

Article Relation of Photochemical Reflectance Indices Based on Different Wavelengths to the Parameters of Light Reactions in Photosystems I and II in Pea Plants

Ekaterina Sukhova * D and Vladimir Sukhov D

Department of Biophysics, N.I. Lobachevsky State University of Nizhny Novgorod,

603950 Nizhny Novgorod, Russia; vssuh@mail.ru

* Correspondence: n.catherine@inbox.ru; Tel.: +7-929-040-2938

Received: 23 March 2020; Accepted: 19 April 2020; Published: 21 April 2020



Abstract: Measurement and analysis of the numerous reflectance indices of plants is an effective approach for the remote sensing of plant physiological processes in agriculture and ecological monitoring. A photochemical reflectance index (PRI) plays an important role in this kind of remote sensing because it can be related to early changes in photosynthetic processes under the action of stressors (excess light, changes in temperature, drought, etc.). In particular, we previously showed that light-induced changes in PRIs could be strongly related to the energy-dependent component of the non-photochemical quenching in photosystem II. The aim of the present work was to undertake comparative analysis of the efficiency of using light-induced changes in PRIs (Δ PRIs) based on different wavelengths for the estimation of the parameters of photosynthetic light reactions (including the parameters of photosystem I). Pea plants were used in the investigation; the photosynthetic parameters were measured using the pulse-amplitude-modulated (PAM) fluorometer Dual-PAM-100 and the intensities of the reflected light were measured using the spectrometer S100. The Δ PRIs were calculated as Δ PRI(band,570), where the band was 531 nm for the typical PRI and 515, 525, 535, 545, or 555 nm for modified PRIs; 570 nm was the reference wavelength for all PRIs. There were several important results: (1) Δ PRI(525,570), Δ PRI(531,570), Δ PRI(535,570), and Δ PRI(545,570) could be used for estimation of most of the photosynthetic parameters under light only or under dark only conditions. (2) The combination of dark and light conditions decreased the efficiency of Δ PRIs for the estimation of the photosynthetic parameters; Δ PRI(535,570) and Δ PRI(545,570) had maximal efficiency under these conditions. (3) $\Delta PRI(515,570)$ and $\Delta PRI(525,570)$ mainly included the slow-relaxing component of PRI; in contrast, $\Delta PRI(531,570)$, $\Delta PRI(535,570)$, $\Delta PRI(545,570)$, and Δ PRI(555,570) mainly included the fast-relaxing component of PRI. These components were probably caused by different mechanisms.

Keywords: photochemical reflectance index (PRI); modified PRIs; light-induced changes in PRIs; parameters of light reaction in photosystem I; parameters of light reaction in photosystem II; slow-relaxing changes in PRI; fast-relaxing changes in PRI; light curve

1. Introduction

Environmental conditions change over time; in particular, there are fluctuations in light intensity [1–3], temperature [4,5], precipitation [6,7], etc., with durations from less than one second to days and weeks. Plants are very sensitive to these fluctuations in environmental conditions, which can act as stressors, inducing a decrease in photosynthetic activity and plant productivity, as well as other changes in physiological processes [1,3,4,8,9]. Early and remote detection of stressor-induced changes forms the basis of the timely use of methods of plant protection, i.e., it is important for agriculture



and environmental conservation [10]. Optical methods are widely used for this purpose [11,12]; in particular, the measurement of plant reflectance is a perspective solution to target this problem [12–15].

There are numerous investigations that analyze the relations of the modifications of reflectance with the responses of physiological processes (including photosynthesis and its regulation [16–20], growth [21], water exchange [14,22,23], isoprene emission [20,24,25], electrical activity [26–28], etc.), the changes in biochemical content (including concentrations of chlorophylls [29–31], carotenoids [32–34], nitrogen compositions [35,36], etc.), or the plant damage caused by abiotic [17,20] and biotic stressors [11,12,15]. The calculation of numerous reflectance indices, which are often based on measurements of changes in intensity of the reflected light at specific wavelengths in comparison with one at a reference wavelength, can strongly improve the analysis of plant reflectance [10,13–15]. It is shown that these reflectance indices can be used for the effective remote sensing of plants in short-term [13,35,37–39], seasonal [20,40–42], and multi-year observations [39,43,44].

A photochemical reflectance index (PRI), which was proposed by Gamon et al. (1992) [35], is a perspective tool for the remote sensing of plants at different spatial scales (leaf, canopy, ecosystem) [17,20] and time (seconds, minutes, hours, days, seasons, years) scales [13,17,20,43,44]. Typically, a PRI is calculated in accordance with Equation (1) [17,31,39]:

$$PRI(531,570) = \frac{R_{531} - R_{570}}{R_{531} + R_{570}} \tag{1}$$

where R_{531} is the intensity of the green reflected light at 531 nm, and R_{570} is intensity of the yellow reflected light at 570 nm. The reflected light at 531 nm is used as the center of a green shift in reflectance, which is related to the de-epoxidation of violaxanthin to zeaxanthin via antheraxanthin [35,39,45]. The de-epoxidation is induced by acidification of the chloroplast lumen and is an important photosynthetic protective mechanism under excess light [46,47]. The reflected light at 570 nm is typically used as reference light [17], because reflectance at 570 nm is weakly changed under illumination [35,45].

However, de-epoxidation or epoxidation develop for time periods from several minutes to tens of minutes [48–50]; in contrast, changes in PRI can be observed after 1–2 minutes or even after seconds of illumination [16,37,38,51]. Additionally, long-term PRI changes (up to seasons and years) can be also observed [42–44,52]. This means that other mechanisms can also participate in the changes in PRIs. The long-term changes in PRIs are probably caused by changes in carotenoid/Chl pigment ratios [34,41,44], which are related to changes in the total xanthophyll pigment pool size. It is interesting that this pool size can also influence the changes in PRIs in a range of minutes induced by the action of light with high intensity [31], i.e., long-term changes in PRIs can be related to short-term changes in this reflectance index.

The fast changes in PRI (seconds and minutes) are probably caused by light scattering changes (with a maximum at about 535–545 nm) associated with chloroplast lumen acidification, which induces chloroplast shrinkage [45,53,54]. The strong relations between the changes in the PRI and the changes in light scattering at 535 nm for 2–4 minutes after the start of illumination support this hypothesis [38]. Additionally, it cannot be fully excluded that electrochromic shift (with a maximum at about 515–520 nm and a time range up to seconds [38]) can participate in these fast changes in PRIs.

As a result, PRI is strongly related to acidification of the chloroplast lumen (in particular, by means of the de-epoxidation of violaxanthin to zeaxanthin and chloroplast shrinkage); it is known that this acidification can influence dominant photosynthetic processes [47]. It is very probable that changes in PRI are related to photosynthetic processes [17,20,39,41,54,55]; however, it can be also expected that this relation to photosynthetic parameters can be intricate. Our previous meta-analysis showed that correlations between PRIs and widely-used photosynthetic parameters (a quantum yield of photosystem II (γ (PSII)), a non-photochemical quenching of chlorophyll fluorescence (NPQ), and a photosynthetic light using efficiency) can vary strongly [56]. In particular, these correlations are sensitive to illumination conditions and the scale of measurement, as well as to the parameters of

stressor-induced changes in photosynthetic processes [37,56]. The use of light-induced changes in PRIs (Δ PRIs) can strongly decrease errors of the PRI value; however, correlation coefficients between Δ PRI and photosynthetic parameters can be also varied [31,57–64].

Another potential way of improving of relation between PRIs and photosynthetic parameters can be based on the use of modified methods of PRI calculation. It is important that the typical PRI is calculated on the basis of the reflected light at 531 nm [35]. In accordance with Gamon et al. (1990, 1997) [45,54], changes in the intensity of the reflected light at 531 nm are related to two components of change in reflectance. The first component of the reflectance change has a maximum at about 526 nm (the "526 nm component"); this component is mainly related to the de-epoxidation of violaxanthin to zeaxanthin [54]. The second component of the reflectance change has a maximum at about 535–545 nm (the "545 nm component"); this component is likely related to changes in the light scattering by chloroplasts [45,54]. It is probable that the 526 nm component of the reflectance change is the major component of the changes in the typically calculated PRI [54].

However, it can be expected that changes in PRI-like reflectance indices (term "modified PRIs" was used in our work), which are calculated as PRI(band,570) (where the "band" is the wavelength, in the green range which differs from 531 nm), should be dependent on the 526 nm and 545 nm components of change in reflectance in different manners. It is very probable that the use of bands of the green light less than 531 nm should increase the participation of the 526 nm component in changes of the modified PRIs. In contrast, the use of bands of the green light more than 531 nm should increase the participation of the 545 nm component in changes of the modified PRIs. The question "Can a change in the wavelength increase the relations between the changes in PRIs and photosynthetic parameters?" is not clear at present. Gamon et al. (1997) [54] showed that the 545 nm component of change in reflectance is weakly related to changes in some photosynthetic parameters (in particular, γ (PSII)); in contrast, the 526 component is strongly related to these parameters. However, some works [41] show that PRI(545,570) can be strongly correlated with NPQ; i.e., the 545 nm component of change in reflectance is likely also related to photosynthetic parameters. As a result, determining the relations of modified PRIs based on different wavelengths to photosynthetic parameters requires further investigation. The complex analysis of photosynthetic parameters (in particular, analysis of the main parameters of light reactions in both photosystem I (PSI) and photosystem II (PSII)) can be more effective for such investigation.

