Photosystem II efficiency in response to diurnal and seasonal variations in photon flux density and air temperature for green, yellow-green, and purple-leaved cultivars of sweet potato [*Ipomoea batatas* (L.) Lam]

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Abstract

This study aimed to investigate the impact of diurnal and seasonal variations in photon flux density (PPFD) and air temperature on PSII efficiency in three sweet potato leaf-color cultivars: green (G), yellow-green (Y), and purple (P). The cultivars were exposed to full sunlight and measurements were taken from November to March. The maximal quantum yield of PSII photochemistry for the dark-adapted state (F_v/F_m) indicated Y's increased sensitivity to low temperatures at predawn, followed by G and P. Both quantum yield of PSII photochemistry for the dark and light-adapted state ($\Delta F/F_m'$) depressions were correlated with increased PPFD, with regression slopes in the order of Y > G > P. On high-light and low-temperature days, F_v/F_m values deviated below regression lines, with differences ranked as Y > G > P. These findings suggest that Y exhibits the highest sensitivity to high light and low temperatures, followed by G and then P in terms of PSII efficiency.

Introduction

There are usually multiple varieties having differing leaf colors in vegetables and foliage plants. Different leaf colors are due to physical leaf structures (Chen *et al.* 2017) and pigment content chemistry (Li *et al.* 2019). Leaf color is typically green when chlorophyll (Chl) predominates in plant leaves. When leaves have less Chl content, they are usually yellow-green (Peng *et al.* 2002, Weng *et al.* 2011, Yang *et al.* 2020). Leaves containing anthocyanins

Highlights

- Predawn F_v/F_m in sweet potatoes: yellow-green (Y) variety is the most sensitive
- Noon and 17:00 h: F_v/F_m and $\Delta F/F_m'$ dips with high light, Y > G > P
- On high light days, F_v/F_m values deviated below regression lines, with differences ranked as Y > G > P

appear red or purple (Lin *et al.* 2019, Stetsenko *et al.* 2020). Leaf pigment content not only affects appearance but also affects photosynthetic performance (Peng *et al.* 2002, Weng *et al.* 2011, Yang *et al.* 2020). The quality of light, referring to the wavelength and spectral components, exerts profound effects on the process of photosynthesis in plants (Lazar *et al.* 2022). The color of plant leaves impacts their utilization of the light spectrum because the pigments influencing leaf color also affect the absorption and utilization of light.

Chl is an essential pigment for photosynthesis, as it absorbs light energy and converts it to chemical energy, especially in the red and blue ranges of the spectrum (Gitelson *et al.* 2003). Red light primarily participates in photosynthesis, promoting biomass synthesis. Blue light is effective in stomata regulation, as well as biomass accumulation (Li and Kubota 2009). However, when leaves absorb more energy than they can utilize, this excessively absorbed energy may lead to photosystem damage (Demmig-Adams and Adams 1996, Kato *et al.* 2003, Adams *et al.* 2004). Plants can employ several mechanisms to protect the photosystem, such as xanthophyll-dependent nonphotochemical quenching of the excited Chl states (NPQ), to dissipate the excess energy as heat (Demmig-Adams and Adams 1996, Jahns *et al.* 2009), and utilize antioxidants to reduce the oxidative stress caused by reactive oxygen species from excessively absorbed energy (Smirnoff 2000). Carotenoids also absorb blue light, causing around 90% attenuation of blue and red light in the top 20% of the leaf, while a significant portion of green light penetrates deeper (Cui *et al.* 1991, Lazar *et al.* 2022). Anthocyanins are water-soluble, nonphotochemically active pigments belonging to the flavonoid family of plant secondary metabolites. Anthocyanins are ubiquitous and can be found in flowers, fruits, stems, and leaves in the vegetative and reproductive organs of plants (Tanaka and Ohmiya 2008). Anthocyanins typically exhibit their absorption peaks in the blue-light region of the visible spectrum. Anthocyanins play a vital regulatory role in plant photosynthesis and survival by providing additional light absorption (Gould 2004). The function of anthocyanin in plant leaves involves protection against various biotic and abiotic stresses. Conditions such as high irradiance and low temperature can cause damage to plant PSII, anthocyanin contributes to plant protection by both diminishing excess light through absorption and by acting as an antioxidant to shield the photosystem from oxidative damage (Zhang *et al.* 2019, Stetsenko *et al.* 2020).

Whether excess energy is absorbed depends on the amount of light energy absorbed and consumed. When light intensity increases, a cell can decrease the fraction of absorbed energy to protect itself against photodamage. When CO_2 exchange is inhibited, such as during low temperatures, energy consumption is reduced (Baker 1993, Leegood 1995). Thus, both high irradiance and low temperature can cause damage to the PSII of plants (Weng *et al.* 2013, Huang *et al.* 2016, Lin *et al.* 2021). A reduction of Chl content reduces the ability of leaves to absorb photons as well as changes in the light-harvesting

apparatus and the efficiency with which absorbed photons are subsequently used in photosynthesis (Peng *et al.* 2002, Goh *et al.* 2009, Yang *et al.* 2020). However, the photoprotective mechanisms of leaf-color mutants are diverse. Some mutants show more efficiency in photoprotection than wild types of the same species. Yet, the opposite trend is also found in other mutants (Peng *et al.* 2002, Goh *et al.* 2009, Yang *et al.* 2020). Our previous studies (Weng *et al.* 2011) found that compared to green cultivars of the same species yellow-green foliage cultivars of four vegetables showed lower Chl and total carotenoid values, smaller PSII antenna size, and lower photosynthetic capacities. Due to a lower ability in xanthophyll cycle-dependent energy quenching (q_E), yellow-green sweet potato (*Ipomoea batatas* L.) cultivars showed more photoinhibition under high irradiance, particularly in low temperatures (Weng *et al.* 2011, Lin *et al.* 2021).

Sweet potato is an important root crop and leafy vegetable in the subtropical and tropical regions of the world. It is relatively tolerant to environmental stresses; however, it is sensitive to low-temperature conditions (Noh *et al.* 2009). In Taiwan (21°53'N–25°18'N), sweet potato is often cultivated through the winter. In subtropical regions, chilling temperatures (*i.e.*, < 10°C) coincide frequently with bright mornings as a result of rapid heat dissipation by the emission of long-wave radiation during clear nights. Thus, high-light and low-temperature stresses may occur simultaneously. In addition, high-light irradiances exert adverse effects on sweet potato plants during warmer seasons. Temperature and light changes evoke a variety of photosynthetic responses that can vary among different sweet potato species with different leaf colors (Weng *et al.* 2011, Lin *et al.* 2021). Such photo- and temperature responses are of practical importance in recent plant cultivation technologies. There are many sweet potato cultivars with different leaf colors, including yellow-green, green, and purple. Our previous studies indicated that the PSII efficiency of a yellow-green cultivar was more sensitive to low temperature and high light than the green cultivar. It was reported that in many cases, seasonal variation in PSII efficiency can reflect the capacity of photosynthetic CO_2 exchange (Corcuera *et al.* 2005, Weng *et al.* 2005), and seasonal variations in lutein, neoxanthin, and β-carotenoid can reflect the capacity of photoprotection (Corcuera *et al.* 2005). In addition, under dynamic or diurnal variations in light intensity, photosynthetic rates are closely correlated with the value obtained by light intensity × PSII efficiency (Wong *et al.* 2012, Sun and Wang 2018, Alemu and Roro 2020, He and Qin 2020, Huang *et al.* 2021).

