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 lightflashes 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. $Y(NO) \times PPFD$] 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; $E_{photov}$, 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); $F_v$ ($F'_v$), variable fluorescence in the fully relaxed state (in the light); $F_s$ ($F'_s$), steady-state fluorescence in the light; $F_{v0}$ ($F'_{v0}$), variable fluorescence in the fully relaxed state (in the light); $F_s - F_o$ ($F'_s - F'_o$); $F_v/F_{v0}$, maximum photochemical efficiency of photosynthesis in PSII of dark-adapted leaves; $k_{pi}$, rate constant of photodamage; $k_{rec}$, rate constant of repair; $K_{rec}$, 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$_{LIAos}$, maximum PPFD at the campsite; PPFD$_{LIAa}$, maximum PPFD for the imaginary completely open site at the same geographical location; PPFD$_{LIAa-sample}$, PPFD at the imaginary completely open site at the same location as the sampling site; PPFD$_{sample}$, PPFD at the completely open site; PPFD$_{sample}$, PPFD of the sampling site; Q$_{10}$, temperature coefficient; $q_L$, quenching coefficient of the lake model; $q_P$, photochemical quenching coefficient of the puddle model; ROS, reactive oxygen species; TSR, total shortwave radiation; $Y(II)$, quantum yield of PSII photochemistry of the lake model; $Y(NO)$, yield of non-photochemical energy dissipation of the lake model; $\Phi_{PSII}$, 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 (Ögren 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).
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Kok (1956) expressed the photodamage and repair of PSII as first-order reactions. Using these equations, the rate constants for the photodamage (k$_\text{pi}$) and repair reaction (k$_\text{rec}$) have been estimated (Kok 1956, Kato et al. 2002a). k$_\text{pi}$ and k$_\text{rec}$ 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$_\text{pi}$ decreased with the increase in growth irradiance in *Cucurbita pepo* L. In contrast, Lee et al. (2001) indicated that k$_\text{rec}$ increased with the increase in growth irradiance in *Capsicum annuum* L. For k$_\text{rec}$, both studies indicated that k$_\text{rec}$ 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$_\text{pi}$ and k$_\text{rec}$ using field plants have never been made. Do plants in the field show k$_\text{pi}$, k$_\text{rec}$ and other photosynthetic parameters different from those in laboratory-grown plants?

Using field-grown plants such as *Vinca minor* L., Demmig-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 (EY), and growth irradiance. qP, NPQ and EY 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 EY 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. ‘Tora’), Miyata et al. (2012) showed that, at medium irradiances, NPQ and EY 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 (Tikkkanen 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$_\text{pi}$, k$_\text{rec}$ and various fluorescence parameters are influenced by temperature (Tsonve and Hikosaka 2003), we compared the data obtained in warm and cool seasons. We also made temperature corrections to estimate k$_\text{rec}$ 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$_\text{pi}$, k$_\text{rec}$ and PSII fluorescence parameters to daily PPFD in cucumber leaves

For brief experimental procedures, see the legend of Fig. 1A (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$_\text{pi}$, k$_\text{rec}$ 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$_\text{pi}$ at 400 and 1,200 μmol m$^{-2}$ s$^{-1}$ decreased with the increase in daily PPFD (Fig. 1A). k$_\text{pi}$ 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$_\text{rec}$ at 400 and 1,200 μmol m$^{-2}$ s$^{-1}$ increased with daily PPFD (Fig. 1B). k$_\text{rec}$ values at 1,200 μmol m$^{-2}$ s$^{-1}$ were smaller than those at 400 μmol m$^{-2}$ s$^{-1}$. k$_\text{pi}$ and k$_\text{rec}$ at 400 and 1,200 μmol m$^{-2}$ s$^{-1}$ in the plants grown outdoors were near the regression lines (Fig. 1A).