Thus, the aim of the present work was to conduct a comparative analysis of the relation of light-induced changes in PRIs based on different wavelengths (515, 525, 531, 535, 545, and 555 nm; 570 was the reference wavelength) to the main parameters of light reactions in PSI and PSII. It was important that we investigated both the typical Δ PRI(531,570) and the modified Δ PRIs, including Δ PRI(515,570), Δ PRI(525,570), Δ PRI(535,570), Δ PRI(545,570), and Δ PRI(555,570).

2. Materials and Methods

2.1. Materials

Pea seedlings were used in this investigation because these seedlings offered a suitable model object in our earlier investigations [26,37,38,62,64]. In particular, we previously showed that a reference wavelength equal to 570 nm is optimal for PRI calculation in this object [26], and that light-induced changes in PRIs (ΔPRIs) were more effective for the estimation of NPQ in peas than the absolute values of these indices [62,64]. Additionally, it was known that durations of de-epoxidation and epoxidation in the xanthophyll cycle were about 10 min or more in peas [49]; in contrast, changes in the chloroplast light scattering were observed for about 1–2 min after the initiation or termination of illumination [38,65]. This means that we were able distinguish changes in PRIs related to activity of the xanthophyll cycle (slow-relaxing changes), and ones related to modifications of light scattering (fast-relaxing changes) on basis of a simple analysis of PRI relaxation without actinic light.

Plants were cultivated hydroponically (a half-strength Hoagland–Arnon medium) in a Binder KBW 240 climatic chamber (Binder GmbH, Tuttlingen, Germany) at 23 °C under a 16/8 light/dark photoperiod. The measurements were performed on 2–3-week-old plants. The reflectance and photosynthetic parameters were investigated in the second mature leaves.

2.2. The Procedure of the Measurements of the Photosystem II Fluorescence, Photosystem I Light Absorption, and Reflected Light Intensity in Pea

Figure 1a shows the schema of simultaneous measurements of the intensity of the reflected light, fluorescence of PSII, and light absorption of PSI in pea leaves, which was previously described in detail [64]. The leaves were fixed in the measuring system before the experimental procedure.

The PSII fluorescence and PSI absorption measurements were performed using a standard Dual-PAM-100 measuring system (Heinz Walz GmbH, Effeltrich, Germany). The system analyzed photosynthetic parameters on basis of method of the pulse-amplitude-modulated (PAM) fluorometry [66–68] and its modification for measurement of the PSI light absorption [69]. Weak pulses (2.5 μ s in length) of two types of measuring light (ML) were used; light with a maximum at 460 nm for the measurements of the PSII fluorescence and dual-wavelength light with maximums at 830 and 870 nm for the measurements of the PSI light absorption. Saturation pulses (SPs) of red light (maximum intensity at 630 nm, 10,000 μ mol m⁻² s⁻¹, 300 ms pulse length) were used for the saturation of the chloroplast electron transport chain; a red actinic light (AL; maximum intensity at 630 nm, intensities varied) was used for supporting the photosynthetic activity. It can see that the angle between the leaf surface and the direction of the AL, SP, and ML was about 60°.

Plants were adapted under dark conditions for 15 min before measurements. After that, the first SP was used for measurement of the dark (F_0) and maximal (F_m) fluorescence yields [66–68]. The second SP, which was generated 60 s after the first one, was used for measurement of maximal change in the P700 signal (P_m) of PSI, showing maximal P700 oxidation [69]. The next SPs were also generated every 60 s for the entire experimental period; the steady-state (F) and maximal (F_m') fluorescence yields [66–68] and the steady-state (P) and maximal (P_m') P700 signals [68] were measured at each SP generation.

Four illuminations by red actinic light with different intensities (131, 344, 830, and 1599 μ mol m⁻² s⁻¹) were used in the experiments. The duration of each illumination was 5 min; the time intervals between illuminations (without AL) were 2 min, and the time intervals before the first illumination and after the last illumination were 5 min.

A compact wide-range S100 spectrometer (SOLAR Laser Systems, Minsk, Belarus) with a fiberoptic cable was used for measuring the reflected light (RL). The distance from the leaf surface to the fiberoptic surface (Figure 1a) was about 1.5 cm. The angle between the leaf surface and the input of the fiberoptic cable was about 30°. The spectral range and spectral resolution of the S100 spectrometer were 190–1050 nm and about 1 nm, respectively; the integration time for each spectral measurement was 5 s. Only the absolute intensities of the reflected light were measured; the reflectance was not estimated.

In accordance with the method proposed in our previous work [64], green-yellow light (GYL) pulses were used for measurement of the intensities of the reflected light at 515, 525, 531, 535, 545, and 555 nm, and the reference reflected light at 570 nm. This method can decrease the errors caused by the different intensities of actinic light [64].

The white TDS-P003L4U14 LED (TDS Lighting Co., Ltd., Jiangsu, China), which was equipped by standard yellow- (Y-1,4x) and yellow-green (YG-2x)-colored glass bandpass filters, was used as the GYL source. The angle between the leaf surface and the direction of the GYL was about 30°. Figure 1b shows the spectrum of GYL; the intensity of the green-yellow light was 240 μ mol m⁻² s⁻¹ (in the zone of the leaf). The duration of the GYL pulses was 30 s (Figure 1c). The pulses were generated every minute for the entire experimental period, and each pulse was terminated 5 s before the SP.



Figure 1. (a) A schema of the measurements of the reflected light (RL), the photosystem II (PSII) fluorescence, and the photosystem I (PSI) light absorption in pea leaves. ML was the weak measuring light, AL was the red actinic light, GYL were the pulses of the measuring green-yellow light, and SPs were the pulses of saturation red light. (b) A light spectrum of the green-yellow light source. (c) A schema of the measurement of the intensities of the reflected light (R) at the investigated wavelengths of the GYL pulse. R^{BG} and R^{Averaged} were the reflected light intensity before the GYL pulse and the averaged reflected light intensity during this pulse, excluding the first (R^{First}) and the last (R^{Last}) values. (d) A scheme of the calculation of the fast- and slow-relaxing components of a photochemical reflectance index (PRI). AL on and AL off were the initiation and termination of the illumination by actinic light, respectively.

Figure 1c also shows the calculation of the intensities of the reflected light at the green-yellow pulse (the method was described in our previous work [64] in detail): the reflected light intensity before the GYL pulse (R^{BG}) and the averaged intensity during the GYL pulse, excluding the first and the last values ($R^{Averaged}$), were measured for each investigated wavelength. After that, the intensity of the reflected light (R) was calculated as $R^{Averaged} - R^{BG}$.

In accordance with the procedure described in our previous work [64] in detail, we used the 18% grey card QPcard 101 Calibration Card ver. 3 (Argraph Corp., Carlstadt, NJ, USA) as a standard for initial calibration under the GYL pulses. This calibration eliminated the influence of the difference between the intensities of GYL at 515, 525, 531, 545, 555, and 570 nm from our light source on the values of the typical and the modified PRIs.

2.3. Calculation of the Photosynthetic Parameters and the Photochemical Reflectance Index and Data Analysis

We calculated the main parameters of PSI and PSII, including the effective quantum yields of PSI (γ (PSI)) and PSII (γ (PSI)), the nonphotochemical quenching of chlorophyll a (NPQ), the coefficient of photochemical quenching (qP), the fraction of the overall PSI that was oxidized in a given state due to its donor side limitation (γ (ND)), and the fraction of the overall PSI that could not be oxidized by a SP in a given state due to a lack of acceptors (γ (NA)). These parameters were automatically calculated by software of the Dual-PAM-100 on basis of Equations (2)–(7) [66–69]:

$$\gamma(PSI) = \frac{P_m' - P}{P_m} \tag{2}$$

$$\gamma(PSII) = \frac{F_m' - F}{F_m'} \tag{3}$$

$$NPQ = \frac{F_m - F_m'}{F_m'} \tag{4}$$

$$qP = \frac{F_{m}' - F}{F_{m}' - F_{0}'}$$
(5)

$$\gamma(ND) = \frac{P}{P_m} \tag{6}$$

$$\gamma(NA) = \frac{P_m - P_m'}{P_m} \tag{7}$$

where F_0' refers to the minimum fluorescence yield in the light-adapted state, which was calculated in accordance with Equation (8):

$$F_0' = \frac{F_0}{\frac{F_m^- F_0}{F_m} - \frac{F_0}{F_m'}}$$
(8)

The PRIs were calculated on the basis of the intensities of the reflected light at the same reference wavelength (570 nm) and at different investigated wavelengths (band); Equation (9) was used:

$$PRI(band, 570) = \frac{R_{band} - R_{570}}{R_{band} + R_{570}}$$
(9)

where R_{band} refers to the intensities of the reflected light at 515, 520, 525, 535, 545, 550, and 555 nm for modified PRIs and at 531 nm for the typical PRI; and R_{570} refers to the intensity of the reflected light at 570 nm. All intensities of the reflected light were averaged within about 2 nm spectral bands.