PSII efficiency can be assessed quickly and easily by Chl fluorescence quenching analysis and is a reliable method for assessing changes in the functioning of PSII under different environmental and physiological conditions (Roháček and Barták 1999, Maxwell and Johnson 2000). It has been widely used to monitor functional changes such as seasonal and diurnal variations in the photosynthesis apparatus under different conditions, as well as in responses to stresses (Demmig-Adams

and Adams 1996, Verhoeven *et al.* 1999, Close *et al.* 2001, Weng *et al.* 2005, Li *et al.* 2021). However, most experiments with sweet potato cultivars having different leaf colors have been made under artificial conditions. Moreover, the purple cultivar has not been explored (Weng *et al.* 2011, Lin *et al.* 2021). The response in the PSII efficiency of sweet potato cultivars to natural diurnal and seasonal variations in air temperature and PPFD under natural conditions is not clear, so in this study, we used three cultivars having green, yellow-green, and purple leaves to elucidate these variable responses.

Materials and methods

Plant materials and growth conditions: Three sweet potato [*Ipomoea batatas* (L.) Lam] cultivars having green (Taoyuan No. 2), yellow-green (cv. CN1927), and purple leaves (CYY 8467) were used. Plant materials propagated from cuttings were planted into pots (16-cm diameter, 12-cm depth) filled with sandy loam and placed outdoors to receive regular water and fertilizers (1/2 strength of Hoagland's nutrient solution) and full sunlight on the campus of National Chung-Hsing University, Taichung, Taiwan (24°08'N, 120°40'E, 70 m a.s.l.). The mean monthly air temperature was 17–26°C during the growing season (October–March).

Air temperature and photosynthetic photon flux density: PPFD and air temperature were measured with an *LI-190SA* sensor (*LI-COR*, Lincoln, NE, USA) and copper constantan thermocouples, respectively. Sensors were connected to a data logger (*CR10*, *Campbell Scientific*, Logan, UT, USA) that collected data automatically every 2 min, and the averaged values for each hour were recorded.

Leaf pigments: Leaves were collected in November for the determination of Chl content using the methods described by Yang *et al.* (1998). Leaf discs were excised using a standard hole punch, immediately sealed in prelabeled aluminum envelopes, and placed in liquid nitrogen. Sixteen pieces of each color leaf were selected for measuring pigments. Tissues were stored at –80°C until analysis and then extracted in 80% (v/v) aqueous acetone. Supernatants were obtained by centrifuging mixtures at 3,000 rpm for 10 min. Supernatants were assayed for the absorbance of Chl *a* and Chl *b* at 440.5, 663.6, and 646.6 nm in a 10-mm quartz cuvette (*Hellma*, Germany), with a *U-2000* type spectrophotometer (*Hitachi*, Tokyo, Japan). Chl *a*, Chl *b*, and carotenoid (Car) concentrations were calculated using the following equations (Yang *et al.* 1998):

Chl *a* [µg m^{-2}] = (12.25 A$_{663.6}$ – 2.55 A$_{646.6}$) × volume of supernatant [mL]/leaf area [m^2]

Chl *b* [µg m^{-2}] = (20.31 A$_{646.6}$ – 4.91 A$_{663.6}$) × volume of supernatant [mL]/leaf area [m^2]

Car [µg m^{-2}] = (4.69 A$_{440.5}$ × volume of supernatant [mL]/ leaf area [m^2]) – 0.267 Chl (*a*+*b*) [µg m^{-2}]

The anthocyanin content of extracts was measured based on the protocol of Mancinelli *et al.* (1975). A mixture of 99% methanol containing 1% HCl (v/v) was added to powdered samples and incubated for 1 h at room temperature. The mixture was then centrifuged at 4°C and 3,000 rpm for 5 min to obtain a supernatant, followed by measuring absorbances at 530 and 657 nm on a spectrophotometer. The following equation (Mancinelli *et al.* 1975) was used:

Anthocyanin [mmol m^{-2}] = (A$_{530}$ – 0.33 × A$_{657}$/31.6) × volume of supernatant [mL]/leaf area [m^2]

Chl fluorescence measurements: Chl fluorescence was determined by a *PAM-2000* fluorometer (*Walz*, Effeltrich, Germany) with a low-intensity beam from light-emitting diodes (excitation wavelength at 655 nm, detection above 700 nm, 1.6 kHz). The minimum fluorescence intensities (F$_0$, dark-adapted state; F$_0$', light-adapted state) and the maximum fluorescence intensities induced by a brief saturation light [0.8 s, > 5,000 µmol(photon) m^{-2} s^{-1}] (F$_m$, dark-adapted state; F$_m$', light-adapted state) were detected, respectively, and used to calculate the quantum yield of photochemistry as F$_v$/F$_m$ = (F$_m$ – F$_0$)/F$_m$ and ΔF/F$_m$' = (F$_m$' – F$_0$')/F$_m$' (Kitajima and Butler 1975, Genty *et al.* 1989). F$_v$/F$_m$ – ΔF/F$_m$' represents the difference between the maximal quantum yield of PSII photochemistry for the dark-adapted state at predawn and the efficient quantum yield of PSII photochemistry for the light-adapted state at noon. Nonphotochemical quenching (NPQ) was calculated as F$_m$/F$_m$' – 1 (Maxwell and Johnson 2000). From November to March, the maximal quantum yield of PSII photochemistry for the dark-adapted state (F$_v$/F$_m$) at predawn, noon, and dusk, and the efficient quantum yield of PSII photochemistry for the light-adapted state (ΔF/F$_m$') at noon, were measured every 1 to 5 d. At predawn, potted materials were moved to a dark room before sunrise (5:40 h). Then F$_v$/F$_m$ was measured at 6:00 h. After the measurement of F$_v$/F$_m$, potted materials were put outdoors to receive full sunlight until noon. At noon (12:00–12:20 h), the ΔF/F$_m$' was measured by the same fluorometer under sunlight. Materials were then moved to a darkroom for 20 min, and then F$_v$/F$_m$ was measured from the same leaves that were used to measure ΔF/F$_m$'. Measurements were taken in the darkroom to avoid underestimating F$_v$/F$_m$, because large F$_0$ values could have resulted from the high leaf temperatures that could have occurred when leaves were clipped under high illumination (Weng 2006). After the measurement of F$_v$/F$_m$, materials were moved outdoors again until measurements of F$_v$/F$_m$ were taken at dusk. At 17:00 h, potted materials were moved to the darkroom for 20 min and then F$_v$/F$_m$ was measured.

Statistical analysis: In this study, one leaf from each pot was designated as a replicate, with four replicates measured in total. The study aimed to compare the pigment content [Chl (*a*+*b*), carotenoid, and anthocyanin] across three leaf colors. The data were analyzed using analysis of variance (*ANOVA*), followed by the Least Significant Difference (LSD) test, with significance determined at

$p<0.05$. Chl fluorescence data were examined through linear or logarithmic regression models, depending on statistical significance ($p<0.05$). Correlation coefficients (r for linear models) and coefficients of determination (r^2 for nonlinear models) were calculated using *Sigma Plot 9.01* (*Systat Software*, Point Richmond, CA, USA). The normality of the data was assessed using the *Kolmogorov–Smirnov*'s test.

