Rate Constants of PSII Photoinhibition and its Repair, and PSII Fluorescence Parameters in Field Plants in Relation to their Growth Light Environments

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The extent of photoinhibition of PSII is determined by a balance between the rate of photodamage to PSII and that of repair of the damaged PSII. It has already been indicated that the rate constants of photodamage ($k_{pi}$) and repair ($k_{rec}$) of the leaves differ depending on their growth light environment. However, there are no studies using plants in the field. We examined these rate constants and fluorescence parameters of several field-grown plants to determine inter-relationships between these values and the growth environment. The $k_{rec}$ values were strongly related to the excess energy, $E_v$, of the puddle model and non-regulated energy dissipation, $Y(NO)$, of the lake model, both multiplied by the photosynthetically active photon flux density (PPFD) level during the photoinhibitory treatment. In contrast, the $k_{rec}$ values corrected against in situ air temperature were very strongly related to the daily PPFD level. The plants from the fields showed higher NPQ than the chamber-grown plants, probably because these field plants acclimated to stronger light flecks than the averaged growth PPFD. Comparing chamber-grown plants and the field plants, we showed that $k_{pi}$ is determined by the incident light level and the photosynthetic capacities such as in situ rate of PSII electron transport and non-photochemical quenching (NPQ) [e.g. $Y(NO) \times \text{PPFD}$] and that $k_{rec}$ is mostly determined by the growth light and temperature levels.

Keywords: Chlorophyll fluorescence • Excess energy hypothesis • Jaljale Himal • Non-regulated energy dissipation • Photoinhibition • Two-step hypothesis.

Abbreviations: $a$, fraction of active PSII; $E_{\text{photov}}$, average photon energy of PAR; $E_v$, yield of excess energy of the puddle model; $F_m$ ($F_m^*$), maximum fluorescence in the fully relaxed state (in the light); $F_o$ ($F_o^*$), minimum fluorescence in the fully relaxed state (in the light); $F_r$ ($F_r^*$), steady-state fluorescence in the light; $F_v$ ($F_v^*$), variable fluorescence in the fully relaxed state (in the light); $F_o$, $F_v$, $F_r$, variable fluorescence in the fully relaxed state (in the light); $F_{m-0}$, $F_{v-0}$, $F_{r-0}$, $F_{v-0}$, $F_{r-0}$; $F_o/F_{m}$, maximum photochemical efficiency of photochemistry in PSII of dark-adapted leaves; $k_{pi}$ photodamage constant of photodamage; $k_{rec}$ rate constant of repair; $k_{rec}^{\text{corrected}}$, corrected $k_{rec}$; LED, light-emitting diode; NPQ, non-photochemical quenching of the puddle model; OSR, open sky ratio; PAR, photosynthetically active radiation; PPFD, photosynthetically active photon flux density; PPFD$_{\text{LIA}}$, maximum PPFD at the campsite; PPFD$_{\text{LIAos}}$, maximum PPFD for the imaginary completely open site at the same geographical location; PPFD$_{\text{LIAos-sample}}$, PPFD at the imaginary completely open site at the same location as the sampling site; PPFD$_{\text{sample}}$, PPFD at the completely open site; Q$_{10}$, temperature coefficient; qL, quenching coefficient of the lake model; qP, photochemical quenching coefficient of the puddle model; ROS, reactive oxygen species; TSR, total shortwave radiation; Y(I), quantum yield of PSII photochemistry of the lake model; Y(NPQ), yield of non-photochemical energy dissipation of the lake model; Y(NO), non-regulated energy dissipation of the lake model; $\Phi_{\text{PSII}}^{\text{quantum yield of PSII photochemistry of the puddle model}}$.

Introduction

Light is the ultimate energy source for photosynthesis, and is thereby indispensable to plants. At the same time, light inhibits photosynthesis, and this has been called photoinhibition (Kok 1956). The primary target of photoinhibition is PSII (Powles 1984), and the degree of the PSII photoinhibition in vivo is determined by the balance of two reactions, photodamage and repair (Greer et al. 1986, Aro et al. 1993b). There are two main hypotheses for the mechanisms of photoinhibition. One is the excess energy hypothesis (Øgren et al. 1984, Vass et al. 1992); this includes two different mechanisms: the acceptor side (Vass et al. 1992, Vass 2011) and the donor side inhibitions (Callahan et al. 1986, Aro et al. 1993b, Vass 2011). The other is the two-step hypothesis, claiming that the manganese cluster is primarily damaged by UV and/or blue light (Hakala et al. 2005, Ohnishi et al. 2005). Both mechanisms appear to operate in the photodamage of PSII in vivo, but the major mechanism would differ depending on various conditions (Oguchi et al. 2009).
Kok (1956) expressed the photodamage and repair of PSII as first-order reactions. Using these equations, the rate constants for the photodamage ($k_{pi}$) and repair reaction ($k_{rec}$) have been estimated (Kok 1956, Kato et al. 2002a). $k_{pi}$ and $k_{rec}$ differ depending not only on the incident PPFD (photosynthetic photon flux density) during the photoinhibition treatment but also on the growth irradiance (Tyystjärvi et al. 1992).

Tyystjärvi et al. (1992) indicated that $k_{pi}$ decreased with the increase in growth irradiance in *Cucurbita pepo* L. In contrast, Lee et al. (2001) indicated that $k_{rec}$ increased with the increase in growth irradiance in *Capsicum annuum* L. For $k_{rec}$, both studies indicated that $k_{rec}$ increased with the increase in growth irradiance. In many studies examining the effects of growth irradiance, artificial light sources such as fluorescence tubes were used and the irradiance was kept constant in the daytime. Measurements of $k_{pi}$ and $k_{rec}$ using field plants have never been made. Do plants in the field show $k_{pi}$ and $k_{rec}$ and other photosynthetic parameters different from those in laboratory-grown plants?

Using field-grown plants such as *Vinca minor* L., Demming-Adams et al. (1996) examined the relationships between the puddle model fluorescence parameters, such as the photochemical quenching coefficient ($qP$), non-photochemical quenching (NPQ) and excess energy ($E_Y$), and growth irradiance. $qP$, NPQ and $E_Y$ are the fraction of the open PSII, the parameter for regulated heat dissipation and the fraction of energy transferred to the closed PSII, respectively. When measured at the same PPFD level, $qP$ and NPQ increased with the increase in growth irradiance, and thereby $E_Y$ decreased with the increase in growth irradiance. In *Vicia faba* L. ‘Minpo’ plants grown in a growth chamber, Stefanov and Terashima (2008) showed the same trends. With chamber-grown spinach plants (*Spinacia oleracea* L. ‘Tori’), Miyata et al. (2012) showed that, at medium irradiances, NPQ and $E_Y$ were smaller in high-light plants than in low-light plants, although the differences become obscure at higher irradiances.

NPQ was identified as a fluorescence parameter affecting both the rate of photodamage and the rate of repair of the photodamage (Li et al. 2002, Takahashi et al. 2009). According to the excess hypothesis, NPQ decreases the rate of photodamage, whereas, according to the two-step hypothesis, NPQ maintains the rate of repair by suppressing the ROS (reactive oxygen species) level (Takahashi et al. 2009).

