DOI: 10.1007/s11099-016-0225-7 PHOTOSYNTHETICA 54 (3): 331-339, 2016

Photosynthetic activity of variegated leaves of *Coleus* × *hybridus* hort. cultivars characterised by chlorophyll fluorescence techniques

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Abstract

Different pigments often occur together and affect photosynthetic characteristics of the respective leaf portions. In this study, photosynthetic activity in variegated leaves of five cultivars of the ornamental and medicinal plant, *Coleus* × *hybridus* hort., was estimated by image analysis and point data measurements of major chlorophyll (Chl) fluorescence parameters and related to the amount of photosynthetic pigments measured with a Chl meter or spectrophotometrically in leaf extracts. Significant differences in Chl and carotenoid (Car) contents were noticed among differentially pigmented sectors of a leaf and among the cultivars. Although the higher Chl concentration was noticed in purple parts compared to green parts of the leaves, the values of minimal and maximal fluorescence yield at the dark- and light-adapted state (F_0, F_m, F_0', F_m', respectively) were a little lower than those in the green sectors, indicating photoprotective effects provided by anthocyanins and Car, more abundant in the red parts. The lowest Chl and Car content was detected in creamy-yellow and pink sectors and this contributed to low F_0, F_m, and F_m', maximal quantum yield of PSII photochemistry, and nonphotochemical and photochemical quenching but high PSII maximum efficiency and effective quantum yield of PSII photochemistry. Both methods of Chl fluorescence analysis revealed heterogeneity in capture, transfer, and dissipation of excitation energy but Chl fluorescence imaging was more suitable in examining very narrow pigmented leaf areas.

Additional key words: carotenoids; chlorophyll imaging; pigments.

Introduction

Coleus × *hybridus* hort. [syn. *Coleus blumei* Benth.; *Plectranthus scutellarioides*] is an ornamental garden plant. There are more than 500 varieties and cultivars of the species cultivated all over the world (Lebowitz 1985, Soni and Singhai 2012, Osman 2013). In recent years, it has become more popular due to a large number of cultivars with highly decorative and variegated leaves. The colours of *Coleus* foliage range from green, yellow, orange, and brown through purple to red and pink (Chung and Choi 2008, Osman 2013). Moreover, the leaves may differ in patterns (*e.g.* spots, flecks, mosaics), number of coloured sectors (*e.g.* bicoloured, tricoloured), shapes (*e.g.* narrow, wide, round, ovate), size, and even fancy edges. In addition to their decorative value, the plants may have medicinal properties thank to the presence of rosmarinic acid, an important phenolic compound exhibiting antiviral, antibacterial, anti-inflammatory, and antioxidant activity (Park *et al.* 2008).

Coleus leaf colour depends on the presence of pigments including Chls, Cars, flavonoids, anthocyanins, and betalains (Alkema and Seager 1982, Tanaka *et al.* 2008). In higher plants, Chl *a* and *b* and Cars are the pigments responsible for light harvesting, while Chl *a* passes absorbed light energy into the electron transport chain. Cars are accessory photosynthetic pigments involved in direct light harvesting (Młodzińska 2009, Khoo *et al.* 2011) and photoprotective functions (Bartley and Scolnik 1995, Yamamoto and Bassi 1996, Maxwell and

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Received 10 April 2015, *accepted* 23 March 2013, *published as online-first* 12 April 2016.

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Abbreviations: CA – Camaroon; Car(s) – carotenoid(s); CF – chlorophyll fluorescence; Chl – chlorophyll; DD – Dappled Down; F_0, F_m – minimal and maximal fluorescence yield at the dark-adapted state; F_0', F_m' – minimal and maximal fluorescence yield at the light-adapted state; F_t – steady-state fluorescence yield; F_v – variable fluorescence at the dark-adapted state; F_v/F_m – maximal quantum yield of PSII photochemistry; FM – fresh mass; SD – standard deviation; NPQ – Stern-Volmer nonphotochemical quenching coefficient; NR – Neon Rose; q_P – photochemical quenching coefficient; RU – Ruby; SR – Sunlover Red Ruffles; Φ_{PSII} – effective quantum yield of PSII photochemistry.

Aknowledgements: The study was supported by the University of Agriculture in Krakow, DS 3113.

Johnson 2000). In autumn, they are also responsible for yellow colour of leaves normally masked by Chls. Based on their structure, Cars are divided into two major groups: orange-red carotenes, composed exclusively of carbon and hydrogen, and yellow-orange xanthophylls that also contain oxygen atoms (Goodwin 1980, Młodzińska 2009).