Results

The pigment contents of the yellow-green (Y), green (G), and purple (P) sweet potato leaves are shown in Table 1. The Chl ($a+b$), Car, and Ant values observed in P leaves were 0.41 g m^{-2}, 0.058 g m^{-2}, and 11.8 mmol m^{-2}, respectively. Significantly lower Chl ($a+b$) and Car contents in Y leaves were compared to the others, and P had the highest Chl ($a+b$), followed by G. The Car of P and G was higher than that in Y, but there was no significant difference between P and G. Y and G were not tested for Ant, so no such data are presented. For the Chl a/b ratio, the Y leaves had the highest values (4.99), followed by G leaves (3.2) (Table 1). The different contents of Chl, Chl a/b, Car, and Ant may lead to variations in photosynthetic efficiency among three colored leaves.

During the experiment period (19 November to March), predawn temperatures ranged from 18.9°C (27 December) to 7.5°C (6 March). Temperatures drop significantly when cold fronts strike in winter in Taiwan. The temperature at noon was between 31.7°C (19 November) and 12.9°C (3 March). The average PPFD of 11:00–12:00 h was the highest at 1,440 µmol m^{-2} s^{-1} (6 March), and the lowest was 35 µmol m^{-2} s^{-1} (3 March) (Fig. 1A,B). PPFD was mainly affected by the amount of cloud cover at that time. Because sunlight raises temperatures during the day, a day with strong sunlight has a large day–night temperature difference. During the experiment, the difference between daily maximum and minimum temperatures ranged from 19.3°C (6 March) to 1.7°C (2 February). Fig. 1C–E shows the F_v/F_m of the three leaf-color sweet potato cultivars at predawn, noon, and dusk, and the $\Delta F/F_m'$ under light at noon. The results show that, in the morning, F_v/F_m decreased with increasing PPFD and recovered gradually with decreasing PPFD in the afternoon. The predawn F_v/F_m ranges of Y, G, and P were 0.32–0.85, 0.65–0.84, and 0.77–0.85, respectively. At noon, these values were 0.37–0.80, 0.63–0.81, and 0.75–0.82, and at dusk were 0.28–0.82, 0.59–0.81, and 0.73–0.83, respectively. The variation range of F_v/F_m was the largest for Y and

smallest for P. It is worth noting that Y showed a higher F_v/F_m value at noon than at predawn on 6 March, when there was a cold predawn as well as a bright and warmer noon. The noon $\Delta F/F_m'$ of the three leaf-color sweet potato cultivars were 0.11–0.76, with Y showing the lowest values (0.11–0.73), then G (0.14–0.74), and P the highest (0.23–0.76).

The predawn F_v/F_m values of the three leaf-color sweet potato cultivars showed a significant positive logarithmic curve with the daily minimum temperature ($r = 0.851$–0.927, $p<0.001$) (Fig. 2). In warmer predawns, the F_v/F_m of the three sweet potato cultivars were all above 0.8. Y dropped slowly below 16°C and then dropped sharply below 10°C. G and P decreased slowly below 14°C and 12°C, respectively. G dropped sharply below 8°C, while P did not drop sharply until 7.5°C. This shows that the F_v/F_m of the three sweet potato cultivars decreased when the predawn temperature dropped to a certain level. The temperature at which the F_v/F_m begins to decrease, and the degree of decrease, was the highest for Y, then G, and then P. The F_v/F_m of the three sweet potato cultivars decreased with the increase in PPFD at noon, and there was a significant negative linear correlation ($r = 0.770$–0.841, $p<0.001$, Fig. 3, *triangle symbols*). The noon F_v/F_m values of the three sweet potato cultivars were around 0.8 for the low PPFD. However, the slope of the F_v/F_m decrease was as follows: Y was the largest (0.169×10^{-3}), G second (0.887×10^{-4}), and P the lowest (0.380×10^{-4}) as PPFD increased. The F_v/F_m of Y showed more drastic depressions on 1 January and 5 March, as those two days had low temperatures ($< 20°C$) and high PPFD values ($> 1,000$ µmol m^{-2} s^{-1}). The relationship between the PPFD and F_v/F_m of G and P was not affected by low temperatures at noon (Fig. 3B,C).

The noon $\Delta F/F_m'$ decreased linearly with the increase in PPFD (Fig. 3, *circle symbols*), but the difference in the slope of the decline between the different leaf colors was about 1.2 times (0.263–0.308×10^{-3}). The difference in the slope of F_v/F_m decreasing with the increase in PPFD was about 4.4 times (0.380×10^{-5}–0.169×10^{-4}). The $\Delta F/F_m'$ was still decreasing under 100 µmol m^{-2} s^{-1} low light, and the degree of decrease was Y > G > P. The dusk F_v/F_m showed a positive linear regression with PPFD (16:00–17:00 h), its decreasing slope being Y > G > P (Fig. 4). However, the F_v/F_m was much lower than the regression line on the two days having high light and low temperature (1 January and 5 March), and the degree of deviation between the F_v/F_m value and the regression line was Y > G > P. These results were similar to Fig. 3A. At low PPFD, the F_v/F_m

Table 1. Contents of chlorophyll ($a+b$), carotenoids, and anthocyanin in yellow-green, green, and purple sweet potato leaves. Each point represents the mean of four leaves. Data are mean ± SE. *Different letters* indicate significant differences in the LSD analyses of Chl ($a+b$) and Car.

