

Temperature optimum, stress temperature range and thermal limits of quantum yield of PSII in tropical versus temperate plants

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ABSTRACT

Temperature response (-5 to +60 °C) of effective ($\Delta F/F_m'$) and maximum (F_v/F_m) quantum yield of PSII was assessed in temperate and tropical plants in a comparative eco-physiological approach. Besides thermal limits, optimum temperatures (T_{opt}) and optimum, sub- and supraoptimal temperature ranges were assessed. The temperature response of $\Delta F/F_m'$ followed a distinct optimum curve. T_{opt} was higher by 8 K in tropical (28.9 °C) vs. temperate (20.9 °C) plants, ranging from 23.5 to 39.5 °C (16 K) and 9.8 to 33.3 °C (23.7 K), respectively, matching with growth temperature. T_{opt} increased with increasing irradiation intensity (*Musa* sp. 0.3 K/100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). The suboptimal temperature range was much larger (25.4 K) in tropical than in temperate species (16.8 K). Frost damage commenced in tropical species close to ice nucleation (LT_{10} -1.9 °C), and in temperate plants at -7.0 °C (LT_{10}). The supraoptimal temperature range was narrower (12.0-12.7 K). The threshold for supraoptimal temperatures was 39.5 °C in tropical and 33 °C in temperate plants. Heat induced PSII impairment was not observed before 51.5 °C and 45.7 °C in tropical and temperate plants, respectively. In darkness F_v/F_m was unaffected by temperature over more than 40 K. Significant changes occurred only upon ice formation and shortly (3.3-7.3 K)

above the heat limit of the optimum range of $\Delta F/F_m'$. Tropical plants, in addition to having significantly different thermal limits of PSII appeared to have a higher optimum temperature but a narrower optimum temperature range than temperate species. Particularly within the broader suboptimal temperature range, tropical plants experienced a more severe reduction of $\Delta F/F_m'$ than temperate species. Temperature response curves of $\Delta F/F_m'$ allow assessing the growth temperature conditions of a leaf via its peculiar thermal adaptation.

KEYWORDS: chlorophyll fluorescence, heat stress, ice nucleation, maximum photosystem II efficiency, frost resistance

ABBREVIATIONS

$\Delta F/F_m'$ effective quantum yield of PSII photochemistry
 F_0 minimal fluorescence of dark-adapted state
 F_m maximal fluorescence of dark-adapted state
 F_m' maximal fluorescence of light-adapted state
 F_s steady state fluorescence
 F_v variable fluorescence
 F_v/F_m maximal quantum yield of PSII photochemistry
 LT_{10} temperature at 10% heat (frost) damage
PPFD photosynthetic photon flux density
PSII photosystem II
 T_{cf} critical low temperature threshold of PSII
 T_{ch} critical high temperature threshold of PSII
 T_{opt} optimum temperature of $\Delta F/F_m'$

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INTRODUCTION

The temperature response of photosynthesis is characterized by the optimum temperature range and its thermal limits. Although many factors contribute to the overall shape of the temperature response of photosynthesis, one of the main targets of low or high temperature stress [1] and of thermal acclimation of photosynthetic temperature optimum has been shown to be associated with the thylakoid membrane, and photosystem II (PSII) in particular [2, 3]. In plants from different biomes, thermal adaptation of photosynthesis to the unique temperature climate should be manifested in both the temperature optimum and the thermal limits of PSII, leaving characteristic low and high temperature stress ranges. Comparisons of photosynthetic performances of plants grown under natural field conditions in various biomes and in relation to plant distribution have a long tradition, aiming to compare and contrast photosynthetic properties in very different ecosystems with the same mechanistic base [4]. While the temperature response of photosynthetic CO₂ gas exchange is well assessed in this respect, comparatively little information exists on the primary processes of photosynthesis.

High temperature limits of photosynthetic light reactions have been studied to some extent at a global scale comparing plants from different biomes [5, 6, 7, 8]. Low temperature limits of photosynthetic light reactions have been studied in temperate species to a lesser extent [1, 9] and only rarely in subtropical and tropical species. Information on the chilling temperature range, i.e. for suboptimal temperatures lower than +15 °C, is particularly scarce. Optimum temperatures of effective quantum yield of PSII, $\Delta F/F_m'$, have been investigated in plant species from various biomes of the world (Antarctic plants [10, 11], temperate plants [10, 12], alpine plants [13], Mediterranean sclerophylls [14], subtropical and tropical plants [10, 15, 16]). The optimum temperatures obtained in these studies indicate significant differences between plants from different biomes with an increasing temperature optimum from Antarctic plant species of about 12 °C [10, 11] to tropical plant species of 30 °C or higher [10, 15, 16]. However, comparisons between the results of these studies are difficult as the experimental setups differ significantly between these studies

in terms of the investigated temperature span (20 K-75 K), the temperature exposure duration including cooling and heating rates and the irradiation intensities employed (125-1,600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). This is a particularly significant drawback affecting comparability as for e.g. optimum temperatures of CO₂ gas exchange shift towards higher temperatures with increasing PPFD [17, 18]. So far, an effect of PPFD on optimum temperatures of effective quantum yield of PSII has only been shown in an alpine field study [13]. Another significant drawback of these studies on the temperature optimum of $\Delta F/F_m'$ is that thermal thresholds for PSII and for survival were mostly not included, which impedes the assessment of the sub- and supraoptimal stress temperature ranges. On the other hand, studies having a focus on high or low temperature limits of photosynthetic functions miss information on the optimum temperature ranges.