Anthocyanins are nonphotosynthetic pigments, but numerous studies have pointed out their photoprotective role. They are accumulated in vacuoles of adaxial epidermal or palisade parenchyma cells and act as a photoshield, especially in developing and senescing leaves as well as during exposure to intense light in combination with environmental stresses, such as low temperature or low nutrient availability (Gould 1995, Henry *et al.* 2012, Liakopoulos and Spanorigas 2012, Logan *et al.* 2015).

Noninvasive and rapid Chl fluorescence (CF) techniques offer highly accurate measurements that illustrate photosynthetic performance of plants. CF analysis is often used to investigate a plant response to various abiotic (in particular, high-light intensity, heavy metals, drought, low temperature) and biotic stresses (*e.g.* herbivore or pathogen attack), and to assess plant stress tolerance (Nedbal *et al.* 2000, Pineda *et al.* 2008, Takayama *et al.* 2011, Borek *et al.* 2012, Dos Anjos *et al.* 2012, Muniz *et al.* 2014). The aim of this study was to determine the actual state of photosynthetic apparatus in differentially pigmented leaves. As fluorescence imaging also offers the possibility to screen gradients and irregularities of CF signatures over the whole leaf area (Lichtenthaler *et al.* 2000, Borek *et al.* 2012), its use seemed to be justified in the research on differentially pigmented leaves.

Given the specificity of multicoloured leaves of *Coleus* × *hybridus* hort. cultivars, our goal was to investigate the mechanisms of capture, transfer, and dissipation of excitation energy within an individual leaf with green, red, pink, and creamy-yellow coloration. This was achieved by employing CF imaging and point data measurements.

Materials and methods

Plant material and its detailed characteristics: Plants of five variegated *Coleus x hybridus* hort. cultivars: 'Dappled Down' (DD), 'Camaroon' (CA), 'Ruby' (RU), 'Sunlover Red Ruffles' (SR), and 'Neon Rose' (NR) were used in all the experiments presented in this study. DD plants had medium-large, green leaves with creamy-yellow or white sectors near the veins. The edges of their foliage were surrounded by arrow-shaped tips. The specimens of CA had medium-size leaves with creamy centres and wide, green outer margins. Plants of RU had purple foliage with narrow pink centres near the main vein and large, green borders. SR specimens were similar to RU in their pigmentation, although their pink sectors were larger than those of RU, and the green fragments formed very narrow outline located mainly at the base of the leaf and/or on the arrow-shaped tips. The leaves of NR had purple centres surrounded by a wide, two-tone border of bright green and yellow (Fig. 1).

Growth conditions: The plants were produced from cuttings and grew in pots filled with a commercial garden soil under natural light [14-h day with a natural light intensity of 400–500 μmol(quanta) m^{-2} s^{-1}], in a greenhouse. The temperature was 20–23ºC (day) and 17–20ºC (night), and the relative humidity (RH) was approx. 30%. All specimens were free from any disease or nutrient shortage.

Measurements and analyses: All the measurements and analyses were performed on the leaf areas differing in pigmentation, in 5–7 biological replicates (one plant was used for all types of analyses, and the number of plants was 5–7). Green, creamy-yellow, purple, pink, and yellow-green sectors were studied. Photosynthetic activity was estimated based on the analysis of images and numeric data of major CF parameters. Physiological background to these data were the analyses of the main photosynthetic pigments.

Chl content: Relative Chl content was measured photometrically with a portable Chl meter (*Cl-01*, *Hansatech,* UK), and determined using dual wavelength optical absorbance (620 and 920 nm). The results were expressed as "chlorophyll index" or "greenness index" (Cassol *et al.* 2008).

Photosynthetic pigment content assay: Chl *a*, Chl *b*, and Car content assays were performed according to Lichtenthaler (1987). Tissue samples of the same pigmentation were cut with a razor from mature (developed but not senescing) variegated leaves, weighed, and frozen in liquid nitrogen. The samples (50 mg) were homogenized in 5 ml of 96% ethanol and stored on ice, in darkness. The homogenates were centrifuged at 4ºC for 5 min (3,000 × *g*), and the supernatants were used for the spectrophotometric analysis (*Rayleigh UV-1800 UV/VIS,* China). The absorbance was determined at 649 nm, 664 nm, and 470 nm.

