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Plasticity of photosynthesis after the ‘red light syndrome’ in cucumber

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ABSTRACT

The quantum efficiency of photosynthesis of leaves is wavelength dependent and peaks in red (620–670 nm). However when cucumber plants are raised under pure red light, leaf photosynthesis becomes severely impaired. This “red light syndrome” has been characterized before by a low F_v/F_m, unresponsive stomatal conductance (g_s), and a low photosynthetic capacity (A_{max}). It is not known if the syndrome also occurs in fully developed leaves that are exposed to pure red light after reaching maturity and if initially injured leaves can recover from the syndrome. This study investigates the plasticity leaf photosynthetic apparatus after inducing or releasing the “red light syndrome” in leaves of young cucumber plants. The plants were grown under pure red (R) or mixed red/blue (RB; 70%R) LED light and subsequently exposed to RB (R/RB) and R (RB/R) light (100 μmol PPFD m^{-2} s^{-1}, 16 h photoperiod) or kept at their initial growth spectrum (R/R) and (RB/RB). Acclimation of fully developed leaves was monitored with gas exchange and chlorophyll fluorescence (CF) over a period of 8–10 days after the shift. After switching to RB, R injured leaves recovered from photodamage within 4 days. Photosynthetic capacity (A_{max}) and g_s partly recovered, but did not restrict the net CO_2 assimilation rate at growth irradiance (A_{100}), which increased to the same level as in healthy (RB/RB) leaves. After imposing injurious R to healthy mature leaves, they transiently developed signs of the red light-syndrome: a slightly decreased F_v/F_m and more severely reduced A_{max} and g_s. However, A_{100} did not significantly decrease. CF quenching analysis revealed an potentially harmful increased quantum yield of non-regulated non-photochemical energy loss in PSII under R, which was higher in leaves that developed under R than in leaves that were exposed to R after reaching maturity. We conclude that exposure to pure red light is harmful to photosynthetic systems in both developing and developed leaves of cucumber, but the effect on CO_2 assimilation rate and F_v/F_m is much more severe in developing leaves than in mature leaves at low growth irradiance. Chloroplasts of previously R light injured leaves can recover within a few days after releasing from R light, while stomatal conductance and other (partial) morphologically determined leaf factors do not completely acclimate.

1. Introduction

Light is an indispensable energy source for plant growth and is usually supplied by the Sun. Artificial light, however, is used as an energy source for plants in certain situations, such as in greenhouses at high latitudes where natural sunlight severely limits plant growth in the late autumn – early spring period (Trouwborst et al., 2010), or in growth cabinets for research purposes or in plant factories for urban farming. Many different lamp types are used to generate artificial light for plant growth and most of them produce a broad emission spectrum within the PAR range, although this is often superimposed upon distinct emission lines such as in the case of gas discharge lamps or fluorescent tubes (McCree, 1972b). All lamps have in common that they are spectrally very different from sun light, and this considerably influences plant morphology (Hogewoning et al., 2010a). Today, also light emitting diodes (LEDs)

Abbreviations: A_{100}, net photosynthetic rate at growth irradiance; A_n, net photosynthetic rate; A_{mg}, maximum gross photosynthetic rate; A_{max}, maximum net photosynthetic rate; CF, chlorophyll fluorescence; C_i, internal CO_2 concentration; ETR, electron transport rate; F_v/F_m, maximum quantum efficiency of PSII photochemistry in the dark; F_v'/F_m', maximum quantum efficiency of PSII photochemistry in the light; g_s, stomatal conductance; J_{max}, maximum electron transport rate; LMA, leaf mass per area; N_{org}, organic nitrogen within the leaf ($g\,m^{-2}$); NPQ, non-photochemical quenching; PAR, photosynthetic active radiation; PNUE, photosynthetic nitrogen use efficiency; Q_A, primary quinone acceptor of PSII; q_P, PSII efficiency factor; R_D, dark respiration rate; V_{Cmax}, maximum carboxylation rate; α, initial quantum efficiency; θ, parameter for curvature; Φ_{PSII}, PSII operating efficiency; Φ_{NPQ}, regulated energy dissipation; Φ_{NO}, non regulated energy dissipation including fluorescence emission.

