Chapter 5

Light-Response Curves in Land Plants

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Abstract

Light-response curves reveal the photosynthetic properties of plants. Depending upon the methodology selected they can be used to characterize CO_2 assimilation, photochemistry, photoacclimation, photoinhibition, and kinetics of photoprotective mechanisms in response to changing light conditions. They are widely used to describe the ontogeny and range in physiological plasticity of plants. Here we describe methods for acquiring light-response curves using CO_2 gas exchange and chlorophyll *a* fluorescence measurements that are applicable to a wide range of land plants.

Key words Light-response curves, Gas exchange, Chlorophyll *a* fluorescence

1 Introduction

Growth of autotrophic land plants is intrinsically linked to light, the driving force for photosynthesis. There is a well-defined relationship between the amount of irradiance absorbed by a leaf, the electron transport rate (ETR), and the rate of RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) carboxylation or oxygenation reactions [1]. As the quantity (quantum flux density) and quality (spectral composition) of light are dynamic properties, it is desirable to gain insights into the characteristics and adaptation of photosynthesis to the light environment, and/or probe the physiological properties of different species. This can be achieved by measuring light-response curves.

Photosynthetic light-response curves (*P*-*I*) describe the net CO_2 assimilation (P) as a function of irradiance (I). While various mathematical models have been used to describe the relationship, physiologists frequently use a non-rectangular hyperbola equation [2–5]

$$\theta P^2 - (\varphi I + P_{\max})\, P - \varphi I\, P_{\max} = 0$$

where P is the photosynthetic rate, I is the absorbed irradiance, φ is the maximum quantum yield for CO_2 uptake, θ is the convexity of

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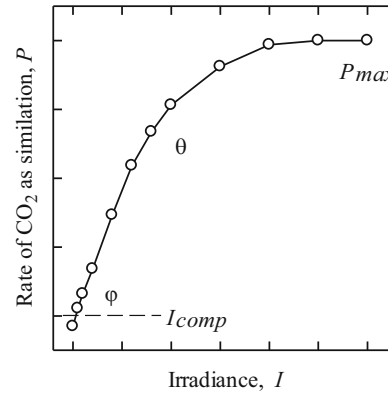

Fig. 1 Idealized light-response curve of photosynthesis

the curve, and P_{max} is the light-saturated rate of photosynthesis. The initial portion of the curve is characterized by the light compensation point (I_{comp}), the value of I at which CO_2 assimilated by photosynthesis is equal to the CO_2 produced by respiration (Fig. 1). Beyond this point there is a supposed linear response of P to I up to approximately 200 μmol m^{-2} s^{-1}, which is used to calculate φ. This is followed by a region of nonlinear die-off described by θ before reaching a semi-plateau (P_{max}) where further increases in I do not lead to an increase in P. Occasionally, after reaching P_{max} there is a decline in P resulting from photoinhibition. Interpreting the mechanistic basis of the P-I response is not straightforward. It is difficult to pinpoint experimentally the transition between RuBP-regenerated and RuBisCO-limited photosynthesis, two major rate limitations of photosynthesis which are important for understanding the component processes controlling the response of photosynthesis to light. The model does not account for all the physiological or biochemical processes of the leaf [6], nor do all parameters derived from it, such as θ, have an obvious physiological meaning [7–9]. Despite this, P-I response curves provide a means with which to characterize CO_2 assimilation in response to light.

More recently, measurements of chlorophyll *a* fluorescence have been established as a useful and informative indicator characterizing the light reactions of photosynthesis. Light energy absorbed by chlorophylls associated with photosystem II (PSII) can be used to drive photochemistry in which an electron (e^-) is transferred from the reaction center chlorophyll of PSII (P_{680}) to the primary quinine acceptor of PSII (Q_A). Alternatively, absorbed light energy can be lost from PSII as chlorophyll fluorescence or as heat. These three processes are in direct competition with each other; if the rate of one process increases, then the rates of the other two will decrease. Thus, the yield of chlorophyll fluorescence

emission gives us valuable information about the quantum efficiency of photochemistry and heat dissipation [10].

Combining gas exchange with chlorophyll *a* fluorescence means it is possible to simultaneously characterize the dark and light reactions of photosynthesis and gain greater insights into the photosynthetic processes. In this chapter we present methods for measuring light-response curves using gas exchange and chlorophyll *a* fluorescence. These are the most commonly employed techniques and offer the greatest flexibility in order to investigate photosynthetic performance in response to light.