In the field, irradiance incident on a leaf fluctuates dynamically with time due to clouds and other structures including the leaf canopy. The fluctuating light induces PSII photoinhibition (Tikkanen et al. 2010, Kono et al. 2014). On the other hand, it has been shown that the high irradiance components of the fluctuating light enhance NPQ capacity in a manner depending on the frequency and duration of the high light periods (Alter et al. 2012).

In this study, we used leaves of cucumber plants grown in a growth chamber at three constant irradiance levels as the control materials. We also grew cucumber plants outdoors. Moreover, we used several herbaceous plants on the University of Tokyo campus and some alpine plants in the Himalayas. Through measuring the rate constants of PSII photoinhibition and repair, and various fluorescence parameters, we examined the influence of growth light environment in the field on the photosynthetic reactions, photoinhibition and repair. In the present study, we calculated fluorescence parameters based not only on the puddle model but also on the lake model (Hendrickson et al. 2004, Kramer et al. 2004). For the parameters of the puddle and lake models, see the Materials and Methods. For detailed definitions and inter-relationships of the parameters, see Klughammer and Schreiber (2008) and Kasajima et al. (2009). As $k_{pi}$, $k_{rec}$ and various fluorescence parameters are influenced by temperature (Tsoncev and Hikosaka 2003), we compared the data obtained in warm and cold seasons. We also made temperature corrections to estimate $k_{rec}$ in situ. There have been warnings that conditions inducing stomatal closure or occlusion, and/or thickening of the leaf boundary layer accelerate photoinhibition even at low PPFD levels (Kato et al. 2002b). We carefully avoided such artifacts during the photoinhibitory treatment.

**Results**

**Relationships of $k_{pi}$, $k_{rec}$ and PSII fluorescence parameters to daily PPFD in cucumber leaves**

For brief experimental procedures, see the legend of Fig. 1 (for full details, see the Materials and Methods). Data for the leaves of the cucumber plants grown in the growth chamber or outdoors, photoinhibited at 400 or 1,200 μmol m$^{-2}$ s$^{-1}$, are shown in Fig. 1. $k_{pi}$, $k_{rec}$ and various fluorescence parameters calculated according to both the puddle and lake models are plotted against the mean daily PPFD. For the leaves grown outdoors, the data are plotted against the mean daily PPFD for 7 or 14 d before the sampling. Regression lines are drawn for the data obtained with the three groups of cucumber plants grown in continuous light at 35, 170 and 500 μmol m$^{-2}$ s$^{-1}$ for 14 h per day in the growth chamber.

$k_{pi}$ at 400 and 1,200 μmol m$^{-2}$ s$^{-1}$ decreased with the increase in daily PPFD (Fig. 1A). $k_{pi}$ values at 1,200 μmol m$^{-2}$ s$^{-1}$ were greater than those at 400 μmol m$^{-2}$ s$^{-1}$ by 2-fold (Fig. 1A). $k_{rec}$ at 400 and 1,200 μmol m$^{-2}$ s$^{-1}$ increased with daily PPFD (Fig. 1B). $k_{rec}$ values at 1,200 μmol m$^{-2}$ s$^{-1}$ were smaller than those at 400 μmol m$^{-2}$ s$^{-1}$. $k_{pi}$ and $k_{rec}$ at 400 and 1,200 μmol m$^{-2}$ s$^{-1}$ in the plants grown outdoors were near the regression lines (Fig. 1A).

$\Phi_{psii}$ of the puddle model, which is identical to $\gamma(U)$ of the lake model, in cucumber leaves at both 400 and 1,200 μmol m$^{-2}$ s$^{-1}$ increased with daily PPFD, and $\Phi_{psii}$ of the outdoor leaves also showed similar trends (Fig. 1C).

PSII fluorescence parameters analyzed by the puddle model are $qP$, $E_Y$ and NPQ. $qP$ at 400 μmol m$^{-2}$ s$^{-1}$ decreased with the increase in daily PPFD for the chamber-grown plants (Fig. 1D). On the other hand, NPQ at 1,200 μmol m$^{-2}$ s$^{-1}$ increased slightly with daily PPFD. The values of NPQ were greater at 1,200 μmol m$^{-2}$ s$^{-1}$. The NPQ values at both 400 and 1,200 μmol m$^{-2}$ s$^{-1}$ of the 2-week-old outdoor leaves in July and October were significantly higher than the regression
qP increased with daily PPFD, and qP of the outdoor leaves lay near the regression line (Fig. 1E). Values of qP at 1,200 μmol m⁻² s⁻¹ were generally lower than those at 400 μmol m⁻² s⁻¹. Eₐ decreased with daily PPFD. Eₐ of the outdoor leaves was close to the regression line (Fig. 1F). Absolute values of Eₐ at 1,200 μmol m⁻² s⁻¹ tend to be greater than those at 400 μmol m⁻² s⁻¹.

Y(NPQ) of the lake model showed a pattern similar to that of NPQ (Fig. 1G). Y(NPQ) values at 1,200 μmol m⁻² s⁻¹ were greater than those at 400 μmol m⁻² s⁻¹. The Y(NPQ) decreased slightly with daily PPFD. Y(NPQ) in the 2-week-old outdoor leaves in July and October were significantly higher than the regression line (Fig. 1G).

qL of the lake model corresponds to qP of the puddle model, and the trend of qL was similar to that of qP (Fig. 1H). Y(NO) includes the fraction corresponding to Eₐ. Y(NO) at 400 μmol m⁻² s⁻¹ did not change with daily PPFD, while Y(NO) at 1,200 μmol m⁻² s⁻¹ decreased with daily PPFD (Fig. 1I). Y(NO) values of the outdoor leaves at both 400 and 1,200 μmol m⁻² s⁻¹ were lower than the regression lines (Fig. 1I).
The rate constants of photodamage and repair in Erigeron philadelphicus, Fagopyrum dibotrys, Hottuynia cordata, Persicaria chinensis, Plantago asiatica and Polygonum longisetum on the University of Tokyo campus

Whether the photoinhibitory treatments were conducted at 400 or 1,200 μmol m$^{-2}$ s$^{-1}$, $k_p$ values of these field plants had no significant relationships with the OSR (open sky ratio) and daily PPFD (Fig. 2A–D). $k_p$ values at 1,200 μmol m$^{-2}$ s$^{-1}$ were greater than those at 400 μmol m$^{-2}$ s$^{-1}$. When the treatment was conducted at 400 μmol m$^{-2}$ s$^{-1}$, all plants gave similar $k_p$ values. At 1,200 μmol m$^{-2}$ s$^{-1}$, the data points were more scattered. In contrast, $k_{rec}$ values obtained at 400 and 1,200 μmol m$^{-2}$ s$^{-1}$ were both positively related to OSR and daily PPFD (Fig. 2E–H). The absolute $k_{rec}$ values at 1,200 μmol m$^{-2}$ s$^{-1}$ were somewhat smaller than those at 400 μmol m$^{-2}$ s$^{-1}$. There were some data that did not conform to these trends. For example, $k_{rec}$ values in Po. longisetum obtained at 400 μmol m$^{-2}$ s$^{-1}$ in November 2013 and in F. dibotrys obtained at 1,200 μmol m$^{-2}$ s$^{-1}$ in October 2014 were much lower than the regression lines, and those of P. asiatica from 52.7% OSR at 400 μmol m$^{-2}$ s$^{-1}$ in July 2014 and P. asiatica from 3.3 μmol m$^{-2}$ d$^{-1}$ daily PPFD at 400 μmol m$^{-2}$ s$^{-1}$ in October 2014 were higher than the regression lines (Fig. 2E–H). $k_{rec}$ values measured in warm months tended to be lower than those measured in cool months.

