Journal of Plant Physiology 167 (2010) 1130–1136

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Sucrose-induced hypocotyl elongation of *Arabidopsis* seedlings in darkness depends on the presence of gibberellins

Yongqiang Zhang, Zhongjuan Liu, Liguang Wang, Sheng Zheng, Jiping Xie, Yurong Bi*

Key Laboratory of Arid and Grassland Agroecology (Ministry of Education), School of Life Sciences, Lanzhou University, Lanzhou Gansu 730000, People's Republic of China

ARTICLE INFO

Article history:
Received 6 November 2009
Received in revised form 14 March 2010
Accepted 15 March 2010

Keywords:
Arabidopsis
Dark
Gibberellin
Hypocotyl elongation
Sucrose

ABSTRACT

In this study, the effects of sucrose on hypocotyl elongation of *Arabidopsis* seedlings in light and in dark were investigated. Sucrose suppressed the hypocotyl elongation of *Arabidopsis* seedlings in light, but stimulated elongation in dark. Application of paclobutrazol (PAC, a gibberellin biosynthesis inhibitor) impaired the effects of sucrose on hypocotyl elongation, suggesting that endogenous GAs is required for sucrose-induced hypocotyl elongation in the dark. Exogenous GA_3 application reversed the repression caused by PAC application, indicating that exogenous GA_3 could substitute, at least partially, for endogenous GAs in sucrose-induced hypocotyl elongation. In addition, we found that *GA 3-oxidase 1* (*GA3ox1*), encoding a key enzyme involved in endogenous bioactive GA biosynthesis, was up-regulated by sucrose in the dark, whereas *GIBBERELLIN INSENSITIVE DWARF 1a* (*AtGID1a*), encoding a GA receptor and playing an important role during GAs degradation to DELLA proteins (DELLAs, repressors of GA-induced plant growth), was down-regulated. These results imply that endogenous bioactive GA levels are expected to be enhanced, but the degradation of DELLAs was inhibited by sucrose in dark. Thus, our data suggest that the sucrose-induced hypocotyl elongation in the dark does not result from GA-induced degradation of DELLAs. We conclude that sucrose can stimulate hypocotyl elongation of *Arabidopsis* seedlings in the dark in a GA-dependent manner.

Introduction

Sugars, the end products of photosynthesis, have a vital signaling function and modulate a range of important processes during plant growth and development, including seed germination, floral transition, fruit ripening, embryogenesis, and senescence (Rolland et al., 2002; León and Sheen, 2003). Responses to sugars in plants are closely integrated with many response pathways of environmental factors, among which, light is one of the important components (Gibson, 2005; Rook et al., 2006). Generally, in the presence of light, high sugar concentration inhibits seedling development, represses expression of photosynthetic genes and induces expression of storage metabolism genes (Rook et al., 2006).

Light is probably one of the most influential environmental cues. It not only provides the source of energy for plant life, but as an informational signal, also affects plant growth and development throughout the entire life cycle from germination to flowering (Lee et al., 2007). The development of plants in the light is referred to as photomorphogenesis, whereas development in the absence of light is referred to as skotomorphogenesis. The latter is characterized by an etiolated appearance of seedlings with a fast-growing hypocotyl or epicotyl, but the growth of the hypocotyl or epicotyl is slowed when light triggers photomorphogenetic development (Alabadí et al., 2004; Chen et al., 2004). The transition between the two development pathways is tightly regulated. Thus far, the CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1)-based complex is considered to play a critical role in the light-dependent repression of photomorphogenesis in the dark (Lee et al., 2007). COP1, as an E3 ubiquitin ligase, can constantly degrade a number of transcription factors that are required for development in light, such as the bZIP transcription factor LONG HYPOCOTYL 5 (HY5), but allows accumulation of others that promote etiolated growth, such as PHYTOCHROME INTERACTING FACTOR 1 (PIF1), PIF3, and PIF4 (Alabadí et al., 2008).

Hypocotyl elongation is one of the most prominent morphological features accompanying dark-triggered etiolation, and in addition to light, endogenous gibberellins (GAs) can also control the hypocotyl elongation (Peng and Harberd, 1997; Vandenbussche et al., 2005; Achard et al., 2007). The production of bioactive GAs in plants involves GA 20-oxidases (GA20ox), GA 2-oxidases (GA2ox)

Abbreviations: AtGID1, *Arabidopsis* GIBBERELLIN INSENSITIVE DWARF 1; COP1, CONSTITUTIVELY PHOTOMORPHOGENIC 1; GA, gibberellin; GA2ox, GA 2-oxidases; GA3ox, GA 3-oxidases; GA20ox, GA 20-oxidases; GAI, GA INSENSITIVE; HY5, LONG HYPOCOTYL 5; HYH, HY5 HOMOLOG; PAC, paclobutrazol; PIF, PHYTOCHROME-INTERACTING FACTOR; RGA, REPRESSOR OF GA1-3; SLY1, SLEEPY1.