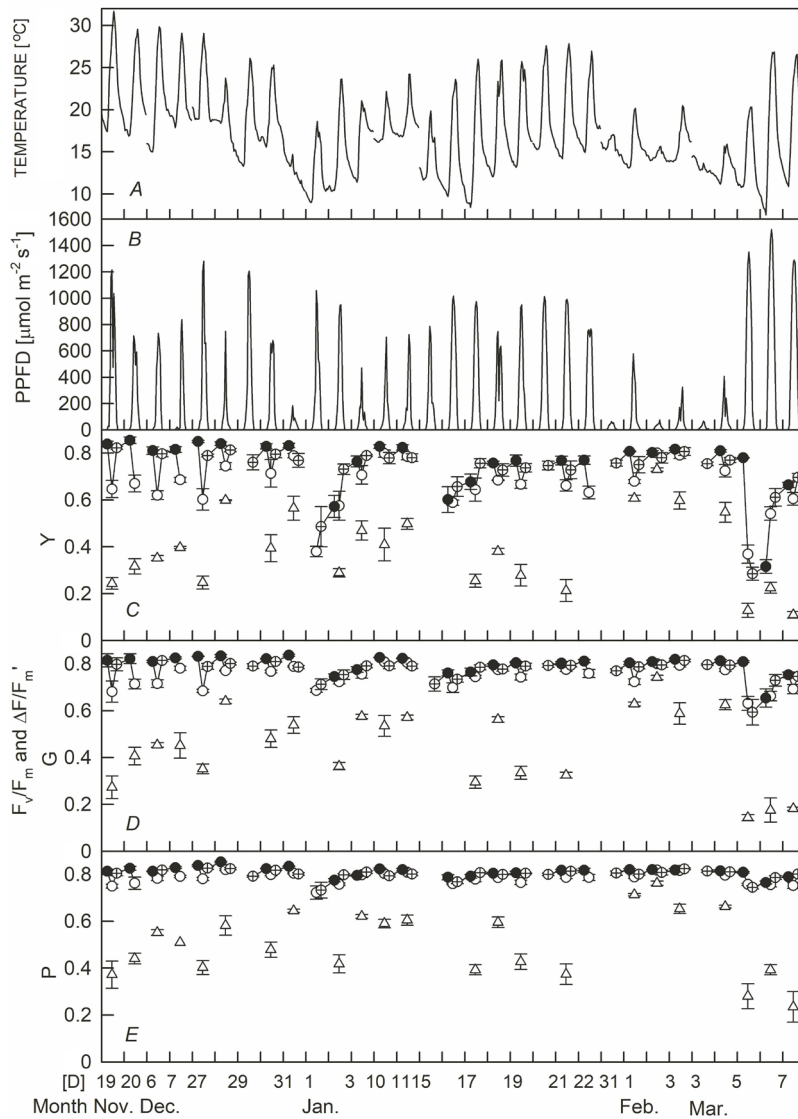| Leaf color	Chl ($a+b$) [g m^{-2}]	Chl a/b ratio	Carotenoids [g m^{-2}]	Anthocyanin [mmol m^{-2}]
Yellow-green	0.096 ± 0.007[c]	4.99 ± 0.89[a]	0.037 ± 0.002[b]	Not determined
Green	0.337 ± 0.024[b]	3.20 ± 0.25[b]	0.060 ± 0.002[a]	Not determined
Purple	0.413 ± 0.019[a]	2.58 ± 0.18[c]	0.058 ± 0.003[a]	11.802 ± 0.910

Fig. 1. Temperature (*A*), photosynthetic photon flux density (*B*), and photosystem II efficiency of yellow-green (*C*), green (*D*), and purple-leaf sweet potato (*E*) at predawn (●), noon (○ and △), and dusk (⊕). *Circle* and *triangle symbols* indicate the quantum yield of PSII photochemistry for dark (F_v/F_m) and light-adapted ($\Delta F/F_m'$) state, respectively. The error bar is SE (*n* = 4).

values of the three cultivars were restored to nearly 0.8. In addition, the decrease of PSII in sweet potato leaves was also investigated by NPQ dissipation in this study. Fig. 5 shows that both $\Delta F/F_m'$ and $F_v/F_m - \Delta F/F_m'$ values were negatively correlated with NPQ (*r* = –0.781 and –0.912) in the leaves of sweet potato, respectively.

Discussion

Seasonal variation of F_v/F_m at predawn: When leaves are exposed to light, PSII efficiency decreases with increasing irradiance and decreasing temperature (Weng *et al.* 2013, Acebron *et al.* 2021, Lin *et al.* 2021). In prior research concerning Chl fluorescence induction parameters, the use of mean and standard deviation for expressing parameters has been discouraged (Lazár and Nauš 1998). Therefore, in this study, data measured from a single leaf were utilized to perform analyses using linear or logarithmic regression models. In the present study, our work revealed a negative correlation between $\Delta F/F_m'$

and NPQ in sweet potato. Similarly, Lin *et al.* (2021) also indicated that NPQ is negatively correlated with F_v/F_m and $\Delta F/F_m'$, respectively, in sweet potato under different light intensities. These results suggested that the depression in PSII efficiency in sweet potato under high light is mainly due to xanthophyll-dependent NPQ dissipating excess energy as heat (Demmig-Adams and Adams 1996, Jahns *et al.* 2009, Lin *et al.* 2021). Within the xanthophyll cycle, violaxanthin (V) is de-epoxidized first into antheraxanthin (A) and then into zeaxanthin (Z) (Hager and Holocher 1994). Nevertheless, PSII efficiency gradually recovers when sunshine mellows in the afternoon (Verhoeven *et al.* 1999, Santanoo *et al.* 2019, Acebron *et al.* 2021) due to the conversion of Z to A, and then to V (Demmig-Adams and Adams 1996, Verhoeven *et al.* 1999). However, after high-light illumination, particularly at low temperatures, high contents of A + Z may be maintained for a long time. Therefore, at predawn in winter, large amounts of A + Z are retained and low values of intrinsic PSII efficiency are sustained (Verhoeven *et al.* 1999, Adams *et al.* 2004,
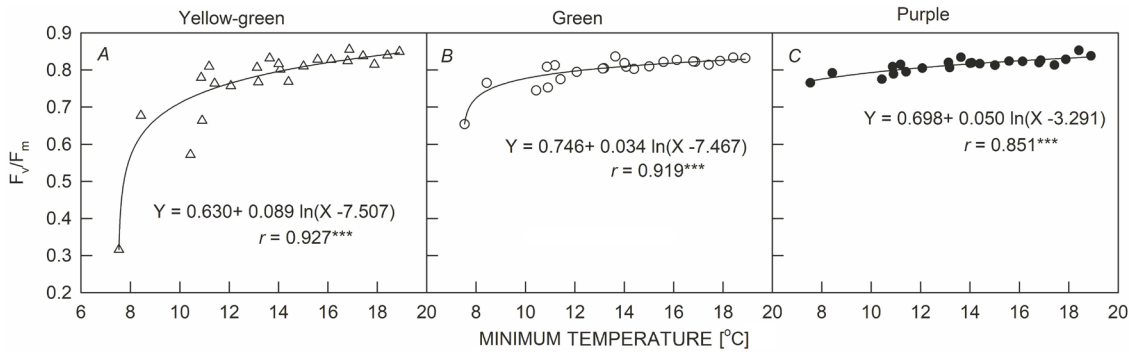
Fig. 2. Relationship between the maximal quantum yield of PSII photochemistry for dark-adapted state (F_v/F_m) and daily minimum air temperature at predawn on sweet potato plants having different leaf colors (△: yellow-green leaves, ○: green leaves, ●: purple leaves). Each line represents the mean of 23 points. ***: $p<0.001$.
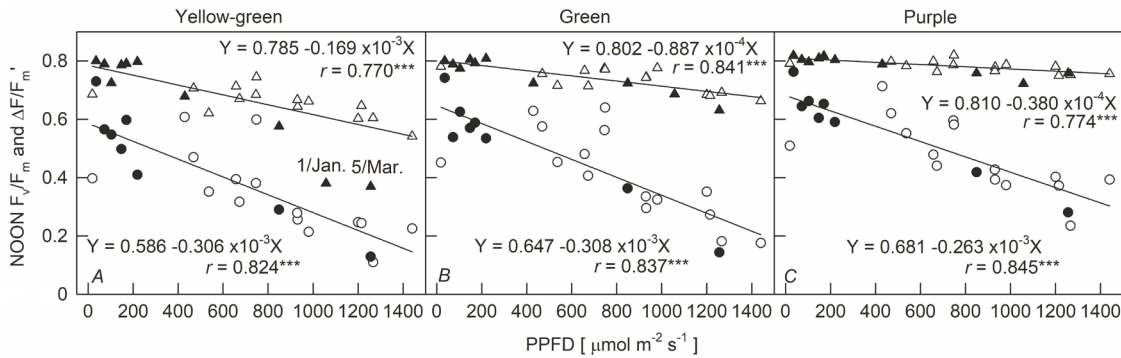

Fig. 3. Relationship between the maximal quantum yield of PSII photochemistry for dark-adapted state (F_v/F_m) and photosynthetic photon flux density (PPFD) for sweet potatoes with different leaf colors at noon. *Triangle* and *circle symbols* are for F_v/F_m and $\Delta F/F_m'$ at noon, respectively. *Closed* and *open symbols* indicate that the average temperature (11:00–12:00 h) was below and above 20°C, respectively. Each line represents the mean of 24 points. ***: $p<0.001$.

