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A comparison between yellow-green and green cultivars of four vegetable species in pigments, ascorbate, photosynthesis, energy dissipation, and photoinhibition

J.-H. WENG[*, **, +], L.-F. CHIEN[**], C.-Y. JIANG[**], F.-C. SHIH[**], and H.-Y. CHEN[**]

Graduate Institute of Ecology and Evolutionary Biology, China Medical University, Taichung, Taiwan[*]
Department of Life Science, National Chung-Hsing University, Taichung, Taiwan[**]

Abstract

Yellow-green foliage cultivars of four vegetables grown outdoors, *i.e.*, Chinese mustard (*Brassica rapa*), Chinese kale (*Brassica oleracea* var. *alboglabra*), sweet potato (*Ipomoea batatas*) and Chinese amaranth (*Amaranthus tricolor*), had lower chlorophyll (Chl) (*a*+*b*) (29–36% of green cultivars of the same species), total carotenoids (46–62%) and ascorbate (72–90%) contents per leaf area. Furthermore, yellow-green cultivars had smaller photosystem II (PSII) antenna size (65–70%) and lower photosynthetic capacity (52–63%), but higher Chl *a/b* (107–156%) and from low (60%) to high (129%) ratios of de-epoxidized xanthophyll cycle pigments per Chl *a* content. Potential quantum efficiency of PSII (F_v/F_m) of all overnight dark-adapted leaves was *ca*. 0.8, with no significant difference between yellow-green and green cultivars of the same species. However, yellow-green cultivars displayed a higher degree of photoinhibition (lower F_v/F_m after illumination) when they were exposed to high irradiance. Although vegetables used in this study are of either temperate or tropical origin and include both C_3 and C_4 plants, data from all cultivars combined revealed that F_v/F_m after illumination still showed a significant positive linear regression with xanthophyll cycle-dependent energy quenching (q_E) and a negative linear regression with photoinhibitory quenching (q_I). F_v/F_m was, however, not correlated with nonphotochemical quenching (NPQ). Yet, a higher degree of photoinhibition in yellow-green cultivars could recover during the night darkness period, suggesting that the repair of PSII in yellow-green cultivars would allow them to grow normally in the field.

Additional key words: *Amaranthus tricolor*; ascorbate-deficient; *Brassica oleracea* var. *alboglabra*; *Brassica rapa*; chlorophyll-deficient; energy dissipation; *Ipomoea batatas*; photoinhibition; photosynthesis.

Introduction

Many higher plants have mutants that are depleted in Chl *a* and/or *b* and have light- or yellow-green foliage (*e.g.* Gilmore *et al.* 1996, Goh *et al.* 2009, Dall'Osto *et al.* 2010). While most of these mutants either do not survive or only grow very slowly, a few are able to photosynthesize and grow as rapidly as the wild type (Keck *et al.* 1970, Lin *et al.* 2003). In some Asian countries, yellow-green foliage varieties of Chinese mustard (*Brassica rapa* L. Chinensis Group), Chinese kale (*Brassica oleracea* L. var. *alboglabra* (Bailey) Musil) and Chinese amaranth (*Amaranthus tricolor* L.) are very popular and important vegetables. In addition, sweet potato [*Ipomoea batatas* (L.) Lam.] of green or yellow-green foliage is also cultured as leaf vegetable. They have lighter coloured homogeneous leaves when compared with the corresponding green cultivars. Some of them have been produced by seed company and could grow rapidly in the field (*e.g.* http://www.knownyou.com/en_index.jsp).

It has been known that in strong irradiance, leaves absorb more photons than they can utilize, and this excessively absorbed energy enhances the formation of reactive oxygen species (ROS) that could damage many cellular components, including photosystems (Demmig-

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[+]Corresponding author; fax: +886 4 22071507, e-mail: jhweng@mail.cmu.edu.tw

Abbreviations: A − antheraxanthin; Chl – chlorophyll; F_m − maximum fluorescence value of dark-adapted leaves; F_m' − maximum fluorescence value of light-exposed leaves; $F_m^d - F_m$ value measured at 2 min after dark recovery; $F_m^t - F_m$ value measured at 30 min after dark recovery; F_v/F_m − potential quantum efficiency of PSII; F_0 – minimal fluorescence value of dark-adapted leaves; NPQ − nonphotochemical quenching; PPFD − photosynthetic photon flux density; PSII − photosystem II; ROS – reactive oxygen species; q_E − xanthophyll cycle-dependent energy quenching; q_I − photoinhibitory quenching; q_T − state-transition quenching; V – violaxanthin; Z − zeaxanthin.

