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BRIEF COMMUNICATION

Characteristics of gas exchange and chlorophyll fluorescence in red and green leaves of *Begonia semperflorens*

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Abstract

To determine the effects of leaf colour on gas exchange and chlorophyll fluorescence, two genotypes of *Begonia semperflorens* with green leaves or red leaves were compared. The red leaves showed a high accumulation of anthocyanins and high absorbance at 282 and 537 nm while the green leaves exhibited a higher net photosynthetic rate and lower thermal dissipation of light energy. It seems likely that anthocyanins in the vacuoles restricted the absorption of green light to the chloroplasts, leading to a decrease in the efficiency of excitation capture by open PS 2 centres, photochemical quenching and CO_2 assimilation.

Additional key words: anthocyanins, net photosynthetic rate, non-photochemical quenching, photochemical quenching, Rubisco.

Plants with colored leaves have attracted great commercial attention in recent years. Anthocyanins are mainly responsible for red-to-blue coloration of leaves. Anthocyanins are usually synthesised in unmatured juvenile leaves and disappear when photosynthetic tissue matured or in matured leaves of some evergreen species in response to harsh climatic conditions (Hughes *et al.* 2007, Hughes and Smith 2007). Meanwhile, plant accumulates anthocyanins in leaves in response to biotic and abiotic stresses such as low temperature (Close *et al.* 2004), nutrient deficiency (Peng *et al.* 2008), wounding (Reyes and Cisneros 2003), herbivory (Lev-Yadun and Gould 2007), pathogen (Karageorgou and Manetas 2006), UV-B or high irradiance (Pfündel *et al.* 2007).

Several studies showed that leaves with anthocyanins display shade characteristics and are less prone to photoinhibition. Purified anthocyanin fractions and the red leaves typically absorb more green light than do green leaves (Neill and Gould 1999). The absorption of green light by anthocyanins has been postulated to protect chloroplasts from photoinhibition by intercepting energy quanta that would otherwise be absorbed by chlorophylls (Nishio 2000). Meanwhile, anthocyanin productions can also modify the photosynthetic performance of leaves. Red leaves usually show lower net photosynthetic rate than green leaves although there were some exceptions (Gould *et al.* 2002). Anthocyanins are frequently accumulated in the cell vacuole, and therefore physically separated from the chloroplasts. They, therefore, work as simple light filters, impacting directly on photosynthesis by reducing the quanta absorbed by chlorophyll. Importantly, the selective absorption of green light would impair the photosynthesis (Terashima *et al.* 2009). Up to date, information about how light absorption, light energy transfer and CO_2 assimilation are influenced by the anthocyanin accumulations is scarce.

Begonia semperflorens is one of the most popular bedding plants. To examine the relation between anthocyanin accumulations and photosynthesis, the cultivars with green leaves (cv. Super Olympia) and red

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Abbreviations: F_v'/F_m' - efficiency of excitation capture by open PS 2 centers; J_{fD} - rate of energy dissipation *via* constitutive thermal dissipation and fluorescence; J_{max} - maximum potential rate of electron transport contributed to Rubisco regeneration; J_{NPQ} - rate of energy dissipation *via* light-dependent thermal dissipation; J_{PS2} - electron flux in PS 2; NPQ - non-photochemical quenching coefficient; P_{Nmax} - light saturated net photosynthetic rate; PS 2 - photosystem 2; q_p - photochemical quenching coefficient; V_{cmax} - maximum carboxylation velocity of Rubisco; Φ_{fD} - quantum efficiency of constitutive thermal dissipation and fluorescence; Φ_{NPQ} - quantum efficiency of light-dependent thermal dissipation; Φ_{PS2} - quantum efficiency of PS 2.

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leaves (cv. Cocktail) were used for the present study. Seedlings of *B. semperflorens* were grown in pots with 2 dm^3 of peat as growth medium in a greenhouse and watered or fertilized when necessary. Chlorophylls (Chls) were extracted with 80 % (v/v) cold acetone and determined spectrophotometrically by reading the absorbance at 662 and 644 nm according to Arnon (1949). Anthocyanins were extracted with 80 % ethanol and measured by reading the absorbance at 530 and 653 nm according to Murray and Hackett (1991). The spectral characteristics of pigment were analysed by scanning the leaf extracts in mixture of acetone and ethanol (1:1, v/v) and measured from 250 to 700 nm (Pomar and Ros Barceló 2007).