We analyzed the light-induced changes in the PRIs (Δ PRIs) because elimination of the variety of initial levels of the PRIs strongly decreased the standard errors of the measured values in our experiments (data not shown); the result was in full accordance with our early works [26,38,62]. The initial level of the PRIs was measured 2 min before initiation of the first illumination by AL during the experiment. The relation of Δ PRI(band,570) with the photosynthetic parameters was investigated in three variants of analysis: (1) analysis of all of the values of Δ PRI(band,570) and the photosynthetic parameters; (2) analysis of the "light" values of Δ PRI(band,570) and the photosynthetic parameters (it included only time points measured under the actinic light); and (3) analysis of the "dark" values of Δ PRI(band,570) and the photosynthetic parameters (including only the time points measured without actinic light). In the last variant, only some photosynthetic parameters (γ (PSI), γ (PSII), and NPQ) were analyzed, because these parameters should be related to slow-relaxing changes in PSI and PSII (in particular, with photodamages). The estimation of the relations between Δ PRI(band,570) and the photosynthetic parameters was based on the description of these relationships using linear regressions and the calculation of the Root Mean Squared Error (RMSE), determination, and correlation coefficients.

Additionally, a dark relaxation of Δ PRI(band,570) was investigated. Figure 1d shows that changes in the PRIs could be divided into fast-relaxing changes (duration of the changes' relaxation was 1–2 min) and slow-relaxing changes (relaxation was not observed). The slow-relaxing changes induced by actinic light were calculated as the difference between the last PRI value before initiation of the AL illumination (PRI₁) and the second value of the PRI after termination of the AL illumination (PRI₂). The fast-relaxing changes induced by actinic light were calculated as the difference between the second value of the PRI after termination of the AL illumination (PRI₂) and the last PRI value before termination of the AL illumination (PRI₃).

2.4. Statistics

A separate pea seedling was used for each experiment. Mean values, standard errors, and determination coefficients are presented in the figures. Scatter plots, regression equations, and determination and correlation coefficients are presented in the Supplementary Materials. The RMSE for all regression equations are shown in the table. Student's *t*-test was used to identify significant differences. The significance of the linear correlation coefficients was estimated on basis of the standard table of critical values for Pearson correlation.

3. Results

3.1. Light-Induced Changes in the Photochemical Reflectance Index and Photosynthetic Parameters

The parameters of light reactions in PSI and PSII under actinic light with different intensities were firstly investigated. Figure 2 shows that γ (PSI), γ (PSII), γ (NA), and qP decreased with an increase of the intensity of the actinic light; in contrast, γ (ND) and NPQ increased with an increase of the intensity of the actinic light. Most of these photosynthetic parameters were not saturated in the investigated range of intensities of actinic light; however, the values of γ (NA) were similar under the intensities of actinic light equal to 344, 830, and 1599 μ mol m⁻² s⁻¹.

Figure 3 shows light curves for the investigated Δ PRIs, including Δ PRI(515,570), Δ PRI(525,570), Δ PRI(531,570), Δ PRI(535,570), Δ PRI(545,570), and Δ PRI(555,570). An increase of the intensity of the actinic light stimulated changes in all of the investigated PRIs; however, there were differences in these light curves for different indices. First, changes in Δ PRI(515,570) and Δ PRI(555,570) were very weak; a decrease of these indices was mainly observed under high-intensity actinic light (mostly, 1599 µmol m⁻² s⁻¹). In contrast, significant changes in the other Δ PRIs could be observed under the actinic light intensities of 344 µmol m⁻² s⁻¹ or more. It should be additionally noted that the rates of changes in the Δ PRIs increased with an increase of the wavelength: changes in Δ PRI(515,570) were the fastest (they were observed at least for 5 min); in contrast, changes in Δ PRI(555,570) were the fastest (they were reached for 1 min).



Figure 2. Averaged light curves for the parameters of the light reactions in photosystems I and II (PSI and PSII, respectively) in pea leaves (n = 6). (**a**) the quantum yield of PSI (γ (PSI)); (**b**) the quantum yield of PSII (γ (PSII)); (**c**) the fraction of overall P700 that is oxidized in a given state (γ (ND)); (**d**) the fraction of overall P700 that cannot be oxidized by the saturation pulse in a given state due to a lack of acceptors (γ (NA)); (**e**) the non-photochemical quenching (NPQ); (**f**) the photochemical quenching (qP). PAR was the intensity of the actinic light. The photosynthetic parameters were measured every minute (every saturation pulse).



Figure 3. Averaged light curves for the Δ PRIs, which were calculated on the basis of the intensities of the actinic light at different wavelengths, including Δ PRI(515,570) (**a**), Δ PRI(525,570) (**b**), Δ PRI(531,570) (**c**), Δ PRI(535,570) (**d**), Δ PRI(545,570) (**e**), and Δ PRI(555,570) (**f**) in pea leaves (*n* = 6). PAR was the intensity of the actinic light. The Δ PRIs were measured every minute (every GYL pulse).

Second, dark relaxations of the investigated Δ PRIs differed strongly: the relaxation of Δ PRI(515,570) was fully absent (moreover, a decrease of the PRI was observed after termination of the illumination by actinic light), the relaxation of Δ PRI(525,570) was weak, the relaxations of Δ PRI(531,570)

and Δ PRI(535,570) were moderate, and the relaxations of Δ PRI(545,570) and Δ PRI(555,570) were very expressive.

Third, the values of the Δ PRIs under actinic light with maximal intensity (1599 µmol m⁻² s⁻¹) differed for different indices (Figure 4). It was interesting that Δ PRI(531,570) and Δ PRI(535,570) were maximal; Δ PRI(525,570) and Δ PRI(545,570) were also expressive. In contrast, Δ PRI(515,570) and Δ PRI(555,570) were weak under high-intensity light.



Figure 4. Averaged maximal magnitudes of Δ PRI(515,570), Δ PRI(525,570), Δ PRI(531,570), Δ PRI(535,570), Δ PRI(545,570), and Δ PRI(555,570) in pea leaves under actinic light with an intensity equal to 1599 µmol m⁻² s⁻¹ (*n* = 6). The magnitudes were measured before termination of the illumination by actinic light. * indicates that the Δ PRI was significant (*p* < 0.05).

3.2. Relations between Changes in the Photochemical Reflectance Index and the Photosynthetic Parameters

Furthermore, we analyzed the relationship between the photosynthetic parameters and the investigated Δ PRIs. The determination coefficients of the linear regressions and the significance are shown in Figures 5–7; The RMSE values are shown in Table 1; and all variants of the scatter plots, linear regressions, and correlation coefficients are presented in the Supplementary Materials (Figures S1–S18).

Figure 5 and Figure S1–S6 (Supplementary Materials) show the results of the analysis based on a combination of dark (without actinic light) and light (under actinic light) averaged experimental values of the Δ PRIs and the photosynthetic parameters. It was shown that the relations of Δ PRI(band,570) to most of the investigated photosynthetic parameters (γ (PSI), γ (ND), γ (PSII), qP, and NPQ) increased with an increase of the wavelength, which was used for the calculation of the Δ PRIs (band). In particular, Δ PRI(515,570) and Δ PRI(525,570) were weakly related to all of the investigated photosynthetic parameters. In contrast, Δ PRI(535,570), Δ PRI(545,570), and Δ PRI(555,570) were strongly linearly related to most of the investigated photosynthetic parameters (excluding γ (NA)); furthermore, maximal determination coefficients were observed for Δ PRI(545,570). The relations of Δ PRI(531,570) (the typical PRI) to the photosynthetic parameters were also strong; however, they were weaker than these relations for Δ PRI(535,570), Δ PRI(545,570).





(c) *qP ηP ηP





Figure 5. Determination coefficients (R²) for the linear regressions, which were calculated on the basis of all of the averaged experimental values of the photosynthetic parameters and Δ PRI(515,570) (**a**), Δ PRI(525,570) (**b**), Δ PRI(531,570) (**c**), Δ PRI(535,570) (**d**), Δ PRI(545,570) (**e**), and Δ PRI(555,570) (**f**). The coefficients were calculated on the basis of the results from Figures 2 and 3; details of the relations are shown in Figures S1–S6 (Supplementary Materials). * indicates that the relation was significant (*p* < 0.05 for the correlation coefficients between these values).





(c)







(f)

(d)



Figure 6. Determination coefficients (R²) for the linear regressions, which were calculated on the basis of light averaged experimental values (only time points measured under actinic light) of the photosynthetic parameters and Δ PRI(515,570) (**a**), Δ PRI(525,570) (**b**), Δ PRI(531,570) (**c**), Δ PRI(535,570) (**d**), Δ PRI(545,570) (**e**), and Δ PRI(555,570) (**f**). The coefficients were calculated on the basis of the results from Figures 2 and 3; the details of the relations are shown in Figures S7–S12 (Supplementary Materials). * indicates that the relation was significant (*p* < 0.05 for the correlation coefficients between these values).



