Photosynthesis Research (2021) 149:57–68
https://doi.org/10.1007/s11120-020-00780-5

ORIGINAL ARTICLE

Do rapid photosynthetic responses protect maize leaves against photoinhibition under fluctuating light?

Mei-Yu Qiao[1,2] · Ya-Jun Zhang[1,2] · Li-An Liu[1] · Lei Shi[1] · Qing-Hu Ma[1] · Wah Soon Chow[3] · Chuang-Dao Jiang[1]

Received: 24 March 2020 / Accepted: 30 July 2020 / Published online: 11 August 2020

Abstract

Plants in their natural environment are often exposed to fluctuating light because of self-shading and cloud movements. As changing frequency is a key characteristic of fluctuating light, we speculated that rapid light fluctuation may induce rapid photosynthetic responses, which may protect leaves against photoinhibition. To test this hypothesis, maize seedlings were grown under fluctuating light with various frequencies (1, 10, and 100 cycles of fluctuations/10 h), and changes in growth, chlorophyll content, gas exchange, chlorophyll *a* fluorescence, and P700 were analyzed carefully. Our data show that though the growth and light-saturated photosynthetic rate were depressed by rapidly fluctuating light, photosynthesis induction was clearly speeded up. Furthermore, more rapid fluctuation of light strikingly reduced the chlorophyll content, while thermal dissipation was triggered and enhanced. The chlorophyll *a* fluorescence induction kinetics and P700 absorption results showed that the activities of both photosystem II and photosystem I decreased as the frequency of the fluctuating light increased. In all treatments, the light intensities of the fluctuating light were kept constant. Therefore, rapid light fluctuation frequency itself induced the acceleration of photosynthetic induction and the enhancement of photoprotection in maize seedlings, which play important roles in protecting photosynthetic apparatus against fluctuating high light to a certain extent.

Keywords Light environment · Photosynthetic acclimation · Gas exchange · Chlorophyll *a* fluorescence

Abbreviations

DW	Dry weight
FW	Fresh weight
P_n	Net photosynthetic rate
G_s	Stomatal conductance
PPFD	Photosynthetic photon flux density
PS II	Photosystem II
PS I	Photosystem I
F_o	Minimum fluorescence intensity in the dark-adapted state
F_m	Maximum fluorescence intensity in the dark-adapted state
F_v/F_m	Maximum quantum yield of photosystem II
ψ_o	Efficiency at which a trapped exciton can move an electron further than Q_A^- in the electron transport chain
φ_{Eo}	Probability that an absorbed photon will move an electron into the electron transport chain
F_s	Steady-state fluorescence intensity in the light-adapted state
F_m'	Maximum fluorescence intensity in the light-adapted state
F_o'	Minimum fluorescence intensity in the light-adapted state
F_v'/F_m'	Efficiency of excitation captured by open PSII centers in the light-adapted state
q_p	Photochemical quenching coefficient of PSII in the light-adapted state
Φ_{PSII}	Actual efficiency of PSII in the light-adapted state
NPQ	Non-photochemical quenching

Mei-Yu Qiao and Ya-Jun Zhang (Current organization: Shanxi Agricultural University) have contributed equally to this work.

✉ Chuang-Dao Jiang
jcdao@ibcas.ac.cn

[1] Key Laboratory of Plant Resources, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, People's Republic of China

[2] University of Chinese Academy of Sciences, Beijing 100049, China

[3] Division of Plant Sciences, Research School of Biology, The Australian National University, Acton, Canberra ACT 2601, Australia

Introduction

Light is not only a driving force for photosynthesis, but also an important environmental factor affecting plant growth. In the field, plants are often exposed to a fluctuating light environment for periods extending from seconds to hours because of self-shading and cloud movements (Evans 2013; Smith and Berry 2013; Slattery et al. 2018). Though the light intensity and frequency are key characteristics of fluctuating light, more attention has been paid to influences of the transient changes in light intensity on photosynthesis (Külheim et al. 2002; Soleh et al. 2016; Kaiser et al. 2018; Slattery et al. 2018; Yamamoto and Shikanai 2019). Upon the transition of plants from high to weak light, the decay of photosynthesis occurs within seconds (Soleh et al. 2016; Kaiser et al. 2018; Slattery et al. 2018). When high light is regained after a period of weak light, leaf photosynthetic electron transport responds almost instantaneously, while stomatal conductance and activities of enzymes show lagging responses (Lawson et al. 2012; Kaiser et al. 2018). The delay in stomata opening and enzyme activation reduces photosynthetic rate below the steady-state potential (Lawson et al. 2012; Kaiser et al. 2018).

As the rate of photosynthesis is lower under fluctuating light than steady-state conditions, it can result in an increase of absorbed excitation energy that cannot be utilized by photochemistry (Lawson et al. 2012; Shimakawa and Miyake 2018). When plants capture more light than they can utilize in photosynthesis, the excessive excitation energy may induce photoinhibition of photosynthesis (Takahashi et al. 2009; Zhang et al. 2012; Dietz 2015; Kaiser et al. 2015). To avoid photoinhibition, higher plants dissipate excessive excitation energy through non-photochemical quenching (NPQ) that responds quickly to changes in light (Logan et al. 1998; Dietz 2015; Kaiser et al. 2015; Vialet-Chabrand et al.2017). Mutants lacking the photosystem II subunit S protein (PsbS) are specifically defective in NPQ, are more sensitive to high light upon transition of plants from weak to high light, and show decreased fitness under fluctuating light conditions (Külheim et al. 2002). In addition, the reduction in NPQ due to decreasing cyclic electron transfer in *pgr5* mutants exacerbates their sensitivity to the transition of plants from weak to high light, and stunts their growth (Suorsa et al. 2012; Yamamoto and Shikanai 2019).

Beside transient changes in light intensity, the other key characteristic of a fluctuating light environment is the frequency at which the fluctuation occurs. More frequent fluctuating light breaks the continuity of high light exposure and shortens the duration of steady-state photosynthesis, which may further restrict the photosynthetic carbon fixation. More importantly, leaf photosynthetic rate does not rapidly recover to its maximum steady-state level when high light returns after a period of weak light, but increases gradually over several minutes to approach a new steady state (Soleh et al. 2016; Kaiser et al. 2018; Slattery et al. 2018). During this process, a large amount of excessive excitation energy has to be safely dissipated by photoprotective mechanisms as quickly as possible. Otherwise, photoinhibition would occur unavoidably. It seems that rapid photosynthetic responses to transient changes in light intensity may benefit plants in acclimating to a fluctuating light environment. Accordingly, we wondered whether more frequent fluctuating light could induce a more rapid response of photosynthesis in plants, and whether the rapid photosynthetic responses play important role in protecting photosynthetic apparatus against fluctuating high light. To test this hypothesis, the effects of various frequencies of light fluctuation on photosynthetic induction and photoprotective mechanisms were investigated in maize seedlings.

Materials and methods

Plant materials and light treatments

Experiments were carried out between May and June in 2018 and 2019 at the Institute of Botany, Chinese Academy of Sciences. The germinated maize (zhendan 958) seeds were sown in pots (21 cm in diameter, 21 cm in height), and about three quarters of each pot was filled with a mixture of loess and peat (1:1). The seedlings (1 plant per pot) were first cultured for 1-week outdoors, where the light intensity at midday was about 1600 ± 200 μmol photons m^{-2} s^{-1}, and a mean day/night temperature was about 33/23 °C. Then, the 1-week seedlings (4–5 leaf stage) were transferred to a greenhouse with heavy shading (< 2% light transmittance).