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

PSII fluorescence parameters analyzed by the puddle model are NPQ, qP and EY. NPQ 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
Fig. 1 Relationships of the rate constants for photodamage \((k_{pi})\) and repair \((k_{rec})\), and PSII fluorescence parameters to daily PPFD in *Cucumis sativus* leaves. Blue symbols and lines, leaves treated at 400 \(\mu\)mol m\(^{-2}\) s\(^{-1}\); red symbols and lines, leaves treated at 1,200 \(\mu\)mol m\(^{-2}\) s\(^{-1}\). The values of \(k_{pi}\) and \(k_{rec}\) were obtained with the cucumber plants grown outdoors in May, July and October 2014, and those grown in a growth chamber at three different PPFDs. White LEDs were used as photoinhibitory light and the actinic light for fluorescence measurements. For estimation of \(k_{pi}\), the leaves were fed with 1 mM lincomycin for half a day to a whole day in the dark. Photoinhibitory treatments lasted for 30, 45 and 60 min. For estimation of \(k_{rec}\), leaves without lincomycin treatment were used. Before the \(F_{v}/F_{m}\) measurements after the photoinhibitory treatments, the leaves were kept in the dark for 30 min. Other fluorescence parameters were measured in leaves with no lincomycin pre-treatment at 10 min after the onset of the actinic light illumination. During the photoinhibitory treatment, the laminae were kept in air while the petioles were kept in water or 1 mM lincomycin solution. Fans were used to increase the boundary layer conductance. Filled circles, *Cucumis sativus* grown in the growth chamber; open diamonds, open circles and grey circles, *C. sativus* grown outdoors on the campus in May, July and October 2014. ○ and ○ denote 1- and 2-week-old first leaves, respectively, of outdoor cucumber plants measured in July 2014. Regression lines are drawn for the data obtained with cucumber leaves that were grown in continuous light at 35, 170 and 500 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) for 14 h d\(^{-1}\). For regression lines, see Supplementary Table S2. Asterisks indicate significant differences at 5% between the outdoor cucumber leaves and the regression lines for the chamber-grown cucumber leaves. Means ± SD (\(n \geq 5\)) and means ± SD (\(n \geq 3\)) are shown for the leaves from the plants grown in the growth chamber and for those from the plants grown outdoors, respectively.

\(q_{L}\) increased with daily PPFD, and \(q_{P}\) of the outdoor leaves lay near the regression line (Fig. 1E). Values of \(q_{P}\) at 1,200 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) were generally lower than those at 400 \(\mu\)mol m\(^{-2}\) s\(^{-1}\). \(E_{V}\) decreased with daily PPFD. \(E_{V}\) of the outdoor leaves was close to the regression line (Fig. 1F). Absolute values of \(E_{V}\) at 1,200 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) tend to be greater than those at 400 \(\mu\)mol 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 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) were greater than those at 400 \(\mu\)mol 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). \(q_{L}\) of the lake model corresponds to \(q_{P}\) of the puddle model, and the trend of \(q_{L}\) was similar to that of \(q_{P}\) (Fig. 1H). \(Y(NO)\) includes the fraction corresponding to \(E_{Y}\). \(Y(NO)\) at 400 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) did not change with daily PPFD, while \(Y(NO)\) at 1,200 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) decreased with daily PPFD (Fig. 1I). \(Y(NO)\) values of the outdoor leaves at both 400 and 1,200 \(\mu\)mol 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, \(k_{\text{rec}}\) 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 \(k_{\text{rec}}\) 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, \(k_{\text{rec}}\) 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 mol 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). \(k_{\text{rec}}\) 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). \(k_{\text{rec}}\) 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). \(k_{\text{rec}}\) 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

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**Fig. 2** Relationships of the rate constants for photodamage (\(k_p\)) and repair (\(k_{\text{rec}}\)) to OSR or daily PPFD in the field plants in Tokyo. Rate constants of photodamage (\(k_p\)) in the field plants treated at 400 μmol m\(^{-2}\) s\(^{-1}\) (A and C) or 1,200 μmol m\(^{-2}\) s\(^{-1}\) (B and D) and rate constants of repair (\(k_{\text{rec}}\)) in the field plants treated at 400 μmol m\(^{-2}\) s\(^{-1}\) (E and G) or 1,200 μmol m\(^{-2}\) s\(^{-1}\) (F and H) are shown. Filled circles, *Erigeron philadelphicus*; plus signs, *Fagopyrum dibotrys*; filled triangles, *Hottuynia cordata*; filled squares, *Persicaria chinensis*; crosses, *Plantago asiatica*; and filled triangles, *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); blue symbols, October 2014 (19.1 °C); and indigo symbols, November 2013 (13.5 °C). For regression lines, see Supplementary Table S3. Means ± SD (n ≥ 3) are shown.
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). $\Phi_{PSII}$ showed trends similar to those in Fig. 1 (Figs. 3C, 4C). $\Phi_{PSII}$ 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). Eₚ values of the field plants at 400 μmol m⁻² s⁻¹ were mostly above the regression line (Fig. 3F). On the other hand, Eₚ 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(NO) 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(NO) showed trends fairly different from those of Eₚ (Figs. 3I, 4I). Most Y(NO) values were below the regression lines, while Eₚ values of the field plants lay near the regression line. Y(NO) values of P. asiatica at 28.3 mol m⁻² d⁻¹
in July 2014 at both 400 and 1,200 μmol m⁻² s⁻¹ and of *H. cordata* in October 2014 at 400 μmol m⁻² s⁻¹ were, however, near the regression lines (Figs. 3I).

