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Variations in light energy dissipation in *Woodfordia fruticosa* leaves during expansion

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Abstract

Young leaves of tropical trees frequently appear red in color, with the redness disappearing as the leaves mature. During leaf expansion, plants may employ photoprotective mechanisms to cope with high light intensities; however, the variations in anthocyanin contents, nonphotochemical quenching (NPQ), and photorespiration during leaf expansion are poorly understood. Here, we investigated pigment contents, gas exchange, and chlorophyll (Chl) fluorescence in *Woodfordia fruticosa* leaves during their expansion. Young red leaves had significantly lower Chl content than that of expanding or mature leaves, but they accumulated significantly higher anthocyanins and dissipated more excited light energy through NPQ. As the leaves matured, net photosynthetic rate, total electron flow through PSII, and electron flow for ribulose-1,5-bisphosphate oxygenation gradually increased. Our results provided evidence that photorespiration is of fundamental importance in regulating the photosynthetic electron flow and CO_2 assimilation during leaf expansion.

Additional key words: anthocyanin; leaf expansion; nonphotochemical quenching; gas exchange; photosynthetic electron flow; photorespiration.

Introduction

Leaf expansion is a crucial process for plant growth, particularly with regard to the development of photosynthetic machinery (Yan *et al.* 2012, Chondrogiannis and Grammatikopoulos 2016). The newly formed leaves are often on the topmost parts of the canopy, and consequently receive more light energy than that of leaves lower down the plant (Zhu *et al.* 2016). Excess light energy may decrease stomatal conductance (g_s) and generate reactive oxygen species (ROS) (Nishiyama *et al.* 2011). Several researches have indicated that ROS production can damage cellular components by a photooxidative damage and result in photoinhibition of PSI and PSII (Carpentier 1997, Murata *et al.* 2007, Oukarroum *et al.* 2015). To prevent photodamage, plants have evolved several photoprotective mechanisms that dissipate excess light energy.

Young leaves on tropical trees frequently appear red in color, with the redness disappearing as the leaves mature (Lee *et al.* 1987, Cai *et al.* 2005, Karageorgou and Manetas, 2006). The red color is caused by the accumulation of anthocyanins (Close and Beadle 2003, Ranjan *et al.* 2014), which play important roles in light attenuation (Zhang *et al.* 2013a, Ranjan *et al.* 2014), ultraviolet B screening (Lee and Lowry 1980), and protection from herbivory (Numata *et al.* 2004, Karageorgou and Manetas 2006). Furthermore, with regard to lower photosynthetic efficiency, the young leaves of tropical forest trees are highly vulnerable to photoinhibition (Krause *et al.* 1995). It has been reported that the foliar anthocyanins can

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Abbreviations: Abs – mean spectral absorption; AQE – apparent quantum efficiency; ARI – anthocyanin reflectance index; Car – carotenoid; Chl – chlorophyll; C_i – intercellular CO_2 concentration; EL – expanding leaf; F_v/F_m – maximum quantum yield of PSII; F_v'/F_m' – maximum quantum yield of PSII after light adaptation; g_m – mesophyll conductance; g_s – stomatal conductance; J_c – electron flow for RuBP carboxylation; J_{max} – maximum rate of RuBP regeneration; J_o – electron flow for RuBP oxygenation; J_{o-max} – light-saturated rate of J_o; J_T – total electron flow through PSII; LSP – light-saturation point; ML – mature leaf; NPQ – nonphotochemical quenching; P_{Nmax} – light-saturated net photosynthetic rate; P_N – net photosynthetic rate; q_P – photochemical quenching coefficient; R_{Red}/R_{Green} – ratio of anthocyanin to chlorophyll based on spectral traits; R_D – dark respiration; ROS – reactive oxygen species; RuBP – ribulose-1,5-bisphosphate; TPU – triose phosphate utilization; V_{cmax} – maximum rate of RuBP carboxylation; WWC – water-water cycle; YL – young red leaf; Φ_{PSII} – actual photochemical efficiency of PSII.
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act as ideal photoprotective agents and antioxidants to scavenge ROS (Gould *et al.* 2002, Zhu *et al*. 2016). Therefore, anthocyanin accumulation is an adaptive strategy in young leaves (Manetas *et al.* 2002).

Nonphotochemical quenching (NPQ) and photorespiratory pathway have been suggested to be two main mechanisms involved in the dissipation of excess excited light energy, particularly in C_3 plants (Osmond and Grace 1995, Niyogi 1999, Hao *et al.* 2011, Zhang *et al.* 2016). The activation of NPQ is achieved *via* a high proton gradient across the thylakoid membrane (ΔpH) (Munekage *et al.* 2002, Breštič *et al*. 2015), which decreases the fraction of light energy that is used for the photochemistry and contributes to the efficient photoprotection (Hao *et al.* 2011). A considerable proportion of excess excitation energy is dissipated as heat through molecular vibrations (Müller *et al.* 2001), thereby minimizing ROS generation (Takahashi *et al.* 2009). Thus, NPQ is a key photoprotection in PSI and PSII (Niyogi 1999, Breštič *et al*. 2015). Furthermore, the consumption of photosynthetic excited electrons in the oxygenation of ribulose-1,5-bisphosphate (RuBP) is twice as high as that in the carboxylation of RuBP (Laisk and Edwards 1998). Excess photochemical energy, such as ATP and NADPH, can be consumed in the photorespiratory pathway (Osmond *et al.* 1997). Enhanced photorespiration can prevent over-reduction of the photosynthetic electron flow and D_1 protein synthesis in the acceptor side of PSII, thereby avoiding photoinhibition and photo-oxidative damage to PSII (Foyer *et al.* 2009). Meanwhile, photorespiration can alleviate the overaccumulation on the acceptor side of PSI, thus, preventing photoinhibition of PSI (Huang *et al*. 2014). Under high light intensities, enhanced photosynthetic excited energy to photorespiration can effectively maintain linear electron flow (LEF) (Osmond and Grace 1995), which can help maintain a high P_N value (Huang *et al.* 2014, Zhang *et al*. 2016). As leaves mature, the contents of pigments increase and mesophyll conductance (g_m) increases; meanwhile, the light-saturated net photosynthetic rate (P_{Nmax}) and total electron flow through PSII (J_T) increase gradually (Cai *et al*. 2005, Yan *et al*. 2012, Chondrogiannis and Grammatikopoulos 2016). However, the role of the photorespiratory pathway in regulating CO_2 assimilation and photosynthetic electron flow during leaf expansion is poorly understood.

