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REGULAR PAPER

Relationships between the photochemical reflectance index (PRI) and chlorophyll fluorescence parameters and plant pigment indices at different leaf growth stages

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Abstract This study aimed to evaluate the photochemical reflectance index (PRI) for assessing plant photosynthetic performance throughout the plant life cycle. The relationships between PRI, chlorophyll fluorescence parameters, and leaf pigment indices in *Solanum melongena* L. (aubergine; eggplant) were studied using photosynthetic induction curves both in short-term (diurnal) and long-term (seasonal) periods under different light intensities. We found good correlations between PRI/non-photochemical quenching (NPQ) and PRI/electron transport rate (ETR) in the short term at the same site of a single leaf but these relationships did not hold throughout the life of the plant. In general, changes in PRI owing to NPQ or ETR variations in the short term were <20 % of those that occurred with leaf aging. Results also showed that PRI was highly correlated to plant pigments, especially chlorophyll indices measured by spectral reflectance. Moreover, relationships of steady-state PRI/ETR and steady-state PRI/photochemical yield of photosystem II (Φ_{PSII}) measured at uniform light intensity at different life stages proved that overall photosynthesis capacity and steady-state PRI were better correlated through chlorophyll content than NPQ and xanthophylls. The calibrated PRI index accommodated these pigments effects and gave better correlation with NPQ and ETR than PRI. Further studies of PRI indices based on pigments other than xanthophylls, and studies on PRI mechanisms in different species are recommended.

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Introduction

Chlorophyll fluorescence analysis is the most powerful non-destructive technique to assess the photosynthetic performance of plants (Bilger and Björkman 1990; Genty et al. 1989; Govindjee 1995; Krause and Weis 1991; Maxwell and Johnson 2000; Omasa 2011; Papageorgiou and Govindjee 2004). Excessive light dissipated as heat through non-photochemical quenching (NPQ) of chlorophyll fluorescence, via mechanisms stabilized by xanthophyll cycles, are used as a proxy to estimate photosynthesis capacity of plants. In the violaxanthin cycle, excessive absorbed light causes the de-epoxidation of violaxanthin to zeaxanthin via antheraxanthin, leading to an increase in NPQ that is an indicator of the proportion of the absorbed radiation not used for electron transport in photosynthesis. Description of this mechanism has been presented by many researchers (Adams and Demmig-Adams 1994; Bilger and Björkman 1990; de Bianchi et al. 2010; Demmig-Adams and Adams 1992).

However, using chlorophyll fluorescence analysis to assess photosynthesis activities of plants at larger scales is difficult. Studies carried out using a portable laboratory radiometer have identified an absorbance change at 505 nm (Bilger et al. 1989) and a reflectance change at 531 nm (Gamon et al. 1990) related to the conversion of violaxanthin to zeaxanthin in an individual leaf. Using this concept, the photochemical reflectance index (PRI) was developed based on the changes in reflectance at 531 nm to a reference band at around 570 nm, for daily evaluation of photosynthesis via detection of epoxidation state of

pigments in xanthophyll cycle (Gamon et al. 1990, 1992). Thus PRI has emerged as a good alternative to chlorophyll fluorescence analysis in remote sensing as it is able to detect short term (diurnal) changes of photosynthesis that are not captured in conventional remotely sensed vegetation indices such as normalized difference vegetation index (NDVI). PRI has been found to be well correlated to NPQ and photosynthetic light use efficiency (LUEp) at both leaf and canopy levels (Filella et al. 1996; Gamon et al. 1992, 1997; Penuelas et al. 1995, 2011; Richardson and Berlyn 2002; Trotter et al. 2002). At ecosystem levels, PRI was correlated with total crop field CO_2 uptake (Penuelas and Inoue 2000) and LUEp in the boreal forest (Nichol et al. 2002), and also has been used to estimate the ecosystem carbon uptake (Nichol et al. 2002, 2006; Rahman et al. 2004, 2001). A meta-analysis of PRI data can be found in Garbulsky et al. (2011).

However, the relationship between PRI and LUEp has been found to vary quite widely between different studies, so that canopy measurement of PRI often failed to quantify photosynthetic efficiency (Methy 2000) or was greatly affected by seasonal changes in canopy structure (Filella et al. 2004; Nakaji et al. 2006). Canopy level PRI is significantly affected by the solar illumination and viewing angle, and PRI values are more sensitive to changes in leaf area index (LAI) than LUEp (Barton and North 2001; Suárez et al. 2009) and vary greatly between species with the same photosynthetic capacity (Guo and Trotter 2004). To reduce the effects of canopy structure, Hernández-Clemente et al. (2011) used different formulations to calculate PRI for conifer forests. Jones and Vaughan (2010) also pointed out that interpretation of spectral changes associated with xanthophyll epoxidation can become complicated by other light-induced changes in leaf absorbance, scattering and reflectance caused by processes such as chloroplast movement. Recent studies on PRI indicate that when PRI is applied over longer time periods (seasons, years) or across species, its variations appear to assess relative composition of chlorophylls and carotenoids (Filella et al. 2004; Sims and Gamon 2002; Stylinski et al. 2002). On the other hand, spatiotemporal changes of PRI and NPQ at leaf level indicated that differences in xanthophyll pigments during photosynthesis induction are too small to be detected by PRI in some instances (Koneshi et al. 2008). Also 3D lidar image analysis using computer graphics techniques indicated that when photosynthetic activities of *Helianthus annuus* were inhibited by application of a herbicide, changes in chlorophyll fluorescence intensity was large whereas changes in PRI and plant pigments changes, were small (Omasa et al. 2007).

