



Plant stress detection through a chlorophyll content-dependent relationship between F_v/F_m and the photochemical reflectance index

D. OH*, J.-H. RYU**, K.-M. KIM*, H. JEONG***, H.-D. MOON*^{##}, H. KIM*^{###}, S. CHOI*, B.-K. KIM*^{##}, H. WON*, H. YANG*, and J. CHO*^{##,§,†} 

*Department of Applied Plant Science, Chonnam National University, 77 Yongbong-ro, 61186 Gwangju, Republic of Korea**

*National Agricultural Satellite Center, National Institute of Agricultural Science, Rural Development Administration, 310 Nongsaeangmyeong-ro, 54875 Deokjin-gu, Jeonju-si, Republic of Korea***

*Smart Agricultural Technology Division, National Institute of Crop Science, Rural Development Administration, 20 Jeompiljae-ro, 50424 Miryang-si, Republic of Korea****

Interdisciplinary Program for Agriculture & Life Science, Chonnam National University, 77 Yongbong-ro, 61186 Gwangju, Republic of Korea[#]

BK21 Four Center for IT-Bio Convergence System Agriculture, Chonnam National University, 77 Yongbong-ro, 61186 Gwangju, Republic of Korea^{##}

Crop Environment Research Division, National Institute of Crop Sciences, Rural Development Administration, 181 Hyeoksin-ro, Iseo-myeon, 55365 Wanju-gun, Republic of Korea^{###}

AgriBio Institute of Climate Change Management, Chonnam National University, 77 Yongbong-ro, 61186 Gwangju, Republic of Korea[§]

Abstract

Optical sensing for plant stress detection requires physiologically relevant indices. F_v/F_m , representing the maximum quantum yield of photosystem II photochemistry, quantifies stress across species but requires dark adaptation, limiting field application. Photochemical reflectance index (PRI), a reflectance-based indicator of xanthophyll cycle activity, can be remotely measured, but its irregular variation complicates interpretation. Paddy rice was grown in temperature-gradient field chambers to evaluate F_v/F_m , PRI, and SPAD (soil-plant analysis development). Changes in F_v/F_m were associated with net leaf photosynthesis and showed sensitivity to stress affecting PSII, as its maximum value decreased under high-temperature stress. PRI exhibited a diurnal pattern similar to that of F_v/F_m , while the linear PRI- F_v/F_m relationship varied with SPAD values throughout the season. Utilizing this relationship, variation in F_v/F_m can be inferred from PRI and chlorophyll content, enabling spatial and temporal scaling and quantitative stress assessment. These findings support remote sensing-based plant stress diagnosis.

Keywords: chlorophyll content; F_v/F_m ; photochemical reflectance index; photosynthetic efficiency; plant stress.

Introduction

When plants experience stress due to biotic or abiotic factors, certain functions are reduced; however, stress,

unlike damage, is reversible (Lichtenthaler 1996). For crops, if stress is not relieved and these reductions continue to accumulate, growth and development are inhibited, and cells and tissues become damaged, ultimately leading to

Highlights

- High temperature reduced the maximum quantum yield of photosystem II
- Photochemical reflectance tracked diurnal photosystem II dynamics
- Reflectance and chlorophyll estimated photosystem II efficiency

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*Corresponding author

e-mail: chojaeil@gmail.com

Abbreviations: ETR – electron transport rate; F_0 – minimum chlorophyll fluorescence yield (all PSII reaction centers open in the dark-adapted state); F_m – maximum chlorophyll fluorescence yield (all PSII reaction centers closed under saturating light); F_v/F_m – maximum quantum yield of PSII photochemistry; NPQ – nonphotochemical quenching; PAR – photosynthetically active radiation; P_N – leaf net photosynthesis; PRI – photochemical reflectance index; SPAD – soil-plant analysis development; TGFC – temperature-gradient field chamber.

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lower quality and reduced productivity (Behmann *et al.* 2014, Ahmad *et al.* 2016, Dresselhaus and Hückelhoven 2018). Thus, monitoring physiological stress and growth of field crops should provide a primary basis for agricultural decision-making, particularly under variable climatic conditions (Berger *et al.* 2022, Wu *et al.* 2023, Dong *et al.* 2024). Indeed, smart farming systems use remote sensing-based crop monitoring to implement data-driven farm operations aimed at optimizing and improving sustainability in agricultural production (Katsoulas *et al.* 2016).

