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Far-red light is needed for efficient photochemistry and photosynthesis

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ABSTRACT

The efficiency of monochromatic light to drive photosynthesis drops rapidly at wavelengths longer than 685 nm. The photosynthetic efficiency of these longer wavelengths can be improved by adding shorter wavelength light, a phenomenon known as the Emerson enhancement effect. The reverse effect, the enhancement of photosynthesis under shorter wavelength light by longer wavelengths, however, has not been well studied and is often thought to be insignificant. We quantified the effect of adding far-red light (peak at 735 nm) to red/blue or warm-white light on the photosynthetic efficiency of lettuce (*Lactuca sativa*). Adding far-red light immediately increased quantum yield of photosystem II (Φ_{PSII}) of lettuce by an average of 6.5 and 3.6% under red/blue and warm-white light, respectively. Similar or greater increases in Φ_{PSII} were observed after 20 min of exposure to far-red light. This longer-term effect of far-red light on Φ_{PSII} was accompanied by a reduction in non-photochemical quenching of fluorescence (NPQ), indicating that far-red light reduced the dissipation of absorbed light as heat. The increase in Φ_{PSII} and complementary decrease in NPQ is presumably due to preferential excitation of photosystem I (PSI) by far-red light, which leads to faster re-oxidization of the plastoquinone pool. This facilitates reopening of PSII reaction centers, enabling them to use absorbed photons more efficiently. The increase in Φ_{PSII} by far-red light was associated with an increase in net photosynthesis (P_n). The stimulatory effect of far-red light increased asymptotically with increasing amounts of far-red. Overall, our results show that far-red light can increase the photosynthetic efficiency of shorter wavelength light that over-excites PSII.

1. Introduction

Photosynthesis is dependent on both the quantity and quality of light that reaches the chloroplasts. While the most common measure of photosynthetic radiation, photosynthetic photon flux density (*PPFD*), weighs photons within the 400–700 nm wavelength range equally, photosynthetic responses to light are wavelength-dependent (Emerson and Lewis, 1943; Evans, 1987; Hogewoning et al., 2012; Hoover, 1937; Inada, 1976; McCree, 1972a). The efficiency of photons to drive photosynthesis, measured as the amount of O_2 evolved or CO_2 fixed per mole of absorbed photons, is highest for red photons (roughly 600–680 nm), followed by blue and green photons (Inada, 1976; McCree, 1972a). Yield photon flux (*YPF*) is an alternate measure of photosynthetic radiation which takes into account the spectral dependence of photosynthesis, weighing photons in the 360–760 nm range according to their relative quantum efficiency obtained by McCree (1972a) (Barnes et al., 1993). Photons with different wavelengths are treated as independent and additive in the calculation of *YPF* and *PPFD*.

Far-red light ($\lambda > 700$ nm) has long been considered to make a minimal contribution to photosynthesis, due to its poor absorption by leaves and low quantum yield of photosynthesis (McCree, 1972a). Emerson and Lewis (1943) first described the 'red drop', a sharp decline in quantum yield of O_2 evolution at wavelengths above 685 nm. They observed that the quantum yield at 700 nm was less than half of that at 685 nm (Emerson and Lewis, 1943). Emerson and coworkers subsequently found that the photosynthetic rate under simultaneous illumination of long- ($\lambda > 685$ nm) and short-wavelength lights was greater than the sum of the rates from applying the two lights separately (Emerson et al., 1957; Emerson and Rabinowitch, 1960; Myers, 1971). With the assumption that the quantum yield of photosynthesis at shorter wavelengths is maximum and constant, Emerson and others ascribed this synergistic effect on photosynthesis to the enhancement of quantum

Abbreviations: Φ_{PSII}, quantum yield of PSII; LED, light emitting diode; LHCII, light harvesting complex II; NPQ, non-photochemical fluorescence quenching; PPFD, photosynthetic photon flux density; PSI, photosystem I; PSII, photosystem II; qE, high energy-dependent quenching; YPF, yield photon flux.

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yield of longer wavelength light by shorter wavelengths (Duysens and Amesz, 1962; Emerson et al., 1957; Emerson and Rabinowitch, 1960; Myers and Graham, 1963). This interpretation has been widely accepted by other researchers (Govindjee and Govindjee, 1964; Myers and Graham, 1963). The reverse effect, the enhancement of quantum yield of shorter wavelength light by far-red light, has not received much attention and sometimes is thought not to be present (McCree, 1972b).

It is now known that the low quantum yield of photosynthesis under far-red light is caused by unbalanced excitation of the two photosystems, PSI and PSII, which operate in series to carry out photochemical reactions (Duysens and Amesz, 1962; Hill and Bendall, 1960; Myers, 1971). To achieve optimal efficiency of photochemistry (electron transport from PSII to PSI and ultimately to ferredoxin), the two photosystems should be equally excited by light and operate at matching rates (Duysens and Amesz, 1962; Myers, 1971). Far-red light preferentially excites PSI, while shorter wavelengths (about 400–670 nm) generally excite PSII *Psi*I more than PSI (Evans, 1987; Hogewoning et al., 2012). Since PSI tends to be under-excited relative to PSII under shorter wavelength light, this limits the overall rate of photochemistry and subsequently CO_2 assimilation. When shorter wavelength light is supplemented with far-red light that preferentially excites PSI, the excitation balance between the two photosystems can be restored. This can synergistically increase photochemistry and photosynthesis.

Plants can dynamically adjust their photosynthetic apparatus in response to ambient light conditions to optimize photosynthetic efficiency. When the two photosystems are unequally excited, a mobile pool of light harvesting complex II (LHCII) moves to the under-excited and thus rate-limiting photosystem to rebalance the excitation energy between the two photosystems (Allen, 1992, 2003; Chow et al., 1981). This process is termed state transitions and takes place within minutes (Haldrup et al., 2001). Longer-term adjustment of photosystem stoichiometry can also take place on a time scale of days to correct unbalanced excitation of the two photosystems (Chow et al., 1990; Fujita, 1997; Haldrup et al., 2001).

