S35-012

Photoprotection under low temperatures: Reorganization of pigment-protein complexes in leaves of an Australian mistletoe *Amyema miquelii*.

S Matsubara[1], AM Gilmore[1], WS Chow[1], MC Ball[1], JM Anderson[1], CB Osmond[2]

[1]*Research School of Biological Sciences, Australian National University, GPO Box 475, Canberra, ACT 2601, Australia. Fax: 61-2-6125-8056, Email:* matsubara@rsbs.anu.edu.au

[2]*Biosphere 2 Center, Columbia University, Box 689, Oracle, AZ 85623, USA.*

Keywords: cold acclimation, lutein epoxide, photoprotection, 77K fluorescence spectra, xanthophyll cycle

Introduction

For evergreens during winter, under a combined stress of low temperature and high light, protection of the photosynthetic membranes by balancing absorption, transfer and utilization of light energy is of crucial importance apart from the repair system for recovery from damage. Seasonal reorganization of pigment-protein complexes of the thylakoid membrane seems to occur in leaves of evergreen species (Ottander *et al*. 1995, Gilmore and Ball 2000). The pigment-protein complexes of higher plants contain a number of carotenoids besides chlorophyll *a* and *b*. Carotenoids are *sine qua non* for photosynthesis as well as for photoprotection: (1) light harvesting and energy transfer to chlorophylls, (2) quenching of the triplet excited state of chlorophylls to prevent the formation of singlet oxygen, and (3) the dissipation of the singlet excited state of chlorophylls to avoid overexcitation of photosynthetic apparatus under high light (Demmig-Adams *et al*. 1996, Niyogi 1999). Thus, long-term acclimation to the light environment and short-term adjustment of excitation energy transfer to reaction centres both involve modifications of the composition and the relative levels of carotenoids.

In this study, we investigated the pigment composition and the excitation energy transfer within the pigment-protein complexes in leaves of a hemiparasitic mistletoe *Amyema miquelii*. This species has an unusual auxiliary xanthophyll cycle with lutein epoxide and lutein along with the common violaxanthin cycle (Bungard *et al*. 1999, Matsubara *et al*. 2001) and is an interesting material to study photoprotective mechanisms. Sun and shade leaves of *A. miquelii* exhibit dynamic rearrangement of photosynthetic pigments and undergo transition of the photosynthetic apparatus to enhance photoprotection during winter acclimation.

Materials and methods

In situ Chl *a* fluorescence measurements were performed with a PAM-2000 (Walz, Effeltrich, Germany) on sun and shade leaves of six mistletoes, *Amyema miquelii* (Lehm. ex Miq.) Tiegh., growing on host eucalypts in Tharwa, ACT, Australia. After the pre-dawn measurements of F_v/F_m, a sample disc was removed from each leaf and frozen in liquid nitrogen for the pigment analysis. Pigments were assayed by HPLC as described in Gilmore and Yamamoto (1991).

Leaves for laboratory experiments were collected early in the morning in April, June and July from a plant of the same species growing in Canberra, ACT, Australia. All leaves were dark-adapted at 4^{o}C for at least 1 h and vacuum-infiltrated with 500μM 3-(3,4-

dichlorophenyl)-1,1'-dimethylurea (DCMU), 0.3M glucose, HEPES (pH7.6) at room temperature in dark for 30 min prior to the measurements. Fluorescence excitation spectra (detected at 687 nm) and emission spectra (excitation by 435 nm or 470 nm) at 77K were measured at the F_m level after DCMU treatment (Jennings *et al.* 1993) by using an SLM-8100 spectrofluorimeter (Spectronic, Rochester, NY) fitted with a liquid nitrogen cold-finger dewar. The excitation and emission slit widths were 8 and 4 mm, respectively; resolution was 2 nm mm^{-1}. Data were normalized at 440 nm for excitation spectra and at 742 nm for emission spectra.