2 Materials

A variety of commercial infrared gas exchange analyzers (IRGAs) and chlorophyll fluorometers capable of pulse-amplitude-modulated (PAM) measurements [11–13] are available. Discussion, comparison, and detailed operation instructions are beyond the scope of this chapter and so the reader is referred to the user manuals and wider literature.

3 Methods

3.1 Light-Response Curve Strategy

There are several strategies for acquiring light-response curves each with its own biological interpretation. The two most common are the following (*see* **Note 1**):

1. Steady-state light-response curves (LRCs): These are designed to characterize the potential response of steady-state photosynthesis under a range of light conditions (photoacclimation) (*see* **Note 2**).
2. Rapid light-response curves (RLCs): These are designed to characterize the dynamic response of the photosynthetic apparatus in a rapidly changing light environment. They are used to detect short-term adjustments in the functioning of the photosynthetic apparatus including the activation and deactivation of carbon metabolism, operation of photoprotective mechanisms, and photoinhibition (*see* **Note 3**).

3.2 Photosynthetic Light-Response Curves

3.2.1 Operational Considerations

1. Set up the gas exchange system according to the manufacturer's instructions.
2. A CO_2 concentration of 400 μmol mol^{-1} and 21% O_2 is representative of ambient conditions (*see* **Note 4**).
3. A constant leaf temperature should be used. For most experiments a temperature of 25 °C is recommended (*see* **Note 5**).

4. Where possible, a relative humidity (RH) similar to the growth environment should be used in order to avoid stomatal effects (*see* **Note 6**).
5. A constant airflow should be used; 500 μmol m^{-2} s^{-1} is suggested (*see* **Note 7**).
6. A standard protocol for C_3 plants is a photosynthetic photon flux density (PPFD) of 1500, 1000, 500, 250, 120, 60, 40, 20, 10, and 0 μmol m^{-2} s^{-1} (*see* **Notes 8** and **9**). A maximum light intensity of 2000 μmol m^{-2} s^{-1} is suggested for C_4 plants or 500 μmol m^{-2} s^{-1} for C_3 or C_4 plants grown in the shade (*see* **Note 10**).
7. If plants were in the dark prior to starting the curves, then they should be equilibrated for 15–20 min at a light intensity representative of the natural growth environment (*see* **Note 11**).
8. For steady-state light-response curves (LRCs) leaves should be equilibrated at each light intensity for 3–5 min (*see* **Note 12**). For rapid light-response curves (RLCs) the leaves should be equilibrated at high light for a minimum of 5 min (*see* **Note 13**) and then the light intensity should be decreased in intervals of 200 μmol m^{-2} s^{-1} every 1–3 min (*see* **Note 14**).
9. Typically, during gas exchange measurements, a CO_2 zero point is obtained every hour or after changing the CO_2 concentration. When acquiring light-response curves this is only important at the start of each curve and does not need to be repeated.

3.2.2 Methods

1. Set starting light intensity, CO_2 concentration, and flow.
2. Insert leaf (*see* **Note 15**).
3. Set leaf temperature and humidity.
4. Program and start the desired light-response curve protocol.
5. When the protocol has finished remove the leaf. If measuring other leaves, go back to **step 1**.
6. Download data, graph, and analyze data (*see* **Notes 16** and **17**).
7. I_{comp} is calculated using least square regression analysis of the intercept of the initial part of the curve (PPFD <200 μmol m^{-2} s^{-1}).
8. φ is calculated using least square regression analysis to the initial slope (PPFD <200 μmol m^{-2} s^{-1}).
9. The convexity of the response (θ) curve can be calculated using curve fitting routines (*see* **Note 16**), although frequently curve shapes are simply compared.
10. P_{max} can be extracted from the dataset if light saturation of photosynthesis is reached.
11. R_d, respiration rate in the dark, can be estimated from rates of P at a PPFD of 0 μmol m^{-2} s^{-1} (*see* **Note 18**).