Fig. 4. Relationship between the maximal quantum yield of PSII photochemistry for dark-adapted state (F_v/F_m) in sweet potatoes having different leaf colors and photosynthetic photon flux density (PPFD) at dusk. *Closed* and *open symbols* indicate that the average temperature (16:00–17:00 h) was below and above 20°C, respectively. Each line represents the mean of 24 points. * and ***: $p<0.02$ and 0.001, respectively.
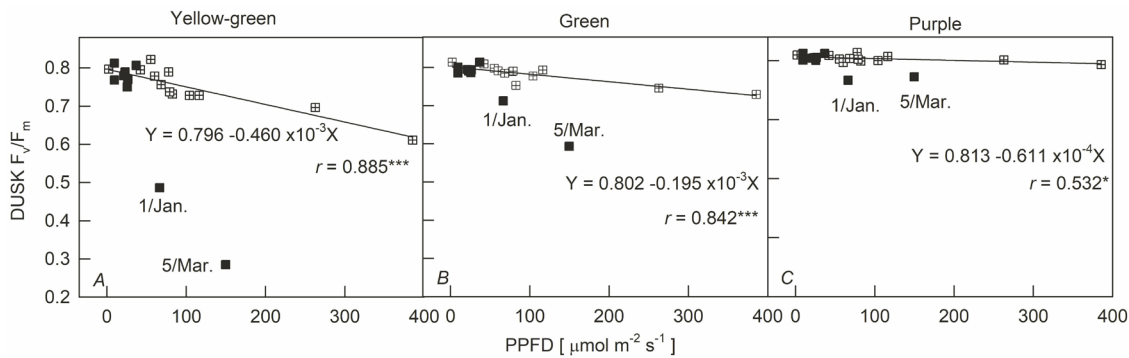
Weng *et al.* 2006). The depression of predawn F_v/F_m during winter is due to chronic photoinhibition and is related to a slowly reversible loss of PSII reaction center functioning (Long *et al.* 1994, Close *et al.* 2001). Previous studies (Blennow *et al.* 1998, Weng *et al.* 2005, 2006) have pointed out that predawn F_v/F_m in several plant species is closely related to the daily minimum temperature. We also found that, in subtropical Taiwan, tropical species show

a greater depression in predawn F_v/F_m, and accumulate more A + Z with decreasing temperature, than species distributed over a broad altitude range (Weng *et al.* 2006). The same tendency is observed in Fig. 2. On warmer days, predawn F_v/F_m was above 0.8, indicating that no chronic photoinhibition occurred in PSII (Björkman and Demmig 1987, Bolhàr-Nordenkampf *et al.* 1989). However, lower F_v/F_m was observed in the cool predawn, and predawn
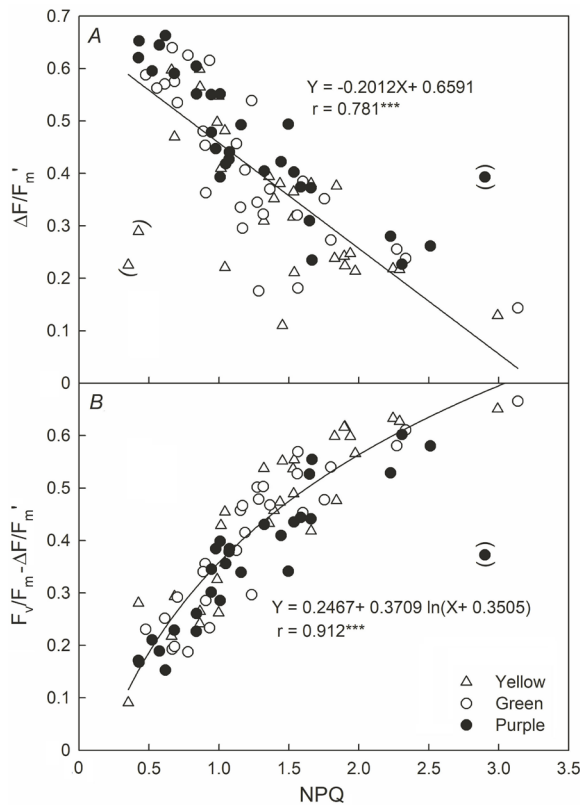
Fig. 5. Relationship between nonphotochemical quenching (NPQ) and the efficient quantum yield of PSII photochemistry for the light-adapted state ($\Delta F/F_m'$) in sweet potatoes having different leaf colors. ***: $p<0.001$. *The brackets* indicate that the measurements were taken during a cold current, resulting in extremely low temperatures at dawn and high light but low temperatures at noon (the data was not included in the calculation).

F_V/F_m values of Y were most sensitive to low temperature, followed by G and then P. This may be due to the low night temperature delay of the epoxidation of Z to A and V, and lead to the downregulation of PSII (Verhoeven *et al.* 1999, Adams *et al.* 2004, Weng *et al.* 2006).

Effect of light intensity and air temperature on F_v/F_m and $\Delta F/F_m'$ at noon: Generally, the factors that reduce the efficiency of PSII include increasing light intensity and low temperature. PSII efficiency is the highest in the early morning before sunrise, then decreases with increasing light intensity, and reaches a minimum mostly at noon (Demmig-Adams *et al.* 1996, Verhoeven *et al.* 1999, Santanoo *et al.* 2019, Yang *et al.* 2020, Acebron *et al.* 2021). The decrease in PSII efficiency with increasing light intensity occurs drastically in low temperatures (Huang *et al.* 2016, Santanoo *et al.* 2019). This same tendency is also seen in Figs. 1 and 3.

Under illumination, the depression of PSII efficiency is correlated with an increase in NPQ (Demmig-Adams *et al.* 1996, Müller *et al.* 2001). At least four different components of NPQ are defined by their formatting and dark relaxation kinetics. The fastest and most important

component of NPQ is q_E (Müller *et al.* 2001, Johnson and Ruban 2011). This is closely related to: (*1*) the thylakoid membrane proton gradient (ΔpH), (*2*) the xanthophylls pool ratio change, and (*3*) the PsbS protein of PSII (Müller *et al.* 2001, Lavaud and Lepetit 2013). Nilkens *et al.* (2010) reported that after the completion of the reaction in subunit PsbS, zeaxanthin (Z) is combined with PsbS to dissipate H^+. Therefore, the part after the reaction of q_E is called q_Z and is similar to the slower NPQ response identified by Maxwell and Johnson (2000). The third component of NPQ is q_T (phosphorylation shift-dependent quenching) (Quick and Stitt 1989), which shows the phosphorylation shift of the light-harvesting complex (LHC) II between PSII and PSI. The slowest reaction component of NPQ is q_I (photoinhibitory quenching), which is a slowly inducible and reversible component, takes several hours to relax and is related to the photoinhibition of photosynthesis (Müller *et al.* 2001). It is also known as sustained NPQ and is associated with zeaxanthin retention (Demmig-Adams *et al.* 2006).

