.98^{b}	29.95^{a}	25.81^{a}	21.58^{b}	22.00^{b}	21.88^{b}
Specific energy fluxes expressed per active PSII RC																
ABS/RC	1.00^{c}	1.51^{a}	1.27^{b}	1.48^{a}	1.44^{c}	1.89^{b}	2.22^{ab}	2.46^{a}	1.12^{b}	1.51^{a}	1.19^{b}	1.35^{ab}	1.56^{bc}	1.88^{a}	1.68^{b}	1.75^{ab}
DI_0/RC	0.15^{b}	0.31^{a}	0.21^{b}	0.30^{a}	0.21^{c}	0.42^{b}	0.46^{b}	0.59^{a}	0.18^{b}	0.29^{a}	0.19^{b}	0.26^{a}	0.24^{b}	0.37^{a}	0.26^{b}	0.29^{ab}
TR_0/RC	0.85^{b}	1.20^{a}	1.06^{ab}	1.19^{a}	1.23^{c}	1.47^{b}	1.76^{a}	1.87^{a}	0.94^{b}	1.21^{a}	1.01^{b}	1.10^{ab}	1.32^{b}	1.50^{a}	1.43^{a}	1.47^{a}
ET_0/RC	0.51^{b}	0.56^{ab}	0.62^{a}	0.66^{a}	0.67^{b}	0.75^{ab}	0.86^{a}	0.76^{ab}	0.63^{a}	0.58^{a}	0.63^{a}	0.71^{a}	0.75^{a}	0.68^{a}	0.76^{a}	0.73^{a}
RE_0/RC	0.19^{b}	0.19^{b}	0.23^{ab}	0.27^{a}	0.24^{b}	0.31^{ab}	0.35^{a}	0.30^{b}	0.24^{a}	0.20^{a}	0.21^{a}	0.26^{a}	0.27^{a}	0.23^{a}	0.24^{a}	0.24^{a}
Quantum yields and probabilities																
ψ_{E0}	0.60^{a}	0.47^{b}	0.59^{a}	0.56^{a}	0.55^{a}	0.51^{a}	0.49^{a}	0.40^{b}	0.67^{a}	0.48^{b}	0.63^{a}	0.65^{a}	0.57^{a}	0.45^{b}	0.54^{a}	0.50^{ab}
φ_{E0}	0.51^{a}	0.37^{b}	0.49^{a}	0.45^{a}	0.47^{a}	0.40^{b}	0.39^{b}	0.31^{c}	0.56^{a}	0.38^{b}	0.53^{a}	0.53^{a}	0.48^{a}	0.36^{b}	0.45^{ab}	0.42^{ab}
φ_{R0}	0.19^{a}	0.13^{b}	0.19^{a}	0.18^{a}	0.17^{a}	0.16^{a}	0.16^{a}	0.12^{b}	0.21^{a}	0.13^{b}	0.18^{a}	0.19^{a}	0.17^{a}	0.12^{b}	0.14^{ab}	0.14^{b}
δ_{R0}	0.37^{a}	0.35^{a}	0.38^{a}	0.40^{a}	0.36^{a}	0.41^{a}	0.40^{a}	0.40^{a}	0.38^{a}	0.34^{a}	0.34^{a}	0.36^{a}	0.36^{a}	0.34^{a}	0.32^{a}	0.33^{a}
Performance indexes																
PI_{abs}	9.20^{a}	2.33^{c}	5.86^{b}	3.95^{bc}	5.36^{a}	1.94^{b}	1.72^{b}	0.91^{c}	10.09^{a}	2.51^{c}	7.70^{b}	6.81^{b}	5.11^{a}	1.80^{c}	3.86^{b}	2.94^{b}
PI_{total}	5.39^{a}	1.27^{c}	3.62^{b}	2.77^{b}	3.04^{a}	1.37^{b}	1.14^{b}	0.60^{c}	6.24^{a}	1.32^{c}	3.96^{b}	3.98^{b}	2.84^{a}	0.93^{c}	1.78^{b}	1.42^{b}

