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BRIEF COMMUNICATION

Diurnal cycle of chlorophyll fluorescence in *Phalaenopsis*

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Abstract

Chlorophyll (Chl) fluorescence of warm day/cool night temperature exposed *Phalaenopsis* plants was measured hourly during 48 h to study the simultaneous temperature and irradiance response of the photosynthetic physiology. The daily pattern of fluorescence kinetics showed abrupt changes of photochemical quenching (q_P), non-photochemical quenching (NPQ) and quantum yield of photosystem II electron transport (Φ_{PSII}) upon transition from day to night and *vice versa*. During the day, the course of Φ_{PSII} and NPQ was related to the air temperature pattern, while maximum quantum efficiency of PSII photochemistry (F_v/F_m) revealed a rather light dependent response. Information on these daily dynamics in fluorescence kinetics is important with respect to meaningful data collection and interpretation.

Additional key words: Crassulacean Acid Metabolism (CAM), irradiance, photosynthesis, temperature.

Chl fluorescence is a subtle reflection of the primary reaction of photosynthesis and has become ubiquitous in ecophysiological studies. The development of compact, field portable and affordable fluorometers together with the ease of operation and advanced instrument software has led to the wide spread use of Chl fluorescence analysis to assess plant responses to environmental stresses (Adams and Demmig-Adams 2004). Unfortunately, the method still deals with ambiguity in interpretation of fluorescence data. One major reason is the daily dynamics in fluorescence kinetics which are species specific.

In this contribution, a case study was made of *Phalaenopsis*, which is a CAM-plant (Sayed 2001), aiming to accurately investigate the diurnal photosynthetic physiology in response to simultaneous changes in temperature and irradiance. Therefore, a greenhouse-climate chamber transfer experiment was performed subjecting 26-month-old plants of the *Phalaenopsis* hybrid 'Isis' to a warm day/cool night temperature regime for 48 h. Air temperature was measured with a thermocouple (type *T*, *Omega Engineering*, Stamford, USA). The realised daytime and night time air temperature averaged 37.2 ± 1.8 °C (greenhouse) and 18.4 ± 0.2 °C (climate chamber), respectively (Fig. 1*A*). A 12-h photoperiod (greenhouse) was supplied and irradiance was determined immediately above the plant canopy with a PAR quantum sensor (*QS, Delta-T Devices*, Cambridge, UK). A shade screen closed automatically at 200 µmol m^{-2} s^{-1} to avoid photodamage. As a result, PAR peaked at 199 µmol m^{-2} s^{-1} and 156 µmol m^{-2} s^{-1} on day one and day two, respectively (Fig. 1*A*). Since greenhouse PAR intensity was dependent on natural irradiance and day two was less sunny, maximum PAR-value was about 50 µmol m^{-2} s^{-1} lower on the second day as compared to day one. Chl fluorescence measurements were carried out hourly for 48 h on the youngest fully developed leaf ($n = 3$). The centre of the adaxial surface of the leaf, about 1 cm away from the main vein, was measured after 5 min of illumination with continuous red, non-saturating actinic light (<150 µmol m^{-2} s^{-1}) using a portable fluorometer (*PAM-2000, Walz*, Effeltrich, Germany).

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Abbreviations: Chl – chlorophyll; F_v/F_m – maximum quantum efficiency of photosystem II photochemistry; NPQ – non-photochemical quenching; q_P – photochemical quenching; Φ_{PSII} – quantum yield of photosystem II electron transport.

