Journal of Experimental Botany, Vol. 70, No. 15 pp. 3705–3708, 2019
doi:10.1093/jxb/erz164 Advance Access Publication 19 April, 2019
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eXtra Botany

Viewpoint

A mechanistic view of the reduction in photosynthetic protein abundance under diurnal light fluctuation

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Received 10 January 2019; Editorial decision 25 March 2019; Accepted 27 March 2019

Editor: Tracy Lawson, University of Essex, UK

Leaves adapted to diurnal fluctuating light (FL) tend to have reduced photosynthetic parameters in comparison with those grown under constant light but intercepting the same daily photon integral (DPI). This reduction may result from a non-linear relationship between photosynthetic protein synthesis rate (PPSR) and incident photosynthetically active radiation (PAR). Models incorporating the PPSR–PAR relationship have quantitatively predicted the effects of FL reported in the literature. Further simulations suggest that the degree of this reduction varies with the FL pattern, DPI level and parameters describing the PPSR–PAR relationship.

Obtaining an understanding of the physiological responses of the plant to a FL regime has gained increasing attention in the past few years since FL reflects a more realistic situation for plants growing under natural conditions (Kaiser *et al.*, 2018; Matsubara, 2018; Burgess *et al.*, 2019). By hypothesizing that photosynthetic capacity (A_{max}) is determined by a mechanism leading towards maximal carbon assimilation, a higher A_{max} would be expected under FL (Retkute *et al.*, 2015). However, this hypothesis leads to an overestimation of A_{max} by more than 50% under frequent light fluctuation, suggesting a more complex underlying mechanism. Recently, Vialet-Chabrand *et al.* (2017) have experimentally demonstrated that the daily carbon assimilation of plants grown under FL was lower in comparison with plants grown under a square wave light (SQ) regime but intercepting the same DPI (mol m^{-2} d^{-1}). They highlighted the influence of diurnal light fluctuations on photosynthetic acclimation and photosynthetic capacity. One of their findings is that plants grown under FL had reduced photosynthetic parameters, particularly maximal electron transport rate (J_{max}) and leaf absorptance of incident PAR. Biochemically, this reduction is due to a decrease in the photosynthetic protein abundance by 3–15%. However, the physiological mechanisms resulting in this difference in protein abundance between FL and SQ remain unknown. Here, we seek explanations for this reduction in photosynthetic proteins under FL by applying an hourly based dynamic model for photosynthetic acclimation.

Protein abundance is regulated by the orchestration of multiple mechanisms and is the outcome of protein turnover: the continuous dynamics of protein synthesis and degradation (Kristensen *et al.*, 2013; Nelson and Millar, 2015). Based on the concept of protein turnover, we have recently presented a mechanistic model describing photosynthetic acclimation (Pao *et al.*, 2019) in which the experimental data suggested a non-linear relationship between the PPSR and PAR (see Supplementary Fig. S1 at *JXB* online). PPSR increases almost linearly with PAR under low light conditions (up to 200 µmol PAR m^{-2} s^{-1} for Rubisco and electron transport proteins), and then the slope of the PPSR–PAR curve decreases and the protein synthesis rate approaches a saturation level at high PAR (around 900 µmol PAR m^{-2} s^{-1}). This form of a non-linear relationship is not surprising since it has been observed in the cause–effect relationships of many other biological phenomena. Of note is its implication that protein synthesis rate under FL conditions (ranging between 0 and 1500 µmol PAR m^{-2} s^{-1} in Vialet-Chabrand *et al.*, 2017) is saturated occasionally during the course of the day and the protein synthesis per day per unit DPI is consequently less than that under SQ, the non-saturating condition (constantly at 460 µmol PAR m^{-2} s^{-1} for 12 h). By applying this PPSR–PAR relationship (Fig. 1A), it is possible to assess the differences in photosynthetic protein abundance between plants grown under FL and SQ.