Adams and Adams 1992, Osmond and Grace 1995). Plants can employ several mechanisms, such as xanthophyll-dependent nonphotochemical quenching (NPQ), to dissipate the excess energy as heat (Demmig-Adams and Adams 1996, Dreuw *et al.* 2003, Jahns *et al.* 2009), and utilize antioxidants to reduce the oxidative stress caused by ROS from excessively absorbed energy (Smirnoff 2000). Within the xanthophyll cycle, violaxanthin (V) is de-epoxidized first into antheraxanthin (A) and then to zeaxanthin (Z) by violaxanthin de-epoxidase (Hager 1969). According to their dark relaxation kinetics, NPQ can be divided into at least three different components, namely, q_E, q_T, and q_I (Horton and Hague 1988, Müller-Moulé *et al.* 2001). Among them, q_E is the fastest component which relaxes within seconds to minutes (Müller-Moulé *et al.* 2001, Schansker *et al.* 2006). It is related to the pH of the lumen and sensitive to zeaxanthin. q_T, a state-transition quenching which relaxes within tens of minutes in vascular plants, was interpreted to represent the inactivation kinetics of ferredoxin-NADP+-reductase (Schansker *et al*. 2006). It is generally the smallest component of NPQ. q_I, with very slow relaxation in the range of hours, is photoinhibitory quenching which is caused by photoinhibition (Müller-Moulé *et al.* 2001).

It has been shown that ascorbate is a cofactor of violaxanthin de-epoxidase and an important antioxidant in chloroplasts (Hager 1969, Smirnoff 2000). Carotenoids are not only involved in the xanthophyll cycle, but can also prevent the harmful effects of singlet oxygen (Demmig-Adams and Adams 1996, Dreuw *et al.* 2003, Jahns *et al.* 2009). Mutants lacking carotenoids cannot survive exposure to even very low level of light (Sager and Zalokar 1958, Anderson and Robertson 1960). *Arabidopsis* mutants with lower ascorbate content are sensitive to high light, and exhibit limited NPQ of Chl fluorescence (Noctor *et al.* 2000, Müller-Moulé *et al.* 2002, 2004).

Reduction of Chl content reduces the ability of leaves to absorb photons. However, works on mutants lacking or deficient in Chls in a number of plant species have indicated that changes in components and organization of the light-harvesting apparatus could also change the efficiency with which absorbed photons are subsequently used in photosynthesis (Peng *et al.* 2002, Lin *et al.* 2003, Henriques 2008, Goh *et al.* 2009). Previous studies have found that some Chl-deficient mutants had lower carotenoids content and were more sensitive to high light than the wild type (Peng *et al.* 2002, Lin *et al.* 2003, Henriques 2008, Goh *et al.* 2009); yet, little or no such difference was observed in some other mutants (Peng *et al.* 2002). Chl-deficient mutants insensitive to high light have been found with high capacity of photo- and/or antioxidative protection (Peng *et al.* 2002, Lin *et al.* 2003, Štroch *et al.* 2004).

Previous studies on this subject used Chl-, carotenoid- or ascorbate-deficient mutants which could not grow as healthily or rapidly as wild types, especially under strong lighting (Sager and Zalokar 1958, Anderson and Robertson 1960, Müller-Moulé *et al.* 2004). There is hitherto no published work which investigates the effect of deficiency of all these pigments. Recently, we found that sweet potato cultivars with yellow-green foliage (Chl-deficient) had lower carotenoids and ascorbate contents than green-foliage cultivars (Jiang 2007). In the present study, yellow-green foliage cultivars of four vegetables growing normally in the field have been used as materials to elucidate the characteristics of photosynthesis, energy dissipation and photoinhibition as related to deficiency in Chl, carotenoid, and ascorbate.

Materials and methods

Plant materials: A green-foliage cultivar and a yellow-green foliage cultivar for each of four vegetables, *i.e.*, Chinese mustard, Chinese kale, sweet potato and Chinese amaranth, were used as materials. Detailed information of these cultivars is given in Table 1. Among them, Chinese mustard and Chinese kale are of temperate origin, and sweet potato and Chinese amaranth are of tropical origin. In addition, Chinese amaranth is a C_4 plant and the others are C_3 plants.

Sweet potato propagated from cuttings and the other three vegetables propagated from seeds were planted in 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.). During the growth period of the plants (Sept.–Oct. 2005), mean daily air temperature was about 26–27°C.

Measurements of photosynthesis and Chl fluorescence under artificial illumination: In October, about a month after sowing or cutting, photosynthesis, Chl fluorescence, leaf pigments and ascorbate of fully expanded youngest leaves were measured. Net CO_2 exchange rates were measured on attached fully expanded youngest leaves using a portable, open-flow gas-exchange system (*LI-6400, LI-COR Inc.*, Lincoln, NE, USA) with a LED light source (*6400-02, LI-COR Inc.*, Lincoln, NE, USA) under near saturating (1,500 μmol m^{-2} s^{-1}) photosynthetic photon flux density (PPFD), 25°C, 60–75% relative humidity and atmospheric CO_2 concentration (350–400 μmol mol^{-1}). Then the plants were dark-adapted overnight in a room (air temperature about 25°C). On the next day, Chl fluorescence of these plants was measured in a growth cabinet, and the surfaces of the same leaves used for photosynthesis measurement were illuminated with 1,000 μmol m^{-2} s^{-1} (sweet potato only) and

Table 1. Cultivars of four vegetables species used in this study.

