Photosynthetic activity of red and green leaf sectors in *Coleus blumei* plants as sensed by chlorophyll fluorescence

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Abstract

The changes and responses in the chlorophyll (Chl) fluorescence (ChlF) components were studied on the red and green parts of leaves in three *Coleus blumei* varieties under different light intensities. Chl *a*, carotenoids (Car), and SPAD were linearly, positively, and significantly correlated with total Chl [*i.e.*, Chl (*a*+*b*)] of red and green leaves. Significant differences in Chl and Car contents were noted between differentially pigmented sectors of the leaf, but no anthocyanin content was found in the green parts compared to red leaves. Values of minimal and maximal fluorescence yields in dark- and light-adapted states in both red and green leaves were all positively and significantly correlated with Chl contents under dark conditions and all PPFD treatments for 20 min. Assessing the ChlF parameters under PPFD variations is an important tool for finding improved photosynthetic productivity of plants.

Additional key words: light intensity; light response; photosynthetic efficiency; photosynthetic photon flux density.

Introduction

Chloroplasts are a major target of many environmental stress factors. Plants respond to sudden and sustained fluctuations in light intensities partly *via* their chloroplast molecular redox-signaling transduction mechanisms, which initiate marked modulations in chloroplast components, ultimately leading to acclimation of the photosynthetic apparatus (Anderson *et al.* 1997). Light intensity is one of the major factors that affect the growth, leaf form, floral morphology, and biochemical characteristics of plants, and it is also associated with the photosynthetic efficiency of plants (Montgomery and Givnish 2008). At high light intensities, photosynthetic carbon fixation increases, but the excess light is a stressor and therefore causes depression of the photosynthetic efficiency (Powles 1984). The study of photosynthesis–irradiance relationships is a basic aspect of plant ecophysiological research and is important for managing rare species; photosynthetic light-response curves are used to assess the ability to capture light and understand optimal habitat light conditions of plants (Aleric and Kirkman 2005). Insufficient light can limit photosynthesis, which causes reductions in net carbon gains and plant growth. Contrary, under high irradiance, during the light reaction, plants can absorb more photons than can be used by carbon fixation reactions, *e.g.*, by leaves in the upper canopy layer exposed to the sun and

also by shade leaves exposed to sunflecks. The excessive absorbed energy can lead to reductions in the efficiency of photosystems, especially PSII (Adams *et al.* 2004).

High contents of pigments in the leaves can be attributed to a wide occurrence of those plants under high light conditions (de la Rosa-Manzano *et al.* 2015). Elevated Chl contents in red-leafed plants were associated with lower Chl *a/b* ratios similar to shade-adapted plants (Liakopoulos and Spanorigas 2012). Variations in the Chl *a/b* ratio occur due to differences in PSI/PSII ratios as well as the size and composition of light-harvesting complexes associated with each photosystem and containing different amounts of Chl *b* (Borek *et al.* 2016). Stress decreases the ability of photosynthetic systems to utilize incident photons, thus leading to photoinhibition, and reduced quantum yields of photochemistry and chlorophyll fluorescence (ChlF). Photoinhibition causes inhibition of PSII, while also increases thermal deexcitation of excited Chl (Demmig-Adams *et al.* 1996). Electrons transferred from PSII to PSI are used by downstream electron sink pathways, including photosynthetic carbon fixation and photorespiration. When carbon fixation becomes saturated, photosynthesis is unable to use all of the energy absorbed by plants under high light (Dewir *et al.* 2015). Plants adapt photosynthesis to a certain degree in response to the prevailing environment, and the sensitivity of photosynthesis to stress varies among plant species and cultivars. ChlF,

Received 27 August 2018, accepted 10 December 2018.