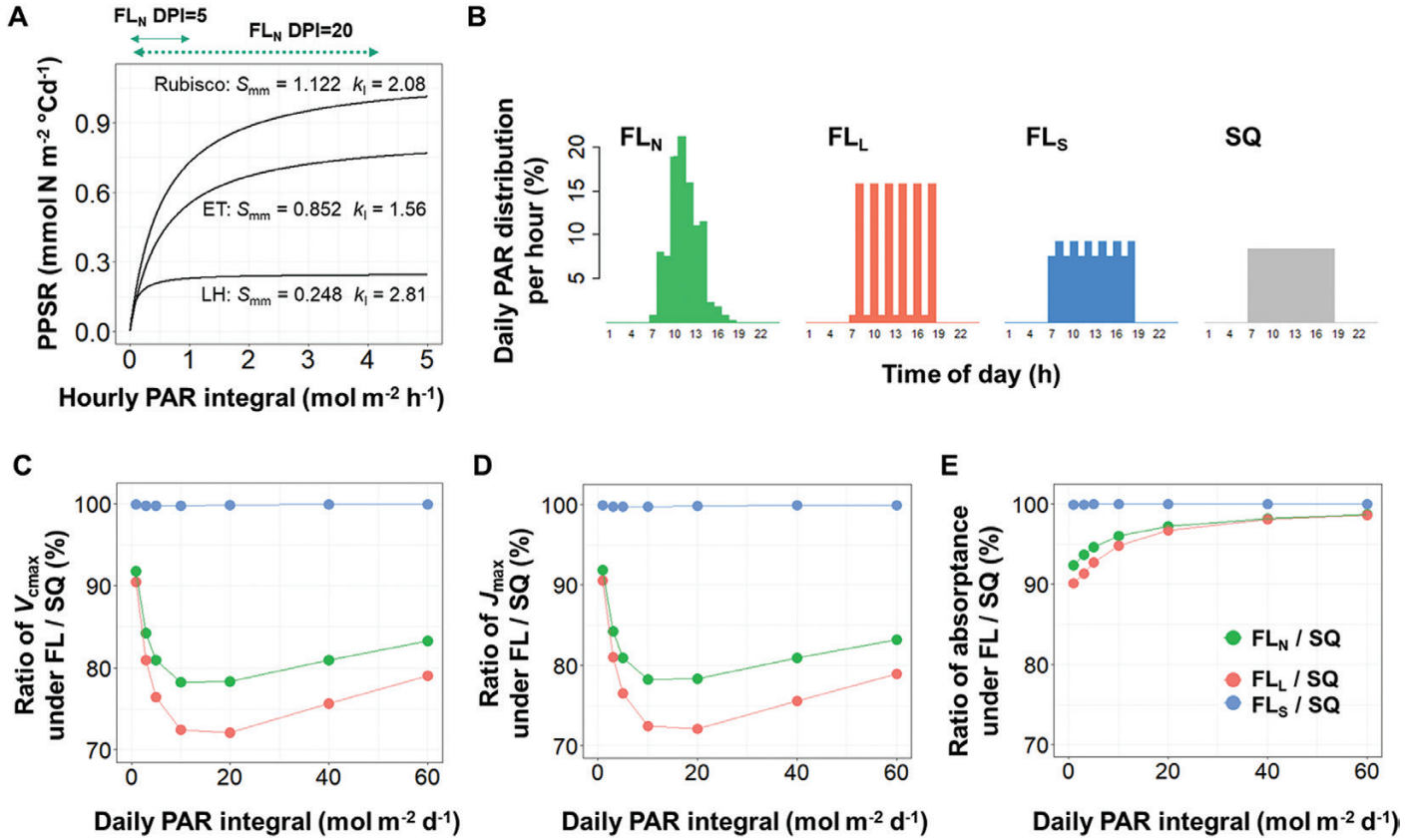

Fig. 1. (A) The response curves of photosynthetic protein synthesis rate (PPSR) to photosynthetically active radiation (PAR) in Pao *et al.* (2019) are different between Rubisco, electron transport proteins, and light harvesting proteins, depending on the maximum synthesis rate (S_{mm}) and the curvature (k_i) of each functional protein group. The effect of fluctuating light (FL) on the photosynthetic parameters under different daily photon integral (DPI) and 12 h photoperiod was simulated with different diurnal FL patterns. (B) Daily PAR distribution (%) per hour for natural diurnal fluctuation (FL_N; large fluctuation (FL_L), small fluctuation (FL_S), and square wave (SQ) light regimes. (C–E) The ratio of maximum carboxylation rate (V_{cmax}) (C), maximum electron transport rate (J_{max}) (D), and leaf absorptance of PAR (E) between FL and SQ under different DPI levels. The solid and dotted green lines with arrows above (A) indicate the ranges of PAR under FL_N pattern at DPI level of 5 and 20 mol m^{-2} d^{-1}, respectively. (B) Adapted from Vialet-Chabrand *et al.*, 2017. Importance of fluctuations in light on plant photosynthetic acclimation. Plant Physiology 173, 2163–2179 (www.plantphysiol.org), 'Copyright American Society of Plant Biologists.'

To simulate the effect of the diurnal light fluctuation, we first converted the parameters in the model of Pao *et al.* (2019) to an hourly basis by assuming a 12 h photoperiod and zero protein synthesis in the dark. Then, three FL patterns (Fig. 1B) and SQ with DPI between 1 and 60 mol m^{-2} d^{-1} were used as light input to simulate the abundance of Rubisco, electron transport, and light harvesting proteins (Pao *et al.*, 2019), which were then converted to maximal Rubisco carboxylation rate (V_{cmax}), J_{max}, and leaf PAR absorptance, respectively. Constants converting the amount of nitrogen in each functional protein pool into the corresponding capacities are used according to Buckley *et al.