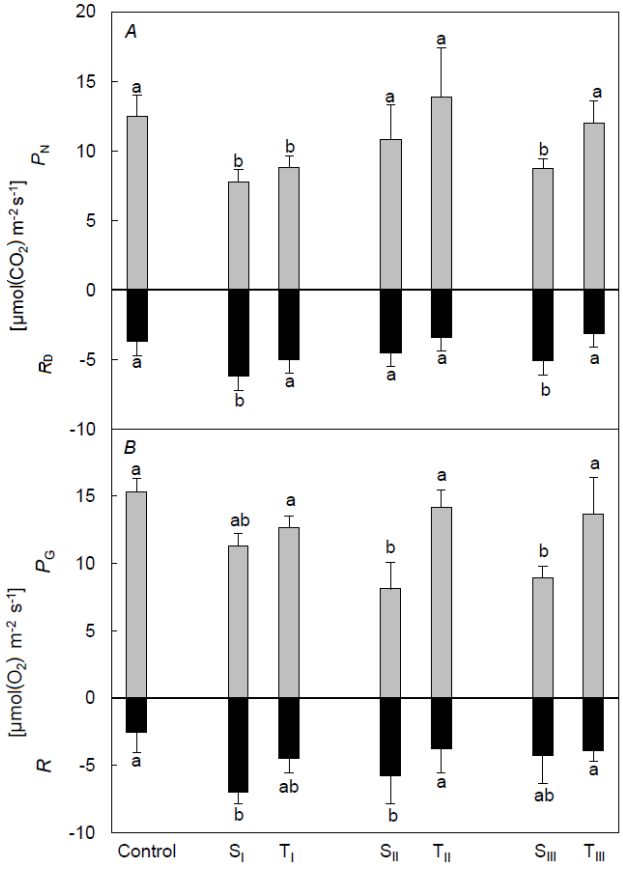

Fig. 4. Net photosynthesis rate and dark respiration (P_N and R_D) (*A*), gross photosynthesis rate and respiration intensity (P_G and *R*) (*B*) of nonsporulating leaves (control) and in the sporophilic (S_I, S_{II}, S_{III}) and trophophilic (T_I, T_{II}, T_{III}) parts of *Platycerium bifurcatum* leaves in individual stages of sporulation, $n = 4$.

PSII between the electrons leaving the RC acceptor and the electrons donated by the donor side (Oukarroum *et al*. 2007). In addition, the time shift of the maximum L band indicates a slower energy transfer from the antennas to the RC (Kalaji *et al*. 2018). In turn, these bands were significantly lower or even absent in the trophophilic part, indicating fewer ungrouped PSII units in photosynthetic membranes and probably higher OEC activity (Guissé *et al*. 1995).

In both sporophilic and trophophilic parts of the leaf, the parameters determining the viability of the plant (PI_{ABS} and PI_{total}) decreased; both parts also combined the efficiency of energy conversion in PSII and PSI (PI_{total}). During spore development, however, the decline was systematic only in subsequent stages S_I–S_{III}. This should be explained by the susceptibility of PI_{total} to changes in the antenna properties manifested in the efficiency of electron trapping and their transport outside of Q_A (Kalaji *et al*. 2014). Until now, this process has been observed in *P. bifurcatum* and other species under environmental stress (Živčák *et al.* 2008, Miszalski *et al.* 2016, Oliwa and Skoczowski 2019a).

The visible increase in the J–P phase of the fast Chl *a* FL curve represented energy transport from Q_A to PSI and reflected a decrease in the electron transport chain (Schansker *et al*. 2005, 2006). In the trophophilic part of the leaf, these changes were only temporary (stage I), but in the sporophilic part (bottom of the leaf), they lasted longer.

The Chl *a* FL values confirm a greater efficiency of the photosynthetic apparatus in the trophophilic part of the leaf than that in the sporophilic part, where spores are developed (Table 1). Other observations to confirm this phenomenon include, just like in abiotic and biotic stresses, the decrease in the maximum photochemical efficiency of PSII (F_v/F_M), the probability of electron transport from Q_A^- to PQ (φ_{E0}), and the pool of available PSII acceptors (A_M) in following stages of sporulation (Maxwell and Johnson 2000, Bąba *et al*. 2016). However, energy streams in a single active RC (ABS/RC, TR_0/RC, ET_0/RC, RE_0/RC) usually increased in subsequent stages S_I–S_{III} and T_I–T_{III}, suggesting an increase in the rate of the electron transport chain. Such an increase may be related to the high energy demand that leaves have during sporulation and to the need to use effectively all the available energy. Thus, the formation of spores on the lower side of the leaf blade in the sporophilic part also leads to changes in the absorption and transport of light energy both on the upper side of the leaf blade and in the trophophilic part. It is not clear, however, why the DI_0/RC ratio also increases in this case, indicating the conversion of absorbed light into heat energy.