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Abbreviations: Ant – anthocyanins; Car – carotenoids; Chl – chlorophyll; ChlF – chlorophyll fluorescence; F_0 – minimum fluorescence yield in a dark-adapted state; F_0' – minimum fluorescence yield in a light-adapted state; F_m – maximum fluorescence yield in a dark-adapted state; F_m' – maximum fluorescence yield in a light-adapted state; F_v/F_m – maximum photochemical quantum yield; NPQ – nonphotochemical quenching; SPAD – soil-plant analysis development.

a noninvasive technique, offers highly accurate measurements that illustrate the photosynthetic performance of plants, and is often used to investigate a plant's response to various abiotic and biotic stresses in controlled environments and in the field (Nedbal *et al.* 2000, Pineda *et al.* 2008, Rahbarian *et al.* 2011, Takayama *et al.* 2011, Dos Anjos *et al.* 2012, Wilson and Jacobs 2012, Huang *et al.* 2013, Muniz *et al.* 2014). Measuring ChlF is a simple, rapid, and accurate method that can be performed with portable equipment, during which no materials are consumed (Molina-Bravo *et al.* 2011). However, functioning of the photosynthetic apparatus has not been examined yet by ChlF in *Coleus blumei* plants under different light intensities. Understanding the photosynthetic characteristics of *C. blumei* would benefit field cultivation management.

Coleus blumei, a member of the Lamiaceae, is an ornamental garden plant that has become popular due to a large number of cultivars with highly decorative and variegated leaves. The colors of *Coleus* foliage range from green, yellow, orange, and brown through purple to red and pink (Osman 2013). *Coleus* leaf colors depend on the presence of pigments including Chls, anthocyanins (Ant), carotenoids (Car), flavonoids, and betalains (Tanaka *et al.* 2008). *Coleus* plants are sensitive to environmental changes and may exhibit changes in leaf structure and physiological conditions of their leaves (Padri and Umponstira 2017). Furthermore, this species is also sensitive to changes in light intensity and has a rapid phenotypic response to it, but there is limited information available regarding the photosynthetic activity of these plants. The hypothesis of this research was that some of the ChlF components might exhibit distinguishable differences between green and red parts of leaves in plants under different light intensities since pigment composition of the leaves is different. The aim of this study was to determine the actual state of the photosynthetic apparatus in differentially pigmented leaves of multicolored leaves of three *Coleus* cultivars. The mechanisms of the capture, transfer, and dissipation of excitation energy were detected by ChlF measurements within an individual leaf with green and red coloration in response to various light intensities. ChlF can be considered a selection index to examine the growth of *Coleus* plants under broad ranges of light intensity. Relationships of ChlF indices with Chl and other pigments can be used for ecophysiological research in *C. blumei* plants. Precise management of the photosynthetic capacity in response to PPFD can potentially be used to maximize the efficiency of the growth and development of *Coleus* plants grown in controlled environments for economic benefits.

Materials and methods

Plant material and culture practice: Plants of three *C. blumei* cultivars, 'Blumei Benth' (BB), 'Wizard Rose' (WR), and 'Solenostemon Scutellaroides' (SS) (Fig. 1S, *supplement*), were purchased from local flower shops in Taipei (Taiwan), and were used in all experiments undertaken in this study. BB plants have medium-large, yellowish-green leaves with red sectors near the main

veins. The edges of the foliage are surrounded by arrow-shaped tips. WR leaves have large and wide red centers surrounded by narrow outer margins of light-green and white. SS plants have large leaves with red centers, while bright-green fragments form a very wide and large outline located mainly at the base of the leaf borders. Plants (16–20 cm tall) were transplanted into 20-cm plastic pots (1,444 mL) containing commercial potting soil with a 1:1:1 (v/v/v) mixture of sand, peat moss, and perlite, and were placed in a greenhouse. Plants were evenly spaced to promote similar growth rates and sizes. Plants were watered daily, and optimal amounts of a liquid fertilizer solution of 0.1% NH_4NO_3 and K_2HPO_4 (50 mL) were applied weekly. Plants were grown for 1 month, and those with a uniform size of 30–40 cm in height were selected and randomly separated into three groups for subsequent experiments. During the period of plant growth, average day/night temperatures were 30/22°C, and the average day length was 14 h under a natural light intensity of 500–700 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ in a greenhouse, and the relative humidity was approximately 60%. All plants were free from any disease or nutrient shortage.