For an enhancement of photosynthesis to occur when combining two lights, 1) either of the lights alone should provide unequal excitation of the two photosystems, and 2) the two lights should complement each other, *i.e.* one light over-excites PSI and the other light over-excites PSII (Myers, 1971). It is thus expected that neither of the two lights would be optimal for photosynthesis when applied alone, and that the interaction between the two lights would be synergistic: photosynthetic efficiency of both lights would be improved by each other. Therefore, ignoring the synergistic effects among wavelengths (*e.g.* in the calculation of *YPF* and *PPFD*) or exclusion of far-red light (*e.g.* in the integration of *PPFD*) can lead to inaccurate measures of the photosynthetic activity of light.

Chlorophyll fluorescence has long been used to study the light reactions of photosynthesis. Chlorophyll fluorescence decreases in response to increases in the efficiency of photochemistry and/or thermal dissipation of the absorbed light energy (estimated as non-photochemical quenching (NPQ) using chlorophyll fluorescence analyses) (Baker et al., 2007; Maxwell and Johnson, 2000). Enhancement of photosynthesis when combining long- and short-wavelengths is evident from the corresponding, rapid changes in chlorophyll fluorescence: the fluorescence yield under the combination of far-red light and shorter wavelength light is less than additive of that produced by the two lights separately (Govindjee et al., 1960). Fluorescence yield under shorter wavelength light decreases rapidly when far-red light is added (Bonaventura and Myers, 1969; Butler, 1962; Myers, 1971). The rapid decrease in fluorescence yield is thought to be caused by increases in the efficiency of photochemistry (Bonaventura and Myers, 1969; Myers, 1971). Although early work has shown that far-red light affects chlorophyll fluorescence, researchers were unable to use these measurements to quantify the quantum yield of photosystem II (Φ_{PSII}). Genty et al. (1989) were the first to describe how fluorescence measurements can be used to determine Φ_{PSII}. The subsequent development of pulse amplitude modulation fluorometry has made measurements of Φ_{PSII} fast and easy.

Understanding the wavelength-dependence of photosynthesis and the interaction among wavelengths is of particular importance for optimizing photosynthetic lighting provided by electric light sources in controlled environment agriculture. Electric lights, such as light emitting diodes (LEDs), are increasingly used to supplement sunlight in greenhouses or as the sole lighting source for indoor production of high value crops. Production costs, however, can increase substantially due to the high electrical consumption of lighting. One way to improve the photosynthetic lighting efficiency is to provide light with a spectral distribution that most efficiently drives photosynthesis. Currently, the optimization of photosynthetic light spectra is primarily based on the action or quantum yield spectra of photosynthesis developed by McCree (1972a), assuming that different wavelengths affect photosynthesis independently and additively and that far-red has little photosynthetic efficiency. The synergistic effects of different wavelengths are largely overlooked. In this study, we revisited the Emerson enhancement effect, with a focus on the enhancement of photosynthetic efficiency of shorter wavelength light by long-wavelength light. Instead of measuring photosynthetic action or quantum yield of photosynthesis under low light conditions, as was done by McCree (1972a), we focus on the first steps of the light reactions of photosynthesis: the quantum yield of PSII (Φ_{PSII}), which is the fraction of light absorbed by the leaves that is used for photochemical electron transport (Maxwell and Johnson, 2000). We specifically wanted to answer the following questions, which have not been addressed in past research: 1) how does far-red light affect Φ_{PSII} and NPQ (*i.e.* heat dissipation of the absorbed light energy) and does this depend on the intensity of the far-red light?, 2) how does far-red light affect Φ_{PSII} and NPQ when added to different intensities of *PPFD*?, and 3) does the effect of far-red light depend on the spectrum of the *PPFD* (red/blue vs. white light)? Our goal is to provide a better understanding of the interactive effects of light with different wavelengths on Φ_{PSII} and photosynthesis.

2. Materials and methods

2.1. Plant material and growing conditions

Lettuce (*Lactuca sativa* 'Green Towers') plants were grown inside a greenhouse at the University of Georgia (Athens, GA, USA) in 1.7 L, round, plastic containers filled with a soilless substrate (Fafard 2P; 60% peat and 40% perlite; Sun Gro Horticulture, Agawam, MA, USA). Plants were placed on ebb-and-flow benches and sub-irrigated daily with a nutrient solution containing100 mg L^{-1} nitrogen, made with a water-soluble fertilizer (15N-2.2P-12.45 K Cal-Mag; Everris, Marysville, OH, USA). During the growing period (31 October 2015–7 January 2016), the average daily temperature and vapor pressure deficit were 20.3 ± 0.9 °C and 1.0 ± 0.4 kPa. Daily light integral ranged from 0.4 to 9.1 mol m^{-2} d^{-1} with an average of 4.6 mol m^{-2} d^{-1}. No Supplemental lighting was provided. Three groups of lettuce were seeded on the following dates: 30 October, 13 November, and 29 November 2015. Measurements were made on plants that were 31–39 days old.

2.2. LED lights

After plants reached maturity, plants were moved into an enclosed chamber where chlorophyll fluorescence and photosyn-

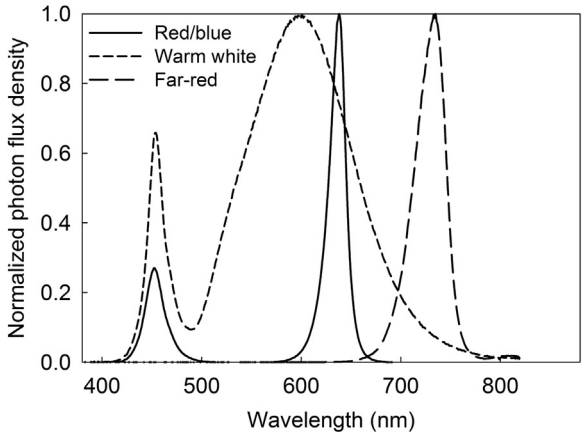

Fig. 1. Normalized spectral distribution of red/blue, warm-white, and far-red light emitting diodes.

thesis measurements were made under different LED lights. Three types of LEDs – red/blue (54 W; PopularGrow, Shenzhen Houyi Lighting, Shenzhen, China), warm-white (Bridgelux, Livermore, CA) and far-red (Epistar, Hsinchu, Taiwan) were used. The spectral distributions of the LEDs were measured using a spectroradiometer (SS-110; Apogee Instruments, Logan, UT, USA) and normalized to their respective peaks (Fig. 1). Around 90% of the photons from red/blue LED light were within the 433–473 nm and 618–658 nm wavelength ranges, centered at 453 nm (blue) and 638 nm (red), respectively. The blue (400–500 nm):green (501–600 nm):red (601–700 nm):far-red (701–800 nm) ratio (B:G:R:FR ratio) of the red/blue LED light was 23.3:0.9:75.8:0 (each component expressed as% of total photons). The warm-white light had a primary peak at 599 nm and a secondary peak at 453 nm, and 4.4% of its total photons were >700 nm. The B:G:R:FR ratio of the warm-white light was 12.1:42.9:40.6:4.2. The far-red LED light (peak wavelength at 735 nm) had 90% of its photons within the 701–769 nm wavelength range, and 8.2% of its total photons were between 601 and 700 nm. Yield photon flux was calculated by the spectroradiometer software.