3.3 Chlorophyll a Fluorescence Light-Response Curves

3.3.1 Operational Considerations

1. Set up the chlorophyll fluorescence system according to the manufacturer's instructions.
2. The distance between the leaf and sensor should be optimized to ensure that a strong fluorescence signal is obtained (*see* **Note 19**).
3. A constant leaf temperature should be used. For most experiments a temperature between 25 and 30 °C is recommended.
4. Where possible, measurements should be made at a RH similar to the growth environment in order to avoid stomatal effects.
5. The user needs to decide whether a period of dark adaption is necessary before making the first measurement (*see* **Note 20**) as this may affect the experimental setup.
6. A standard protocol for C_3 plants is a PPFD of 0, 200, 400, 600, 800, 1000, 1200, 1400, and 1500 μmol m^{-2} s^{-1} (*see* **Note 21**), acclimating the leaf at each actinic light intensity for between ~20 s (RLCs) (*see* **Note 22**) and 20 min (LRCs). Consideration of the type of light-curve strategy is discussed above (*see* Subheading 3.1). A maximum light intensity of 2000 μmol m^{-2} s^{-1} is recommended for C_4 plants or 500 μmol m^{-2} s^{-1} for C_3 or C_4 plants grown in the shade (*see* **Note 10**).

3.3.2 Methods

1. Dark-adapt the leaf for a minimum of 5–30 min (*see* **Note 23**). This can be achieved using the manufacturer's leaf clips or the user's own protocol.
2. Switch off the measuring beam and apply the fluorescence detector to ensure that the reading is zero.
3. Switch on the measuring beam. Exposing the leaf to a weak modulated measuring beam (PPFD of 0.1 μmol m^{-2} s^{-1}) results in the minimal level of fluorescence (F_o). Applying a pulse of weak far-red light prior to the measurements of F_o is recommended (*see* **Note 24**).
4. Expose leaves to a saturating light pulse (>4000 μmol m^{-2} s^{-1}) for a duration of 0.6–0.8 s (*see* **Note 25**); Q_A will be maximally reduced and the maximal fluorescence level (F_m) will be observed. The difference between F_m and F_o is defined as the variable fluorescence (F_v). The ratio of F_v/F_m is used to estimate the maximum quantum yield of Q_A reduction (*see* **Note 26**).
5. Switch on the actinic light.
6. When steady state is reached, measure fluorescence emission (F').
7. Apply a saturating pulse and attain maximum fluorescence in the light (F_m').

8. If dark-adapted measurement are not required, then you can directly apply the light-response curve protocol after inserting the leaf. Start from **step 2** above (*see* **Note 27**).
9. Remove leaf.
10. Download data, graph, and analyze data (*see* Table 1 for a list of formulae).
11. The maximum quantum yield of PSII photochemistry in the dark (F_v/F_m) is calculated as $(F_m - F_o)/F_m$.

Table 1
Chlorophyll fluorescence parameters and equations frequently used in studies of photosystem II photochemistry

Parameter	Definition	Formula	Description
F_v/F_m	Maximum quantum efficiency of PSII photochemistry	$(F_m - F_o)/F_m$	Maximum efficiency at which light absorbed by PSII is used for reduction of Q_A
$\Delta F/F_m'$	PSII operating efficiency	$(F_m' - F')/F_m'$	Quantum efficiency of PSII electron transport in the light. Also known as φII or F_q'/F_m'
F_v'/F_m'	PSII maximum efficiency	$(F_m' - F_o')/F_m'$	Maximum efficiency of PSII photochemistry in the light, which is the PSII operating efficiency if all the PSII centers were "open" (Q_A oxidized)
ETR	Photosynthetic electron transport rate of PSII	PPFD × 0.84 × 0.5 × ($\Delta F/F_m'$)	Electron transport rate through photosystem II
NPQ	Nonphotochemical quenching	$(F_m/F_m') - 1$	Estimates the apparent rate constant for heat loss from PSII
q_P	PSII efficiency factor	$(F_m' - F')/(F_m' - F_o)$	Relates PSII maximum efficiency to operating efficiency. Nonlinearly related to the proportion of PSII centers that are "open" (Q_A oxidized). *See* q_L
q_L	Fraction of PSII centers that are "open"	$(\Delta F/F_v')(F_o'/F') = [(F_m' - F')/(F_m' - F_o')] \times (F_o'/F')$	Estimates the fraction of "open" PSII centers (with Q_A oxidized) on the basis of a lake model [11] for the PSII photosynthetic apparatus
q_N	Coefficient of nonphotochemical quenching	$(F_m - F_m')/(F_m - F_o)$	Estimated from the proportion of quenching of the variable fluorescence F_v, $(F_m - F_o)$. On a scale of 0–1

12. The effective quantum yield of PSII, $\Delta F/F_m'$, is calculated as $(F_m' - F')/F_m'$, where F' is fluorescence yield of the light-adapted sample and F_m' is the maximum light-adapted fluorescence yield (*see* **Note 28**).
13. The photosynthetic electron transport rate (ETR) of PSII is calculated as PPFD $\times$ 0.84 $\times$ 0.5 $\times$ $(\Delta F/F_m')$ (*see* **Note 29**).
14. Nonphotochemical quenching (NPQ) is calculated as $(F_m/F_m') - 1$ (*see* **Note 30**).
15. The PSII efficiency factor (q_P) is calculated as $(F_m' - F')/(F_m' - F_o)$ (*see* **Note 31**).
16. The fraction of PSII centers that are "open" (q_L) is obtained as $(\Delta F/F_v')(F_o'/F') = [(F_m' - F')/(F_m' - F_o')] \times (F_o'/F')$ (*see* **Note 32**).