Owing to rain-shadow effects of the mountains in Southwest China, the valleys between the mountains have a dry and hot climate with high irradiance (Jin and Ou 2000). These valleys are dominated by deciduous trees that are usually exposed to full sunlight, with new leaves appearing red at first, then gradually turning green as the leaves mature. Because young leaves have a low light-saturation point (LSP) and are susceptible to photoinhibition under high light intensities (Ranjan *et al*. 2014, Zhu *et al.* 2016), young red leaves may be an adaptive strategy to respond to high light. *Woodfordia fruticosa* (L.) Kurz. is a dominant tree species in the Chinese savanna. It establishes easily and grows quickly in dry and hot valleys in Southwest China, and it is used for vegetation restoration and preventing soil erosion in degraded Chinese savannas (Jin and Ou 2000). Zhang *et al*. (2014) reported that the activity of PSII is resistant to seasonal drought in the leaves of this species, but the photoprotection mechanisms involved during leaf expansion are not fully understood. Here, we investigated the changes of pigment contents, photosynthesis, and photoprotection in *W. fruticosa* leaves during their expansion, *i.e*., in flat young red leaves (YL), expanding green leaves (EL), and fully mature green leaves (ML). The aims of the study were to (*1*) determine the role of anthocyanin accumulation, NPQ, and photorespiration in light energy dissipation during leaf expansion and (*2*) ascertain whether the photorespiratory pathway regulates photosynthetic efficiency during the leaf expansion.

Materials and methods

Study site: The study was conducted at Yuanjiang Savanna Ecosystem Research Station (YSERS), Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Yuanjiang County, Yunnan province, Southwest China (23°27′N, 102°10′E, and 481 m a. s. l.). The climate is characterized by two distinct seasons, a rainy season (May to October) and a dry season (November to April). Based on YSERS meteorological records dating from 2012 to 2015, the mean annual temperature was 24.9°C. The total mean annual precipitation was 694.7 mm, with 80.4% of the precipitation occurring during the rainy season. All of the measurements were taken in June and July 2015. During the experiment, the mean day/night temperature was 32/23°C, with a mean relative air humidity of 60%. The maximum PPFD was 2,200 μmol(photon) m^{-2} s^{-1} at midday.

Plant material: Five adult *W. fruticosa* individuals were selected from a long-term monitoring plot (100 m × 100 m) at YSERS that was established in 2011 (23°28′25″N, 102°10′38″E, and 565 m a. s.l.). The diameter at breast height (DBH) of the sample trees was 5–10 cm, with an approximate height of 3–5 m. Each sample tree was measured as a replicate, and there were five replicates. We did not follow single leaves during the process of leaf expansion; instead, YL, EL, and ML from each sample tree were chosen for measurements (Yan *et al.* 2012). All of the measurements were taken from the third leaf from the top of the branch. The leaf areas of YL and EL were 25–32% and 50–65%, respectively, that of ML (100%). The predawn leaf water potential was –0.2 MPa (measured using a pressure chamber, *PMS*, Corvallis, OR, USA). Judging by the leaf water potential, the sample leaves were not under water stress.

Pigment determination: Chlorophyll (Chl) and carotenoids (Car) were extracted from leaf sections (0.2 g) using 95% ethanol. Pigment extraction was performed by adding liquid nitrogen and a small amount of $CaCO_3$ to facilitate extraction in dim light [<2 μmol(photon) m^{-2} s^{-1}]. The supernatant absorbance was measured at 665, 649, and 470 nm with a spectrophotometer (*UV-2550*, *Shimadzu*, Tokyo, Japan). These were compared to a 95% ethanol blank. Chl *a*, Chl *b*, total Chl, and Car contents were calculated according to the methods described by Wellburn (1994).

Spectral traits and estimation of anthocyanin accumulation: A *USB-4000* spectrometer (*Ocean Optics*, Florida, USA) was used to measure leaf spectral traits. Spectral reflectance (R, %) and spectral transmittance (T, %) were measured at wavelengths ranging from 400 to 700 nm, which is the wavelength range of photosynthetic active radiation. The mean spectral absorption (Abs, %) between 400 and 700 nm was calculated using the following formula:

$$Abs = 1 - (R + T) \quad (1)$$

An anthocyanin reflectance index (ARI) was used to determine the anthocyanin content (Gamon and Surfus 1999, Zhang *et al.* 2013a). The R_{Red}/R_{Green} ratio represents the ratio of anthocyanin to Chl (Gamon and Surfus 1999). These techniques allow for a rapid and accurate estimation of anthocyanin accumulation in leaves (Gamon and Surfus 1999, Gitelson *et al.* 2001). Both ARI and R_{Red}/R_{Green} were calculated using the following equations:

$$ARI = (R_{550})^{-1} - (R_{700})^{-1} \quad (2)$$
$$R_{Red}/R_{Green} = (R_{600-699})/(R_{500-599}) \quad (3)$$

The subscripts refer to specific wavelengths or wavelength ranges.