The warm-white and far-red LEDs were mounted on aluminum heat sinks with cooling fans added on top of the heat sinks. Two DC power supplies (PPS2320A, Circuit Specialists, Tempe, AZ and E3631A, Agilent Technologies, Santa Clara, CA, USA) were used to power the LEDs. The intensity of the LEDs was controlled by adjusting the current output from DC power supplies.

2.3. Treatments and measurements

2.3.1. Dark measurements

Plants were placed in the dark chamber for at least an hour prior to data collection to dark-adapt them. Chlorophyll fluorescence measurements were made on the upper-most fully expanded leaves using a pulse-amplitude modulated fluorometer (Mini-PAM; Heinz Walz, Effeltrich, Germany). Minimal fluorescence level (F_o) in the dark was recorded five times per second by the fluorometer. A saturating light pulse was applied to the dark-adapted leaves to transiently close all the PSII reaction centers, resulting in the maximal fluorescence (F_m) (Maxwell and Johnson, 2000). Net photosynthetic rate (P_n) was measured on the same leaves using a photosynthesis system (CIRAS-2; PP Systems, Amesbury, MA, USA). The CO_2 concentration within the leaf cuvette was maintained at 394 ± 12.5 μmol mol^{-1}. Cuvette temperature was set at 25 °C. Vapor pressure deficit inside the cuvette was 1.7 ± 0.3 kPa.

2.3.2. Far-red light effects on photochemistry and photosynthesis under different intensities of red/blue or warm white light

Seven intensities of red/blue (or warm-white) light intensity, ranging from a *PPFD* of 50–750 μmol m^{-2} s^{-1}, were used to simulate the wide range of light levels that plants were exposed to inside the greenhouse. After the measurements in dark-adapted leaves were taken, red/blue (or warm-white) LEDs were switched on to a *PPFD* of 50 μmol m^{-2} s^{-1}. Similar to the chlorophyll fluorescence measurements made on the dark-adapted leaves, steady-state fluorescence yield (F_t) in the light was recorded five times per second throughout the rest of the data collection period. After plants were given 15–20 min to acclimate to the light level and F_t was steady, a saturating pulse was applied to determine the maximal fluorescence in the light (Fm′). Quantum yield of PSII (Φ_{PSII}) of light-adapted leaves and non-photochemical quenching of chlorophyll fluorescence (NPQ) were calculated as $\Phi_{PSII} = (Fm' - F_t)/Fm'$ and $NPQ = (F_m - Fm')/Fm'$, respectively (Genty et al., 1989; Maxwell and Johnson, 2000). P_n was also measured after plants had acclimated to the given light level. After that, far-red light (110 μmol m^{-2} s^{-1} in the 700–770 nm wavelength range) was added to the red/blue (or warm-white) light, and Φ_{PSII} and NPQ were determined immediately (within 1 min). Plants were then allowed to acclimate for about 20 min under the red/blue (or warm-white) plus far-red light before the stabilized Φ_{PSII}, NPQ, and P_n were determined. Far-red light was then switched off, and the intensity of red/blue (or warm-white) light was increased to the next level. Φ_{PSII}, NPQ, and P_n were again taken under the increased intensity of red/blue (or warm-white) light after plants were allowed to acclimate, and then with the addition of far-red light in a similar manner as described above (Φ_{PSII} and NPQ were determined both immediately and 20 min after the addition of far-red light). Measurements were taken in this fashion until the highest red/blue (or warm-white) light level of 750 μmol m^{-2} s^{-1} was reached. P_n/*PPFD* was calculated as the ratio of steady-state P_n to incident *PPFD* at each of the seven levels of *PPFD* used in this study.

This entire procedure was replicated five times using five different plants under red/blue light, and three times using three different plants under warm-white light. Note that leaf temperature measured inside the gas exchange cuvette increased by 4.3 and 3 °C from dark condition to when 750 μmol m^{-2} s^{-1} of red/blue and warm-white light, respectively, was given.

2.3.3. Do different intensities of far-red light affect photochemistry and photosynthesis under constant red/blue light?

Six levels of far-red light intensity, ranging from 0 to 90 μmol m^{-2} s^{-1} within the 700–770 nm wavelength range, were added to red/blue light with a *PPFD* of 200 μmol m^{-2} s^{-1}. Only red/blue light was used for these studies, because the results from part 1 had indicated that far-red light enhanced photosynthetic efficiency of red/blue light more than that of white light. Data were collected in a similar manner as described above. F_o, F_m, and P_n were first determined in the dark. Red/blue light was then switched on, and F_t was recorded five times per second throughout the data collection. Φ_{PSII}, NPQ, and P_n were determined under red/blue light only. After that, Φ_{PSII} and NPQ were measured immediately after each increase in the intensity of far-red light. Stabilized Φ_{PSII}, NPQ and P_n were determined after plants were given about 20 min to acclimate after each increment of far-red light until the highest intensity of far-red light (*i.e.* 90 μmol m^{-2} s^{-1}) was added. This entire procedure was replicated four times using four different plants. Leaf temperature increased by 1.4 °C when increasing far-red intensity from 0 to 90 μmol m^{-2} s^{-1}.

2.4. Statistical analysis

Data were analyzed using regression (polynomial, multiple linear, and exponential rise to maximum) and ANOVA in Statistical Analysis Systems (SAS Institute, Cary, NC, USA). Mean separation was performed using Fisher's protected least significant difference (LSD, $P = 0.05$).