The aim of this study was to assess the whole temperature response of quantum yield of PSII including optimum, sub- and supraoptimal temperatures as well as thermal limits of PSII, and the effect of PPFD on temperature optimum of $\Delta F/F_m'$ in a comparative eco-physiological approach using plants from contrasting biomes-tropical versus temperate species under comparable experimental conditions.

MATERIALS AND METHODS

Plant material

The temperate plant species investigated include three spring geophytes. *Allium ursinum* L. and *Ranunculus ficaria* L. naturally occupy the understorey of the Arboretum of the Botanical Garden of the University of Innsbruck. *Narcissus cyclamineus* DC. plants were bought as potted plants and cultivated under greenhouse conditions. *Taraxacum* sect. *Ruderalia* KIRSCHNER, H. ØLLG & ŠTĚPÁNEK, a widespread perennial herbaceous species in the temperate zone was also measured. In contrast to the temperate species, three tropical plants, two C3 species (*Musa* sp., *Carica papaya* L.) and the C4 plant *Saccharum officinarum* L. that are all cultivated in the tropical greenhouse of the Botanical Garden of the University of Innsbruck were investigated. The tropical species *Musa* sp., *C. papaya* and *S. officinarum* are cultivated in a mixture of

various substrates (40% humus, 25% peat moss, 20% sand and 15% lavalit; pH = 6.5). *A. ursinum*, *Taraxacum* and *R. ficaria* grow in the Botanical Garden in natural soil (pH = 6-7); *N. cyclamineus* was potted in a commercial soil mixture for bulb plants. Plants were sufficiently supplied with water throughout the experimental period. In the tropical greenhouse, air humidity was between 80 and 95%, and in the field during sunny days, approximately 60%. The spectral transmittance of the roofing (Makrolon® multi UV, ThyssenKrupp Plastics, Austria) of the tropical greenhouse allows transmittance of all irradiation classes by approximately 80%. The temperate species were exposed to full natural sunlight in the Botanical Garden as the deciduous trees of the Arboretum were still defoliated during experiments and flushing is usually not completed before the end of May. Leaf samples of all species were healthy (no disease or pest) and fully developed. Growing temperature conditions differed significantly between the two species groups. While the tropical species were exposed to a 30/20 °C day/night temperature cycle in the tropical greenhouse, the temperate species were exposed to the natural daily temperature fluctuations of air from a night mean minimum of 6.4 ± 3.9 °C to a daytime mean maximum of 20.2 ± 5.0 °C (one week prior to measurements). Leaves were collected from the plants between April and the beginning of June of 2010 and 2011 immediately before the onset of measurements. After detachment the leaves were put into a temperature controlled box and transferred inside to the laboratory.

Temperature treatment

Controlled temperature treatment was conducted inside the cold storage compartment of a commercial freezer (GT 2102, Liebherr, Lienz, Austria). The compartment is fully temperature controllable in the temperature range between -27 °C and +60 °C by a control system described in detail by Hacker and Neuner (2007) [19]. During measurement detached leaves were placed on wet paper towels to keep them fully turgid. Leaf temperatures were recorded with copper-constantan thermocouples (solder junction diameter: 0.3 mm) that were closely attached to the lower leaf surface. The thermocouples were connected to a data logger (CR10X, Campbell Scientific Instruments, Logan, UT) and leaf temperatures were recorded every

second. Leaf temperature was either lowered from +25 °C down to -10 °C or increased from +25 °C to +60 °C at cooling or heating rates of 12 K h⁻¹. Temperatures are presented in °C and temperature differences in Kelvin (K) as is the custom in bioclimatology [20].

Upon ice formation in the leaf tissue, freezing exotherms were detectable. From the temperature-time plot, ice nucleation temperatures were determined graphically as the lowest temperature immediately before the sudden temperature increase during the freezing exotherm.

During the temperature treatment leaves were either kept in darkness or illuminated using a lighting unit (FL-460, Walz, Effeltrich, Germany). Usually a PPFD of 400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ was used. But if the effect of PPFD on the temperature response of effective quantum yield of PSII was studied, various irradiation intensities between 0 and 1,600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ were applied using the FL-460 lighting unit.

Chlorophyll fluorescence measurements

Five PAM chlorophyll fluorometers (three PAM 101, one PAM-2000 and one Mini-PAM; Walz, Effeltrich, Germany) were employed in order to yield five chlorophyll fluorescence measurements during each temperature run. Chlorophyll fluorescence signals were continuously recorded on a data logger (CR10X, Campbell Scientific, Logan, UT, USA). Before the start of a temperature run and during temperature exposure in darkness, maximal quantum yield of PSII photochemistry, F_v/F_m , was determined for each leaf as $(F_m - F_0)/F_m$, where F_0 is the minimal fluorescence in the dark-adapted state and F_m is the maximal fluorescence in the dark-adapted state upon application of a saturating light pulse. When temperature exposure was conducted under irradiation the steady-state fluorescence, F_s , was continuously recorded and upon application of a saturating light pulse the maximal fluorescence in the light-adapted state, F_m' , was measured to assess effective quantum yield of PSII photochemistry, $\Delta F/F_m'$ ($\Delta F = (F_m' - F_s)$) [21]. Critical temperature thresholds for heat (T_{ch}) and frost (T_{cf}) induced disturbances of PSII were determined according to Neuner and Pramsöhler (2006) [22].