* Corresponding author. Tel.: +86 931 8912561; fax: +86 931 8912561.
E-mail address: yrbi@lzu.edu.cn (Y. Bi).

0176-1617/$ – see front matter. Crown Copyright © 2010 Published by Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2010.03.007

and GA 3-oxidases (GA3ox), among which GA2ox can inactivate most active GAs (Yamaguchi, 2008). Previous studies have shown that correct GA homeostasis in etiolated seedlings is essential to properly control the transition between skotomorphogenesis and photomorphogenesis (Vandenbussche et al., 2005; Alabadí et al., 2008). Plants defective in either GA biosynthesis or GA signaling are not able to fully repress photomorphogenesis after germination in the dark, and the seedlings appear to be partially de-etiolated (Achard et al., 2003, 2007; Alabadí et al., 2004, 2008; Vriezen et al., 2004). Thus, GA plays an important role during skotomorphogenetic development in the dark, including dark-induced hypocotyl elongation (Alabadí et al., 2004).

GA signal transduction involves DELLA proteins, which are nuclear growth repressors, a subset of the GRAS family of candidate transcriptional factors (Yamaguchi, 2008). There are five types of DELLAs in *Arabidopsis*. Among these, RGA (encoded by *Repressor of GA1*) and GAI (encoded by *GA Insensitive*) are the main repressors controlling hypocotyl and stem elongation (Achard et al., 2007; Vandenbussche et al., 2007; Feng et al., 2008; Hartweck, 2008; Yamaguchi, 2008). DELLAs restrain plant growth, whereas GAs promote growth by overcoming DELLA-mediated growth restraint (Achard et al., 2007; Hartweck, 2008). GAs relieve DELLA restraint by promoting the degradation of nuclear DELLAs (Silverstone et al., 2001; Fleck and Harberd, 2002). In *Arabidopsis*, GAs are perceived by the GA receptor GIBBERELLIN INSENSITIVE DWARF 1 (AtGID1) (Nakajima et al., 2006). Binding of GA to AtGID1 promotes interaction of AtGID1 with the DELLAs, which promotes interaction with the F-box protein SLEEPY1 (SLY1), polyubiquitination of these proteins by the SCF^{SLY1} ligase complex, and eventual degradation of DELLAs in the 26S proteasome (Nakajima et al., 2006; Hartweck, 2008). It has recently been found that, while light inhibits hypocotyl growth via GA decrease and in turn DELLA accumulation, the induction of hypocotyl elongation in dark is associated with GA accumulation (Achard et al., 2007). Hence, GA is a key component of the light signaling pathway regulating hypocotyl growth (Alabadí et al., 2004; Achard et al., 2007).

In comparison to the repression effect on plant growth in the presence of light (Gibson, 2005), the role of sugar in the dark remains less clear. In this study, we found that sugars stimulated *Arabidopsis* hypocotyl elongation in the dark and that GAs were essential for this process.

Materials and methods

Plant materials and growth conditions

The seeds of *Arabidopsis* wild type Col-0 were surface-sterilized with 20% (v/v) bleach solution for about 13 min. Seeds were extensively rinsed with sterile water and sown on agar medium in Petri dishes with half strength Murashige and Skoog (MS) salts containing 0.8% (w/v) agar and without any sugars. Agar plates were kept at 4 °C in the dark for 3 days, and then transferred to a growth chamber maintained at 23 °C under continuous white light (about 60–70 $\mu mol\, m^{-2}\, s^{-1}$) for 4 days before treatment. For study of hypocotyl elongation, the 4-day-old seedlings were transferred to dark or still kept in light with or without sugars or other chemicals (GA_3, PAC) in the media for the indicated time shown in each figure, and then hypocotyl lengths were measured.

Hypocotyl length measurement

After the indicated time of growth and treatment, at least 25 seedlings were laid horizontally on an agar plate, digital pictures were taken, and hypocotyl length was measured using a standard 4 mm scaled ruler with ImageJ software (Alabadí et al., 2004).