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are considered as viable sources of plant growth light (Hogewoning et al., 2007; Massa et al., 2006, 2008; Trouwborst et al., 2010). In contrast to the broad spectrum light sources LEDs emit light in a narrow wavelength band (typically 25–50 nm half-power bandwidth).

Of particular interest is the influence of wavelength on leaf photosynthesis. Early work on the spectral quantum efficiency for leaf photosynthesis has shown it is highest in the red region of the spectrum (Evans, 1987; Inada, 1976; McCree, 1972a). However, highest instantaneous photosynthesis does not necessarily result in optimal photosynthesis and growth in the long term. Leaves of cucumber plants that were grown under pure red LED-light ($100\,\mu mol\,m^{-2}\,s^{-1}$; 640 nm; R-grown leaves) developed a low dark-adapted F_v/F_m (Hogewoning et al., 2010b), which is indicative for photodamage (Baker, 2008). This 'red light syndrome' is further characterized by unresponsive stomata, a low photosynthetic capacity, low photosynthetic nitrogen use efficiency, a low leaf mass per area and impaired growth (Hogewoning et al., 2010b). Similar results were observed with tomato (unpublished results), but none of these effects occurred in leaves that were grown under mixed red (640 nm) and blue (450 nm) light (RB-grown leaves). It appears therefore that exposure to red light alone during leaf development influences photosynthesis at different functional levels extending from the thylakoid to the whole leaf level. It is unknown if the adverse effects of red light during leaf development are structural and persist after a change in light spectrum or can be partially or completely overcome. Based on how photosynthesis acclimates to changes in light intensity, different extents of acclimation due to changes in light spectrum might be expected at thylakoid and whole leaf level.

The objective of this study was to investigate the plasticity of photosynthesis at different functional levels (thylakoid to whole leaf) in response to the induction and release of the 'red light syndrome' in cucumber. This was done by changing the spectrum of incident light from pure red (640 nm) to mixed red and blue light (640 and 450 nm) on leaves with the 'red light syndrome', and vice versa on healthy leaves (without the 'red light syndrome').

At the thylakoid level we investigated changes in fate of excitation energy in PSII using chlorophyll fluorescence (Cailly et al., 1996; Genty et al., 1996; Hendrickson et al., 2004) before, during and after changes in light spectrum to assess changes in energy dissipation between photosynthetic electron transport (Φ_{PSII}) and regulated (Φ_{NPQ}) and constitutive energy dissipation processes (Φ_{NO}). At the leaf level we used gas exchange measurements to determine changes in photosynthetic light- and CO_2-response curves before, during and after changes in light spectrum, and we measured several leaf anatomical parameters.

2. Materials and methods

2.1. Plant material and growth conditions

One week old seedlings (*Cucumis sativus* "Hoffmann's Giganta") were transplanted to a hydroponic growth system in a climate chamber as described in Hogewoning et al. (2010b) and further grown horizontally to avoid shading of older by younger leaves. Immediately after transplanting, the plants were subjected to the following light treatments: 100% red LED (638 nm dominant wavelength) light (R) or a mixture of 70% red and 30% blue LED (450 nm dominant wavelength) light (RB) to allow full leaf development under distinct different light spectra. After three weeks, when the second leaves were fully expanded, half of the plants per light treatment were changed to the other light spectrum, resulting in 4 light treatments: 2 with a distinct change in spectral composition (RB/R and R/RB) and 2 controls (R/R or RB/RB). Photosynthetic photon flux density (PPFD) and duration of photoperiod were the same for all light treatments: $100 \pm 5\,\mu mol\,m^{-2}\,s^{-1}$ for 16 h a day (Li-190, Li-Cor Inc., Lincoln, NE, USA). Temperature and relative humidity inside the climate room were respectively 25 °C and 70%.