Determination of Chl, Car, and Ant contents: Contents of Chls and Car in red and green sectors of leaves were determined using methods described by Lichtenthaler *et al.* (1987). In brief, leaf discs were excised using a standard hole punch, immediately sealed in prelabeled aluminum envelopes, and placed in liquid nitrogen. Tissues were stored at -80°C until analysis, and then extracted in a solvent mixture of acetone, methanol, and water (80:15:5, v/v/v) at 4°C overnight. The supernatant was obtained by centrifuging the mixture at $13,000 \times g$ for 10 min. Supernatants were assayed for the absorbance of Chl *a* and Chl *b* in acetone at 663 and 647 nm, respectively, with a U-2000 type spectrophotometer (Hitachi, Tokyo, Japan). Chl *a*, Chl *b* and Car concentrations were calculated using the following equations:

$$\text{Chl } a = 0.01373 \times \text{OD}_{663} - 0.000897 \times \text{OD}_{537} - 0.003046 \times \text{OD}_{647},$$

$$\text{Chl } b = 0.02405 \times \text{OD}_{647} - 0.004359 \times \text{OD}_{537} - 0.005507 \times \text{OD}_{663},$$

$$\text{Car} = [\text{OD}_{470} - (17.1 \times (\text{Chl } a + \text{Chl } b) - 9.479 \times \text{Ant})] / 119.26.$$

Ant contents of the extracts were measured based on the protocol of Mancinelli and Rabino (1984). Red and green sectors of leaf discs were immersed in boiling methanol/ $\text{H}_2\text{O}/\text{HCl}$ (90:10:1, v/v/v) for 10 min and incubated for 1 h at room temperature. The mixture was then centrifuged at 3,000 rpm and 4°C for 5 min to obtain the supernatant, followed by measuring the absorbances at 530 (A_{530}) and 657 nm (A_{657}) by a spectrophotometer. The following equation was used: $\text{Ant} = A_{530} - (0.25 \times A_{657})$.

In addition, total Chl contents of red and green parts of leaves were determined using a nondestructive Chl estimation by SPAD analyzer (SPAD-502, Konica Minolta, Tokyo, Japan). Measurements were collected from 16 mature, healthy, and fully expanded leaves from the middle to upper

portion of each plant and averaged. SPAD values were determined using dual wavelength optical absorbances at 650 and 940 nm (Bonneville and Fyles 2006). Leaf veins were avoided during the measurement.

Leaf spectral measurement: After SPAD measurements, reflectance was measured on red and green sectors of leaves in a plant with different total Chl [*i.e.*, Chl (*a+b*)] contents using a bifurcated fiber optic cable and a leaf-clip of a spectrometer (CI-700, CID Inc., Vancouver, WA, USA) according to Xue *et al.* (2014). Tested leaves were irradiated from one side with a tungsten halogen lamp in the spectrometer using the bifurcated fiber cable. The experimental setup provided a spectral range of 400–700 nm. Transmittance was measured according to the method of Konoplyova *et al.* (2008). The absorbance (*A*) was calculated as: $A = 1 - (\text{Reflectance} + \text{Transmittance})$.