* (2013). Under natural diurnal light fluctuation (FL_N in Fig. 1B) and light intensity (DPI = 10 and 20 mol PAR m^{-2} d^{-1}) similar to the FL experiment in Vialet-Chabrand *et al.* (2017), the model predicted the effects of FL on photosynthetic parameters: V_{cmax} and J_{max} were reduced by 21–22% (Fig. 1C, D) and leaf PAR absorptance by 2–4% (Fig. 1E). This prediction is within the range reported for leaf PAR absorptance (3–5%) but is different from that for the V_{cmax} (8–10%) and J_{max} (11–15%) found in Arabidopsis (Vialet-Chabrand *et al.*, 2017). These differences could be due to the lack of protein synthesis in the dark assumed in the simulations (see below) or due to the fact that their model was parameterized using greenhouse cucumber (*Cucumis sativus*), which might have different PPSR–PAR responses from Arabidopsis. However, both experimental and model studies suggest that V_{cmax} and J_{max} were more affected by FL than leaf PAR absorptance. This can be explained by the fact that the synthesis rate of light harvesting proteins reaches saturation at a lower PAR level than Rubisco and electron transport proteins (Fig. 1A). Therefore, the effects of FL on light harvesting proteins under high DPI were almost negligible.

The different effects of FL on V_{cmax}, J_{max}, and leaf absorptance (Fig. 1C–E) imply that the characteristics of the PPSR–PAR curve determine the impact of light fluctuation on the abundance of photosynthetic proteins. Hence, we further examined the extent to which the PPSR–PAR curve parameters, the maximum protein synthesis rate (S_{mm}, equal to 0.1, 0.5, or 2.5) and the curvature (k_i, equal to 0.5 or 5), affect the photosynthetic acclimation under natural diurnal light fluctuation (FL_N in Fig. 1B) with DPI levels between 1 and 60 mol m^{-2} d^{-1} in combinations with nitrogen supply levels (2–10 mM) and leaf