3.4 Combined Gas Exchange and Chlorophyll a Fluorescence Light-Response Curves

Combining chlorophyll fluorescence with gas exchange enables correlation of PSII photosynthetic efficiency directly with CO_2 assimilation. This is achieved by installing a fluorescence measurement head to the infrared gas analyzer (*see* **Note 33**).

4 Notes

1. When selecting a light curve strategy careful consideration needs to be given to the response you want to measure; typically this will be either the short-term responses of photosynthesis to rapid changes in light conditions or the response of photosynthesis to steady-state light conditions.
2. LRCs measure steady-state conditions which are not typically found in the natural environment. They are used to investigate plasticity and inherent properties of photosynthesis. They can be slow to acquire, and so, to save time, light curves can be performed on multiple leaves equilibrated at different light intensities. Caution is required in order to avoid introducing variability into measurements by using leaves with different ages or adapted to different conditions due to their position and orientation within the canopy. Leaves of similar physiological development should be selected from a single plant or multiple representative plants.
3. RLCs are relatively fast to acquire and can be used to mimic natural light conditions. They are typically used to investigate short-term responses to rapid changes in the light environment. To overcome light dosage effects, non-sequential light-response curves can be acquired, where curves are based upon

independent measurements at each light level on different leaves taken from a population in the same physiological state.

4. It is important that a constant CO_2 and O_2 concentration is maintained to avoid confounding the effects of CO_2 with light and/or O_2.
5. Photosynthesis is strongly affected by temperature and exhibits a thermal optimum of ~25 °C. Thermal acclimation changes this thermal optimum and so the temperature of the growth environment needs to be considered before measurements are made. The mechanisms controlling temperature response in C_3 and C_4 plants are numerous and varied [14] and so care should be taken when deciphering temperature responses.
6. Excessive humidity (>85%) will lead to condensation and erroneous measurements in some instruments. Humidity will change alongside conductance and transpiration and so very high (>85%) or low (<15%) values should be avoided in order to accommodate these changes.
7. In some gas-exchange systems, flow rate will affect the speed of detection: the higher the flow rate the faster the gas mixtures reach the infrared gas analyzers. Flow rate is also important for partially controlling humidity; at high flow rates, air inside the cuvette is replenished quickly with dry air and humidity will remain low, even if the transpiration rate is high. Flow rates should be decreased when CO_2 assimilation or stomatal conductance is low as the difference between the CO_2 concentration in the incoming and outgoing air will be very small and high flow rates under these conditions may lead to measurement error.
8. LED actinic light source with peak wavelengths of ~655 ± 15 nm and a smaller proportion of light at 465 ± 5 nm are used in commercial systems. It is possible to perform a light-response curve without a light source by using neutral density filters.
9. LRCs can be performed by increasing light intensity, but the responses of stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) will be different to those under decreasing light and A may be limited by g_s as stomata can be relatively slow to respond to changes in I.
10. Ideally, light-saturated photosynthesis (P_{max}) or maximum quantum efficiency (F_v/F_m or F_v'/F_m') should be determined before any measurements are made. Exposing plants to light intensities above those experienced in the normal growth environment will lead to photoinhibition and photodamage; this will adversely affect subsequent measurements of light response. C_4 species have a higher light use efficiency than C_3

species and so high irradiances can be used for the light curve. Shade-adapted species tend to have lower dark respiration rates, lower compensation points, and lower maximum photosynthetic rates than sun-adapted leaves.