The results proved that changes in the photosynthesis and respiration intensities of the sporophilic part of the leaf, represented by the P_N, P_G, and *R* parameters, are associated with the development of sporangia. In the early sporulation stages, photosynthesis intensity in this part of the leaf was lower than that in the trophophilic part; a difference was even greater in the later stages. The maturation process of sporangia changed the functioning of the photosynthetic apparatus of the whole plant, as reflected in the decrease in the Chl *a* FL parameters (*e.g*., PI_{total}). These changes probably resulted from the decrease in Chl content in this part of the leaf. In the final stage of spore maturation, the Chl content in the sporophilic part decreased even more clearly (unpublished data), resulting in significant disturbances in the functioning of the photosynthetic apparatus.

In the juvenile stage of sporulation (S_I), the sporophilic part of the leaf had lower photosynthesis intensity (represented by P_N and P_G) than did the trophophilic part, likely a result of the hindered diffusion of CO_2 and O_2 (caused by the developing sporangia), CO_2 reassimilation processes, and dense tomentose. Differences in photosynthesis intensity measured by the amount of CO_2 uptake or O_2 evolved may indicate that atmospheric CO_2 was not the only substrate for photosynthesis in the sporophilic part of the *P. bifurcatum* leaf. Another likely substrate – at least partially – for photosynthesis transferred to chloroplasts formed in sporangia was CO_2 present in phloem sap. Nevertheless, it seems atmospheric CO_2 and respiration are two primary sources of this gas, CO_2 produced by the latter being reassimilated to a varying extent (Hibberd and Quick 2002, Wittmann *et al.* 2006, Millar *et al.* 2011).

The respiration intensities of individual parts of the

sporotrophophyll leaf during sporangium development varied significantly. The increased respiration in the sporophilic part of the leaf blade in the S_I stage was probably due to the increased energy demand, which energy is required for protein synthesis and the transport of organic compounds and ions necessary for the formation of sporangia and spores (Wittmann *et al.* 2006, Millar *et al.* 2011). For this reason, the respiration rate clearly decreased in the spore area during the final stage of sporulation, down to the level observed in the trophophilic part of the leaf.

The research is the first to show changes in the photosynthesis process and leaf dark respiration during sporulation in ferns.

Conclusion: The paper reports the results of experiments carried out in the sporophilic and trophophilic parts of sporotrophophyll leaves of *Platycerium bifurcatum*. They showed that the sporotrophophyll is a heterogeneous structure. The approach we took helped us analyze and understand physiological processes taking place within individual parts of the leaf. Similar observations were made in the study of physiological processes occurring within the leaf blade and vascular bundles in *Mesembryanthemum crystallinum* (Kuźniak *et al*. 2016).

Our experiments indicate that the formation of spores on the bottom side of the leaf blade in the sporophilic part leads to changes in the absorption and transport of light energy also on the upper side of the leaf blade and in the trophophilic part of the leaf. Differential OJIP curves for the photosynthetic apparatus undergoing ontogenetic changes showed similar bands to those observed in plants under stress. This observation provides new opportunities to use the JIP-test to monitor the state of PSII and photosynthetic activity, which – especially when combined with other methods for measuring photosynthesis – can help better understand the processes of plant growth and development.

References

Ambrožič-Dolinšek J., Camloh M.: Gametophytic and sporophytic regeneration from bud scales of the fern *Platycerium bifurcatum* (Cav.) C. Chr. *in vitro*. – Ann. Bot.-London **80**: 23-28, 1997.

Aspiras R.A.: Sporophyte and gametophyte development of *Platycerium coronarium* (Koenig) Desv. and *P. grande* (Fee) C. Presl. (Polypodiaceae) through *in vitro* propagation. – Saudi J. Biol. Sci. **17**: 13-22, 2010.

Bąba W., Kalaji H.M., Kompała-Bąba A., Goltsev V.: Acclimatization of photosynthetic apparatus of tor grass (*Brachypodium pinnatum*) during expansion. – PLoS ONE **11**: e0156201, 2016.

Blankenship R.E.: Molecular Mechanisms of Photosynthesis. 2nd Edition. Pp. 312. Wiley Blackwell, Chichester 2014.

Camloh M., Gogala N.: *In vitro* culture of *Platycerium bifurcatum* gametophytes. – Sci. Hortic.-Amsterdam **51**: 343-346, 1992.