The field plants collected in October and November 2013, and July and October 2014 were photoinhibited at 400 μmol m$^{-2}$ s$^{-1}$ and those collected in September and October 2013, and July and October 2014 were photoinhibited at 1,200 μmol m$^{-2}$ s$^{-1}$ (Figs. 3, 4). The daily PPFDs for the field plants are mean values for 14 d before the respective sampling days. The regression lines are drawn for the data obtained with the chamber-grown cucumber leaves (note that regression lines in Fig. 2 are for the field plants). $k_p$ values of the field plants at 400 μmol m$^{-2}$ s$^{-1}$ were lower than the regression line (Fig. 3A). $k_{rec}$ values of the field plants at 400 μmol m$^{-2}$ s$^{-1}$ were scattered but tended to increase with daily PPFD (Fig. 3B).

When $k_p$ values at 1,200 μmol m$^{-2}$ s$^{-1}$ are plotted against daily PPFD, some plants such as H. cordata, P. longisetum and P. asiatica in September 2013, P. asiatica in October 2013 and P. asiatica in July 2014 were near the regression line (Fig. 4A). The $k_p$ values of the other plants were considerably lower than the regression line (Fig. 4A). $k_{rec}$ values of the field plants at 1,200 μmol m$^{-2}$ s$^{-1}$ were scattered but tended to increase with daily PPFD (Fig. 4B).

Relationships between PSII fluorescence parameters and daily PPFD in the plants sampled on the University of Tokyo campus

These field plants collected in July and October 2014 were photoinhibited at 400 or 1,200 μmol m$^{-2}$ s$^{-1}$. Various
fluorescence parameters at 400 and 1,200 μmol m\(^{-2}\) s\(^{-1}\) are plotted against the daily PPFD. The daily PPFDs for the field plants are mean values for 14 d before the respective sampling days (Figs. 3, 4). \(\Phi_{\text{PSII}}\) showed trends similar to those in Fig. 1 (Figs. 3C, 4C). \(\Phi_{\text{PSII}}\) values of the field plants were mostly lower than the regression line, although some data points of \(P. asiatica\) were near or above the lines (Figs. 3C, 4C). NPQ values of the field plants were significantly above the regression lines, except for two data points of \(P. asiatica\) (Figs. 3D, 4D). NPQ values of the field plants in July 2014 were lower than the regression lines, except for \(P. asiatica\) at 28.3 mol m\(^{-2}\) d\(^{-1}\) daily PPFD (Fig. 3E, 4E). qP values of the field plants in October 2014 were somewhat lower or around the regression lines, except for \(H. cordata\) at 400 μmol m\(^{-2}\) s\(^{-1}\) and \(P. asiatica\) at 11.7 mol m\(^{-2}\) d\(^{-1}\) daily PPFD at 1,200 μmol m\(^{-2}\) s\(^{-1}\) (Figs. 3E, 4E). \(Y_{(\text{NO})}\) values of the field plants at 400 μmol m\(^{-2}\) s\(^{-1}\) were mostly above the regression line (Fig. 3F). On the other hand, \(E_{\gamma}\) values at 1,200 μmol m\(^{-2}\) s\(^{-1}\) of the field plants in October 2014 were lower than the regression line, except for \(H. cordata\) (Fig. 4F). \(Y_{(\text{NPQ})}\) showed a pattern similar to that of NPQ (Figs. 3G, 4G). For qL, the trend was similar to that of qP (Fig. 3H, 4H). \(Y_{(\text{NO})}\) showed trends fairly different from those of \(E_{\gamma}\) (Figs. 3I, 4I). Most \(Y_{(\text{NO})}\) values were below the regression lines, while \(E_{\gamma}\) values of the field plants lay near the regression line. \(Y_{(\text{NO})}\) values of \(P. asiatica\) at 28.3 mol m\(^{-2}\) d\(^{-1}\)
in July 2014 at both 400 and 1,200 mol m\(^{-2}\) s\(^{-1}\) and of \textit{H. cordata} in October 2014 at 400 mol m\(^{-2}\) s\(^{-1}\) were, however, near the regression lines (Figs. 3I, 4I).

**Relationships of \(k_{\text{pi}}\) to daily PPFD, incident PPFD, excess energy and non-regulated energy dissipation during the photoinhibitory treatment**

The \(k_{\text{pi}}\) data for the field plants photoinhibited at both 400 and 1,200 mol m\(^{-2}\) s\(^{-1}\) were plotted together against the daily PPFD. We also plotted \(k_{\text{pi}}\) data against the incident PPFD, excess energy and non-regulated energy dissipation during the photoinhibitory treatments. The excess energy has been defined as \(E_{\text{v}} \times \text{incident PPFD} \times \text{leaf absorptance} \times \text{energy partition ratio to PSII} \) (Kato et al. 2003). \(Y(\text{NO})\) includes the rate constants of fluorescence and non-radiative decay (Hendrickson et al. 2004). In this study, for excess energy and non-regulated energy dissipation, we simply used \(E_{\text{v}} / \text{incident PPFD} \) and \(Y(\text{NO}) / \text{incident PPFD} \) [\(Y(\text{NO}) / \text{PPFD}\)].

Strong negative relationships were obtained between the \(k_{\text{pi}}\) values of cucumber plants and the daily PPFD, whereas the relationships between the \(k_{\text{pi}}\) of the field plants and the daily PPFD were weak (Fig. 5A). When the \(k_{\text{pi}}\) values were plotted

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**Fig. 4** Relationships of the rate constants for photodamage (\(k_{\text{pi}}\)) and repair (\(k_{\text{rec}}\)), and PSII fluorescence parameters measured at 1,200 mol m\(^{-2}\) s\(^{-1}\) to daily PPFD in the field plants in Tokyo. The \(k_{\text{pi}}\) and \(k_{\text{rec}}\) were obtained for the leaves of the field plants collected in September 2013, October 2013, July 2014 and October 2014, and the cucumber leaves grown at three different PPFDs in the growth chamber. Filled black circles, chamber-grown \textit{Cucumis sativus}; filled coloured circles, \textit{Erigeron philadelphicus}; plus signs, \textit{Fagopyrum dibotrys}; filled diamonds, \textit{Hottuynia cordata}; filled squares, \textit{Persicaria chinensis}; crosses, \textit{Plantago asiatica}; and filled triangles, \textit{Polygonum longisetum}. Yellow symbols, July 2014 with the monthly average temperature of 26.8°C; orange symbols, September 2013 (25.2°C); purple symbols, October 2013 (19.8°C); and blue symbols, October 2014 (19.1°C). Means ± SD (\(n = 3\)) are shown. Regression lines are drawn for the data obtained with cucumber leaves that were grown in continuous light at 35, 170 and 500 mol m\(^{-2}\) s\(^{-1}\) for 14 h d\(^{-1}\). Asterisks indicate significant differences at 5% between the field plant leaves and the regression lines for the chamber-grown cucumber leaves.
against the incident PPFD and $E_Y \times \text{PPFD}$, not only cucumber plants but also all field plants showed strong relationships (Fig. 5B, C). The strongest relationship was obtained with $Y(\text{NO}) \times \text{PPFD}$ (Fig. 5B–D).