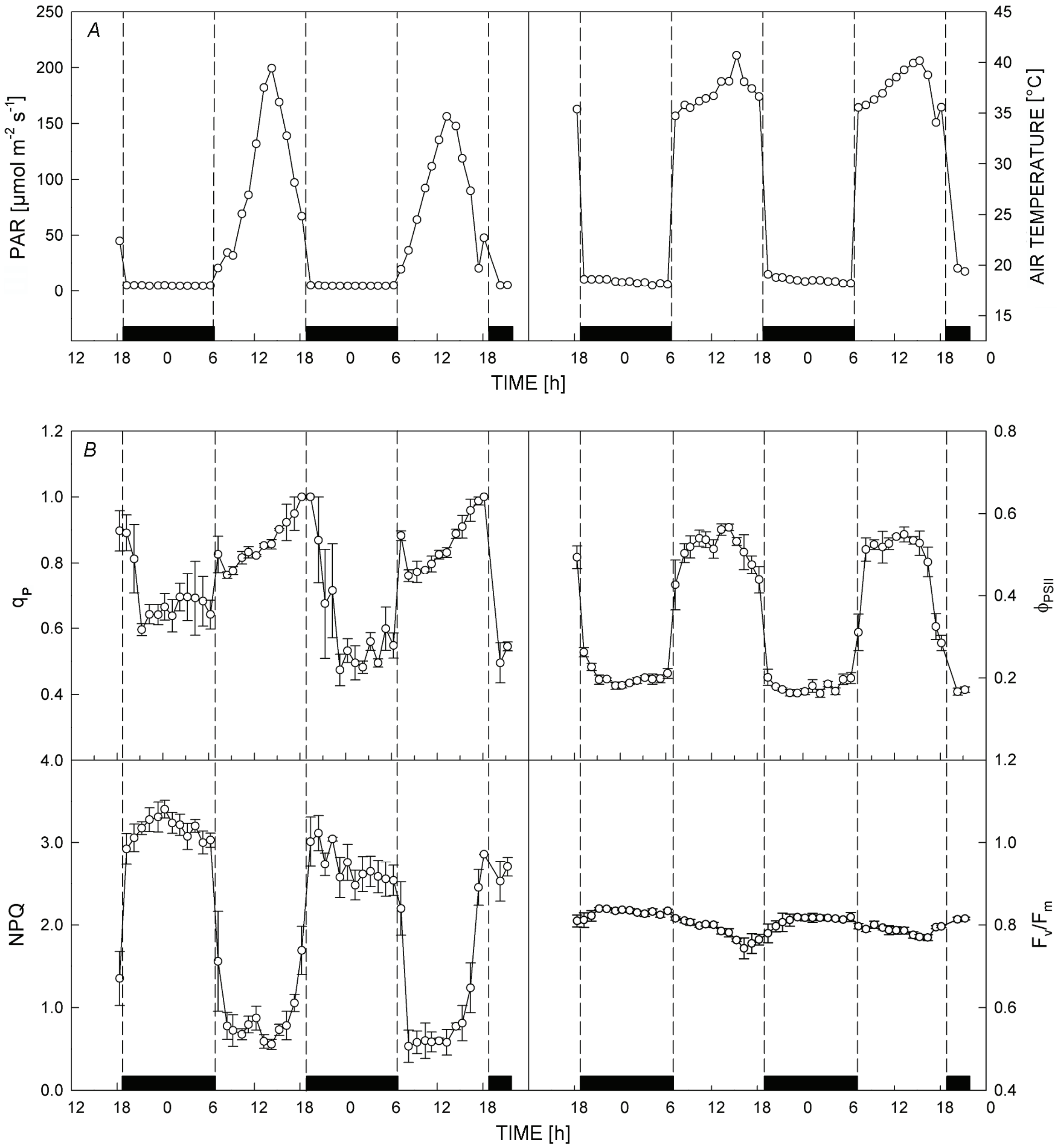

Fig. 1. *A*: Evolution of photosynthetically active radiation (PAR) and air temperature and *B*: the diurnal cycle of photochemical quenching (q_P), non-photochemical quenching (NPQ), quantum yield of photosystem II electron transport (Φ_{PSII}) and maximum quantum efficiency of photosystem II photochemistry (F_v/F_m) for *Phalaenopsis* 'Isis' subjected to a warm day/cool night temperature regime and a 12-h photoperiod. Fluorescence data represent the mean of three individual plants (±SE).

Leaves were allowed to dark adapt for 20 min (Genty *et al.* 1989).

Photochemical quenching (q_P) and the quantum yield of photosystem II electron transport (Φ_{PSII}) were strongly affected by temperature and light (Fig. 1*B*). When plants were exposed to cool night conditions the q_P component decreased during 3–5 h. Transferring plants to warm day conditions resulted in an immediate increase in q_P followed by a further rise which initially was slow and increased in rate towards the end of the day. Also Φ_{PSII} showed a rapid decrease and increase at the transition times but more importantly, during the day, the course of Φ_{PSII} paralleled the air temperature pattern. Φ_{PSII} responses matched the slight air temperature increase from 7 h until 15 h and the gradual decline and steep decrease at the end of day one and day two, respectively. The influence of temperature and light on the photosynthetic physiology was also clearly reflected in the trend of the non-photochemical quenching component (NPQ) (Fig. 1*B*). Transition from cool night to warm day conditions was accompanied with a rapid decrease of NPQ and *vice versa*. Besides, NPQ was inversely related to Φ_{PSII}, and as such to air temperature, showing initially a steady rise and then a dramatic increase at the end of day one and day two, respectively. Warm day/cool night transitions were not clearly reflected in the maximum quantum efficiency of PS II photochemistry (F_v/F_m). However, F_v/F_m decreased slowly and slightly during the day and attained a minimum after the irradiance peaked (Fig. 1*B*). During the night F_v/F_m remained constant and averaged 0.82.