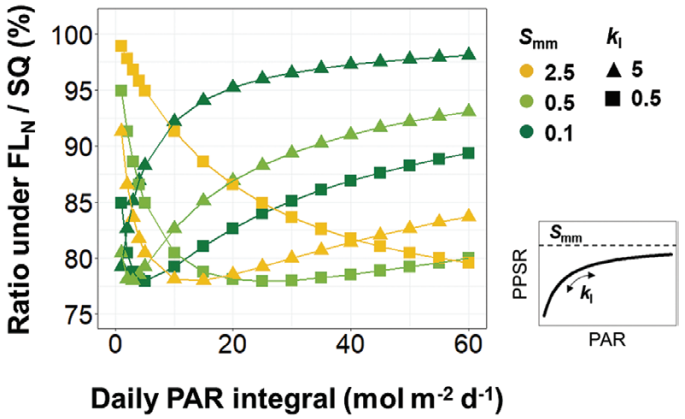

Fig. 2. The effects of FL_N (Fig. 1B) on photosynthetic protein abundance depend on the values of maximum synthesis rate (S_{mm}, equal to 0.1, 0.5, or 2.5) and the curvature (k_l, equal to 0.5 or 5) of the PPSR–PAR response curves. For abbreviations see Fig. 1.

age (5–45 d). Age and nitrogen levels had no influence (<1%) on the impact of FL_N (data not shown). The reduction of protein abundance due to FL was up to 22%, depending on the combinations of DPI, S_{mm}, and k_l (Fig. 2). Three types of response curves can be identified: (i) the combination of high k_l and low S_{mm} results in the strongest reduction under low light and this reduction decreases with DPI, resembling the light harvesting proteins (Fig. 1A); (ii) combining high k_l and S_{mm} or low k_l and S_{mm} shows the strongest reduction under low–intermediate DPI, resembling Rubisco and electron transport proteins (Fig. 1A); (iii) combining low k_l and high S_{mm}, the reduction in protein abundance increases with DPI. The third type of these response curves indicates that the PPSR will not be saturated even under high light conditions, probably an unfavorable strategy under natural selection. Altogether, these results suggest that variations in the parameters of the PPSR–PAR curve can form an explanation of the different acclimatory responses to FL between plant functional types, as reported by Watling *et al.* (1997).

Mathematically, the hyperbolic characteristics of the PPSR–PAR response (Fig. 1A) suggest that the strongest impact of light fluctuation can be expected if the incident PAR fluctuates largely across the vertex of the PPSR–PAR curve and the impact of FL becomes smaller when it mostly fluctuates within the nearly linear range of the PPSR–PAR curve. This non-linear characteristic has two biological implications. Firstly, the influence of FL can be expected to be small under low or saturating PAR levels. In our simulation, the reductions of V_{cmax} and J_{max} increase with DPI under low light level (DPI < 10 mol m^{-2} d^{-1}, Fig. 1C, D). This result is similar to the observation in Arabidopsis that the impact of FL on the electron transport rate is stronger under a DPI of 5.1 than one of 3.6 mol m^{-2} d^{-1} (Alter *et al.*, 2012). Also, in *Alocasia macrorrhiza* no reduction in A_{max} was observed under FL at a very low DPI level (1.4 mol m^{-2} d^{-1}; Sims and Pearcy, 1993) while A_{max} tended to be 15% lower than SQ when DPI was 7 mol m^{-2} d^{-1} (Watling *et al.*, 1997). Secondly, photosynthetic protein abundance will be more strongly affected by the large fluctuation (FL_L in Fig. 1B) than by the small fluctuation (FL_S in Fig. 1B, C–E). This agrees with the observations in Arabidopsis that, in comparison with SQ (85 μmol PAR m^{-2} s^{-1}), FL_L (ranging between 50 and 1250 μmol PAR m^{-2} s^{-1}) reduces electron transport rate by 28%, while FL_S (ranging between 50 and 650 μmol PAR m^{-2} s^{-1}) reduces electron transport rate by only 8% (Alter *et al.*, 2012). Grown under the FL_S pattern (ranging between 30 and 525 μmol PAR m^{-2} s^{-1}), A_{max} and nitrogen per unit leaf area (N_{area}, a proxy of photosynthetic protein abundance) of *Shorea leprosula* leaves were not different from those of leaves grown under SQ (170 μmol PAR m^{-2} s^{-1}; Leakey *et al.*, 2002), but in their following study Leakey *et al.* (2003) showed that A_{max} and N_{area} of the same species grown under FL_L (ranging between 0 and 1700 μmol PAR m^{-2} s^{-1}) were 20–30% lower than those of their counterparts grown under FL_S (ranging between 0 and 750 μmol PAR m^{-2} s^{-1}).