**Relationship between $k_{\text{rec}}$ and daily PPFD**

The $k_{\text{rec}}$ of cucumber plants and other field plants increased with the increase in the daily PPFD (Fig. 6A). However, $k_{\text{rec}}$ is greatly affected by temperature (Tsonev and Hikosaka 2003). Therefore, we corrected $k_{\text{rec}}$ values using a temperature coefficient, $Q_{10}$, calculated from Tsonev and Hikosaka (2003). The corrected $k_{\text{rec}}$ values are called $K_{\text{rec}}$ values. We used daily average air temperatures in Tokyo provided by the Japan Meteorological Agency. The daily average air temperatures in the preceding 14 d before the sampling of the leaves were used, except for the outdoor cucumber plants grown in July 2014.

Because the first leaves of these plants expanded in 7 d in July 2014, the mean of the day average air temperatures for the preceding 7 d was used. $K_{\text{rec}}$ values were also obtained for the chamber-grown cucumber because the air temperature in the growth chamber was 23°C. The $K_{\text{rec}}$ values of all plants increased with the increase in the daily PPFD (Fig. 6B). For all the data, the determination coefficient ($R^2$) for $k_{\text{rec}}$ vs. $K_{\text{rec}}$ at 400 and 1,200 $\mu$mol m$^{-2}$ s$^{-1}$ was 0.54 vs. 0.70 and 0.52 vs. 0.82, respectively (Fig. 6A, B). The regression line for all the plants of $K_{\text{rec}}$ at 400 $\mu$mol m$^{-2}$ s$^{-1}$ had a steeper slope than that at 1,200 $\mu$mol m$^{-2}$ s$^{-1}$ (Fig. 6B).

**The rate constants of photodamage and repair in relation to light environments in the Jaljale Himal**

When the photoinhibitory treatment was conducted at 1,200 $\mu$mol m$^{-2}$ s$^{-1}$, $k_{\text{pi}}$ and $k_{\text{rec}}$ values of the alpine plants

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**Fig. 5** Rate constants of photodamage ($k_{\text{pi}}$) plotted against daily PPFD, incident PPFD, $E_Y \times \text{PPFD}$ and $Y(\text{NO}) \times \text{PPFD}$ during the photoinhibitory treatment in cucumber and in the field plants in Tokyo. Filled circles and blue solid lines, cucumbers grown in the chamber photoinhibited at 400 $\mu$mol m$^{-2}$ s$^{-1}$; open circles and red solid lines, cucumbers grown in the chamber photoinhibited at 1,200 $\mu$mol m$^{-2}$ s$^{-1}$; black solid lines, cucumbers grown in the chamber photoinhibited at 400 or 1,200 $\mu$mol m$^{-2}$ s$^{-1}$; filled diamonds, all the field plants including cucumbers grown outdoors photoinhibited at 400 $\mu$mol m$^{-2}$ s$^{-1}$; open diamonds, all the field plants including cucumbers grown outdoors photoinhibited at 1,200 $\mu$mol m$^{-2}$ s$^{-1}$; dotted lines, all the field plants including cucumbers grown outdoors; and dashed lines, all the plants. For regression lines, see Supplementary Table S4.
showed weak relationships with the OSR (Fig. 7A, C). However, the \( k_{\text{pi}} \) and \( k_{\text{rec}} \) showed much stronger and statistically significant relationships with daily PPFD. With the increase in daily PPFD, not only \( k_{\text{pi}} \) but also \( k_{\text{rec}} \) increased (Fig. 7B, D).

For these alpine plants, the measurements were conducted at in situ temperatures of around 10 °C. \( k_{\text{pi}} \) and \( k_{\text{rec}} \) ranged from 0.0059 to 0.013 min⁻¹ and from 0.0089 to 0.14 min⁻¹, respectively. The \( k_{\text{pi}} \) and \( k_{\text{rec}} \) values of alpine plants measured in situ were higher and much lower, respectively, than those of chamber-grown cucumber and the field plants measured at 25 °C. If the changes in \( k_{\text{pi}} \) and \( k_{\text{rec}} \) with temperature in these alpine plants followed the changes reported for Chenopodium album (Tsonev and Hikosaka, 2003), \( k_{\text{pi}} \) and \( k_{\text{rec}} \) of these alpine plants at 25 °C would be about 0.70 and 5.4 times the values measured in situ.

**Discussion**

**Relationships of \( k_{\text{pi}} \) and \( k_{\text{rec}} \) to daily PPFD**

Several studies have shown that the increase in growth irradiance causes the decrease in \( k_{\text{pi}} \) and the increase in \( k_{\text{rec}} \) (Tyystjärvä et al. 1992, Aro et al. 1993a, Kato et al. 2002a, Miyata et al. 2012). However, for high-light- and low-light-grown C. annuum L., an opposite trend of \( k_{\text{pi}} \) has been reported (Lee et al. 2001). Their results appear to support the hypothesis proposed by Anderson and Aro (1994) claiming that the shade-type thylakoids would be more resistant to photoinhibition because photoactivated PSII in the shade-type thylakoids dissipated excess energy more efficiently than those in the high-light leaves. In the cucumber plants used in the present study, however, whether they were grown in the growth chamber or outdoors, \( k_{\text{pi}} \) decreased and \( k_{\text{rec}} \) increased with the increase in growth irradiance at both photoinhibitory PPFDs (Fig. 1A, B). \( k_{\text{rec}} \) values at 1,200 \( \mu \)mol m⁻² s⁻¹ were lower than those at 400 \( \mu \)mol m⁻² s⁻¹ (Figs. 1B, 6A). This trend is consistent with the previous studies showing that \( k_{\text{rec}} \) has a peak against the photoinhibitory PPFD probably due to inhibition of the repair system by enhanced ROS production (He and Chow 2003, Takahashi and Badger 2011, Miyata et al. 2012).

For the field plants on the University of Tokyo campus, \( k_{\text{rec}} \) values increased with the OSR or daily PPFD (Figs. 2, 6). The high \( k_{\text{rec}} \) values of \( P. \) asiatica in October 2013 by the photoinhibition treatment at 1,200 \( \mu \)mol m⁻² s⁻¹ were noteworthy (Figs. 3B, 4B). This would be attributed to the characteristics of \( P. \) asiatica as a typical sun plant, showing high \( \Phi_{\text{PSII}} \) in high light.

For the plants in the Jaljale Himal, the relationships between these rate constants, \( k_{\text{pi}} \) and \( k_{\text{rec}} \), and OSR were very weak (Fig. 7A, C). The stronger relationships were obtained with the daily PPFD (Fig. 7B, D). Even for a given OSR, the daily PPFD differs depending on the topography of the site, time of the year, and the local climate and weather. Thus, calculation of the daily PPFD would be preferable to the direct use of OSR. It is worth stressing that we can fairly precisely calculate the daily PPFD using hemispherical photos, the software (CanopOn 2 and LIA32) and solar radiation data from nearby meteorological stations.