Gas exchange and Chl fluorescence were measured simultaneously using a *LI-6400XT* portable photosynthesis system with a *6400-40* fluorescence chamber (*Li-Cor*, Lincoln, NE, USA). All of the measurements were taken between 08:30 and 11:30 h on sunny days. The leaves were first acclimated to the dark for at least 30 min before the ground fluorescence yield (F_o) was determined. The maximum fluorescence yield (F_m) was measured using a 0.8-s saturating pulse at 7,000 μmol(photon) m^{-2} s^{-1}. Light-response curves were measured after at least 20 min of light adaptation under a PPFD of 1,000 μmol(photon) m^{-2} s^{-1}. Light-response curves of gas exchange and Chl fluorescence were measured using the following nine PPFD gradients provided by a blue (10%) and red (90%) light-emitting diode light source: 2,000; 1,500; 1,000; 500, 200, 100, 50, 20, and 0 μmol(photon) m^{-2} s^{-1} at 30°C and a relative humidity of 80%, using a reference CO_2 concentration of 400 μmol(CO_2) mol^{-1}. Based on previously described methods (Genty *et al.* 1989, Kramer *et al.* 2004), we determined the minimum (F_o') and maximum (F_m') Chl fluorescence of light-adapted leaves, as well as steady-state fluorescence under actinic light (F_s).

A nonrectangular hyperbola was fitted for each data set. The LSP, P_{Nmax}, and apparent quantum efficiency (AQE) were calculated from the light-response curves using P_N/C_i and P_N/PPFD *Response Curve Analysis s*oftware *version 1.0* (*Li-Cor*, 2/2008). The photochemical quenching coefficient (q_P) and the actual proportion of absorbed light energy used by PSII photochemistry (Φ_{PSII}) were calculated according to formulae 4–6 (Genty *et al.* 1989). J_T was estimated according to formula 7 (Krall and Edwards 1992).

$$q_P = \frac{F_m' - F_s}{F_m' - F_o'} \quad (4)$$
$$F_v'/F_m' = \frac{F_m' - F_o'}{F_m'} \quad (5)$$
$$\Phi_{PSII} = 1 - \frac{F_s}{F_m'} \quad (6)$$
$$J_T = 0.5 \times Abs \times PPFD \times \Phi_{PSII} \quad (7)$$

It was assumed that absorbed photons were distributed equally in PSI and PSII. A factor of 0.5 was used in the calculation of the electron flow, and the electron flow for both RuBP carboxylation (J_c) and oxygenation (J_o) were calculated according to the following formulae (Valentini *et al.* 1995):

$$J_C = \frac{1}{3} \times [J_T + 8 \times (P_N + R_D)] \quad (8)$$
$$J_O = \frac{2}{3} \times [J_T - 4 \times (P_N + R_D)] \quad (9)$$

where P_N is the net photosynthetic rate under each PPFD gradient and R_D is dark respiration. Under low light intensity, P_N is limited by light intensity, and linearly positively correlated with light intensity (Zhang *et al.* 2009). In this study, R_D was calculated using light-response curves at the low light levels of 20–100 μmol(photon) m^{-2} s^{-1}.

Response curves of leaf gas exchange to intercellular CO_2 concentration (P_N/C_i) were determined using 400, 200, 100, 50, 800; 1,200; 1,600; 2,000 μmol(CO_2) mol^{-1} under a PPFD of 1,000 μmol(photon) m^{-2} s^{-1}. Following the method described by Long and Bernacchi (2003), the maximum rate of RuBP regeneration (J_{max}), maximum rate of RuBP carboxylation (V_{cmax}), and triose phosphate utilization (TPU) were calculated based on the P_N/C_i response curves.

Determination of mesophyll conductance: Mesophyll conductance (g_m) was calculated at a PPFD of 1,000 μmol(photon) m^{-2} s^{-1} using the following formula:

$$g_m = \frac{P_N}{C_i - \Gamma^* \times [J_T + 8 \times (P_N + R_D)]/[J_T - 4 \times (P_N + R_D)]} \quad (10)$$

The P_N, R_D, C_i, and J_T are described above. Γ^* is the CO_2-compensation point in the absence of respiration (Brooks and Farquhar 1985), and was assumed to be 43.9 at 30°C (Bernacchi *et al.* 2002).

Statistical analyses: The statistical analyses were performed using *SPSS16.0* for *Windows* (*SPSS Inc*., Chicago,

IL, USA). The results are reported as means ± standard error (SE). A one-way analysis of variance (*ANOVA*) was used to test for significant differences at a significance level of $p<0.05$, and multiple comparison of *Tukey*'s HSD test was used for post-hoc analysis of YL, EL, and ML in order to indentify statistically homogenous groups.

Results

Pigments and spectral traits: As the leaves matured, Chl *a*, Chl *b*, and total Chl contents gradually increased, with significant differences between the leaves at different developmental stages (Fig. 1*A*–*C*). The Car content increased slightly during leaf expansion, but no significant difference was found between YL, EL, and ML (Fig. 1*D*). YL had a significantly higher Car/Chl ratio than that of EL or ML (Fig. 1*E*). The ARI was 19- and 95-fold higher in YL than that in EL and ML, respectively (Fig. 1*F*), and the R_{Red}/R_{Green} ratio was 1.9- and 2.2-fold higher in YL than that in EL and ML, respectively (Fig. 1*G*). These results showed that anthocyanins accumulated in YL. In the 400–700 nm range of photosynthetic active radiation, the Abs progressively increased as the leaves matured. There was a significant difference in Abs between YL, EL, and ML (Fig. 1*H*).