In the greenhouse, the light intensity, which was controlled by LED (Philips) using a programmable controller (DELIXI, China), fluctuated between two irradiances, following a square wave function (Fig. 1). The high light was, in each case, 1600 μmol photons m^{-2} s^{-1}; the weak light was 50 μmol photons m^{-2} s^{-1}. Maize seedlings (4–5 leaf stage) were divided into 3 treatments. In the first treatment ($T_{1CF/10\ h}$), seedlings were exposed alternately to high light (1600 μmol photons m^{-2} s^{-1}) for 300 min and then to weak light (50 μmol photons m^{-2} s^{-1}) for 300 min (1 cycle of fluctuation/10 h); in the second treatment ($T_{10CF/10\ h}$), seedlings were subjected alternately to high light and weak light every 30 min (10 cycles of fluctuations/10 h); in the third treatment ($T_{100CF/10\ h}$), seedlings were exposed alternately to high light and weak light every 3 min (100 cycles of fluctuations/10 h). The day

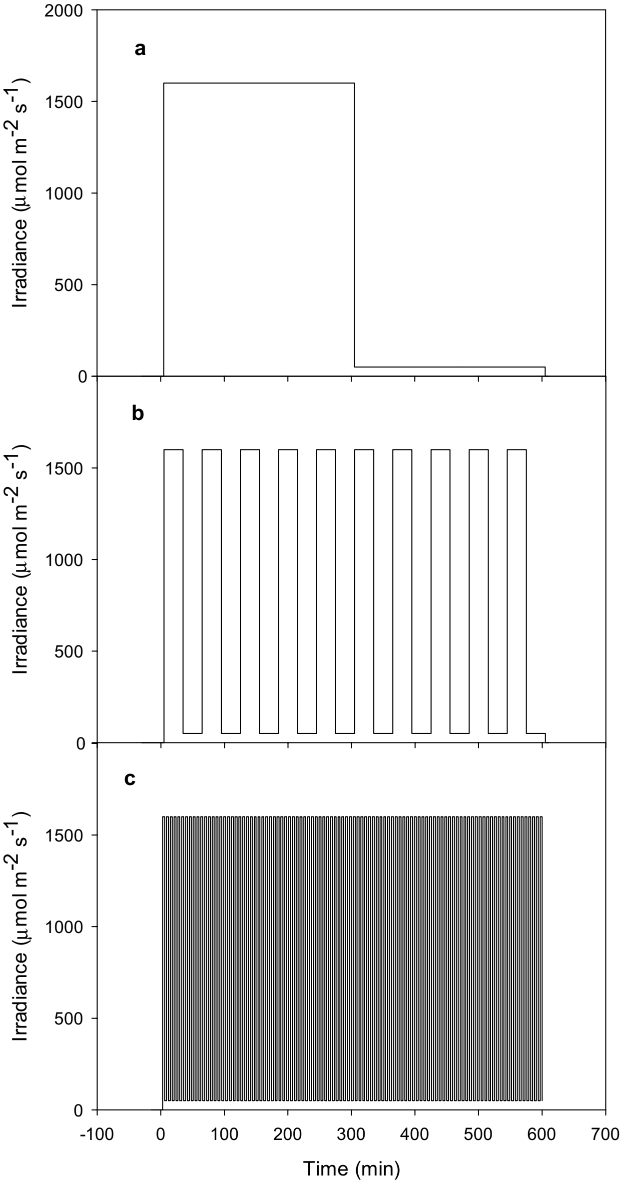

Fig. 1 Fluctuating light regimes. During the 10 h photoperiod, photosynthetic photon flux density was switched between 1600 and 50 µmol photons m^{-2} s^{-1} by a programmable controller. The duration of the high and low light periods was 300 (**a**), 30 (**b**), and 3 (**c**) min with equal duration of the high light and the low light

length in all experiments was 10 h and the temperature was controlled at 32 ± 2 °C during the daytime and 22 ± 2 °C during the night time. The ambient relative humidity was 70%. In the experiments, water and slow-release fertilizer were supplied sufficiently throughout to avoid any potential nutrient and drought stresses. Every treatment had at least ten replicates. Two weeks later, newly fully expanded leaves of maize seedlings (8–9 leaf stage) were used for all measurements.

Measurement of plant growth and chlorophyll content

Whole seedlings were carefully washed out of pots and fresh weigh (FW) was determined first, and then dry weights (DW) was measured after drying in an oven at 80 °C for 48 h. Leaf area was determined using an AM 100 leaf area meter (ADC, Bioscientific Ltd., Hoddesdon, UK).

Twenty leaf disks of 6 mm in diameter were collected from the newly fully expanded leaves. Chlorophylls *a* and *b* were extracted with 80% acetone and quantified using the method of Arnon (1949) and a UV-120 system spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan).

Measurement of gas exchange

With maize seedlings, gas exchange was measured at ambient CO_2 concentration (380–400 µmol mol^{-1}), 70–80% relative humidity and ambient temperature using a CIRAS-2 portable photosynthesis system (PP Systems, USA). Photosynthetic photon flux density (PFD) was fixed every 10 min in a sequence of 2000, 1600, 1200, 800, 600, 400, 300, 200, 150, 100, 50, and 0 µmol photons m^{-2} s^{-1}. Light intensity was controlled by the automatic control device of the CIRAS-2 photosystem. The net photosynthetic rate (P_n) and other parameters were recorded.

Gas exchange was recorded every 5 s during photosynthetic induction under high light (1600 µmol·m^{-2}·s^{-1}). The recording was continued until the photosynthetic rates leveled off as a maximum steady-state photosynthetic rate was reached. Then, the high light was interrupted by weak light (50 µmol photons m^{-2} s^{-1}) for 3 or 30 min. Thereafter, high light (1600 µmol photons m^{-2} s^{-1}) was turned on to induce photosynthesis. In these studies, at least six replicates were measured for each treatment.

Measurement of chlorophyll *a* fluorescence induction kinetics

After finishing light treatments ($T_{1CF/10\ h}$, $T_{10CF/10\ h}$ and $T_{100CF/10\ h}$) as described above, chlorophyll *a* fluorescence induction kinetics was measured by a plant efficiency analyzer (HandyPEA, UK) in dark-adapted (30 min) samples with high red light (3000 µmol photons m^{-2} s^{-1}). Fluorescence transients were recorded during a 1 s light pulse. Each polyphasic rise of fluorescence transient (OJIP curve) was analyzed according to the JIP-test (Strasser et al. 1995). The following original data were retained: maximum fluorescence intensity (F_m), the fluorescence intensity at 20 µs considered as minimum intensity (F_o), the fluorescence intensity at 2 ms (J step) and 30 ms (I step). The variable chlorophyll fluorescence yield (F_v, defined as $F_m - F_o$), the maximum quantum yield of photosystem II ($F_v/F_m = 1 - F_o/F_m$), the efficiency at which a trapped exciton can move an electron further than Q_A^- in the electron transport chain (ψ_o) and the probability that an absorbed photon will move an electron into the electron transport chain (φ_{Eo}) were calculated according to Strasser et al. (1995).

Measurement of chlorophyll *a* fluorescence quenching

Chlorophyll *a* florescence quenching was measured at room temperature (25 °C) using a Pulse-modulated Fluorimeter (FMS, Hansatech, UK) with attached maize leaves. The maximum quantum yield (F_v/F_m) was determined in fully dark-adapted (2 h) leaves. After determining the initial chlorophyll fluorescence yield (F_o) under low modulated measuring light, a 0.7 s pulse of saturating "white light" (> 8000 μmol photons m^{-2} s^{-1}) was applied to obtain the maximum chlorophyll yield (F_m) and F_v/F_m. The fluorescence measurement protocol was as follows: dark-adapted leaves were continuously illuminated by actinic light from the FMS-2 light source. The actinic light intensity was 1600 μmol photons m^{-2} s^{-1}. The steady-state fluorescence levels (F_s) and the maximum chlorophyll yield in the light-adapted state (F_m') during exposure to actinic light were recorded; after the actinic light had been switched off, far-red light was applied for the determination of the F_o'. The efficiency of excitation captured by open PSII centers (F_v'/F_m', defined as $(F_m' - F_o')/F_m'$), photochemical quenching coefficient (q_p, defined as $(F_m' - F_s)/(F_m' - F_o')$), actual PSII efficiency (Φ_{PSII}, defined as $(F_m' - F_s)/F_m'$) and non-photochemical quenching (NPQ, defined as $F_m/F_m' - 1$) were calculated according to Genty et al. (1989) and Maxwell et al. (2000).