Determination of ChlF variables: In order to understand better the ecophysiology of *C. blumei* plants, the relationship of the photosynthetic efficiency with Chl (*a+b*) content was analyzed by determining the ChlF index. The potted plants were moved to a dark room overnight, and the middle portions of both red and green leaves of a plant were used for the measurements. The overnight dark-adapted plants were exposed to light stepwise from low to high levels of PPFD (*i.e.*, 200, 400, 800; 1,200; and 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$), by a slide projector with a tungsten halogen lamp for 20 min. After illumination, leaves were adapted in the dark for 20 min. The ChlF parameters of dark-adapted (before and after the illumination) and light-exposed leaves were measured at ambient temperature with a portable fluorometer (PAM-2000, Heinz Walz, Effeltrich, Germany) (Weng *et al.* 2010). All measurements were taken before 11:00 h to avoid the midday depression in photosynthesis. Values of the minimal ChlF (F_0) and maximal ChlF (F_m) of dark-adapted samples were respectively determined using modulated irradiation of a weak light-emitting diode (LED) beam (measuring light) and saturating pulse. We then calculated the maximum photochemical quantum yield (F_v/F_m), where F_v , the yield of variable fluorescence, was calculated as ($F_m - F_0$). When measuring F_v/F_m , samples were first acclimated to dark conditions to ensure that all reaction centers were in an open state, and there was minimal nonphotochemical dissipation of excitation energy. F_v/F_m is an index of the photosynthetic potential as well as the potential for photochemical dissipation, and reveals the percentage of PSII that is open and its effectiveness in capturing complexes from photoenergy and later transfer to quanta (Fracheboud and Leipner 2003). The actual PSII efficiency (F_v'/F_m') was calculated as $(F_m' - F_0')/F_m'$. F_0' and F_m' are the actual and maximal levels of fluorescence during illumination, respectively; the former was determined at each PPFD level, and the latter was determined by applying a saturating flash. From these data, nonphotochemical quenching (NPQ) could be computed based on the modulated fluorescence kinetics: $\text{NPQ} = (F_m - F_m')/F_m'$ (Porcar-Castell *et al.* 2008). Measurements were recorded with WinControl-3 software (Heinz Walz).

Statistical analysis: All measurements and analyses were performed on leaf areas with different pigmentation in three biological replicates. One plant was used for all types of analyses, and the number of plants was 16–30. All experiments were arranged in a completely randomized design. All parameters were subjected to a one-way analysis of variance (ANOVA), with a significance level of $p \leq 0.05$ using CoStat statistical software (Cohort Berkeley, Monterey, CA, USA). Regression analyses were used to examine relationships among Chl (*a+b*), pigments, and Ant. In addition, model datasets were based on at least 48 leaves from each PPFD level, and ChlF parameters were calculated using Chl (*a+b*) data from the model validation datasets. Several models were tested, with the nonlinear regression model being selected as the best interpretation of the relationship between ChlF parameters and the PPFD. All models were evaluated for goodness of fit by graphical analysis of residuals, and by computing the correlation coefficient (*r*) and determination coefficient (r^2). Each experiment was performed twice independently with a randomized design for the growth environment, sampling day, and ChlF analyses. All graphs were created with Sigmaplot 10.0 (Systat Software, San Jose, CA, USA).

Results

Changes in spectral characteristics and Chl contents in different leaf colors: Leaf pigments and contents affect the leaf absorbance spectra. Leaves of *C. blumei* had widely varying Chl contents and exhibited variable leaf optical properties of transmittance and reflectance (Fig. 1). In the blue range, reflectance of leaves was high and widely varied among Chl contents in all leaves. In contrast, transmittance of all leaves converged to a narrow range of around 0.03 with a smaller variability. In the green range, the transmittance and reflectance showed higher variability in green parts of leaves compared to red parts. However, in the red range, the reflectance showed higher variability in red parts of leaves compared to green parts. Therefore, the transmittance and reflectance of green parts of leaves (Fig. 1A,C) was strikingly higher than that of red parts (Fig. 1B,D) at 500–600-nm wavebands. In the visible light range (400–700 nm), the transmittance and reflectance gradually increased with a decrease in the Chl content in green parts of leaves. In the red light range, the transmittance and reflectance gradually increased with a decrease in the Chl content in red parts of leaves. Nevertheless, the reflectance and transmittance of leaves at 690–850-nm wavebands did not show any trend between spectra and Chl content in leaf colors.

Relationships among Chl, Car, and Ant: Relationships among Chl (*a+b*) vs. SPAD, Car, Car/Chl, Ant, Chl *a*, and Chl *a/b* in green and red portions of leaves are presented in Fig. 2. Regression analyses showed that Chl *a*, Car, and SPAD were significantly and positively correlated with Chl (*a+b*) at $r = 0.99$ – 0.996 , 0.855 – 0.882 , and 0.962 – 0.985 in green and red parts of leaves, respectively (Fig. 2A–C). However, calibration curves of Chl *a/b* and Car/Chl were nonlinear to Chl (*a+b*) (Fig. 2D,E). In particular, the

Fig. 1. Changes in the spectral reflectance and transmittance with different total Chl [*i.e.*, Chl (*a+b*)] contents in green sectors (A, C) and red sectors (B, D) of leaves of *Coleus blumei* plants.