Despite shortcomings discussed above, PRI is increasingly being applied to assess plant photosynthetic capacity at different scales. However, the actual correlation between PRI and NPQ varies with species and seasonal changes in canopy structure so that it may not explain photosynthetic performance in all conditions and at different temporal and spatial scales. It seems that PRI variation may be related to changes in the levels of xanthophyll pigment cycle in the short term (diurnal) and changes in chlorophyll and carotenoid contents in the long term (seasonal).

To the authors' best knowledge, no report dealing with the PRI relationship to chlorophyll fluorescence during photosynthetic induction in the short term and over leaf development in the long term is available. Also relatively few studies have been conducted on the relationship between PRI and plant pigments other than the xanthophylls. The objective of this study is to evaluate the relationship between PRI and chlorophyll fluorescence parameters and leaf pigment indices at both short-term and long-term (different life stages of the leaf) periods under different light intensities to have a better insight into the applicability of PRI for assessing plant photosynthetic performance over the plant life cycle.

Materials and methods

Experimental conditions

Plant materials and growth conditions

Solanum melongena L. (aubergine; eggplant) was grown in a growth chamber for 120 days. The plants were illuminated by a halogen lamp for 12 h each day at a PPF of 500 μmol m^{-2} s^{-1}. The growth chamber air temperature was 28 °C during the day and 22 °C at night with relative humidity of about 50 %. Plants were watered daily with a nutrient solution (1:1000 dilution of HYPONex).

Chlorophyll fluorescence and reflectance measurement

Chlorophyll fluorescence parameters were measured using the pulse amplitude modulated (PAM) chlorophyll fluorometer acquisition system (PDA-100 Heinz Walz, Germany, joined to PAM 101/102/103). This system was installed in a dark room and actinic light for photosynthesis and saturation pulse that cause a transient saturation of photosynthetic electron transport were provided with a 150-W halogen lamp (LS-DWL, Sumita Optical Glass, Inc., Japan) and the PAM101/102/103 system with FL103, respectively. Spectral reflectance was measured by a spectrometer (USB 4000, Ocean Optics Inc, USA). A fiber optics (101F, Heinz Walz, Germany) was used to connect the two light sources (actinic light source and saturation pulse light source), and then the leaf area was illuminated by light from the fiber optics. The chlorophyll fluorescence

was also measured through the fiber optics. The attached leaves (under growth conditions) were set horizontally using a leaf holder in the way that both leaf surfaces could exchange CO_2 and water vapor with the air. The fiber optics for light source and chlorophyll fluorescence measurement were set vertically to the leaf surface from the upper position. Another fiber optics for reflectance measurement was set at about 45° to the leaf surface and the light source fiber optics cable to avoid direct reflection of light from the leaf. The distance between leaf surface and fiber optics surface for reflectance and fluorescence measurement was about 0.5 cm. Actinic and saturation pulse lights were illuminated to the leaf area about 1.5 cm in diameter. The center area was measured. In this area, the changes in PPF were ± 0.3 % of actinic light intensities. The saturation light pulse was about 4,000 μmol m^2 s^{-1} ± 5 %. Two standard reflectors were used to estimate exact leaf reflectance. One was the standard white board ($BaSO_4$) with reflectance over 95 % and another was an 18 % gray card (R-27, Kodak). The spectrometer was calibrated by the standard light source (LS-1-CAL, Ocean Optics Inc, USA) before the experiment. First, the spectral reflectance ratio of the 18 % gray card to the standard white board ($BaSO_4$) was obtained, because the reflectance of the 18 % gray card is similar to leaf reflectance and that of the standard white board is very high with respect to the leaf. The spectral reflection intensities from leaf and 18 % gray card were measured by the USB 4000 spectrometer under actinic light conditions before saturation pulse illumination for PAM measurement in the experiments. The spectral leaf reflectance was calculated from the measured leaf and 18 % gray card reflection intensities using the spectral reflectance ratio of 18 % gray card to the standard white board ($BaSO_4$). Experiments were performed using plant leaves during different life stages under different light intensities. Actinic lights of 100, 500, 1,000 and 1,500 μmol m^{-2} s^{-1} PPF were considered as low, normal, strong, and very strong light intensities, respectively. Leaves of *Solanum melongena* have relatively flat and broad area between main veins. The area of attached leaf was measured under the condition in which the leaf was set horizontally. Therefore, effects of morphological and anatomical variations may be negligible although these effects cannot be perfectly removed. We also measured leaves with low water stress growing under enough water supply.

After dark adaptation for 30 min, the maximum yield of chlorophyll fluorescence in dark (F_m) was acquired under saturation pulse light. Then, after the start of actinic light illumination, maximum yield of chlorophyll fluorescence in light (F_m') and minimal yield of chlorophyll fluorescence in light (F_t) were measured during photosynthetic induction experiments lasting 20 min, and spectral reflectance (from 350 nm to 1050 nm) was measured on the same area of the leaf after 0, 1, 2, 3, 4, 5, 6, 8, 10, 15, and 20 min. The photosynthetic parameters NPQ, photochemical yield of photosystem II (Φ_{PSII}) and electron transport rate (ETR) as overall photosynthetic capacity were calculated using fluorescence parameters of F_m, F_t, F_m', employing the following equations:

$$\mathrm{NPQ} = (F_m - F'_m)/F_m \quad (1)$$

$$\phi_{PSII} = (F'_m - F_t)/F'_m \quad (2)$$

$$\mathrm{ETR} = \phi_{PSII} \times \mathrm{PPF} \times 0.84 \times 0.5. \quad (3)$$

Retrieved spectral reflectances of the plant leaves at all time intervals (0, 1, 2, 3, 4, 5, 6, 8, 10, 15, and 20 min) were used to calculate PRI using the following equation:

$$\mathrm{PRI} = (R_{531} - R_{570})/(R_{531} + R_{570}), \quad (4)$$

where R_{531} and R_{570} are the reflectance at 531 and 570 nm.