Measurement of the redox state of P_{700}

After finishing light treatments ($T_{1CF/10\ h}$, $T_{10CF/10\ h}$ and $T_{100CF/10\ h}$) as described above, the redox state of P700 was determined in vivo in dark-adapted (30 min) maize leaves under growth temperature and ambient O_2 and CO_2 conditions using a PAM-101 modulated fluorometer with a dual-wavelength emitter-detector ED-P700DW unit and PAM-102 units (Klughammer and Schreiber 1994) as described in detail by Schreiber and Klughammer (2008). Far-red light was provided by an FL-101 light source. The redox state of P700 was evaluated as the absorbance change around 820 nm in a custom-designed cuvette.

Statistical analysis

Data were analyzed by one-way ANOVA using SPSS (version 22). The significant differences between the means were determined through least significant difference (Tukey and Nonparametric) test. Unless otherwise indicated, significant differences among different treatments were given at $P < 0.05$. Plots and curves were performed using SigmaPlot 12.5.

Results

Effects of frequent fluctuation of light intensity on growth and chlorophyll content

With increasing frequency of light fluctuation (from 1 to 100 cycles of fluctuations/10 h), the growth of maize seedlings decreased gradually, reflected by plant height and biomass. Compared with $T_{1CF/10\ h}$, the decrease in growth was not statistically significant under $T_{10CF/10\ h}$ treatment (Fig. 2a–c), while $T_{100CF/10\ h}$ induced significant declines in plant height and biomass. Under $T_{100CF/10\ h}$ treatment, the dry weight was decreased by 50% (Fig. 2c). These data indicate that increasing frequency of fluctuating light resulted in a distinct decrease in plant growth. Yet, there was no statistically significant in leaf area of maize seedlings as the frequency of the fluctuating light increased (Fig. 2d).

With more frequent light fluctuation, the chlorophyll *a*, chlorophyll *b* and total chlorophyll contents decreased significantly, while the ratio of chlorophyll *a/b* increased slightly (Fig. 3). Therefore, increasing the frequency of fluctuating light noticeably reduced the chlorophyll content, especially that of chlorophyll *b*.

Effects of frequent fluctuation of light intensity on photosynthetic capacity

As shown in photosynthetic light-response curves (Fig. 4), maize seedlings grown under fluctuating light had a gradual decrease in the light-saturated photosynthesis rate as the frequency of fluctuation increased. Compared with $T_{1CF/10\ h}$, the light-saturated photosynthetic rate (P_{sat}) decreased by 11% and 18% in $T_{10CF/10\ h}$ and $T_{100CF/10\ h}$ treatments, respectively (Fig. 4). These data demonstrate that more frequent light fluctuation limits the photosynthetic capacity of maize seedlings.

Effects of frequent fluctuation of light intensity on photosynthetic induction

In addition to photosynthetic capacity, we also determined photosynthetic induction of maize seedlings grown under various light treatments. After reaching their steady-state photosynthetic rate under high light, maize leaves in all treatments were exposed to weak light for 3-min, and then photosynthesis was re-induced by high light. Photosynthesis induction curves are shown in Fig. 5a. To exactly exhibit

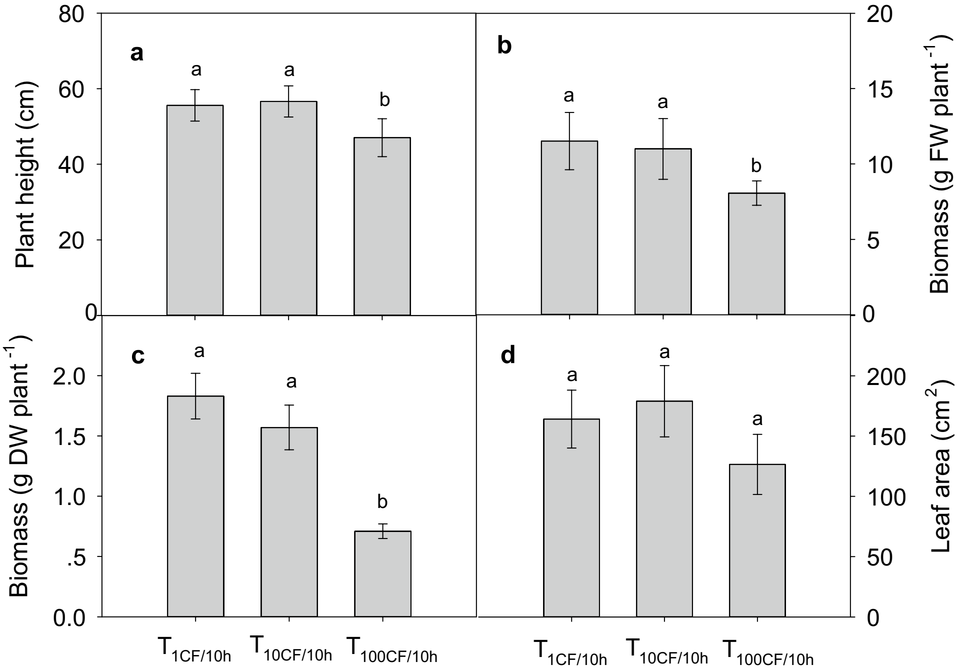

Fig. 2 Effects of the rate of light intensity fluctuation on **a** plant height, **b** fresh weight, **c** dry weight, and **d** leaf area in maize seedlings. $T_{1CF/10\,h}$: Seedlings were exposed alternately to high light (1600 µmol photons $m^{-2}\,s^{-1}$) for 300 min and then to weak light (50 µmol photons $m^{-2}\,s^{-1}$) for 300 min (1 cycle of fluctuation/10 h); $T_{10CF/10\,h}$: Seedlings were exposed alternately to high light and weak light every 30 min (10 cycles of fluctuations/10 h); $T_{100CF/10\,h}$: Seedlings were exposed alternately to high light and weak light every 3 min (100 cycles of fluctuations/10 h). Mean ± SE of six replicates are presented. Different letters denotes statistically significant ($P < 0.05$) differences between treatments

