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Relationship between flavonoids and photoprotection in shade-developed *Erigeron breviscapus* transferred to sunlight

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Abstract

Flavonoids are thought to participate in protection of the photosynthetic apparatus against photoinhibition under excessive light. Flavone glycoside, scutellarin, is a main active ingredient extracted from *Erigeron breviscapus*, the plant used in Chinese medicine. Shade-developed leaves of *E. breviscapus* were transferred from shade to full sunlight to quantify a relationship between the concentration of leaf scutellarin and tolerance to high radiation stress or the recovery from photoinhibition. The maximal quantum yield of PSII photochemistry showed a diurnal fluctuation in both shaded and sunlit leaves throughout the day. It indicated dynamic photoinhibition in the leaves of *Erigeron*, *i.e.*, higher photoinhibition at solar noon and lower one in the morning and late afternoon. The sun-developed leaves reached the higher scutellarin content and values of nonphotochemical quenching coefficient with a lower degree of photoinhibition than the shade-developed leaves. When the shade-developed leaves were transferred to full sunlight, the content of scutellarin was declining continuously for 10 d and then was increasing for 15 d. After 50 d, all leaves became the sun-developed leaves with their scutellarin contents of about 138.5 ± 5.2 mg g^{-1}(dry mass, DM) which was significantly higher than that of the shade-developed leaves [107.8 ± 9.8 mg g^{-1}(DM)]. During acclimatization, the degree of photoinhibition was negatively correlated with the scutellarin content. Our results demonstrated a synchronous fluctuation between the flavonoid content and degree of protection against photoinhibition.

Additional key words: chlorophyll fluorescence; flavonoid; phenolics; protection; photodamage.

Introduction

Secondary metabolites have been long recognized to play multiple roles in defense mechanisms of higher plants against pathogens and predators (Dixon and Paiva 1995, Harborne and Williams 2000, McNally *et al.* 2003). Based on this, numerous theories have been proposed for observed variations in a type and amount of secondary metabolites produced by plants under different environmental conditions (Berenbaum 1995, Koricheva 2002), such as, *e.g.*, the resource availability hypothesis (Coley *et al.* 1985, Bazzaz *et al.* 1987) and the carbon-nutrient balance (CNB) hypothesis (Bryant *et al.* 1983, 1991). Further, secondary metabolites in plants are also known to offer protection from ultraviolet radiation or to be carbon sinks that absorb excess photosynthetic carbon (*i.e.*, when plants experience stress, the synthesized carbon from photosynthesis surpasses growth needs and is used for the synthesis of secondary metabolites) (Waterman *et al.* 1984, Reuber *et al.* 1996, Bassman 2004). More recently, flavonoids, which are phenolic secondary metabolites, have been hypothesized to perform antioxidant functions in leaves exposed to high sunlight (Close and McArthur 2002, Majer *et al.* 2014).

Under high light conditions, the light energy absorbed exceeds photosynthetic capacity. Even the most rapidly growing plants with the highest rates of photosynthesis do not use more than about half of the light absorbed by their leaves at noon (Björkman and Demmig 1987, Demmig-Adams and Adams 1996a). The excess energy absorbed causes the formation of highly reactive chemical species, particularly triplet chlorophyll (Chl), singlet oxygen, and hydroxyl radicals (Niyogi 1999). These oxidizing molecules can cause oxidative damage to the photosynthetic apparatus (Asada 1994, Foyer and Harbinson 1994), which decreases the efficiency and/or

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Abbreviations: Chl – chlorophyll; CNB – carbon-nutrient balance; DAT – days after transfer; DM – dry mass; F_m – maximal fluorescence yield of the dark-adapted state; F_v – variable fluorescence; F_v/F_m – maximal quantum yield of PSII photochemistry; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; SH – shade treatment; SU – sunlit treatment.
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maximum rate of photosynthesis (*i.e.*,photoinhibition) (Niyogi 1999). Plants developed various photoprotective mechanisms to avoid photoinhibition and protect from damage. Some plants produce a range of antioxidant molecules and scavenging enzymes that can quench reactive chemical species, thereby minimizing potential photodamage. These compounds include ascorbate (Conklin *et al.* 1996, 1997), carotenoids (Frank and Cogdell 1993, Demmig-Adams and Adams 1996b, Havaux *et al.* 1998), and enzymes superoxide dismutases (Asada 1994). The addition of antioxidants *in vitro* decreased the extent of photoinactivation (Barényi and Krause 1985, Tschiersch and Ohmann 1993, Tjus *et al.* 2001) and minimized the potential for photodamage in *Euglena gracilis* and *Spinacia oleracea* (Tschiersch and Ohmann 1993, Tjus *et al.* 2001). Moreover, a lack of antioxidants was associated with a higher sensitivity to light treatments (Danna *et al.* 2003). Flavonoids have been shown to scavenge effectively free radicals both *in vitro* (Rice-Evans *et al.* 1996, Yamasaki *et al.* 1997, Gardner *et al.* 1998) and *in vivo* (Pérez *et al.* 2002, Agati *et al.* 2007), and are reported to act as physiological antioxidants in plants exposed to excess light (Rice-Evans *et al.* 1996, Gould and Lister 2006, Agati *et al.* 2007, 2009, 2013). However, there is a lack of studies on the relationship between the concentration of leaf flavonoids and tolerance to high radiation stress or the recovery from photoinhibition.