The steady-state values of NPQ, Φ_{PSII}, ETR, and PRI obtained 20 min after illumination were used to compare long-term changes in these parameters over the life cycle of leaves. Similarly, steady-state reflectance spectra of leaves 20 min after illumination were used to retrieve leaf pigment content indices. For chlorophyll content estimation, five common indices including red-edge normalized difference vegetation index ($mNDVI_{705}$) (Datt 1999; Gamon and Surfus 1999; Gitelson and Merzlyak 1994; Sims and Gamon 2002), modified red-edge normalized difference vegetation index (mND_{705}) (Sims and Gamon 2002), NDVIgreen (Datt 1999; Gitelson et al. 1996), modified Red-edge Ratio (mSR_{705}) (Sims and Gamon 2002) and combinations of wavelengths ratios (R_{750}/R_{700} ratio) (Gitelson and Merzlyak 1996, 1997) were calculated. For Carotenoids content estimation, four indices namely carotenoid reflectance index (CRI_{550}, CRI_{700} and $CRI_{550\text{-}780}$) (Gitelson et al. 2002) and modified carotenoid reflectance index (mCRI) (Gitelson et al. 2006) and for anthocyanins content estimation, two indices including anthocyanin reflectance index (ARI) (Gitelson et al. 2001) and modified anthocyanin reflectance index (mARI) (Gitelson et al. 2006) were calculated using equations given below:

$$\mathrm{mNDVI}_{705} = (R_{750} - R_{705})/(R_{750} + R_{705}) \quad (5)$$

$$\mathrm{mND}_{705} = (R_{750} - R_{705})/(R_{750} + R_{705} - 2R_{445}) \quad (6)$$

$$\mathrm{NDVI}_{green} = (R_{750} - R_{550})/(R_{750} + R_{550}) \quad (7)$$

$$\mathrm{mSR}_{705} = (R_{750} - R_{445})/(R_{705} + R_{445}) \quad (8)$$

$$\mathrm{Ratio} = (R_{750}/R_{700}) \quad (9)$$

$$\mathrm{CRI}_{550} = (R_{510})^{-1} - (R_{550})^{-1} \quad (10)$$

$$\mathrm{CRI}_{700} = (R_{510})^{-1} - (R_{700})^{-1} \quad (11)$$

$$\mathrm{CRI}_{550-780} = \left[(R_{510})^{-1} - (R_{780})^{-1}\right] - m\left[(R_{550})^{-1} - (R_{780})^{-1}\right] \quad (12)$$

$$\text{mCRI} = \left[(R_{510})^{-1} - (R_{550})^{-1}\right] \times R_{780} \quad (13)$$

$$\text{ARI} = (R_{550})^{-1} - (R_{700})^{-1} \quad (14)$$

$$\text{mARI} = \left[(R_{550})^{-1} - (R_{700})^{-1}\right] \times R_{780}, \quad (15)$$

where terms with different R subscripts indicate reflectance at the corresponding wavelengths. In Eq. 12 the value of m was considered to be 0.75 (Gitelson et al. 2002). The indices used to estimate pigment contents have been confirmed to have good relationships by comparing with destructive measurements of chlorophyll content, carotenoid content, etc., from a lot of previous papers and our own other experiments. Therefore, we did not use the destructive measurements in this experiment.

Results

Relationships between PRI and chlorophyll fluorescence parameters at both short-term and long-term under different light intensities

Short-term variation in PRI, NPQ, Φ_{PSII}, and ETR with time in an induction curve for a matured single green leaf (short-term) at 3 different light intensities is presented in Fig. 1. PRI was almost unchanged over time (0–20 min) at low light intensity but at higher light intensity a rapid reduction in PRI was observed for 5 min followed by a steady state attained for the remainder of the period (Fig. 1a). Chlorophyll fluorescence showed an inverse but otherwise similar trend in NPQ with increasing light intensity, reflecting the rate and change in Φ_{PSII} (Fig. 1c) and ETR (Fig. 1d) as calculated from Eq. 1–3 above. PRI/NPQ and PRI/ETR relationships over time in the matured single green leaf at different light intensities are presented in Fig. 2. PRI was inversely well correlated with both NPQ and ETR ($R^2 = 0.87$; $P < 0.0001$ and $R^2 = 0.85$; $P < 0.0001$, respectively) when data from all light intensities were pooled.

These experiments were repeated with leaves of different ages throughout the plant life cycle. All data for the relationships between PRI/NPQ and PRI/ETR during induction experiments with twenty-seven leaf samples at different growth stages from mature (dark green) to old (yellow) leaves are presented in Fig. 3, where samples tested at different light intensities are plotted separately for clarity. In this figure, leaf chlorophyll content generally declines from top to bottom as leaves age. Although linear and well correlated relationships between PRI/NPQ (Fig. 3a, c, e) and PRI/ETR (Fig. 3b, d, f) were found for each leaf, overall these parameters were very poorly correlated over the whole growth cycle. Data points for mature leaves with higher chlorophyll content are clustered at the top section of each graph in Fig. 3 where chlorophyll content generally declined with leaf age from top to bottom. Correlations between PRI/NPQ and PRI/ETR were better at higher chlorophyll contents, but at later stages of the plant growth, the relationships were not very good (lower part of each graph in Fig. 3). In general, the range of changes in PRI values with NPQ or ETR for induction curves with individual leaves were found to be <20 % of overall decline in PRI values with leaf age (top to bottom of all graphs in Fig. 3).