In this study, $\Delta F/F_m'$ was measured under sunlight, and F_v/F_m was measured after 20 min of dark adaption. By noon, leaves had been exposed to sunlight for several hours, thus, the depression of $\Delta F/F_m'$ included the formation of q_E, $q_Z + q_T$, and q_I. While the depression of F_v/F_m was mainly due to the q_I, F_v/F_m is widely used as an indicator of photoinhibition (Adams *et al.* 2004, Gorbe and Calatayud 2012). Fig. 3 shows that the intercept of the F_v/F_m–PPFD regression in the three cultivars was nearly 0.8. This indicates that, under low PPFD, no chronic photoinhibition can be found (Björkman and Demmig 1987, Bolhàr-Nordenkampf *et al.* 1989) for these three cultivars. However, the slope of the F_v/F_m–PPFD regression was Y > G > P. As a result, under high PPFD, Y showed the greatest degree of photoinhibition, followed by G and then P. For the response of F_v/F_m to temperature among the three cultivars, only the F_v/F_m of Y showed a more drastic depression to lower temperature (< 20°C) at higher PPFD (> 1,000 µmol m^{-2} s^{-1}). The other two cultivars had no notable differences between high and low temperatures in their F_v/F_m–PPFD relationships. Fig. 3 also shows that under all levels of PPFD, the depression of $\Delta F/F_m'$ was Y > G > P. This indicates that Y shows the highest degree of $q_E + (q_Z + q_T) + q_I$, followed by G and then P. It could be considered that, under the same level of PPFD, the difference between $\Delta F/F_m'$ and F_v/F_m values is mainly due to the components of $q_E + (q_Z + q_T)$. The q_E is the pH- (energy-) dependent nonphotochemical quenching of Chl fluorescence (ChlF). The q_Z is nonphotochemical quenching of ChlF dependent on zeaxanthin, and the q_T is lowering of ChlF due to the state-transition (Müller *et al.* 2001, Nilkens *et al.* 2010). Fig. 3 shows that, at low PPFD, the degree of $q_E + (q_Z + q_T)$ is Y > G > P, but it is P ≈ G > Y at high PPFD.

Our previous studies indicate that, compared to G, Y shows lower abilities in q_E and PSII efficiency under high irradiances, particularly during low temperatures (Weng *et al.* 2011, Lin *et al.* 2021). The results shown in Fig. 3 generally agree with the results of our previous study (Weng *et al.* 2011, Lin *et al.* 2021). In addition, Fig. 3

shows that P has the lowest q_I, and $q_E + (q_Z + q_T)$, as does G under high light. Fig. 1 also shows that G has the highest F_v/F_m in the cool predawn. These results indicate that P had the lowest photoinhibition in high-light levels and low temperatures. The leaves of P contain anthocyanin (Table 1), which plays a light attenuation role and/or antioxidant role in protecting the photosystem (Zhang *et al.* 2019, Stetsenko *et al.* 2020). The protective effects of anthocyanin are related to its ability, *via* screening and internal light trapping, to reduce the amount of excessive solar radiation reaching the photosynthetic apparatus (Liakopoulos *et al.* 2006, Gitelson *et al.* 2009). Anthocyanins absorb mostly green light, they also absorb blue and red light, decreasing the absorption of Chl (Landi *et al.* 2021). In addition, a high content of Ant in young leaves is correlated with low pools of xanthophyll-cycle components (Manetas *et al.* 2002). In this study, P resisted photoinhibition whether it was a shading or a protective effect in this study.

Generally, F_v/F_m is lower at noon than at predawn (Demmig-Adams *et al.* 1996, Verhoeven *et al.* 1999, Santanoo *et al.* 2019, Yang *et al.* 2020, Acebron *et al.* 2021). However, the F_v/F_m of Y was higher at noon than predawn on 6 March. Fig. 1 shows that the temperature was as low as 7.5°C in the predawn on 6 March due to radiation cooling at night on 5 March. In Taiwan, air temperature varies considerably in the winter, with chilling temperatures (*i.e.*, < 10°C) coinciding frequently with bright mornings (*i.e.*, high incident sunlight) because of rapid heat dissipation by emission of long-wave radiation during clear nights. Nevertheless, air temperatures can increase to almost 25°C at midday in winter. Fig. 1*B* shows that 6 March was a clear sunny day, the temperature rose to 27.8°C at noon (Fig. 1*A*), and the F_v/F_m increased to the general level (corresponding to the F_v/F_m–PPFD regression shown in Fig. 3). This indicates that the depression of F_v/F_m is related to the reversible loss of the PSII function in a cold predawn (Long *et al.* 1994, Close *et al.* 2001).

Recovery of F_v/F_m at dusk: Afternoon PSII efficiency recovers gradually with decreasing light intensity, and reaches similar predawn values at sunset in warmer seasons (Verhoeven *et al.* 1999, Santanoo *et al.* 2019, Acebron *et al.* 2021). In this study, leaves were dark-treated at 17:00 h, although the sun had not yet set. F_v/F_m was still significantly negatively correlated with PPFD at this time (Fig. 4). The slope of the F_v/F_m–PPFD regression was Y > G > P. At high light and low temperature on 1 January and 5 March, the F_v/F_m of the three cultivars was lower than the regression line (Fig. 4). The differences between measured values and regression lines were Y > G > P. From the (*1*) slopes of the F_v/F_m–PPFD regressions and (*2*) differences between measured values and regression lines, the sensitivities of F_v/F_m to light intensity and low temperature at dusk were Y > G > P. These also indicated that the response of predawn F_v/F_m values to temperature was also Y > G > P (Fig. 1). However, the F_v/F_m of Y deviated downward while G and P did not at noon on 1 January and 5 March (Fig. 3).

Conclusion: The results of this study indicated that the pigment analysis showed that P leaves had the highest Chl (*a+b*) and carotenoid contents, while Y leaves exhibited significantly lower contents. Notably, Y leaves possessed the highest Chl *a/b* ratio. Predawn temperature fluctuations significantly impacted PSII efficiency (F_v/F_m) across the leaf types. F_v/F_m values decreased with lower predawn temperatures and increased with higher PPFD at noon. Y leaves demonstrated the most significant F_v/F_m variation, suggesting greater sensitivity to temperature and light changes than G and P leaves. A significant negative correlation between nonphotochemical quenching (NPQ) and F_v/F_m indicated that high light-induced PSII efficiency depression in sweet potato leaves was primarily due to xanthophyll-dependent NPQ, which dissipates excess energy as heat. The study also found that P leaves' F_v/F_m was the least affected under high light and low temperatures, likely attributable to the protective role of anthocyanin. Conversely, Y leaves showed an unusual increase in F_v/F_m at noon compared to predawn under specific conditions of cold predawns followed by bright, warmer noons, implying a reversible loss of PSII function. In summary, this research sheds light on the adaptive responses of sweet potato leaves to environmental stressors, emphasizing the intricate relationship between pigment content, temperature, light intensity, and PSII efficiency. These findings contribute to a deeper understanding of plant photoprotective mechanisms and offer valuable information for optimizing sweet potato cultivation practices.