3. Results and discussion

3.1. Far-red light effects on photochemistry and photosynthesis under different intensities of red/blue or warm white light

3.1.1. Changes in chlorophyll fluorescence

3.1.1.1. Effects of actinic light intensity on chlorophyll fluorescence yield. Chlorophyll fluorescence yield was minimal in the dark (F_o) and rose to a maximum (F_m) when a brief saturating pulse was applied, transiently closing all the PSII reaction centers (Fig. 2A). Upon transitioning from darkness to actinic light (*i.e.* red/blue or warm-white light) with a *PPFD* of 50 μmol m^{-2} s^{-1}, fluorescence yield exhibited a typical Kautsky effect (Butler, 1962; Maxwell and Johnson, 2000), sharply increasing within seconds, and then slowly decaying over a time-scale of minutes back to a steady-state level (F_t), which was higher than F_o (Fig. 2A). The increase in the fluorescence yield under light-adapted steady-state (F_t) compared to that in the dark (F_o) is indicative of less efficient photochemistry. This likely resulted from a partial closure of the PSII reaction centers, not photoinhibitory damage to the reaction centers, under the relatively low levels of light used in this study (Maxwell and Johnson, 2000). A similar rapid increase in fluorescence yield followed by relaxation to a steady-state level that was higher than F_o was observed when increasing the intensity of actinic light to a *PPFD* of 100 μmol m^{-2} s^{-1} (Fig. 2A) or higher (data not shown). Maximal fluorescence under actinic light (Fm′), *e.g.* at *PPFD* of 50 μmol m^{-2} s^{-1}, was consistently lower than that in the dark (F_m), most likely resulting from the light-induced up-regulation of non-photochemical fluorescence quenching (NPQ) in the form of heat dissipation mediated by the xanthophyll cycle (Baker et al., 2007; Demmig-Adams et al., 1996; Maxwell and Johnson, 2000).

3.1.1.2. Far-red light effects on chlorophyll fluorescence. Fluorescence yield decreased immediately when far-red light was added to red/blue (or warm-white) light and reached a minimum within 10–15 s (Fig. 2A and B). A decrease in fluorescence yield can be caused by an increase in the efficiency of photochemistry (*i.e.*, Φ_{PSII}) or NPQ: a greater proportion of the excitation energy/absorbed light is being used for photochemistry or quenched through non-photochemical processes, including xanthophyll cycle-dependent heat dissipation, photoinhibitory damage to PSII reaction centers (not likely to have occurred as discussed above), and state transitions (Baker et al., 2007; Krause and Weis, 1991; Maxwell and Johnson, 2000; Roach and Krieger-Liszkay, 2014; Ruban, 2015). The rapid decrease in fluorescence yield upon adding far-red was most likely attributable to increased Φ_{PSII} rather than an increase in NPQ, as regulation of NPQ, either through the xanthophyll cycle or state transition, is a relatively slow process involving enzymatic reactions and typically requires at least minutes to occur (Roach and Krieger-Liszkay, 2014; Ruban, 2015). Efficiency of photochemistry, on the other hand, can change on a millisecond time scale, driven by changes in the proportion of open reaction centers (Ruban, 2015). Under light conditions that over-excite PSII, the plastoquinone (PQ) pool, which is the intermediate electron transporter between PSII and PSI, gradually becomes reduced as electrons from PSII are being moved faster into the PQ pool than they can leave it (Allen, 2003). Reduction of the PQ pool, especially Q_A – the primary electron