Fig. 2. Relationships between Chl (*a+b*) content and Chl *a* (A), carotenoids (Car) (B), soil-plant-analysis-development (SPAD) (C), Chl *a/b* (D), Car/Chl (E), and anthocyanins (Ant) (F) of red and green parts of leaves of *Coleus blumei* plants. Each symbol represents the average of one plant with three leaves, and 16–30 plants were randomly selected from each treatment. Each Chl pigment index was calculated using red (black circle) and green (white circle) leaf data ($n=48-90$) from the model's validation datasets. The correlation coefficient (r) and significance of the regression are shown (* – $p<0.05$; *** – $p<0.001$).

relationship of Chl (*a+b*) and Chl *a/b* in red parts of leaves was weak ($r=0.395$, $p<0.05$). Ant contents in green parts of leaves were undetectable, and a no distinct relationship was observed between Ant and Chl (*a+b*) in red parts of leaves, indicating that the red parts mainly resulted from the Ant accumulation (Fig. 2F). In addition, the analysis of photosynthetic pigments showed differences in amounts of Chl and Car, as well as in their ratios within variegated leaves of the tested plants. Green areas had

much higher ratios of Chl *a/b* and Car/Chl than the red areas. Car contents in red and green parts of leaves did not significantly differ, and changes in the Car/Chl ratio were therefore attributed to the total Chl content in the leaves.

Correlations between Chl contents and ChlF measurements at different leaf parts in response to PPFD treatments: Responses of F_0 , F_m , and F_v/F_m to Chl (*a+b*) in red and green parts of leaves of *C. blumei* plants

under different PPFDs showed that PPFD effects on the ChlF parameter differed (Fig. 3A,H,O). However, responses of F_0' , F_m' , and F_v'/F_m' to Chl ($a+b$) in red and green parts of leaves of *C. blumei* plants showed different ChlF parameters under different PPFDs (Fig. 3B–G, 3I–N, and 3P–U). There were strongly significant correlations ($r = 0.772$ – 0.843 , $p < 0.001$) among Chl ($a+b$) and F_0 (or F_0') in response to all PPFD levels and the dark (Fig. 3A–G). Trends of F_m (or F_m') against Chl ($a+b$) in both red and green parts of leaves were similar to those of F_0 (or F_0') in response to PPFD (Fig. 3H–N). A high PPFD-induced decreases in F_m' , and the value of

F_m (or F_m') was found to remain in a narrow range in both green and red parts of leaves. Compared to low PPFDs (*i.e.*, 0 – $400 \mu\text{mol m}^{-2} \text{s}^{-1}$), high PPFD (*i.e.*, $1,200$ and $2,000 \mu\text{mol m}^{-2} \text{s}^{-1}$) yielded relatively lower values of F_m' from 10 to 50 readings of Chl ($a+b$). Nevertheless, F_v'/F_m' was neither strongly correlated with Chl ($a+b$) at PPFD of 200 and $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($r = 0.457$ – 0.561 , $p < 0.05$ and 0.01 , respectively; Fig. 3P,Q), nor correlated with Chl ($a+b$) at PPFD of 0 , 800 , $1,200$; and $2,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ and the dark (Fig. 3O,R–U). In general, F_v'/F_m' in dark-adapted red parts of leaves was higher than that of green parts of leaves (Fig. 3U). Moreover, under different PPFD