Chl *a* fluorescence imaging: Fluorescence images of the leaf surface were obtained using Chl fluorescence imaging system (*FluorCam 701 MF*, *PSI*, Brno, Czech Republic). Leaves of different plants ($n = 5–7$) of each cultivar were cut and immediately placed in darkness inside a measuring chamber. CF images were captured at 22°C according to an experimental protocol consisting of 20 min dark adaptation and measurements of F_0 (minimal fluorescence – CF yield after dark adaptation, when all of the PSII reaction centres and electron acceptor molecules are fully oxidised), and F_m [maximum fluorescence yield in dark-adapted samples measured after light-saturating pulse of about 1,800 μmol(photon) m^{-2} s^{-1} provided by halogen light source]. Then, orange actinic light (620 nm) of 1,000 μmol(photon) $m^{-2} s^{-1}$ was switched on for 400 s. Saturating pulses [approx. 1,800 μmol(photon) $m^{-2} s^{-1}$] were repeated every 25 s, which was sufficient to stabilize steady-state fluorescence yield (F_t), and F_m' (maximum fluorescence yield in light-adapted samples) was measured during the last pulse. F_0' (minimal fluorescence of light-adapted leaves) was recorded after 3 s of far red light illumination after the actinic light had been switched off. The images were processed by the internal software of the equipment (*Fluorcam v. 5.0*). Maximum efficiency of PSII photochemistry was calculated as F_v/F_m, where $F_v = F_m - F_0$. PSII antenna trapping efficiency was calculated as F_v'/F_m', where $F_v' = F_m' - F_0'$. Effective quantum yield of PSII photochemistry (Φ_{PSII}) was calculated according to Maxwell and Johnson (2000) as $(F_m' - F_t)/F_m'$, where F_t = the steady state fluorescence under actinic light prior to a saturating pulse. Photochemical quenching coefficient was calculated according to Schreiber *et al.* (1986): $q_P = (F_m' - F_t)/(F_m' - F_0')$. Stern-Volmer nonphotochemical quenching was expressed as $NPQ = (F_m - F_m')/F_m'$ (Bilger and Björkman 1991). The most representative images were presented.

Chl *a* fluorescence point data measurements: To confirm the results of CF imaging, numeric CF parameters were measured by *FMSII* pulse-amplitude modulated fibre-optic system (*Hansatech*, Kings Lynn, UK). The calculations for the parameters were the same as in the case of CF imaging. Leaf clips (with a 5-mm diameter hole) were fastened on the leaf areas with the same pigmentation on the same plants used for CF imaging. After 20 min of dark adaptation, F_0 was measured and a saturating-light pulse [10,000 μmol(photon) $m^{-2} s^{-1}$ for 0.9 s] was used to determine F_m. Next, the leaf was irradiated with the actinic light [1,500 μmol(photon) $m^{-2} s^{-1}$] for 270 s, which was sufficient to measure steady state fluorescence (F_t). Then, the saturating-light pulse was used again to determine the F_m'. After turning the actinic light off, F_0' was measured by immediately irradiating the leaf for 3 s with a far red emitting diode (radiation of about 15 W m^{-2}). The source of a modulation beam (duration pulses 1.8 μs, 2.3 kHz) was an amber LED [peak wavelength 594 nm, PFD *ca.* 0.05 μmol(photon) $m^{-2} s^{-1}$]. Actinic and pulse irradiations were provided by a halogen lamp (20 W). The signal detector was a *PIN* photodiode with a long-pass filter (>700 nm), and the sampling rate was 10–20 kHz (depending on the instrument mode).

Statistical analysis: All the measurements were performed on 5–7 replicates (n = 5–7). The data were subjected to an analysis of variance (*ANOVA*). Differences between means were compared using the *Student*'s *t*-test (for two means) and *Duncan*'s test (for three means). The differences were considered significant if P was at least ≤ 0.05. All the analyses were carried out using *Microsoft Office Excel 2003* and the *Statistica 9.0*.

Results

The analysis of the photosynthetic pigments showed differences in the amount of Chls and Cars, as well as in their ratios within variegated leaves of *Coleus* plants of the different cultivars (Table 1). As expected, the green parts of the DD and CA leaves had much higher content of total Chl than the creamy-yellow areas. It was confirmed by both leaf greenness index (measured with a Chl meter) and the spectrophotometric analyses, although the difference range reflected by the Chl index was much greater than the values of spectrophotometrically measured Chl *a*+*b* (Chl index: the values 31–140 times higher, spectrophotometric assays: 3–4 times higher in favour of the green areas). The Chl *a*/*b* ratio in the creamy-yellow sectors was approximately five times lower compared to the green parts, and this was caused by a greater amount of Chl *b* in relation to Chl *a*. Besides, the total Car content was below detection limits.

Interestingly, the purple areas of RU and SR leaves contained 2–4 times greater amount of total Chls than that of the green ones, as revealed by both methods (Table 1). The differences in the Chl index were larger than those obtained from the extraction method but not as large as in the case of green and creamy-yellow cultivars. In the pink areas, Chls were present at very low concentrations (extremely low when the Chl index was measured), the total Car content was undetectable, and similarly to the creamy-yellow areas (of DD and CA), the pink ones had a higher concentration of Chl *b* than Chl *a*.

In the purple leaf areas of NR plants, the Chl index was four times higher than in the yellow-green ones, which was partially confirmed by the spectrophotometric analyses and was due to the higher amount of Chl *a* in the purple areas (Table 1).