Species	Foliage color	Cultivar
Chinese mustard (*Brassica rapa*)	Green	Yu-tsai-sum
	Yellow-green	Speedy (Funshan)
Chinese kale (*Brassica oleracea* var. *alboglabra*)	Green	Hei Chiehlan
	Yellow-green	Huang Chiehlan
Sweet potato (*Ipomoea batatas*)	Green	Taoyuan No.1
	Yellow-green	CH-1
Chinese amaranth (*Amaranthus tricolor*)	Green	Hunshien
	Yellow-green	Baishien

2,000 μmol m^{-2} s^{-1} (for all four species) PPFD at 10°C (for all four species), 25°C (Chinese kale and sweet potato only) or 35°C (Chinese kale and sweet potato only) for 30 min with cool light source (halogen light source plus optical fiber), followed by a 30-min dark recovery period at each measured temperature. Chl fluorescence was measured with a portable pulse amplitude-modulated fluorometer (*PAM-2000, Walz*, Effeltrich, Germany) immediately before illumination and every 2–5 min during illumination until the end of dark recovery. The potential quantum efficiency of PSII (F_v/F_m) was calculated from $(F_m - F_0)/F_m$. The light energy dissipated through NPQ was calculated from $F_m/F_m' - 1$, the light energy dissipated through formation of zeaxanthin from xanthophyll cycle (q_E) was calculated from $(F_m^d - F_m')/F_m'$, and photoinhibitory quenching (q_I) was calculated from $(F_m - F_m^t)/F_m'$. F_0, the minimal fluorescence of dark-adapted leaves, was determined by applying a weak pulse of red light [<0.1 μmol(quantum) m^{-2} s^{-1}]. F_m and F_m' are the maximum fluorescence values of dark-adapted and light-exposed leaves, respectively; these values were determined by applying a 1-s pulse of saturating flashes of approximately 6,000 μmol(quantum) m^{-2} s^{-1}. F_m^d and F_m^t are F_m values measured at 2 min and 30 min respectively after dark recovery (Demmig-Adams and Adams 1996, Müller-Moulé *et al.* 2002).

Pigments, ascorbate, and PSII antenna size: When measurements of gas exchange and Chl fluorescence were completed, the measured leaves were removed for determination of Chl and carotenoid contents. Three fresh leaf disks (0.84 cm^2) were extracted with 80% acetone and contents of Chl *a*, *b* and total carotenoids were determined by a spectrophotometer (*U-2000, Hitachi Ltd.*, Tokyo, Japan) using the absorbance at 440.5, 645, and 663 nm by the equations of Arnon (1949) and von Wettstein (1957).

In addition, ascorbate and PSII antenna size were determined using fully expanded youngest leaves harvested at predawn. Ascorbic acid and dehydroascorbate were extracted and quantified from samples of fully expanded youngest leaves. Frozen leaf material (1 g) was ground to fine powder in a mortar prechilled with liquid N_2, and 2 ml 10% (w/v) trichloroacetic acid was added to the homogenate. After centrifugation for 15 min at 13,000 × *g* (4°C), the supernatant was transferred to a new reaction vessel on ice for immediate assays of ascorbic acid and dehydroascorbate (Kampfenkel *et al.* 1995).

Antenna size of PSII was estimated from the total area enclosed between the fluorescence induction curve, the vertical axis at time zero, and the maximal fluorescence (F_m) horizontal line of DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea]-poisoned leaves (Malkin *et al.* 1981, Maury *et al.* 1993). Detached fully expanded youngest leaves were infiltrated with 50 mM DCMU for 30 min in darkness. DCMU was initially dissolved in a small amount of ethanol, and then diluted in water containing 0.1% Tween 20 (Yi *et al*. 2005). The area over the fluorescence induction curve was detected using a *Handy PEA* fluorometer (*Plant Efficiency Analyzer*; *Hansatech Ltd.*, King's Lynn, Norfolk, UK) run by a *Handy PEA* software (Aksmann and Tukaj 2008) under red actinic light intensity of 3,000 μmol m^{-2} s^{-1} (Yusuf *et al*. 2010).

Leaves for analysis of xanthophyll cycle pigments were harvested at noon of a clear day, and rapidly frozen by liquid N_2 and stored at –80°C until use. Frozen samples of 20–30 cm^2 were homogenized in a mortar prechilled with liquid N_2, and pigments were extracted with 5 ml of pure acetone. After centrifugation (30,000 × *g*, 4°C for 30 min), pigments were quantified by high-pressure liquid chromatography (*HPLC, L-7100* and *L-7200, Hitachi Ltd.*, Tokyo, Japan) adopted from Gilmore and Yamamoto (1991).

Chl fluorescence under midday sunlight: From October to January, F_v/F_m of Chinese kale and sweet potato was measured every 1 to 5 days at noon. The potted materials were put outdoors to receive full sunlight until noon, and then they were moved to a darkroom without air-conditioning for 30 min to avoid an underestimation of F_v/F_m, since a large F_0 value could result from the high leaf temperature when the leaf was clipped under high illumination (Weng 2006). Chl fluorescence was measured by a fluorometer (*PAM-2000*).