Correlations between steady-state PRI and NPQ, Φ_{PSII}, and ETR measured at the end of 20-min induction curves from leaves at different growth stages are presented in Table 1. This table shows two important relationships. First, steady-state PRI values at low light intensity are better correlated with Φ_{PSII} and ETR than with NPQ values. Second, the significance of correlations with both Φ_{PSII} and ETR decreased in assays at higher light intensity. Considering data at all light intensities together, it is found that only steady-state Φ_{PSII} shows significant relationship with steady-state PRI. There was no relationship between steady-state PRI and NPQ at different leaf growth stages.

Relationship between PRI and chlorophyll fluorescence parameters and leaf pigment indices in different leaf growth stages

Relationships between steady-state PRI, NPQ, Φ_{PSII} and ETR and plant pigment indices at different growth stages (from dark green to yellow) for all 27 leaf samples are presented in Table 2. PRI was found to be non-linearly (logarithmically) related to all chlorophyll indices where the highest R^2 values corresponded to $mNDVI_{705}$ and mND_{705}, respectively. For carotenoid content indices, steady-state PRI had better logarithmic relationships with $CRI_{550-780}$ and CRI_{550}, respectively whereas its relationships with mCRI and CRI_{700} were poor. Steady-state PRI was inversely related to anthocyanin indices with mARI having the highest linear R^2 value. In contrast to PRI which was highly and significantly correlated with leaf pigment indices especially chlorophyll indices, the three chlorophyll fluorescence parameters were poorly correlated with leaf pigment indices. NPQ was found to be unrelated to any of the leaf pigment indices; Φ_{PSII} had weak but significant relationships with only three chlorophyll indices ($mNDVI_{705}$, mND_{705} and $NDVI_{green}$) and no significant relationships with the other two pigment indices. ETR had a poor relationship with only CRI_{550} and showed no correlation with other plant pigment indices. The relationships

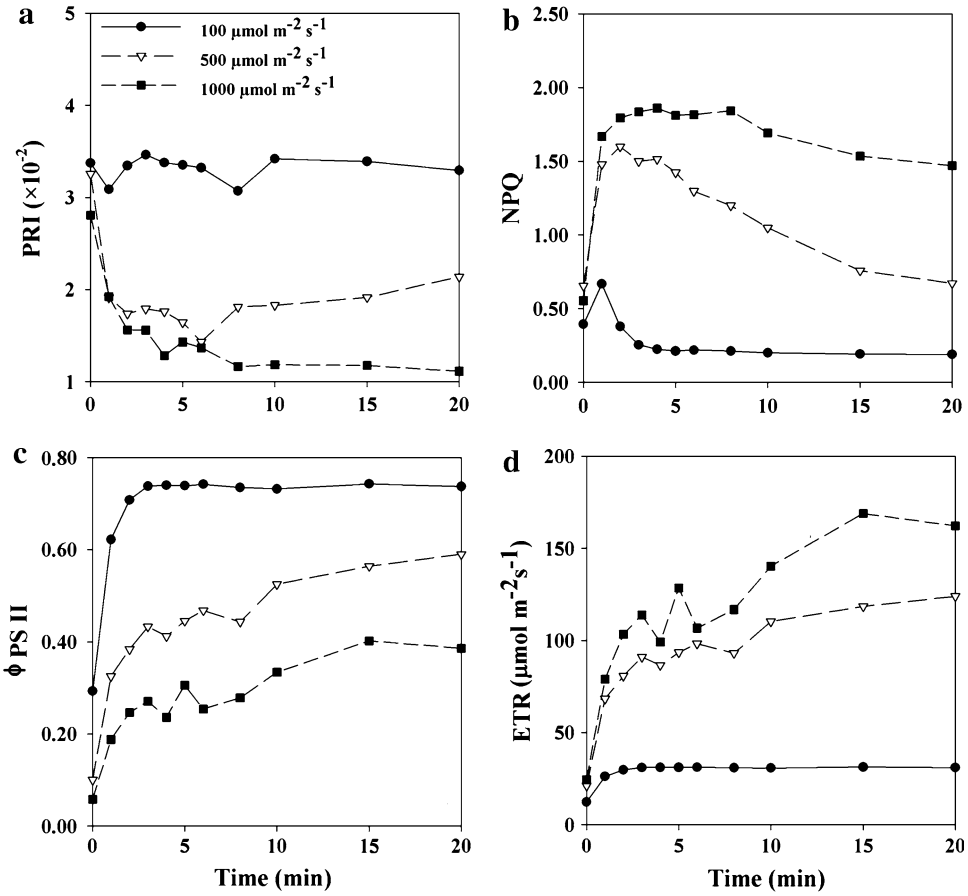

Fig. 1 Changes in **a** PRI, **b** NPQ, **c** Φ_{PSII}, and **d** ETR of a matured single green leaf (short term) at three different light intensities (100, 500, and 1,000 µmol m^{-2} s^{-1}) after dark-light transition