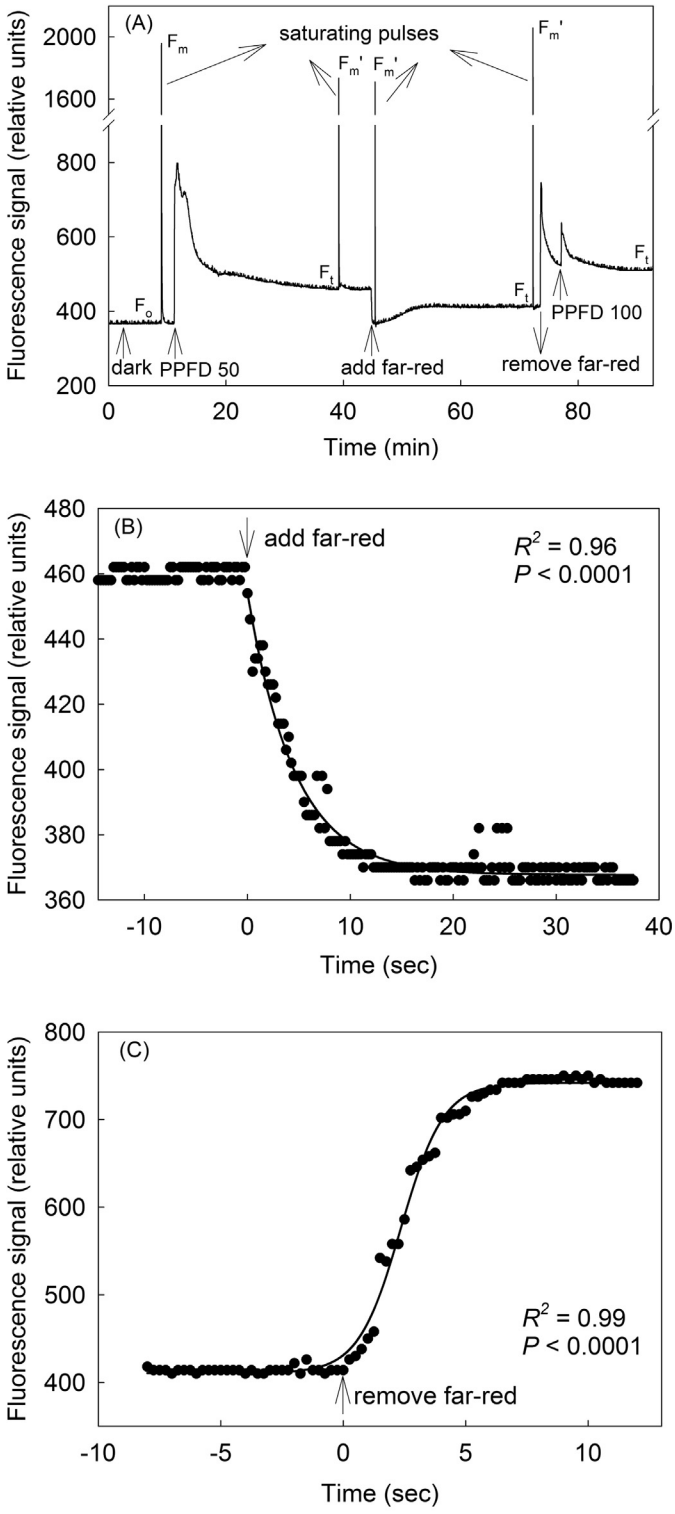

Fig. 2. A typical fluorescence trace of a lettuce leaf under changing light conditions illustrates changes in fluorescence yield upon transitioning of the leaves from dark to different intensities of red/blue light, and when far-red light (110 μmol m^{-2} s^{-1} in the 700–770 nm wavelength range) was added to or removed from red/blue light (A). Saturating light pulses were applied to determine the quantum yield of photosystem II (Φ_{PSII}) and non-photochemical quenching (NPQ). PPFD 50 and PPFD 100 represent switching on or increasing red/blue light intensity to a photosynthetic photon flux density (*PPFD*) of 50 or 100 μmol m^{-2} s^{-1}. F_m and Fm′ are the maximal fluorescence yields in the dark and light, respectively. F_0 is the minimal fluorescence yield in the dark, and F_t is the steady-state fluorescence in the light. The changes in fluorescence yield as affected by the addition (B) or removal (C) of far-red light from the same dataset are shown on a much a shorter time scale.

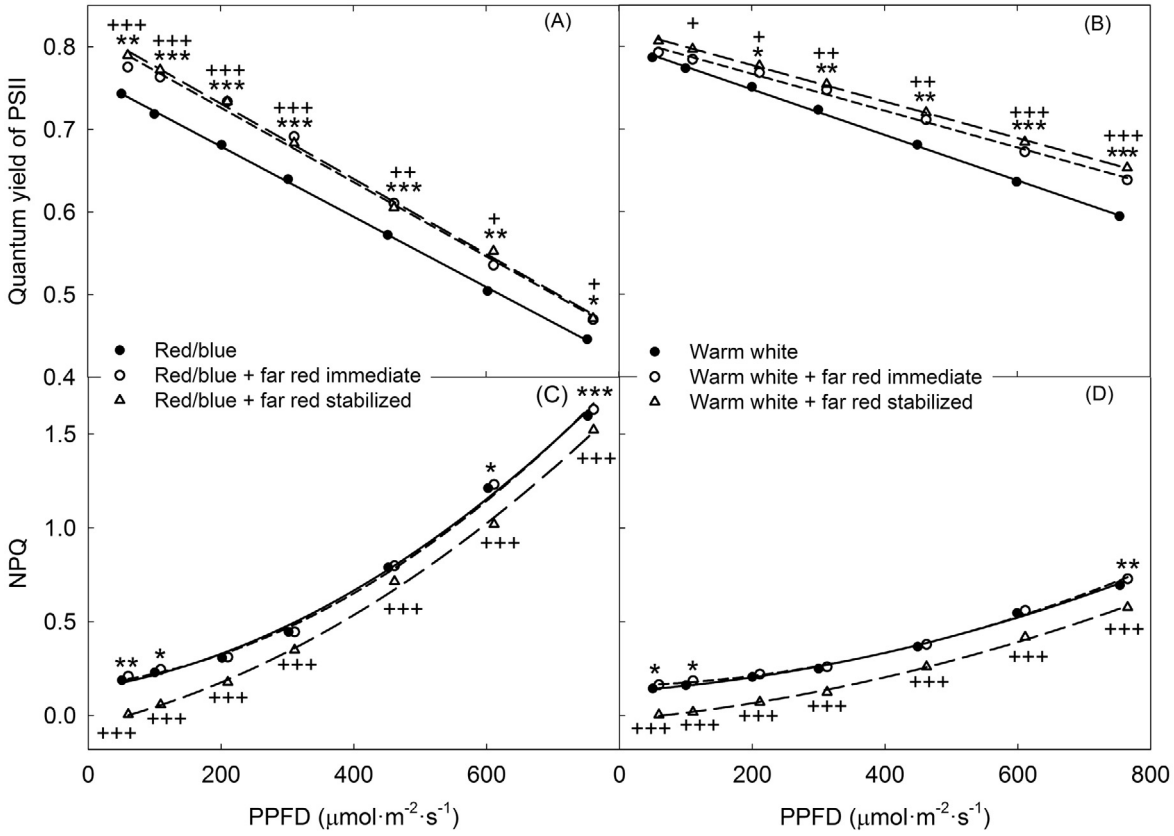

Fig. 3. Quantum yield of photosystem II (Φ_{PSII}) (A, B) and non-photochemical quenching (NPQ) (C, D) of chlorophyll fluorescence as affected by adding far-red light to different intensities of red/blue (A, C) or warm-white light (B, D). Data points in A and C (red/blue) represent means from 5 replicates; data points in B and D (warm-white) represent means from 3 replicates. Within each *PPFD* level, ***, **, and * (or $^{+++}$, $^{++}$, and $^{+}$) indicate significant differences between light without added far-red and the immediate (or stabilized) response to the addition of far-red light at $P < 0.001$, $P < 0.001$, and $P < 0.05$, respectively.

acceptor of PSII, prevents transfer of electrons away from PSII. Thus the PSII reaction centers become 'closed', *i.e.* incapable of using light for photochemistry (Maxwell and Johnson, 2000). Far-red light can increase the proportion of open PSII reaction centers through preferential excitation of PSI (Evans, 1987; Hogewoning et al., 2012), which leads to faster re-oxidation of plastoquinones (Bonaventura and Myers, 1969; Ruban, 2016). Re-oxidized plastoquinones can accept electrons from excited PSII reaction center chlorophylls and thus help to re-open PSII reaction centers more quickly (Bonaventura and Myers, 1969; Baker, 2008; Maxwell and Johnson, 2000), resulting in increased Φ_{PSII} (see Fig. 3A and B) and a consequent drop in fluorescence yield. The observed decrease in fluorescence yield and increase in Φ_{PSII} when adding far-red light to red/blue (Fig. 3A) or warm-white (Fig. 3B) LED light suggests that excitation energy is unequally distributed between the two photosystems under red/blue or warm-white light, with PSI being under-excited and thus limiting the overall rate and efficiency of photosynthesis.