Fig. 3. Relationships between Chl ($a+b$) concentrations and Chl fluorescence (ChlF) parameters of the minimum fluorescence yield in a dark-adapted state (F_0) (A), the actual level of fluorescence during illumination (F_0') (B–G), maximum fluorescence yield in a dark-adapted state (F_m) (H), the maximal level of fluorescence during illumination (F_m') (I–N), the maximum photochemical quantum yield (F_v/F_m) (O), and the actual PSII efficiency (F_v'/F_m') (P–U) of red and green parts of leaves of *Coleus blumei* plants with respect to different light intensity treatments (PPFD of 0, 200, 400, 800; 1,200; and 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for 20 min, and dark conditions (20 min). Each symbol represents the average of one plant with three leaves, and 16 plants were randomly selected from each treatment. Each Chl pigment index was calculated using red (black circle) and green (white circle) leaf data ($n = 48$) from the model's validation datasets. The correlation coefficient (r) and significance of the regression are shown (* – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$; ns – insignificant).

treatments, the F_v/F_m' values of leaves with different Chl contents were similar.

The relationship between the PPFD and F_0' slope showed no detectable difference in any leaves (Fig. 4A). Nevertheless, the regression analysis showed that the F_0' intercept and F_m' slope and intercept were significantly correlated with PPFD, with $r = 0.986$, 0.996 , and 0.993 ($p < 0.001$), respectively, and a significant decrease in the PPFD level was also observed for the F_0' intercept and F_m' slope and intercept (Fig. 4B–D). PPFD variations in response to NPQ at red and green parts of leaves are demonstrated in Fig. 5. Insignificant correlations were detected between NPQ and Chl ($a+b$) at all PPFD treatments. NPQ values of leaves with the higher Chl contents were almost the same as those of leaves with lower Chl contents in response to different PPFD treatments. High PPFD (Fig. 5E) resulted in higher NPQ compared to low PPFD (Fig. 5A,B).

Fig. 4. Photosynthetic photon flux density (PPFD) treatment (0, 200, 400, 800; 1,200; and 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for 20 min in response to changes in minimum fluorescence yield in a light-adapted state (F_0') slope (A), maximum fluorescence yield in a light-adapted state (F_m') slope (B), F_0' intercept (C), and F_m' intercept (D) from all leaves of tested plants. The correlation coefficient (r) and significance of the regression curve are shown (** – $p < 0.01$; *** – $p < 0.001$; ns – insignificant).

Discussion

The reflectance and transmittance of green leaves was different from that of red leaves at 400–700 nm (Fig. 1), and the reflectance and transmittance decreased when both Chl and Car contents were enhanced in green leaves (Fig. 2B). In red leaves, the reflectance and transmittance of 550 nm was much lower compared to the green leaves, but it peaked around 650 nm, suggesting that the light absorbance of Ant in red leaves resulted in the lower reflectance and transmittance at 550 nm (Merzlyak *et al.* 2003, Gitelson and Merzlyak 2004). Although changes in variegated leaf colors are complex, the light absorbance increased with the enhanced Chl content, especially in green portions of leaves. However, the light absorbance of multicolored leaves was not affected by the increasing Chl content at 690–850 nm (Carter and Young 1993). This study provides deeper insights into the interception of light by photosynthetic and photoprotective pigments as a function of light intensity conditions, which is important for plant biology as well as knowledge-driven selection of spectral bands for non-

invasive pigment estimation models.

Fig. 2 illustrated that red and green leaf sectors of *C. blumei* plants had wide variations in pigment contents, clearly suggesting that SPAD can be used as more precise metrics for developing leaf Chl estimation algorithms due to high correlations. In addition, the high SPAD of leaves indicated that more leaves had higher Chl concentrations. In our study, the Chl a/b ratio in the red parts of leaves was lower than that of green parts, suggesting that it could be an indicator for estimating relative photosystem stoichiometry (Pfannschmidt *et al.* 1999). Plants grown under controlled ozone treatments appeared to synthesize more Chl a because it has a wider spectrum compared to that of Chl b , and Chl a is the molecule that makes photosynthesis possible (Calatayud *et al.* 2004). Furthermore, a change in the Chl a/b ratio is usually positively correlated with variations in PSII light-harvesting antenna size (Leong and Anderson

1984). In our study, SPAD measurements were performed at 650 and 940 nm, *i.e.*, at wavelengths not absorbed by Ant, whose peak absorbance is at 500–550 nm (Landi *et al.* 2015). Moreover, no correlation of Ant with Chl ($a+b$) in red parts of leaves was observed. This was because the color of all adaxial parts was red, and content of Ant in red leaf was not affected by the Chl content.