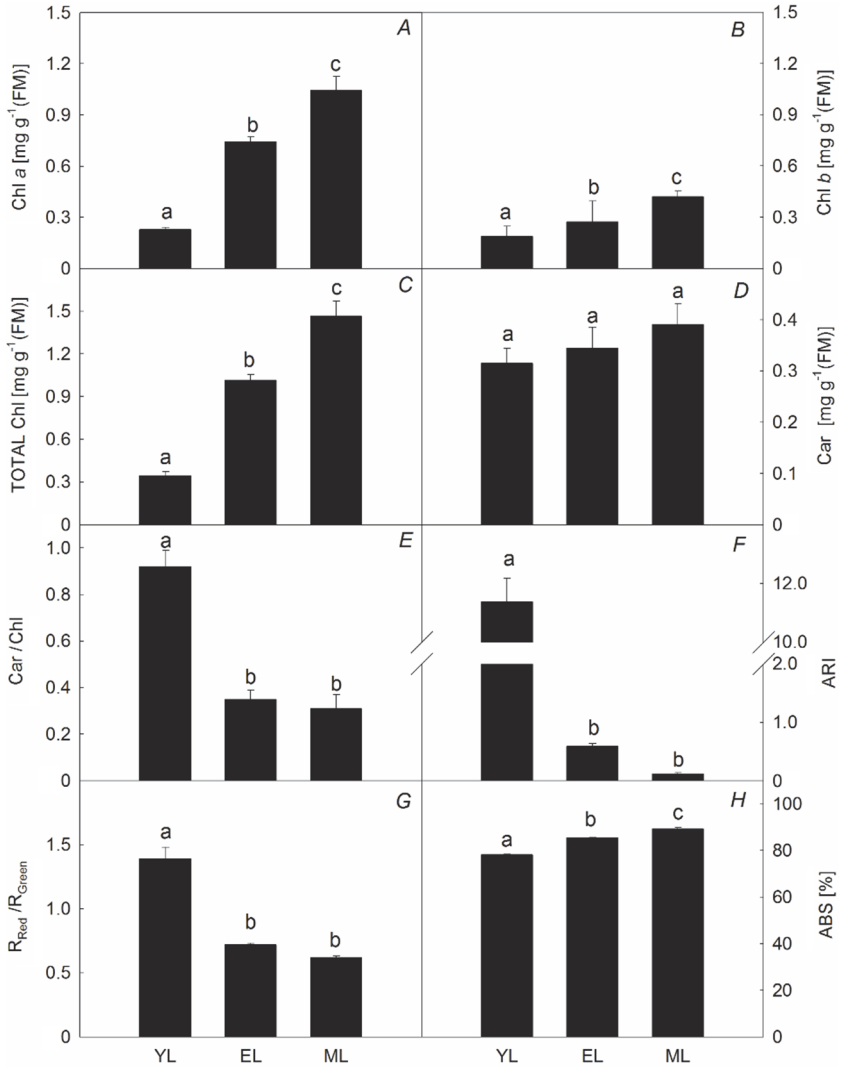

Fig. 1. Pigment content and reflectance characteristics of *Woodfordia fruticosa* leaves during expansion. Different small letters above the bars indicate significant differences between the three developmental stages (*ANOVA*, *Tukey*'s HSD test, $p<0.05$).

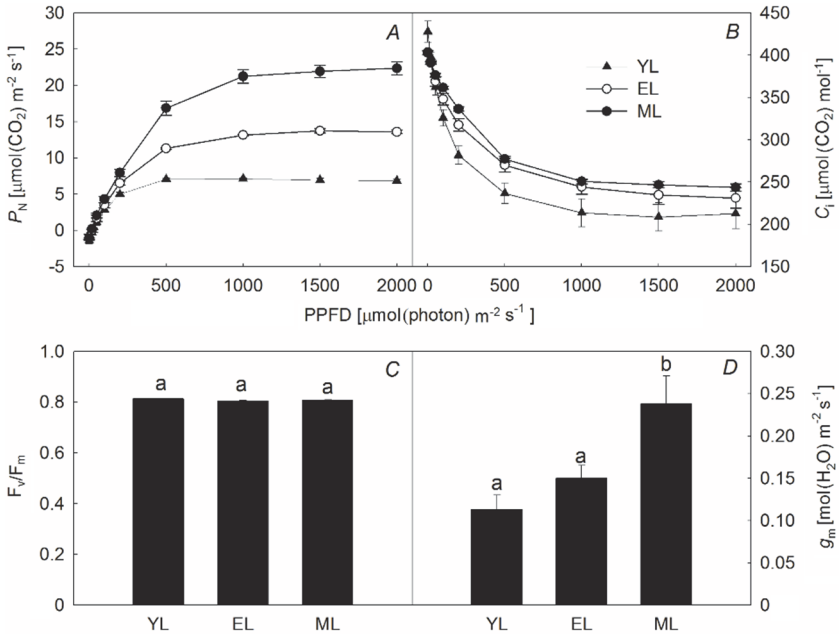

Fig. 2. Response curves of the net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), maximum quantum yield of PSII (F_v/F_m), and mesophyll conductance (g_m) in *Woodfordia fruticosa* leaves during expansion. Different small letters above the bars indicate significant differences between the three developmental stages (*ANOVA*, *Tukey*'s HSD test, $p<0.05$).