Photosynthesis is a fundamental process underlying plant physiological function. Thus, stress in crop plants is often identified through changes in photosynthetic efficiency (Muhammad *et al.* 2021). When chlorophyll absorbs light energy for photosynthesis, it is distributed among three concurrent processes: photosynthetic charge separation, which drives electron transport through the electron carriers shown in the Z-scheme (e.g., Govindjee *et al.* 2017) and leads to the reduction of NADP^+ to NADPH, ultimately contributing to the formation of chemical energy (ATP and NADPH) required for carbon fixation (Blankenship 2014), dissipation as heat, and emission of fluorescent light (Baker 2008, Papageorgiou and Govindjee 2014). This release occurs because energy exceeding the plant's photophosphorylative capacity can cause damage, for example, through the photo-oxidation of cellular components and the photosynthetic apparatus (Demmig-Adams and Adams 1996a). Thus, under conditions in which absorbed light exceeds the capacity for photochemical utilization, stressed plants will give off more energy as heat and fluorescence than healthy plants (Krause and Weis 1991). Therefore, the amount of these two leaked energy types can be used to measure not only photosynthetic efficiency but also plant stress (Porcel *et al.* 2015). Indeed, two indices, the photochemical reflectance index (PRI) (Gamon *et al.* 1992) and the maximum quantum yield of PSII photochemistry (F_v/F_m) (Kitajima and Butler 1975), have been developed to detect physiological stress conditions in crops (Baker and Rosenqvist 2004, Katsoulas *et al.* 2016, Ihuoma and Madramootoo 2017).

Xanthophyll cycle pigments, particularly zeaxanthin formed *via* the de-epoxidation of violaxanthin, contribute to the dissipation of excess excitation energy as heat in plant leaves in conjunction with other components such as PsbS and lumen acidification (Demmig-Adams and Adams 1996a,b; Gilmore 1997). In situations where a plant must dissipate more excitation energy as heat, such as under physiological stress, increases in the pigment zeaxanthin in leaves – along with the involvement of the PsbS protein – result in a rise in spectral reflectance at 531 nm (Gamon and Surfus 1999). The PRI was defined as the ratio of a plant's reflectance at 531 nm to its reflectance at 570 nm, a wavelength that acts as a reference and thus responds to the relative amount of zeaxanthin. Previous studies have shown that the PRI effectively indicates photosynthetic efficiency, with lower PRI values generally reflecting reduced photosynthetic efficiency (Gamon *et al.* 1997, Barton and North 2001, Styliniski *et al.* 2002, Guo and Trotter 2004, Chou *et al.* 2017, Jiang *et al.* 2023).

While, due to its ease of measurement using spectral sensors, the PRI enables continuous monitoring across various spatial scales using the remote sensing method, the data are often difficult to interpret due to irregular variation. The energy unused in photophosphorylation is lost as both heat and fluorescence. Thus, the PRI, which is associated with heat dissipation processes, shows limited applicability depending on the growth stage and species of the plant. However, the F_v/F_m is easier to interpret. F_v/F_m measurements require 15–20 min of prior dark adaptation, during which photochemical processes are inactive and nonphotochemical energy dissipation is minimal, resulting in fluorescence emission primarily from open reaction centers (Genty *et al.* 1989, Maxwell and Johnson 2000, Baker 2008). In this process, the heat dissipation mechanism becomes inactive in leaves that are maintained in darkness.

Thus, chlorophyll fluorescence measurements after dark adaptation, such as F_v/F_m , provide an estimate of the maximum quantum yield of PSII photochemistry, which reflects the photochemical capacity of PSII. In F_v/F_m , F_v is defined as $F_m - F_o$, representing the difference between chlorophyll fluorescence when all PSII reaction centers are closed (F_m) and when they are open (F_o), whereas F_m represents the maximum fluorescence emitted when all reaction centers are closed under saturating light conditions. The F_v/F_m ratio typically ranges around ~0.8–0.85 across plant species and is widely used as an indicator of PSII photochemical efficiency. An F_v/F_m value of ~0.85 corresponds to the maximum quantum yield of PSII under non-stressed conditions (Demmig and Björkman 1987, Stirbet *et al.* 2018), and a value of 0.4, for example, indicates that PSII photochemical efficiency is reduced to approximately half of its maximum, without directly reflecting CO_2 assimilation.