The ratio of variable fluorescence (F_v) to maximal fluorescence yield of the dark-adapted state (F_m), *i.e.*, F_v/F_m, represents the maximal quantum yield of PSII photochemistry in a dark-acclimated state. A decline in F_v/F_m indicates a reduction in the quantum yield of PSII photochemistry and a disturbance or damage to the photosynthetic apparatus (Demmig-Adams and Adams 1992, Bertamini and Nedunchezhian 2004). Therefore, F_v/F_m is widely used to estimate the degree of PSII photoinhibition or damage of plants exposed to high light (Jifon and Syvertsen 2003, Bertamini and Nedunchezhian 2004).

In the shade-developed leaves of *Fagus crenata* and seedlings of three tropical rain forest species, *Garcinia*, the F_v/F_m decreased after sudden exposure to high light during the first 2–7 d followed by a subsequent increase up to about 30 d (Guo *et al.* 2006, Naramoto *et al.* 2006). This suggested that the capacity to protect or recover (PSII repair) from photoinhibition could increase gradually with plant acclimatization.

If flavonoids participate in photoprotection, plants that contain flavonoids in leaves should show a synchronous dynamic trend in the leaf flavonoid content similar to F_v/F_m during the process of acclimation from shade to full sunlight. In addition, the flavonoid content should be negatively correlated with the degree of photoinhibition. If synchronous fluctuations cannot be observed, we must rethink the hypothesized relationship between flavonoids and photoprotection.

Erigeron breviscapus (vant.) Hand-Mazz (Asteraceae), also known as dengzhanxixin, is used in Chinese medicine to treat cardiovascular and cerebral vessel diseases and is officially listed in the Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission 2005). The active components identified from this herb include flavonoids, caffeoyl derivatives, coumarins, and erigesides (Zhang *et al.* 2007). Among these, scutellarin, which is a flavone glycoside, is the most abundant flavone (Chinese Pharmacopoeia Commission 2005, Zhang *et al.* 2007) and it has been reported to scavenge reactive oxygen *in vitro* (Liu *et al.* 2002).

This study tested the hypothesis that flavonoids play a role in protection against photoinhibition. For this purpose, the relationship between the concentration of leaf scutellarin and the capacity of protection against photoinhibition was investigated during the acclimation process of shade-developed *E. breviscapus* transferred from shade to full sunlight. The degree of photoinhibition and the concentration of scutellarin were expected to fluctuate synchronously, but with contrasting trends.

Materials and methods

Study site: This study was carried during the spring (April–May) in 2011 at Kunming, Southwest China. The climate in this region is dominated by the southwest monsoon from the Indian Ocean. Mean temperatures were 17.3 and 19.2°C and mean precipitations were 31.2 and 29.1 mm in April and May, respectively (data from CMA Public Meteorological Service Center).

Exposure to shade was achieved by using a shade screen, which transmits only about 30% of incident light. Photosynthetic photon flux density (PPFD) was measured with a *LiCor* quantum sensor (*LI-COR Inc.*, Lincoln, NE, USA) from 08:00 to 18:00 h on three typical sunny days at the study site (Fig. 1). Sensors were placed under the shade screen and in full sunlight.

Plant material and treatments: About 200 plantlets with two or four leaves, which were produced from a single seedling by tissue culture, were transplanted into pots (0.12 m diameter, 0.15 m high) containing soil obtained from the field, with one plantlet per pot. Among these, 170 plantlets were placed under the shade screen with about 30% sunlight, and 30 plantlets were placed under full sunlight (sunlit treatment, SU). The pots were watered 3–4 times per week to maintain steady soil moisture. A slow-release chemical fertilizer (N:P:K = 10:10:5) was applied at the rate of 2 g per pot every 30 d. The pots were also rotated randomly every 14 d. After 60 d, 150 pots were moved from the shade screen to full sunlight (transferred treatment), while 20 pots were left under the shade screen (shade treatment, SH).