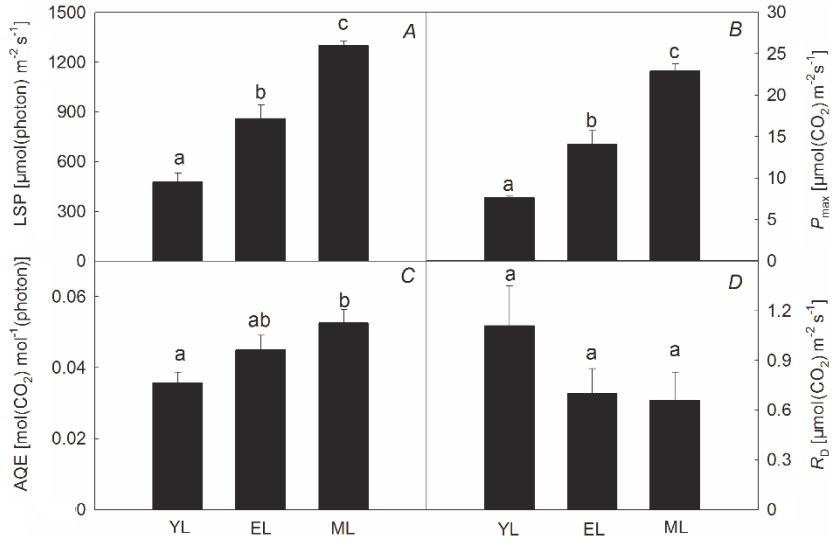

Fig. 3. Light-saturation point (LSP), light-saturated net photosynthetic rate (P_{Nmax}), apparent quantum efficiency (AQE), and dark respiration (R_D) in *Woodfordia fruticosa* leaves during expansion. Different small letters above the bars indicate significant differences between the three developmental stages (*ANOVA*, *Tukey*'s HSD test, $p<0.05$).

Photosynthetic properties and photochemical efficiency: The light-response curves at 30°C and a CO_2 concentration of 400 μmol(CO_2) mol^{-1} suggested that P_N and the intercellular CO_2 concentration (C_i) were significantly higher in ML than that in YL or EL when the PPFD was greater than 200 μmol(photon) m^{-2} s^{-1} (Fig. 2*A*,*B*). Regardless of a developmental stage, the maximum quantum yield of PSII (F_v/F_m) remained above 0.8, with no significant difference in F_v/F_m between YL, EL, and ML (Fig. 2*C*). Under a PPFD of 1,000 μmol(photon) m^{-2} s^{-1} and a CO_2 concentration of 400 μmol(CO_2) mol^{-1}, the estimated values of g_m were 0.113, 0.150, and 0.238 mol(H_2O) m^{-2} s^{-1} in YL, EL, and ML, respectively. The g_m value in ML was significantly higher than that in YL or EL (Fig. 2*D*).

As the leaves matured, the LSP and P_{Nmax} significantly increased (Fig. 3*A*, *B*). The values of LSP were 477, 861, and 1,301 μmol(photon) m^{-2} s^{-1} for YL, EL, and ML, respectively (Fig. 3*A*), and the corresponding P_{Nmax} values were 7.6, 14.1, and 22.9 μmol(CO_2) m^{-2} s^{-1}, respectively

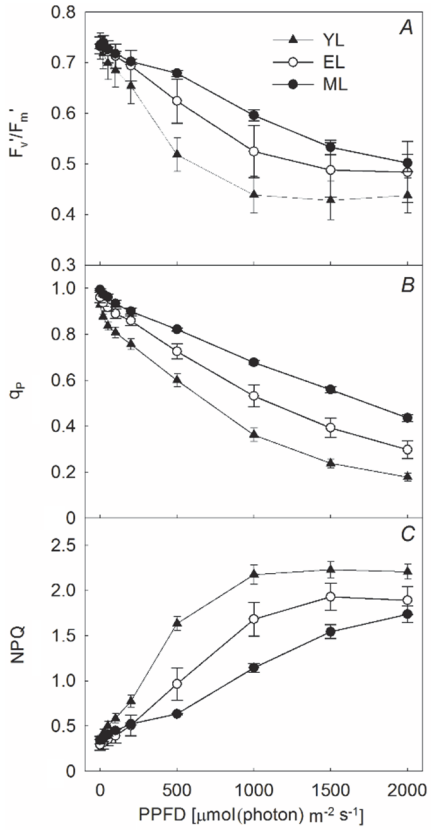

Fig. 4. Response curves of the maximum quantum yield of PSII after light adaptation (F_v'/F_m'), photochemical quenching coefficient (q_P), and nonphotochemical quenching (NPQ) in *Woodfordia fruticosa* leaves during expansion.

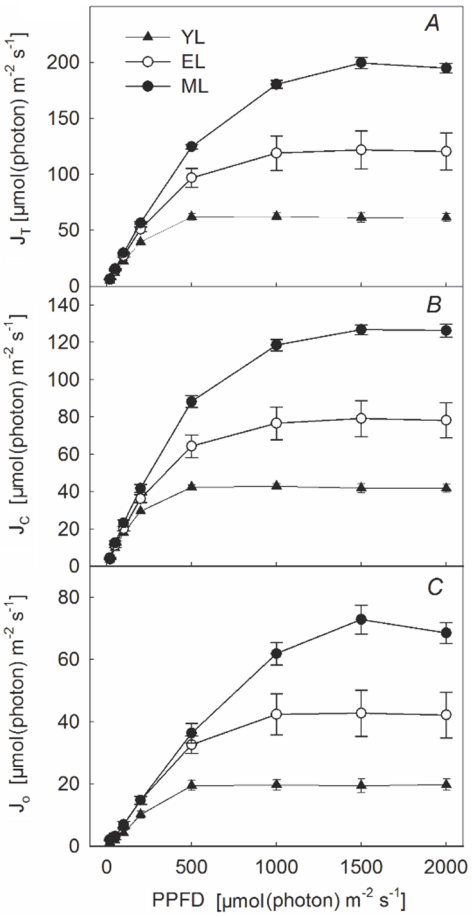

Fig. 5. Response curves of the total electron flow through PSII (J_T), electron flow for RuBP carboxylation (J_c), and electron flow for RuBP oxygenation (J_o) to the incident photosynthetic photon flux density (PPFD) in *Woodfordia fruticosa* leaves during expansion.

(Fig. 3*B*). The AQE increased as the leaves developed, with ML having a significantly higher value than that in YL (Fig. 3*C*). YL had a relatively higher R_D value than those of EL or ML, but no significant difference in R_D was detected among the three stages (Fig. 3*D*).