Calculation of the optimum temperatures of $\Delta F/F_m'$

Optimum temperature of $\Delta F/F_m'$, T_{opt} , was calculated according to the method described by Battaglia *et al.* (1996) [23], that used a parabolic function for calculation of optimum temperatures of photosynthetic gas exchange. Using P.Fit software (Fig.P Software Corporation, Durham, NC, USA) this parabolic function ($\Delta F/F_{m'(T)} = \Delta F/F_{m'(max)} - b(T-T_{opt})^2$) was fitted to the $\Delta F/F_{m'(T)}$ versus temperature (T) data. A typical fit is exemplarily shown for results obtained on leaves of *N. cyclamineus* (Fig. 1). The optimum temperature, T_{opt} , the maximum effective quantum yield, $\Delta F/F_{m'(max)}$, and the parameter b could then be directly read from the curve fitting protocol.

Calculation of the optimum and stress temperature ranges of $\Delta F/F_m'$

The optimum temperature range was assessed by a statistical procedure. Threshold temperatures for temperature induced reduction from optimum values were calculated by determination of significant differences using ONEWAY ANOVA and Duncan-Multiple Range post hoc test at $P < 0.01$ between mean values for respective temperature classes and the mean value of $\Delta F/F_m'$ at T_{opt} . All $\Delta F/F_m'$ data obtained for a species were taken together

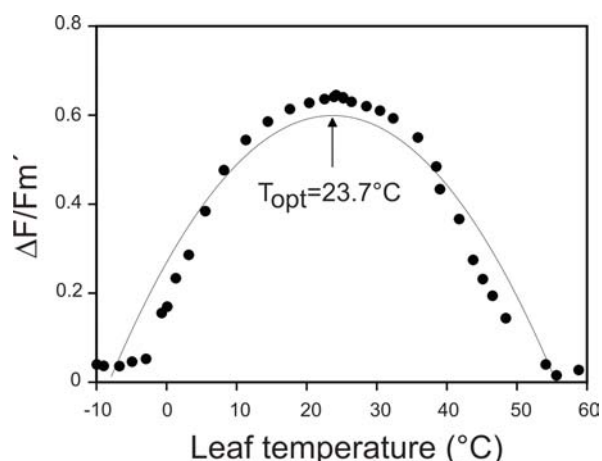


Fig. 1. Determination optimum temperature, T_{opt} , of effective quantum yield of PSII photochemistry ($\Delta F/F_m'$) measured on leaves of *N. cyclamineus* under an irradiation of $400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ by fitting a parabolic function: $\Delta F/F_{m'(T)} = \Delta F/F_{m'(max)} - b(T-T_{opt})^2$ to the data using P. Fit software.

and grouped into 3 K temperature classes. Then the mean values were calculated for each temperature class and tested for significant differences from $\Delta F/F_m'$ at T_{opt} .

To assess threshold temperatures for the stress temperature ranges, $\Delta F/F_m'$ values at T_{opt} were taken to be 100%. All other $\Delta F/F_m'$ values grouped into 3 K temperature classes were expressed in % to determine the temperatures at 50% and 80% reduction, respectively.

Determination of heat and frost resistance

For determination of tissue heat resistance a standard test procedure was used [24]. Leaves were exposed to a controlled heat treatment in temperature controlled water baths using thermostats (CC1, Huber, Offenburg, Germany). Leaves, inside heat resistant polyethylene bags, were plunged into the water baths with preset temperatures between $+38^\circ\text{C}$ and $+60^\circ\text{C}$ in steps of 2 K. This ensured a fast adjustment to the target temperature. After 30 min exposure to the target temperature the leaves were immediately removed from the water bath and cooled down to room temperature.

For determination of frost resistance, leaves were exposed on wet paper towels that were enclosed in polyethylene bags. Wrapped up in this manner, the samples were put inside the freezing compartments of computer-controlled commercial freezers as described in Neuner and Buchner (1999) [25]. Cooling and thawing rates below 0°C were 3 K h^{-1} . Samples remained exposed to target temperatures for 4 h. Target temperatures differed by 4 K and ranged from $+4$ to -20°C . Treatment temperatures in both the heat and frost test were chosen such that the highest temperature should cause no damage and the lowest temperature should kill the leaves.

After the temperature treatment the leaves remained exposed on wet paper towels inside polyethylene bags for one week. Heat and frost damage were then assessed by rating the percentage damage to the leaf blades. Temperature damage was usually clearly visible as distinct discoloration on the leaf blade. If not, the viability of leaves was determined by chlorophyll fluorescence measurements using a FluorCAM (PSI, Brno, Czech Republic). Percentage damage was then plotted against the treatment temperature. A classical

logistic function was fitted to the data using P.Fit (Fig.P Software Corporation, Durham, NC, USA). The temperature causing 10% damage (LT₁₀) was then calculated via the logistic function.