References

Acebron K., Matsubara S., Jedmowski C. *et al.*: Diurnal dynamics of nonphotochemical quenching in *Arabidopsis npq* mutants assessed by solar-induced fluorescence and reflectance measurements in the field. – New Phytol. **229**: 2104-2119, 2021.

Adams W.W., Zarter C.R., Ebbert V., Demmig-Adams B.: Photoprotective strategies of overwintering evergreens. – BioScience **54**: 41-49, 2004.

Alemu S.T., Roro A.G: Ultraviolet-B, end of day light and exclusion effect on photosynthetic efficiency of sweet potato (*Ipomoea batatas* L.) based on altitude. – J. Hortic. Postharvest Res. **3**: 1-10, 2020.

Baker N.R.: Chilling stress and photosynthesis. – In: Foyer C.H., Mullineaux P.M. (eds.): Cause of Photooxidative Stress and Amelioration of Defense Systems in Plants. Pp. 127-154. CRC Press, New York 1993.

Björkman O., Demmig B.: Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. – Planta **170**: 489-504, 1987.

Blennow K., Lang A.R.G., Dunne P., Ball M.C.: Cold-induced photoinhibition and growth of seedling snow gum (*Eucalyptus pauciflora*) under differing temperature and radiation regimes in fragmented forests. – Plant Cell Environ. **21**: 407-416, 1998.

Bolhàr-Nordenkampf H.R., Long S.P., Baker N.R. *et al.*: Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. – Funct. Ecol. **3**: 497-514, 1989.

Chen Y.-S., Chesson P., Wu H.-W. *et al.*: Leaf structure affects a plant's appearance: combined multiple-mechanisms

intensify remarkable foliar variegation. – J. Plant Res. **130**: 311-325, 2017.

Close D.C., Beadle C.L., Hovenden M.J.: Cold-induced photoinhibition and foliar pigment dynamics of *Eucalyptus nitens* seedlings during establishment. – Aust. J. Plant Physiol. **28**: 1133-1141, 2001.

Corcuera L., Morales F., Abadía A., Gil-Pelegrín E.: Seasonal changes in photosynthesis and photoprotection in a *Quercus ilex* subsp. *ballota* woodland located in its upper altitudinal extreme in the Iberian Peninsula. – Tree Physiol. **25**: 599-608, 2005.

Cui M., Vogelmann T.C., Smith W.K.: Chlorophyll and light gradients in sun and shade leaves of *Spinacia oleracea*. – Plant Cell Environ. **14**: 493-500, 1991.

Demmig-Adams B., Adams W.W.: The role of xanthophyll cycle carotenoids in the protection of photosynthesis. – Trends Plant Sci. **1**: 21-26, 1996.

Demmig-Adams B., Adams W.W., Barker D.H. *et al.*: Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. – Physiol. Plantarum **98**: 253-264, 1996.

Demmig-Adams B., Ebbert V., Mellman D.L. *et al.*: Modulation of PsbS and flexible *vs* sustained energy dissipation by light environment in different species. – Physiol. Plantarum **127**: 670-680, 2006.

Genty B., Briantais J.-M., Baker N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – BBA-Gen. Subjects **990**: 87-92, 1989.

Gitelson A.A., Chivkunova O.B., Merzlyak M.N.: Nondestructive estimation of anthocyanins and chlorophylls in anthocyanic leaves. – Am. J. Bot. **96**: 1861-1868, 2009.

Gitelson A.A., Gritz Y., Merzlyak M.N.: Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. – J. Plant Physiol. **160**: 271-282, 2003.

Goh C.-H., Jang S., Jung S. *et al.*: Rice *phot1a* mutation reduces plant growth by affecting photosynthetic responses to light during early seedling growth. – Plant Mol. Biol. **69**: 605-619, 2009.

Gorbe E., Calatayud A.: Applications of chlorophyll fluorescence imaging technique in horticultural research: a review. – Sci. Hortic.-Amsterdam **138**: 24-35, 2012.

Gould K.S.: Nature's Swiss army knife: the diverse protective roles of anthocyanins in leaves. – J. Biomed. Biotechnol. **2004**: 314-320, 2004.

Hager A., Holocher K.: Localization of the xanthophyll-cycle enzyme violaxanthin de-epoxidase within the thylakoid lumen and abolition of its mobility by a (light-dependent) pH decrease. – Planta **192**: 581-589, 1994.

He J., Qin L.: Growth and photosynthetic characteristics of sweet potato (*Ipomoea batatas*) leaves grown under natural sunlight with supplemental LED lighting in a tropical greenhouse. – J. Plant Physiol. **252**: 153239, 2020.

Huang M.-Y., Wong S.-L., Weng J.-H.: Rapid light-response curve of chlorophyll fluorescence in terrestrial plants: relationship to CO_2 exchange among five woody and four fern species adapted to different light and water regimes. – Plants-Basel **10**: 445, 2021.

Huang W., Hu H., Zhang S.-B.: Photosynthesis and photosynthetic electron flow in the alpine evergreen species *Quercus guyavifolia* in winter. – Front. Plant Sci. **7**: 1511, 2016.

Jahns P., Latowski D., Strzalka K.: Mechanism and regulation of the violaxanthin cycle: The role of antenna proteins and membrane lipids. – BBA-Bioenergetics **1787**: 3-14, 2009.

Johnson M.P., Ruban A.V.: Restoration of rapidly reversible photoprotective energy dissipation in the absence of PsbS protein by enhanced ΔpH. – J. Biol. Chem. **286**: 19973-19981, 2011.

Kato M.C., Hikosaka K., Hirotsu N. *et al.*: The excess light energy that is neither utilized in photosynthesis nor dissipated by photoprotective mechanisms determines the rate of photoinactivation in photosystem II. – Plant Cell Physiol. **44**: 318-325, 2003.

Kitajima M., Butler W.L.: Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. – BBA-Bioenergetics **376**: 105-115, 1975.

Landi M., Agati G., Fini A. *et al.*: Unveiling the shade nature of cyanic leaves: a view from the 'blue absorbing side' of anthocyanins. – Plant Cell Environ. **44**: 1119-1129, 2021.

Lavaud J., Lepetit B.: An explanation for the inter-species variability of the photoprotective non-photochemical chlorophyll fluorescence quenching in diatoms. – BBA-Bioenergetics **1827**: 294-302, 2013.

Lazár D., Nauš J.: Statistical properties of chlorophyll fluorescence induction parameters. – Photosynthetica **35**: 121-127, 1998.

Lazar D., Stirbet A., Björn L.O., Govindjee G.: Light quality, oxygenic photosynthesis and more. – Photosynthetica **60**: 25-58, 2022.