When the light intensities were greater than 500 μmol (photon) m^{-2} s^{-1}, F_v'/F_m' and q_P values were significantly higher in ML than that in YL or EL (Fig. 4*A*, *B*). The highest and lowest NPQ values were in YL and ML, respectively. Under a PPFD of 2,000 μmol(photon) m^{-2} s^{-1}, the NPQ values were 2.21, 1.89, and 1.74 for YL, EL, and ML, respectively. NPQ was significantly higher in YL than that in ML (Fig. 4*C*).

Photosynthetic electron flow: The light-response curves indicated that J_T, J_c, and J_o did not differ significantly between YL, EL, and ML under light intensities that were lower than 200 μmol(photon) m^{-2} s^{-1} (Fig. 5). However, when the PPFD was greater than 500 μmol(photon) m^{-2} s^{-1}, J_T, J_c, and J_o were significantly higher in ML than that in YL or EL. The maximum J_o values were 20.2, 44.4, and 79.7 μmol(photon) m^{-2} s^{-1} in YL, EL, and ML, respectively (Fig. 5*C*). The changes to the maximum J_o suggest that the photorespiratory pathway was enhanced as the leaves developed.

When C_i was greater than 100 μmol(CO_2) mol^{-1}, the P_N/C_i curves at a PPFD of 1,000 μmol(photon) m^{-2} s^{-1} and 30°C revealed that ML had higher values of P_N than YL or EL (Fig. 6*A*). The maximum P_N to incident CO_2 concentration were 16.3, 21.6, and 28.4 μmol(CO_2) m^{-2} s^{-1} for YL, EL, and ML, respectively, when the PPFD maintained at 1,000 μmol(photon) m^{-2} s^{-1}.

According to the P_N/C_i curves, when produced at PPFD of 1,000 μmol(photon) m^{-2} s^{-1} and 30°C, the highest and lowest values of J_T, J_c, and J_o were in ML and YL, respectively (Fig. 6*B–D*). Both J_T and J_c increased linearly with increasing C_i (Fig. 6*B*,*C*). In contrast, J_o decreased rapidly and linearly with an increase in C_i (Fig. 6*D*), but only when C_i was lower than 400 μmol(CO_2) mol^{-1}. With a C_i value of below 100 μmol(CO_2) mol^{-1}, J_o was 31.3, 41.9, and 78.8 μmol(photon) m^{-2} s^{-1} for YL, EL, and ML, respectively. J_{max}, V_{cmax}, and TPU significantly increased during leaf expansion (Fig. 7). The J_{max}/V_{cmax} ratios were 0.92, 0.91, and 0.91 in YL, EL, and ML, respectively. No significant difference in the J_{max}/V_{cmax} ratio was observed among the three stages of leaf development (Fig. 7*C*).

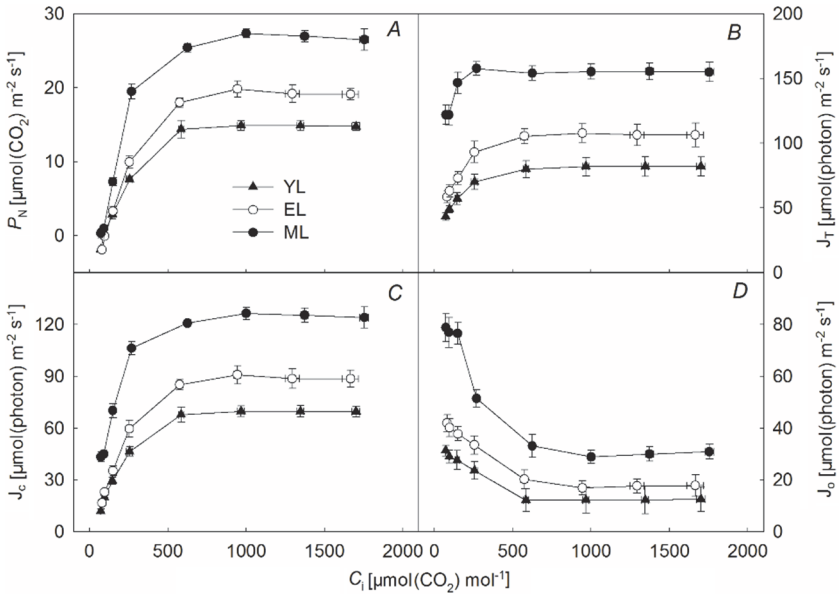

Fig. 6. Response curves of net photosynthetic rate (P_N), total electron flow through PSII (J_T), electron flow for RuBP carboxylation (J_c), and electron flow for RuBP oxygenation (J_o) to the incident CO_2 concentration in *Woodfordia fruticosa* leaves during expansion.

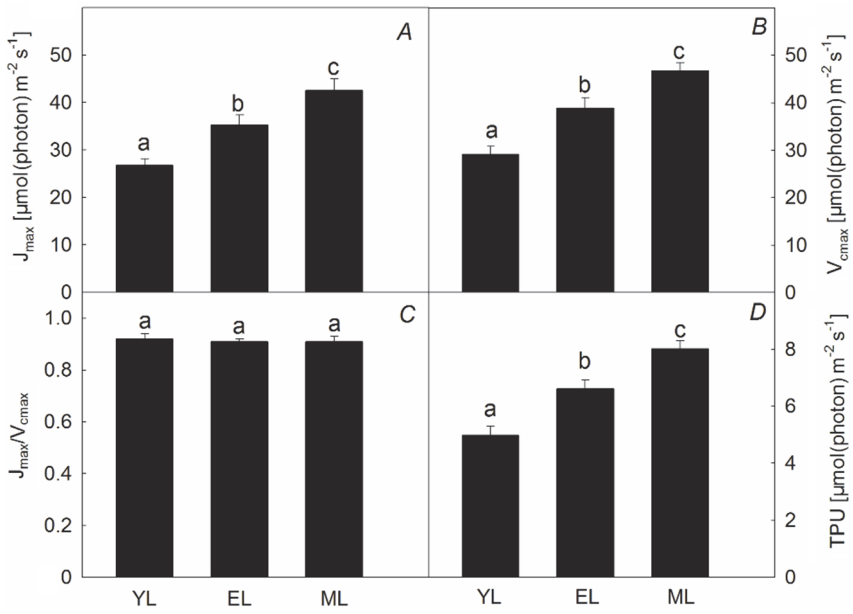

Fig. 7. Maximum rate of RuBP regeneration (J_{max}), maximum rate of RuBP carboxylation (V_{cmax}), the J_{max}/V_{cmax} ratio, and triose phosphate utilization (TPU) in *Woodfordia fruticosa* leaves during expansion. Different small letters above the bars indicate significant differences between the three developmental stages (*ANOVA*, *Tukey*'s HSD test, $p<0.05$).