Results and discussion

Seasonal changes in the pigment composition

Distinct seasonal changes were observed in the pigment composition of sun and shade mistletoe leaves in the field (Table 1). Sun leaves always exhibited much lower chlorophyll concentrations per unit leaf area with slightly higher chlorophyll *a* to *b* ratios (Chl *a/b*) than shade, which could suggest downsizing of light harvesting antenna to avoid absorption of excess light (Anderson 1986). The Chl *a/b* ratios were the lowest in winter when a marked decrease in chlorophyll concentrations was observed (-42% and -27% compared to summer for sun and shade, respectively).

The levels of carotenoid pigments on a chlorophyll basis were remarkably high in sun leaves. In addition to light harvesting, most of these pigments, except for neoxanthin (Neo), play important rolls in photoprotection, such as quenching of the triplet excited state of chlorophyll molecules by β-carotene (β-caro) or thermal dissipation of excess light energy by the xanthophyll cycle. It has been demonstrated that an auxiliary xanthophyll cycle (Lx cycle) involving lutein epoxide (Lx) and lutein (Lut) (Bungard *et al*. 1999) is operating in *A. miquelii* in parallel with the common xanthophyll cycle (V cycle) with violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z) under short-term diurnal light regime in the field (Matsubara *et al*. 2001). There was a significant (P<0.0001 for the slope and the intercept) correlation between the changes in the pool size of the Lx- and the V-cycle in mistletoe leaves (Fig. 1), which would support the assumption of the common regulation of the two cycles in long-term acclimation to the light environment. The estimated y-axis intercept of 105 could be ascribable to a pool of Lut, which is not involved in the light-dependent cycle.

Table 1. Seasonal changes in pigment concentrations in sun and shade leaves of mistletoe at dawn.

		Chl *a+b*	Chl *a/b*	β-caro	Neo	Lx	Lut	V	A	Z	DPS	F_v/F_m
sun	winter	346	3.32	381	32	8	292	44	59	90	0.77	0.27
	spring	364	3.62	376	32	17	244	134	20	7	0.16	0.63
	summer	594	3.54	174	29	21	120	134	8	3	0.07	0.71
shade	winter	538	3.05	184	32	5	194	40	33	34	0.63	0.53
	spring	432	3.53	237	32	20	210	115	12	3	0.11	0.69
	summer	740	3.49	139	29	20	99	103	4	1	0.04	0.70

The concentrations of chlorophylls are on a leaf area basis (μmol m^{-2}) and those of carotenoid pigments are on a chlorophyll basis (mmol mol^{-1} Chl *a+b*). Each value is a mean of 6 samples.

The components of the two xanthophyll cycles showed very similar patterns of seasonal variations in sun leaves, reaching the largest pool size in winter under low temperatures (Table 1). Interestingly, the pre-dawn concentrations of Lx and V were extremely low in winter in both sun and shade with concomitantly higher concentrations of de-epoxidized xanthophylls (Lut, A and Z), with which dissipation of excess light energy is associated. This sustained de-epoxidation of the xanthophyll-cycle pigments in winter leaves, as has been observed in many light-stressed leaves (Adams *et al.* 1994), becomes more obvious when expressed as de-epoxidation state [DPS=(A+Z)/(V+A+Z)]. The strikingly high DPS values maintained overnight were accompanied by low PSII efficiency (F_v/F_m), which may be indicative of marked down-regulation of PSII and/or damage in the winter leaves. Both DPS and F_v/F_m exhibited a recovery from winter to spring. The other two abundant carotenoids, Lut (Pogson *et al*. 1998) and β-caro, are also likely to contribute to photoprotection in leaves of the parasite exposed to the severe excitation pressure under low temperatures.

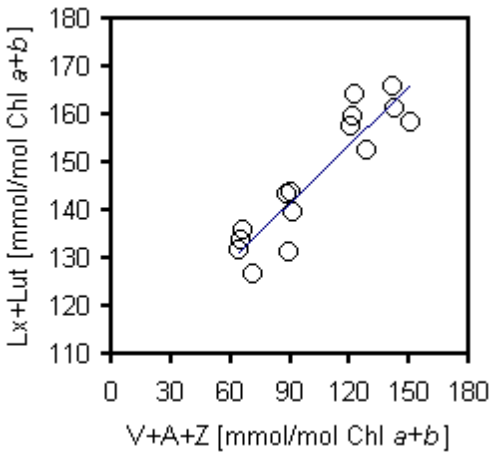

Fig. 1. The correlation between the V-cycle and the Lx-cycle components in sun and shade leaves of mistletoe (r^2=0.8314, P<0.0001).