Air temperature and PPFD were measured by copper-

constantan thermocouples and *LI-190SA* sensor (*LI-COR Inc.*, Lincoln, NE, USA), respectively. The sensors were connected to a data-logger (*CR10, Campbell Scientific Inc.*, Logan, UT, USA), and data were collected automatically every 2 min and the averaged values of each hour were recorded.

Statistics: Four leaves were measured in each treatment, and data from each leaf was taken as one replicate in statistical analyses. Data were analyzed by unpaired t-test or linear regression (*SigmaPlot version 9.01; Systat Software, Inc.*, Point Richmond, CA, USA).

Results

When compared with the corresponding green cultivars, yellow-green cultivars of all four species contained less Chl (*a*+*b*) (29–36% of green cultivars in the same species), Chl *a* (31–37%), Chl *b* (20–32%), total carotenoids (46–62%) and ascorbate (72–90%) per leaf area. Furthermore, yellow-green cultivars had smaller PSII antenna size (65–70%) and lower photosynthetic capacity (52–63%). However, they had higher Chl *a*/*b* (107–156%) and PSII antenna size/Chl *a* (189–209%) ratios, and had low (60%) to high (129%) ratios of de-epoxidized xanthophyll cycle pigments (A+Z) per Chl *a* content. In addition, the yellow-green cultivars had (A+Z)/(V+A+Z) ratios close to 91–97% of their corresponding green cultivars, except that the yellow-green cultivars of sweet potato showed a lower ratio (56%) [Table 2, Chl *a* and *b* contents could be calculated from Chl (*a*+*b*) contents and Chl *a*/*b* ratios].

F_v/F_m of all overnight dark-adapted leaves was *ca*. 0.8, with no significant difference between yellow-green and green cultivars of the same species (Fig. 1). However, when leaves were treated with artificial illumination, F_v/F_m decreased with the decline of temperature and the increase of light intensity. Under high irradiance (2,000 μmol m^{-2} s^{-1}), all tested yellow-green cultivars measured at 10, 25, and 35°C showed significantly lower F_v/F_m than the green foliage cultivars of the same species. Under medium irradiance (1,000 μmol m^{-2} s^{-1}), only sweet potatoes were measured and there was a significant difference between yellow-green and green cultivars only at 15°C, but not at 25 and 35°C. When data obtained from different levels of temperature and irradiance are merged, the difference of F_v/F_m between green and yellow-green cultivars of the same species was higher at lower temperature and higher irradiance (Fig. 1).

At outdoors, in spite of a higher variation of air temperature (13–29°C at noon), F_v/F_m at noontime showed a significant (p<0.001) negative linear (both cultivars of sweet potato and green Chinese kale) or curve-linear (yellow-green Chinese kale) correlation with PPFD (Fig. 2). At low level of irradiance, both green and yellow-green cultivars could maintain a high level of F_v/F_m. However, the slope of the F_v/F_m-PPFD regression line was steeper in yellow-green foliage cultivars for low to high (sweet potato) or at high (Chinese kale) irradiance. Therefore, there was no significant difference in F_v/F_m between green and yellow-green foliage cultivars at low level of irradiance. But at higher irradiance, *i.e.* PPFD>250 μmol m^{-2} s^{-1} for sweet potato, and PPFD> 1,000 μmol m^{-2} s^{-1} for Chinese kale, yellow-green foliage

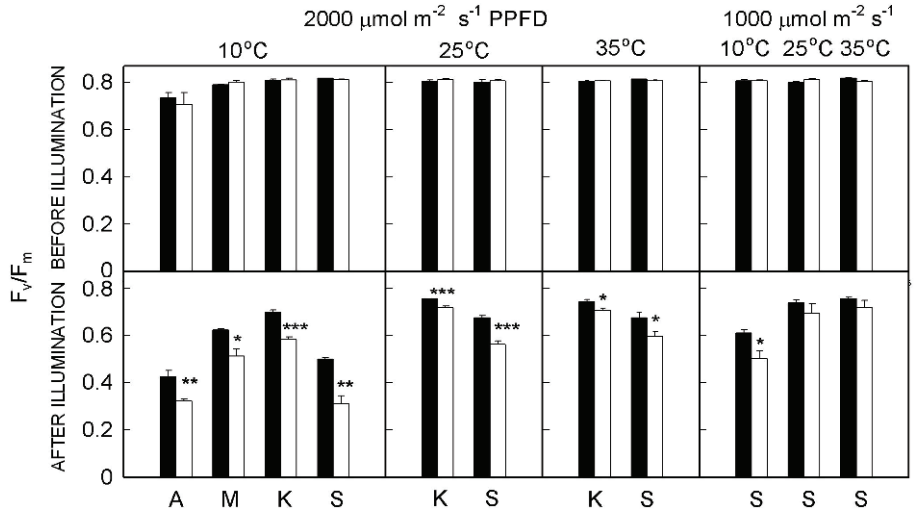

Fig. 1. The potential quantum efficiency of PSII (F_v/F_m, obtained before illumination and after 30 min of artificial illumination and a subsequent dark recovery for 30 min) for green (■) and yellow-green (□) foliage cultivars of 4 vegetable species at varied light intensity and temperature. A – Chinese amaranth; M – Chinese mustard; K – Chinese kale; S – sweet potato. *Vertical bars* indicate standard errors (n = 4); *, ** and *** – significant differences between green and yellow-green foliage cultivars of the same species at p<0.05, p<0.01 and p<0.001, respectively, based on unpaired t-test.