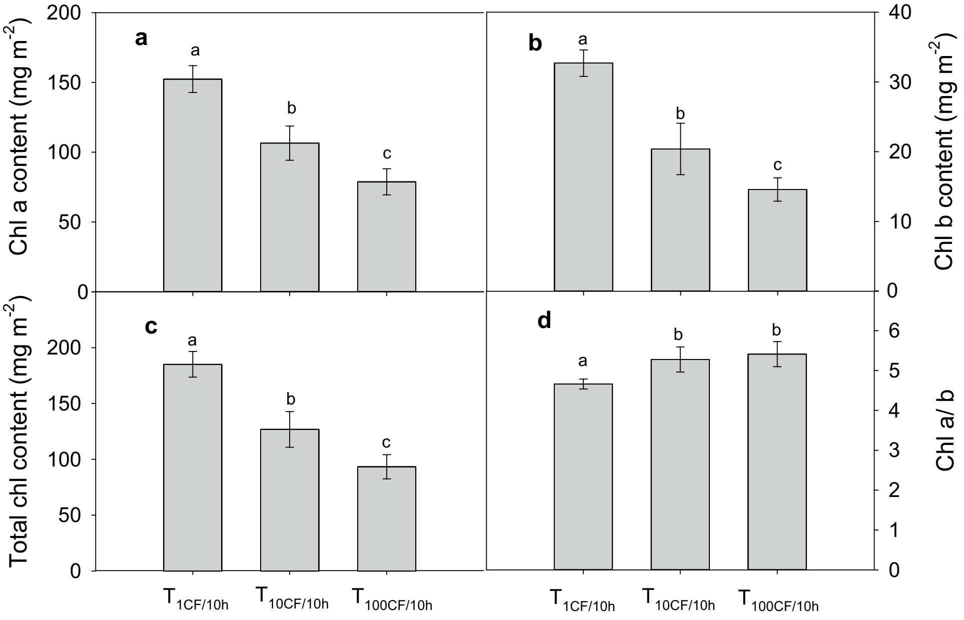

Fig. 3 Effects of the rate of light intensity fluctuation on chlorophyll content in maize seedlings. **a** Chlorophyll *a* content; **b** chlorophyll *b* content; **c** total chlorophyll content; **d** chlorophyll *a/b* ratio. Mean ± SE of 20 replicates are presented. Different letters denotes statistically significant ($P < 0.05$) differences between treatments

the state of photosynthesis, the induction state of photosynthesis was calculated by expressing photosynthetic rate at any given time as a percentage of the steady-state and light-saturated photosynthetic rate (Fig. 5b). As shown in Fig. 5, photosynthetic induction in maize leaves of $T_{1CF/10\,h}$ treatment was the slowest among all treatments after 3-min weak light break; as the frequency of fluctuation increased, the induction of photosynthesis was speeded up visibly. In $T_{10CF/10\,h}$ and $T_{100CF/10\,h}$ treatments, it took about 2 and 1 min to reach the maximum photosynthetic rate, respectively (Fig. 5b). Moreover, the difference in photosynthetic induction times was statistically significant. In addition, our data also show that the induction of photosynthetic rate was more rapid than stomatal conductance after a 3-min weak light break (Fig. 5c): for a given G_s, P_n was greater at a greater frequency of fluctuating light.

To further test the above results, steady-state photosynthesis in all treatments was induced by high light after transferring maize seedlings from 30-min weak light. For all treatments, photosynthesis was re-induced gradually to

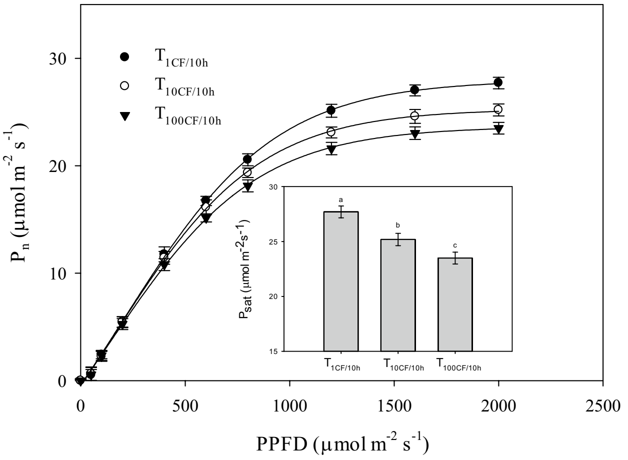

Fig. 4 Effects of the rate of light intensity fluctuation on net photosynthetic light-response curves (P_n-PPFD) in maize seedlings. The inset shows the light-saturated photosynthetic rate (P_{sat}) in various treatments. Mean ± SE of six replicates are presented. Different letters denotes statistically significant ($P < 0.05$) differences between treatments

their maximum values (Fig. 6). In $T_{1CF/10\ h}$ and $T_{10CF/10\ h}$ treatments, it took about 10 and 6 min to reach the maximum values, respectively; while the maximum photosynthetic rate was induced in about 4 min in the $T_{100CF/10\ h}$ treatment (Fig. 6b). Thus, photosynthetic induction time in $T_{10CF/10\ h}$ and $T_{100CF/10\ h}$ was shortened by 40% and 60%, respectively, compared with $T_{1CF/10\ h}$. We noted that the difference in photosynthetic induction times was statistically significant. As shown in Fig. 6c, after a 30-min weak light break, the photosynthetic rate was induced more rapidly than stomatal conductance.

Clearly, all these results demonstrate that more frequent light fluctuation appreciably accelerated photosynthesis induction.

Effects of frequent fluctuation of light intensity on excitation energy distribution

To analyze the excitation energy distribution of PSII, chlorophyll *a* fluorescence quenching was measured in maize seedlings grown under fluctuating light conditions. As shown in Fig. 7, the efficiency of excitation energy capture by open PSII reaction centers (F_v'/F_m') decreased gradually when dark-adapted maize leaves were subjected to high light, while the photochemical quenching coefficient (q_p), actual PSII efficiency (Φ_{PSII}), and non-photochemical quenching (NPQ) increased steadily. After turning off the light, all these parameters recovered rapidly. Cleary, more rapid light fluctuation resulted in a distinct decrease in F_v'/F_m', q_p, and Φ_{PSII}. However, NPQ increased gradually with increasing frequency of light fluctuation. The maximum value of NPQ was induced by the $T_{100CF/10\ h}$ treatment, which was statistically significant compared with $T_{1CF/10\ h}$ and $T_{10CF/10\ h}$ treatments. (Fig. 7). Therefore, more frequent fluctuation of light resulted in a distinct increase in dissipation of excess excitation energy.

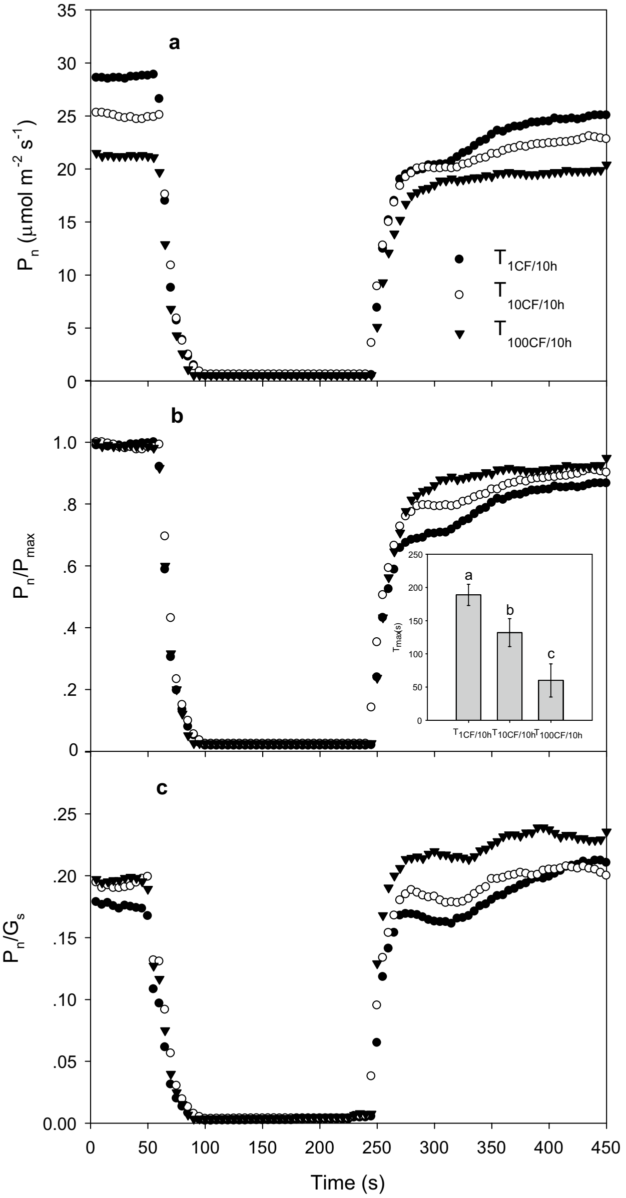

Fig. 5 Effects of the rate of light intensity fluctuation on induction of photosynthesis: **a** photosynthetic rate (P_n); **b** normalized photosynthetic rate (P_n/P_{max}); **c** ratio of P_n to G_s. First, the maximum steady-state photosynthesis was induced by high light. And then, the high light was interrupted by 3-min weak light. Thereafter, the high light was turned on to induce photosynthesis. The inset in **b** shows the time of photosynthetic induction (T_{max}) required to reach the maximum photosynthetic rate in various treatments. Mean ± SE of six replicates are presented. Different letters denotes statistically significant ($P < 0.05$) differences between treatments