The images of minimal fluorescence yield for dark- and light-adapted leaves (F_0 and F_0', respectively) in the DD and CA plants showed significant differences between the green and creamy-yellow sectors (Fig. 1), and this was confirmed by the numeric data (Table 2). These parameters were very low in the creamy parts when compared to the green ones. Additionally, the images of F_0' revealed that the values of this parameter were heterogeneously distributed within the green sectors of the CA leaves, with the highest intensity recorded near the top of the leaf (Fig. 1). Similar results were obtained for F_m and F_m' (Table 2, Fig. 1).

The images of F_v/F_m in the DD and CA leaves revealed lower values in the creamy-yellow sectors than in the green ones (Fig. 1). In the case of DD, the CF imaging results were not confirmed by the point data measurements as the

Table 1. The Chl index and the content of photosynthetic pigments and Chl *a/b* ratio in variegated leaves of plants of *Coleus* cultivars. Mean ± SD, $n = 5–7$. The means labelled with *the same letter* are not significantly differentiated ($P<0.05$; paired *Student*'s *t*-test or *Duncan*'s test). DD – Dappled Down, CA – Camaroon, RU – Ruby, SR – Sunlover Red Ruffles, NR – Neon Rose, [1] – below detection limits.

Cultivar	Leaf colour	Chl index [relative unit]	Chl (*a*+*b*) [$\mu g\ g^{-1}$(FM)]	Chl *a* [$\mu g\ g^{-1}$(FM)]	Chl *b* [$\mu g\ g^{-1}$(FM)]	Car [$\mu g\ g^{-1}$(FM)]	Chl *a*/*b* [$\mu g\ g^{-1}$(FM)]
DD	Green	2.81 ± 0.54^{a}	332.72 ± 42.98^{a}	232.37 ± 32.50^{a}	100.36 ± 11.26^{a}	56.38 ± 7.02	2.31 ± 0.13^{a}
	Creamy-yellow	0.02 ± 0.03^{b}	78.78 ± 27.85^{b}	23.85 ± 9.11^{b}	54.93 ± 18.84^{b}	$\approx 0^{1}$	0.43 ± 0.03^{b}
CA	Green	7.74 ± 2.26^{a}	284.32 ± 31.54^{a}	207.63 ± 21.27^{a}	76.69 ± 11.13^{a}	54.08 ± 5.21	2.72 ± 0.19^{a}
	Creamy-yellow	0.25 ± 0.24^{b}	86.03 ± 59.18^{b}	35.56 ± 15.30^{b}	50.47 ± 30.98^{b}	$\approx 0^{1}$	0.70 ± 0.12^{b}
RU	Green	2.17 ± 0.32^{b}	351.55 ± 28.31^{b}	245.47 ± 19.95^{b}	106.08 ± 9.76^{b}	59.83 ± 6.09^{a}	2.32 ± 0.13^{a}
	Purple	8.06 ± 0.67^{a}	446.61 ± 40.52^{a}	295.48 ± 24.56^{a}	151.13 ± 16.08^{a}	62.01 ± 6.18^{a}	1.96 ± 0.05^{b}
	Pink	0.28 ± 0.12^{c}	67.18 ± 1.32^{c}	26.76 ± 2.99^{c}	40.42 ± 2.30^{c}	$\approx 0^{1}$	0.67 ± 0.11^{c}
SR	Green	3.44 ± 0.81^{b}	290.91 ± 49.54^{b}	198.49 ± 40.34^{b}	92.42 ± 9.77^{b}	47.26 ± 11.23^{b}	2.13 ± 0.25^{a}
	Purple	6.99 ± 0.37^{a}	375.34 ± 24.37^{a}	253.27 ± 11.47^{a}	122.07 ± 16.81^{a}	57.73 ± 4.93^{a}	2.11 ± 0.29^{a}
	Pink	0.04 ± 0.04^{c}	62.11 ± 18.80^{c}	22.12 ± 8.02^{c}	39.99 ± 12.36^{c}	$\approx 0^{1}$	0.56 ± 0.16^{b}
NR	Yellow-green	0.84 ± 0.38^{b}	212.68 ± 66.96^{a}	124.93 ± 18.26^{b}	81.76 ± 50.93^{a}	36.03 ± 3.68^{a}	1.54 ± 0.57^{a}
	Purple	3.27 ± 0.74^{a}	292.63 ± 14.93^{a}	177.82 ± 11.09^{a}	91.80 ± 1.94^{a}	41.00 ± 5.14^{a}	1.93 ± 0.17^{a}