Table 2. Leaf chlorophyll (Chl), carotenoid, relative content of de-epoxidized xanthophylls [(A+Z)/(V+A+Z) and (A+Z)/Chl *a* at noon of a clear day] and ascorbate contents, as well as antenna size and photosynthetic capacity (P_N, measured under 1,500 μmol m^{-2} s^{-1} PPFD) of green (G) and yellow-green (YG) foliage cultivars of 4 vegetable species. *, ** and *** – significant differences between green and yellow-green foliage cultivars of the same species at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, based on unpaired *t*-test. Data are means ± standard errors, $n = 4$ leaves.

Species	Foliage color	Chl (*a*+*b*) [g m^{-2}]	Chl *a*/*b*	Total carotenoids [g m^{-2}]	Ascorbate [μmol g^{-1}]	(A+Z)/(V+A+Z)	(A+Z)/Chl *a* [mmol mol^{-1}]	P_N [μmol m^{-2} s^{-1}]	Antenna size	Antenna size/Chl *a*
Sweet potato	G	0.337±0.024(100)	3.20±0.07(100)	0.060±0.002(100)	7.01±0.50(100)	0.718±0.017(100)	128.4±10.5(100)	22.18±3.08(100)	217.4± 6.9(100)	847(100)
	YG	0.096±0.007(29)**	4.99±0.24(156)**	0.037±0.002(62)**	5.04±0.34(72)**	0.401±0.051(56)**	134.0±22.4(105)ns	13.66±2.89(62)**	141.3± 8.3(65)**	1,767(209)
Chinese kale	G	0.307±0.026(100)	2.65±0.11(100)	0.062±0.004(100)	20.32±1.33(100)	0.832±0.080(100)	158.2±37.8(100)	17.62±2.73(100)	433.8±11.4(100)	1,946(100)
	YG	0.104±0.011(34)**	2.83±0.21(107)ns	0.034±0.002(55)**	16.68±1.16(82)*	0.809±0.067(97)ns	203.4±26.2(129)ns	9.12±1.69(52)**	290.5± 5.9(67)**	3,780(194)
Chinese mustard	G	0.278±0.019(100)	3.14±0.10(100)	0.059±0.002(100)	11.79±0.48(100)	0.878±0.036(100)	132.2±11.4(100)	18.73±2.14(100)	265.8±16.7(100)	1,261(100)
	YG	0.099±0.003(36)**	3.70±0.24(118)*	0.036±0.001(61)**	8.53±0.69(72)**	0.840±0.040(94)ns	139.3±17.3(105)ns	11.78±1.94(63)**	186.0±13.3(70)**	2,387(189)
Chinese amaranth	G	0.291±0.031(100)	2.52±0.25(100)	0.078±0.006(100)	26.36±0.57(100)	0.942±0.010(100)	133.1± 4.9(100)	27.57±4.87(100)	196.7±13.0(100)	944(100)
	YG	0.094±0.013(32)**	3.75±0.45(149)**	0.036±0.003(46)**	23.59±0.39(90)*	0.857±0.027(91)*	79.8± 3.2(60)***	16.32±3.21(59)**	136.8±16.3(70)**	1,843(195)

cultivars showed significantly lower F_v/F_m than green foliage cultivars, and the difference in F_v/F_m between green and yellow-green foliage cultivars increased with increasing irradiance (Fig. 2).

Under artificial illumination, NPQ values of yellow-green cultivars of Chinese amaranth, Chinese mustard and Chinese kale were higher than those of their green cultivars (Fig. 3*I–L*). For sweet potato, there was no significant difference in NPQ between the green and yellow-green cultivars at 25°C and 35°C, but yellow-green cultivars showed lower NPQ than green-foliage cultivars at 10°C. When compared with the green cultivars of the same species, four yellow-green cultivars always showed either higher q_I or lower q_E, or both (Fig. 3*A–H*). When data from all tested species, illumination and temperature were merged, F_v/F_m after illumination showed a significant positive linear regression with q_E (r^2 = 0.613, p<0.001) and a negative one with q_I (r^2 = 0.613, p<0.001); F_v/F_m, however, was not correlated with NPQ (r^2 = 0.168, p>0.05) (Fig. 4).