However, the requirement for dark adaptation limits the applicability of the F_v/F_m measurement method. Measuring F_v/F_m requires over 15 min of dark adaptation and a specific optical instrument, such as a pulse-amplitude-modulated (PAM) fluorometer, which uses a saturating light pulse to close all PSII reaction centers, making it impossible to use with drones or satellites. Thus, the spatial scale of F_v/F_m measurement cannot be expanded from the leaf level to the canopy level (Cendrero-Mateo *et al.* 2016).

Previous studies have reported relationships between PRI and chlorophyll fluorescence parameters, such as electron transport rate (ETR) and nonphotochemical quenching (NPQ), although these relationships may vary depending on conditions (Rahimzadeh-Bajgiran *et al.* 2012). It is assumed that there is a relationship between the PRI and F_v/F_m because PRI is associated with energy dissipation, whereas F_v/F_m reflects changes in PSII photochemical efficiency influenced by various physiological factors during the light-dependent reactions of photosynthesis. The expected relationship suggests that difficulties in interpreting the PRI can be addressed through F_v/F_m , and conversely, the challenges in observing F_v/F_m can be overcome through the PRI. This study aims

to analyze the relationship between PRI and F_v/F_m in rice plants subjected to high-temperature and light stress during two growing seasons.

Materials and methods

Experimental facility and rice cultivation: The study was carried out using the temperature-gradient field chamber (TGFC) facility of Chonnam National University, Gwangju, Republic of Korea. The TGFCs are special-purpose heating units based on field conditions that consist of one heater and three ventilators (Oh *et al.* 2023). Their air-temperature control system is described in detail in Ryu *et al.* (2022). The innermost air temperature of each TGFC is constantly maintained to be approximately 3°C higher than the ambient temperature, while the temperature near the entrance is controlled to match the ambient temperature. Plants located closer to the interior of the chamber experience greater high-temperature stress, particularly when ambient temperatures are already high during the rice-growing season, due to the elevated temperature, which adversely affects their photosynthetic activity (Ryu *et al.* 2022).

In the TGFC, paddy rice was cultivated from 2016 to 2018, and various plant responses to different levels of high-temperature stress were observed. In this study, *Oryza sativa* L. *japonica* 'Ilmi', a mid-to-late maturing cultivar and one of the leading Korean rice cultivars, was used. After sowing the seeds, plants were transplanted into the TGFC on 3 June 2016 (DOY 155) and 1 June 2018 (DOY 152) and harvested on 13 October 2016 (DOY 287) and 27 September 2018 (DOY 270), respectively. Three rice plants were planted per clump, with a clump spacing of 15 × 30 cm. Fertilizer treatments of 845.2 g of N (nitrogen), 972 g of P (phosphorus), and 547.2 g of K (potassium) per TGFC, based on an N:P:K of 9:4.5:5.7 kg per 10 acres, were applied at planting. Two more supplemental fertilizations were applied based on a 5:2:3 ratio.

Optical sensing: Leaf-scale measurements of F_v/F_m , PRI, and SPAD values were performed five times during each growing season, one each at the tillering, booting, early grain-filling, mid grain-filling, and late grain-filling stages. For measurements of F_v/F_m and PRI, the 10 topmost, fully expanded south-facing leaves were used. As F_v/F_m measurement requires an intense flash of light to saturate the photosynthetic reaction center complex and electron transfer chain of PSII, continuous measurements of F_v/F_m may impose additional light stress on the sample leaf under repeated measurements. To prevent the inclusion of photodamaged leaf samples in the data, observations were conducted at 1- to 2-h intervals from before sunrise until sunset. Additionally, measurements were performed on the same leaf in the order PRI, SPAD, and then F_v/F_m within 3–4 min to avoid potential effects of saturating light pulses on spectral reflectance measurements, as optical signals may also be influenced by chloroplast movement induced by blue light (Nauš *et al.* 2010).