Measuring the yield of ChlF gives specific information about the photochemical efficiency and heat dissipation. ChlF components can be used to measure different functional levels of photosynthesis, and changes in ChlF can be also used to quickly assess plant physiological responses during stress (Laing *et al.* 2000). F_v/F_m (or F_v'/F_m') shows the actual photochemical ability of PSII under light conditions and has a linear relationship with the CO_2 fixation rate (Cui *et al.* 2006). Studies of the effects of light intensities on ChlF components of *C. blumei* plants in regard to their ability of photosynthetic activity are scarce. F_0 (or F_0') and F_m (or F_m') indices showed gradual increases as Chl ($a+b$) increased at different PPFDs. Significant correlations were observed between PPFD and the F_0 intercept

Fig. 5. The correlation coefficient between Chl (*a+b*) concentrations and the nonphotochemical quenching (NPQ) index was calculated under PPFD treatments of 200 (A), 400 (B), 800 (C); 1,200 (D); and 2,000 (E) $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 20 min.

and between the F_m slope and intercept in *C. blumei* plants. Consequently, F_0 and F_m are more comprehensively applicable to nondestructive estimates of Chl contents of plant leaves. F_0 and F_m indices respond to slight changes in Chl and also give accurate estimates of changes in the photosynthetic flux of *C. blumei* canopies, since Chl pigments are closely related to PSII photochemical efficiency and dissipation of light energy not used in photosynthesis (Frank *et al.* 1994, Gitelson and Merzlyak 1994, Björkman and Demmig-Adams 1995). The different colors of leaves exhibit their abilities and specificities of ChlF in response to PPFD. As a result, different leaf colors show different responses, and they can be used as a PPFD-plant model to investigate pigment composition under PPFD exposure. Moreover, red and green parts of leaves of *C. blumei* plants developed at a given PPFD could be separated on the basis of F_0 and F_m at a given light intensity, indicating that ChlF can monitor PS efficiency of red and green leaves under various PPFDs.

In our study, Chl (*a+b*) was positively and significantly correlated to F_0' (Fig. 3G) and F_m' (Fig. 3N) of red and

green leaves under dark conditions for 20 min. However, insignificant difference was found between Chl (*a+b*) and F_v'/F_m' (Fig. 3U) of red and green leaves in response to dark for 20 min. The slope of F_0 (F_0') against Chl (*a+b*) in both red and green leaves was between 166.21–107.29 (Fig. 3A–F) which did not change too much as PPFD increased. Nevertheless, the slope of F_m (F_m') against Chl (*a+b*) in both parts of leaves declined from 710.67 to 139.88 as PPFD increased (Fig. 3H–M), indicating that F_m' was less affected by Chl (*a+b*) content under high PPFDs (*i.e.*, 800–2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Furthermore, exposure to high PPFDs might have caused decreases in F_v'/F_m' values of both green and red parts of leaves in *C. blumei* plants. All plants showed low F_v'/F_m' of 0.2–0.7 with high PPFDs. Exposure of plant leaves to illumination that exceeds their photosynthetic capacity leads to photoinhibition of the electron transport system. The susceptibility of photosynthesis to photoinhibition strongly depends on growth light intensities to which the plant is exposed. The excess energy in PSII increases, leading to increase of F_0 and decrease of F_m values due to greater photoinhibition when plants are exposed to higher PPFDs. Lower F_v'/F_m' values might be obtained in a plant at higher PPFDs. Experiencing higher PPFDs destabilizes the PSII, resulting in a separation between LHCII and PSII reaction centers that increase F_0 and F_m at different rates (Makarenko *et al.* 2016).