Figure 7. Determination coefficients (R²) for the linear regressions, which were calculated on the basis of dark averaged experimental values (only time points measured without actinic light) of the photosynthetic parameters and Δ PRI(515,570) (**a**), Δ PRI(525,570) (**b**), Δ PRI(531,570) (**c**), Δ PRI(535,570) (**d**), Δ PRI(545,570) (**e**), and Δ PRI(555,570) (**f**). The coefficients were calculated on the basis of the results from Figures 2 and 3; the details of the relations are shown in Figures S13–S18 (Supplementary Materials). * indicates that the relation was significant (*p* < 0.05 for the correlation coefficients between these values).

Type of Analysis	Type of ∆PRI	γ(PSI)	γ(PSII)	γ(ND)	γ(NA)	NPQ	qP
Analysis of both light and dark values	ΔPRI(515,570)	0.00183	0.00188	0.00191	0.00165	0.00189	0.00188
	ΔPRI(525,570)	0.00197	0.00221	0.00237	0.00276	0.00224	0.00218
	ΔPRI(531,570)	0.00143	0.00172	0.00196	0.00296	0.00177	0.00168
	ΔPRI(535,570)	0.00102	0.00128	0.00156	0.00294	0.00135	0.00126
	ΔPRI(545,570)	0.00056	0.00066	0.00090	0.00222	0.00070	0.00065
	ΔPRI(555,570)	0.00034	0.00042	0.00053	0.00101	0.00043	0.00039
Analysis of light values	ΔPRI(515,570)	0.00070	0.00068	0.00076	0.00122	0.00063	0.00065
	ΔPRI(525,570)	0.00069	0.00072	0.00082	0.00235	0.00072	0.00073
	ΔPRI(531,570)	0.00066	0.00072	0.00084	0.00283	0.00075	0.00074
	ΔPRI(535,570)	0.00064	0.00070	0.00082	0.00298	0.00073	0.00075
	ΔPRI(545,570)	0.00048	0.00052	0.00065	0.00244	0.00050	0.00052
	ΔPRI(555,570)	0.00033	0.00034	0.00041	0.00116	0.00032	0.00032
Analysis of dark values	ΔPRI(515,570)	0.00066	0.00116	-	-	0.00157	-
	ΔPRI(525,570)	0.00079	0.00141	-	-	0.00223	-
	ΔPRI(531,570)	0.00089	0.00120	-	-	0.00199	-
	ΔPRI(535,570)	0.00051	0.00092	-	-	0.00153	-
	ΔPRI(545,570)	0.00037	0.00049	-	-	0.00078	-
	ΔPRI(555,570)	0.00031	0.00034	-	-	0.00043	-

Table 1. Values of Root Mean Squared Error (RMSE), which were calculated on the basis of the linear regressions describing the relation of the Δ PRIs to the photosynthetic parameters. The linear regressions were shown in Figures S1–S18 (Supplementary Materials). The light values were measured under actinic light; the dark values were measured without actinic light.

Table 1 shows that the values of the RMSE for the linear regressions, which were calculated on the basis of both light and dark values, decreased with an increase of the wavelength, which were used for the calculation of the Δ PRIs. In particular, these values for Δ PRI (515,570) were about 0.0015–0.0020; these values were similar to the magnitudes of changes in these PRIs under the action of maximal actinic light (Figure 4). In contrast, these values for Δ PRI (555,570) were about 0.0003–0.0005 (excluding relation to γ (NA)); these values were much lower than the magnitudes of changes in these PRIs under the action of actinic light.

It should be noted that the relations of all of the investigated Δ PRIs to γ (NA) were weak; however, the determination coefficient for the linear regression describing the relation of Δ PRI (545,570) to γ (NA) was maximal (about 0.15) and significant. In contrast, the determination coefficient for the linear regression describing the relation of Δ PRI (525,570) to γ (NA) was minimal (about 0). It is interesting that a significant determination coefficient for the linear regression describing the relation of Δ PRI (515,570) to γ (NA) was observed; however, the determination coefficients for the linear regression describing the relation of Δ PRI (515,570) to γ (NA) was observed; however, the determination coefficients for the linear regression describing the relation of Δ PRI (515,570) to γ (NA) was observed; however, the determination coefficients for the linear regression describing the relations of Δ PRI (515,570) to the other photosynthetic parameters were about 0.

Figure 6 and Figure S7–S12 (Supplementary Materials) show the results of the analysis based on only light averaged experimental values of the Δ PRIs and the photosynthetic parameters, which were measured under actinic light. Figure 6 shows that most of the photosynthetic parameters (γ (PSI), γ (ND), γ (PSII), qP, and NPQ) were significantly linearly related to the investigated Δ PRIs. The determination coefficients for the linear regression describing the relations of Δ PRI(515,570) to these photosynthetic parameters were about 0.4–0.5. In contrast, the determination coefficients for the other Δ PRIs were about 0.9 or more; i.e., the relation of these Δ PRIs to γ (PSI), γ (ND), γ (PSII), qP, and NPQ were very strong in the analysis of only the light values. However, it should be noted that the linear regressions describing the relations of Δ PRI(545,570) to most of the photosynthetic parameters had maximal determination coefficients (at least about 0.95).

Table 1 shows that values of the RMSE for the linear regressions, which were calculated on the basis of the light values, decreased with an increase of the wavelength, which were used for the calculation of the Δ PRIs (excluding the RMSE for the regressions describing the relations of the Δ PRIs

to γ (NA)). In particular, these values for Δ PRI(515,570) were about 0.0006–0.0007; in contrast, these values for Δ PRI(555,570) were about 0.0003–0.0005. However, it is important that all these RMSE values were much lower than magnitudes of the changes in these PRIs under the action of the actinic light.

It should be noted that the relations of all investigated Δ PRIs to γ (NA) were weak in the analysis of only the light experimental values. These relations were significant for Δ PRI(531,570), Δ PRI(535,570), and Δ PRI(545,570); however, the determination coefficients for the linear regression describing the relation of these Δ PRIs to γ (NA) were low.

Additionally, we analyzed only the dark averaged experimental values of the Δ PRIs and the photosynthetic parameters (without actinic light). It should be noted that only γ (PSI), γ (PSII), and NPQ were analyzed, because these parameters under dark conditions could be connected with PSII photodamage (NPQ) or with suppression of the maximal efficiency of PSI (γ (PSI)) and PSII (γ (PSII)).

Figure 7 and Figure S7–S12 (Supplementary Materials) show that the relations of Δ PRI(515,570), Δ PRI(525,570), Δ PRI(531,570), Δ PRI(535,570), and Δ PRI(545,570) to γ (PSI) and γ (PSII) were strong; in particular, the determination coefficients for the linear regression describing these relations were about 0.8–0.9. In contrast, these coefficients were low for Δ PRI(555,570).

The relations of Δ PRI(band,570) to NPQ decreased with an increase of the wavelength, which was used for calculation of the Δ PRIs (band). In particular, the determination coefficient for linear regression describing the relation of Δ PRI(515,570) to NPQ was about 0.6; in contrast, the determination coefficient for the linear regression describing the relation of Δ PRI(555,570) to NPQ was about 0.

Table 1 shows that the values of the RMSE for the linear regressions, which were calculated on the basis of the dark values, were maximal for Δ PRI(525,570) and Δ PRI(531,570). It is probable that this effect caused the maximal magnitudes of changes Δ PRI(525,570) and Δ PRI(531,570) under dark conditions (up to about 0.7; see Fig. 3).

3.3. The Fast- and Slow-Relaxing Components of the Photochemical Reflectance Index

It is probable that different relations of different Δ PRIs to the photosynthetic parameters can be connected with different participation levels of the 526 and 545 nm components of change in reflectance [54] in the forming of light-induced changes in these Δ PRIs. We analyzed this hypothesis on the basis of the investigation of the slow- and fast-relaxing components in the investigated variants of PRIs, because the 526 nm component is traditionally related to changes in activity of the xanthophyll cycle (time of dark relaxation was about 10 min or more in peas [49]) and the 545 nm component is probably related to changes in chloroplast light scattering (time of dark relaxation was about 1–2 min in peas [65]).

Figure 8 shows that the fast- and slow-relaxing components of the light-induced changes in the investigated Δ PRIs were weak under low and moderate intensities of actinic light (131 and 344 µmol m⁻² s⁻¹); however, these components greatly increased under the high intensities of actinic light (830 and 1599 µmol m⁻² s⁻¹). It is interesting that a significant small increase of the fast-relaxing component was even observed under 131 µmol m⁻² s⁻¹; in contrast, significant changes in the slow-relaxing component were not shown under this intensity of actinic light.

The maximum of the fast-relaxing component was observed in the PRIs that were calculated at 535 and 545 nm. It is very probable that Δ PRI (535,570) and Δ PRI(545,570) were mainly connected with the 545 nm component of changes in leaf reflectance. In contrast, the slow-relaxing component had maximal magnitudes in the PRIs calculated at 515 and 525 nm; thus, Δ PRI (515,570) and Δ PRI (525,570) are probably connected with the 526 nm component of leaf reflectance.