Relationship between P_N and photorespiration: There was a significant, positive correlation between P_{Nmax} and the light-saturated rate of J_o (J_{o-max}), according to values calculated from the light-response curves at 30°C and a CO_2 concentration of 400 μmol(CO_2) mol^{-1} ($R^2 = 0.91$, $p<0.001$; Fig. 8).

Discussion

A number of significant changes occurred to YL under high light intensity. They had a low Chl content and photochemical efficiency, as suggested by lower F_v'/F_m' and q_P values, a significantly higher ARI, and a higher NPQ value than that of EL or ML (Figs. 1, 4). These results support the hypothesis that anthocyanin accumulation and enhanced NPQ promote photoprotection in YL.

It is well-known that pigment content and

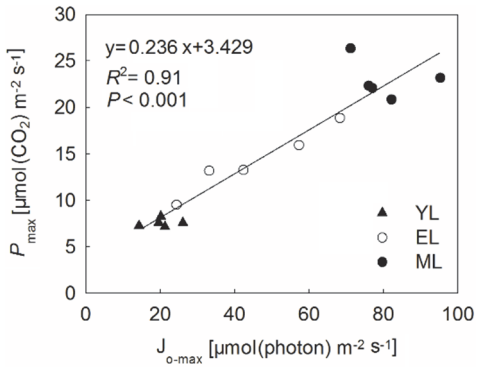

Fig. 8. Relationship between the light-saturated net photosynthetic rate (P_{Nmax}) and the light-saturating rate of the electron flow for RuBP oxygenation ($J_{o–max}$) in *Woodfordia fruticosa* leaves during expansion.

photoprotective enzyme activity are low in young leaves because of photosynthetic immaturity in their structure and physiological function (Dodd *et al.* 1998, Neill *et al.* 2002). Swoczyna *et al*. (2010) revealed that significantly lower Chl content was closely related to weak photosynthetic efficiency. In this study, YL exhibited a lower total Chl and photosynthetic efficiency than that of EL and ML (Figs. 1*C*, 2*A*). However, YL had a significantly higher ARI than that in EL and ML (19- and 95-fold higher, respectively). This finding is consistent with those from previous studies, which reported that anthocyanin accumulation is inversely related to photosynthetic immaturity (Cai *et al.* 2005, Ranjan *et al*. 2014, Zhu *et al*. 2016). During the initial stages of leaf expansion, anthocyanin accumulation can compensate for immature photopigments. Anthocyanins can minimize light capture, resulting in a lower Abs of visible light (Smillie and Hetherington 1999). Indeed, YL had a lower Abs than either EL or ML for wavelengths of the visible spectrum (Fig. 1*H*). Furthermore, anthocyanins are closely associated with an increased antioxidant capacity in YL, enabling them to scavenge ROS in the chloroplasts and preventing photo-oxidative damage under high light intensities (Neill *et al.* 2002, Zhu *et al*. 2016). Chl *a* fluorescence measurements can be used to assess to photosynthetic apparatus efficiency, and the values of F_v/F_m can indicate the photosynthetic activity of PSII (Swoczyna *et al*. 2010, Tuba *et al*. 2010). The significant decrease of F_v/F_m has been shown to be indicative of an impaired PSII photosynthetic efficiency, which inhibits the redox reaction after primary quinine electron acceptors of PSII (Q_A) (Oukarroum *et al*. 2015). Although the ROS-scavenging capacity of anthocyanins was not analyzed in this study, the high maximum quantum yield of PSII (F_v/F_m= 0.813) in YL was similar to that in EL and ML (Fig. 2*C*). This suggests that YL exhibited almost the same PSII activity as EL and ML and anthocyanin accumulation may be an adaptive response to high light intensities by YL.

Because of photosynthetic immaturity, YL had lower g_m and C_i values than those of EL or ML, and consequently a reduced CO_2 assimilation capacity (Fig. 2). YL had a low LSP value [477 μmol(photon) m^{-2} s^{-1}, Fig. 3*B*], but the maximum PPFD increases to 2,200 μmol(photon) m^{-2} s^{-1} at midday in the Chinese savanna. It is evident that more excess light energy is needed to dissipate safely in YL than in EL or ML. With regard to NPQ, previous studies have reported that it is greater in young leaves than that in mature ones (Cai *et al.* 2005, Yan *et al*. 2012, Ranjan *et al*. 2014). The higher NPQ capacity in young leaves suggests a higher ΔpH across the thylakoid membrane. The generation of ΔpH is mainly dependent on cyclic electron flow and the water-water cycle (WWC) activity in higher plants (Makino *et al*. 2002, Munekage *et al*. 2002, Huang *et al*. 2016). Furthermore, it has been reported that ΔpH is the result of H^+ accumulation and its consumption for ATP synthesis (Breštič *et al*. 2015). In young leaves, a low photochemical efficiency indicates a low consumption of ATP supplied from these two flexible pathways. As a result, the higher content of anthocyanins is thought to be related to the higher ΔpH *via* the WWC activity. Thus, a high ΔpH activates high NPQ level in young leaves. Indeed, in this study, young anthocyanin-pigmented leaves had high NPQ and dissipated excess light energy as heat (Fig. 4*C*). The enhancement of ΔpH is essential for photoprotection in PSI *via* "photosynthetic control", which controls the electron flow from cytochrome b_6/f complex to PSI and thus prevent the over-reduction of PSI electron carriers (Suorsa *et al*. 2012, Tikkanen and Aro 2014, Yamori and Shikanai 2016, Yamori *et al*. 2016). High NPQ regulates and protects the photosynthetic apparatus under high light intensities, in which the absorbed light energy exceeds the amount used for CO_2 assimilation (Müller *et al.* 2001). Therefore, high NPQ may play an important role in photoprotection during the initial stages of leaf expansion.