After the rapid drop in fluorescence yield upon adding far-red light, fluorescence yield gradually increased over a time period of 6–8 min, until a steady state value was reached (Fig. 2A). Bonaventura and Myers (1969) similarly observed a gradual increase in fluorescence yield following an initial rapid drop when adding far-red light (710 nm) to shorter wavelength light (645 nm). They ascribed the slow increase in fluorescence yield to state transitions. We postulate that under red/blue or warm-white LED light, a mobile pool of LHCII moved from PSII to PSI to redistribute more light energy to PSI. The detachment of LHCII from PSII decreases the antenna absorption cross section of PSII and excitation energy transfer to PSII (Allen and Mullineaux, 2004), therefore possibly contributing to the slow decay of PSII fluorescence observed after switching on actinic light (Fig. 2A). As far-red light preferentially excites PSI, the addition of far-red can restore the balance of excitation between the two photosystems, or may even cause PSI to be overexcited relative to PSII when a large amount of far-red is added. It is likely that LHCII gradually migrated back to PSII from PSI after far-red light was added to red/blue or warm-white light, thus increasing the PSII antenna absorption cross section and the amount of light received by PSII, which in turn increased PSII chlorophyll fluorescence over a 6–8 min period following the addition of far-red light (Fig. 2A). F_t in the presence of far-red light was greater than F_o, but lower than F_t under red/blue (or warm-white) light only (Fig. 2A). The decrease in steady-state fluorescence yield following the addition of far-red light indicates less efficient chlorophyll fluorescence, thus more energy was partitioned to photochemistry or NPQ processes (Maxwell and Johnson, 2000). However, the stabilized Fm′ was higher (thus NPQ was reduced) after the addition of far-red light (see Fig. 3C and D).

In contrast to the changes in fluorescence yield induced by adding far-red light, removal of far-red light from the actinic light caused a transient increase in fluorescence yield, which reached a maximum after 5–6 s and then slowly relaxed over a timescale of minutes (Fig. 2A and C). This transient increase in fluorescence yield after removal of far-red indicates that the efficiency of photochemistry was decreased, presumably due to the reduction of the PQ pool as a consequence of PSI operating slower than PSII under red/blue or warm-white light. The reduction of the PQ pool in turn leads to closure of PSII reaction centers and less efficient photochemistry (Maxwell and Johnson, 2000). The magnitude of this transient fluorescence rise upon removal of far-red light was much greater

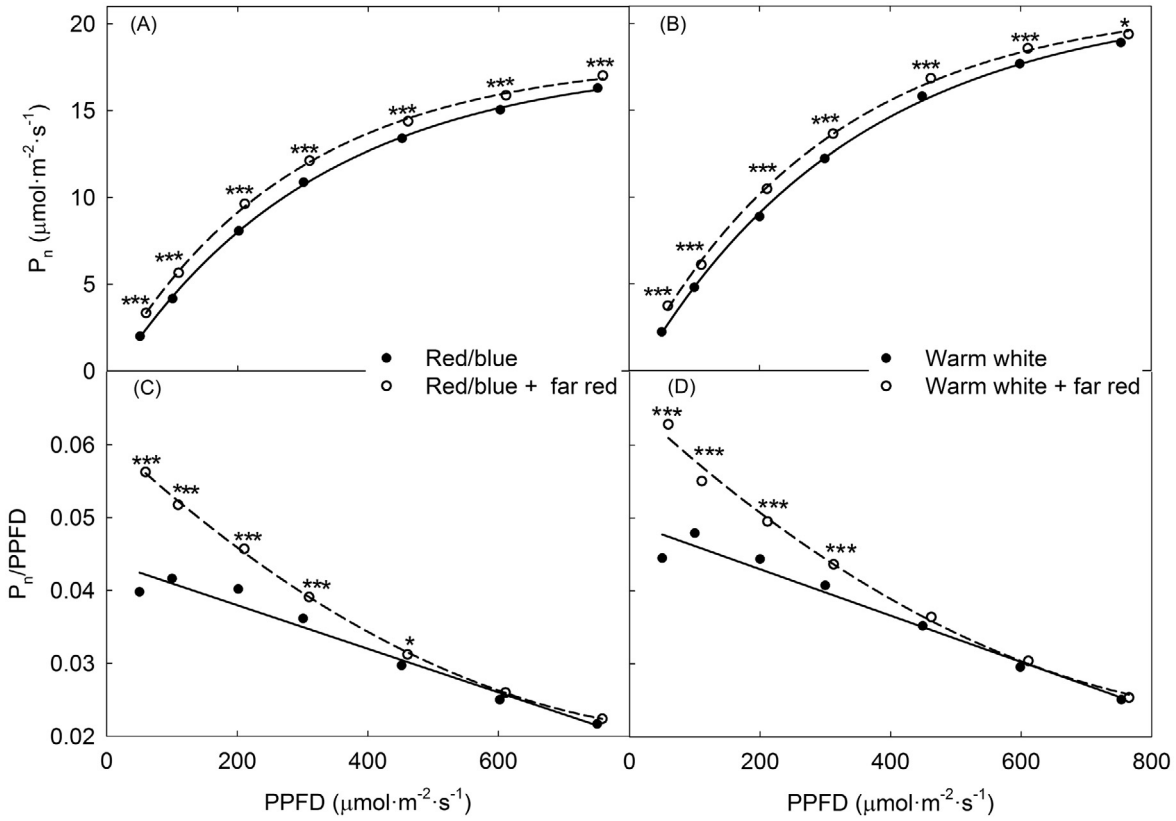

Fig. 4. The effect of adding far-red light to different intensities of red/blue or warm-white light on net photosynthetic rate (Pn) (A, B) and Pn/*PPFD* (C, D). Data points in A and C (red/blue) represent means from 5 replicates; data points in B and D (warm-white) represent means from 3 replicates. Within each *PPFD* level, *** and * indicate significance at $P < 0.001$ and $P < 0.05$.

than that of the transient drop in fluorescence when adding far-red light (Fig. 1A). This differential change in fluorescence following the addition and removal of far-red light is thought to be indicative of the occurrence of state transitions (Haldrup et al., 2001; Lunde et al., 2000). The gradual relaxation of fluorescence after the initial rapid rise upon removal of far-red (Fig. 2A) is likely caused by the movement of LHCII back to PSI, redirecting more excitation energy to PSI.