F_v/F_m measurement: Chlorophyll fluorescence parameters were measured using a portable fluorometer (FP100, Photon Systems Instruments, Drásov, Czech Republic) with leaf dark clips, which provide dark adaptation of the measurement area on the leaf. All measurements were performed after at least 20 min of dark adaptation. The F_v/F_m is defined as (Kitajima and Butler 1975):

$$F_v/F_m = (F_m - F_o)/F_m \quad (1)$$

where F_m is measured during the first saturation flash after dark adaptation; F_o is the minimum fluorescence in the dark-adapted state; and F_v represents the difference between F_m and F_o . Accordingly, the F_v/F_m ratio represents the maximum quantum yield of PSII photochemistry (Kitajima and Butler 1975). The value decreases according to changes in photosynthetic efficiency under stress conditions (Valladares and Pearcy 1997).

PRI measurement: In this study, the mean of five measurements taken using a portable PRI meter (PRI-200, Photon Systems Instruments, Drásov, Czech Republic) at the same part of the selected leaf was used to determine each PRI value. The PRI was calculated as (Gamon *et al.* 1992):

$$PRI = (R_{531} - R_{570})/(R_{531} + R_{570}) \quad (2)$$

where R_{531} indicates the reflectance at 531 nm, and R_{570} indicates the reflectance at 570 nm. Specifically, the 531-nm wavelength is indicative of xanthophyll cycle pigment conversion (Demmig-Adams and Adams 1996a, Gilmore 1997). During the xanthophyll cycle, which facilitates the thermal dissipation of excess energy through the conversion of violaxanthin to zeaxanthin (and its reverse *via* epoxidation), an increase in zeaxanthin leads to a decrease in R_{531} , while R_{570} remains almost unchanged, resulting in a decrease in the PRI. Changes in PRI can be associated with variations in photosynthetic activity and may also reflect plant stress under certain conditions, although the relationship depends on physiological and environmental factors.

SPAD measurement: Chlorophyll is a dominant factor controlling the leaf optical properties of healthy green plants and is the basis for optical measurements of chlorophyll content, such as SPAD readings (Süß *et al.* 2015). Previous studies have characterized the relationship between leaf chlorophyll content and SPAD values (Gianquinto *et al.* 2004, León *et al.* 2007, Uddling *et al.* 2007, Shah *et al.* 2017), which are calculated from the transmittance of red and near-infrared wavelength bands representing the spectral characteristics of chlorophyll at the leaf scale, although SPAD readings can also be affected by chloroplast movement (Nauš *et al.* 2010). In this study, SPAD values were measured for all leaf samples using a hand-held chlorophyll meter (SPAD-502, Minolta Corp., Osaka, Japan) and used as a proxy for chlorophyll content.

Leaf net photosynthesis: Leaf net photosynthesis (P_N) measurements were conducted on selected leaves using

a LI-6400 portable system with an infrared gas analyzer (LiCor Inc., Lincoln, NE, USA) and a 2×3 cm clear-top chamber (LiCor Inc.) to quantify photosynthesis driven by natural photosynthetically active radiation [PAR, $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$]. After P_N measurements under natural PAR, the same leaves were dark-adapted using leaf clips and subsequently used for F_v/F_m measurements. All measurement data were recalculated based on the actual leaf area of all samples measured in the interior area (6 cm^2) of the leaf cuvette. Measurements were performed over 1- to 2-h intervals between 06:00 and 18:00 h on clear days, considering the natural PAR. Air temperature and relative humidity inside the leaf cuvette were maintained near atmospheric conditions, and the reference CO_2 concentration was maintained at near $400 \mu\text{mol mol}^{-1}$ using 12-gram CO_2 cylinders (LiCor Inc.).

Results and discussion

Relationship between leaf P_N and F_v/F_m : P_N showed a significant polynomial response to increasing PAR ($p < 0.01$), with a coefficient of determination (R^2) of 0.85 (Fig. 1A). It increased with PAR, then leveled off once PAR reached saturation. This leveling off occurs at high PAR intensities because photoinhibition reduces the photosynthetic rate under excessive light, not solely due to instantaneous light intensity (Powles 1984, Li *et al.* 2009). This photosynthetic response curve is generally consistent with the findings of previous studies (Edwards and Baker 1993, Ögren and Evans 1993, Zhang *et al.* 2020, Harbinson *et al.* 2022). There was no significant correlation between P_N and F_v/F_m . The P_N value is determined by the proportion of absorbed energy that is utilized for carbon fixation (Rosati and DeJong 2003).