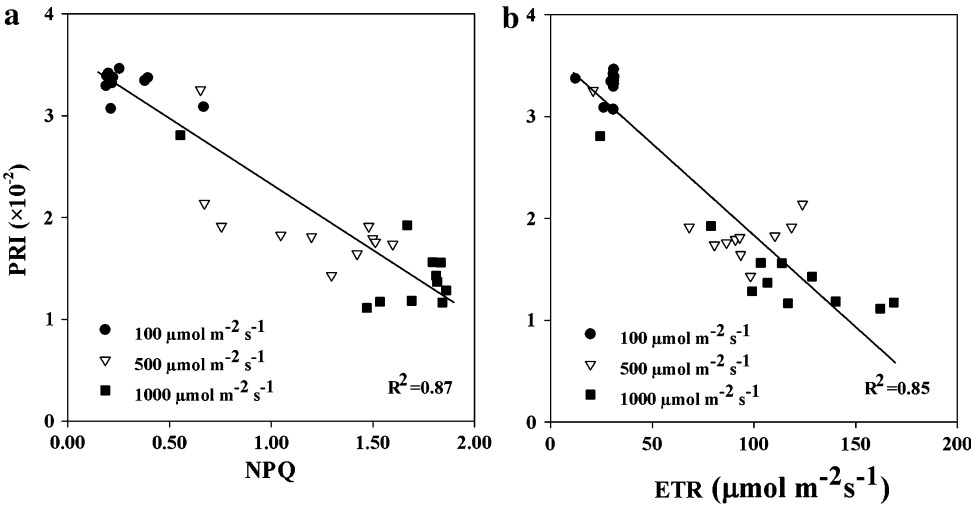

Fig. 2 Relationships of **a** PRI/NPQ and **b** PRI/ETR of the matured single green leaf presented in Fig. 1 (symbols for light intensities are the same as those in Fig. 1). PRI is inversely correlated with both NPQ and ETR ($R^2 = 0.87$; $P < 0.0001$ and $R^2 = 0.85$; $P < 0.0001$, respectively)

between PRI and some plant pigments having the highest coefficients of determination are shown in Fig. 4. As it can be observed form Table 2 and Fig. 4, although spectral reflectances of these samples were measured at different light intensities, there were strong relationships between plant pigment indices especially chlorophyll indices and steady-state PRI.

Calibration of PRI with plant pigment indices

As PRI was found to be strongly correlated with plant pigment indices, it seemed necessary to incorporate the effect of plant pigments on PRI to improve PRI and NPQ relationships in the long-term. In this section, a correction method was applied to calibrate PRI values to improve the