Statistical data analysis

Statistical analyses were made using the PASW Statistics 18 software (formerly SPSS, IBM Corporation, New York, USA). The significant difference between the investigated species with respect to means of T_{opt}, ice nucleation temperatures and thermal thresholds was assessed by ONE WAY ANOVA and Duncans Multiple Range post hoc test at P < 0.05.

RESULTS

Temperature optimum of $\Delta F/F_m'$, T_{opt}

The temperature dependency of $\Delta F/F_m'$ from -5 to +60 °C shows an optimum curve response and reveals distinct differences between tropical and temperate species. This difference is particularly evident when comparing *Musa sp.* and *N. cyclamineus* (Fig. 2). The mean T_{opt} of $\Delta F/F_m'$ was +20.3 ± 0.7 °C in *N. cyclamineus* and +27.3 ± 0.6 °C in *Musa sp.* T_{opt} was significantly higher in tropical species than in temperate plants (Table 1) and was found to range from +27.3 to +30.9 °C in tropical species (mean 28.9 °C) and from +20.2 °C to +21.8 °C in temperate plants (mean 20.9 °C).

PPFD had a significant effect on the optimum temperature (T_{opt}) of $\Delta F/F_m'$ (Fig. 3) for the two species for which its effect was tested. While T_{opt} of $\Delta F/F_m'$ in leaves of *Musa sp.* increased linearly with increasing PPFD by 0.3 K/100 $\mu\text{mol photons}$

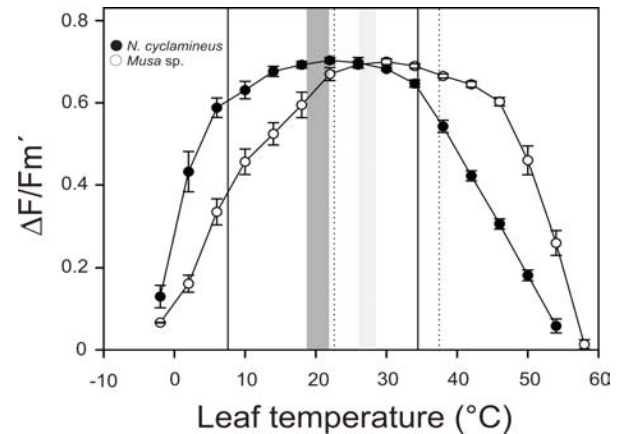


Fig. 2. Temperature response of effective quantum yield of PSII photochemistry ($\Delta F/F_m'$) measured during temperature exposure under an irradiation of 400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ on leaves of the spring geophyte (●) *N. cyclamineus* as compared to the tropical crop species (○) *Musa sp.* The values are means ± SE. The vertical lines indicate the optimum temperature range for (solid line) *Musa sp.* and (dotted line) *N. cyclamineus*. T_{opt} is given by a dark grey (*Musa sp.*) and light grey (*N. cyclamineus*) vertical bar.

Table 1. Optimum temperatures, T_{opt}, and the optimum temperature ranges of $\Delta F/F_m'$ (400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and of F_v/F_m were investigated in tropical plants (*C. papaya*, *Musa sp.*, and *S. officinarum*) in comparison to temperate plants (*A. ursinum*, *N. cyclamineus*, *R. ficaria* and *Taraxacum*). Different capital letters indicate significant differences between optimum temperatures tested by ANOVA at P < 0.05.

Species	$\Delta F/F_m'$			F_v/F_m				
	T _{opt} (°C ± SE)*	T _{opt} range (°C)**		T _{opt} span (K)	T _{opt} range (°C)**		T _{opt} span (K)	
		min	max		min	max		
Tropical	<i>C. papaya</i>	28.5 ± 1.8 ^{A,B}	22.5	40.5	18	-0.5	41.5	42
	<i>Musa sp.</i>	27.3 ± 0.6 ^A	22.5	37.5	15	-1.5	40.5	42
	<i>S. officinarum</i>	30.9 ± 0.6 ^B	25.5	40.5	15	-4.5	46.5	51
Temperate	<i>A. ursinum</i>	21.3 ± 0.3 ^C	7.5	34.5	27	-4.5	40.5	45
	<i>N. cyclamineus</i>	20.3 ± 0.7 ^C	7.5	34.5	27	-1.5	40.5	42
	<i>R. ficaria</i>	20.2 ± 0.7 ^C	13.5	31.5	18	-1.5	40.5	42
	<i>Taraxacum</i>	21.8 ± 1.2 ^C	10.5	31.5	21	-2.5	39.5	42

*Calculation by fitting a parabolic function; **temperature threshold by Duncan Multiple Range test at P < 0.01.

