Journal of Photochemistry & Photobiology, B: Biology 165 (2016) 157–162

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Deficiency in phytochrome A alters photosynthetic activity, leaf starch metabolism and shoot biomass production in tomato

Eros Kharshiing *, Shriravi Prasad Sinha

Department of Botany, St. Edmund's College, Meghalaya 793 003, India

ARTICLE INFO

Article history:
Received 15 June 2016
Received in revised form 20 October 2016
Accepted 21 October 2016
Available online 24 October 2016

Keywords:
Photoreceptors
Photosynthesis
Phytochrome A
Tomato

ABSTRACT

Photosynthesis is a key process that promotes plant growth and development. Light provides photosynthetic organisms with a major source of energy to fix carbon dioxide into organic matter. Of the entire visible light spectrum, red/blue light are known to maximise photosynthetic performance and are thus essential for proper growth and development of plants. Red and blue light stimulate synthesis of chlorophyll and orchestrate the positioning of leaves and chloroplasts for optimal utilisation of light, both of which are critical for photosynthesis. The response of plants to external light cues is accomplished via finely tuned complex photoreceptors and signaling mechanisms which enable them to continually monitor light availability and quality for optimal utilisation of light energy towards enhancing their growth. Higher plants contain a suite of photoreceptor proteins that allow them to perceive red, blue/UV-A and UV-B light. Analyses of the *phyA* mutant of tomato deficient in the red-light photoreceptor phytochrome A (phyA), showed reduced photosynthetic activity of isolated chloroplasts along with decreased shoot biomass in adult plants. The regulation of leaf transitory starch in the mutant was also altered as compared to the wild type (cv Moneymaker). Our results suggest a possible role for phyA in these processes in tomato.

1. Introduction

Photosynthesis is a key process that promotes plant growth and development. Light provides photosynthetic organisms with energy to fix carbon dioxide into organic matter and release oxygen as a byproduct. Visible light is the most pivotal source of light energy that drives plant biomass production through photosynthesis. Of the entire visible light spectrum, red and blue light induce maximal photosynthetic activity and are essential for proper growth and development, even though green light has been suggested to drive photosynthesis [51]. Red and blue light stimulate synthesis of chlorophyll [36] and orchestrate the positioning of leaves and chloroplasts for optimal utilisation of light [43,29] both of which are critical for photosynthesis. The response of plants to external light cues is accomplished via finely tuned complex photoreceptors and signaling mechanisms which enable them to continually monitor light availability and quality for optimal utilisation of light energy towards enhancing their growth. In plants, at least four diverse families of photoreceptors have been elucidated, viz.; red/far-red absorbing phytochromes [34], blue/UV-A absorbing cryptochromes [55], and phototropins [7] and UV-B absorbing UVR-8 [42]. These photoreceptor families contain more than one member, encoded by different genes with a high degree of similarity among the individual members of the same family. Higher plants are known to contain multiple phytochromes, three cryptochromes, two phototropins and one UVR8 photoreceptor [32].

One of the most significant influences of the environment on plants is the alteration of their growth and form by light. In natural conditions, plants have to overcome complex changes in light availability and signaling status by adopting pathways that enable them to adapt to such changes. In tomato, alterations in the gene families encoding photoreceptor proteins, drastically affect the developmental responses of the plants [31,53,39,24,47]. Loss-of-function tomato mutants for phytochrome and cryptochrome show longer hypocotyls and reduced anthocyanin production in leaves [31,53,39], while transgenic lines overexpressing the cryptochrome gene *CRY2*, show elevated levels of carotenoids, anthocyanin and chlorophyll [24]. Apart from mutants defective in genes encoding for photoreceptor proteins, chromophore mutants of tomato have also been isolated which are either lacking or deficient in phytochrome and have drastically reduced chlorophyll and anthocyanin levels [33]. Plant photoreceptors have been linked with photosynthetic capacity in Arabidopsis [50,6,16], potato [52,5] and maize [44], while in tomato they are reported to regulate transcripts and proteins involved in photosynthesis [35,20]. However, while information on the role of photoreceptors in photosynthesis of tomato is still scant, there are reports which suggest a role of phytochrome in mobilisation of storage material in germinating tomato seeds [56].