Dąbrowski P., Baczewska A.H., Pawluśkiewicz B. *et al.*: Prompt chlorophyll *a* fluorescence as a rapid tool for diagnostic changes in PSII structure inhibited by salt stress in Perennial ryegrass. – J. Photoch. Photobio. B **157**: 22-31, 2016.

Guissé B., Srivastava A., Strasser R.J.: The polyphasic rise of the chlorophyll *a* fluorescence (O-K-J-I-P) in heat-stressed leaves. – Arch. Sci. Genève **48**: 147-160, 1995.

Hibberd J.M., Quick W.P.: Characteristics of C_4 photosynthesis in stems and petioles of C_3 flowering plants. – Nature **415**: 451-454, 2002.

Holtum J.A.M., Winter K.: Degrees of crassulacean metabolism in tropical epiphytic and lithophytic ferns. – Aust. J. Plant Physiol. **26**: 749-757, 1999.

Joliot P., Joliot A.: Excitation transfer between photosynthetic units: the 1964 experiment. – In: Govindjee, Beatty J.T., Gest H., Allen J.F. (ed.): Discoveries in Photosynthesis. Pp. 187-191. Springer-Verlag, Berlin-Heidelberg 2005.

Kalaji H.M., Bąba W., Gediga K. *et al.*: Chlorophyll fluorescence as a tool for nutrient status identification in rapeseed plants. – Photosynth. Res. **136**: 329-343, 2018.

Kalaji M.H., Jajoo A., Oukarroum A. *et al*.: The use of chlorophyll fluorescence kinetics analysis to study the performance of photosynthetic machinery in plants. – In: Parvaiz A., Rasool S. (ed.): Emerging Technologies and Management of Crop Stress Tolerance. Pp. 347-384. Elsevier Academic Press, San Diego 2014.

Kluge M., Avadhani P.N., Goh C.J.: Gas exchange and water relations in epiphytic tropical ferns. – In: Lüttge U. (ed.): Vascular Plants as Epiphytes: Evolution and Ecophysiology. Pp. 87-108. Springer-Verlag, Berlin-Heidelberg-New York-London-Paris-Tokyo-Hong Kong, 1989.

Kreier H., Schneider H.: Phylogeny and biogeography of the staghorn fern genus *Platycerium* (Polypodiaceae, Polypodiidae). – Am. J. Bot. **93**: 217-225, 2006.

Kuckenberg J., Tartachnyk I., Noga G.: Temporal and spatial changes of chlorophyll fluorescence as a basis for early and precise detection of leaf rust and powdery mildew infections in wheat leaves. – Precis. Agric. **10**: 34-44, 2009.

Kuźniak E., Kornas A., Kaźmierczak A. *et al.*: Photosynthesis-related characteristics of the midrib and the interveinal lamina in leaves of C_3-CAM intermediate plant *Mesembryanthemum crystallinum*. – Ann. Bot.-London **117**: 1141-1151, 2016.

Lazár D.: The polyphasic chlorophyll *a* fluorescence rise measured under high intensity of exciting light. – Funct. Plant Biol. **33**: 9-30, 2006.

Liao Y.K., Wu Y.H.: *In vitro* propagation of *Platycerium bifurcatum* (Cav.) C. Chr. *via* green globular body initiation. – Bot. Stud. **52**: 455-463, 2011.

Makgomol K.: Morphology of fern spores from Phu Phan National Park. – Kasetsart J. **40**: 116-122, 2006.

Maxwell K., Johnson G.N.: Chlorophyll fluorescence – a practical guide. – J. Exp. Bot. **51**: 659-668, 2000.

Millar A.H., Whelan J., Soole K.L., Day D.A.: Organization and regulation of mitochondrial respiration in plants. – Annu. Rev. Plant Biol. **62**: 79-104, 2011.

Miszalski Z., Skoczowski A., Silina E. *et al*.: Photosynthetic activity of vascular bundles in *Plantago media* leaves. – J. Plant Physiol. **204**: 36-43, 2016.

Oliwa J., Kornas A., Skoczowski A.: Morphogenesis of sporotrophophyll leaves in *Platycerium bifurcatum* depends on the red/far-red ratio in the light spectrum. – Acta Physiol. Plant. **38**: 247, 2016.

Oliwa J., Kornas A., Skoczowski A.: A low ratio of red/far-red in the light spectrum accelerates senescence in nest leaves of *Platycerium bifurcatum*. – Acta Biol. Cracov. Bot. **59**: 67-80, 2017.