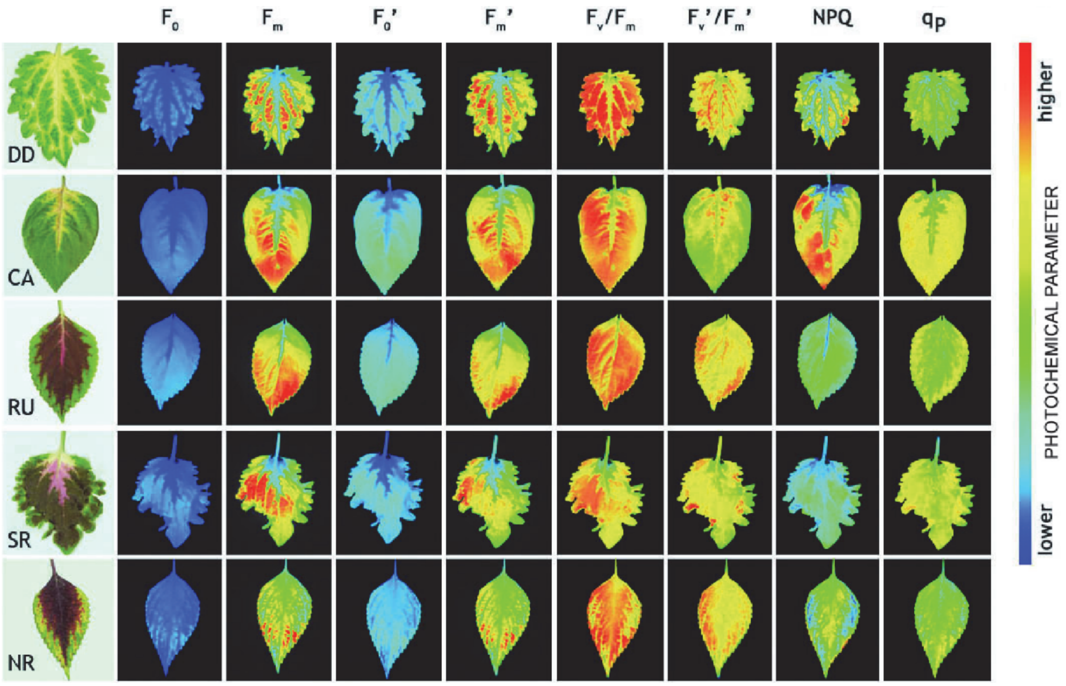

Fig. 1. Chl fluorescence images of F_0, F_m, F_0', F_m', F_v/F_m, F_v'/F_m', NPQ, and q_P in variegated leaves of *Coleus* × *hybridus* hort. All images are representative for all studied plants ($n = 5$). DD – 'Dappled Down', CA – 'Camaroon', RU – 'Ruby', SR – 'Sunlover Red Ruffles', NR – 'Neon Rose'. Coloured bar parallel to the images shows the range of values and how they mapped to the colour palette.

difference was statistically insignificant (Table 2). On the other hand, the image analysis of F_v'/F_m' in the DD and CA plants showed that higher PSII antenna trapping efficiency was detected mainly on the border between the green and yellow-pigmented sectors (Fig. 1).

In the case of NPQ, distinct differences in CF between the green and creamy-yellow areas were noticed in the leaves of DD and CA (Fig. 1, Table 1). In the CA leaves, the NPQ image resembled that of F_m and F_m', revealing heterogeneity of the parameters within the green area. q_P in the DD leaves was low, as indicated by both CF imaging and measurements, especially in the case of the creamy-yellow sectors (Fig. 1, Table 1). q_P in the CA leaves was higher than in DD ones, and higher in the green parts than in the creamy-yellow areas (Fig. 1, Table 2). The green sectors of the CA leaves showed also the highest NPQ and

Table 2. Chlorophyll *a* fluorescence parameters of variegated leaves of *Coleus* × *hybridus* hort. Mean ± SD, $n = 5–7$. The means labelled with *the same letter* are not significantly differentiated ($P<0.05$; paired *Student*'s t-test or *Duncan*'s test). DD – 'Dappled Down', CA – 'Camaroon', RU – 'Ruby', SR – Sunlover Red Ruffles, NR – 'Neon Rose'. In the case of SR plants it was impossible to pin the fluorescence clip to the narrow green leaf fragments embedded into the green ones. nm – not measurable. F_0, F_m – minimal and maximal fluorescence yield at the dark-adapted state; F_0', F_m' – minimal and maximal fluorescence yield at the light-adapted state; F_v – variable fluorescence at the dark-adapted state; F_v/F_m – maximal quantum yield of PSII photochemistry; Φ_{PSII} – effective quantum yield of PSII photochemistry. Means ± SD.