High-intensity light conditions can cause physiological stress, decreasing F_v/F_m (Gauslaa and Solhaug 2000). However, because F_v/F_m represents the maximum photochemical efficiency rather than actual photosynthetic rate, it does not directly correspond to leaf net photosynthesis, which depends on both light availability and its utilization. Accordingly, a significantly positive correlation ($p < 0.01$) was observed between $F_v/F_m \times \text{PAR}$ and P_N , with an R^2 value of 0.84 (Fig. 1B). The product

$\text{PAR} \times F_v/F_m$ can be interpreted as a proxy for effective photochemical energy because F_v/F_m was measured for each leaf under the prevailing in situ light-stress conditions and therefore varied among individual P_N measurements, allowing $\text{PAR} \times F_v/F_m$ to incorporate both incident light availability and variation in PSII photochemical efficiency (Sun and Wang 2018). Although this does not imply that $\text{PAR} \times F_v/F_m$ fundamentally changes the saturating nature of the photosynthetic light response, the differing relationship between P_N and $\text{PAR} \times F_v/F_m$ and between P_N and PAR alone reflects the variation in F_v/F_m associated with the prevailing light-stress conditions during measurement.

Responses of F_v/F_m and the PRI to stress: Fig. 2 shows the response of F_v/F_m and PRI to air temperature. The upper bound of F_v/F_m values began to decline at approximately 35°C , indicating high-temperature stress, and fell below 0.8 (Fig. 2A). Although various stress factors may contribute to reductions in F_v/F_m values, in the current study, the decline is interpreted as evidence of damage to PSII caused by elevated temperatures. Previous studies have reported that high-temperature stress can influence photosynthetic efficiency through changes in electron transport, photoprotection, and photooxidative balance, particularly under combined high light and temperature conditions (Mathur *et al.* 2014, Zahra *et al.* 2023), all of which are known to influence PSII function and are therefore reflected in F_v/F_m . The variability observed at similar temperatures further suggests that factors other than temperature also influence F_v/F_m under field conditions. The F_v/F_m values found below the maximum line are influenced by other factors affecting photosynthetic activity, such as high solar radiation in addition to temperature, suggesting that the distribution of F_v/F_m may deviate from normality and vary with stress intensity (Lazár and Nauš 1998).

On the other hand, the response of the PRI to temperature was more complicated. In Fig. 2B, the PRI observations are divided by month, with July representing the tillering stage of rice, August representing the booting stage, and September representing the grain-filling stage. As growth progressed, the minimum and maximum

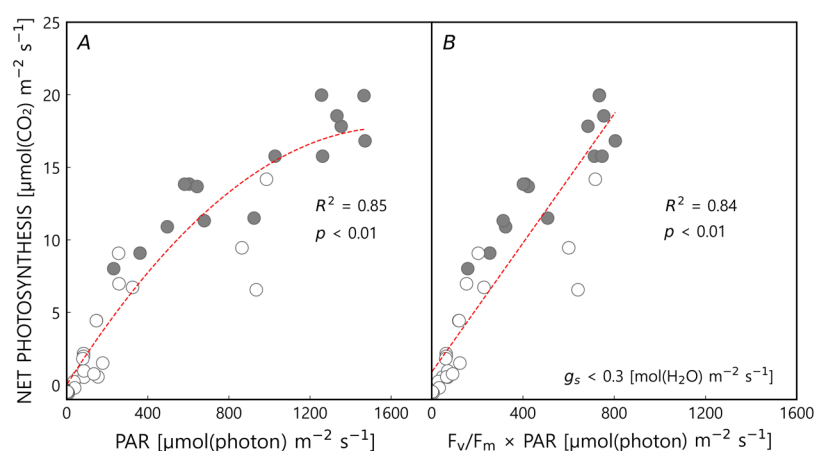


Fig. 1. Responses of leaf net photosynthesis to (A) photosynthetically active radiation (PAR) and (B) maximum photochemical energy (F_v/F_m measured after dark adaptation) $\times$ PAR. Open circles represent lower ($< 0.3 \text{ mol m}^{-2} \text{s}^{-1}$) stomatal conductance. The regression analyses included both open and closed circles.