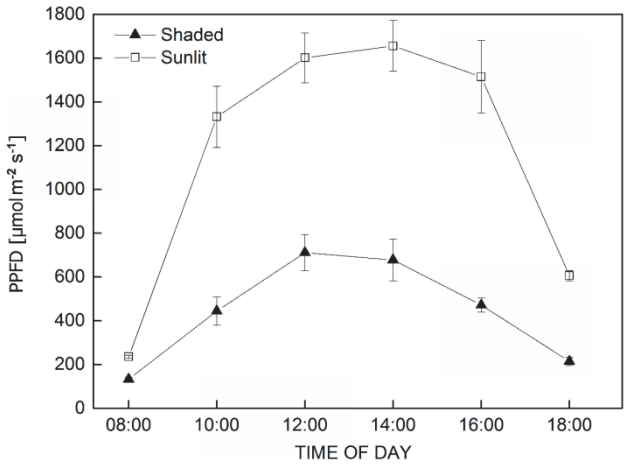

Fig. 1. Daily course of photosynthetic photon flux density (PPFD, μmol m^{-2} s^{-1}) under sun (*open square*) or shade (*closed triangles*) conditions at the study site in three typical sunny days (mean ± SD, n = 3), measured with a *LiCor* quantum sensor.

Extraction and analyses of scutellarin: Scutellarin was extracted according to the protocol from the Pharmacopoeia (Chinese Pharmacopoeia Commission 2005). All leaves from *E. breviscapus* were dried at 80°C, weighed, and powdered using a mortar and pestle. About 40–50 mg of leaf powder was extracted with 20 mL of 50% methanol for 25 min in an ultrasonic bath. The solution was filtered and 1 mL of the extract was added to 10 mL of 50% methanol and the absorbance was measured at 335 nm, using a spectrophotometer (*UV-1800*, *Shimadzu*, Japan). Scutellarin was the main compound absorbing at 335 nm in the leaf extracts (Qu *et al.* 2001, Xu *et al.* 2003, Gao *et al.* 2007, Zhang *et al.* 2007). Scutellarin concentration in the solution was calculated using the absorption coefficient ($E^{1\%}_{1cm}$) of 570 mL mg^{-1} cm^{-1} (Xu *et al.* 2003). The scutellarin content of leaves was calculated from concentration of the extract and the DM of leaves.

On days 1, 2, 4, 5, 10, 15, 20, 25, 40, and 50 after transfer (DAT) and the day before transfer (day 0), following the F_v/F_m measurement at 16:00 h, the leaves of 10 plants of each treatment group were harvested to analyse the scutellarin content. We also randomly harvested fully sun-developed leaves from another group of SU plants to measure the scutellarin concentration at 08:00, 12:00, and 17:00 h to observe the daily variations in the scutellarin content (n = 10).

Leaf Chl fluorescence was measured using a portable fluorometer (*PAM 2100*, *Heinz Walz GmbH*, Germany) according to the PAM-2100 Handbook (2003). The F_v/F_m ratio was measured after *ca*. 20 min when plants were kept in the dark. The light-response curve of q_N was measured according to the *PAM2100 Standard Experiment Run 9* (PAM-2100 Handbook 2003), over a range of PPFD values between 0 and 1,600 μmol m^{-2} s^{-1}; the leaf was illuminated at each intensity for 3 min.

Daily variations of F_v/F_m in shaded and sunlit leaves: Ten SH and ten SU plants were exposed to full sunlight on a typical sunny day; F_v/F_m was measured on a fully developed leaf of each of the plants at 06:00, 08:00, 10:00, 12:00, 14:00, 16:00, and 18:00 h.

Photoinhibition and recovery under full sunlight and q_N in shaded and sunlit leaves: Five plants grown under the shade screen and five under full sunlight were transferred from outdoors into a room on the evening before the experiment. The next day at 07:00 h, F_v/F_m was measured on a fully developed leaf on each of the five plants. Thereafter, the light-response curve of q_N was measured at 09:00 h on a fully developed leaf on each of the five plants. At 11:00 h, the plants were transferred to the outdoors and exposed to full sunlight [1,700 μmol(photon) m^{-2} s^{-1}] for 2 h; they were then transferred from the outdoors into the room again and F_v/F_m was measured on a fully developed leaf on each of the five plants in complete darkness for 100 min. The degree of photoinhibition was quantified as the ratio of decrease in F_v/F_m relative to the value at dawn.

Time course of photoinhibition and q_N in transferred plants: In the morning, F_v/F_m was measured on a fully developed leaf on each of the 150 plants grown under the shade screen before transfer into full sunlight. All 150 plants were then transferred into full sunlight. Plants (n = 10) were chosen randomly from the 150 plants at 16:00 h on 1, 2, 4, 5, 10, 15, 20, 25, 40, and 50 DAT and the day before transfer (day 0). They were kept in the dark for 40 min and then F_v/F_m was measured on a fully developed leaf of each plant.