Cultivar	Leaf colour	Fluorescence parameter								
		F_0	F_m	F_0'	F_m'	F_v/F_m	F_v'/F_m'	NPQ	q_P	Φ_{PSII}
DD	Green	317.2 ± 47.9^{a}	1,934.4 ± 196.4^{a}	261.0 ± 54.9^{a}	703.2 ± 168.7^{a}	0.840 ± 0.007^{a}	0.724 ± 0.012^{a}	1.628 ± 0.229^{a}	0.102 ± 0.040^{a}	0.064 ± 0,024^{b}
	Creamy-yellow	12.0 ± 6.6^{b}	90.3 ± 66.3^{b}	15.3 ± 0.6^{b}	55.7 ± 4.6^{b}	0.851 ± 0.033^{a}	0.626 ± 0.018^{b}	0.621 ±0.035^{b}	0.025 ±0.001^{b}	0.511 ± 0.168^{a}
CA	Green	307.7 ± 24.8^{a}	1,796.1 ± 94.4^{a}	229.3 ± 19.4^{a}	500.7 ± 58.6^{a}	0.828 ± 0.011^{a}	0.540 ± 0.021^{b}	2.623 ± 0.386^{a}	0.321 ± 0.111^{a}	0.125 ± 0.026^{b}
	Creamy-yellow	27.8 ± 5.2^{b}	124.8 ± 30.9^{b}	33.6 ± 5.8^{b}	101.4 ± 22.4^{b}	0.763 ± 0.023^{b}	0.666 ± 0.021^{a}	0.232 ± 0.188^{b}	0.213 ± 0.047^{b}	0.212 ± 0.009^{a}
RU	Green	290.4 ± 26.4^{a}	1,620.4 ± 150.8^{a}	218.6 ± 16.3^{a}	510.0 ± 46.7^{a}	0.821 ± 0.012^{a}	0.571 ± 0.018^{b}	2.182 ± 0.198^{a}	0.168 ± 0.035^{b}	0.085 ± 0.006^{b}
	Purple	184.2 ± 27.6^{b}	1,087.0 ± 158.4^{b}	147.8 ± 23.7^{b}	365.2 ± 79.6^{b}	0.830 ± 0.014^{a}	0.590 ± 0.031^{b}	1.884 ± 0.133^{a}	0.621 ± 0.048^{a}	0.098 ± 0.016^{b}
	Pink	13.2 ± 2.5^{c}	43.0 ± 21.8^{c}	12.6 ± 4.4^{c}	35.8 ± 9.7^{c}	0.631 ± 0.053^{b}	0.638 ± 0.027^{a}	0.187 ± 0.051^{b}	0.149 ± 0.012^{a}	0.452 ± 0.037^{a}
SR	Green	nm	nm	nm	nm	nm	nm	nm	nm	nm
	Purple	119.3 ± 34.1^{a}	1,123.6 ± 182.4^{a}	155.4 ± 28.1^{a}	401.4 ± 95.0^{a}	0.830 ± 0.008^{a}	0.607 ± 0.039^{a}	1.865 ± 0.419^{a}	0.563 ± 0.150^{a}	0.134 ± 0.018^{b}
	Pink	13.0 ± 2.2^{b}	41.3 ± 10.5^{b}	14.0 ± 3.2^{b}	39.3 ± 92.0^{b}	0.678 ± 0.058^{b}	0.642 ± 0.041^{a}	0.048 ± 0.041^{b}	0.229 ± 0.071^{b}	0.360 ± 0.093^{a}
NR	Yellow-green	159.3 ± 40.3^{a}	1,115.9 ± 211.5^{a}	135.7 ± 30.3^{a}	345.1 ± 74.6^{a}	0.862 ± 0.012^{a}	0.606 ± 0.006^{a}	1.819 ± 0.256^{b}	0.237 ± 0.045^{a}	0.138 ± 0.031^{a}
	Purple	123.4 ± 35.6^{b}	800.3 ± 232.7^{b}	85.8 ± 28.9^{b}	206.4 ± 79.9^{b}	0.840 ± 0.006^{b}	0.592 ± 0.017^{a}	2.517 ± 0.368^{a}	0.203 ± 0.039^{a}	0.129 ± 0.031^{b}

q_P amongst the studied cultivars (Fig. 1, Table 2).

The images of the pink sectors of the RU and SR cultivars indicated low values of individual parameters when compared to the green and/or purple parts (Fig. 1), except for the F_v'/F_m' parameter. These data were usually confirmed by the point data measurements (Table 2).

An image-based comparison of the green and purple sectors in the RU and SR plants showed no differences in F_0 and F_0', but the point data measurements revealed that these parameters had higher values in the green parts than in the purple ones (Table 2). In the RU plants, similar results were obtained in the case of F_m and F_m'. In the SR leaves, the imaging of F_m and F_m' revealed heterogeneity within the leaf but it was impossible to compare the images with the numeric data on CF parameters because the green leaf sectors were embedded into the purple ones and too narrow for the clip to be properly positioned.