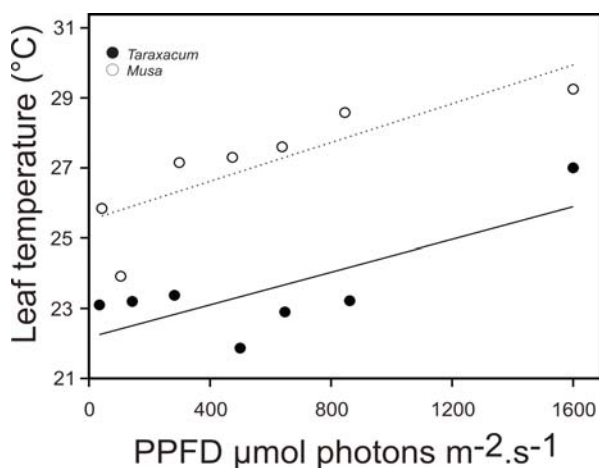


Fig. 3. Effect of irradiation intensity (PPFD) on the optimum temperature, T_{opt} , of effective quantum yield of PSII photochemistry ($\Delta F/F_m'$) determined on leaves of the tropical plant *Musa* sp. as compared to the temperate species *Taraxacum*. Values are means ($n = 4$).

$m^{-2} s^{-1}$, in the temperate *Taraxacum*, T_{opt} of $\Delta F/F_m'$ varied little at PPFDs less than $1000 \mu mol photons m^{-2} s^{-1}$ but had increased significantly by $1,600 \mu mol photons m^{-2} s^{-1}$.

Optimum temperature range

The optimum temperatures of effective quantum yield of PSII were found to range at mean between $+22.5$ and $+40.5$ °C (15-18 K) in tropical and between $+9.8$ and $+33.3$ °C (18-27 K) in temperate plant species as within this temperature span the $\Delta F/F_m'$ values were not significantly different at $P < 0.01$ (Table 1, dark grey bar in Fig. 4).

Maximum quantum yield of PSII photochemistry, F_v/F_m , remained remarkably unaffected by temperature over a broad temperature range of 42.8 K in the tropical species and of 40.3 K in the temperate plants (hatched bar in Fig. 4). Significant reductions occurred only below -2.2 °C and above $+42.8$ °C in tropical and below -2.5 °C and above $+40.3$ °C in temperate plants.

Low temperature stress and critical thresholds of PSII

Low temperature inactivation of F_v/F_m started in both species groups at a quite similar temperature and was closely linked to ice formation. In contrast, significant differences between the two species

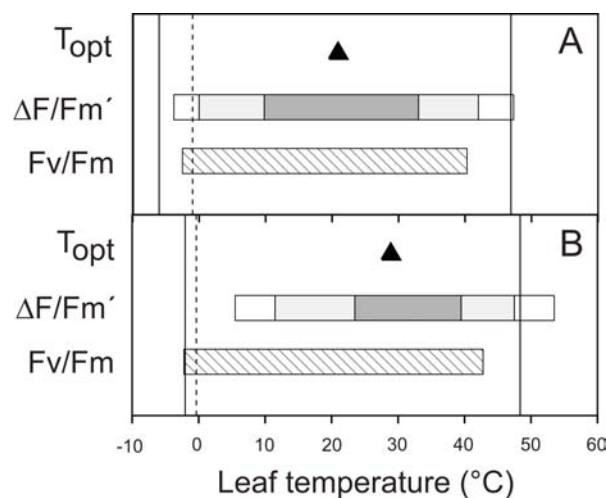


Fig 4. Optimum temperature (\blacktriangle ; T_{opt}), optimum temperature range (dark grey horizontal bar), sub- and supraoptimal temperatures (till 50% $\Delta F/F_m'$: light grey horizontal bar), high and low temperature stress range (till 20% $\Delta F/F_m'$: open bars) for $\Delta F/F_m'$ and optimum temperature range for F_v/F_m (hatched horizontal bar) compared between (A) temperate ($n = 4$) and (B) tropical ($n = 3$) plants. Dotted lines indicate ice formation and solid lines indicate heat and frost resistance.

groups were observed in low temperature tolerance. While the temperate species got frost damaged between -5.8 and -7.9 °C, the tropical plants were ice susceptible and got frost damaged already between -0.5 and -1.7 °C (LT_{10} , Table 2). Except for *Musa* sp. that froze at similar temperatures as *A. ursinum* and *R. ficaria*, in the tropical species ice nucleation occurred at significantly higher temperatures just below 0 °C. Significant differences were observed with respect to low temperature inactivation of $\Delta F/F_m'$ in the suboptimal temperature range (chilling temperature range 0 to $+15$ °C). The differences in the response of photosynthesis to low suboptimal temperatures of tropical versus temperate plants come evident in Fig. 5. While a 50% reduction of $\Delta F/F_m'$ is found in tropical species at $+11.5$ °C, this is not observed before 0 °C in temperate plant species. While the temperate species showed an 80% reduction of $\Delta F/F_m'$ only under exposure to subzero temperatures (-1.5 to -7.5 °C), this was observed in the tropical species at temperatures distinctly above 0 °C, depending on the species between $+10.5$ to $+1.5$ °C and at mean at $+5.5$ °C (Table 2).

Table 2. Ice nucleation temperatures, frost resistance (LT_{10} ; $^{\circ}C \pm SE$), the difference between ice nucleation and 10% frost damage ($\Delta T_{ice-LT10}$), temperatures at 80% reduction of $\Delta F/F_m'$ and low temperature thresholds for PSII, T_{cf} ($^{\circ}C$), determined for tropical plants (*C. papaya*, *Musa* sp. and *S. officinarum*) and temperate plants (*A. ursinum*, *N. cyclamineus*, *R. ficaria* and *Taraxacum*). Different capital letters indicate significant differences tested by ANOVA at $P < 0.05$ ($n = 3-10$).