11. Many photosynthetic enzymes are light regulated and require an activation period before reaching steady state.
12. Sufficient time is required to allow stomatal conductance to adjust and equilibrate to each light intensity. This can be determined by monitoring C_i (intercellular CO_2 concentration) and waiting for it to stabilize (typically this takes 3–5 min at each light intensity). On some machines, this wait time is defined by the user at the start of the program, and so care must be taken to ensure that it is long enough for C_i to stabilize. Otherwise the curve must be repeated. If the resulting *P-I* curve is not smooth, then this is an indication that the wait time was not long enough.
13. High light can lead to the onset of photoinhibition and decreases in A and φ; recovery can take minutes to hours depending upon the mechanism involved [15].
14. Stomata will not be able to adjust and equilibrate to each new light intensity because the acclimation time at each intensity is too short. Pre-adaptation to a high light intensity will ensure that stomata are fully open and do not limit CO_2 assimilation. This means that as the light intensity is decreased, g_s and C_i will be higher than under increasing light. While values will be higher, response between leaves can still be compared.
15. CO_2 assimilation is typically expressed on a leaf area basis (μmol m^{-2} s^{-1}) so a known leaf area is required.
16. Curve fitting routines for light-response curves are not as straightforward as those for photosynthetic CO_2 response curves. Microsoft Excel-based tools have been developed [16, 17] but use of these requires the user to possess sound statistical and mathematical knowledge as well as the biological understanding to interpret the results. Using the published tools on your own datasets and taking time to understand how they work is the best way to acquire this knowledge.
17. Typical values: φ = 0.425–0.5 mol e^- (mol absorbed PAR photon)$^{-1}$; θ = 0.7–0.96; ETR_{max} = 50–200 μmol e^- m^{-2} s^{-1}; R_d = 0.1–3.0 μmol CO_2 m^{-2} s^{-1}; I_{comp} 0–50 μbar; and P_{max} = <50 μmol CO_2 m^{-2} s^{-1}.
18. In the absence of photorespiration, R_d reflects rates of mitochondrial respiration; measurements are best made predawn to ensure that there is no photorespiratory CO_2 release. Measurements should be made and averaged for a period of 30 min. The values will be negative as they represent CO_2 loss;

however, these are typically expressed as positive values in final data presentation.

19. If the leaf is positioned too far from the fluorometer this may result in a weak fluorescence signal. The leaf should be held between 90 and 45° horizontal to the light source in order to provide homogeneous illumination and must not be changed during measurements. If using an open system, then measurements may be affected by strong fluctuations in ambient irradiance.
20. The decision whether to dark-adapt a leaf or not depends on the objectives of the experiment and the time and equipment available. Dark adaptation is essential for studying the kinetics of nonphotochemical quenching (NPQ) and for quenching analysis. Dark adaptation is not necessary if you are only interested in chlorophyll kinetics in the light.
21. For chlorophyll *a* fluorescence light-response curves, light intensity is always increased; it is not possible to decrease light intensity because high irradiance leads to the onset of photoinhibitory mechanisms which can take minutes to hours to relax [15].
22. Always check whether there is photoinhibition resulting from the repeated application of saturating pulses over short time periods by monitoring F_m. When photoinhibition occurs, F_m declines following sequential pulses and may not recover.
23. If the dark-adaptation period is not long enough, then Q_A may not become maximally oxidized. To establish the length of dark adaptation, non-stressed leaves acclimated in the light at steady-state conditions should be dark-adapted and F_v/F_m measured every 5–10 min until a value of ca. 0.83 is reached.
24. A pulse of weak far-red light should be applied prior to the measurement of F_o to excite photosystem I (PSI) and remove electrons from Q_A. If the chlorophyll fluorescence analyzer does not feature an intrinsic far-red light source, then Q_A may not maximally oxidize leading to overestimation of F_o; thus care should be taken in interpreting the data.
25. Saturating pulses of ~2000 μmol m^{-2} s^{-1} are sufficient to obtain dark-adapted F_m in most situations, but may be too low to produce a true maximum fluorescence (F_m') under high actinic illumination. Most commercial instruments provide saturating light pulses with an intensity of between 4000 and 8000 μmol m^{-2} s^{-1}. C_4 plants will require a higher saturating pulse than C_3 plants and the intensity required will vary according to the light environment in which the plants have been grown. Users must monitor F_m to ensure that the intensity of the pulse is strong enough to fully saturate PSII but does not lead to quenching as standard parameters cannot be given.