Neither the images of F_v/F_m, F_v'/F_m', and NPQ nor their corresponding numeric data showed differences between the green and purple areas of the RU plants. However, the green parts had higher CF imaging and point data values of q_P than the other ones (Fig. 1, Table 2).

In the SR plants, heterogeneity of F_v'/F_m' ratio within both areas was noticed (Fig. 1), but the difference was insignificant (Table 2). Such heterogeneity was also observed in the case of NPQ and q_P, but their values were usually higher in the red than in the pink areas (Fig. 1), and the point data measurements confirmed the differences (Table 2).

The values of F_0, F_m, F_0', F_m', and F_v/F_m in the yellow-green areas of the NR cultivar were higher as compared to the purple parts (Table 2, Fig. 1). Although the point data measurements of F_v'/F_m' and q_P parameters in the leaves of this cultivar did not show differences between the examined areas (Table 2), the results of CF imaging indicated higher values in the yellow-green areas than in the purple ones, and in the case of NPQ an opposite result was obtained (Fig. 1).

The values of the effective quantum yield of PSII photochemistry (Φ_{PSII}) were much higher in the creamy-yellow (DD, CA) and purple (RU) parts of the leaves than in the green ones (Table 2). They were also higher within the pink areas of SR compared to the purple ones. Contrary to RU, a slight difference in favour of the yellow-green NR parts *vs*. the purple ones was also noticed (Table 2).

Discussion

The Chl index and standard Chl spectrophotometric assay revealed differences between different areas of *Coleus* leaves, but some discrepancies in the range of these differences were obtained. It has been known, however, that the relationship between these two methods is nonlinear, and may vary upon the anatomical features of the leaves, the amount of chloroplasts within the mesophyll and nonuniform distribution of radiation across the leaf (Uddling *et al.* 2007, Cassol *et al.* 2008). Not only the "sieve effect", but also interference from the anthocyanins to the Chl index measurements is possible. Although *CL-01* measurements were performed at 620 and 940 nm, *i.e.* at the wavelengths not absorbed by these pigments, whose peak absorbance is 500–550 nm (Landi *et al.* 2015), it has been proved that absorption of anthocyanins *in vivo* can be shifted toward the region above 600 nm (Merzlyak *et al.* 2008, Hlavinka *et al.* 2013). For correcting the readings of SPAD and NDVI Chl meters in order to eliminate such contributions to measurements, a model was proposed by Hlavinka *et al.* (2013).

Irrespective of the discrepancy between the methods, the results indicated that Chl occurred in all variegated sectors of *Coleus* leaves but in different amounts and different Chl *a*/*b* ratios. The lowest Chl content was noticed in the creamy-yellow and pink parts, irrespective of the method. Similar results were obtained by Hung and Xie (2009) in pale yellow plants regenerated from a variegated *Epipremnum aureum.* The highest contents of Chls were present not in the green parts but in their purple counterparts, similarly to differentially pigmented varieties of *Pelargonium* × *hortorum* (Liakopoulos and Spanorigas 2012). An unusually high amount of Chl *a* and *b* and anthocyanins in the surface layers of the mesophyll was also found in black-pigmented leaves of *Ophiopogon planiscapus* 'Nigrescens' (Hatier and Gould 2007).

Numerous studies indicated that the increased Chl content in red-leafed plants is associated with the lower Chl *a*/*b* ratio similarly to the shade-adapted ones (Björkman 1981, Anderson 1986, Porra 2002, Hughes and Smith 2007, Nielsen and Simonsen 2011, Liakopoulos and Spanorigas 2012). The variation in the Chl *a*/*b* ratio is due to the differences in PSI to PSII ratio as well as the size and composition of the LHCs associated with each photosystem and containing different amounts of Chl *b* (Zhu *et al.* 2003, Wentworth *et al.* 2006). In the case of the purple-pigmented sectors, our experiments revealed similar result only for the RU plants, but it should be emphasised that *Coleus* is a species requiring not low but high irradiance (Garland 2009, and personal observations, unpublished). In the creamy-yellow and pink sectors, the Chl *a*/*b* ratio was markedly lower and within the small pool of Chls, Chl *b* was more abundant than Chl *a*, which is unusual (Kim *et al.* 2009). The presence of protochlorophyllides may be an explanation, because they have the absorbance spectra similar to Chl *b* (Belyayeva and Litvin 2011, Gabruk *et al.* 2015).