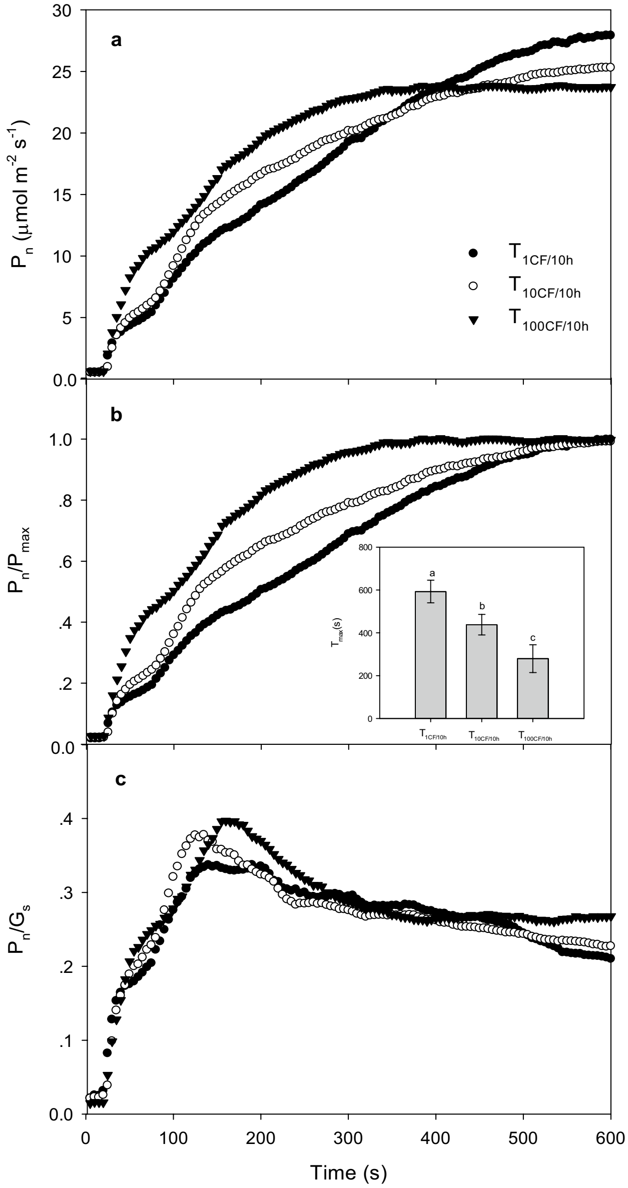

Fig. 6 Effects of the rate of light intensity fluctuation on induction of photosynthesis: **a** photosynthetic rate (P_n); **b** normalized photosynthetic rate (P_n/P_{max}); **c** ratio of P_n to G_s. First, the maximum steady-state photosynthesis was induced by high light. And then, the high light was interrupted by 30-min weak light. Thereafter, the high light was turned on to induce photosynthesis. The inset in **b** shows the time of photosynthetic induction (T_{max}) required to reach the maximum photosynthetic rate in various treatments. Mean ± SE of six replicates are presented. Different letters denote statistically significant ($P < 0.05$) differences between treatments

Effects of frequent fluctuation of light intensity on photosystem II activity

With faster rates of light fluctuation, the shape of chlorophyll *a* fluorescence kinetics curve was noticeably altered (Fig. 8a). To further analyze the differences in fluorescence induction kinetics curves, the maximum photochemical efficiency of the photosystem II (F_v/F_m), the efficiency with which a trapped exciton can move an electron further than Q_A^- in the electron transport chain (ψ_o) and the probability that an absorbed photon will move an electron into the electron transport chain (φ_{Eo}) were calculated. As shown in Fig. 8b, the value of F_v/F_m in $T_{10CF/10\ h}$ and $T_{100CF/10\ h}$ was 2% and 12% lower than that in $T_{1CF/10\ h}$, respectively. Compared to $T_{1CF/10\ h}$, under $T_{10CF/10\ h}$ and $T_{100CF/10\ h}$ treatments, ψ_o decreased by 14% and 38%, and φ_{Eo} decreased by 16% and 45%, respectively. Although there was only a slight difference in F_v/F_m, the values of ψ_o and φ_{Eo} decreased obviously as the light fluctuation rate increased. Therefore, the electron transport activity of PSII was reduced in maize seedlings grown at a high rate of light intensity fluctuation.

Effects of frequent fluctuation of light intensity on photosystem I activity

Changes in P700 absorption were also measured in maize seedlings grown under various light regimes (Fig. 9). As shown in Fig. 9a, the typical absorption curve of P700 first rose slowly until it reached a peak and then fell rapidly. Compared with the $T_{1CF/10\ h}$ treatment, the peak values of absorption curves of P700 decreased in maize seedlings grown under fluctuating light (Fig. 9a). P_m is the maximum oxidation state of P700, which reflects the number of active PSI reaction centers. Figure 9b shows that the value of P_m in $T_{10CF/10\ h}$ and $T_{100CF/10\ h}$ was 17% and 27% lower than that in $T_{1CF/10\ h}$, respectively. Accordingly, increasing the frequency of fluctuating light depressed PSI activity to a certain extent in maize seedlings.

Discussion

Multiple influences of frequency of fluctuating light on carbon assimilation

In this study, more frequent light fluctuation induced a larger decrease in the activity of PSII and PSI (Figs. 8, 9). Therefore, the lowered activities of photosystems may result in the decrease in photosynthetic capacity to a certain extent under fluctuating light conditions. It is likely that this is an important reason why maize seedlings had lower photosynthetic capacity under fluctuating light. Although photosynthetic capacity of maize seedlings was lowered under more frequent light fluctuation conditions (Fig. 4), we also noticed that more frequent light fluctuation accelerated photosynthetic induction (Figs. 5, 6). Clearly, the quick photosynthetic induction may help to reduce the loss in carbon assimilation under fluctuation light conditions. However, in $T_{10CF/10\ h}$ treatment (10 cycles of fluctuations/10 h), it took