Analysis of the 77K fluorescence spectra

The fluorescence excitation and emission spectra provide information about the *in vivo* excitation energy transfer from excited pigments to the fluorescing Chl *a* in the pigment-protein complexes. The 77K excitation spectra of mistletoe leaves in April, June and July revealed a decrease in the intensity of the broad band within 460-500 nm comprising excitation by Chl *b*, β-caro and xanthophylls (Thorne and Boardman 1971) relative to the major band at 440 nm (excited by Chl *a*) (Figs. 2A-C). The drastically diminished fluorescence intensity within 460-500 nm for sun leaves in July may indicate reduced efficiency of excitation energy transfer from Chl *b* molecules in the light harvesting antenna to the core Chl *a*-protein complexes (Špunda *et al.* 1998).

The 77K emission spectra for the same samples are shown in Figs. 2D-F. The minor bands at 685 nm and 695 nm originate from the PSII core antenna complex (Shen and Vermaas 1994, Govindjee 1995). The lower intensity within 685-695 for shade leaves

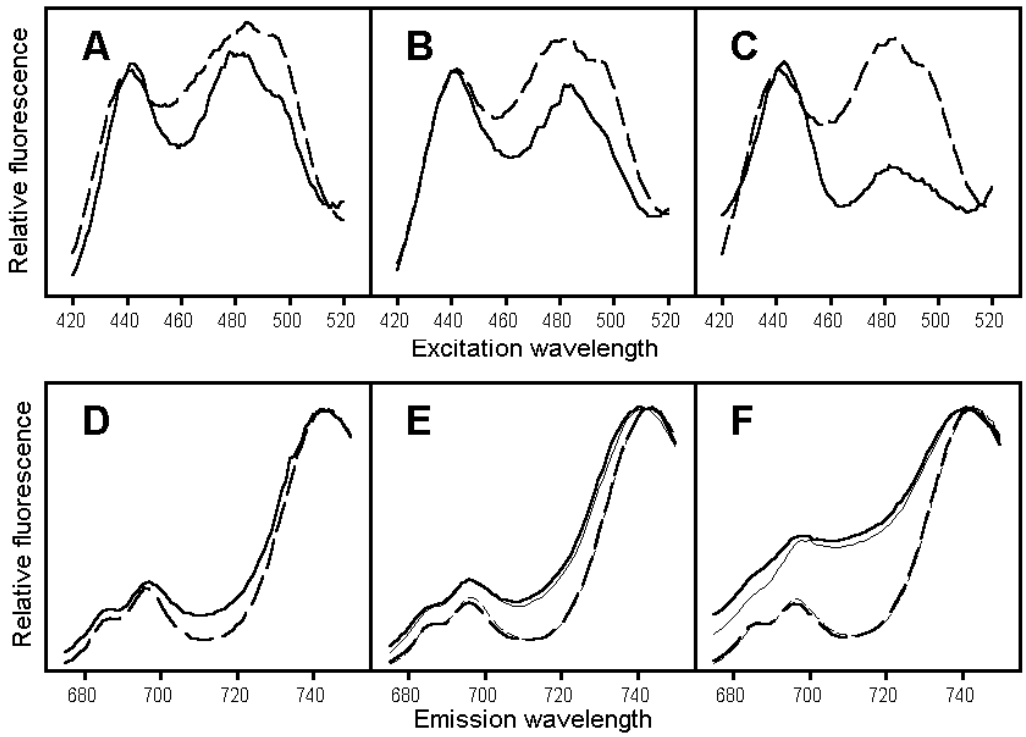

Fig. 2. 77K fluorescence excitation (A-C) and emission (D-F) spectra of sun (solid) and shade (dash) leaves of mistletoe. For A-C, the emission wavelength was 687 nm and data were normalized at 440 nm. For D-F, the excitation wavelength was 435 nm (bold) or 470 nm (thin) and data were normalized at 742 nm. A, D=April, B, E=June, C, F=July. Each line is a mean of 2 or 3 samples.