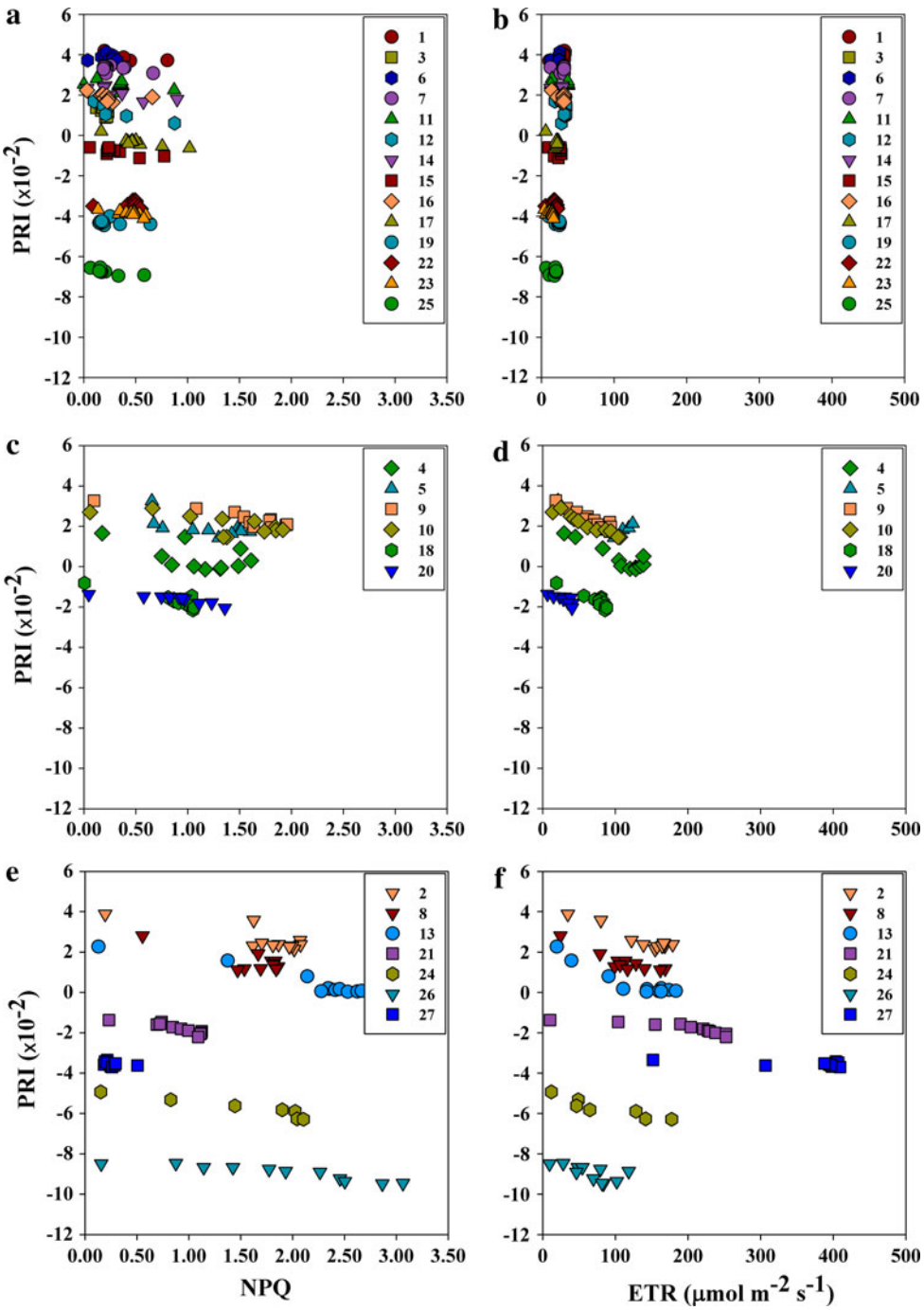

Fig. 3 Relationships between PRI/NPQ (left) and PRI/ETR (right) during 20-min induction time (similar to those in Fig. 1) at different leaf growth stages (long term; 27 samples). Leaf age increased from sample 1 to 27. Leaf color changed from green (mature leaf) to yellow (old leaf). Chlorophyll content values as represented by $mNDVI_{705}$ for the 27 samples are (1) 0.54, (2) 0.5, (3) 0.50,(4) 0.50, (5) 0.50, (6) 0.49, (7) 0.49, (8) 0.48, (9) 0.47, (10) 0.45, (11) 0.43, (12) 0.43, (13) 0.43, (14) 0.39, (15) 0.37, (16) 0.32, (17) 0.28, (18) 0.27, (19) 0.26, (20) 0.21, (21) 0.19, (22) 0.18, (23) 0.16, (24) 0.16, (25) 0.16, (26) 0.13, and (27) 0.12. The light intensity used for samples 1, 3, 6, 7, 11, 12, 14, 15, 16, 17, 19, 22, 23 and 25 was 100 µmol m^{-2} s^{-1} (Fig. 3a, b), whereas 500 µmol m^{-2} s^{-1}, was used for samples 4, 5, 9, 10, 18, and 20 (Fig. 3c, d). Sample 8 was exposed to 1,000 µmol m^{-2} s^{-1} and samples 2, 13, 21, 24, 26 and 27 were exposed to 1,500 µmol m^{-2} s^{-1} (Fig. 3e, f). PRI was found to be weakly correlated with NPQ and ETR using all 27 samples data (linear $R^2 = 0.01$; $P = 0.1277$ and $R^2 = 0.02$; $P = 0.05$, respectively)

relationship between PRI and NPQ for leaves during different growth stages.

First, a multiple regression analysis was performed with steady-state PRI as a function of three plant pigment indices having the highest coefficients of determination as previously determined (see Table 2). As steady-state PRI was found to be logarithmically correlated to $mNDVI_{705}$ and $CRI_{550–780}$ but linearly to mARI, the multiple regression analysis involved ln $mNDVI_{705}$ and ln $CRI_{550–780}$ as variables as well as linear mARI. The results of such an analysis are presented in Table 3 where only $mNDVI_{705}$ remained in the model and the two other indices were excluded. Therefore, the calibration process was continued using only $mNDVI_{705}$ as the variable.

Using Eq. 16 derived from the relationship between steady-state PRI and $mNDVI_{705}$ $(y = 0.645 * \ln(x) + 0.0688)$ the calibrated PRI (CPRI) was developed:

Table 1 Linear relationships between steady-state PRI and steady-state NPQ, Φ_{PSII} and ETR for samples with the same light intensities (light intensities are 100 μmol m^{-2} s^{-1} for samples 1, 3, 6, 7, 11, 12, 14, 15, 16, 17, 19, 22, 23, and 25, 500 μmol m^{-2} s^{-1} for samples, 4, 5, 9, 10, 18, and 20, and 1,500 μmol m^{-2} s^{-1} for samples 2, 13, 21, 24, 26, and 27) and for all samples together (data from Figs.3, 4)

Light intensity (μmol m^{-2} s^{-1})	NPQ				Φ_{PSII}				ETR (μmol m^{-2} s^{-1})			
	100	500	1,500	All intensities	100	500	1,500	All intensities	100	500	1,500	All intensities
R^2 value	0.04	0.00	0.10	0.12	0.58	0.46	0.06	0.33	0.53	0.43	0.06	0.02
SE	0.035	0.022	0.046	0.033	0.023	0.016	0.0466	0.0291	0.026	0.016	0.046	0.035
P value	0.4832	0.9174	0.5521	0.0786	0.0016**	0.1441	0.6428	0.0017**	0.003**	0.1589	0.6330	0.4441

** Significant at 1 % error

Table 2 Relationships between steady-state PRI, NPQ, Φ_{PSII}, and ETR from induction curves 20 min after the dark-light transition and plant pigment indices at different leaf growth stages (27 samples)

Leaf pigment indices	PRI		NPQ		Φ_{PSII}		ETR (μmol$m^{-2}s^{-1}$)	
	R^2	*P* value	R^2	*P* value	R^2	*P* value	R^2	*P* value
Chlorophylls								
$mNDVI_{705}$	0.82	<0.0001**	0.05	0.5097	0.23	0.0413*	0.15	0.1427
mND_{705}	0.82	<0.0001**	0.04	0.5843	0.24	0.0385*	0.11	0.2493
mSR_{705}	0.75	<0.0001**	0.02	0.7491	0.21	0.0601	0.03	0.7086
Ratio	0.78	<0.0001**	0.02	0.7722	0.22	0.0585	0.07	0.3973
$NDVI_{green}$	0.76	<0.0001**	0.04	0.6440	0.25	0.0316*	0.00	0.8896
Carotenoids								
$CRI_{550\text{-}780}$	0.41	0.0003**	0.01	0.8471	0.20	0.0736	0.22	0.0544
CRI_{550}	0.32	0.0022**	0.01	0.8481	0.15	0.1453	0.25	0.0332*
CRI_{700}	0.14	0.0506	0.02	0.7935	0.12	0.2283	0.20	0.0696
mCRI	0.29	0.0041**	0.01	0.8401	0.07	0.4358	0.22	0.0531
Anthocyanins								
ARI	0.52	<0.0001**	0.01	0.8793	0.05	0.5566	0.08	0.3865
mARI	0.54	<0.0001**	0.02	0.8249	0.07	0.4315	0.19	0.0772

The samples are the same as those in Figs. 3 and 4. The R^2 values represent best fit relationships (logarithmic between PRI and chlorophyll and carotenoid indices; linear between PRI and anthocyanin indices and quadratic polynomial for the rest of the table)

* Significant at 5 % error; ** significant at 1 % error

$$\mathrm{CPRI} = \mathrm{PRI} - (0.645 * \ln(\mathrm{mNDVI}_{705}) + 0.0688) \quad (16)$$

To evaluate the performance of CPRI, the relationships between CPRI and NPQ and ETR during 0–20 min after dark-light transition in different growth stages of the leaves are presented in Fig. 5. Comparing Fig. 3a, c, e and Fig. 5a one can easily observe that the relationship between CPRI and NPQ is better than that between PRI and NPQ. Also by comparing Fig. 3b, d and f and Fig. 5b, CPRI shows a better relationship with ETR than that observed for PRI/ETR. Therefore, it seems that CPRI can be a better index than PRI to be used for assessing plant photosynthetic performance.