Discussion

There are reportedly two types of Chl mutants. In one mutant type, synthesis of both Chl *a* and *b* was restricted and in another type, all Chl *b* synthesis was inhibited (Gilmore *et al.* 1996, Lin *et al.* 2003). Results of the present study indicate that Chl *a* and *b* contents of yellow-green cultivars of 4 vegetable species were 31–37% and 20–32%, respectively, of those of the corresponding green-foliage cultivars [Chl *a* and *b* contents could be calculated from Chl (*a*+*b*) contents and Chl *a*/*b* ratios shown in Table 2]. This implies that the 4 yellow-green cultivars tested in the present study were depleted of both Chl *a* and *b* contents. The physiological characteristics of this type of Chl mutants have been studied widely, and showed polymorphism among mutants. For example, when compared to the wild type, barley *chlorina* mutant f_{104} had about 50% less Chl (*a*+*b*); it showed little difference in thermal dissipation and photoinhibition. The mutant contained a high level of de-epoxidation states of xanthophylls, and required around 2.5 times higher concentration of these xanthophylls relative to Chl (*a*+*b*) to obtain the same levels of xanthophyll cycle-dependent fluorescence quenching (Gilmore *et al.* 1996, Peng and Glimore 2002). A Syrian barley landrace, Tadmor, had about 30% less in the Chl (*a*+*b*) and carotenoid contents; it had a higher ability of converting violaxanthin to zeaxanthin, and a lower degree of photoinhibition in strong light (Tardy *et al.* 1998). A Chl-deficient rice mutant showed a lower photon absorption rate, and a stronger xanthophyll cycle capacity and a lower degree of photoinhibition (Dai *et al.* 2003). On the other hand, some Chl-deficient rice mutants displayed a higher degree of photoinhibition, either having lower abilities of thermo-dissipation and antioxidative protection (Goh *et al.* 2009) or a higher level of de-epoxidation states of xanthophylls (Chen *et al.* 2008). The results of the present study indicated that, although vegetables used in this study are of either temperate or tropical origin and include C_3 and C_4 plants, 4 yellow-green cultivars showed the same tendency on changes in energy dissipation and photoinhibition (Figs. 1–3). However, these results are not entirely consistent with other reported Chl-deficient mutants. In addition, even though yellow-green cultivars displayed a higher degree of photoinhibition when they were exposed to high irradiance, they could recover during the night darkness period (Fig. 2). These physiological characteristics may probably explain why the yellow-green cultivars could grow normally in the field.

It has been reported that several Chl mutants could change their efficiency of energy dissipation and antioxidative protection in order to adapt to high irradiance (Peng *et al.* 2002, Štroch *et al.* 2004, Chen *et al.* 2008, Goh *et al.* 2009).

The same tendency has also been found in mutants with low ascorbate content (Noctor *et al.* 2000, Müller-Moulé *et al.* 2002, 2004). Moreover, the size of light-harvesting complex as well as the capacity of photo- and

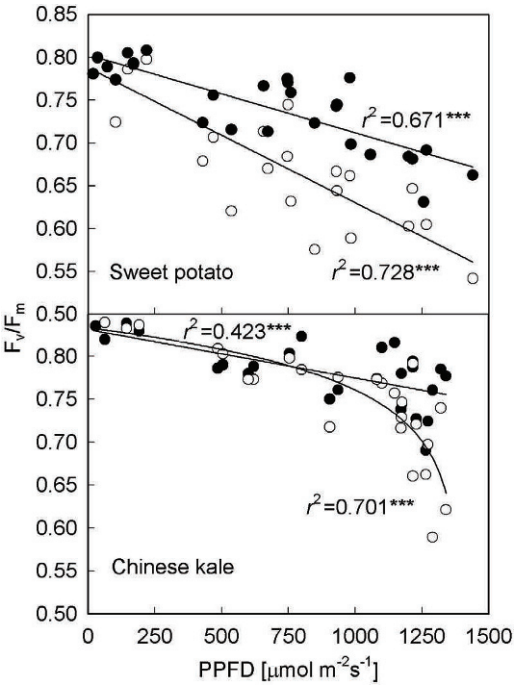

Fig. 2. Effect of sunlight at noon (averaged PPFD from 11:00–12:00 h) on potential quantum efficiency of PSII (F_v/F_m) of green (●) and yellow-green (○) foliage cultivars in both sweet potato and Chinese kale. *** – p<0.001. Significant differences between green and yellow-green foliage cultivars for sweet potato when PPFD>250 μmol m^{-2} s^{-1} (p<0.001), and for Chinese kale when PPFD>1,000 μmol m^{-2} s^{-1} (p<0.01), based on unpaired *t*-test.