Net photosynthetic rate (P_N) was measured with an infrared gas analyzer (*LI-6400*, *Li-COR* Lincoln, NE, USA). The air temperature was maintained at 25 ± 2 °C, relative air humidity at 80 %, CO_2 concentration at 400 $\mu mol\ mol^{-1}$, and photosynthetic photon flux density (PPFD) at 800 $\mu mol\ m^{-2}\ s^{-1}$, respectively. Measurement and estimation of maximum carboxylation rate by Rubisco (V_{cmax}) and maximum potential rate of electron transport contributed to RuBP regeneration (J_{max}) were calculated according to Ethier and Livingston (2004). Chl fluorescence was measured as previously described (Xia *et al.* 2009) using an *Imaging-PAM* chlorophyll fluorometer comprising a computer-operated *PAM*-control unit (Walz, Effeltrich, Germany). Photochemical quenching coefficient (q_p), efficiency of excitation capture by open PS 2 centers (F_v'/F_m') and non-photochemical quenching coefficient (NPQ) were calculated as described by Van Kooten and Snel (1990). Utilization of photons absorbed by the PS 2 antennae were assessed from the quantum efficiency of photochemical energy dissipation (Φ_{PS2}), light-dependent (Φ_{NPQ}), and constitutive (Φ_{fD}) thermal dissipation and fluorescence, respectively, with $\Phi_{PS2} + \Phi_{NPQ} + \Phi_{fD} = 1$ (Hendrickson *et al.* 2004). The rate of energy dissipation *via* each process (J_{PS2}, J_{NPQ}, $J_{f,D}$) was calculated by multiplying respective quantum efficiency (Φ) with irradiance and α, where α is a constant which depends on the molar ratio of PS 2 to PS 1 in the thylakoid membranes and the efficiency of absorption of light by the leaf under a specific conditions (Harley *et al.* 1992, Hendrickson *et al.* 2004). The data with at least four replicates per treatment were analyzed. All results were subjected to analysis of variance (*DPS 3.01*) and Duncan's multiple range test at $P \le 0.05$ was used to ascertain the significance of differences among treatments.

The acetone-methanol extract from green leaves exhibited a typical characteristic of photosynthetic pigments with three absorption peaks at 324, 441 nm and 665 nm while the extract from red leaves showed five absorption peaks at 282, 324, 441, 537 and 665 nm respectively (Fig. 1). Biochemical analysis showed that anthocyanin content in red leaves [696.2 mg m^{-2}] was about 10 times higher than that [70.5 mg m^{-2}] in green leaves.. The high absorption at 282 and 537 nm was largely attributed to anthocyanins. Apparently, anthocyanins absorb more and reflect less in green light (500 - 600 nm), while absorb less and reflect more in red light (640 - 660 nm) as compared to Chls. Total chlorophyll contents in green (484.7 mg m^{-2}) and red (469.8 mg m^{-2}) leaves were similar, however, V_{cmax} (25.4 $\mu mol\ m^{-2}\ s^{-1}$) and J_{max} (62.8 $\mu mol\ m^{-2}\ s^{-1}$) for the green leaves were significantly higher than those for the red leaves (18.3 $\mu mol\ m^{-2}\ s^{-1}$ and 47.9 $\mu mol\ m^{-2}\ s^{-1}$, respectively).

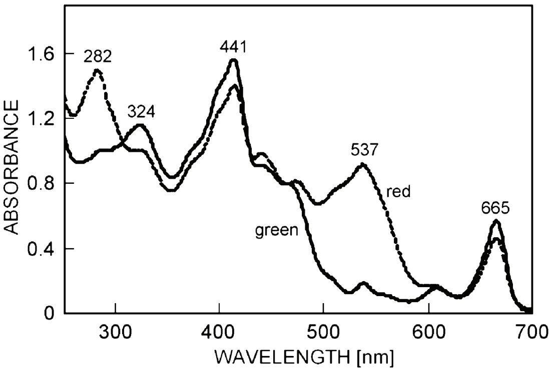

Fig 1. Absoption spectrum for acetone-ethanol extracts from green and red leaves of *B. semperflorens*.