Reverse transcription (RT)-PCR assays

Seedlings were harvested in liquid nitrogen, ground, and RNA extracted using TRIzol reagent (Invitrogen). The amount of mRNA was analyzed using semi-quantitative RT-PCR. The complementary DNA (cDNA) was then synthesized using a random hexamer primer and Moloney murine leukemia virus (M-MuLV) reverse transcriptase (Fermentas) at 42 °C for 60 min. An equal amount of cDNA, estimated using reactions with *ACTIN2* primers, was used as template in the following PCR reaction: 25 μL reaction mixture containing 2 mM $MgCl_2$, 0.2 mM each dNTP, 0.5 μM each gene-specific primer, and 1 units Taq DNA polymerase together with the manufacturer's buffer using the following protocol: 5 min denaturation at 94 °C followed by indicated cycles with each cycle composed of 94 C for 30 s, 57.8, 59.5, 61, 55.5, 55, 53, 53.5 and 60 °C, for genes *ACTIN2*, *GA20ox1*, *GA3ox1*, *AtGID1a*, *AtGID1c*, *SLY1*, *RGA*, and *GAI*, respectively for 30 s, 72 °C for 1 min, and 10 min at 72 °C. PCR products were visualized by electrophoresis on agarose gels containing ethidium bromide. The sequences of the PCR primers used in this study were the following: *ACTIN2*F (5′-GTT GGG ATG AAC CAG AAG GA-3′), *ACTIN2*R (5′-CTT ACA ATT TCC CGC TCT GC-3′); *GA20ox1*F (5′-CAG CCA TTT GGG AAG GTG TATC-3′), *GA20ox1*R (5′-CAA GCA GCT CTT GTA TCT ATC GT-3′); *GA3ox1*F (5′-CCG AAG GTT TCA CCA TCA CTG-3′), *GA3ox1*R (5′-GAG GCG ATT CAA CGG GAC TAA C-3′); *AtGID1a*F (5′-GTT TGG TGG GAA TGA GAG AAC G-3′), *AtGID1a*R (5′-CTA AAC GCC TCA CTG TTC TTC C-3′); *AtGID1c*F (5′-ACC GTC ATC TCG CAG AGT TT-3′), *AtGID1c*R (5′-TCC TTG ACT CAA CCG CTC TT-3′); *SLY1*F (5′-GCG CAG TAC TAC CGA CTCTG-3′), *SLY1*R (5′-CTT AGT GAA ACT CAT CTT CTC G-3′); *RGA*F (5′-CGG AAA CGC GAT TTA TCA GT-3′), *RGA*R (5′-GTC GTC ACC GTC GTT CCT AT-3′); *GAI*F (5′-AGC GTC ATG AAA CGT TGA GTC AGT G-3′), *GAI*R (5′-TGC CAA CCC AAC ATG AGA CAG C-3′).

Results

Induction of hypocotyl elongation by sucrose in the dark

To test the effect of sugars on hypocotyl growth, *Arabidopsis* Col-0 seedlings grown in continuous white light for 4 days (4-day-old seedlings) were treated with or without 90 mM sucrose in the dark or in light. As shown in Fig. 1A and B, the hypocotyls of control seedlings in the dark significantly elongated as treatment time increased, and application of sucrose to the media further stimulated the hypocotyl elongation. For example, the hypocotyl length in the presence of sucrose was 50%, 90%, and 152% longer than that of control at days 1, 2, and 3, respectively (Fig. 1B). These results clearly suggest that sucrose plays a positive role in stimulating hypocotyl elongation in the dark. However, sucrose exhibited a slight repression effect on hypocotyl growth in the light. For example, the hypocotyl length of seedlings in the presence of sucrose was only 85% of that of the control (without sucrose) at day 3 (Fig. 1A and B). In the presence of different concentrations of sucrose, the hypocotyl length of Col-0 seedlings increased when sucrose concentration increased from 0 to 60 mM after growing in the dark for 2 days, while higher concentrations of sucrose (60–150 mM) had almost no effect in terms of increasing hypocotyl length (Fig. 1C). Thus, 90 mM sucrose was sufficient to induce significant hypocotyl elongation in the dark. In addition to sucrose, glucose and fructose also stimulated hypocotyl elongation in the dark, whereas sorbitol had no statistically significant effect on hypocotyl elongation and mannitol repressed hypocotyl elongation (Fig. 2). In addition, all the carbohydrates tested here