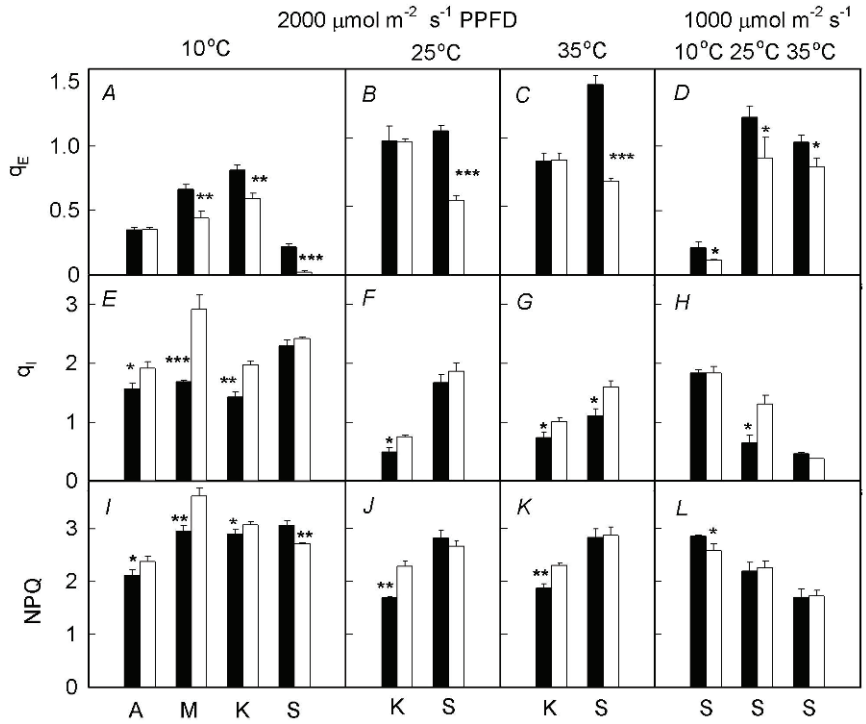

Fig. 3. Xanthophyll cycle-dependent energy quenching (q_E), photoinhibitory quenching (q_I) and nonphotochemical quenching (NPQ) for green (■) and yellow-green (□) foliage cultivars of 4 vegetable species at varied illumination and temperature for 30 min. A – Chinese amaranth; M – Chinese mustard; K – Chinese kale; S – sweet potato. *Vertical bars* indicate standard errors (n = 4); *, **, and *** – significant differences between green and yellow-green foliage cultivars of the same species at p<0.05, p<0.01 and p<0.001, respectively, based on unpaired t-test.

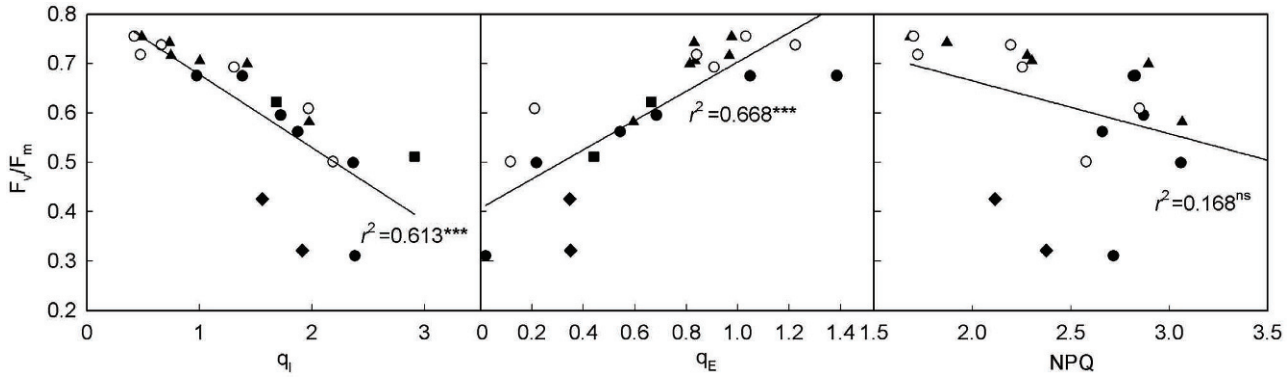

Fig. 4. The relationships of the degree of photoinhibition (F_v/F_m, obtained at artificial illumination for 30 min and then dark recovery for 30 min) to xanthophyll cycle-dependent energy quenching (q_E), photoinhibitory quenching (q_I) and nonphotochemical quenching (NPQ) (data from all tested species and cultivars at varied illumination and temperature were merged). ◆ – Chinese amaranth; ■ – Chinese mustard; ▲ – Chinese kale; ○ and ● – sweet potato (*open* and *closed symbols* indicate measurements at 1,000 and 2,000 μmol m^{-2} s^{-1} PPFD, respectively). *** and ns – p<0.001 and p>0.05, respectively.

antioxidative protection can be modulated in response to changes of lighting in the environment (Kurasová *et al.* 2002, Štroch *et al.* 2004, Chen *et al.* 2008). Therefore, light sensitivity of some Chl- deficient mutants varied with the light condition of growth (Štroch *et al.* 2004).