Plants (n = 5) were chosen randomly at 07:00 h on 1, 5, 10, 20, and 40 DAT and the light-response curve of q_N was measured on each plant.

Statistical methods: The experiments were set up in a completely randomized design with ten plants per treatment. All statistical analyses were performed using *SPSS 13.0* for *Windows* (*SPSS*, Chicago, IL, USA). Significant differences at $P \leq 0.05$ were assessed by analysis of variance (*ANOVA*). The value of t was determined by the t-test. Correlation and regression analysis between the scutellarin content and the degree of photoinhibition were examined. Values were considered significant when $P \leq 0.05$.

Results

Scutellarin content in shade and sunlit leaves: The amount of scutellarin was considerably affected by the amount of light and was significantly higher (37%) in SU leaves [147.9 ± 10.7 mg g^{-1}(DM)] than that in the SH leaves [107.8 ± 9.8 mg g^{-1}(DM)] of *E. breviscapus*.

Daily variations of F_v/F_m in shade and sunlit leaves: In the morning, F_v/F_m in the leaves grown under shade and full sunlight were 0.825 ± 0.07 and 0.803 ± 0.005, respectively. The SH leaves had a higher F_v/F_m than those under SU, conforming to the general knowledge. There was a clear fluctuation in F_v/F_m in both SH and SU leaves under full sunlight throughout the day with lower values at solar noon and greater values in the morning and late afternoon (Fig. 2). In the SU leaves, F_v/F_m decreased after sunrise and was significantly lower at 14:00 and 16:00 h than at 06:00 h. In the SH leaves, F_v/F_m was significantly higher at 06:00 h, and significantly lower at 12:00, 14:00, and 16:00 h than that at other times. Compared to dawn, F_v/F_m at 16:00 h decreased by 4% in the SU leaves and 9% in SH leaves. At 18:00 h, the F_v/F_m in the SU leaves was not significantly different from that at 06:00 h and seemed to have entirely recovered from photoinhibition, although the leaves in shade did not recover.

Photoinhibition and recovery after exposure to full sunlight in shade and sunlit leaves: At 13:00 h after exposure to full sunlight for 2 h, photoinhibition was observed in both SH and SU leaves (Fig. 3). F_v/F_m decreased by about 24 and 11% in the SH- and SU-developed leaves, respectively, although both were dark-adapted for 20 min. The degree of photoinhibition in the SU leaves was lower than that in the SH leaves. By 40 min, the SU leaves almost entirely recovered from photoinhibition, whereas the SH leaves did not completely recover even after 100 min.

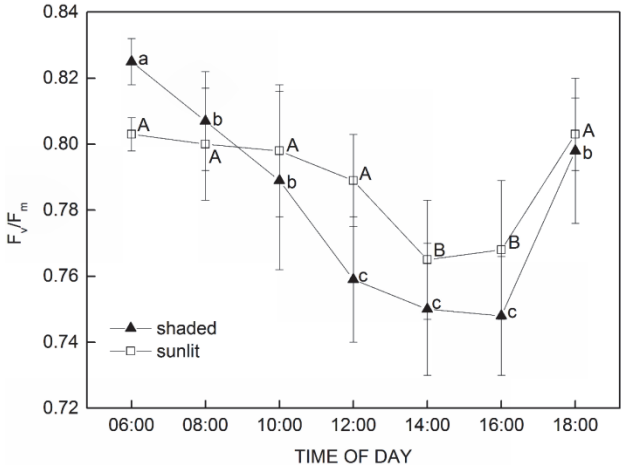

Fig. 2. Daily variations in maximal quantum yield of photosystem II photochemistry (F_v/F_m) in shaded (*closed triangles*) and sunlit (*open squares*) leaves under full sunlight (mean ± SD, n = 10). *Different small and capital letters* denote significant differences between F_v/F_m at different times in shaded and sunlit leaves, respectively, at P<0.05; *different capital letters* denote significant differences between F_v/F_m at different time in sunlit leaves at P<0.05.