**Relationships of *k*ₚᵢ to daily PPFD, incident PPFD, excess energy and non-regulated energy dissipation during the photoinhibitory treatment**

The *k*ₚᵢ data for the field plants photoinhibited at both 400 and 1,200 μmol m⁻² s⁻¹ were plotted together against the daily PPFD. We also plotted *k*ₚᵢ data against the incident PPFD, excess energy and non-regulated energy dissipation during the photoinhibitory treatments. The excess energy has been defined as \( E_{Y} \times \text{incident PPFD} \times \text{leaf absorbance} \times \text{energy partition ratio to PSII} \) (Kato et al. 2003). \( Y(NO) \) 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 \( E_{Y} \times \text{incident PPFD} \) and \( Y(NO) \times \text{incident PPFD} \).

Strong negative relationships were obtained between the *k*ₚᵢ values of cucumber plants and the daily PPFD, whereas the relationships between the *k*ₚᵢ of the field plants and the daily PPFD were weak (Fig. 5A). When the *k*ₚᵢ values were plotted...
against the incident PPFD and $E_Y \times \text{PPFD}$, not only cucumber plants but also the 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 $K_{0,\text{rec}}$ 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. $K_{0,\text{rec}}$ values of the 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. $K_{0,\text{rec}}$ at 400 and 1,200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was 0.54 vs. 0.70 and 0.52 vs. 0.82, respectively (Fig. 6A, B). The regression line for all the plants of $K_{0,\text{rec}}$ at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ had a steeper slope than that at 1,200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (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 $\mu\text{mol m}^{-2} \text{s}^{-1}$, $k_{\text{pi}}$ and $k_{\text{rec}}$ values of the alpine plants
Fig. 6 Rate constants of repair (krec) and corrected rate constants of repair (Krec) plotted against daily PPFD in cucumber and in the field plants in Tokyo. Rate constants of repair (krec,A) and corrected rate constants of repair (Krec,B) for all the plants including the chamber-grown cucumber and the field plants treated at 400 or 1200 μmol m⁻² s⁻¹. Filled squares and blue dashed and dotted lines, all plants exposed to 400 μmol m⁻² s⁻¹; open squares and red dashed and dotted lines, all plants exposed to 1,200 μmol m⁻² s⁻¹; and black dashed lines, all the plants. For regression lines, see Supplementary Table S5.

showed weak relationships with the OSR (Fig. 7A, C). However, the kpi and krec showed much stronger and statistically significant relationships with daily PPFD. With the increase in daily PPFD, not only kpi but also krec increased (Fig. 7B, D).

For these alpine plants, the measurements were conducted at in situ temperatures of around 10°C. kpi and krec ranged from 0.0059 to 0.013 min⁻¹ and from 0.0089 to 0.14 min⁻¹, respectively. The kpi and krec 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 kpi and krec with temperature in these alpine plants followed the changes reported for Chenopodium album (Tsonev and Hikosaka, 2003), kpi and krec of these alpine plants at 25°C would be about 0.70 and 5.4 times the values measured in situ.

Discussion

Relationships of kpi and krec to daily PPFD

Several studies have shown that the increase in growth irradiance causes the decrease in kpi and the increase in krec (Tyystjärvi 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 kpi 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 photoinactivated 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, kpi decreased and krec increased with the increase in growth irradiance at both photoinhibitory PPFDs (Fig. 1A, B). krec values at 1,200 μmol m⁻² s⁻¹ were lower than those at 400 μmol m⁻² s⁻¹ (Figs. 1B, 6A). This trend is consistent with the previous studies showing that krec has a peak against the photochemical inhibition 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, krec values increased with the OSR or daily PPFD (Figs. 2, 6). The high krec values of P. asiatica in October 2013 by the photo-inhibition treatment at 1,200 μmol 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 ΦPSII in high light.