**Relationships of \( k_{\text{pi}} \), \( k_{\text{rec}} \) and PSII fluorescence parameters to daily PPFD**

NQZ values of the cucumber leaves grown outdoors for 1 week in July 2014 were near the regression line for the cucumber leaves grown at three PPFD levels in the growth chamber. In contrast, the 2-week-old leaves in July 2014 and the leaves in October 2014 showed NQZ values higher than the regression line (Fig. 1D). It has been shown that, in Arabidopsis thaliana...
Photoinhibition-treated field plants at 1,200 μmol m$^{-2}$ s$^{-1}$, Bergenia purpurascens blue hemmed marks, Jaljale Himal–Tin Pokhari, August 2012, lower repair lines, see Cremanthodium reniforme. Bistorta milletioides crosses, Berteroa incana relationships between the rate constants for photodamage ($k_{pi}$ and repair ($k_{rec}$) of the chamber-grown cucumber leaves, except for several data points including those of P. asiatica (Figs. 3, 4). Reflecting these trends, E$_Y$ levels in the field plants were similar to those in the chamber-grown cucumber. According to the excess energy hypothesis, the similar E$_Y$ levels would bring about similar $k_{pi}$ values. However, $k_{pi}$ values in the field plants on the campus tended to be lower than those of cucumber leaves (Figs. 3, 4). In October 2014, qP values of the field plants were not necessarily lower than the regression line, and thereby E$_Y$ levels were lower than the regression line.

$k_{pi}$ values were strongly related to the incident PPFD (Fig. 5B). The strong relationships between the $k_{pi}$ values and incident PPFD have been already pointed out (Tyystjärvi and Aro 1996). However, the regression line of $k_{pi}$ on the incident PPFD for the field plants had a positive intercept at 100 μmol m$^{-2}$ s$^{-1}$ on the $x$-axis (Fig. 5B). The regression line for the chamber-grown cucumber showed a small negative intercept on the incident PPFD at $-3.8$ μmol m$^{-2}$ s$^{-1}$. This difference would be attributed to the fact that the field plants had much greater NPQ capacity (Fig. 5B, C). There are some studies showing strong relationships between $k_{pi}$ and excess energy (Kato et al. 2003, Miyata et al. 2012). In the present study also, the relationship between $k_{pi}$ and the excess energy was strong. However, the regression lines of $k_{pi}$ to excess energy had negative intercepts on the $x$-axis. For example, the negative intercept on the $x$-axis for all the plants was $-82.1$ μmol m$^{-2}$ s$^{-1}$. The determination coefficients were comparable between the incident PPFD and the excess energy (Fig. 5B, C).

In the lake model of Hendrickson et al. (2004), Y(NO) is described as ($k_i + k_d$)($k_i + k_d + k_{NPQ} + k_{pi}$), where $k_i$, $k_d$, $k_{NPQ}$ and $k_{pi}$ are the rate constants of fluorescence, non-radiative decay, non-photochemical quenching and photochemistry, respectively. Y(NO) × PPFD is comprised not only of the excess energy but also of the energy approaching the open PSIIL centers but not used in photochemistry. An estimate for the probability of excitation transfer from a closed center to a neighboring open center in plants is about 0.6 (Lázár 1999). Laišk et al. (2012) even claimed that the transfer hardly occurs. Then, the excitation energy delivered to the closed PSIIL would be most harmful as the excess energy hypothesis claims. However, photoinhibition occurred even under the conditions where PSIIL was hardly closed (Oguchi et al. 2009). Moreover, the regression lines of $k_{pi}$ on the excess energy shown in Kato et al. (2003) and Tsonev and Hikosaka (2003) also showed negative intercepts on the $x$-axis. Thus, the excess energy delivered to open PSIIL but not used in photochemistry could be also harmful. When $k_{pi}$ values of all the plants were plotted against the Y(NO) × PPFD, the determination coefficient was much higher than those for $k_{pi}$ vs. incident PPFD and for $k_{pi}$ vs. the excess energy (Fig. 5B–D). These indicate that Y(NO) rather than excess energy or incident light should be more directly responsible for photoinhibition.
According to the two-step hypothesis, NPQ affects photo-inhibition through preventing $k_{\text{rec}}$ from decreasing by suppressing ROS production (Murata et al. 2012). The increase in ROS production with the increase in irradiance may explain the fact that $k_{\text{rec}}$ was smaller at 1,200 μmol m$^{-2}$ s$^{-1}$ than at 400 μmol m$^{-2}$ s$^{-1}$ (Fig. 6A), which implies indirectly the importance of NPQ as a determinant of $k_{\text{rec}}$. However, when we plotted $k_{\text{rec}}$ against NPQ for all the plants, no clear relationships can be seen (not shown). In contrast, $k_{\text{rec}}$ showed a strong relationship to the daily PPFD (Fig. 6A). It has been reported that the $k_{\text{rec}}$ values are greater in high-light-grown plants than in low-light-grown plants (Tyystjärvi et al. 1992, Kato et al. 2002a, Miyata et al. 2012). Moreover, our previous simulation indicated that doubling the $k_{\text{rec}}$ value does not lead to a marked increase in the daily photosynthetic gain, whereas halving the value causes a dramatic decrease in the photosynthetic gain (Miyata et al. 2012). In this study, we propose that the $k_{\text{rec}}$ value is determined depending mainly on daily PPFD to keep the activity of the photosynthetic machinery at an appropriate level.

It is likely that the excess energy would lead to production of ROS. However, ROS would be scavenged by various scavenging systems (Asada 1999). Probably, the plants grown in the field have higher capacities for dissipation of excess energy, in addition to those for NPQ. For example, β-carotene quenches O$_2$ generated via P680 (Asada 2006, Vass 2011). If these activities are different among the sample plants, excess energy may not be very strongly related to $k_{\text{pi}}$. Indeed, in H. cordata and F. dibotrys, $k_{\text{pi}}$ values were lower than those of cucumber plants, although $E_Y$ levels were similar to those of cucumber plants (Figs. 3, 4).

As already mentioned, Y(NO) × PPFD would be a better determinant. Given that Y(II) + Y(NPQ) + Y(NO) = 1, Y(NPQ) and Y(II) should be important determinants of $k_{\text{pi}}$. This challenges the two-step hypothesis that claims that $k_{\text{pi}}$ is proportional to absorbed irradiance and that $k_{\text{pi}}$ is not related to NPQ. To test these two hypotheses of the photoinhibition mechanisms, of course, we need to conduct more precise experimental studies.

Recently, it has been clarified that a fraction of the ‘apparent’ NPQ is caused by the changes in the leaf optics due to chloroplast re-location, which occurs in response to blue light and is mediated by phototropins (Cazzaniga et al. 2013, Dall’Osto et al. 2014). Because we used a white light-emitting diode (LED) containing a blue light component in the present study, NPQ detected in this study was not purely the energy-dependent quenching. Y(NPQ) was also overestimated. By using red actinic light, we can suppress chloroplast re-location (Tholen et al. 2008, Cazzaniga et al. 2013, Kono et al. 2014). However, this also suppresses the photoinhibition mechanisms claimed by the two-step hypothesis. Thus, we used a white LED.