	Species	Ice nucleation ($^{\circ}C \pm SE$)	LT_{10} ($^{\circ}C \pm SE$)	$\Delta T_{ice-LT10}$ (K)	80% of $\Delta F/F_m'$ ($^{\circ}C$)	T_{cf} ($^{\circ}C$)
Tropical	<i>C. papaya</i>	-0.6 ± 0.2^A	-1.2 ± 0.0^A	-0.6	4.5	-3.5 ± 0.0
	<i>Musa</i> sp.	$-1.7 \pm 0.4^{A,B}$	-3.4 ± 0.2^B	-1.7	1.5	-7.4 ± 0.6
	<i>S. officinarum</i>	-0.5 ± 0.1^A	-1.3 ± 0.0^A	-0.7	10.5	-5.5 ± 0.1
Temperate	<i>A. ursinum</i>	$-1.3 \pm 0.3^{A,B}$	-5.8 ± 0.0^C	-4.5	-7.5	-7.3 ± 0.7
	<i>N. cyclamineus</i>	-3.2 ± 0.9^C	-7.3 ± 0.8^D	-4.1	-4.5	-5.5 ± 0.1
	<i>R. ficaria</i>	$-1.6 \pm 0.5^{A,B}$	-7.9 ± 0.1^D	-6.3	-1.5	-2.1 ± 0.6
	<i>Taraxacum</i>	$-2.2 \pm 0.7^{B,C}$	n.d.*	n.d.*	-1.5	-3.9 ± 0.6

*n.d. = not determined.

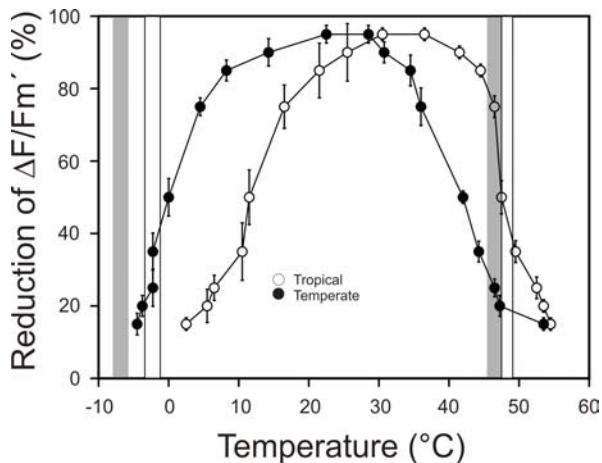


Fig. 5. Reduction of $\Delta F/F_m'$ (in % of $\Delta F/F_m'$ at T_{opt}) as affected by temperature: (○) tropical are compared to (●) temperate plants. Vertical bars indicate killing temperatures (LT_{10}) for (dark grey) temperate and (open) tropical plants.

High temperature stress and critical thresholds of PSII

At high temperatures in both species groups, F_v/F_m' gets significantly reduced already under moderate heat, i.e. even below the temperature where $\Delta F/F_m'$ is reduced to 50%. This means that there is a direct heat effect in darkness on maximum efficiency of PSII in both species groups. The tropical species showed a significantly higher temperature where an 80% reduction of $\Delta F/F_m'$

took place. Also the parameter $(T_{ch} + T_{ph})/2$ which is usually close to the heat killing temperatures indicated a higher heat stability of PSII in tropical plants. In the tropical species $(T_{ch} + T_{ph})/2$ varied between 50.4 and 52.9 $^{\circ}C$; in the temperate plants this was distinctly lower and occurred between 44.3 and 47.2 $^{\circ}C$ (Table 3). In the temperate species the supraoptimal temperature range was 12.7 K, at mean from 33 $^{\circ}C$ (supraoptimal threshold temperature) until 45.7 $^{\circ}C$ ($(T_{ch} + T_{ph})/2$). The supraoptimal temperature range similarly was 12.0 K in the tropical species, but occurred at higher temperatures, i.e. at mean from 39.5 $^{\circ}C$ (supraoptimal threshold temperature) until 51.5 $^{\circ}C$ ($(T_{ch} + T_{ph})/2$). In tropical species LT_{10} corresponded with T_{ch} , the critical temperature for heat induced increases in basic chlorophyll fluorescence; in temperate plants LT_{10} was recorded at a 4.1-5.5 K higher temperature.

DISCUSSIONS

Temperature optimum of photosynthetic gas exchange in plants from all major biome types [4, 26] occurs at temperatures that highly correlate with the temperature of the growing season determined by their geographical origin [1, 4, 18]. The temperature optimum of a plant species can be shifted downward or upward by growing the plants at cool or hot temperatures, respectively, usually by one third to one half of the shift in

Table 3. Heat resistance (LT_{10} ; °C \pm SE), temperature at 80% heat reduction of $\Delta F/F_m'$, high temperature thresholds for PSII, T_{ch} and T_{ph} (°C), $(T_{ch} + T_{ph})/2$, and the difference between $(T_{ch} + T_{ph})/2$ and 10% heat damage (ΔT_h) determined for tropical in comparison to temperate plants. Different capital letters indicate significant differences between means (n = 3-10) tested by ANOVA at P < 0.05.