For the plants in the Jaljale Himal, the relationships between these rate constants, kpi and krec, 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 kpi, krec and PSII fluorescence parameters to daily PPFD

NPQ 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 NPQ values higher than the regression line (Fig. 1D). It has been shown that, in Arabidopsis thaliana,
photoinhibition-treated field plants at 1,200 m
air temperature site (7.9 °C)  (Fig. 5B, C). In
October 2014, qP values of the field plants were not necessarily lower than the regression line, and thereby Eγ levels were lower than the regression line.

kτ values were strongly related to the incident PPFD (Fig. 5B). The strong relationships between the kτ values and incident PPFD have been already pointed out (Tyystjärvi and Aro 1996). However, the regression line of kτ 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 kτ and excess energy (Kato et al. 2003, Miyata et al. 2012). In the present study also, the relationship between kτ and the excess energy was strong. However, the regression lines of kτ 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 (kτ + kτd)/ (kτ + kτd + kNPQ + kτp), where kτ, kτd, kNPQ and kτp 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 PSII 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 (Lažár 1999). Laisk et al. (2012) even claimed that the transfer hardly occurs. Then, the excitation energy delivered to the closed PSII would be most harmful as the excess energy hypothesis claims. However, photoinhibition occurred even under the conditions where PSII was hardly closed (Oguchi et al. 2009). Moreover, the regression lines of kτ 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 PSII but not used in photochemistry could be also harmful. When kτ values of all the plants were plotted against the Y(NO) × PPFD, the determination coefficient was much higher than those for kτ vs. incident PPFD and for kτp vs. the excess energy (Fig. 5B). These indicate that Y(NO) rather than excess energy or incident light should be more directly responsible for photoinhibition.

Fig. 7 Relationships of the rate constants for photodamage (kτ) and repair (kτd) to OSR or daily PPFD in the Jaljale Himal. Rate constants of photodamage (kτ, A and B) and repair (kτd, C and D) were for the photoinhibition-treated field plants at 1,200 μmol m−2 s−1. Open diamonds, Bergenia purpurascens; open squares, Rheum acuminatum; crosses, Bistorta milletioides; open circles, Cremanthodium oblongatum; plus signs, Cremanthodium pinnatifilm; and open triangles, Cremanthodium reniforme. Red hemmed marks, Jaljale Himal, August 2012, higher air pcv107-F7 temperature site (10.9 °C); and blue hemmed marks, Jaljale Himal–Tin Pokhari, August 2012, lower air temperature site (7.9 °C). Regression lines are drawn for the data obtained with all the field plants in the Jaljale Himal. For regression lines, see Supplementary Table 56. Means ± SD (n = 3) are shown.
According to the two-step hypothesis, NPQ affects photo-inhibition through preventing $k_{rec}$ 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 $k_{rec}$ 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 $k_{rec}$. However, when we plotted $k_{rec}$ against NPQ for all the plants, no clear relationships can be seen (not shown). In contrast, $k_{rec}$ showed a strong relationship to the daily PPFD (Fig. 6A). It has been reported that the $k_{rec}$ 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 $k_{rec}$ 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 $k_{rec}$ 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 3P680* (Asada 2006, Vass 2011). If these activities are different among the sample plants, excess energy may not be very strongly related to $k_{pi}$. Indeed, in H. cordata and F. dibotrys, $k_{pi}$ 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, $Y(NO) \times$ 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 $k_{pi}$. This challenges the two-step hypothesis that claims that $k_{pi}$ is proportional to absorbed irradiance and that $k_{pi}$ is not related to NPQ. To test these two hypotheses of the photo-inhibition 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 photo-inhibition mechanisms claimed by the two-step hypothesis. Thus, we used a white LED.