The strong correlation of Φ_{PSII} and NPQ with air temperature as well as the light dependent response of F_v/F_m reveals the importance of a proper specification of the environmental conditions to allow a correct and meaningful interpretation of fluorescence data and the matching of different fluorescence studies. The fluorescence parameters also demonstrate the main difference between the day and night photosynthetic physiology of *Phalaenopsis*. The steep decrease of q_P and Φ_{PSII}, and increase of NPQ, at the day-to-night transition corresponds to the termination of photosynthetic electron transport due to Calvin cycle inactivation. At night, the actinic light illumination reduces PSII reaction centres and non-photochemical processes, in particular thermal dissipation, are triggered. During the day, decarboxylation of malic acid takes place and CO_2 is refixated behind closed stomata into the Calvin cycle (Dodd *et al.* 2002). Towards the late afternoon, the internal pool of malic acid seems to be consumed leading to a decrease of Φ_{PSII}, an increase of NPQ, and a steeper increase of q_P (Adams *et al.* 1989). For q_P, a decrease could be expected but the controlled dissipation of energy through non-photochemical processes together with the involvement of alternative electron sinks (Ort and Baker 2002) allowed the primary electron acceptor of PSII to reach an highly oxidative state. A similar diurnal course of Chl fluorescence was found by Winter and Demmig (1987) and Adams *et al.* (1989) for the CAM-plant *Kalanchoë daigremontiana* and two CAM-operating cacti (*Nopalea* and *Opuntia*), respectively. However, fluorescence measurements were only performed during the day. Observations of the fluorescence induction kinetics over a 24-h period were carried out by Everson *et al.* (1983) for *K. daigremontiana* and *Ananas comosus*, both CAM plants, under similar warm day/cool night temperature conditions (*i.e.* 30 °C/16 °C). Unfortunately, only the variable fluorescence was assessed. Nevertheless, identical abrupt changes in fluorescence intensities upon changing from night to day and day to night were demonstrated. Our findings are also consistent with Roháček and Šiffel (1995) who performed 24-h fluorescence measurements for the C_3 tree, *Picea abies*, under day/night temperatures of 20 °C/11 °C. The diurnal photosynthetic activity cycle has consequences with respect to the collection and interpretation of fluorescence data. Variations in the photosynthetic physiology between different times of measurement might result in significant variations of a particular fluorescence parameter. According to our results, the malic acid pool depletion seems to be a critical point with respect to the late afternoon (*i.e.* after 15 h) changes of q_P, Φ_{PSII} and NPQ.

Environmental conditions that alter the nocturnal built up and daytime decomposition of malic acid in CAM plants, such as temperature, irradiance, water availability or CO_2 concentrations (Lüttge 2006; Hsu *et al.* 2006), might likely lead to a time displacement of the critical point, affecting the daytime dynamics of q_P, Φ_{PSII} and NPQ. Detecting stress by comparing with control values obtained from non-stressed plants or discriminating stressed from more stressed plants will only be reasonable when determining fluorescence before the critical point. In conclusion, Chl fluorescence measurements require taking into account the diurnal cycle of photosynthetic activity as well as environmental conditions.

References

Adams, W.W., III., Demmig-Adams, B.: Chlorophyll fluorescence as a tool to monitor plant responses to the environment. – In: Papageorgiou, G.C., Govindjee (ed.): Chlorophyll *a* Fluorescence: a Signature of Photosynthesis. Pp. 583-604. Springer, Dordrecht 2004.

Adams, W.W., Diaz, M., Winter, K.: Diurnal changes in photochemical efficiency, the reduction state of Q, radiationless energy-dissipation, and non-photochemical fluorescence quenching in cacti exposed to natural sunlight in northern Venezuela. – Oecologia **80**: 553-561, 1989.

Dodd, A.N., Borland, A.M., Haslam, R.P., Griffiths, H., Maxwell, K.: Crassulacean acid metabolism: plastic, fantastic. – J. Exp. Bot. **53**: 569-580, 2002.

Everson, G., Chen, S.S., Black, C.C.: Diurnal variations in leaf fluorescence induction kinetics – variable fluorescence in Crassulacean Acid Metabolism plants. – Plant Physiol. **72**: 455-460, 1983.

Genty, B., Briantais, J.M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. Biophys. Acta **990**: 87-92, 1989.

Hsu, C.-C., Lin, T.-C., Chiou, W.-L., Lin, S.-H., Lin, K.-C., Martin, C.E.: Canopy CO_2 concentrations and Crassulacean acid metabolism in *Hoya carnosa* in a subtropical rain forest in Taiwan: consideration of CO_2 availability and the evolution of CAM in epiphytes. – Photosynthetica **44**: 130-135, 2006.

Lüttge, U.: Photosynthetic flexibility and ecophysiological plasticity: questions and lessons from *Clusia*, the only CAM tree, in the neotropics. – New Phytol. **171**: 7-25, 2006.

Ort, D.R., Baker, N.R.: A photoprotective role for O_2 as an alternative electron sink in photosynthesis? – Curr. Opin. Plant Biol. **5**: 193-198, 2002.

Roháček, K., Šiffel, P.: Temperature and light dependences of chlorophyll fluorescence *in vivo* for Norway spruce seedlings.

– In: Mathis, P. (ed.): Photosynthesis: from Light to Biosphere. Vol. V. Pp. 937–940. Kluwer Academic Publish., Dordrecht – Boston – London 1995.

Sayed, O.H.: Crassulacean acid metabolism 1975-2000, a check list. – Photosynthetica **39**: 339-352, 2001.

Winter, K., Demmig, B.: Reduction state of Q and nonradiative energy-dissipation during photosynthesis in leaves of a Crassulacean Acid Metabolism plant, *Kalanchoë daigremontiana* Hamet et Perr. – Plant Physiol. **85**: 1000-1007, 1987.