Leegood R.C.: Effects of temperature on photosynthesis and photorespiration. – In: Smirnoff N (ed.): Environment and Plant Metabolism: Flexibility and Acclimation. Pp. 45-62. BIOS Scientific Publishers, Oxford 1995.

Li Q., Kubota C.: Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. – Environ. Exp. Bot. **67**: 59-64, 2009.

Li S., Zhao L., Sun N. *et al.*: Photosynthesis product allocation and yield in sweetpotato with different irrigation levels at mid-season. – Agr. Water Manage. **246**: 106708, 2021.

Li Y., Sun Y., Jiang J., Liu J.: Spectroscopic determination of leaf chlorophyll content and color for genetic selection on *Sassafras tzumu*. – Plant Methods **15**: 73, 2019.

Liakopoulos G., Nikolopoulos D., Klouvatou A. *et al.*: The photoprotective role of epidermal anthocyanins and surface pubescence in young leaves of grapevine (*Vitis vinifera*). – Ann. Bot.-London **98**: 257-265, 2006.

Lin H.-H., Lin K.-H., Jiang J.-Y. *et al.*: Comparisons between yellow and green leaves of sweet potato cultivars in chlorophyll fluorescence during various temperature regimes under high light intensities. – Sci. Hortic.-Amsterdam **288**: 110335, 2021.

Lin K.H., Huang M.Y, Weng J.H., Kao S.C.: Photosynthetic activity of red and green leaf sectors in *Coleus blumei* plants as sensed by chlorophyll fluorescence. – Photosynthetica **57**: 659-667, 2019.

Long S.P., Humphries S., Falkowski P.G.: Photoinhibition of photosynthesis in nature. – Annu. Rev. Plant Physiol. Plant Mol. Biol. **45**: 633-662, 1994.

Mancinelli A.L., Yang C.P.H., Lindquist P. *et al.*: Photocontrol of anthocyanin synthesis: III. The action of streptomycin on the synthesis of chlorophyll and anthocyanin. – Plant Physiol. **55**: 251-257, 1975.

Manetas Y., Drinia A., Petropoulou Y.D.: High contents of anthocyanins in young leaves are correlated with low pools of xanthophyll cycle components and low risk of photoinhibition. – Photosynthetica **40**: 349-354, 2002.

Maxwell K., Johnson G.N.: Chlorophyll fluorescence – a practical guide. – J. Exp. Bot. **51**: 659-668, 2000.

Müller P., Li X.-P., Niyogi K.K.: Non-photochemical quenching: A response to excess light energy. – Plant Physiol. **125**: 1558-

1566, 2001.

Nilkens M., Kress E., Lambrev P.: Identification of a slowly inducible zeaxanthin-dependent component of non-photochemical quenching of chlorophyll fluorescence generated understeady-state conditions in *Arabidopsis*. – BBA-Bioenergetics **1797**: 466-475, 2010.

Noh S.A., Park S.H., Huh G.H. *et al*.: Growth retardation and differential regulation of expansin genes in chilling-stressed sweetpotato. – Plant Biotechnol. Rep. **3**: 75-85, 2009.

Peng C.-L., Duan J., Lin G., Gilmore A.M.: Correlation between photoinhibition sensitivity and the rates and relative extents of xanthophyll cycle de-epoxidation in *chlorine* mutants of barley (*Hordeum vulgare* L.). – Photosynthetica **40**: 503-508, 2002.

Quick W.P., Stitt M.: An examination of factors contributing to non-photochemical quenching of chlorophyll fluorescence in barley leaves. – BBA-Bioenergetics **977**: 287-296, 1989.

Roháček K., Barták M.: Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. – Photosynthetica **37**: 339-363, 1999.

Santanoo S., Vongcharoen K., Banterng P. *et al*.: Seasonal variation in diurnal photosynthesis and chlorophyll fluorescence of four genotypes of cassava (*Manihot esculenta* Crantz) under irrigation conditions in a tropical savanna climate. – Agronomy **9**: 206, 2019.

Smirnoff N.: Ascorbic acid: metabolism and functions of a multifaceted molecule. – Curr. Opin. Plant Biol. **3**: 229-235, 2000.

Stetsenko L.A., Pashkovsky P.P., Voloshin R.A. *et al*.: Role of anthocyanin and carotenoids in the adaptation of the photosynthetic apparatus of purple- and green-leaved cultivars of sweet basil (*Ocimum basilicum*) to high-intensity light. – Photosynthetica **58**: 890-901, 2020.

Sun D., Wang Q.: Linear relationships between photosynthetic rate and photochemical energy expressed by PAR × F_v/F_m. – Am. J. Plant Sci. **9**: 125-138, 2018.

Tanaka Y., Ohmiya A.: Seeing is believing: engineering anthocyanin and carotenoid biosynthetic pathways. – Curr. Opin. Biotech. **19**: 190-197, 2008.

Verhoeven A.S., Adams W.W., Demmig-Adams B.: The xanthophyll cycle and acclimation of *Pinus ponderosa* and *Malva neglecta* to winter stress. – Oecologia **118**: 277-287, 1999.

Weng J.-H.: Underestimate of PS2 efficiency in the field due to high leaf temperature resulting from leaf clipping and its amendment. – Photosynthetica **44**: 467-470, 2006.

Weng J.-H., Chen Y.-N., Liao T.-S.: Relationships between chlorophyll fluorescence parameters and photochemical reflectance index of tree species adapted to different temperature regimes. – Funct. Plant Biol. **33**: 241-246, 2006.

Weng J.-H., Chien L.-F., Jiang C.-Y. *et al*.: A comparison between yellow-green and green cultivars of four vegetable species in pigments, ascorbate, photosynthesis, energy dissipation and photoinhibition. – Photosynthetica **49**: 361-370, 2011.

Weng J.-H., Liao T.-S., Sun K.-H. *et al*.: Seasonal variation in photosynthesis of *Picea morrisonicola* grown in sub-alpine of subtropical Taiwan. – Tree Physiol. **25**: 973-979, 2005.

Weng J.-H., Wong S.-L., Lin R.-J., Jhaung L.-H.: Quantitative effects of temperature and light intensity on PSII efficiency of mango leaves under artificial and natural conditions. – J. For. Res. **18**: 371-378, 2013.

Wong S.-L., Chen C.-W., Huang H.-W., Weng J.-H.: Using combined measurements of gas exchange and chlorophyll fluorescence to investigate the photosynthetic light responses of plant species adapted to different light regimes. – Photosynthetica **50**: 206-214, 2012.

Yang C.-M., Chang K.-W., Yin M.-H., Huang H.-M.: Methods for the determination of chlorophylls and their derivatives. – Taiwania **43**: 116-122, 1998.

Yang S.H., Wei J.J., Yan F. *et al*.: Differences in leaf anatomy, photosynthesis, and photoprotective strategies in the yellow-green leaf mutant and wild type of *Rosa beggeriana* Schrenk. – Photosynthetica **58**: 1167-1177, 2020.

Zhang Q., Zhai J., Shao L. *et al*.: Accumulation of anthocyanins: an adaptation strategy of *Mikania micrantha* to low temperature in winter. – Front. Plant Sci. **10**: 1049, 2019.