**Influence of temperature on $k_{\text{pi}}$, $k_{\text{rec}}$ and PSII fluorescence parameters**

The effects of measuring temperature on $k_{\text{pi}}$, $k_{\text{rec}}$ and the puddle model PSII fluorescence parameters were studied in *C. album* L. (Tsonev and Hikosaka 2003). $k_{\text{pi}}$ at 10°C was greater than that at 25°C by 1.4-fold, while $k_{\text{rec}}$ at 10°C was about 20% of that at 25°C (Tsonev and Hikosaka 2003). Because $k_{\text{pi}}$ is greater at low temperatures, PSII is damaged more frequently at low temperatures. However, considerable $k_{\text{rec}}$ will recover the damage. $k_{\text{rec}}$ in the plants on the university campus showed the same tendency. In the season when the daytime outdoor air temperature was higher than the photoinhibitory temperature of 25°C, the $k_{\text{rec}}$ values obtained in the laboratory would be lower than $k_{\text{rec}}$ operating in situ (Figs. 3B, 4B). On the other hand, in cool seasons where the outdoor air temperature was lower, $k_{\text{rec}}$ values obtained in the laboratory would be greater than the $k_{\text{rec}}$ in situ (Figs. 3B, 4B). Probably, these facts explain why the $k_{\text{rec}}$ values measured in July were lower than those measured in October or November. Therefore, we corrected $k_{\text{rec}}$ ($K_{\text{rec}}$) of all the plants with a temperature coefficient, $Q_{10} = 3.07$, calculated based on the data in Tsonev and Hikosaka (2003). The corrected rate constant of repair ($K_{\text{rec}}$) plotted against daily PPFD showed a higher determination coefficient than that before correction, and the regression line passed through near the origin (Fig. 6A, B).

$E_Y$ and Y(NO) did not show a marked difference depending on the seasons (Figs. 3F, 1F, 4F, 1F). These tendencies are consistent with the findings of Tsonev and Hikosaka (2003), although in their study the fluorescence parameters according to the puddle model were measured. With the increase in measuring temperature, $E_Y$ decreased and $\Phi_{\text{PSII}}$ and $qP$ increased.

The photosynthetic capacity, which can be assessed by PSII fluorescence parameters, acclimates to the long-term air temperature and is probably unaltered in short-term temperature fluctuations. The decrease in the long-term air temperature suppresses the photosynthetic rate, namely $\Phi_{\text{PSII}}$. However, plants can keep a proportion of open PSII ($qP$ or $qL$) by increasing NPQ. These explanations are relevant irrespective of the puddle and the lake models.

When we measured $k_{\text{pi}}$ and $k_{\text{rec}}$ of the plants in the Jaljale Himal, the temperature was roughly at 10°C (Fig. 7). If $k_{\text{rec}}$ in the plants in the Jaljale Himal had been measured at 25°C and if a $Q_{10}$ of 3.07 for *C. album* grown at 25/20°C was relevant to these alpine plants, the $k_{\text{rec}}$ obtained would be 5.4-fold larger. Actually, the corrected $k_{\text{rec}}$ of the alpine plants ranged from 0.048 to 0.71 min$^{-1}$. With the corrected $k_{\text{rec}}$ of the alpine plants at around 10 mol m$^{-2}$ d$^{-1}$ daily PPFD, *B. milletioides* showed the highest value of 0.45 min$^{-1}$ while in the field plants on the campus, *P. asiatica* at 11.7 mol m$^{-2}$ d$^{-1}$ daily PPFD showed the highest value of only 0.071 min$^{-1}$. With the corrected $k_{\text{rec}}$ of the alpine plants at around 20 mol m$^{-2}$ d$^{-1}$ daily PPFD, *R. acuminatum* showed the highest value of 0.71 min$^{-1}$ while in the field plants on the campus, *P. asiatica* at 18.3 mol m$^{-2}$ d$^{-1}$ daily PPFD showed the highest value of only 0.10 min$^{-1}$. These differences were remarkable. This indicates that plants growing under low temperature would have large repair capacities against photo-inhibition to maintain photosynthesis. However, because the alpine plants in the Jaljale Himal probably adapted and acclimated to low temperatures, the corrections using the $Q_{10}$ values for *C. album* grown at 25/20°C in Tsonev and Hikosaka (2003) should be regarded as crude trials.
Acclimation to light environments

In the field, plants would adjust their NPQ capacity to the growth PPFD levels depending on the duration and frequency of light-flecks (Alter et al. 2012, Kono and Terashima 2014). At low temperatures, NPQ would be further increased. The high NPQ decreases $\Phi_{PSII}$. The decreased electron flow to PSI would suppress production of $\text{H}_2\text{O}_2$. We propose that $k_{\text{NPQ}}$ changes in response to short-term light parameters, such as $Y(\text{NO}) \times \text{PPFD}$. Thus, the effects of acclimation of the capacities for the electron transport rate, NPQ and other dissipation mechanisms on growth irradiance and temperature should be very important, because such capacities are important determinants of $Y(\text{NO})$ or $E_{\text{V}}$. In this study, we propose that $Y(\text{NO}) \times \text{PPFD}$ is strongly related to $k_{\text{NPQ}}$. $Y(\text{NO})$ includes the energy transferred to closed PSI and the energy approaching the open PSI but not used in photochemistry. The former corresponds to the excess energy of the paddle model. At first, effects of these energy fractions must be separately examined. In this regard, neither the paddle model nor the lake model is perfect.

On the other hand, $k_{\text{rec}}$ responds to the growth light environment. $k_{\text{rec}}$ is also very sensitive to temperature. We should study effects of growth temperatures and instantaneous temperatures on the changes in $k_{\text{rec}}$ and in $k_{\text{PI}}$ as well.

Materials and Methods

Plant materials on the University of Tokyo campus

Plants occurring in clusters of $>50$ cm x 50 cm on the campus of the University of Tokyo (35°42'N, 139°45'E, 23 m a.s.l.) were used. Samplings were made from September 7 to November 12 in 2013, and from 16 to 23 July and 20 to 31 October in 2014. Monthly average air temperatures and total precipitations for September, October and November 2013 were 25.2°C and 231.5 mm, 19.8°C and 44.0 mm and 26.0 mm, respectively. Those of July and October 2014 were 26.8°C and 105.5 mm and 19.1°C and 384.5 mm, respectively. Monthly average air temperatures and precipitations in Tokyo were provided by the Japan Meteorological Agency (http://www.data.jma.go.jp/tenki/index.html).

The soil on the university campus was andosol (kurobokude) of volcanic ash origin. The plants used were *Erigeron philadelphicus* L. (Asteraceae), *Fagopyrum dibotrys* H. Harra (Polygonaceae), *Hottotinia cordata* Thunb. (Saururaceae), *Parsicaria chinensis* H. Gross (Polygonaceae), *Plantago asiatica* L. (Plantaginaceae) and *Polygonum longistatum* Brujin (Polygonaceae). The leaves that were most recently expanded were used. All plants have amphistomatous leaves. For the plant species used in the present study, see Supplementary Table S1.