We further examined the changes in P_N, q_p, F_v'/F_m' and NPQ in the two cultivars after exposure to different PPFD (Table 1). P_N and NPQ increased whilst q_p and F_v'/F_m' decreased with the increase in PPFD in both genotypes similarly as was observed in *Colocasia esculenta* (Chowdhury *et al.* 2009). In comparison to red leaves, green leaves showed higher P_N, q_p and F_v'/F_m' and lower NPQ at PPFD range 400 - 800 $\mu mol\ m^{-2}\ s^{-1}$. Reduced P_N for red leaves was also observed in other plant species. In *Quintinia serrata,* a 23 % reduction in CO_2 assimilation of red versus green leaves was found although there was no significant difference in the apparent quantum efficiency between two kind leaves as observed in our study (Gould *et al.* 2002). When PPFD increased from 800 to 1000 $\mu mol\ m^{-2}\ s^{-1}$ or higher, green leaves showed a reduction in P_N while red leaves showed a further increase in P_N, leading to less difference in P_N between the two genotypes (Table 1). Accordingly, green leaves had lower light saturation point than red leaves. Similar trends were also observed for Φ_{PS2}, q_p and NPQ parameters. Additional experiment showed that both genotypes experienced photoinhibition when they were exposed to PPFD 1200 $\mu mol\ m^{-2}\ s^{-1}$ for 1 day as indicated from the sharp reduction of the maximum photochemical efficiency (F_v/F_m) from 0.84 to 0.63 for green leaves and to 0.69 for red leaves, respectively. There are evidences that leaves rich in anthocyanins may acclimate to high PPFD better and reduce the risk of photoinhibition (Manetas *et al.* 2006, Hughes and Smith 2007, Shao *et al.* 2007, Merzlyak *et al.* 2008). Anthocyanins may also serve as antioxidants and alleviate oxidative stress produced by excess irradiance, leading to less photoinhibition in leaves (Shao *et al.* 2007).

Table 1. Changes of net photosynthetic rate, P_N [µmol m^{-2}s^{-1}], photochemical quenching coefficient (q_p), efficiency of excitation energy capture by open PS 2 reaction centre (F_v'/F_m'), non-photochemical quenching coefficient (NPQ), quantum efficiency and flux [µmol m^{-2}s^{-1}] of photochemical energy dissipation (Φ_{PS2}, J_{PS2}), light-dependent (Φ_{NPQ}, J_{NPQ}) and constitutive ($\Phi_{f,D}$, $J_{f,D}$) thermal dissipation and fluorescence, respectively, with the changes in PPFD for *B semperflorens* green leaves (cv. Super Olympia) and red leaves (cv. Cocktail). Means (*n* = 4) within a row followed by different letters are significantly different at $P \leq 0.05$.

Parameters	Leaves	PPFD [µmol m^{-2} s^{-1}]								
		50	100	150	200	400	600	800	1000	1200
P_N	green	0.61j	2.47i	4.04gh	5.34f	7.83cd	8.6b	9.57a	8.47bc	-
	red	0.73j	2.29i	3.55h	4.46g	6.64e	7.30de	7.43d	7.84cd	-
q_p	green	0.98a	0.96a	0.93abc	0.89cd	0.73e	0.63f	0.52g	0.35h	0.28i
	red	0.96ab	0.94abc	0.90bc	0.84d	0.64f	0.47g	0.37h	0.28i	0.23i
F_v'/F_m'	green	0.69ab	0.69ab	0.69ab	0.69ab	0.61c	0.55d	0.51de	0.45f	0.43fg
	red	0.71a	0.70a	0.69ab	0.65bc	0.50e	0.44f	0.42fg	0.39gh	0.37h
NPQ	green	0.28g	0.29g	0.40g	0.64f	1.24e	1.74d	2.03c	2.28b	2.57a
	red	0.28g	0.29g	0.31g	0.43g	1.56d	2.24b	2.58a	2.57a	2.70a
Φ_{PS2}	green	0.69a	0.66ab	0.64ab	0.61b	0.48c	0.38d	0.27ef	0.16gh	0.14hi
	red	0.68ab	0.66ab	0.62b	0.54c	0.33de	0.22fg	0.16gh	0.12hi	0.09i
Φ_{NPQ}	green	0.07j	0.08j	0.10ij	0.15h	0.29g	0.40f	0.49e	0.58c	0.62bc
	red	0.07j	0.08j	0.09j	0.14hi	0.41f	0.54d	0.60bc	0.63ab	0.67a
Φ_{fD}	green	0.24c	0.26bc	0.25bc	0.24c	0.23c	0.23c	0.24c	0.26bc	0.24c
	red	0.25bc	0.26bc	0.29ab	0.32a	0.26bc	0.24c	0.23c	0.25c	0.25bc
J_{PS2}	green	14.44f	27.60ef	40.34de	51.17cd	80.43ab	93.96a	89.65a	66.57bc	67.53bc
	red	14.25f	27.90ef	39.00de	45.75de	55.29cd	56.45cd	54.39cd	50.95cd	45.93de
J_{NPQ}	green	1.41k	3.17k	6.34k	12.39k	47.61j	99.08h	163.39f	243.63d	310.97b
	red	1.50k	3.19k	5.64k	11.51k	68.72i	135.29g	202.96e	265.89c	337.11a
J_{fD}	green	5.02h	10.95gh	15.90g	19.89fg	38.86e	57.31d	80.76c	107.06b	122.21a
	red	5.27h	10.94gh	18.41fg	26.80f	44.13e	60.45d	78.91c	103.49b	121.36a