4. Discussion

An increase of the crop of agricultural plants is a global problem of modern humanity. In particular, the increase can be based on the development of methods of remote sensing, which constitute the basis of "precision agriculture" and are an important tool for crop protection under the action of stressors [9]. Using optical methods, which are very sensitive to plant changes under the action of

stressors and are relatively simple, is a potential way of plant remote sensing [10,12]. Measurements of the reflectance of leaves are a widely-used method of analysis of physiological processes in plants [11–15,67]. It is important that intensities of the reflected light can be used for the calculation of reflectance indices, because analysis based on these indices has low errors in comparison with the analysis of absolute values of the reflected light [10]. It is known that reflectance indices permit to estimate different characteristics of plants, including the growth of biomass [21], the photosynthetic efficiency and photosynthetic stress responses [16–20,23,33,58,70,71], the changes in biochemical compositions [29–36], the transpiration [72,73], the isoprene emission [20,24,25], etc.

Measurements of the photochemical reflectance index are a potential tool of the remote sensing of plants, which can be used in agriculture and ecological monitoring [10]. A PRI is considered to be related to transitions in the xanthophyll cycle [32–35] and changes in chloroplast shrinkage [53]; both processes are stimulated by excess lumen acidification, which is often induced by the action of stressors on plants [35,39,54]. The work by Gamon et al. (1997) [54] showed that a change in the reflectance of plant leaves at 531 nm, which is traditionally used for PRI calculation, includes different components (at least, the 526 nm and 545 nm components of the reflectance change). Thus, it can be expected that PRIs, which are calculated on the basis of the intensities of the reflected light at different wavelengths, can have different efficiencies for the estimation of photosynthetic parameters.

In this study, we showed that the relations of the light-induced changes in the PRIs, which were calculated on the basis of the reflected light intensities at different wavelengths ($\Delta PRI(515,570)$, ΔPRI(525,570), ΔPRI(531,570), ΔPRI(535,570), ΔPRI(545,570), and ΔPRI(555,570)), to the parameters of the light reactions in PSI and PSII (γ (PSI), γ (PSII), γ (ND), γ (NA), qP, and NPQ) were dependent on the wavelength used for the PRI calculation (Figures 5–7 and Table 1). It is important that the relations between the Δ PRIs and the photosynthetic parameters were also dependent on the type of analysis. If only the light values, which were measured under actinic light, were analyzed, then most of the Δ PRIs (excluding Δ PRI(515,570)) were strongly related to most of the investigated photosynthetic parameters (excluding γ (NA)). The maximal relations to the photosynthetic parameters were observed for $\Delta PRI(535,570)$ and $\Delta PRI(545,570)$. The relation of $\Delta PRI(555,570)$ to the photosynthetic parameters was also high; however, the values of $\Delta PRI(555,570)$ were very small (Figure 4). In contrast, the analysis of only the dark values, measured without actinic light, showed that $\Delta PRI(555,570)$ was weakly related to the photosynthetic parameters; the other Δ PRIs were strongly related to γ (PSI) and γ (PSII) and were moderately related to NPQ. The maximal relations to the photosynthetic parameters were observed for $\Delta PRI(515,570)$ and $\Delta PRI(525,570)$; however, the absolute values of $\Delta PRI(515,570)$ were very small (Figure 4).

These differences can be caused by different mechanisms of photosynthetic changes under light and dark conditions. Without actinic light, the changes in the photosynthetic parameters are mainly related to slow changes in photosynthetic machinery, including de-epoxidation and epoxidation in the xanthophyll cycle (from several minutes to tens of minutes) [48–50] and the damage of PSI and PSII (from hours to days) [68,74,75]. Δ PRI(515,570) and Δ PRI(525,570) are probably related to the 526 nm component of change in leaf reflectance [54], which is mainly caused by transition in the xanthophyll cycle. As a result, it can be hypothesized that the sensitivity of Δ PRI(515,570) and Δ PRI(525,570) to the photosynthetic parameters under dark conditions is based on changes in the xanthophyll cycle. Figure 8 supports this hypothesis, because the maximal portion of the slow-relaxing component of the PRIs (the component that does not relax for 2 min) was observed in Δ PRI(515,570) and Δ PRI(525,570). In contrast, the photodamage of PSII probably weakly influences the Δ PRIs, because NPQ under dark conditions, which is mainly caused by photodamage [74,75], was moderately related to these indices (Figure 7).



Figure 8. Dependences of the magnitudes of the slow- and fast-relaxing components of the PRIs on the spectral band of the reflected light, which was used for the calculation of the photochemical reflectance index (band) under different intensities of plant illuminations by actinic light (n = 6). (**a**) The magnitudes under 131 µmol m⁻² s⁻¹; (**b**) the magnitudes under 344 µmol m⁻² s⁻¹; (**c**) the magnitudes under 830 µmol m⁻² s⁻¹; (**d**) the magnitudes under 1599 µmol m⁻² s⁻¹.

The photosynthetic changes under actinic light are strongly related to photosynthetic electron flows and, thereby, can be accompanied by changes in the stromal and luminal pH in chloroplasts [76,77]. It is known that acidification of the chloroplast lumen can induce fast changes in a PRI [16,38]; these changes can be related to the 545 nm component of change in leaf reflectance [45,54], which is probably caused by chloroplast light scattering. These changes are likely the basis of the sensitivity of Δ PRI(545,570) to the photosynthetic parameters under light conditions, because they are rather caused by fast photosynthetic changes (minutes and, probably, seconds) under illumination. Figure 8 supports this hypothesis, because the maximal portion of the fast-relaxing component of the PRIs (the component that fully relaxes for 2 min) was observed in Δ PRI(535,570) and Δ PRI(545,570). It is interesting that our results differ from that data of the work by Gamon et al. (1997) [54], which did not observe a relation between the 545 nm component of change in leaf reflectance and the photosynthetic parameters. The following question is particularly important: are there Δ PRIs that are effective for the estimation of the photosynthetic parameters under both light and dark conditions? Our results showed (Figure 5) that Δ PRI(545,570) was most effective in this analysis. The efficiencies of Δ PRI(535,570) and Δ PRI(555,570) were also high. However, the absolute values of Δ PRI(555,570) were very small (Figure 4); in contrast, a light-induced decrease of PRI(535,570) had a maximal magnitude. As a result, it can be concluded that Δ PRI(545,570) and, possibly, Δ PRI(535,570) are the most effective estimators of photosynthetic change under both light and dark conditions.

It is also important that the investigated Δ PRIs were related to the quantum yields of both photosystems; moreover, the relations between the Δ PRIs and γ (PSI) could be stronger than the ones between the Δ PRIs and γ (PSI) (e.g., Figure 5). This result can be explained by the participation of the cyclic electron flow around the PSI in forming Δ pH across the thylakoid membrane [76]—especially under the action of stressors. γ (PSI) is related to both the linear and cyclic electron flows (both flows influence stromal and luminal pH); in contrast, γ (PSII) is mainly related to the linear electron flow [78]. Thus, stimulation of the cyclic electron flow around the PSI, which was observed under high-intensity actinic light [66,68,76,79], probably disrupts the relation between the Δ PRIs and γ (PSII).

Another unexpected result was the low relation of the Δ PRIs to γ (NA) under light or light and dark conditions (Figures 5 and 6). We suppose that this effect is caused by the inactivation of ferredoxin–NADP reductase under dark conditions and its re-activation under light conditions [80,81]; the inactivation and re-activation should induce an increase and a decrease in γ (NA), respectively. Figure 2d shows both an increase of γ (NA) under dark conditions and its decrease under light conditions. It is important that the dependence of the magnitude of changes in γ (NA) on actinic light intensity was saturated at intensities equal to 344 µmol m⁻² s⁻¹ or more; this dependence differs in the dependencies of the other photosynthetic parameters (Figure 2). As a result, the participation of the regulation mechanisms of the electron flow through the acceptor side of the PSI can disturb the relation between γ (NA) and the Δ PRIs.

Thus, our results show that the efficiency of photochemical reflectance indices, which are calculated on the basis of the intensity of the reflected light at various wavelengths, in the estimation of the photosynthetic parameters can differ in different variants of analysis (analysis of only the values under light conditions, analysis of only the values under dark conditions, analysis of the values under both dark and light conditions). Figure 9 summarizes our results and shows that $\Delta PRI(525,570)$, Δ PRI(531,570), Δ PRI(535,570), and Δ PRI(545,570) can be used for the estimation of the photosynthetic parameters under the light only conditions or under the dark only conditions, because the efficiencies of all of these indices are relatively high. However, the efficiency of $\Delta PRI(525,570)$ for the estimation of the photosynthetic parameters is maximal under dark conditions; in contrast, the efficiency of Δ PRI(545,570) for the estimation of the photosynthetic parameters is maximal under light conditions. It is very probable that these differences are related to the decrease of the 526 nm component of the reflectance change (caused by relatively slow de-epoxidation of violaxanthin to zeaxanthin via antheraxanthin [54]) and the increase of the 545 nm component (caused by relatively fast changes in the chloroplast light scattering [54]), which are observed with an increase of the band in Δ PRI(band, 570). It is important that the efficiency of most of the investigated Δ PRIs decreased in the analysis of the values measured under both light and dark conditions; the Δ PRI(545,570) and, possibly, Δ PRI(535,570) are probably the most effective photosynthetic estimators under these conditions. We suppose that measurements of these indices can be a perspective tool for the remote sensing of photosynthetic parameters under changeable light conditions; in particular, they can be used for the remote sensing of fast changes in these parameters under light fluctuations, which can strongly influence the plant photosynthetic machinery [82].