compared to sun was presumably due in part to reabsorption by chlorophylls at these wavelengths. The most striking difference between sun and shade is the emission within 700-720 nm relative to the major band at 742 nm from the PSI antenna complex. The relative fluorescence intensity in this region increased considerably in sun leaves from autumn to winter. Notably, a similar fluorescence spectrum profile has been reported for winter-acclimated leaves of snow gum seedlings, where it was associated with protection against photo-oxidative bleaching during high-light exposure in winter (Gilmore and Ball 2000). Excitation by 470 nm (thin lines in Figs. 2E and F) lowered the emission intensity around 685 nm in sun leaves in July, corresponding to the marked decrease in relative fluorescence intensity around 470 nm in Fig. 2C. No significant difference was found between the 435- and the 470-nm excitation for sun and shade in June or for shade in July.

Concluding remarks

The pronounced accumulation of zeaxanthin and antheraxanthin relative to chlorophylls (Table 1) and reduced efficiency of excitation energy transfer from Chl *b* in the light harvesting antenna to the core Chl *a*-protein complexes (Figs. 2C and F) in sun leaves in winter are consistent with photoprotection by down-regulation of PSII. The features of the 77K fluorescence spectra observed for mistletoe sun leaves in July resemble the “cold-hard band” described for winter-acclimated seedlings of snow gums (Gilmore and Ball 2000) and confirm that this type of reorganization of photosynthetic pigment-protein complexes takes

place in evergreens of different families during winter acclimation. The possible candidates, that enable this type of photoprotection in winter mistletoe leaves, e.g. Psbs protein (CP22) (Ottander *et al*. 1995, Li *et al*. 2000), are objects of future studies.

Acknowledgments

Skilled assistance by Wayne Pippen (Ecosystem Dynamics Group, RSBS) in the field experiments is greatly acknowledged. SM is the recipient of ANU PhD Graduate School Scholarship and Overseas PG Research Scholarship.

References

Adams WWIII, Demmig-Adams B, Verhoeven AS, Barker DH (1994) *Australian Journal of Plant Physiology* **22**, 261-276.
Anderson JM (1986) *Annual Review of Plant Physiology* **37**, 93-136.
Bungard RA, Ruban AV, Hibberd JM, Press MC, Horton P, Scholes JD (1999) *Proceedings of the National Academy of Sciences USA* **96**, 1135-1139.
Demmig-Adams B, Gilmore AM, Adams WWIII (1996) *FASEB Journal* **10**, 403-412.
Gilmore AM, Yamamoto HY (1991) *Journal of Chromatography* **543**, 137-145.
Gilmore AM, Ball MC (2000) *Proceedings of the National Academy of Sciences USA* **97**, 11098-11101.
Govindjee (1995) *Australian Journal of Plant Physiology* **22**, 131-160.
Jennings RC, Garlaschi FM, Bassi R, Zucchelli G, Vianelli A, Dainese P (1993) *Biochimica et Biophysica Acta* **1183**, 194-200.
Li X-P, Björkman O, Shih C, Grossman AR, Rosenquist M, Jansson S, Niyogi KK (2000) *Nature* **403**, 391-395.
Matsubara S, Gilmore AM, Osmond CB (2001) *Australian Journal of Plant Physiology* **28**, 793-800.
Niyogi KK (1999) *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 333-359.
Ottander C, Campbell D, Öquist G (1995) *Planta* **197**, 176-183.
Pogson BJ, Niyogi KK, Björkman O, DellaPenna D (1998) *Proceedings of the National Academy of Sciences USA* **95**, 13324-13329.
Shen G, Vermaas WFJ (1994) *Journal of Biological Chemistry* **269**, 13904-13910.
Špunda V, Čajánek M, Kalina J, Lachetová I, Šprtová M, Marek MV (1998) *Plant Science* **133**, 155-165.
Thorne SW, Boardman NK (1971) *Plant Physiology* **47**, 252-261.