3.1.2. Quantum yield of photosystem II (Φ_{PSII})

The quantum yield of photosystem II (Φ_{PSII}) decreased with increasing red/blue (Fig. 3A) or warm-white (Fig. 3B) light intensity. As more light reaches the leaves, an increasing proportion of PSII reaction centers become closed (Baker, 2008), which in turn leads to decreased Φ_{PSII}. Φ_{PSII} increased within 10–15 s after adding far-red light, with a similar increase (6.5% on average) at all intensities of red/blue light (Fig. 3A). Under warm-white light, however, this immediate increase in Φ_{PSII} was 1% at the lowest PPFD and up to 8% at the highest *PPFD*, with an average increase of 3.6% (Fig. 3B). Similar or slightly greater increases in Φ_{PSII} were observed 20 min after adding far-red light (Fig. 3A and B). Two important differences in Φ_{PSII} under red/blue vs warm-white light were evident: 1) at the same *PPFD*, Φ_{PSII} under warm-white light was consistently higher than that under red/blue light and 2) the percent increase in Φ_{PSII} as induced by far-red light tended to be smaller under warm-white light compared to that under red/blue light, especially at low *PPFD* (Fig. 3A and B). One possible explanation for these observations is that while the red/blue light contains no far-red light, warm-white LEDs contain a small proportion of far-red light (Fig. 1), perhaps allowing for more efficient excitation of PSI. However, even the warm-white light does not contain sufficient amount of far-red to ensure that both photosystems operate at matching rates, as is clear for the increase in Φ_{PSII} when far-red light is added.

In addition, plants grown under red/blue LED light have been shown to use red/blue light more efficiently (*i.e.* have higher Φ_{PSII}) than plants grown under sunlight (unpublished data). This is likely due to longer-term adjustments of photosystem stoichiometry in response to ambient light conditions to optimize photosynthetic efficiency (Chow et al., 1990; Fujita, 1997).

3.1.3. Non-photochemical quenching (NPQ)

A corresponding up-regulation of NPQ was observed alongside the decreasing Φ_{PSII} in response to increasing *PPFD* (Fig. 3C and D). An increase in NPQ is typically observed with increasing *PPFD* (Demmig-Adams et al., 1996; Logan et al., 1998). Under high *PPFD*, accumulation of H^+ in the thylakoid lumen often occurs due to high rates of electron transport (Baker et al., 2007). A low lumen pH triggers the high energy-dependent quenching (qE) of the excess absorbed light as heat, which is a major component of NPQ, through activation of the xanthophyll cycle and protonation of the PsbS protein (Demmig-Adams and Adams, 2006; Ruban 2015, 2016). This process is thought to serve a protective role against photodamage to the photosynthetic apparatus (Demmig-Adams and Adams, 1992, 2006; Ruban, 2015).

Non-photochemical quenching measured immediately after adding far-red light showed a statistically significant increase at several *PPFD* levels (Fig. 3C and D), resulting from a negligibly small, but consistent, decrease in the Fm′ measured right after adding far-red light (Fig. 2A). However, this change was too small to be biologically meaningful. The curves describing the relationship between *PPFD* and NPQ were essentially identical before and immediately after adding far-red light.

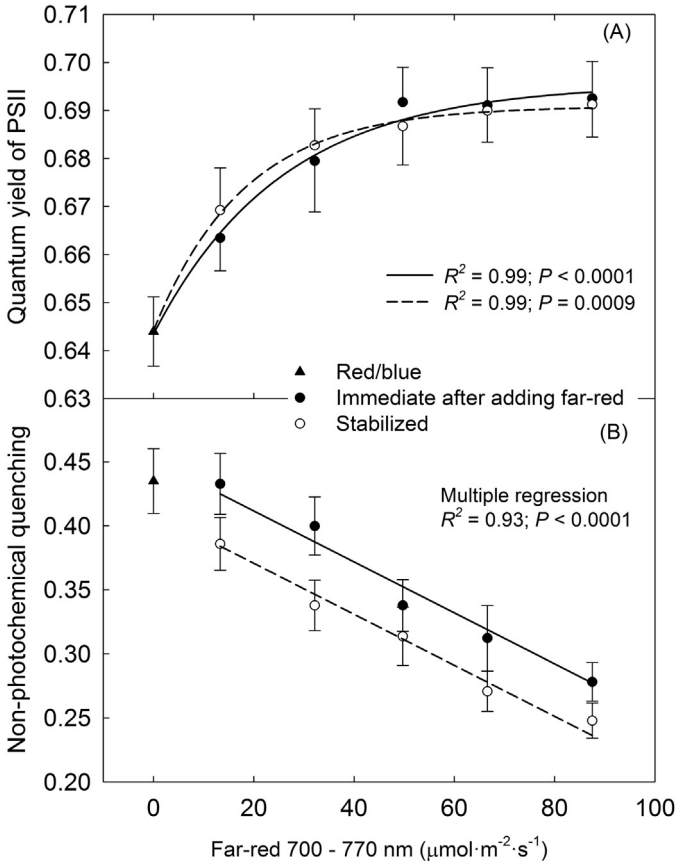

Fig. 5. Quantum yield of photosystem II (Φ_{PSII}) (A) and non-photochemical quenching (NPQ) (B) of chlorophyll fluorescence as a function of the intensities of far-red light added to red/blue light (*PPFD* of 200 μmol m^{-2} s^{-1}). Data points represent means from 4 replicates with error bars representing standard error.

Stabilized NPQ, measured after ~20 min of exposure to far-red light, on the other hand, was significantly lower at all *PPFD* levels (Fig. 3C and D). This relatively slow decrease in NPQ in response to the addition of far-red light may be caused by state transitions: the mobile pool of LHCII moved back to PSII on the order of minutes after adding far-red light, resulting in higher Fm′ (consequently lower NPQ; Fig. 2A) as a bigger PSII antenna captured more light energy, which in turn can increase fluorescence.

The increase in NPQ with increasing *PPFD* was slower with white light than with red/blue light. This is consistent with the higher Φ_{PSII} under warm-white light described above: a more efficient use of photons in photochemistry results in less need to dissipate excess excitation energy through NPQ.