$\triangle PRI(band, 570)$ can be effectively used for estimation of photosynthetic parameters

Figure 9. The hypothetical schema of the effective use of the Δ PRIs (Δ PRI(band,570)), calculated on the basis of the intensities of the reflected light at different wavelengths (band), for the estimation of the photosynthetic parameters at different variants of analysis (values under light conditions, under dark conditions, or under a combination of light and dark conditions). The figure also shows the fast- and slow-relaxing components in the Δ PRIs.

Additionally, it should be noted that the efficiency of Δ PRI(531,570), which is a typically used PRI variant, for the estimation of photosynthetic parameters under both light and dark conditions is lower than that of Δ PRI(535,570) and Δ PRI(545,570); however, it is also relatively high. These efficiencies are also high under light only or dark only conditions. These results support statement that typical PRIs can be an effective tool for the estimation of the parameters of light reactions in PSI and PSII under different light conditions.

5. Conclusions

PRI measurements (especially, Δ PRI) are a perspective spectral method for the early detection of stressor-induced photosynthetic changes in plants, which can contribute to increasing the efficiency of plant cultivation. We analyzed the relations of Δ PRI(515,570), Δ PRI(525,570), Δ PRI(531,570), Δ PRI(535,570), Δ PRI(545,570), and Δ PRI(555,570) to the parameters of the light reaction of PSI and PSII, including γ (PSI), γ (PSII), γ (ND), γ (NA), NPQ, and qP. The following important results were obtained: (1) Δ PRI(525,570), Δ PRI(531,570), Δ PRI(535,570), and Δ PRI(535,570) and Δ PRI(535,570), and Δ PRI(535,570) and Δ PRI(545,570).

of most of the photosynthetic parameters (including the PSI parameters) under light only or under dark only conditions. (2) The combination of dark and light conditions decreased the efficiency of the Δ PRIs used for the estimation of the photosynthetic parameters; PRI(535,570) and Δ PRI(545,570) had maximal efficiency under these conditions. (3) Δ PRI(515,570) and Δ PRI(525,570) mainly included the slow-relaxing component of the PRIs; in contrast, Δ PRI(531,570), Δ PRI(535,570), Δ PRI(545,570), and Δ PRI(555,570) mainly included the fast-relaxing component of the PRIs. The results can be basis of future investigations devoted to the optimization of PRI application in the remote sensing of plants.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-4292/12/8/1312/s1, Figures S1–S18.

Author Contributions: Conceptualization, E.S. and V.S.; formal analysis, E.S. and V.S.; funding acquisition, V.S. and E.S.; investigation, E.S.; methodology, E.S.; project administration, V.S.; supervision, V.S. All authors have read and agreed to the published version of the manuscript.

Funding: The analysis of the relations of light-induced changes in the typical PRI to the parameters of light reactions in photosystems I and II was funded by the Russian Science Foundation, project number 17-76-20032. The analysis of the relations of light-induced changes in modified PRIs to the photosynthetic parameters was funded by the Russian Foundation for Basic Research, project number 20-016-00234 A.

Conflicts of Interest: The authors declare no conflicts of interest.

References

- 1. Rascher, U.; Nedbal, L. Dynamics of photosynthesis in fluctuating light. *Curr. Opin. Plant Biol.* **2006**, *9*, 671–678. [CrossRef] [PubMed]
- 2. Smith, W.K.; Berry, Z.C. Sunflecks? Tree Physiol. 2013, 33, 233–237. [CrossRef] [PubMed]
- 3. Retkute, R.; Smith-Unna, S.E.; Smith, R.W.; Burgess, A.J.; Jensen, O.E.; Johnson, G.N.; Preston, S.P.; Murchie, E.H. Exploiting heterogeneous environments: Does photosynthetic acclimation optimize carbon gain in fluctuating light? *J. Exp. Bot.* **2015**, *66*, 2437–2447. [CrossRef] [PubMed]
- 4. Nievola, C.C.; Carvalho, C.P.; Carvalho, V.; Rodrigues, E. Rapid responses of plants to temperature changes. *Temperature (Austin)* **2017**, *4*, 371–405. [CrossRef] [PubMed]
- 5. Matsubara, S. Growing plants in fluctuating environments: Why bother? *J. Exp. Bot.* **2018**, *69*, 4651–4654. [CrossRef] [PubMed]
- 6. Zeppel, M.J.B.; Wilks, J.V.; Lewis, J.D. Impacts of extreme precipitation and seasonal changes in precipitation on plants. *Biogeosciences* **2014**, *11*, 3083–3093. [CrossRef]
- 7. Teasdale, J.R.; Cavigelli, M.A. Meteorological fluctuations define long-term crop yield patterns in conventional and organic production systems. *Sci. Rep.* **2017**, *7*, 688. [CrossRef]
- Johnová, P.; Skalák, J.; Saiz-Fernández, I.; Brzobohatý, B. Plant responses to ambient temperature fluctuations and water-limiting conditions: A proteome-wide perspective. *Biochim. Biophys. Acta.* 2016, 1864, 916–931. [CrossRef]
- 9. *Crop Stress and its Management: Perspectives and Strategies;* Venkateswarlu, B.; Shanker, A.; Shanker, C.; Maheswari, M. (Eds.) Springer: Dordrecht, The Netherlands, 2012.
- Prabhakar, M.; Prasad, Y.G.; Rao, M.N. Remote Sensing of Biotic Stress in Crop Plants and its Applications for Pest Management. In *Crop Stress and Its Management: Perspectives and Strategies*; Venkateswarlu, B., Shanker, A., Shanker, C., Maheswari, M., Eds.; Springer: Dordrecht, The Netherlands, 2012; pp. 517–545.
- 11. Mahlein, A.-K. Plant disease detection by imaging sensors–parallels and specific demands for precision agriculture and plant phenotyping. *Plant Dis.* **2016**, *100*, 241–251. [CrossRef]
- 12. Mahlein, A.K.; Kuska, M.T.; Behmann, J.; Polder, G.; Walter, A. Hyperspectral sensors and imaging technologies in phytopathology: State of the art. *Annu. Rev. Phytopathol.* **2018**, *56*, 535–558. [CrossRef]
- 13. Eitel, J.U.H.; Long, D.S.; Gessler, P.E.; Hunt, E.R. Combined spectral index to improve ground-based estimates of nitrogen status in dryland wheat. *Agron. J.* **2008**, *100*, 1694–1702. [CrossRef]
- Stagakis, S.; Markos, N.; Sykioti, O.; Kyparissis, A. Monitoring canopy biophysical and biochemical parameters in ecosystem scale using satellite hyperspectral imagery: An application on a *Phlomis fruticosa* Mediterranean ecosystem using multiangular CHRIS/PROBA observations. *Remote Sens. Environ.* 2010, 114, 977–994. [CrossRef]