As the leaves matured, the content of pigments increased, and there was a gradual and concomitant decrease in the Car/Chl ratio and ARI (Fig. 1). This result suggests that the need for photoprotectants decreases as leaves develop (Neill *et al*. 2002, Zhu *et al.* 2016). As the leaves matured, the photochemical efficiency (as indicated by q_P and F_v'/F_m') increased to maintain a high P_N, with a gradual decrease in NPQ also occurring (Figs. 2, 3). This finding is consistent with those from previous studies, which reported that absorbed light energy shifts from nonphotochemical to photochemical as leaves mature (Cai *et al.* 2005, Yan *et al*. 2012).

The q_P, F_v'/F_m', P_{Nmax}, J_T, J_C, and J_o values gradually increased, and reached maximum levels when the leaves matured under saturating light intensities (Figs. 3–5). It is tempting to speculate, therefore, that more photochemical energy, such as NADPH and ATP, was produced as the leaves matured, and was transferred from PSII to PSI through cytochrome b_6/f. However, excess photochemical energy under high light intensities that cannot be utilized in the Calvin cycle may induce an increase in NADPH/$NADP^+$ (Zhang *et al.* 2013b). Because of a lack

of electron acceptors in the photosynthetic electron flow ($NADP^+$), excited electrons with high photochemical energy are transferred to O_2 to produce ROS, such as O_2^- and H_2O_2, with the subsequent photoinhibition of PSII and PSI (Murata *et al.* 2007, Nishiyama *et al*. 2011). Previous reports have suggested that the activation of the photorespiratory pathway can consume excess photosynthetic electrons and dissipate excess photochemical energy (Laisk and Edwards 1998, Takahashi *et al.* 2007, Zhang *et al*. 2016). Additionally, under high light conditions, excess photochemical energy can cause over-acidification of thylakoid lumen (Takahashi *et al*. 2007). Enhanced photorespiration can consume considerable NADPH and ATP, and alleviate this over-acidification (Huang *et al*. 2015). In this study, both the light response and P_N/C_i curves indicated that the photorespiratory pathway was gradually enhanced during the leaf expansion. In particular, J_T and J_o increased simultaneously as the leaves matured, both in the light response and P_N/C_i curves (Figs. 5, 6). The enhanced photorespiration increased the capacity to consume extra NADPH and ATP, which is needed to maintain a low $NADPH/NADP^+$ ratio and the photosynthetic electron flow (Zhang *et al.* 2013b). The gradual increase in J_T from PSII to PSI under saturating light conditions might be caused by an enhanced photorespiratory pathway during leaf expansion. Therefore, enhanced photorespiration promotes the regulating of photosynthetic electron flow under high light conditions, which provides an adaptation to high light with leaves maturing.

It has been reported that g_m constrains the capacity for photosynthetic CO_2 assimilation to occur under saturating light intensities (Ethier *et al.* 2006, Brito *et al.* 2014). In maturing leaves at a PPFD of 1,000 μmol(photon) m^{-2} s^{-1}, g_m increased during leaf expansion (Fig. 2*D*) and promoted a gradual increase in stomatal conductance (data not shown) and C_i under saturating light intensities (Fig. 2*B*). Previous studies have shown that P_{Nmax} is mainly limited by RuBP carboxylation and regeneration (Farquhar *et al.* 1980, Huang *et al.* 2014). Although the J_{max} and V_{cmax} increased as the leaves matured, the J_{max}/V_{cmax} ratio remained at a low value of 0.91, and did not significantly change during leaf expansion (Fig. 7). The low J_{max}/V_{cmax} ratio suggested that the RuBP oxygenation rate exceeded the RuBP regeneration rate (Huang *et al.* 2014); as a result, CO_2 assimilation was limited by RuBP regeneration (Yamori *et al.* 2011). It is important to note that P_{Nmax} was positively correlated with $J_{o\text{-}max}$ during leaf expansion (Fig. 8). Additionally, CO_2 is released by the photorespiratory pathway (Hochberg *et al*. 2013), thus C_i was increased in ML and EL more than that in YL in saturating light (Fig. 2*B*). Therefore, an enhanced photorespiratory pathway helps to maintain a high rate of RuBP regeneration and a high flux of intercellular CO_2, which subsequently promotes the Calvin cycle (Huang *et al.* 2014, Zhang *et al*. 2016). If the photorespiratory pathway was impaired, the ΔpH increased and J_T from PSII to PSI was suppressed (Takahashi *et al*. 2007), thus, the P_N was greatly decreased (Huang *et al*. 2014). Therefore, we propose that a gradual increase in photorespiration promoted the rates of RuBP regeneration and CO_2 assimilation as leaves matured.

In summary, our results showed that anthocyanin accumulation and increased NPQ promoted photoprotection in young red leaves. They also showed that photorespiration gradually increased in order to maintain a high rate of RuBP regeneration and photochemical efficiency as the leaves matured. In addition, photorespiration plays an important role in regulating the photosynthetic electron flow and CO_2 assimilation during leaf expansion.

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