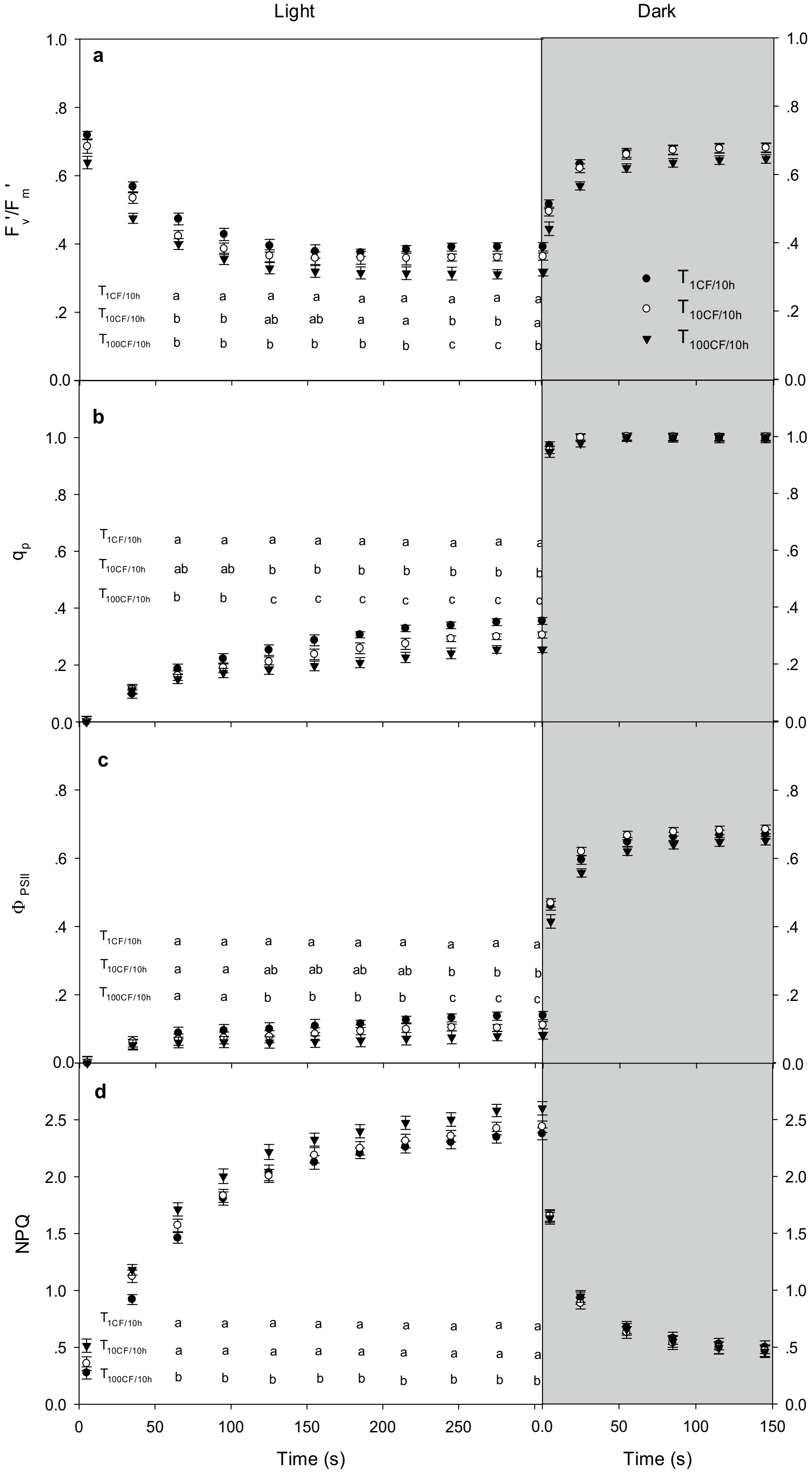

Fig. 7 Effects of the rate of light intensity fluctuation on chlorophyll fluorescence quenching in maize seedlings. **a** Photochemical conversion efficiency of open PSII centers (F_v'/F_m'); **b** photochemical quenching (q_p); **c** actual photosystem II efficiency (Φ_{PSII}); **d** non-photochemical quenching (NPQ). Mean ± SE of six replicates are presented. Different letters denote statistically significant ($P < 0.05$) differences between treatments

6.7 min (400 s) at least for leaf photosynthesis to be fully induced after a 30-min weak light break (Fig. 6). Considering that 10 fluctuations occurred in the $T_{10CF/10\,h}$ treatment, the time duration of steady photosynthesis was reduced by no less than 67 min ($6.7 \times 10 = 67$ min) compared to $T_{1CF/10\,h}$. For the $T_{100CF/10\,h}$ treatment (100 cycles of fluctuations/10 h), full induction of photosynthesis was observed when leaf re-exposed to high light for 1 min (60 s) after a

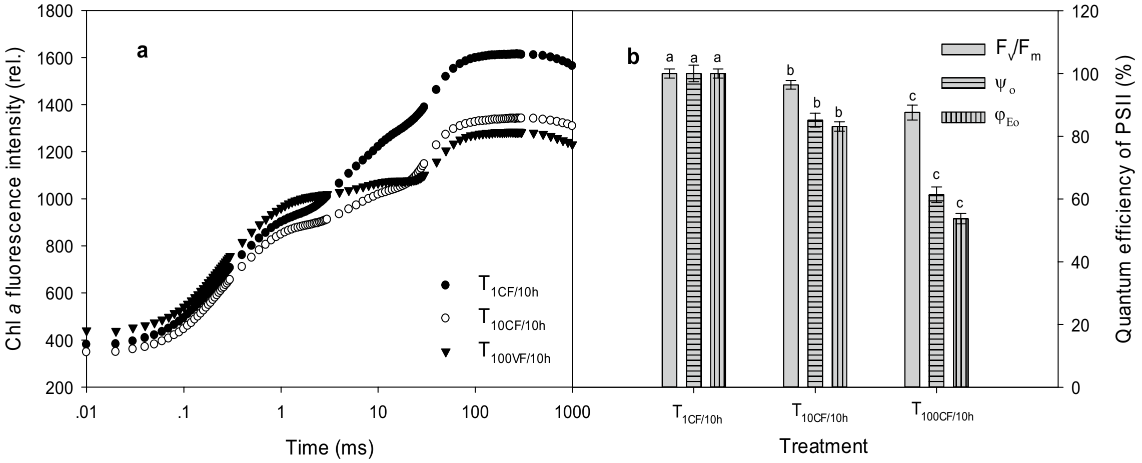

Fig. 8 Effects of the rate of light intensity fluctuation on chlorophyll *a* fluorescence induction kinetics (**a**) and quantum efficiency of PSII (**b**) in maize seedlings. F_V/F_m: the maximum quantum yield of primary photochemistry; ψ_o: the efficiency at which a trapped exciton can move an electron further than Q_A^- in the electron transport chain; φ_{Eo}: the probability that an absorbed photon will move an electron into the electron transport chain. Mean ± SE of 20 replicates are presented. Different letters denote statistically significant ($P < 0.05$) differences between treatments

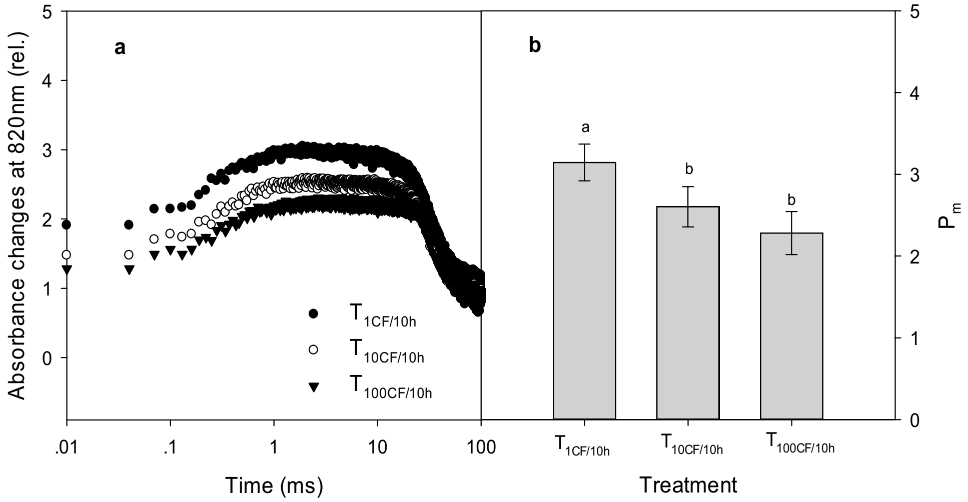

Fig. 9 Effects of the rate of light intensity fluctuation on **a** P700 redox state and **b** P700 maximum oxidation state (P_m) in maize seedlings. Mean ± SE of six replicates are presented. Different letters denote statistically significant ($P < 0.05$) differences between treatments

3-min weak light break. Accordingly, the time duration of steady photosynthesis was diminished by at least 100 min ($1 \times 100 = 100$ min) during the whole high light exposure (Fig. 5). In $T_{10CF/10\ h}$ and $T_{100CF/10\ h}$ treatments, frequent light fluctuations resulted in 22 and 33% decrease in the time duration of steady photosynthesis, respectively. Therefore, the repeated decay and induction of photosynthesis in $T_{10CF/10\ h}$ and $T_{100CF/10\ h}$ treatments significantly reduced the time duration of steady photosynthesis in maize seedlings under fluctuating light conditions, even though the total duration of high light exposure was equivalent to $T_{1CF/10\ h}$. Consequently, the lowered biomass induced by fluctuating light may also result from the clearly reduced duration of steady photosynthesis. Of course, the slight reduction in leaf area may also contribute to the decreased biomass (Fig. 2).