- 15. Mahlein, A.K.; Steiner, U.; Dehne, H.W.; Oerke, E.C. Spectral signatures of sugar beet leaves for the detection and differentiation of diseases. *Precis. Agric.* **2010**, *11*, 413–431. [CrossRef]
- Evain, S.; Flexas, J.; Moya, I. A new instrument for passive remote sensing: 2. Measurement of leaf and canopy reflectance changes at 531 nm and their relationship with photosynthesis and chlorophyll fluorescence. *Remote Sens. Environ.* 2004, *91*, 175–185. [CrossRef]
- 17. Garbulsky, M.F.; Peñuelas, J.; Gamon, J.; Inoue, Y.; Filella, I. The photochemical reflectance index (PRI) and the remote sensing of leaf, canopy and ecosystem radiation use efficiencies. A review and meta-analysis. *Remote Sens. Environ.* **2011**, *115*, 281–297. [CrossRef]
- Peñuelas, J.; Garbulsky, M.F.; Filella, I. Photochemical reflectance index (PRI) and remote sensing of plant CO₂ uptake. *New Phytol.* 2011, 191, 596–599. [CrossRef]
- 19. Weng, J.H.; Wong, S.L.; Lai, K.M.; Lin, R.J. Relationships between photosystem II efficiency and photochemical reflectance index under different levels of illumination: Comparison among species grown at high- and low elevations through different seasons. *Trees-Struct. Funct.* **2012**, *26*, 343–351. [CrossRef]
- Zhang, C.; Filella, I.; Liu, D.; Ogaya, R.; Llusià, J.; Asensio, D.; Peñuelas, J. Photochemical reflectance index (PRI) for detecting responses of diurnal and seasonal photosynthetic activity to experimental drought and warming in a mediterranean shrubland. *Remote Sens.* 2017, *9*, 1189. [CrossRef]
- 21. Rouse, J.W., Jr.; Haas, R.H.; Schell, J.A.; Deering, D.W.; Harlan, J.C. *Monitoring the Vernal Advancement and Retrogradation (Green Wave Effect) of Natural Vegetation*; Type III Final Rep; The National Aeronautics and Space Administration (NASA)/Goddard Space Flight Center (GSFC): Greenbelt, MD, USA, 1974.
- 22. Peñuelas, J.; Filella, I.; Biel, C.; Serrano, L.; Savé, R. The reflectance at the 950–970 nm region as an indicator of plant water status. *Int. J. Remote Sens.* **1993**, *14*, 1887–1905. [CrossRef]
- Peñuelas, J.; Gamon, J.A.; Fredeen, A.L.; Merino, J.; Field, C.B. Reflectance indices associated with physiological changes in nitrogen- and water-limited sunflower leaves. *Remote Sens. Environ.* 1994, 48, 135–146. [CrossRef]
- Peñuelas, J.; Marino, G.; Llusia, J.; Morfopoulos, C.; Farré-Armengol, G.; Filella, I. Photochemical reflectance index as an indirect estimator of foliar isoprenoid emissions at the ecosystem level. *Nat. Commun.* 2013, 4, 2604. [CrossRef] [PubMed]
- 25. Balzarolo, M.; Peñuelas, J.; Filella, I.; Portillo-Estrada, M.; Ceulemans, R. Assessing ecosystem isoprene emissions by hyperspectral remote sensing. *Remote Sens.* **2018**, *10*, 1086. [CrossRef]
- 26. Sukhov, V.; Sukhova, E.; Gromova, E.; Surova, L.; Nerush, V.; Vodeneev, V. The electrical signal-induced systemic photosynthetic response is accompanied by changes in the photochemical reflectance index in pea. *Funct. Plant Biol.* **2019**, *46*, 328–338. [CrossRef] [PubMed]
- 27. Sukhova, E.; Yudina, L.; Akinchits, E.; Vodeneev, V.; Sukhov, V. Influence of electrical signals on pea leaf reflectance in the 400-800-nm range. *Plant Signal Behav.* **2019**, *14*, 1610301. [CrossRef]
- Sukhova, E.; Yudina, L.; Gromova, E.; Nerush, V.; Vodeneev, V.; Sukhov, V. Burning-induced electrical signals influence broadband reflectance indices and water index in pea leaves. *Plant Signal Behav.* 2020, 15, 1737786, [Epub ahead of print]. [CrossRef]
- 29. Gitelson, A.; Merzlyak, M.N. Spectral reflectance changes associated with autumn senescence of *Aesculus hippocastanum L*. and *Acer platanoides L*. leaves. Spectral features and relation to chlorophyll estimation. *Plant Physiol.* **1994**, 143, 286–292. [CrossRef]
- 30. Blackburn, G.A. Quantifying chlorophylls and carotenoids at leaf and canopy scale: An evaluation of some hyperspectral approaches. *Remote Sens. Environ.* **1998**, *66*, 273–285. [CrossRef]
- 31. Gamon, J.A.; Surfus, J.S. Assessing leaf pigment content and activity with a refectometer. *New Phytol.* **1999**, 143, 105–117. [CrossRef]
- 32. Penuelas, J.; Baret, F.; Filella, I. Semiempirical indices to assess carotenoids/chlorophyll a ratio from leaf spectral reflectance. *Photosynthetica* **1995**, *31*, 221–230.
- 33. Filella, I.; Amaro, T.; Araus, J.L.; Peñuelas, J. Relationship between photosynthetic radiation-use efficiency of barley canopies and the photochemical reflectance index (PRI). *Physiol. Plant.* **1996**, *96*, 211–216. [CrossRef]
- Filella, I.; Porcar-Castell, A.; Munné-Bosch, S.; Bäck, J.; Garbulsky, M.F.; Peñuelas, J. PRI assessment of long-term changes in carotenoids/chlorophyll ratio and short-term changes in de-epoxidation state of the xanthophyll cycle. *Int. J. Remote Sens.* 2009, *30*, 4443–4455. [CrossRef]
- 35. Gamon, J.A.; Peñuelas, J.; Field, C.B. A narrow-waveband spectral index that tracks diurnal changes in photosynthetic efficiency. *Remote Sens. Environ.* **1992**, *41*, 35–44. [CrossRef]

- Shrestha, S.; Brueck, H.; Asch, F. Chlorophyll index, photochemical reflectance index and chlorophyll fluorescence measurements of rice leaves supplied with different N levels. *J. Photochem. Photobiol. B Biol.* 2012, 113, 7–13. [CrossRef] [PubMed]
- Sukhov, V.S.; Gromova, E.N.; Sukhova, E.M.; Surova, L.M.; Nerush, V.N.; Vodeneev, V.A. Analysis of correlations between the indexes of light-dependent reactions of photosynthesis and the photochemical reflectance index (PRI) in pea leaves under short-term illumination. *Biochem. Moscow Suppl. Ser. A* 2019, 13, 67–77. [CrossRef]
- 38. Sukhova, E.M.; Yudina, L.M.; Vodeneev, V.A.; Sukhov, V.S. Analysis of changes in photochemical reflectance index (PRI) in relation to the acidification of the lumen of the chloroplasts of pea and geranium leaves under a short-term illumination. *Biochem. Moscow Suppl. Ser. A* **2019**, *13*, 243–252. [CrossRef]
- 39. Peñuelas, J.; Filella, I.; Gamon, J.A. Assessment of photosynthetic radiation-use efficiency with spectral reflectance. *New Phytol.* **1995**, *131*, 291–296. [CrossRef]
- 40. Gamon, J.A. Diverse optical and photosynthetic properties in a neotropical dry forest during the dry season: Implications for remote estimation of photosynthesis. *Biotropica* **2005**, *37*, 547–560. [CrossRef]
- 41. Porcar-Castell, A.; Garcia-Plazaola, J.I.; Nichol, C.J.; Kolari, P.; Olascoaga, B.; Kuusinen, N.; Fernández-Marín, B.; Pulkkinen, M.; Juurola, E.; Nikinmaa, E. Physiology of the seasonal relationship between the photochemical reflectance index and photosynthetic light use efficiency. *Oecologia* **2012**, *170*, 313–323. [CrossRef]
- 42. Stylinski, C.D.; Gamon, J.A.; Oechel, W.C. Seasonal patterns of reflectance indices, carotenoid pigments and photosynthesis of evergreen chaparral species. *Oecologia* **2002**, *131*, 366–374. [CrossRef]
- Garbulsky, M.F.; Peñuelas, J.; Ogaya, R.; Filella, I. Leaf and stand-level carbon uptake of a Mediterranean forest estimated using the satellite-derived reflectance indices EVI and PRI. *Int. J. Remote Sens.* 2013, 34, 1282–1296. [CrossRef]
- 44. Wong, C.Y.; Gamon, J.A. Three causes of variation in the photochemical reflectance index (PRI) in evergreen conifers. *New Phytol.* **2015**, *206*, 187–195. [CrossRef] [PubMed]
- Gamon, J.A.; Field, C.B.; Bilger, W.; Björkman, O.; Fredeen, A.L.; Peñuelas, J. Remote sensing of the xanthophyll cycle and chlorophyll fluorescence in sunflower leaves and canopies. *Oecologia* 1990, 85, 1–7. [CrossRef] [PubMed]
- 46. Demmig-Adams, B. Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. *Biochim. Biophys. Acta.* **1990**, 1020, 1–24. [CrossRef]
- Müller, P.; Li, X.P.; Niyogi, K.K. Non-photochemical quenching. A response to excess light energy. *Plant Physiol.* 2001, 125, 1558–1566. [CrossRef] [PubMed]
- Bilger, W.; Björkman, O.; Thayer, S.S. Light-induced spectral absorbance changes in relation to photosynthesis and the epoxidation state of xanthophyll cycle components in cotton leaves. *Plant Physiol.* **1989**, *91*, 542–551. [CrossRef] [PubMed]
- 49. Jahns, P. The xanthophyll cycle in intermittent light-grown pea plants. Possible functions of chlorophyll a/b-binding proteins. *Plant Physiol.* **1995**, *108*, 149–156. [CrossRef]
- 50. Kress, E.; Jahns, P. The dynamics of energy dissipation and xanthophyll conversion in *Arabidopsis* indicate an indirect photoprotective role of zeaxanthin in slowly inducible and relaxing components of non-photochemical quenching of excitation energy. *Front. Plant Sci.* **2017**, *8*, 2094. [CrossRef]
- 51. Murakami, K.; Ibaraki, Y. Time course of the photochemical reflectance index during photosynthetic induction: Its relationship with the photochemical yield of photosystem II. *Physiol. Plant.* **2019**, *165*, 524–536. [CrossRef]
- 52. Ač, A.; Malenovský, Z.; Urban, O.; Hanuš, J.; Zitová, M.; Navrátil, M.; Vráblová, M.; Olejníčková, J.; Špunda, V.; Marek, M. Relation of chlorophyll fluorescence sensitive reflectance ratios to carbon flux measurements of montanne grassland and norway spruce forest ecosystems in the temperate zone. *Sci. World J.* 2012, 2012, 705872. [CrossRef]
- 53. Zhang, C.; Filella, I.; Garbulsky, M.F.; Peñuelas, J. Affecting factors and recent improvements of the photochemical reflectance index (PRI) for remotely sensing foliar, canopy and ecosystemic radiation-use efficiencies. *Remote Sens.* **2016**, *8*, 677. [CrossRef]
- 54. Gamon, J.A.; Serrano, L.; Surfus, J.S. The photochemical reflectance index: An optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient levels. *Oecologia* **1997**, *112*, 492–501. [CrossRef] [PubMed]