Discussion

Studies of variations in PRI and NPQ with time in photosynthetic induction curves at different light intensities indicated that PRI behavior in a given leaf is similar to that of NPQ. The rapid increase in NPQ seen after 1 min in Fig. 1b presumably reflects the fact that although stomata may close during 30-min dark adaptation, little change in stomatal aperture occurs immediately after irradiation (Pearcy et al. 1994), and that the activities of enzymes involved in photosynthetic CO_2 fixation are low. At low light intensities, the small burst in NPQ presumably involves ΔpH-dependent, xanthophyll-independent processes that may not be detected by PRI, and this is evident

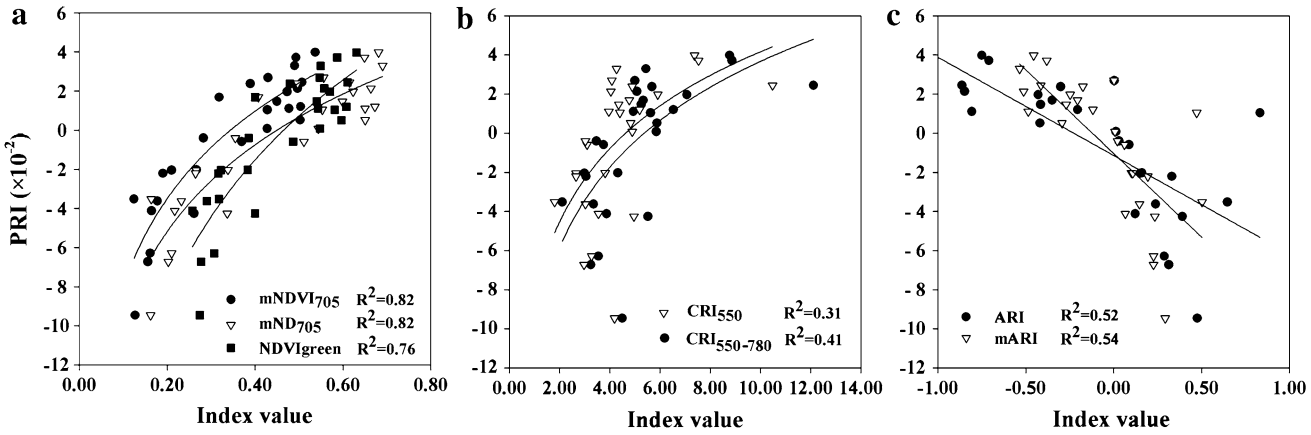

Fig. 4 Relationships between steady-state PRI measured after 20-min exposure to actinic light and some plant pigment indices calculated from the same spectral reflectance data set in each of 27 leaves at different growth stages as presented in Fig. 3. **a** steady-state PRI and chlorophyll indices, **b** steady-state PRI and carotenoid indices and **c** steady-state PRI and anthocyanin indices

Table 3 Results of multiple regression analysis between steady-state PRI and ln ($mNDVI_{705}$), ln ($CRI_{550\text{-}780}$) and mARI (Overall coefficient of determination for the regression analysis, $R^2 = 0.82$ and probability level, $P = 0.000$)

Model	Unstandardized coefficients		Standardized coefficients		
	B	SE	*β*	*t*	*P* value
Constant	0.097	0.031	–	3.106	0.005
mARI	−0.017	0.016	−0.144	−1.057	0.301
$lnmNDVI_{705}$	0.066	0.012	0.930	5.710	0.000
$lnCRI_{550–780}$	−0.017	0.013	−0.180	−1.284	0.212

Data correspond to samples presented in Figs. 3 and 4. Constant is the constant determined for the multiple regression equation

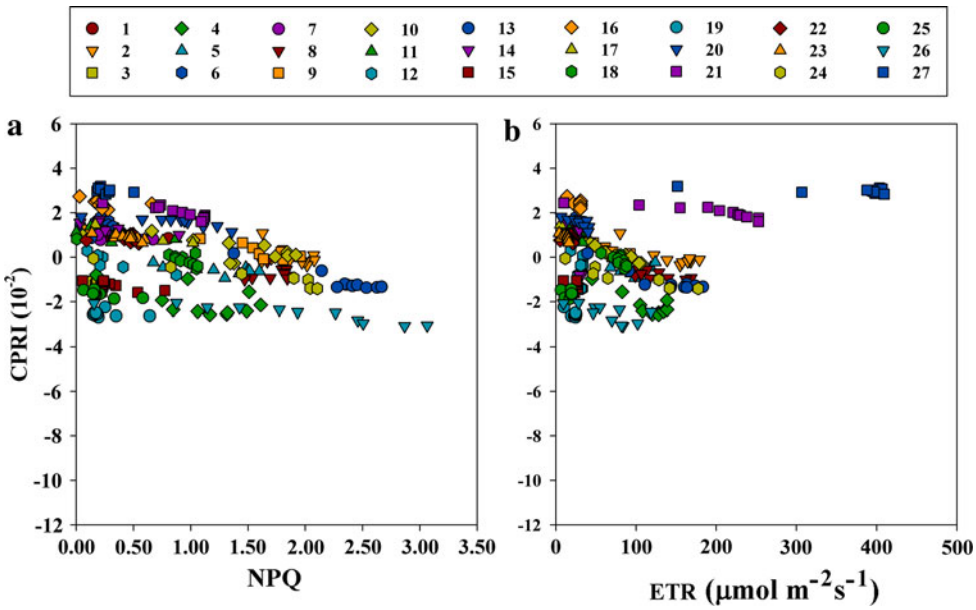

Fig. 5 Relationships of **a** CPRI/NPQ and **b** CPRI/ETR during 0–20 min after dark-light transition in 27 leaf samples (as in Fig. 3) at different growth stages. Color coding is the same as in Fig. 3. The calibration of PRI was conducted using data from Figs. 3 and 4 and significantly improved correlation with NPQ and ETR (linear $R^2 = 0.15$; $P < 0.0001$ and $R^2 = 0.03$; $P = 0.003$, respectively)