As with any other model, this model is a simplification of the real system. For example, it assumes zero protein synthesis rate under darkness, which is unlikely for Rubisco (Ishihara *et al.*, 2015). If a low rate of Rubisco synthesis during the dark period under FL and SQ is assumed (as suggested by Ishihara *et al.*, 2015), the relative impact of FL will be lower than our prediction and thus closer to the reduction measured by Vialet-Chabrand *et al.* (2017). The current model also assumed the same degradation rate constants for different PAR levels although this does not hold true *in planta* especially under high light (Li *et al.*, 2018). The available information is so far restricted for parameterizing this effect (Nelson *et al.*, 2014; Li *et al.*, 2017). Theoretically, if the degradation is enhanced under excess light while the synthesis rate remains stable, it can be expected that the reduction in protein abundance under FL would be even more severe. However, if the synthesis rate is coordinated with the degradation rate as reported for photosystem II subunit D1 protein (Aro *et al.*, 1993), similar results to our simulation could be expected due to restored balance in the net rate of change. In addition, there are still unknown mechanisms involved in the acclimation to FL that are not considered in the model. The effects of the frequency of light fluctuations and the length of individual light events on photoacclimation, as shown in previous studies (Yin and Johnson, 2000; Alter *et al.*, 2012) and implying that protein synthesis does not react to a light signal instantaneously (e.g. Retkute *et al.*, 2015), cannot be reproduced by our model (data not shown). Besides photosynthetic proteins, many physiological processes are also involved in the acclimation mechanism to FL, especially when tackling excess light energy. Photo-oxidative damage caused by the excess light events under FL may increase the need for photoprotection, photorespiration, and cyclic electron flow, which together alter the metabolism and partitioning of nitrogen and carbon (Matsubara, 2018; Annunziata *et al.*, 2018; Schneider *et al.*, 2019). Also, our model does not account for any photoperiodic regulation, which is also known to affect long-term acclimation (Seaton *et al.*, 2018).

In summary, the hyperbolic PPSR–PAR response provides a mechanistic explanation of the reported reduction in photosynthetic protein abundances caused by diurnal light

fluctuation. Our results suggest that the differences in protein abundances between FL and SQ conditions are determined by three components: the pattern of FL, the DPI level, and the species-specific PPSR–PAR curve parameters. Although a model cannot account for all details of the acclimation response under all environmental scenarios, our model delivers a systematic view of this phenomenon and thus can be a useful tool for designing FL scenarios for future experiments (see Supplementary Dataset S1 for the R script of the model). Our analyses point out the avenues for further investigations in the interspecific and genotypic variations of the PPSR–PAR relationship and in the response time to the light signal, as well as photoperiodic regulation and the combined effects of different environmental factors on photosynthetic protein turnover.

Supplementary data

Supplementary data are available at *JXB* online.

Dataset S1. R script of the protein turnover model and input file of light fluctuation patterns.

Fig. S1. Non-linear relationship between photosynthetic protein synthesis rate and light intensity.

Acknowledgements

This work was supported by Deutsche Forschungsgemeinschaft (DFG, Project number 403510751). We are grateful for the valuable comments of the two reviewers.

Keywords: Fluctuating light, light fluctuation pattern, light harvesting, maximum carboxylation rate, maximum electron transport rate, modelling, photosynthetic acclimation, square wave light.

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