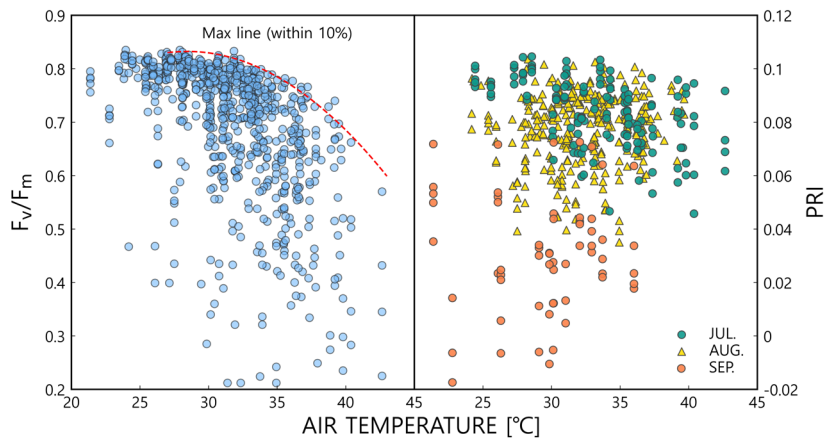


Fig. 2. Responses of (A) maximum quantum yield of PSII photochemistry (F_v/F_m) and (B) photochemical reflectance index (PRI) to air temperature in rice plants over two seasons.

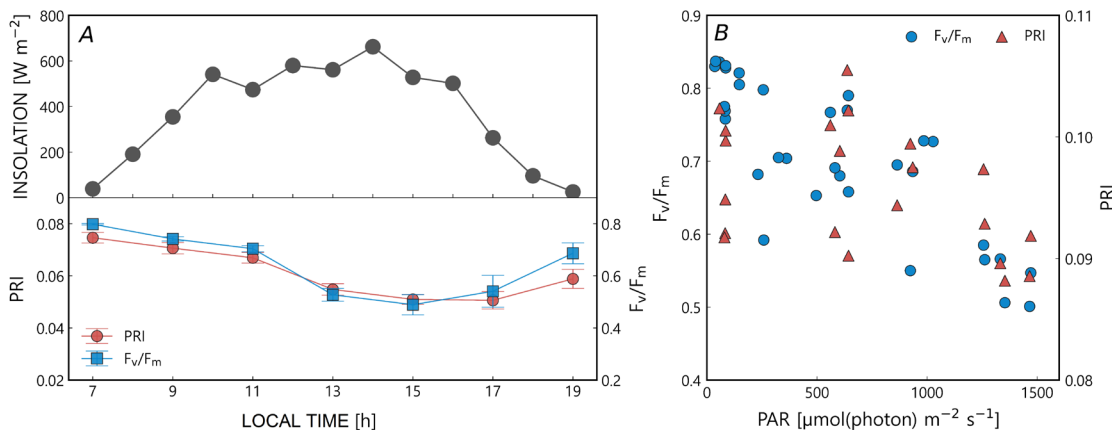


Fig. 3. (A) Hourly incoming solar radiation and diurnal changes in maximum quantum yield of PSII photochemistry (F_v/F_m) and photochemical reflectance index (PRI). (B) The responses of F_v/F_m and PRI to photosynthetically active radiation (PAR).

PRI values decreased, and their variability increased. The F_v/F_m ratio represents changes in the maximum quantum yield of PSII photochemistry under stress conditions, whereas PRI reflects immediate biochemical responses to such conditions. Parameters describing PSII function under light-adapted conditions may also be more closely associated with PRI (Lazár 2015). In this context, the response of PRI to temperature appeared more complex than that of F_v/F_m , and the data shown in Fig. 2 confirm the differences between F_v/F_m and the PRI. Further, F_v/F_m values, which are influenced by multiple physiological and environmental factors, fall within a relatively constrained range (approximately 0.7–0.85 under non-stressed conditions) and represent PSII photochemical efficiency, while PRI exhibits different value ranges depending on plant species and growth stage, making its interpretation more variable.