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

The effects of measuring temperature on $k_{pi}$, $k_{rec}$ and the puddle model PSII fluorescence parameters were studied in *C. album* L (Tsonev and Hikosaka 2003). $k_{pi}$ at 10°C was greater than that at 25°C by 1.4-fold, while $k_{rec}$ at 10°C was about 20% of that at 25°C (Tsonev and Hikosaka 2003). Because $k_{pi}$ is greater at low temperatures, PSII is damaged more frequently at low temperatures. However, considerable $k_{rec}$ will recover the damage. $k_{rec}$ 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 $k_{rec}$ values obtained in the laboratory would be lower than $k_{rec}$ operating in situ (Figs. 3B, 4B). On the other hand, in cool seasons where the outdoor air temperature was lower, $k_{rec}$ values obtained in the laboratory would be greater than the $k_{rec}$ in situ (Figs. 3B, 4B). Probably, these facts explain why the $k_{rec}$ values measured in July were lower than those measured in October or November. Therefore, we corrected $k_{rec}$ (K$^{rec}$) 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$^{rec}$) 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, I, 4F, I). 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 $\Phi_{PSII}$ 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 $\Phi_{PSII}$. 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 $k_{pi}$ and $k_{rec}$ of the plants in the Jaljale Himal, the temperature was roughly at 10°C (Fig. 7). If $k_{rec}$ in the plants in the Jaljale Himal had been measured at 25°C and if a Q10 of 3.07 for *C. album* grown at 25/20°C was relevant to these alpine plants, the $k_{rec}$ obtained would be 5.4-fold larger. Actually, the corrected $k_{rec}$ of the alpine plants ranged from 0.048 to 0.71 min$^{-1}$. With the corrected $k_{rec}$ of the alpine plants at around 10 mol m$^{-2}$ d$^{-1}$ daily PPFD, *B. milleltdioides* 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 $k_{rec}$ 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 Q10 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 PSII would suppress production of $H_2O_2$. We propose that $k_{np}$ changes in response to short-term light parameters, such as Y(NO) × 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(NO) or $E_y$. In this study, we propose that Y(NO) × PPFD is strongly related to $k_{np}$. Y(NO) includes the energy transferred to closed PSII and the energy approaching the open PSII 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, $k_{rec}$ responds to the growth light environment. $k_{rec}$ is also very sensitive to temperature. We should study effects of growth temperatures and instantaneous temperatures on the changes in $k_{rec}$ and in $k_{pi}$ as well.

Materials and Methods
Plant materials on the University of Tokyo campus

Plants occurring in clusters of >50 cm × 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 in April and May 2014 were 15.0°C and 155.0 mm and 20.3°C and 353.5 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 *Bergenia purpurascens* Engl. (amphistomatous leaves, Saxifragaceae) and *Rheum acaule* H. & Thomson ex Hook. (hypostomatous leaves, Polygonaceae) were collected from the sampling site between the Jaljale Himal and Tin Pokhari (27°29’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°C, 7.4°C.

Leaves of *Crematogaster oblongatum* C. B. Clarke (amphistomatous leaves, Asteraceae), *Crematogaster pinifolium* Benth. (hypostomatous leaves, Asteraceae) and *Crematogaster reniforme* Benth. (hypostomatous leaves, Asteraceae) were collected from plants in monospecific clusters near the campsite of 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°C, 5.5°C. The most recently fully expanded leaves were used. Leaf nitrogen contents on a leaf area basis of *R. acaule* and *C. pinifolium* from this area were about 100 mm N 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 (COOLPIX4500 and LC-ER1, NIKON) with the software CanoOn 2 (http://takanaka-akio.okio/etc/cano-pon2/). 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 ($E_{photon}$) was assumed to be $2.17 \times 10^{-19}$ mol m$^{-2}$ (Campbell and Norman 1998). Thus, the daily PPFD at the sampling site was calculated as:

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

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<sub>cs</sub>) was converted to PPFD at the completely open site (PPFD<sub>os</sub>) using the ratio of the maximum PPFD for the imaginary completely open site at the same geographical location (PPFD<sub>IAos</sub>) to maximum PPFD at the campsite (PPFD<sub>IAcs</sub>), both estimated with LIA32 every 5 min for a given calendar day. Namely,

\[
\text{PPFD}_{os} = \text{PPFD}_{cs} \times (\text{PPFD}_{IAos}/\text{PPFD}_{IAcs}).
\]

The PPFD of the sampling site at a given time (PPFD<sub>sample</sub>) was calculated from the PPFD<sub>IAos</sub> PPFD at the imaginary completely open site at the same location of the sampling site (PPFD<sub>IAos-sample</sub>) and PPFD at the sampling site (PPFD<sub>IAsamp</sub>) estimated with LIA32 as:

\[
\text{PPFD}_{sample} = \text{PPFD}_{IAsamp} \times (\text{PPFD}_{IAos} / \text{PPFD}_{IAos-sample}).
\]

