Plant Physiology and Biochemistry 116 (2017) 48–56

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Monochromatic green light induces an aberrant accumulation of geranylgeranyled chlorophylls in plants

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ARTICLE INFO

Article history:
Received 18 November 2016
Received in revised form
3 May 2017
Accepted 4 May 2017
Available online 6 May 2017

Keywords:
Geranylgeranyl reductase
Chlorophyllide
Phytyl chain
LIL3
Light quality
Chlorophyll

ABSTRACT

Light quality is an important environmental factor affecting the biosynthesis of photosynthetic pigments whose production seems to be affected not only quantitatively but also qualitatively. In this work, we set out to identify unusual pigment detected in leaves of barley (*Hordeum vulgare* L.) and explain its presence in plants grown under monochromatic green light (GL; 500–590 nm). The chromatographic analysis (HPLC-DAD) revealed that a peak belonging to this unknown pigment is eluted between chlorophyll (Chl) *a* and *b*. This pigment exhibited the same absorption spectrum and fluorescence excitation and emission spectra as Chl *a*. It was negligible in control plants cultivated under white light of the same irradiance (photosynthetic photon flux density of 240 μmol m^{-2} s^{-1}). Mass spectrometry analysis of this pigment (ions m/z = 889 $[M-H]^-$; m/z = 949 [M+acetic acid-H]$^-$) indicates that it is Chl *a* with a tetrahydrogengeranylgeraniol side chain (containing two double bonds in a phytyl side chain; Chl a_{THGG}), which is an intermediate in Chl *a* synthesis. In plants grown under GL, the proportion of Chl a_{THGG} to total Chl content rose to approximately 8% and 16% after 7 and 14 days of cultivation, respectively. Surprisingly, plants cultivated under GL exhibited drastically increased concentration of the enzyme geranylgeranyl reductase, which is responsible for the reduction of phytyl chain double bonds in the Chl synthesis pathway. This indicates impaired activity of this enzyme in GL-grown plants. A similar effect of GL on Chl synthesis was observed for distinct higher plant species.

1. Introduction

Light is one of the most important factors controlling plant growth and development, photosynthetic rate, and the synthesis of such important molecules as photosynthetic pigments, secondary metabolites, and even enzymes. Green light is included in the visible light spectrum. Its absorption by leaves is reduced in comparison with other wavelengths of visible light (Terashima et al., 2009). Nevertheless, it is not as low as it has been generally assumed (Sun et al., 1998). On the other hand, green light can penetrate deeply into the plant canopy (in contrast to blue and red lights), where it can be used for photosynthesis (Johkan et al., 2012). It is difficult to describe pure green light responses because spectral sensitivity of phytochromes and cryptochromes overlap into the green region of visible light (Folta and Maruhnich, 2007). It is known, though, that green light affects plant morphology and physiology, including leaf growth, stomatal conductance, and early stem elongation (Johkan et al., 2012). Some studies have examined the effect of green light on plant growth and development, e.g. in barley (Lee et al., 2010), lettuce (Muneer et al., 2014), and tomato (Wu et al., 2014). No evidence exists, however, as to the specific effect of green light on the chlorophyll (Chl) synthesis pathway, and particularly on the regulation of related enzyme and the qualitative composition of produced pigments.

The Chl *a* synthesis pathway proceeds mostly without light.

Abbreviations: PP, photosynthetic pigments; PPFD, photosynthetic photon flux density; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; Chl a_{THGG}, chlorophyll *a* with tetrahydrogengeranylgeraniol; Chl a_{GG}, chlorophyll *a* with geranylgeraniol; GGPP, geranylgeranyl diphosphate; GGR, geranylgeranyl reductase; HPLC, high-performance liquid chromatography; MP, mobile phase; MS, mass spectrometry; WL, white light; GL, green light; LIL3, light-harvesting-like proteins; CD, circular dichroism; MW, molecular weight.

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http://dx.doi.org/10.1016/j.plaphy.2017.05.002
0981-9428/

Protochlorophyllide-to-chlorophyllide conversion is however a light-dependent step (Iñigo et al., 2012; Reinbothe et al., 2010; Schoefs, 1999) followed by phytyl chain connection catalyzed by the enzyme Chl synthase (Tanaka et al., 1999). The final step consists in hydrogenation of geranylgeranyl diphosphate (GGPP) into phytyl diphosphate, driven directly by geranylgeranyl reductase (GGR) (Giannino et al., 2004; Park et al., 2010; Wang et al., 2014). During this process, Chls with different degrees of phytyl chain saturation, namely geranylgeraniol (GG; 4 double bonds), dihydrogeranylgeraniol (DHGG; 3 double bonds), tetrahydrogeranylgeraniol (THGG; 2 double bonds), and Chl *a* (1 double bond in the phytyl chain at position C14 = C15), are formed step by step (Tanaka et al., 2010).

Two pathways leading to final production of Chl *a* have been described so far (Shpilyov et al., 2005). Usually, direct GGPP binding to chlorophyllide occurs, followed by gradual double bond hydrogenation of the phytyl chain (Bollivar, 2006). During these processes, a few intermediates with varying numbers of double bonds are produced. Alternatively, hydrogenation of GGPP to phytyl diphosphate occurs before its binding to chlorophyllide (Shpilyov et al., 2005).