*Cucumis sativus* L. ‘Nanshin’ (Cucurbitaceae), purchased from Takii & Co., were grown in a growth chamber with a 14 h light/10 h dark cycle at an air temperature of 23°C for about 20 d. Light was provided by a bank of white fluorescent lamps (FPR96EX-N/A: Toshiba). PPFDs were adjusted with black screen cloths. PPFDs measured just above these plants were 30, 170 and 500 $\mu$mol m$^{-2}$ s$^{-1}$. Seeds were sown in vermiculite in 200 ml pots and supplied with deionized water. After germination, the plants were supplied with 1/500 strength of a commercial nutrient solution, Hypoxen 6-10-5 (Hypoxen Japan), that contained 6.00% total nitrogen (2.90% ammonia-nitrogen and 1.05% nitrate-nitrogen), 10.0% water-soluble phosphate, 5.0% water-soluble potassium, 0.05% water-soluble magnesium, 0.00% water-soluble manganese and 0.00% water-soluble boron. The first true leaves before unfolding of the second true leaves were used in this study. The first true leaves of *C. sativus* are amphistomatous.

Cucumber plants were also grown outdoors on the campus of the University of Tokyo. The cucumber plants, germinated in vermiculite in the 200 ml pots at 500 $\mu$mol m$^{-2}$ s$^{-1}$ in the growth chamber, were moved outdoors on the campus and supplied with 1/500 strength of Hypoxen 6-10-5 every other day. Samplings were made from 23 April to 21 May, from 8 to 31 July and from 3 to 25 October 2014. Monthly average air temperatures and total precipitations in April and May 2014 were 15.0°C and 155.0 mm and 20.3°C and 135.5 mm, respectively. The first true leaves before unfolding of the second true leaves were used in this study. The first true leaves in April–May and October fully unfolded in 2 weeks but those in July unfolded in 1 week from germination. We used not only these fully expanded leaves but also the 2-week-old first true leaves in July 2014. The second true leaves had unfolded by the time of the sampling of 2-week-old first leaves.

**Plant materials in the Jaljale Himal**

We used some alpine plants in stony alpine tundra areas in or near the Jaljale Himal, eastern Nepal. Leaves of *Bergeria purpurascens* Engl. (amphistomatous leaves, Saxifragaceae) and *Rheum acuminatum* Hook. f. & Thomson ex Hook. (hypostomatous leaves, Polygonaceae) were collected from the plants occurring in clusters $>50$ cm x 50 cm. Leaves of *Bistorta millioides* H. Ohba & S. Akiyama (hypostomatous leaves, Polygonaceae) were collected from an even population along a cliff ridge. Leaves of these plants were sampled from 14 to 20 August 2012 near our campsite in the Jaljale Himal (27°27'N, 87°27'E, 4126 m a.s.l.). The average air temperature in daytime from 06:00 to 18:00 h was 10.9°C, and maximum/minimum temperatures in the daytime were 17.2/7.4°C.

Leaves of *Cremanthodium oblongatum* C. B. Clarke (amphistomatous leaves, Asteraceae), *Cremanthodium pinnatifidum* Benth. (hypostomatous leaves, Asteraceae) and *Cremanthodium reniforme* Benth. (hypostomatous leaves, Asteraceae) were collected from plants in monospecific clusters near the campsite between the Jaljale Himal and Tin Pokhari (27°29'N, 87°27'E, 4310 m a.s.l.), from 22 to 26 August 2012. The average air temperature in daytime from 06:00 to 18:00 h was 7.9°C, and maximum/minimum temperatures in the daytime were 11.5/5.5°C. The most recently fully expanded leaves were used. Leaf nitrogen contents on a leaf area basis of *R. acuminatum* and *C. pinnatifidum* from this area were about 100 mM m$^{-2}$, considerable levels for wild herbaceous plants, and the alpine plants including these two species in Jaljale Himal showed high instantaneous nitrogen use efficiencies in situ (Terashima et al. 1993). For specimens of these Himalayan plants, see Supplementary Table S1.

**Light environments in the fields**

The open sky ratio (OSR) is frequently used in ecological and ecophysiological studies. The OSR at each sampling site was evaluated from a hemispherical photograph taken with a camera and a lens (COOLPIX 4500 and LC-ER1, NIKON) with the software CanoOn 2 (http://www.ajku.ac.jp/etc/canoon2/). For each of the sampling sites on the University of Tokyo campus, the daily PPFD was calculated using software (LIA32, http://www.ag.nagoya-u.ac.jp/~shinkan/LIA32/) and the data of the daily total shortwave radiation (TSR) in Tokyo was provided by the Japan Meteorological Agency.

LIA32 gives maximal PPFD values in 5 min intervals on a given calendar day for the site, where the hemispherical photograph was taken, as well as maximal PPFD values of the imaginary completely open site at the same location. The daily TSR data were obtained for the preceding 14 d before the sampling of the leaves. Photosynthetically active radiation (PAR) is assumed to be 43% of the TSR (Basham 1977). The average photon energy of PAR ($E_{\text{photon}}$) was assumed to be $2.17 \times 10^{-17}$ mol$^{-1}$ (Campbell and Norman 1998). Thus, the daily PPFD at the sampling site was calculated as:

\[
\text{Daily PPFD} = (\text{maximal PPFD at the site} / \text{maximal PPFD at the completely open site at the same location}) \times 0.43 \times \text{daily TSR} / E_{\text{photon}}
\]

Daily PPFD was calculated for each of the preceding 14 d before the sampling and averaged.

The PPFD level at the site where cucumber plants were grown outdoors was monitored with a quantum sensor (LI-1000, LI-COR). The PPFD of the site was measured every 1 min every day from 22 April to 20 May 2014. Daily PPFD was averaged for 14 successive days preceding the sampling day. For the cucumber plants grown in July 2014, the PPFD of the site was measured every
5 min every day from 8 to 30 July 2014. Daily PPFD was averaged for seven successive days preceding the sampling day. For 2-week-old leaves, daily PPFD was averaged for 14 d preceding the sampling day. We also estimated the daily PPFD with the above-mentioned method using the hemispherical photo. The ratio of the measured PPFD to the estimated PPFD was 104.9%, indicating very high accuracy of the estimations using the hemispherical photos.

The daily PPFD in the Himalayas was calculated based on the data obtained in the field sites. We measured PPFDs with the quantum sensor at 09:00, 12:00 and 15:00 h every day from 14 to 20 August at the Jaljale Himal campsite and from 22 to 26 August 2012 at the campsite between the Jaljale Himal and Tin Pokhari. The PPFD of the campsite (PPFD_{LIA_{os}}) was converted to PPFD at the completely open site (PPFD_{LIA_{os}}) using the ratio of the maximum PPFD for the imaginary completely open site at the same geographical location (PPFD_{LIA_{os}}) to maximum PPFD at the campsite (PPFD_{LIA_{采}}) both estimated with LIA32 every 5 min for a given calendar day. Namely,

\[
PPFD_{LIA_{采}} = PPFD_{LIA_{os}} \times (PPFD_{LIA_{os}}/PPFD_{LIA_{采}}).
\]

The PPFD of the sampling site at a given time (PPFD_{sample}) was calculated from the PPFD_{LIA_{采}} PPFD at the imaginary completely open site at the same location of the sampling site (PPFD_{LIA_{采-sample}}) and PPFD at the sampling site (PPFD_{LIA_{采-sample}}) estimated with LIA32 as:

\[
PPFD_{sample} = PPFD_{LIA_{采-sample}} \times (PPFD_{LIA_{采}}/PPFD_{LIA_{采-sample}}).
\]

For PPFD_{sample} values from 06:00 to 09:55, from 10:00 to 14:55 h and from 15:00 to 18:00 h, PPFD_{sample} values that were estimated based on PPFD_{LIA_{采}} values measured at 09:00, 12:00 and 15:00 h, respectively, were used to take account of weather changes on each day. Note that LIA32 ver. 0.3781 does not take account of the time zone. We corrected the time difference of 3 h 15 min between Japan and Nepal.