- Guo, J.M.; Trotter, C.M. Estimating photosynthetic light-use efficiency using the photochemical reflectance index: The effects of short-term exposure to elevated CO₂ and low temperature. *Int. J. Remote Sens.* 2006, 27, 4677–4684. [CrossRef]
- 56. Sukhova, E.; Sukhov, V. Connection of the photochemical reflectance index (PRI) with the photosystem II quantum yield and nonphotochemical quenching can be dependent on variations of photosynthetic parameters among investigated plants: A meta-analysis. *Remote Sens.* **2018**, *10*, 771. [CrossRef]
- 57. Elsheery, N.I.; Cao, K.F. Gas exchange, chlorophyll fluorescence, and osmotic adjustment in two mango cultivars under drought stress. *Acta Physiol. Plant.* **2008**, *30*, 769–777. [CrossRef]
- 58. Hmimina, G.; Dufrêne, E.; Soudani, K. Relationship between photochemical reflectance index and leaf ecophysiological and biochemical parameters under two different water statuses: Towards a rapid and efficient correction method using real-time measurements. *Plant Cell Environ.* **2014**, *37*, 473–487. [CrossRef]
- 59. Magney, T.S.; Vierling, L.A.; Eitel, J.U.H.; Huggins, D.R.; Garrity, S.R. Response of high frequency photochemical reflectance index (PRI) measurements to environmental conditions in wheat. *Remote Sens. Environ.* **2016**, *173*, 84–97. [CrossRef]
- Kohzuma, K.; Hikosaka, K. Physiological validation of photochemical reflectance index (PRI) as a photosynthetic parameter using *Arabidopsis thaliana* mutants. *Biochem. Biophys. Res. Commun.* 2018, 498, 52–57. [CrossRef]
- Vilfan, N.; Van der Tol, C.; Yang, P.; Wyber, R.; Malenovský, Z.; Robinson, S.A.; Verhoef, W. Extending Fluspect to simulate xanthophyll driven leaf reflectance dynamics. *Remote Sens. Environ.* 2018, 211, 345–356. [CrossRef]
- Yudina, L.; Sukhova, E.; Gromova, E.; Nerush, V.; Vodeneev, V.; Sukhov, V. A light-induced decrease in the photochemical reflectance index (PRI) can be used to estimate the energy-dependent component of non-photochemical quenching under heat stress and soil drought in pea, wheat, and pumpkin. *Photosynth. Res.* 2020, 1–13, [Epub ahead of print]. [CrossRef]
- 63. Gamon, J.A.; Berry, J.A. Facultative and constitutive pigment effects on the Photochemical Reflectance Index (PRI) in sun and shade conifer needles. *Isr. J. Plant Sci.* **2012**, *60*, 85–95. [CrossRef]
- 64. Sukhova, E.; Sukhov, V. Analysis of light-induced changes in the photochemical reflectance index (PRI) in leaves of pea, wheat, and pumpkin using pulses of green-yellow measuring light. *Remote Sens.* **2019**, *11*, 810. [CrossRef]
- 65. Sukhov, V.; Surova, L.; Morozova, E.; Sherstneva, O.; Vodeneev, V. Changes in H+-ATP synthase activity, proton electrochemical gradient, and pH in pea chloroplast can be connected with variation potential. *Front. Plant Sci.* **2016**, *7*, 1092. [CrossRef] [PubMed]
- Kalaji, H.M.; Schansker, G.; Ladle, R.J.; Goltsev, V.; Bosa, K.; Allakhverdiev, S.I.; Brestic, M.; Bussotti, F.; Calatayud, A.; Dąbrowski, P.; et al. Frequently asked questions about in vivo chlorophyll fluorescence: Practical issues. *Photosynth. Res.* 2014, 122, 121–158. [CrossRef]
- 67. Porcar-Castell, A.; Tyystjärvi, E.; Atherton, J.; van der Tol, C.; Flexas, J.; Pfündel, E.E.; Moreno, J.; Frankenberg, C.; Berry, J.A. Linking chlorophyll a fluorescence to photosynthesis for remote sensing applications: Mechanisms and challenges. *J. Exp. Bot.* **2014**, *65*, 4065–4095. [CrossRef] [PubMed]
- Kalaji, H.M.; Schansker, G.; Brestic, M.; Bussotti, F.; Calatayud, A.; Ferroni, L.; Goltsev, V.; Guidi, L.; Jajoo, A.; Li, P.; et al. Frequently asked questions about chlorophyll fluorescence, the sequel. *Photosynth. Res.* 2017, 132, 13–66. [CrossRef]
- 69. Klughammer, C.; Schreiber, U. Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method. *PAM Appl. Notes* **2008**, *1*, 27–35.
- Middleton, E.M.; Cheng, Y.-B.; Hilker, T.; Black, T.A.; Krishnan, P.; Coops, N.C.; Huemmrich, K.F. Linking foliage spectral responses to canopy-level ecosystem photosynthetic light-use efficiency at a Douglas-fir forest in Canada. *Can. J. Remote Sens.* 2009, *35*, 166–188. [CrossRef]
- 71. Soudani, K.; Hmimina, G.; Dufrêne, E.; Berveiller, D.; Delpierre, N.; Ourcival, J.-M.; Rambal, S.; Joffre, R. Relationships between photochemical reflectance index and light-use efficiency in deciduous and evergreen broadleaf forests. *Remote Sens. Environ.* **2014**, *144*, 73–84. [CrossRef]
- 72. Sarlikioti, V.; Driever, S.M.; Marcelis, L.F.M. Photochemical reflectance index as a mean of monitoring early water stress. *Ann. Appl. Biol.* **2010**, *157*, 81–89. [CrossRef]

- 73. Zarco-Tejada, P.J.; González-Dugo, V.; Berni, J.A.J. Fluorescence, temperature and narrow-band indices acquired from a UAV platform for water stress detection using a micro-hyperspectral imager and a thermal camera. *Remote Sens. Environ.* **2012**, *117*, 322–337. [CrossRef]
- 74. Murata, N.; Takahashi, S.; Nishiyama, Y.; Allakhverdiev, S.I. Photoinhibition of photosystem II under environmental stress. *Biochim. Biophys. Acta.* **2007**, *1767*, 414–421. [CrossRef] [PubMed]
- 75. Goh, C.-H.; Ko, S.-M.; Koh, S.; Kim, Y.-J.; Bae, H.-J. Photosynthesis and environments: Photoinhibition and repair mechanisms in plants. *J. Plant Biol.* **2012**, *55*, 93–101. [CrossRef]
- 76. Johnson, G.N. Physiology of PSI cyclic electron transport in higher plants. *Biochim. Biophys. Acta.* 2011, 1807, 384–389. [CrossRef] [PubMed]
- 77. Ruban, A.V. Nonphotochemical chlorophyll fluorescence quenching: Mechanism and effectiveness in protecting plants from photodamage. *Plant Physiol.* **2016**, *170*, 1903–1916. [CrossRef] [PubMed]
- 78. Sukhov, V.; Surova, L.; Sherstneva, O.; Katicheva, L.; Vodeneev, V. Variation potential influence on photosynthetic cyclic electron flow in pea. *Front. Plant Sci.* **2015**, *5*, 766. [CrossRef] [PubMed]
- 79. Zivcak, M.; Brestic, M.; Balatova, Z.; Drevenakova, P.; Olsovska, K.; Kalaji, H.M.; Yang, X.; Allakhverdiev, S.I. Photosynthetic electron transport and specific photoprotective responses in wheat leaves under drought stress. *Photosynth. Res.* **2013**, *117*, 529–546. [CrossRef] [PubMed]
- 80. Benz, J.P.; Lintala, M.; Soll, J.; Mulo, P.; Bölter, B. A new concept for ferredoxin-NADP(H) oxidoreductase binding to plant thylakoids. *Trends Plant Sci.* **2010**, *15*, 608–613. [CrossRef]
- Mulo, P. Chloroplast-targeted ferredoxin-NADP+ oxidoreductase (FNR): Structure, function and location. *Biochim. Biophys. Acta.* 2011, 1807, 927–934. [CrossRef]
- 82. Sukhova, E.; Khlopkov, A.; Vodeneev, V.; Sukhov, V. Simulation of a nonphotochemical quenching in plant leaf under different light intensities. *Biochim. Biophys. Act—Bioenerg.* **2020**, *1861*, 148138. [CrossRef]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).