Cars are associated with Chls, being bound to the pigment-protein complexes of photosystems, with the majority of xantophylls found in LHCs and β-Car in the core complexes (Yamamoto and Bassi 1996, Matsubara *et al.* 2009). In the present study, Cars were undetectable in the creamy-yellow and pink sectors of *Coleus* leaves that contained only small amounts of Chls. However, it does not necessarily mean that these parts of the leaves are devoid of these pigments. They may contain extremely low concentrations of Car (below detection limit) or/and colourless Car such as phytoene that was found in white sectors of variegated *Arabidopsis thaliana* mutants (Wetzel *et al*. 1994, Wu *et al*. 1999, Foudree *et al*. 2012).

The pigment content is the basis for the interpretation of some CF parameters, and then venturing estimation of the physiological state of photosynthetic apparatus (Lichtenthaler *et al.* 2000, Baker 2008, Bączek-Kwinta *et al.* 2011, Muniz *et al.* 2014). In the creamy-yellow and pink sectors of leaves, the values of F_0, F_m, F_0', and F_m' were very low when compared to the other areas due to the low content of photosynthetic pigments. Other parameters, such as F_v/F_m, q_P, and NPQ, in these parts also remained low indicating diminished photochemical capacity of PSII, low amount of PSII reaction centres, and less energy available due to the lack of Cars or their small amount, respectively (Demmig and Björkmann 1987, Krause and Weis 1991, Sofo *et al.* 2009). These results, together with high Φ_{PSII} and F_v'/F_m' in these areas, may be interpreted as better light energy utilization for photochemistry by the lowered number of functioning PSII reaction centres, as demonstrated in a model by Miyata *et al.* (2012).

Many studies comparing photosynthesis in green and red-leafed plants yielded conflicting results but this could be caused by a different plant (or leaf) age or whether the anthocyanic pigmentation is a feature resulting from the light conditions or, in case of some ornamentals, a result of breeding. For example, red juvenile leaves of *Syzygium* had lower photosynthetic capacity as compared to green ones (Dodd *et al.* 1998). A similar relationship was noticed in the case of red and green leaves of *Quintinia serrata*, but other studies showed higher photosynthetic capacity in the red plants when measured as CO_2 assimilation (Gould *et al.* 1995, 2002; Liakopoulos *et al.* 2006, Nielsen and Simonsen 2011). Our research showed that in the purple, green, and yellow-green parts of *Coleus* leaves the values of most of the studied parameters were usually maintained at a high level. Nevertheless, slight differences between these differentially pigmented sectors were noticed. First of all, although the purple areas contained the highest amount of photosynthetic pigments, F_0, F_m, F_0', and F_m' parameters were a little lower than those in the green and yellow-green sectors due to the high content of Cars and the presence of anthocyanins. Both groups of pigments participate in photoprotection but their mode of action may be either similar or different. While Cars dissipate energy as heat, the effect of anthocyanins is light attenuating (shading) of the photosynthetic tissue (Neill and Gould 2003, Nielsen and Simonsen 2011, Liakopoulos and Spanorigas 2012, Henry *et al.* 2012, Logan *et al.* 2015). In *Coleus*, anthocyanins are accumulated on the adaxial surface of the leaves, and provide photoprotection to LHC (Burger and Edwards 1996, Henry *et al.* 2012, Logan *et al.* 2015). Anthocyanins may participate in photoprotection through their antioxidant capacity, and some are capable of absorbing parts of the UV spectra similarly to the Cars (Neill and Gould 2003, Khoo *et al.* 2011, Takahashi and Badger 2011). In the absence of anthocyanins, green leaves utilise thermal energy dissipation *via* the xanthophyll cycle (Henry *et al.* 2012, Logan *et al.* 2015). In our experiment, it can be reflected by high NPQ values, and according to many authors (Maxwell and Johnson 2000, Montanaro *et al.* 2007, Sofo *et al.* 2009, Bączek-Kwinta *et al.* 2011), it can be related to energy dissipation as heat *via* the xanthophyll cycle and other Cars, as well as conformational changes in PSII antennae (LHC).

Conclusion: Both methods of CF analysis revealed a heterogeneity in leaf CF parameters within a leaf that allowed for distinguishing the mechanisms responsible for capture, transfer, and dissipation of excitation energy in differentially pigmented sectors. In the case of very narrow pigmented leaf areas, CF imaging was more suitable than the point data measurements. Summing up the results, the green parts had the highest q_P and usually high NPQ revealing relatively high light utilisation for photosynthesis but also the involvement of photoprotective mechanism based on thermal energy dissipation by carotenoids. In the red leaf sectors, the Cars content was even higher, and also the shading effect of anthocyanins was manifested by lower q_P despite the high Chl content. The creamy-yellow and pink areas were characterized by the lowest contents of pigments and greatly diminished Chl *a*/*b*, but Φ_{PSII} and F_v'/F_m' were high, indicating better light energy utilisation for photochemistry by diminished number of functioning PSII reaction centres.

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