3.1.4. Net photosynthetic rate (P_n)

Net photosynthetic rate (P_n) gradually increased with increasing red/blue or warm-white light, as more light was available to drive the light reactions of photosynthesis (Fig. 4A and B). At equal *PPFD* levels, white light resulted in higher P_n than red/blue light, consistent with our findings for Φ_{PSII}. However, P_n/*PPFD* at different incident light levels, indicative of the efficiency at which plant uses the incident light for photosynthesis, decreased with increasing *PPFD* (Fig. 4C and D). This response was expected as Φ_{PSII} decreased in response to increasing *PPFD* (Fig. 3), meaning that a smaller fraction of light absorbed by the leaves was used for photochemistry at higher *PPFD*. A greater fraction of the absorbed light energy was lost through NPQ as *PPFD* increased (Fig. 3C and D). Consistent with the increase in Φ_{PSII} by far-red, adding far-red light to the red/blue or warm-white light also increased P_n of lettuce

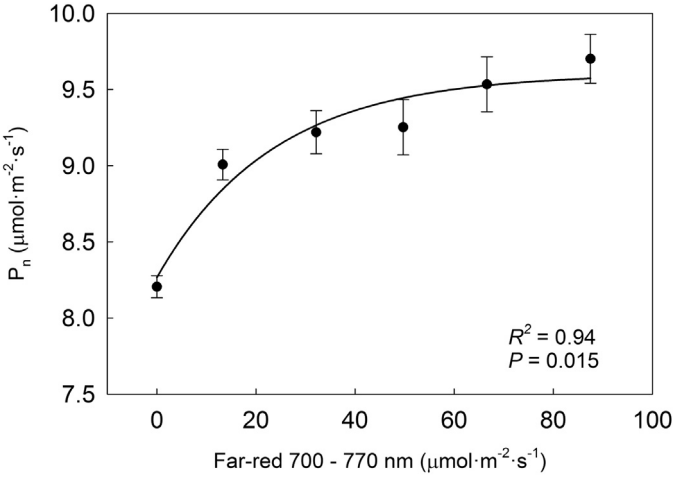

Fig. 6. Net photosynthetic rate (P_n) as a function of the intensities of far-red light added to red/blue light (*PPFD* of 200 μmol m^{-2} s^{-1}). Data points represent means from 4 replicates with error bars representing standard error.

(Fig. 4A and B). The increase in P_n by far-red light was greater than expected based on the increase in *PPFD* from the addition of the far-red light: for each 1% increase in *PPFD* provided by the far-red light, P_n increased by an average of 4% and 3% under the red/blue and warm-white light, respectively. The initial slope of the P_n – *PPFD* curve, which was derived from the fitted exponential rise to maximum function, shows the maximum rate of increase in P_n per unit increase in incident *PPFD*. For both red/blue and warm-white light, the initial slope of the P_n – *PPFD* curve was increased significantly by far-red light, from 0.060 to 0.066 mol CO_2/mol *PPFD* under red/blue light and from 0.071 to 0.078 mol CO_2/mol *PPFD* under warm-white light – a 10% increase for both types of light. The initial slope of P_n was higher under warm-white light than under red/blue light, which is consistent with the higher P_n and Φ_{PSII} under warm-white light observed at the same *PPFD* levels as under red/blue light. This enhancement effect of far-red light on photosynthesis was also reflected in P_n/*PPFD*: far-red light increased P_n/*PPFD* by 41% for both red/blue and warm-white light at the lowest *PPFD* level (50 μmol m^{-2} s^{-1}), and the difference in P_n/*PPFD* with and without far-red light gradually decreased with increasing *PPFD* (Fig. 4C and D).

3.2. Do different intensities of far-red light affect photochemistry and photosynthesis under constant red/blue light?

The amount of far-red light added affected the enhancement of photosynthesis. Φ_{PSII} responded immediately to the addition of far-red light. Φ_{PSII}, both measured within 1 min (immediate) and 20 min (stabilized) after each increase in far-red light, increased asymptotically with increasing far-red light, with a 7.5% increase with the highest amount of far-red light added (Fig. 5A). This response suggests that at a given level of red/blue light (*PPFD* of 200 μmol m^{-2} s^{-1} in this case), only a certain amount of far-red light (~50 μmol m^{-2} s^{-1} in this case) is needed to restore the balance between the excitation of the two photosystems. Far-red light increases Φ_{PSII} when the rate of photochemical reactions is limited by the re-oxidation of the PQ pool due to under-excitation of PSI. Once equal excitation of the two photosystems is reached, however, increasing far-red light induces no further increase in Φ_{PSII}.

Unlike the rapid response of Φ_{PSII} to far-red light, NPQ did not respond immediately to each increase in far-red light: NPQ measured immediately following an increase in far-red light was similar to the stabilized NPQ under the previous far-red light level (Fig. 5B). Instead, NPQ decreased slowly (as shown by the stabilized NPQ)

over time with increasing far-red light (Fig. 5B). This decrease of NPQ by far-red light could be due to the occurrence of state transitions as well as down-regulation of xanthophyll cycle in response to more efficient photochemistry as discussed earlier. The time course of this response is in line with that of state transitions and xanthophyll cycle regulations, which take minutes to occur (Haldrup et al., 2001; Ruban, 2015).

Consistent with the response of Φ_{PSII} to far-red light, P_n similarly increased asymptotically with increasing far-red light (Fig. 6). P_n increased by 18% with a 3% increase in *PPFD* (and 9% increase in *YPF*) at the highest amount of far-red added, suggesting that the increase of P_n by far-red light is not merely a direct effect from an increase in *PPFD* or *YPF*, but rather largely attributable to the increase in Φ_{PSII} (*i.e.* a true enhancement effect, where a greater proportion of absorbed light is used for photochemistry).

4. Conclusions

Our results show that different wavelengths of light can have synergistic effects on photochemistry and photosynthesis. Far-red light is needed for efficient photochemistry, especially under light with wavelengths that over-excite PSII. Adding far-red light to red/blue or warm-white LED light increases Φ_{PSII}, decreases NPQ, and enhances net photosynthetic rate. Chlorophyll fluorescence measurements provide a quick way to identify the interactive effect of different light sources on photochemistry. Both fast (<1 min) and slow (~20 min) changes in fluorescence yield upon altering the light conditions (*i.e.* intensity and quality) have been used to detect and distinguish between changes in photochemical activities and non-photochemical processes (*e.g.* heat dissipation and state transitions). The interactive effects of light of different wavelengths and the photosynthetic enhancement effect of far-red light should be taken into consideration to optimize photochemical efficiency and photosynthesis.

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