	Species	LT_{10} (°C \pm SE)	80 % of $\Delta F/F_m'$ (°C)	T_{ch} (°C)	T_{ph} (°C)	$(T_{ch}+T_{ph})/2$ (°C)	ΔT_h (K)
Tropical	<i>C. papaya</i>	48.7 \pm 1.0 ^A	55.5	49.7 \pm 1.3 ^A	56.2 \pm 0.5 ^A	52.9 \pm 0.7 ^A	4.2
	<i>Musa</i> sp.	48.8 \pm 1.0 ^A	52.5	48.9 \pm 0.8 ^A	53.8 \pm 1.2 ^B	51.1 \pm 1.3 ^{A,B}	2.3
	<i>S. officinarum</i>	47.3 \pm 0.6 ^{A,B}	52.5	46.6 \pm 1.0 ^B	54.3 \pm 0.7 ^{A,B}	50.4 \pm 0.8 ^B	3.1
Temperate	<i>A. ursinum</i>	48.0 \pm 0.3 ^A	46.5	43.9 \pm 0.5 ^C	50.8 \pm 0.5 ^C	47.2 \pm 0.4 ^C	-0.8
	<i>N. cyclamineus</i>	48.1 \pm 1.1 ^A	49.5	43.1 \pm 0.8 ^{C,D}	49.4 \pm 0.4 ^{C,D}	46.1 \pm 1.3 ^{C,D}	-2.0
	<i>R. ficaria</i>	45.3 \pm 1.0 ^B	43.5	40.9 \pm 0.5 ^D	47.8 \pm 0.7 ^D	44.3 \pm 0.5 ^D	-1.0
	<i>Taraxacum</i>	46.3 \pm 0.9 ^{A,B}	49.5	40.8 \pm 0.2 ^D	49.3 \pm 0.6 ^{C,D}	45.0 \pm 0.4 ^{C,D}	-1.3

growth temperature [27, 28, 29]. The capability to adjust the temperature optimum is species specific [30, 31] and most likely the temperature shift of photosynthetic optimum is determined by a plastic response of PSII electron transport. PSII electron transport in winter wheat [3] and in *Quercus* sp. [15] showed maximum rates near the temperature at which the leaves had developed (15, 25 or 35 °C) suggesting that major changes contributing to thermal acclimation of photosynthesis occur at the level of PSII electron transport [31]. T_{opt} of $\Delta F/F_m'$ was calculated by fitting a parabolic equation to the data which has originally been used to model the temperature response of CO₂ gas exchange (net photosynthetic rates) [23, 32] corroborating an 8 K higher T_{opt} of $\Delta F/F_m'$ in tropical (+28.9 °C; 27.3-30.9 °C) vs. temperate (+20.9 °C; 20.2-21.8 °C) plants corresponding very well with roughly the temperature optimum of net photosynthesis in the respective species group [18]. For tropical species optimum temperatures of $\Delta F/F_m'$ between 24 °C (*Gossypium hirsutum*; [10]) and 40 °C (*Shorea platyclados*; [16]), for temperate plants between 15 °C (*Triticum aestivum*; [3]) and 30 °C (*Fagus sylvatica*; [12]) and for Mediterranean plants (*Quercus ilex*; Holm oak) between +14 °C and +28 °C [33] are reported. However, the comparability between these results is limited due to different experimental settings, mainly PPFD employed during testing. Our results clearly show an increase of T_{opt} of $\Delta F/F_m'$ with increasing PPFD. This effect of PPFD is already well documented for net photosynthesis [17, 18]. The effect of

PPFD on the temperature response of $\Delta F/F_m'$ has also considerable consequences for comparisons as different authors used various irradiation intensities during the measurement of the temperature response of $\Delta F/F_m'$ ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$: 125, [10]; 180, [16]; 377, [3]; 400, [15]; 600, [6]; 800, [11]). For example, T_{opt} of *A. ursinum* was found to be 25.1 °C when measurements were conducted under an irradiation of 1,600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ as compared to a T_{opt} of 21.3 °C found in the current investigation under an irradiation of 400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Temperature optimum of photosynthesis may not sufficiently be described by a single optimum temperature. Particularly for species comparisons, it rather appears beneficial to assess the range of temperatures for optimum photosynthesis. The optimum temperature range of photosynthesis has only been loosely defined until now. In the recent review of Sage and Kubien (2007) [34] the optimum temperature range is defined as the temperature range wherein the photosynthetic rate was fully reversible after short-term excursion to non-optimal temperatures. For most plants this range is considered to be roughly about 30 K, between 0-30 °C in cold-adapted, between 7-40 °C in plants from warm habitats and between 15-45 °C in hot environments [34, 35].