2.2. Gas exchange and chlorophyll fluorescence measurements

Measurements on the second fully expanded leaf started on the day at which half of the plants were subjected to the change in light spectrum (day 0). Photosynthetic irradiance–response curves were repeatedly measured over a time course of 10 days with a portable gas analyzer (LI-6400 with fluorescence head and standard LED-irradiance light source; Li-Cor Inc., Lincoln, NE, USA). Photosynthetic CO_2–response (A–C_i) curves were measured on the 1st and the 7th or 8th day of this period. The blue to red ratio of the actinic light in the clamp-on leaf chamber was always set equal to the growth irradiance of the particular leaf subjected to measurements. However above $900\,\mu mol\,m^{-2}\,s^{-1}$ the blue light fraction in the Li-Cor CF measuring head gradually decreased from 30% to 18% at $1600\,\mu mol\,m^{-2}\,s^{-1}$ due to limitations of the blue light source in the leaf chamber. Leaf chamber temperature, air flow speed and the CO_2-concentration were set at 25 °C, $250\,\mu mol\,s^{-1}$, and $380\,\mu mol\,mol^{-1}$ respectively. Air humidity in the leaf chamber was kept similar as during growth. Photosynthetic rates (including correction for diffusion leaks) and chlorophyll fluorescence measurements were determined as described in Trouwborst et al. (2011).

3. Determinations of leaf parameters

At the start and the 7th and 8th day of the experiment, leaf samples were taken to measure chlorophyll, Leaf mass per area (LMA) and organic nitrogen (N_{org}) as described in Trouwborst et al. (2010). The leaf absorptance spectrum was measured in single nanometer steps according to Hogewoning et al. (2010b), and the quantum flux absorbed by the leaf was calculated by multiplying the absorptance spectrum by the growth-light spectrum.

3.1. Calculations and statistics

Maximum PSII efficiency in light (F_v'/F_m'), PSII operating efficiency (Φ_{PSII}), PSII efficiency factor (q_P) (Baker, 2008) and the electron transport rate (ETR) at growth light level were calculated according to Baker et al. (2007). Fo′ was calculated according to Oxborough and Baker (1997). For the calculation of ETR, we assumed an excitation balance of 0.5 and corrected for the measured leaf absorption and Φ_{PSII}. The energy dissipation in PSII was calculated according to Genty et al. (1996) and Cailly et al., (1996). The quantum yield of PSII electron transport is Φ_{PSII}, the quantum yield of regulated thermal dissipation, Φ_{NPQ}, is calculated as $F_s/F_m' - F_s/F_m$ and the quantum yield of non-regulated energy dissipation, Φ_{NO}, is calculated as F_s/F_m (for a more complete consideration of these quantum yields see Kramer et al. (2004), Hendrickson et al. (2004) and Murchie and Harbinson (2014)). A modified version of the Farquhar, Von Caemmerer and Berry (FvCB) model (Farquhar et al., 1980) was fitted to the A–C_i response data. We estimated J_{max} and V_{cmax} normalized to 25 °C using the non-linear fitting procedure NLIN in SAS (release 9.1.3; SAS Institute, Cary, NC, USA) described in detail according to Trouwborst et al. (2011). A non-rectangular hyperbola (Thornley, 1976) was fitted to the photosynthesis irradiance–response data using the non-linear fitting procedure NLIN in SAS to determine dark respiration (R_D), maximum photosynthetic rate (A_{max}), light-limited quantum efficiency of CO_2 assimilation (α) and the scaling constant for the curvature (θ) of the leaves in the different treatments.

All treatments were repeated four times (2–4 plants per replicate). Fisher's LSD was used to make post-hoc multiple