In the field, it is generally believed that the decrease in biomass per plant under close planting may mainly result from the decreased photosynthetic performance (Wu et al. 2019). Actually, close planting often results in a typical fluctuating light environment due to self-shading, which may further reduce the duration of high light, especially at reproductive stage. In this study, the decrease in photosynthetic capacity induced by $T_{100CF/10\ h}$ treatment was less than 18% in maize seedlings, while $T_{100CF/10\ h}$ resulted in 33% decrease in the duration of steady photosynthesis. Probably, the shortened duration of steady photosynthesis may be one of the important causes in the decrease of biomass per plant under close planting conditions.

Accelerated photosynthetic responses depending on frequency of fluctuating light

In this study, photosynthesis induction of maize seedlings grown under $T_{\mathrm{1CF/10\ h}}$ was the slowest among all treatments (Figs. 5, 6). As the light fluctuation became more rapid, photosynthesis induction was speeded up (Figs. 5, 6). This implies that more frequent light fluctuation while still exposing seedlings to the same total duration of high light can induce more rapid induction of photosynthesis. Generally, three phases of photosynthetic induction can be distinguished (Pearcy et al. 1994; Mott and Woodrow 2000; Allen and Pearcy 2000). During the first 1–2 min of suddenly exposing leaves to high light, a fast phase that activates rapidly is associated with limitations in RuBP regeneration. Thereafter, the slow phase of induction is dependent on Rubisco activation, which requires longer illumination at high light. Rubisco limitation is most evident during the period from 1 to 10 min after leaves are exposed to high light. Additionally, stomatal opening is the slowest step in the photosynthetic induction, and reaching full induction can take over an hour. Thus, changes in stomatal conductance also contribute to the slow phase of photosynthetic induction. In this study, after a 3-min weak light break, RuBP regeneration was speeded up by more frequent light fluctuation, reflected by the speeding up of the fast phase during induction of photosynthesis (Fig. 5b). However, our data also show that a more frequent light fluctuation accelerated both the fast and slow phases of the photosynthetic induction after a 30-min weak light break (Fig. 6b), indicating that both RuBP regeneration and Rubisco activation were clearly accelerated under this situation.

It is reported that the decline in stomatal conductance is much slower than carbon fixation when shading occurs under fluctuating light environment (Kaiser et al. 2018). Probably, this is the preliminary condition for more rapid photosynthetic induction. However, in the present study, the induction of photosynthetic rate was more rapid than that of stomatal conductance (Figs. 5c, 6c), demonstrating that more frequent light fluctuation resulted in more distinct stomatal limitation under photosynthetic induction. Moreover, stomatal limitations may occur at any time during induction of photosynthesis. In any case, the quick photosynthetic induction may be one of the important strategies for maize seedlings to reduce the loss in carbon assimilation under fluctuating light conditions.

Enhanced photoprotection depends on the frequency of fluctuating light

Our data show that maize seedlings had a distinct decrease in photosynthetic capacity under fluctuating light conditions (Fig. 4). Moreover, light fluctuation breaks the steady illumination, which in turn results in repeated decay and activation of photosynthesis. During photosynthetic induction, photosynthetic rate was also lower than the maximum value under steady-state conditions (Figs. 5, 6). Therefore, more frequent light fluctuation inevitably induced much more excess excitation energy. We found that more frequent fluctuations resulted in a significant decrease in chlorophyll content and a slight increase in the chlorophyll *a*/*b* ratio (Fig. 3), demonstrating that maize seedlings considerably reduced their light absorption with increasing frequency of fluctuating light.

In the present study, when the light fluctuation was more frequent, thermal dissipation was triggered promptly, thereby avoiding over-reduction of the electron transport chain (Fig. 7d). Thermal dissipation, as one of the important photoprotective mechanisms, can be quickly initiated, and plays a crucial role in helping plants cope with high light (Jiang et al. 2003, 2005; Kaiser et al. 2015; Slattery et al. 2018). More rapid light fluctuation induced an increase in thermal dissipation in the antenna, which could also reduce the light energy capture and excitation energy pressure of PSII (Fig. 7), and thus may reduce the photodamage to the photosynthetic apparatus to a certain extent.

Generally, when the excessive excitation energy is far beyond the plant's photoprotective capacity, damage to the photosynthetic apparatus may occur (Logan et al. 1998; Dietz 2015; Kaiser et al. 2015). In this study, enhancing the frequency of light fluctuation not only resulted in an apparent decrease in the activity of PSII, but also caused a distinct decrease in the activity of PSI (Figs. 8 and 9). We deduced that the lowered q_{p} induced by more frequent light fluctuation, despite increased NPQ (Fig. 7), implied that the over-reduction of the electron transport components in PSII was inevitable, which might induce ROS production. In all treatments, the light intensities of the fluctuating light were kept constant. Therefore, the damage to photosynthetic apparatus under the rapid fluctuating light was dependent on the frequency of fluctuating light in maize seedlings.

It is believed that PSII is stable under fluctuating light conditions, while photosystem I (PSI) is more susceptible (Suorsa et al. 2012; Kono et al. 2014; Liu and Last 2017). In these studies, their plants were grown in constant weak light, and then PSII and PSI activities in leaves were determined under fluctuating high light. However, in our study, maize seedlings grew under light of various fluctuating frequencies, and the activities of both photosystems were measured after their fluctuating light treatments. The rapid light fluctuation accelerated the photosynthetic induction and enhanced photoprotection in maize seedlings (Figs. 5, 6 and 7). The rapid photosynthetic responses to their fluctuating light environment may relieve the photoinhibition of both photosystems. Accordingly, we suggest that the difference in growth conditions may partially explain why there exists a difference between previous studies and our results. Additionally, the photoinhibition of PSI is more dangerous than that of PSII because of the very slow recovery rate of PSI (Kaiser et al. 2015; Zivcak et al. 2015), so photoinhibited PSII might relieve the over-reduce of PSI, thus protect PSI to some certain extent.

Nevertheless, PSI did suffer photoinhibition as indicated by the greater decrease in P_m as the light fluctuation became more rapid (Fig. 9). A working hypothesis to explain our observations is that more rapid light fluctuation meant shorter duration of steady-state photosynthesis and less carbon assimilation which in turn led to increased ROS formation on the acceptor side of PSI, resulting in photodamage of PSI. If the photodamage of PSI occurred largely in bundle sheath chloroplasts, there could be a loss of cyclic photophosphorylation capacity, which would limit the supply of ATP for carboxylation. In turn, this would lead to more ROS formation at PSI, in a positive-feedback manner. The end result is a loss of photosynthetic capacity (Fig. 3).

Conclusions

In maize seedlings, rapid light fluctuation induces the acceleration of photosynthetic induction and the enhancement of photoprotection, which play important roles in protecting the photosynthetic apparatus against fluctuating high light on certain extent.