* Corresponding author.
E-mail address: eros.kharshiing@gmail.com (E. Kharshiing).

http://dx.doi.org/10.1016/j.jphotobiol.2016.10.026
1011-1344/

The phytochrome family of photoreceptors influences many of the responses that plants display in response to changes in their light environment [48]. Tomato contains five *PHY* genes that have been designated *PHYA*, *PHYB1*, *PHYB2*, *PHYE*, and *PHYF* [1], which regulate a variety of photomorphogenic responses including germination [2], de-etiolation [53], shade avoidance [10], flowering [9] and fruit development [27]. Given the importance of phyA in regulating multiple aspects of plant growth and development, we examined whether the loss of phytochrome activity would exert any effect on the photosynthetic capacity of tomato. Here we present experimental data on the photosynthetic capacity, biomass production and starch metabolism in the phyA deficient mutant (*phyA*) of tomato harbouring a mutation in the *PHYA* gene [53].

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The tomato (*Solanum lycopersicum*) genotypes used were *phyA* [53] and its isogenic wild type. Seedlings were sown on wet germination papers in the dark for 3 days at 25 °C ± 2 °C. The germinated seeds were transferred to a mixture of organic potting garden soil mix (Pepper Agro, Bangalore, India) and peat (1:3) and grown at 25 ± 2 °C. In all experiments, the plants were maintained under 16 h light and 8 h dark cycles unless mentioned otherwise. Plants were kept in growth chambers illuminated with 80–100 μmol m^{-2} s^{-1} of light supplied from overhead cool white fluorescent tubes (Philips, India) supplemented with yellow light from fluorescent tubes (Philips, India). For all experiments, the tissues used were harvested between 8 and 8.5 h after onset of light. For dark acclimated plants, tissue was harvested at the same time point as for the light treated plants.

2.2. DCPIP (2,6-Dichlorophenol Indophenol) Reduction Assay

The activity of PSII can be measured in vitro using an artificial electron acceptor such as dichlorophenol indophenol (DCPIP) [18]. Fresh, clean, deveined leaves were weighed and ground in buffer containing 150 mM Tris-HCl (pH 8.8), 10 mM EDTA and protease inhibitor cocktail (P9599, Sigma). The extract was filtered through 3 layers of muslin and centrifuged for 5 min at 4 °C at 1000 × *g*. The resulting pellet was suspended in buffer and stored in ice in dark. The assay was carried out in 3 ml cuvettes containing chloroplasts (normalised to 2 mg/ml of chlorophyll), DCPIP (40 μM) and made up to 2.5 ml with buffer containing 100 mM Tris-HCl (pH 7.5), 500 mM sucrose, 20 mM $MgCl_2 \cdot {}_6H_2O$ and 2 mM EDTA. The cuvette was placed in a customized dark chamber under different light quality and quantity and absorbance was measured at 600 nm for a period of 30 min at 3 min intervals. White (WL), red (RL) and blue (BL) light were obtained using white, red (λ_{max}640) and blue (λ_{max}470) light-emitting diodes (Kwality Photonics, Hyderabad, India). Light intensities were measured using a quantum meter fitted with a sensor (Apogee, USA). Chlorophyll concentration of isolated chloroplasts was estimated according to Arnon [3]. Rates of DCPIP photoreduction were calculated according to Dean and Miskiewicz [18].

2.3. Plant Biomass Assay

Shoots (including leaves) and roots from forty-day-old plants grown under 80–100 μmol m^{-2} s^{-1} WL for 2 weeks were harvested, washed, blotted on filter paper and air dried to remove any excess moisture. Plant parts were weighed and dried to constant weight at 45 °C. The constant dried weight was taken a dry biomass.