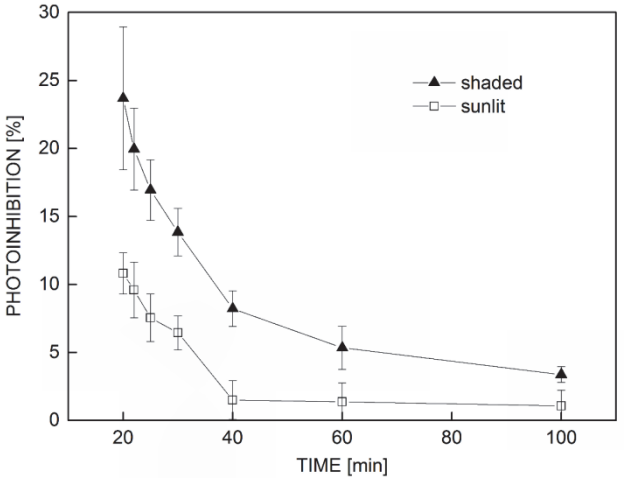

Fig. 3. The course of recovery from photoinhibition in leaves grown under shade (*closed triangles*) and full sunlight (*open squares*) exposed to approximately 1,700 μmol(photon) m^{-2} s^{-1} for 2 h (mean ± SD, n = 5).

Light-response curve of q_N in shade-developed and sun-developed leaves: Below 500 μmol(photon) m^{-2} s^{-1}, no difference was observed in q_N between the SH and SU leaves. But under high sunlight, the SU leaves showed higher q_N values than that of the SH leaves (Fig. 4). Under irradiance of 1,700 μmol(photon) m^{-2} s^{-1}, the

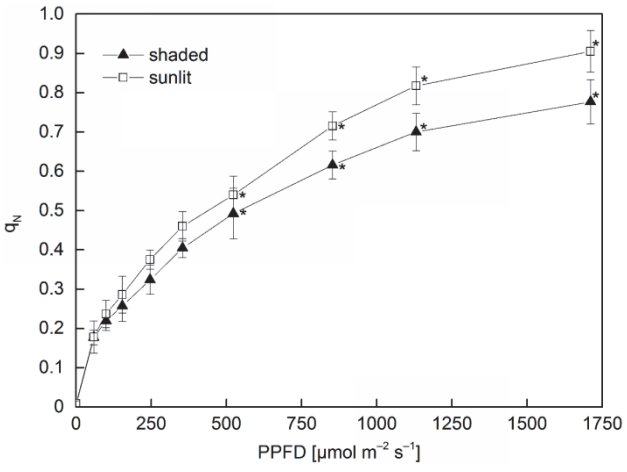

Fig. 4. Nonphotochemical quenching coefficient (q_N) light-response curves of shade-developed (*closed triangles*) and sun-developed (*open square*) leaves (mean ± SD, n = 5). * – significant differences between shade- and sun-developed leaves under the same PPFD.

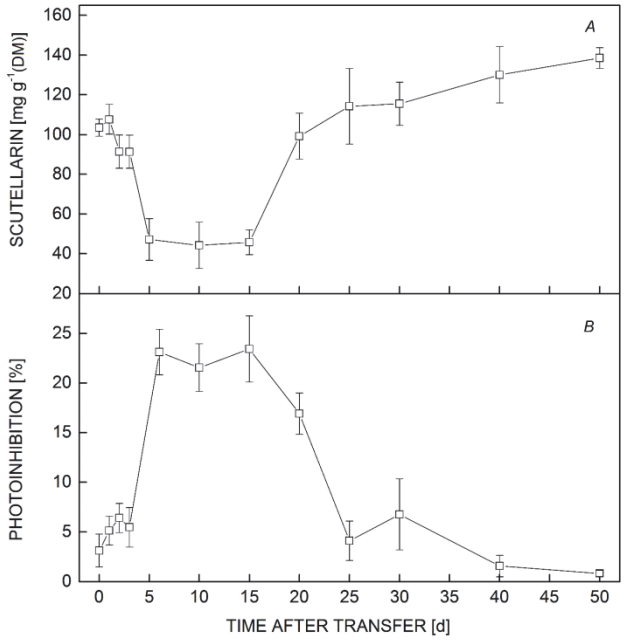

Fig. 5. Time course of the scutellarin content (*A*) and photoinhibition at 16:00 h (*B*) in leaves of transferred plants during the acclimation process from low to high light. The value at day 40 is the average of a mixture of shade-developed and new sun-developed leaves. On day 50, all remaining leaves were sun-developed leaves.

q_N values in the SU leaves were about 16.5% higher than those of the SH leaves.