Acknowledgements This study was supported by the National Natural Science Foundation of China (31571576).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Allen MT, Pearcy RW (2000) Stomatal behavior and photosynthetic performance under dynamic light regimes in a seasonally dry tropical rain forest. Oecologia 122:470–478. https://doi.org/10.1007/s004420050968

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24:1–15. https://doi.org/10.1104/pp.24.1.1

Dietz KJ (2015) Efficient high light acclimation involves rapid processes at multiple mechanistic levels. J Exp Bot 66(9):2401–2414. https://doi.org/10.1093/jxb/eru505

Evans JR (2013) Improving photosynthesis. Plant Physiol 162:1780–1793. https://doi.org/10.1104/pp.113.219006

Genty B, Briantais JM, Baker NR (1989) The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990:87–92. https://doi.org/10.1016/S0304-4165(89)80016-9

Jiang CD, Gao HY, Zou Q (2003) Effects of streptomycin treatment on the chlorophyll fluorescence parameters and the level of the xanthophyll de-epoxidation in maize leaves. Plant Physiol Mol Biol 29(3):221–226. https://doi.org/10.1023/A:1022289509702

Jiang CD, Li PM, Gao HY, Zou Q, Jiang GM, Li LH (2005) Enhanced photoprotection at the early stages of leaf expansion in field-grown soybean plants. Plant Sci 168:911–919. https://doi.org/10.1016/j.plantsci.2004.11.004

Kaiser E, Morales A, Harbinson J, Kromdijk J, Heuvelink E, Marcelis FM (2015) Dynamic photosynthesis in different environmental conditions. J Exp Bot 66(9):2415–2426. https://doi.org/10.1093/jxb/eru406

Kaiser E, Morales A, Harbinson J (2018) Fluctuating light takes crop photosynthesis on a rollercoaster ride. Plant Physiol 176:977–989. https://doi.org/10.1104/pp.17.01250

Klughammer C, Schreiber U (1994) An improved method, using saturating light pulses, for the determination of photosystem I quantum yield via P700+-absorbance changes at 830 nm. Planta 192:261–268. https://doi.org/10.1007/BF00194461

Kono M, Noguchi K, Terashima I (2014) Roles of cyclic electron flow around PSI (CEF-PSI) and O-dependent alternative pathways in regulation of the photosynthetic electron flow in short-term fluctuating light in Arabidopsis thaliana. Plant Cell Physiol 55(5):990–1004. https://doi.org/10.1093/pcp/pcu033

Külheim C, Ågren J, Jansson S (2002) Rapid regulation of light harvesting and plant fitness in the field. Science 297:91–93. https://doi.org/10.1126/science.1072359

Lawson T, Kramer DM, Raines CA (2012) Imroving yield by exploiting mechanisms underlying natural variation of photosynthesis. Curr Opin Biotech 23(2):215–220. https://doi.org/10.1016/j.copbio.2011.12.012

Liu J, Last RL (2017) A chloroplast thylakoid lumen protein is required for proper photosynthetic acclimation of plants under fluctuating light environments. Proc Natl Acad Sci 114:E8110–E8117. https://doi.org/10.1073/pnas.1712206114

Logan BA, Demimig-Adams B, Adams WW, Grace SC (1998) Antioxidants and xanthophyll cycle dependent energy dissipation in *Cucurbita pepo* L. and *Vinca major* L. acclimated to four growth PPFDs in the field. J Exp Bot 49:1869–1879. https://doi.org/10.1093/jxb/49.328.1869

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. J Exp Bot 51:659–668. https://doi.org/10.1093/jxb/51.345.659

Mott KA, Woodrow IE (2000) Modeling the role of *Rubisco activase* in limiting non-steady-state photosynthesis. J Exp Bot 51:399–406. https://doi.org/10.1093/jexbot/51.suppl_1.399

Pearcy RW, Chazdon RL, Gross LJ, Mott KA (1994) Photosynthetic utilization of sunflecks: a temporally patchy resource on a time scale of seconds to minutes. In: Caldwell MM, Pearcy RW (eds) Exploitation of environmental heterogeneity by plants-ecophysiological processes above- and belowground. Academic Press, San Diego, New York, pp 175–208. https://doi.org/10.1016/B978-0-12-155070-7.50011-1

Schreiber U, Klughammer C (2008) New accessory for the DUAL-PAM-100: the P515/535 module and examples of its application. PAM Application Notes, 1–10. https://www.researchgate.net/publication/267549954

Shimakawa G, Miyake C (2018) Changing frequency of fluctuating light reveals the molecular mechanism for P700 oxidation in plant leaves. Plant Direct 1:1–11. https://doi.org/10.1002/pld3.73

Slattery RA, Walker BJ, Weber APM, Ort DR (2018) The impacts of fluctuating light on crop performance. Plant Physiol 176:990–1003. https://doi.org/10.1104/pp.17.01234

Smith WK, Berry ZC (2013) Sunflecks? Tree Physiol 33:233–237. https://doi.org/10.1093/treephys/tpt005

Soleh MA, Tanaka Y, Nomoto Y, Iwahashi Y, Nakashima K, Fukuda Y, Long SP, Shiraiwa T (2016) Factors underlying genotypic differences in the induction of photosynthesis in soybean [*Glycine max* (L) merr]. Plant, Cell Environ 39:685–693. https://doi.org/10.1111/pce.12674

Strasser RJ, Srivastava A, Govindjee (1995) Polyphasic chlorophyll a fluorescence transient in plants and cyanobacterial. Photochem Photobil 61:32–42. https://doi.org/10.1111/j.1751-1097.1995.tb09240.x

Suorsa M, Järvi S, Grieco M, Nurmi M, Pietrzykowska M, Rantala M, Kangasjärvi S, Paakkarinen V, Tikkanen M, Jansson S, Aro EM (2012) PROTON GRADIENT REGULATION5 is essential for proper acclimation of Arabidopsis photosystem I to naturally and artificially fluctuating light conditions. Plant Cell 24:2934–2948. https://doi.org/10.1105/tpc.112.097162

Takahashi S, Milward SE, Fan DY, Chow WS, Badger MR (2009) How does cyclic electron flow alleviates photoinhibition in Arabidopsis? Plant Physiol 149:1560–1567. https://doi.org/10.1104/pp.108.134122

Vialet-Chabrand S, Matthews JS, Simkin AJ, Raines CA, Lawson T (2017) Importance of fluctuations in light on plant photosynthetic acclimation. Plant Physiol 173:2163–2179. https://doi.org/10.1104/pp.16.01767

Wu HY, Zhang YJ, Zhang WF, Wang KR, Li SK, Jiang CD (2019) Photosynthetic characteristics of senescent leaf induced by high planting density of maize at heading stage in the field. Acta Agron Sin 45(2):248–255. https://doi.org/10.3724/SP.J.1006.2019.83042

Yamamoto H, Shikanai T (2019) PGR5-dependent cyclic electron flow protects photosystem I under fluctuating light at donor and acceptor sides. Plant Physiol 179:588–600. https://doi.org/10.1104/pp.18.01343

Zhang Q, Chen YJ, Song LY, Liu N, Sun LL, Peng CL (2012) Utilization of lightflecks by seedlings of five dominant tree species of different subtropical forest successional stages under low-light growth conditions. Tree Physiol 32:545–553. https://doi.org/10.1093/treephys/tps043

Zivcak M, Brestic M, Kunderlikova K, Sytar O, Allakhverdiev SI (2015) Repetitive light pulse-induced photoinhibition of photosystem I severely affects CO_2 assimilation and photoprotection in wheat leaves. Photosynth Res 126:449–463. https://doi.org/10.1007/s11120-015-0121-1