Fig. 3A shows the diurnal patterns of F_v/F_m and PRI values for 10 leaf samples along with diurnal solar radiation. The F_v/F_m ratio decreased to approximately 0.5 in response to increasing solar radiation during the morning period. It began to recover after 15:00 h, when solar radiation started to decrease, reaching approximately 0.7 by 19:00 h. The diurnal pattern of the PRI was highly similar to that of F_v/F_m . The PRI decreased with increasing insolation, but its recovery commenced later than that of

F_v/F_m , and it had not regained even half of the reduction it had experienced over the day by 19:00 h. It is notable that the trends in F_v/F_m and the PRI were comparable, suggesting that both indices reflect photosynthetic activity and respond to plant stress in similar ways.

The diurnal pattern of F_v/F_m was consistent with those seen in previous studies and demonstrates that this index is sensitive to excessive light, which causes photoinhibition (Sweeney *et al.* 1979, Bertamini and Nedunchezian 2003, Murata *et al.* 2007). This can be further illustrated in Fig. 3B, where the leaves' F_v/F_m values generally decrease with increasing incident PAR. Also, driven by increasing PAR, the xanthophyll cycle dissipates excess energy, leading to a decrease in PRI. However, the responses of F_v/F_m and PRI do not consistently correspond under the same PAR conditions, indicating that the two indices capture different aspects of photosynthetic responses to light. Since the amount of xanthophyll pigment varies depending on the plant species and growth stage, previous studies have evaluated plant stress using the daily Δ PRI ($\text{PRI}_{\text{max}} - \text{PRI}_{\text{min}}$) instead of the raw PRI value (Gamon and Berry 2012, Wong and Gamon 2015, Magney *et al.* 2016). The Δ PRI value, shown in Fig. 3A (lower panel), was 0.0292, representing the activity of the xanthophyll cycle in response to stress intensity. However, continuous observation is required to obtain reliable Δ PRI values.

Intercorrelation among F_v/F_m , PRI, and SPAD values:

Three optical measurements, F_v/F_m , PRI, and SPAD values, are shown in Fig. 4. When considering F_v/F_m and PRI, no significant correlation could be found, and the values appeared to be scattered in the correlation plots (Figs. 2 and 3). However, when SPAD values, measuring leaf chlorophyll content, were included, the relationship between F_v/F_m and the PRI at a given SPAD value became clearer. The black lines in Fig. 4 represent negative linear regressions characterizing the relationship between F_v/F_m and the PRI at SPAD values in five ranges: under 24, 24–28, 28–32, 32–36, and over 36.

As SPAD values increased, PRI values also tended to increase, as did the intercepts of PRI to F_v/F_m regression curves. However, the slopes of the regression curves remained relatively constant among the SPAD classes, except when SPAD values dropped below 22. The reflectance at 531 nm, used in PRI calculations, is known to reflect the ratio of the carotenoid to chlorophyll content (Wong and Gamon 2015). In addition, high-temperature stress can induce chloroplast movement, which affects both SPAD-based chlorophyll estimates and leaf reflectance signals (Frolec *et al.* 2010, Nauš *et al.* 2010, Baránková *et al.* 2016). This explains the variation in the regression intercepts observed across different SPAD value ranges in Fig. 4. On the other hand, the similarity of regression slopes across SPAD classes suggests a consistent relationship between F_v/F_m and PRI at a given chlorophyll content. This relationship may partly reflect shared underlying processes, as the xanthophyll cycle has been reported to influence chlorophyll fluorescence parameters, including F_m , through NPQ (Jahns and Holzwarth 2012).

Chlorophyll content, the key factor resolving the relationship between F_v/F_m and PRI, is related to the maximum photosynthetic capacity, whereas F_v/F_m and PRI reveal plant stress by characterizing photosynthetic efficiency. Furthermore, chloroplast movement induced by high-temperature stress may modify apparent chlorophyll content and optical signals, thereby influencing both SPAD values and PRI. Chlorophyll content varies with a plant's growth stage or the leaf's age. Thus, SPAD values varied according to the observation month (Fig. 4). Growth or aging alters not only chlorophyll content but also the carotenoid–chlorophyll ratio, thereby affecting PRI values (Filella *et al.* 2009, Gamon *et al.* 2015). However, while chlorophyll content can influence PRI, it does not affect the maximum quantum efficiency, F_v/F_m . Thus, when stress is at high enough intensities or durations to affect chlorophyll content, the intercept of the linear regression characterizing the relationship between PRI and F_v/F_m decreases, and a new regression line is obtained.