Time course of the scutellarin content in transferred plants during the acclimation process: The scutellarin content was about 103.5 ± 4.4 and 107.7 ± 7.5 mg g^{-1}(DM) in the SH leaves on the day before transfer to sunlight (day 0) and on the first day after transfer, respectively. However, the scutellarin content decreased continuously on the following 2–5 DAT (Fig. 5*A*). At 10 DAT, the content of scutellarin decreased by 58.9% and was about 44.3 ± 11.6 mg g^{-1}(DM), which was significantly lower than that before transfer. The scutellarin content began increasing at 15 DAT and continued until 25 DAT. At 25 DAT, the content of scutellarin was similar to that before the transfer. At 30 DAT, some new leaves began to grow under full sunlight while some SH-developed leaves began wilting. At 40 DAT, the content of scutellarin was about 119.3 ± 8.7 and 140.9 ± 9.4 mg g^{-1}(DM) in the SH and SU leaves, respectively. It should be noted that the value reported in Fig. 5*A* at 40 DAT was the average of a mixture of the SH-developed leaves and new SU-developed leaves. At 50 DAT, all remaining leaves were the SU-developed leaves and their scutellarin content was about 138.5 ± 5.2 mg g^{-1}(DM), which was significantly higher than that in the SH leaves.

Time course of photoinhibition in transferred plants during the acclimation process: The SH-developed leaves prior to sunlight exposure had a high F_v/F_m value (0.825 ± 0.005) at dawn. The degree of photoinhibition at 16:00 h was, on average, approximately 3.13 and 5.13% on the day before transfer (day 0) and the first day after transfer, respectively, and increased continuously for the first 5 DAT (Fig. 5*B*). From 5–15 DAT, the degree of photoinhibition was about 23% and was the highest value observed during acclimatization. The degree of photoinhibition then decreased continuously until 25 DAT. On 25 DAT, the degree of photoinhibition was only about 4.11 ± 1.99% and was only slightly lower than the value reached at the first day (5.13 ± 1.45%). At 40 DAT, the degree of photoinhibition was about 2.7% in the SH leaves and about 0.8% in the newly developed leaves. The value reported in Fig. 5*B* at 40 DAT was the average of a mixture of the SH-developed leaves and new SU-developed leaves. At 50 DAT, all leaves were new and the degree of photoinhibition was about 0.8% and even lower than the values on the first day.

Light-response curve of q_N in transferred plants during the acclimation process: On the first day after transfer of the SH-developed leaves to high sunlight, the q_N value of the SH leaves was about 0.771 under irradiance of 1,700 µmol(photon) m^{-2} s^{-1}. The high light treatment of the SH leaves resulted in an initial reduction in q_N, and at 5 and 10 DAT, q_N was lower than before the transfer. However, at 20 DAT, the leaves had higher q_N than before the transfer. After 20 DAT, the q_N increased continuously and at 40 DAT, the q_N in the SH leaves was 13.6% higher than before the transfer (Fig. 6).

Relationship between degree of photoinhibition and the scutellarin content: Regression analyses showed a statistically significant negative relationship between the degree of photoinhibition and the content of scutellarin in leaves after transfer from shade to sunlight (Fig. 7, $F = 283.05$).

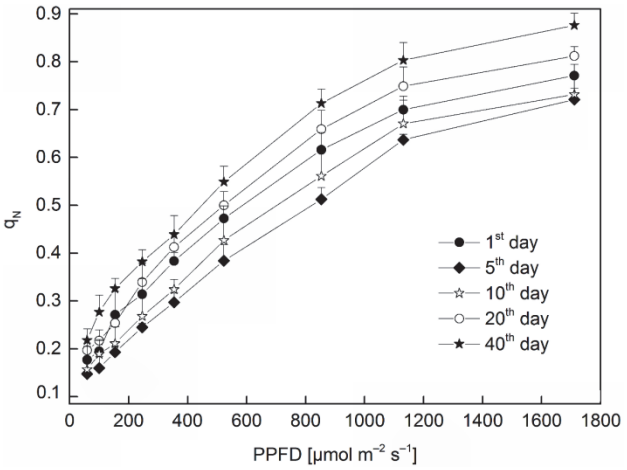

Fig. 6. Light-response curve of nonphotochemical quenching coefficient (q_N) in leaves of transferred plants during the acclimation process from low to high light (mean ± SD), (●, first day; ◆, 5th day; ☆, 10th day; ○, 20th day; ★, 40th day).

Discussion

In most plants exposed to full sunlight, excess light energy is absorbed by leaves at noon and is not entirely used for photosynthesis. The excess energy absorption can disturb or damage photosynthesis, which is demonstrated as a decline in F_v/F_m (Demmig-Adams and Adams 1992, Long *et al.* 1994). Most plants recover from photoinhibition because they possess photoprotection systems (Demmig-Adams and Adams 1992, Long *et al.* 1994, Alves *et al.* 2002, Zhang *et al.* 2007).