Different allocation of energy absorbed by PS 2 antennae to photosynthetic electron transport and to thermal dissipation in response to PPFD was assessed from the quantum efficiency (Φ_{PS2}) and flux (J_{PS2}) of photochemical energy dissipation, light-dependent (Φ_{NPQ}, J_{NPQ}) and constitutive thermal dissipation and fluorescence ($\Phi_{f,D}$, $J_{f,D}$). Φ_{PS2} decreased whilst Φ_{NPQ} increased with the increase in PPFD. However, Φ_{fD} was almost independent on the change in PPFD. There were little differences in Φ_{PS2} and Φ_{NPQ} between two genotypes at PPFD 50 - 200 µmol m^{-2} s^{-1} but green leaves exhibited higher Φ_{PS2} and lower Φ_{NPQ} than red leaves at PPFD 400 - 1000 µmol m^{-2} s^{-1}. Similarly, green leaves showed higher $J_{PS2,}$ and lower J_{NPQ} than red leaves at high PPFD. The J_{PS2}, for example, was 64.9 % higher whereas J_{NPQ} was 19.5 % lower for green leaves than red leaves at PPFD 800 µmol m^{-2} s^{-1}. There was no significant change in J_{fD} between two genotypes at the tested PPFD. All these results suggest that the decrease in Φ_{PS2} and J_{PS2} was largely compensated by the increase in Φ_{NPQ} and J_{NPQ} and light-regulated thermal dissipation was largely responsible for the photoprotection in red leaves as observed in other studies (Hendrickson *et al.* 2004, Zhang *et al.* 2009, Zhou *et al.* 2009, Cai *et al.* 2010).

It is known that anthocyanins modify significantly both the quantity and quality of light incident on chloroplasts (Krol *et al.* 1995). As shown in Fig. 1, acetone-methanol extracts from red leaves had a higher absorbance at the green range and lower absorbance at the red and blue range than those from green leaves. The green light absorbed by anthocyanins, which are mainly attributed in the vacuoles not only contributes little to photosynthesis but also competes with chlorophylls for photon capture (Hughes and Smith 2007). Enhanced reflection of red light can reduce overall absorption in leaf surface. Therefore, anthocyanins are considered as sunscreen and shield chloroplast from effects of red light (Neill and Gould 2003, Hughes and Smith 2007). On the other hand, anthocyanins in the mesophyll restricted absorption of green light to the upmost palisade parenchyma, leading to a decrease in photosynthesis since green light and red light had different effects on leaf photosynthesis (Terashima *et al.* 2009). Consequently, red leaves showed decreased q_p, F_v'/F_m' and Φ_{PS2}. Taken together, our study showed that anthocyanins in the vacuoles restricted the absorption of green light to the chloroplasts, leading to a decrease in the efficiency of energy capture by open PS 2 centers and photochemical quenching followed by a lowered net photosynthetic rate. Further work on how the two genotypes response differently to stress would get insight into the mechanism for the anthocyanin-induced stress tolerance.

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