Oliwa J., Skoczowski A.: Different response of photosynthetic apparatus to high-light stress in sporotrophophyll and nest leaves of *Platycerium bifurcatum*. – Photosynthetica **57**: 147-159, 2019a.

Oliwa J., Skoczowski A.: The high red/far-red ratio supports the acclimation of fern *Platycerium bifurcatum* to high light. –

Biol. Fut. **70**: 185-197, 2019b.
Oukarroum A., El Madidi S., Schansker G., Strasser R.J.: Probing the responses of barley cultivars (*Hordeum vulgare* L.) by chlorophyll *a* fluorescence OLKJIP under drought stress and re-watering. – Environ. Exp. Bot. **60**: 438-446, 2007.
Raghavan V.: Light and planar growth of gametophytes. – In: Raghavan V. (ed.): Developmental Biology of Fern Gametophytes. Pp. 133-143. Cambridge University Press, Cambridge 1989.
Rut G. [Ecophysiology of epiphytic fern *Platycerium bifurcatum* (Cav.) C. Chr.] – Wiadomości Botaniczne **58**: 17-26, 2014. [In Polish]
Rut G., Krupa J., Miszalski Z. *et al.*: Crassulacean acid metabolism in the epiphytic fern *Platycerium bifurcatum*. – Photosynthetica **46**: 156-160, 2008.
Rut G., Krupa J., Rzepka A.: The influence of simulated osmotic drought on functioning of the photosynthetic apparatus in gametophytes of the epiphytic fern *Platycerium bifurcatum*. – Pol. J. Nat. Sci. **Suppl. 1**: 114-115, 2003.
Sanusi R.-A.M., Nuruddin A.A., Hamid H.A.: Leaf chlorophyll fluorescence and gas exchange response to different light levels in *Platycerium bifurcatum*. – Am. J. Agr. Biol. Sci. **6**: 214-220, 2011.
Schansker G., Tóth S.Z., Strasser R.J.: Dark recovery of the Chl *a* fluorescence transient (OJIP) after light adaptation: The q*T*-component of non-photochemical quenching is related to an activated photosystem I acceptor side. – BBA-Bioenergetics **1757**: 787-797, 2006.
Schansker G., Tóth S.Z., Strasser R.J.: Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the Chl *a* fluorescence rise OJIP. – BBA-Bioenergetics **1706**: 250-261, 2005.
Soltis D.E., Soltis P.: The distribution of selfing rates in homosporous ferns. – Am. J. Bot. **79**: 97-100, 1992.
Stirbet A.: Excitonic connectivity between photosystem II units: What is it, and how to measure it? – Photosynth. Res. **116**: 189-214, 2013.
Strasser R.J., Srivastava A., Tsimilli-Michael M.: The fluorescence transient as a tool to characterize and screen photosynthetic samples. – In: Yunus M., Pathre U., Mohanty P. (ed.): Probing Photosynthesis: Mechanism, Regulation and Adaptation. Pp. 443-480. Taylor and Francis, London 2000.
Strasser R.J., Tsimilli-Michael M., Qiang S., Goltsev V.: Simultaneous *in vivo* recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*. – BBA-Bioenergetics **1797**: 1313-1326, 2010.
Strasser R.J., Tsimilli-Michael M., Srivastava A.: Analysis of the chlorophyll *a* fluorescence transient. – In: Papageorgiou G.C., Govindjee (ed.): Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Advances in Photosynthesis and Respiration. Pp. 321-362. Springer, Dordrecht 2004.
Tsimilli-Michael M., Strasser R.J.: Biophysical phenomics: evaluation of the impact of mycorrhization with *Piriformospora indica*. – In: Varma A., Kost G., Oelmüller R.: *Piriformospora indica*. Sebacinales and Their Biotechnological Applications. Pp. 173-190. Springer, Berlin-Heidelberg 2013.
Wittmann C., Pfanz H., Loreto F. *et al.*: Stem CO_2 release under illumination: corticular photosynthesis, photorespiration or inhibition of mitochondrial respiration? – Plant Cell Environ. **29**: 1149-1158, 2006.
Živčák M., Brestič M., Olšovská K., Slamka P.: Performance index as a sensitive indicator of water stress in *Triticum aestivum* L. – Plant Soil Environ. **54**: 133-139, 2008.