in comparisons of Fig. 1a, b. Much larger rapid increases in NPQ were observed at 500 and 1,000 µmol m^{-2} s^{-1} because despite the greater potential activation of photosynthesis, the leaf was quickly exposed to excess light and presumably NPQ was now enhanced by ΔpH-dependent de-epoxidation of xanthophyll pigments that were detected as a decline in PRI. The changes in Φ_{PSII} and ETR flow as expected from changes in Fm′ in these induction

experiments, and so strongly correlated relationships emerge between PRI/NPQ and PRI/ETR with time in the same leaf when assayed at different light intensities.

However, when these assays were repeated as pigments changed during the plant growth cycle, the same relationships were not observed (Fig. 3). Although good correlations were found between PRI/NPQ and PRI/ETR in mature leaves with higher chlorophyll contents, these did not persist to the later stages of the plant growth. Gamon et al. (2001), Filella et al. (2004) and Nakaji et al. (2006) also found that the relationship between PRI and the xanthophyll cycle can break down at different growth stages along with reduction in chlorophyll content. In fact, the relationship between PRI and epoxidation state of xanthophylls and LUEp changes as leaves senesce. The range of PRI variation for a matured single green leaf ($\sim 2.5 \times 10^{-2}$; Fig. 2) is only ~ 20 % of the range for all leaves examined over the life cycle ($\sim 14 \times 10^{-2}$; Fig. 3). Clearly in the longer term, PRI responds to variables other than xanthophylls and it was strongly correlated with chlorophyll indices (Fig. 4). Although previous studies have also shown that PRI variations at short term are mainly influenced by the xanthophyll cycle and changes in contents of carotenoids and chlorophylls (Sims and Gamon 2002; Stylinski et al. 2002), PRI was affected by the pool of carotenoids relative to chlorophylls in Scots pine and Holm oak (Filella et al. 2009).

Relationships between steady-state PRI and NPQ measured at different light intensities were also poor throughout the life cycle of leaves, whereas correlations between PRI and Φ_{PSII} and ETR were much stronger (Table 1). Consistent with this, our studies using thermal and chlorophyll fluorescence imaging techniques, which are capable of simultaneously measuring stomatal conductance and fluorescence parameters, showed that there is a direct relationship between Φ_{PSII} and stomata conductance and CO_2 uptake (Omasa and Takayama 2003). This indicates that we have to look beyond NPQ to discover how to improve the use of PRI as an indicator of overall photosynthetic capacity through its relationship with chlorophyll and carotenoid content, Φ_{PSII}, ETR and LUE.

As shown by the results of this study, chlorophyll content seems to be the main factor affecting PRI in the long-term. Therefore, it seems necessary to correct PRI for the effect of this pigment so that estimations of plant photosynthetic capacity through xanthophyll cycle could be more accurate during the full life cycle of the plant.

We have devised the parameter CPRI (Calibrated PRI) by incorporating the effect of other photosynthetic pigments, especially chlorophyll indices, into understanding the PRI/NPQ relationship in the longer term. We found that CPRI showed better correlation with NPQ when compared with that between PRI and NPQ (Fig. 5). The improvement of overall correlations with NPQ and ETR is small but significant, suggesting that this new index may improve understanding throughout the leaf life cycle. Although CPRI was found to have a better relationship with NPQ than PRI in the long term, it is still a poor index for long term analysis of photosynthetic performance. As mentioned earlier, other studies have confirmed there are many factors, such as changes in canopy structure and LAI, solar illumination and viewing geometry and chloroplast movement which affect the relationship between PRI and NPQ, Φ_{PSII} or ETR and consequently LUE. Therefore, all these factors should be considered while using PRI for estimating photosynthetic performance of vegetation at leaf and canopy scales. Perhaps recently developed remote sensing chlorophyll fluorescence techniques, such as that based on laser-induced fluorescence transients (LIFT) can be applied to further improve correlations between PRI, CPRI, and canopy level photosynthetic parameters (Omasa 1998; Malenovský et al. 2009; Pieruschka et al. 2010). Without further improvement, it seems that PRI is best applied for leaves and canopies with relatively homogeneous chlorophyll content, i.e., to canopies with homogeneous LAI and the same colored leaves.

Conclusions

The present study on the relationship between PRI and chlorophyll fluorescence parameters and leaf pigment indices showed that PRI/NPQ and PRI/ETR were most strongly correlated in the short-term, during photosynthetic induction experiments on mature, chlorophyll-rich leaves under controlled laboratory conditions that engaged energy dissipation via the xanthophyll cycle. Although such relationships were also observed in individual leaves as other pigments changed with leaf age, the numerical value of PRI declined and steady-state PRI was found to be highly correlated with chlorophyll indices. A general relationship between steady-state PRI and Φ_{PSII} and ETR measured at the same light intensity at different life stages of leaves confirmed that overall photosynthesis capacity can be correlated through the relationship of PRI with other plant pigments. The new index (Calibrated PRI; CPRI) suggested in this article showed better correlation with NPQ, confirming that taking into account other factors influencing PRI could significantly improve the performance of this widely used index.

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