Erigeron breviscapus is an herbal heliophyte. It grows in nature mostly with grass under direct sunlight, but it can grow also in shaded environments (Su *et al.* 2001). In our study performed under full sun, the F_v/F_m ratio decreased at noon in the SU-developed leaves of *Erigeron* and recovered in the afternoon, which suggests dynamic photoinhibition in the leaves of *Erigeron*. Excessive energy was delivered to the leaves of *Erigeron* under full sunlight; therefore a protection system was required to quench the excess energy absorbed. After 2-h exposure to high light, the degree of photoinhibition in the SH leaves was higher than that in the SU leaves. After 40 min in the dark, the F_v/F_m in the SU-developed leaves recovered, but it did not completely recover in the SH leaves even after 100 min (Fig. 3). These results suggested that the SU-developed leaves possessed higher capacity for relaxation of dark-reversible components of nonphotochemical dissipation in PSII than the SH-developed leaves.

Plants are protected from photoinhibition or photodamage by several photoprotective mechanisms, such as changing the angle, orientation and surface reflection of leaves to reduce the light absorbed (Demmig-Adams and Adams 1992, Robinson and Osmond 1994) and physiological mechanisms to quench absorbed energy (Krause 1988, Demmig-Adams and Adams 1992, Anderson *et al.* 1994).

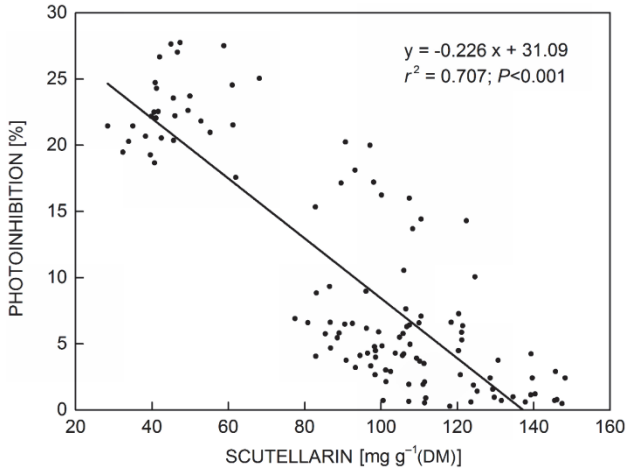

Fig. 7. Linear regression of flavonoid content (based on dry mass (DM)) against photoinhibition of leaves at 16:00 h every day after transfer from shade to full sunlight (P<0.001).

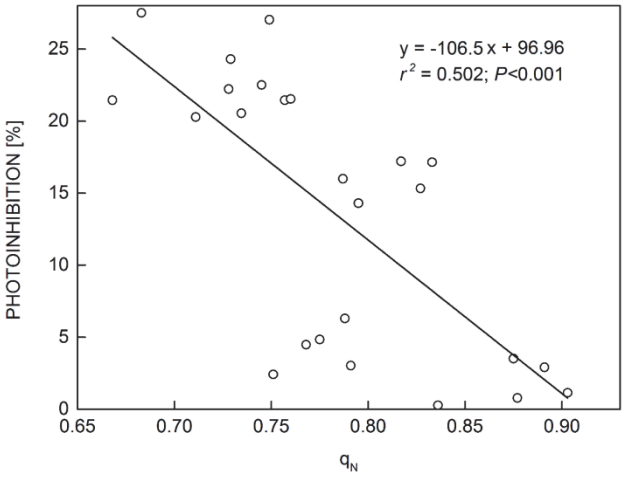

Fig. 8. Linear regression of nonphotochemical quenching coefficient (q_N) against photoinhibition of leaves after transfer from shade to full sunlight (mean ± SD, n = 25, P<0.001).

Quenching the excess energy is an important strategy of photoprotection (Demmig-Adams and Adams 1992). Thermal dissipation, which can be estimated by q_N, is one of the pathways to quench the excess energy. The SU leaves showed the higher q_N values (Fig. 4) and the lower degree of photoinhibition (Fig. 2). After transfer from shade to sunlight, the trend in the variation of q_N values was opposite to the degree of photoinhibition (Fig. 8). The degree of photoinhibition was the highest while the q_N value was the lowest during acclimatization and *vice versa*. These results suggested that the capacity of protection against photoinhibition was attributed to quenching excess energy in the leaves of *Erigeron*. Actually, q_N cannot explain all concerning photoinhibition in *E. breviscapus* (Fig. 8, r^2=0.502); it implies that q_N cannot dissipate all excessive light energy, and there are other photoprotection pathways working.

The nonphotochemical quenching can be divided into at least three different components. The major and most rapid component in most plants is the pH- or energy-dependent component. The pH-dependent Chl fluorescence quenching is promoted by zeaxanthin (Ruban *et al.* 1997) and zeaxanthin might be an important antioxidant (Müller *et al.* 2001) involved in nonphotochemical quenching.