It is known that expression of the gene encoding GGR synthesis is inhibited in dark conditions and stimulated by blue, far-red and mainly red light (Park et al., 2010), increases from morning to evening, decreases at the start of dark, and remains constant through the night (Giannino et al., 2004). Moreover, GGR expression is repressed by ethylene, abscisic acid, and low and high temperature (Liu et al., 2015). GGR regulates not only Chl synthesis but also the synthesis of tocopherols and phylloquinones (Shpilyov et al., 2005; Wang et al., 2014). Giannino et al. (2004) observed high homology of the GGR gene in peach, *Arabidopsis thaliana*, and soybean, which supports the idea that GGR is conserved and has a common ancestor across such various photosynthetic organisms as bacteria, algae, and plants.

In this study, we demonstrate that, although the development of barley leaves under monochromatic green light was not significantly altered regarding CO_2 assimilation efficiency, these plants accumulated a large pool of geranylgeranyl-Chl *a*. This defect in Chl biosynthesis was accompanied by a massive increase in the amount of the GGR enzyme. As we observed similar accumulation of geranylgeranyl-Chl *a* in other plant species, this surprising sensitivity of the GGR enzymatic step to green light appears to be present in another representatives of higher plant species.

2. Materials and methods

2.1. Plant material and cultivation conditions

Spring barley plants (*Hordeum vulgare* L. cv. Bonus) were cultivated in a FytoScope FS130 growth chamber (Photon Systems Instruments, Drasov, Czech Republic) under light emitted by green LEDs (full width at half maximum 35 nm, with maximum at 535 nm) or in HGC 1014 growth chamber (Weiss, Germany) equipped with white light halogen lamps (as a control) at photosynthetic photon flux density (PPFD) of 240 μmol m^{-2} s^{-1} in a 16/8 h light/dark cycle and temperature 22/20 °C. Measurements were made on middle segments of primary barley leaves 7 and 14 days old. To verify the generality of the green light effect, basil (*Ocimum basilicum*), sunflower (*Helianthus annus*), spruce (*Picea abies*), and amaranth (*Amaranthus sp.*) plants were also cultivated under the same conditions. Considering the species-specific cultivation period, the leaves and needles of basil and spruce 21 days old, sunflower 45 days old, and amaranth plants 6 weeks old were used for high-performance liquid chromatography (HPLC) of photosynthetic pigments.

2.2. Spectrophotometric estimation of photosynthetic pigment concentration

An extract of photosynthetic pigments (PP) was prepared in 100% acetone with the addition of a small amount of $MgCO_3$. The extract was centrifuged for 3 min at 3468 RCF (EBA 20, Hettich, Germany) and diluted to a final concentration of 80% acetone. The supernatant was then filtered through a 0.22 μm PTFE filter (Whatman, UK). Pigment concentration was estimated spectrophotometrically using a UV-VIS absorption spectrophotometer (UV/VIS 550, Unicam, UK) according to Lichtenthaler (1987).

2.3. Gas exchange measurements

Light response curves of photosynthetic CO_2 assimilation rate were determined using a Li-6400 open gas-exchange system (Li-Cor Inc., Lincoln, NE, USA) equipped with a 2 × 3 cm broadleaf chamber. Measurements were made on primary barley leaves 14 days old with three leaves clamped in the leaf chamber to cover the entire 6 cm^2 area of the leaf chamber. Light response curves were measured at 380 ppm CO_2 concentration and leaf temperature was kept close to 23 °C. After leaf exposure to saturated red light (670 μmol m^{-2} s^{-1}) for 15–20 min, and once steady state was reached, a light response curve was generated by decreasing PPFD stepwise to zero.

2.4. HPLC-DAD analysis

The PP extracts were analyzed using an Agilent 1200 HPLC-DAD system (Agilent, USA) equipped with a LiChroCART RP-18 (250 × 4 mm, 5 μm) chromatographic column (Merck, Germany). The separation was performed using two mobile phases (MPs): MP A (acetonitrile/methanol/tris, 241/30/1, v/v/v) and MP B (methanol/n-hexane, 4/1, v/v). The separation procedure consisted of a 10 min isocratic elution of MP A followed by a 2 min linear gradient from 100% MP A to 100% MP B and subsequent 8 min isocratic elution of MP B. The column was then flushed with methanol for 5 min and equilibrated for 3 min using MP A. The flow rate was set to 2 ml s^{-1} during the entire separation process. The column was kept at a constant temperature of 22 °C. Compounds were detected at 440 nm and their absorption spectra were recorded within the range of 190–750 nm (with 1 nm resolution). To estimate relative PP quantities in barley leaves, conversion factors published by Färber and Jahns (1998) were used. The qualitative HPLC analysis of PP extracts obtained from other species was performed only to confirm or reject the occurrence of Chl *a* derivative in plants cultivated under green light. Chl *a* and Chl *a* derivative were collected using the fraction collector module of the HPLC system and were used for subsequent spectral analyses (UV-VIS absorption, fluorescence and circular dichroism).

2.5. Optical spectroscopy analyses

Fractions of Chl *a* and Chl derivative were dissolved in methanol:n-hexan solution (4:1, v/v). Absorption spectra were measured using a Specord 250 spectrophotometer (Analytic Jena, Jena, Germany) with a band-pass of 0.5 nm and scanning speed of 120 nm min^{-1}. Chl fluorescence spectra were measured using a Spex Fluorolog 3–22 spectrofluorometer (Horiba Jobin Yvon, Paris, France). The emission spectra were recorded at an excitation wavelength of 436 nm with an integration time of 0.1 s and 5 and 1 nm slit widths of excitation and emission monochromators, respectively. The excitation spectra were measured at an emission wavelength of 725 nm with an integration time of 0.1 s and 2.5 and 5 nm slit widths of excitation and emission monochromators,