2.4. Qualitative and Quantitative Analysis of Leaf Starch

3-week-old light grown plants were dark acclimated for 72 h, and subsequently grown in growth chambers supplemented with 100 μmol m^{-2} s^{-1} light for an additional 48 h. Chlorophyll from the leaves was extracted with hot ethanol, leaves cooled to room temperature, stained with aqueous iodine solution (2% KI and 1% iodine), destained and photographed. For quantification of leaf starch, 3-week-old light grown plants were dark acclimated for 72 h followed by light treatment for one week. Leaves were then detached from the plants, dried to constant weight and powdered. 10 mg of powdered leaf material was used for starch estimation. For each sample, interfering pigments were first extracted with 100% acetone followed by centrifugation at 2000 x *g* for 5 min. Sugars were extracted with 80% ethanol followed by centrifugation at 2000 x *g* for 5 min. 5 ml of 1.1% HCl was added to the residue, mixed and heated at 100 °C for 30 min after which volume was diluted to 10 ml with Milli-Q water. 1 ml was used for starch quantification using Anthrone reagent (Hi-Media, Bangalore, India). Starch was quantified by reading absorbance at 630 nm using a BioSpectrometer (Eppendorf, Germany).

2.5. Gene Expression Analysis

Gene expression analysis was performed by semi-quantitative RT-PCR on cDNA synthesized from total RNA isolated from leaves of light-treated plants. Total RNA from leaves was isolated using Trizol reagent (Invitrogen) and cDNA was reversed transcribed from the total RNA (Takara). Transcript levels of the respective genes studied were then determined by PCR with gene specific oligonucleotides (Supplemental Table 1). Each reaction was carried out for 35 cycles. Transcript levels was ascertained by treating dark acclimated one-month-old grown plants with 100 μmol m^{-2} s^{-1} light for 72 h prior to isolation of total RNA. For total RNA isolation, leaves from similar nodes were taken. Tomato *CAC* was selected as internal control gene for expression analyses [19].

2.6. Statistical Analysis

Unpaired student's *t*-test was used to test the difference in starch content between dark and light-grown samples. The dry shoot and root biomass was also compared between dark and light-grown samples using the student's *t*-test. Student's *t*-test for significance was calculated using the GraphPad *t*-test calculator (http://www.graphpad.com/quickcalcs/ttest1.cfm).

3. Results

3.1. Rates of Electron Transport are Reduced in Isolated Chloroplasts of the Tomato phyA Mutant

Photosynthetic electron transport in chloroplasts involves the transfer of electrons from water to $NADP^+$, which is carried out by photosystem II (PSII) and photosystem I (PSI). The activity of PSII is can be measured in vitro using an artificial electron acceptor such as dichlorophenol indophenol (DCPIP) [18]. When isolated chloroplasts of tomato wild type were irradiated with light of different intensities, there was an observable decline in the reduction of DCPIP with decreasing light intensities (Fig. 1a) consistent with earlier reports of reduced rates of electron transport with decreasing light intensities [17]. A similar trend was also observed for isolated chloroplasts of *phyA* (Fig. 1b). In all light conditions tested, *phyA* chloroplasts showed lesser rates of electron transport in comparison to that of the wild type (Fig. 1c–k). Calculation of rates of photoreduction of DCPIP in isolated chloroplasts of wild type and *phyA*, expectedly showed highest rates of DCPIP photoreduction in RL for both wild type and *phyA* (Table 1). However, when compared with wild type, *phyA* chloroplasts showed reduced photoreduction of DCPIP under the light conditions studied. In comparison to dark controls, the isolated chloroplasts of *phyA* also showed lesser fold change in rates of DCPIP photoreduction at all intensities of WL, BL and RL studied, in contrast to that of wild type chloroplasts (Supplemental Table 2),