For PPFD<sub>sample</sub> values from 06:00 to 09:55, from 10:00 to 14:55 h and from 15:00 to 18:00 h, PPFD<sub>os</sub> values that were estimated based on PPFD<sub>os</sub> 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 (NSPW760CS-K1 RAJIN: Nichia) at a PPFD of 400 or 1,200 μmol m<sup>−2</sup> s<sup>−1</sup> 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<sup>−1</sup> 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 leaves were kept in the air in the presence or absence of lincomycin solution during the photoinhibition treatment while the petioles were soaked in the 1 mM lincomycin solution or deionized water during the photoinhibition treatment. The fan was used to minimize the leaf boundary layer and to keep the leaf temperature near the room temperature of 25 °C. The leaves in the Jaljale Himal were fed with 1 ml lincomycin solution via their petioles in the dark and dry air until they absorbed >1 ml of the 1 mM lincomycin solution g<sup>−1</sup> leaf FW. The feeding of lincomycin in the field took 1 or 2 d. The petioles were soaked in 1 mM lincomycin solution or deionized water during the photoinhibition treatment while the laminae were kept in air. The leaf temperature was stabilized to near the ambient temperature and 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 \( Y(0) \) = \( (F_{m} - F_{o})/F_{m} \) (Genty et al. 1989), non-photothermal quenching, \( NPQ = F_{m}/F_{0} - 1 \) (Bliger and Björkman 1990), photothermal quenching coefficients, \( qP = (F_{m} - F)/F_{m} \) (Schreiber et al. 1994), and the yield of excess energy, \( E_{Y} = (F_{m} - F_{0}) \times (1 - qP) \) (Demming-Adams et al. 1996, Stefanov and Terashima 2008), were calculated according to the pumel model. The yields of non-photothermal energy dissipation, \( Y(\text{NPQ}) = F_{m}/F_{0} \), non-photothermal energy dissipation, \( Y(NO) = F_{m}/F_{0} \), and the quenching coefficient, \( qL = qP \times F_{m}/F_{0} \), according to the lake model (Hendrixson et al. 2004, Kramen et al. 2004, Klughammer and Schreiber 2008), were also calculated. \( F_{m} \) was estimated as \( F_{m} = 1/\sqrt{1/(1/F_{o} - 1/F_{m} + 1/F_{o} + F_{m})} \) according to Oxborough and Baker (1997). Among the quantum yields of the lake model, there is a relationship, namely \( Y(0) = Y(\text{NPQ}) + Y(NO) \). Also note the relationship of the parameters \( Y(\text{NPQ})/Y(NO) = 1 \). The actinic light was white LEDs at 400 or 1,200 μmol m<sup>−2</sup> s<sup>−1</sup>. 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 (PAR-2500, Walz). The saturating pulse at PPFD of 6,250 μmol m<sup>−2</sup> s<sup>−1</sup> 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 \( (F') \) 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 \( (k_{r}) \) were calculated from relative \( F_{i}/F_{m} \) reported by Miyata et al. (2012). \( F_{i}/F_{m} \) where \( F_{i}/F_{m} \) is the maximum quantum yield of PSII (Kitajima and Butler 1975, Krause and Weis 1991). The saturating pulse was the same as described above. \( F_{i}/F_{m} \) 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, Tyyystjärvi et al. 1992). When the active PSII fraction is expressed as \( a \), a change of active PSII fraction per unit time is expressed as \( da/dt \), 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

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

We determined \( k_{p} \) from the time course of the decrease in relative \( F_{i}/F_{m} \) 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 \( F_{i}/F_{m} \) 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:

\[
k_{rec} = 7.58 \times 10^{-4} \times \exp^{0.1177} (11 \leq T \leq 30)
\]

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

\[
Q_{10} = \left( \frac{k_{rec}}{k_{rec}} \right)^{10/12}
\]

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 \( k_{rec} \) at the field temperature. We call \( k_{rec} \) as \( K_{rec} \) as \( K_{rec} \) is expressed as

\[
K_{rec} = k_{rec} \times 3.07^{(10/12)}.
\]

**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.

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