**Photoinhibition treatments**

The sample leaves were exposed to light provided by white LEDs (NSPW70CS-K1 RAJIN: Nichia) at a PPFD of 400 or 1,200 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) to induce PSII photoinhibition. The leaves were exposed to photoinhibitory light for 30, 45 or 60 min, at both PPFD levels. During the exposure to light, the leaves were kept at air temperature (25°C for the plants on the University of Tokyo campus or ambient air temperature in the Jaljale Himal) using a fan to increase boundary layer conductance and stabilize leaf temperature.

To inhibit the repair process of D1 protein in PSII, we used lincomycin, an inhibitor of chloroplast-encoded protein synthesis by the 70S ribosome. In the experiments conducted in the laboratory, the leaves were fed with \(1\) ml of the 1 mM lincomycin solution g\(^{-1}\) leaf FW via their petioles in the dark at 25°C (Miyata et al. 2012). The feeding of lincomycin took from half a day to a whole day. The leaf boundary layer was minimized with a fan.

**Chl fluorescence parameters**

Before the fluorescence measurements, the leaves were kept in the dark for at least 30 min. Quantum yield of PSII photochemistry, \(\Phi_{PSII}\) or \(\eta(0) = \Phi_{PSII}/(\Phi_{PSII} + \Phi_{non})\), non-photochemical quenching, \(NPQ = \Phi_{non}/\Phi_{PSII} - 1\) (Bilger and Björkman 1990), photosynthetic quenching coefficients, \(q_{P} = (\Phi_{PSII} - \Phi_{non})/(\Phi_{PSII} + \Phi_{non})\) (Schreiber et al. 1994), and the yield of excess energy, \(E_{x} = \Phi(\Phi_{PSII}) - 1 + q_{P}\) (Demmig-Adams et al. 1996, Stefanov and Terashima 2008), were calculated according to the pumple model. The yields of non-photochemical energy dissipation, \(Y(NPQ) = F_{m}E_{x} - F_{m}E_{X}\), non-regulated energy dissipation, \(Y(NO) = F_{m}E_{X}\), and the quenching coefficient, \(q_{L} = q_{P}F_{m}/F_{m}E_{X}\), according to the lake model (Hendrickson et al. 2004, Kramer et al. 2004, Klughammer and Schreiber 2008), were also calculated. \(F_{m}\) was estimated as \(F_{m} = 1/(1/F_{o} - 1/F_{m} + 1/F_{X})\) according to Oxborough and Baker (1997). Among the quantum yields of the lake model, there is a relationship, namely \(Y(B) + Y(NPQ) + Y(NO) = 1\). Also note the relationship of the parameters \(NPQ = Y(NPQ)/Y(NO)\). The actinic light was white LEDs at 400 or 1,200 \(\mu\)mol m\(^{-2}\) s\(^{-1}\). These white LEDs were the same ones that were used for the photoinhibitory treatments. The Chl fluorescence parameters were measured 10 min after the onset of the actinic light with a fluorometer (PAM-2500, Walz). The saturating pulse at PPFD of 6,250 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) was given for 0.8 s to obtain \(F_{m}\). To measure the fluorescence parameters with the minimal effects of photoinhibition, we chose 10 min. The fluorescence level \(\langle F'\rangle\) attained steady state in 10 min at 25°C. For measurements of the Chl fluorescence parameters, the petioles of the plant leaves were kept in deionized water and the laminae were kept in air at 25°C. The leaf boundary layer was minimized with a fan.

**Calculation of the rate constants of photodamage and repair**

The rate constants of photodamage \(k_{p}\) and repair \(k_{rec}\) were calculated from relative \(F_{i}/F_{o}\), according to Miyata et al. (2012). \(F_{r}/F_{o}\), where \(F_{r} = F_{m} - F_{o}\) is the maximum quantum yield of PSII (Kitajima and Butler 1975, Krause and Weis 1991). The saturating pulse was the same as described above, \(F_{r}/F_{o}\), was determined after dark treatment for 30 min. We followed the model that photodamage and repair reactions occur concurrently and are described as first-order reactions (Kok 1956, Tyyystärvi et al. 1992). When the active PSII fraction is expressed as \(a\), a change of active PSII fraction per unit time is expressed as \(da/\text{dt}\).

\[
da/\text{dt} = -k_{p}a + k_{rec}(1 - a),
\]

where \(t\) is illumination time. We irradiated the leaf for 30, 45 or 60 min. When lincomycin is present, \(k_{rec}\) is zero. Then, \(a\) is expressed as:

\[
a = \exp(-k_{p}t).\]

We determined \(k_{p}\) from the time course of the decrease in relative \(F_{r}/F_{o}\), up to 60 min in the presence of lincomycin. Then we determined \(k_{rec}\), with the obtained \(k_{p}\), from the time course of the decrease in relative \(F_{r}/F_{o}\), in the absence of lincomycin. The best-fit curves were obtained by the least squares method.

**Temperature correction of \(k_{rec}\)**

We corrected \(k_{rec}\) values measured at room temperature (25°C) with a temperature coefficient, \(Q_{10}\), to estimate \(k_{rec}\) in situ. The \(Q_{10}\) was calculated from the temperature dependence of the \(k_{rec}\) reported by Tsonev and Hikosaka (2003). We fitted an exponential curve to their data from 11 to 30°C to correct out data, because mean outdoor air temperature during our study ranged from 13.5 to 26.8°C. The curve fitted was:

\[
k_{rec} = 7.58 \times 10^{-4} \times e^{0.1127(11 \leq T \leq 30)}
\]

where \(T\) is the temperature in Celsius. \(Q_{10}\), expressed as:

\[
Q_{10} = (k_{rec2}/k_{rec1})^{(T_{2}-T_{1})/10}
\]

was 3.07. When \(T_{1}\) and \(T_{2}\) are the air temperature during the photoinhibition treatment and the air temperature at the field site, respectively, then \(k_{rec2}\) was the \(k_{rec}\) at the field temperature. We call \(k_{rec}\) as \(K_{rec}\), \(K_{rec}\) is expressed as:

\[
K_{rec} = k_{rec} \times 3.07^{(T_{2}-T_{1})/10}.
\]

**Statistical analysis**

The two-sided Welch’s t-test was used to test the significant difference between the chamber-grown cucumber and the outdoor cucumber leaves or the field plants. Asterisks in the figures indicate significant differences at 5% between the outdoor cucumber leaves or the field plants, and the regression lines for the chamber-grown cucumber leaves.
Supplementary data

Supplementary data are available at PCP online.

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Disclosures

The authors have no conflicts of interest to declare.

References