26. F_v/F_m values for non-stressed leaves are remarkably consistent among all species at ca. 0.83 [10].
27. If dark adaptation is not required, then the functional status of PSII will reflect photoacclimation and photoinhibition to the light environment directly prior to the start of measurements.
28. $\Delta F/F_m'$ provides an estimate of the PSII operation efficiency under different light and different environmental conditions. Note that $\Delta F/F_m'$ will be lower than the dark-adapted F_v/F_m value due to quenching of PSII chlorophyll fluorescence by photochemistry and NPQ.
29. Photosynthetic electron transport rate of PSII (ETR) is calculated as $\text{ETR} = I \times A_{\text{leaf}} \times \text{fraction}_{\text{PSII}} \times (\Delta F/F_m')$. The ratio of light absorbed by the leaf, A_{leaf}, is frequently assumed to be 0.84 (84% of incident PPFD is assumed to be absorbed by leaves), although large deviations do occur [10], and so where possible A_{leaf} should be measured with an integrated sphere. The $\text{fraction}_{\text{PSII}}$ is frequently assumed to be 0.5 (equal excitation of both PSII and PSI).
30. Nonphotochemical quenching (NPQ) estimates the apparent rate constant for heat loss from PSII [10].
31. PSII efficiency factor (q_p) estimates the redox state of Q_A.
32. Fraction of PSII centers that are "open," (q_L) estimates the fraction of "open" PSII centers (with Q_A oxidized) on the basis of a lake model [11] for the PSII photosynthetic apparatus. F_o' value can be obtained from a measurement or calculation. The measurement of F_o': following the saturating pulse, the actinic light is switched off and after a few seconds F_o' measured. A pulse of weak far-red light should be applied prior to the measurements of F_o'. The calculation of F_o': $F_o' = F_o/[(F_v/F_m) + (F_o/F_m')]$ [18].
33. Follow the method for *P-I* response curves in Subheading 3.2.1; in **step 6** the light intensity should be increased not decreased (*see* **Note 21**).

References

1. von Caemmerer S (2000) Biochemical models of leaf photosynthesis. CSIRO Publishing, Collingwood, VIC
2. Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149:78–90
3. Marshall B, Biscoe PV (1980) A model for C_3 leaves describing the dependence of net photosynthesis on irradiance. J Exp Bot 31(120):29–39
4. Prioul JL, Chartier P (1977) Partitioning of transfer and carboxylation components of intracellular resistance of photosynthetic CO_2 fixation: a critical analysis of the methods used. Ann Bot 41:789–800
5. Leverenz JW, Jarvis PG (1979) Photosynthesis in Sitka pruce VIII. The effects of light flux density and direction on the rate of net photosynthesis and stomatal conductance of needles. J Appl Ecol 16:919–932

6. Ye ZP (2007) A new model for relationship between irradiance and the rate of photosynthesis in *Oryza sativa*. Photosynthetica 45 (4):637–640
7. Ögren E (1993) Convexity of the photosynthetic light-response curve in relation to intensity and direction of light during growth. Plant Physiol 101:1013–1019
8. Ögren E, Evans JR (1993) Photosynthetic light-response curves. I. The influence of CO_2 partial pressure and leaf inversion. Planta 189:182–190
9. Evans JR, Jakobsen I, Ögren E (1993) Photosynthetic light-response curves. 2. Gradients of light absorptions and photosynthetic capacity. Planta 189:191–200
10. Baker NR (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu Rev Plant Biol 59:89–113
11. Bradbury M, Baker NR (1984) A quantitative determination of photochemical and non- photochemical quenching during the slow phase of the chlorophyll fluorescence induction curve of bean leaves. Biochim Biophys Acta 765:275–281
12. Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and nonphotochemical fluorescence quenching with a new type of modulation fluorometer. Photosynth Res 10:51–62
13. Schreiber U, Bilger W (1993) Progress in chlorophyll fluorescence research: major developments during the last years in retrospect. Progr Bot 54:151–173
14. Sage RF, Kubien DS (2007) The temperature response of C_3 and C_4 photosynthesis. Plant Cell Environ 30:1086–1106
15. Quick WP, Stitt M (1989) An examination of factors contributing to non-photochemical quenching of chlorophyll fluorescence in barley leaves. Biochim Biophys Acta 977:287–296
16. Lobo FA, de Barros MP, Dalmagro HJ et al (2013) Fitting net photosynthetic light-response curves with *Microsoft Excel* - a critical look at the models. Photosynthetica 51(3):445–456
17. Bellasio C, Beerling DJ, Griffiths H (2016) An Excel tool for deriving key photosynthetic parameters from combined gas exchange and chlorophyll fluorescence: theory and practice. Plant Cell Environ 39:1180–1197
18. Oxborough K, Baker NR (1997) Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components – calculation of qP and Fv′/Fm′ without measuring Fo′. Photosynth Res 54:135–142