Aging or long-term stress reduces chlorophyll content, but this response is slower than those of fluorescence and thermal dissipation, and thus those of F_v/F_m and PRI. Therefore, at a given chlorophyll content, variations in F_v/F_m and PRI of leaves can be considered changes caused by short-term stress. This implies that stressors of insufficient intensity and duration to alter chlorophyll content can be addressed using linear regression between F_v/F_m and the PRI. Thus, by utilizing this intercorrelation,

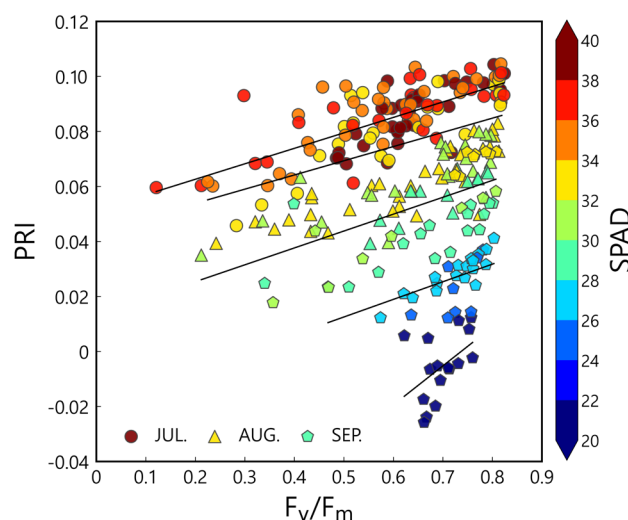


Fig. 4. Intercorrelation among three photosynthetic indicators: maximum quantum yield of PSII photochemistry (F_v/F_m), photochemical reflectance index (PRI), and soil-plant analysis development (SPAD) values. The slope and intercept of associated regression equations varied depending on the SPAD values.

plant stress can be quantitatively assessed by estimating F_v/F_m variation without dark adaptation based on the PRI and chlorophyll content.

Conclusions: This study investigated the relationship between two photosynthesis-related indices, F_v/F_m and PRI. While F_v/F_m typically ranges between 0 and ~0.8 and represents the maximum quantum yield of PSII photochemistry under dark-adapted conditions rather than as a variable parameter under light, its observation and spatial scaling are limited. In contrast, PRI, a reflectance-based vegetation index, allows for remote sensing and continuous monitoring but can be more complex to interpret, as its value range varies depending on plant species and growth conditions. As both indices are involved in the light-dependent reactions of photosynthesis, we assumed a relationship exists between them. Using a temperature-gradient field chamber, rice was grown under increasing thermal stress intensities, and then the F_v/F_m and PRI were measured along with SPAD values, a proxy of chlorophyll content. A linear relationship between F_v/F_m and PRI on the basis of photosynthetic activity and plant stress levels was observed. However, as chlorophyll content increased, the slope of this relationship remained relatively constant while the intercept also increased.

The chlorophyll content proxy used in this study, SPAD, has limited applicability because it represents values at a specific point on a leaf. In addition, it is calculated based on leaf transmittance, not reflectance, making it difficult to apply using satellites or aircraft. These issues are similar to the limitations of F_v/F_m , *i.e.*, both measures are difficult to scale up from the leaf to the canopy level. This issue can be solved by using chlorophyll-related vegetation indices other than the SPAD value, such as the MERIS terrestrial

chlorophyll index or the red-edge chlorophyll index. Further research will be necessary to assess the feasibility of using such vegetation indices. Thus, the findings of this study contribute to the remote sensing-based detection of plant stress at the canopy scale by demonstrating that PRI combined with a chlorophyll content measure can provide indirect information related to variations in F_v/F_m , without requiring dark adaptation.

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