Although F_0 , F_m , F_v/F_m , and NPQ were related through photochemical quenching, F_v/F_m and NPQ were less sensitive to Chl (*a+b*) than were F_0 and F_m in response to PPFD. Under different PPFD treatments, F_v/F_m (F_v'/F_m') and NPQ of leaves of *C. blumei* with higher Chl contents were almost the same as those of leaves with lower Chl contents, suggesting that under visible light illumination, both green and red parts of leaves with lower Chl contents avoid excessive energy absorption through higher reflectance and transmittance, and respond to higher PSII photochemical efficiencies with the higher Chl content. Responses of F_v/F_m (F_v'/F_m') and NPQ to light intensities indicated that the tested plants appeared to be adapted to low-light intensities or were sensitive to high-light intensity conditions, and often suffered serious photoinhibition and photodamage. Protective mechanisms in *C. blumei* plants should prevent leaves from excessive reduction of PSII acceptors in plants.

No significant relationships between NPQ and Chl contents of the tested plant leaves were observed, suggesting that thermal energy dissipation did not take place in antennae.

The PPFD influences the photosynthetic potential of *C. blumei* plants and different responses to Chl (*a+b*) contents in red and green parts of leaves depend on the red and green parts of the leaves. ChlF parameters were PPFD-specific and were not expressed solely in response to increasing excess light energy. In order to characterize the photosynthetic capacity of plants, the light-response curve and the consequent base points produce very important parameters. However, steady-state conditions, which are strictly required, do not occur in nature. So, instantaneous light-response curves, with quick alterations in the PPFD but with a clear risk of encountering non-steady-state

conditions, can be used as an alternative way to achieve insights into light-response characteristics of plants in the field. Our findings suggest that F_0 and F_m were strongly correlated with Chl contents in response to PPFD, and may be used as criteria for differentiating between plants tolerant to high light intensities, and can provide a quantitative assessment for the early screening of high PPFD-tolerant *C. blumei* plants to overcome high light intensity stress. In addition, F_m and F_v/F_m indices can be used as indicators to characterize the physiology of *C. blumei* plants in response to PPFD. In short, these higher ChlF values under high PPFDs can be used to select for the maintenance of photosynthetic capacity with high PPFDs by plant breeding. For instance, the average time required to measure F_0 from a pre-dark-adapted sample is only 1 s. This means that many hundreds of individual plants can be screened per day, providing ample opportunity for the discovery of individuals that manifest quality indicators and exhibit greater seedling quality. Not only can simple evaluations of photosynthesis be made, but the relationships among photosynthetic efficiency, heat dissipation, and fluorescence can also be assessed. Improved high-PPFD tolerance of the photosynthetic apparatus of *C. blumei* plants may, therefore, substantially contribute to improving the performance of the crop in light-intense regions by increasing early vigor and extending the culture period.

We observed that a large number of variegated leaves exist during florescence, but after florescence, the variegated leaves do not occur on new branches. The photosynthetic function of variegated leaves was retained in the early stages of leaf development. More important is that the number of flowers significantly decreased when girdling occurred between variegated leaves and flowers. Therefore, in practice, maintaining or strengthening the photosynthetic capacity of red and green parts of leaves during variegated leaf development may be of great significance for *C. blumei* flowering. Therefore, in field cultivation, choosing a suitable region or using artificial shading should be considered in order to avoid photo-inhibition resulting from high-light intensity exposure. More opportunities exist for modifying Chl contents including broader interest features (*e.g.*, branch and/or node numbers) to floriculture crop producers or to emphasize important traits.

In conclusion, ChlF components were used to measure indirectly the different functional levels of photosynthesis. We showed that different colors of leaves displayed variations in their photosynthetic apparatus associated with Chl contents in a PPFD response. Upon exposure to high PPFDs, F_m' and F_v'/F_m' of Chl contents in leaves were strongly lowered. In addition, both F_0 and F_m can be used to assess Chl contents due to their high correlations, and can prove to be useful in further physiological studies on *C. blumei* plants with high PPFD tolerance. These data reflect the morphological and physiological attributes that contribute to our perception of crop quality and subsequent growth in outdoor planting sites.

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