In the present study, plants were placed outdoors to receive full sunlight. Results indicated that yellow-green cultivars exposed to high irradiance, especially at low temperature, showed a higher degree of photoinhibition (lower F_v/F_m after illumination) than the corresponding green cultivars (Figs. 1–2). Photoinhibition is known to be due to the formation of reactive oxygen species, which is enhanced by excessively absorbed energy, and both high irradiance and low temperature in turn enhance the absorption of excess energy by the plants (Baker 1994, Leegood 1995). Plants could dissipate excess energy and

enhance antioxidative protection to avoid photoinhibition (Demmig-Adams and Adams 1996, Müller-Moulé *et al.* 2004). As described in the introduction, carotenoids and ascorbate are important for energy dissipation and antioxidative protection. Reports have pointed out that mutants with low ascorbate content (Noctor *et al.* 2000, Müller-Moulé *et al.* 2002, 2004), or both low Chl and carotenoid contents (Goh *et al.* 2009) have somewhat reduced NPQ, because of the limitation of violaxanthin to zeaxanthin conversion in conditions of excess light. When compared to green cultivars in the same species, four tested yellow-green cultivars all possessed 46–62% in total carotenoids and 72–90% in ascorbate contents of leaf area level. However, only yellow-green sweet potato had lower (A+Z)/(V+A+Z) ratio (Table 2), and also showed lower NPQ than the green-foliage cultivar at low temperature (Fig. 3*I–L*). The variation of (A+Z)/(V+A+Z) and NPQ of the other three yellow-green cultivars is not in agreement with the results obtained from sweet potato and the pigment mutants mentioned above. It is probable that the higher total carotenoids (130–198%) and ascorbate (196–251%) contents per Chl *a* in the yellow-green cultivars did not pose a limit to the violaxanthin to zeaxanthin conversion.

Although three yellow-green cultivars (Chinese mustard, Chinese kale and Chinese amaranth) showed higher NPQ than, and had (A+Z)/(V+A+Z) ratio close to, their corresponding green cultivars (Table 2 and Fig. 3), all four tested yellow-green cultivars showed a higher degree of photoinhibition than the corresponding green cultivars when they were exposed to high irradiance (Figs. 1, 2). What are the possible causes for this inconsistency? One could be due to the fact that yellow-green cultivars had high PSII antenna size/Chl *a* ratio (189–209%, Table 2) so that they may receive more light energy per reaction center. The other cause was possibly related to the components of NPQ. Among the three components of NPQ, only q_E is related to the xanthophyll cycle-dependent energy quenching, while q_I is photoinhibitory quenching and q_T is a state-transition quenching (Müller-Moulé *et al.* 2001, Kalituho *et al.* 2007). When data from all the tested species, illumination and temperature were merged, the degree of photoinhibition after illumination (F_v/F_m) was closely related to q_E and q_I, but not to NPQ (Fig. 4). These results indicate that, taking into consideration of different effects of temperature and light intensity on the species and cultivars, the degree of photoinhibition was still closely related to xanthophyll cycle-dependent energy quenching and photoinhibitory quenching. Moreover, with NPQ mainly comprising of q_E and q_I, the values of q_E and q_I were reciprocal to each other ($r^2 = 0.632$, $p<0.001$). Therefore, NPQ did not parallel with the energy dissipated through de-epoxidation of xanthophyll cycle pigments, and F_v/F_m showed lower regression with NPQ ($r = –0.410$, $p>0.05$).

When compared with the green cultivars of the same species, four yellow-green cultivars always showed either lower q_E or higher q_I, or both (Fig. 3*A–H*), suggesting that yellow-green cultivars always had lower ability of xanthophyll cycle-dependent energy quenching and higher level of photoinhibitory quenching. This result is not consistent with the observations that (*1*) only yellow-green sweet potato showed lower (A+Z)/(V+A+Z) ratio, and (*2*) (A+Z)/Chl *a* ratio of four tested yellow-green cultivars varied with species (60%–129%) (Table 2). Previous reports have demonstrated that energy dissipation is influenced by both pigments and proteins. For instance, binding of zeaxanthin to PsbS is one of the key factors in controlling the dissipation of excitation energy (Kalituho *et al*. 2007, Dall'Osto *et al*. 2010). The inconsistency between energy dissipation and xanthophyll cycle pigments contents is probably due to the effect of proteins. However, this possibility was not examined in our study, but would certainly warrant future investigation. Moreover, Müller-Moulé *et al*. (2004) pointed out that under high irradiance ascorbate-deficient *Arabidopsis* mutant had a higher level of glutathione, an antioxidant, than the wild type; the higher glutathione content might provide a possible compensation for the lower ascorbate content. Since only ascorbate and carotenoids were analyzed in the present study, the contribution of other antioxidants is still unknown.

The results of the present study indicated that the four yellow-green, carotenoid- and ascorbate-deficient vegetable cultivars all showed a higher degree of photoinhibition than the corresponding green cultivars. Moreover, these yellow-green cultivars always showed either lower xanthophyll cycle-dependent energy quenching (q_E) or higher photoinhibitory quenching (q_I), or both. However, probably due to the effects of the proteins, the energy dissipation and photoinhibition data of these cultivars are inconsistent with the findings of their xanthophyll cycle pigment components as well as their per Chl *a* level of total carotenoids and ascorbate contents. In addition, a higher degree of photoinhibition in yellow-green cultivars due to high irradiance could recover during the night darkness period. It is suggested that the repair of PSII in yellow-green cultivars could allow them to grow normally in the field.

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