However, the determination of threshold temperatures for non-harmful photosynthetic changes may be time-consuming and additionally difficult to determine as it has to be defined what is meant by non-harmful. Other suggestions to take the

temperature at which the activity of the function drops below half its maximal value (photosynthetic thermal window; [35]) may be more useful but may also not allow distinguishing between stress and optimum temperatures. The assessment of the optimum temperatures by significant deviations from the maximum value may reliably allow the determination of the non-stress temperature range of photosynthesis. $\Delta F/F_m'$ was unaffected by temperatures from +23.5 to +39.5 °C (16 K) in tropical vs. the broader range of temperatures from +9.8 to +33.3 °C (23.5 K) in temperate plants, being narrower than the 30 K range proposed earlier [34, 35]. The broader optimum temperature range of temperate species may mirror an adaptation to a naturally occurring higher variability of daily maximum leaf temperatures in temperate environments with stronger deviations of leaf temperature from air temperature on days with full sunshine [17].

In addition to the determination of the optimum temperature ranges, the assessment of stress temperature ranges appears advantageous. Under the experimental conditions, tropical species showed a significant larger suboptimal temperature range as compared to temperate plants (25.4 K vs. 16.8 K). Freezing temperatures were lethal in tropical species immediately upon ice nucleation (-2.2 °C) at -1.9 °C (LT_{10}) but not in the ice tolerant temperate plants that did not show frost damage before -7.0 °C (LT_{10}). Under the experimental conditions, maximum PSII efficiency remained remarkably untouched down to the temperature of ice formation in the leaves between -2.2 and -2.5 °C. T versus $\Delta F/F_m'$ plots have been shown to follow the same pattern as that of quantum yield of CO_2 [12] as both appear linearly correlated. During exposure to suboptimal temperatures under irradiation, excess excitation energy caused a depression of effective quantum yield of PSII. This may be brought about by a reduced utilization of excitation energy in the Calvin cycle leading to an increased proportion of reduced Q_A in the steady state [12]. This may be consistent with the current view that in C3 plants, in the suboptimal temperature range, the P_i regeneration capacity seems to be the major limiting factor for net photosynthesis and P_i regeneration capacity gets enhanced upon acclimation to altered growth

temperatures, at low CO_2 (<300 μ bar) with Rubisco capacity being the predominant limitation [34].

Under the experimental conditions the supraoptimal temperature range, from the upper limit of the optimum temperature range of $\Delta F/F_m'$ to heat induced impairment of PSII ($[T_{ch} + T_{ph}]/2$), ranged quite similar in both species groups, between 12.0-12.7 K. But the starting point of inactivation of PSII, recognized by a heat induced reduction of F_v/F_m , was recognizable at 6.7 K below heat injury in the temperate species and 5.4 K below in the tropical plants. This heat induced decline of F_v/F_m has been documented in tropical [36] and temperate [37] species upon exposure to supraoptimal temperatures. The F_v/F_m decline may have two major sources, as it results from both increasing F_0 , that indicates inhibition of electron transport [1, 37], and decreasing F_m , that likely is caused by detachment of light-harvesting complexes from the core of PSII [37]. For C3 plants at supraoptimal temperatures, electron transport capacity or Rubisco activase have been found to be limiting for net photosynthesis. Also acclimation to increased growth temperature has been shown to increase electron transport capacity or Rubisco activase. However, in C4 plants the control over net photosynthesis at elevated temperatures remained unclear [34]. The observation of inactivation of PSII in the supraoptimal temperature range several degrees below LT_{10} may emphasize the role of PSII in heat stress induced depression of photosynthesis.

While differences between species groups were less pronounced in the supraoptimal temperature range, significant differences in the suboptimal stress temperature range between tropical and temperate plants were clearly recognizable. Mainly the tropical species experienced a more severe reduction of $\Delta F/F_m'$ within the significantly broader suboptimal temperature range as opposed to temperate species. Temperate plants, that additionally showed lower thermal limits of PSII, had a lower optimum temperature but additionally a much broader optimum temperature range than tropical species. Assessment of stress temperature ranges of PSII, in addition to thermal limits and optimum temperature ranges, appears additionally useful for explaining the peculiar thermal adaptation of plants from various biomes to their thermal environment.

CONCLUSIONS

When temperature exposure was conducted in darkness F_v/F_m was fairly unaffected by temperature over a broad temperature range of more than 40 K in both species groups without detectable significant differences. Hence, thermal adaptation of photosynthesis obviously cannot be diagnosed when leaves are exposed in darkness to temperature. The results obtained when leaves were irradiated during the temperature treatment reveal significant differences in temperature optima, low and high temperature thresholds and thermal limits of PSII between plants from temperate and tropical areas. Temperature ranges appeared to describe thermal adaptations of photosynthesis much better than single temperatures. Measurements of the temperature response of $\Delta F/F_m'$ allow diagnosing the whole thermal adaptation of photosynthesis which can nicely be specified by an (1) optimum temperature range, (2) sub- and supraoptimal temperature ranges, wherein temperature induced photoinhibition occurs, and ultimately (3) thermal limits of PSII. Hence, these chlorophyll fluorescence measurements can nicely be employed to describe and assess the peculiar thermal adaptation of photosynthesis of plants from various environments. Care must be taken when comparisons of photosynthetic properties in different ecosystems [4] are intended, as T_{opt} of $\Delta F/F_m'$ is affected and increases with increasing PPFD superimposed to the temperature treatment.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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