Antioxidant systems are other important photoprotection mechanisms. In plants, a wide range of antioxidant molecules have been reported (Asada 1994, Foyer *et al.* 1994, Conklin *et al.* 1996), including flavonoids (Rice-Evans *et al.* 1996, Agati *et al.* 2007). Flavonoid contents were shown to be higher under high sunlight (Grace *et al.* 1998, Karageorgou *et al.* 2002, Agati *et al.* 2007, Guidi *et al.* 2008), which is consistent with our observations in *Erigeron*.

Flavonoids in leaves were considered to be typical UV

radiation-regulated compounds, because higher UV-B radiation leads to enhanced biosynthesis and accumulation through the induction of the phenylpropanoid pathway (Jansen *et al.* 2008, Kim *et al.* 2008, Zhang and Björn 2009) and flavonoids protect plants from UV damage (Ravindran *et al.* 2010). Recent studies showed that flavonoids are antioxidants that neutralize free radicals (Li *et al.* 1993, Agrawal and Mishra 2009, Majer *et al.* 2014). Majer *et al.* (2014) found that higher amounts of flavonols in sun leaves of *Tilia platyphyllos* did not provide better UV-B protection but gave better antioxidant defense against singlet oxygen damage. They suggested that responses to high PPFD and UV were connected and that flavonols played a key role in the successful acclimation to high PPFD by assisting in prevention of singlet oxygen-mediated oxidative stress (Majer *et al.* 2014).

Flavonoid, flavonoidperoxidase, and ascorbic acid reactions are components of a detoxification system (Yamasaki *et al.* 1997, Pérez *et al.* 2002), where flavonoids receive electrons from highly reactive chemical species and are oxidized to flavonoid radicals. The flavonoid radicals regenerate the redox status through the flavonoid-redox cycle and act as antioxidants in the presence of ascorbic acid (Yamasaki *et al.* 1997, Pérez *et al.* 2002). If the cycle works properly, the flavonoid content does not decrease in leaves, but in the absence of ascorbic acid, flavonoids do not switch between the radical and redox states and the flavonoid radicals give rise to polymers. If the flavonoid-redox cycle is broken, flavonoids cannot be regenerated (Takahama and Oniki 1997, Pérez *et al.* 2002). In our study, after transfer from shade to sun, the trend in the variation of q_N values was similar to the scutellarin content. The plants with the high scutellarin content showed a higher capacity to quench the excess energy. The content of flavonoids in the SU-developed leaves of *Erigeron* from another group of plants did not differ in the morning, noon, or afternoon (the scutellarin contents were 107.71 ± 9.81, 106.43 ± 12.73, and 107 ± 7.49 mg g^{-1}(DM), $n = 10$, at 08:00, 12:00, and 17:00 h, respectively). This suggested that the flavonoid-redox cycle in the detoxifying system worked properly in the SU leaves under sunlight. However, in the SH-developed leaves transferred to high light, the flavonoid content decreased continuously during the first 15 d, which indicated that the flavonoid-redox cycle in the SH leaves was damaged and that the flavonoid radicals could not regenerate the proper redox status.

When exposed to high light, *Erigeron* plants did not immediately increase the scutellarin content. After 40 DAT, the scutellarin content increased and reached amounts higher than those before the transfer. Agati *et al.* (2011) found that the concentration of flavonoids in *Ligustrum vulgare* was the highest after 12 d from transfer from 30 to 85% sunlight, which indicates that it took some time for flavonoid synthesis to reach sufficient levels in order to exhibit any protective effect in plants transferred from shade to high light. It is possible that during 40 DAT of acclimatization, the antioxidant system reached sufficient capacity for effective regeneration of the redox state for flavonoid radicals. The data from the first days of the acclimation period suggested that flavonoids themselves might not be effective without a strong antioxidant system.

During the successful acclimation of *Erigeron* leaves to high PPFD, synchronous fluctuations were observed in the content of scutellarin and in photoinhibition (Fig. 5) albeit with contrasting trends. The degree of photoinhibition negatively correlated with the scutellarin content during acclimatization of leaves from low to high light (Fig. 7). These results agree with the hypothesis that flavonoids play a role in protection against photoinhibition. However, it cannot directly prove that the hypothesis is valid, because the contribution of other photoprotective mechanisms such as the role of carotenoids, were not tested in this study. In addition, other possible explanations for the synchronous fluctuations cannot been excluded. Nevertheless, despite the fact that no definitive conclusion about the role of flavonoids in the photoprotection can be drawn from our data, our results pointed out a possible relationship and can serve as a basis for further experiments designed to obtain a decisive answer to this question.

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