ISSN (Print) 1225-9918
ISSN (Online) 2287-3406
Journal of Life Science 2018 Vol. 28. No. 1. 9~16 DOI : https://doi.org/10.5352/JLS.2018.28.1.9

Phytochromes are Involved in the Regulation of Growth and the Gravitropic Response via Ethylene Production in Hypocotyl of *Arabidopsis*

Sang Seung Lee and Soon Young Kim*

Department of Biological Sciences, Andong National University, Andong 760-749, Korea

Received September 25, 2017 / Revised December 12, 2017 / Accepted December 18, 2017

Light is essential to the growth and development of plants, and it is perceived by phytochromes, which are one of the photoreceptors that regulate physiological responses in plants. Ethylene regulates the dormancy, senescence, growth, and development of organs in plants. This research focused on the interaction of phytochromes and ethylene to control hypocotyl growth and gravitropism using phytochrome mutants of *Arabidopsis*, *phyA*, *phyB*, and *phyAB*, under three light conditions: red (R) light, far-red (FR) light, and white light. The mutant *phyAB* exhibited the most stimulation of gravitropic response of all three phytochrome mutants and wild type (WT) in all three light conditions. Moreover, *phyB* in the R light condition showed more negative gravitropism than *phyA*. However, *phyB* in the FR light condition showed less curvature than *phyA*. The hypocotyl growth pattern was similar to the gravitropic response in several light conditions. To explain the mechanism of the regulation of gravitropic response and growth, we measured the ethylene production and activities of *in vitro* ACS and ACO. Ethylene production was reduced in all the mutants grown in white light in comparison to the WT. Ethylene production increased in the *phyA* grown in R light and *phyB* grown in FR light in comparison to the other mutants. The ACS activity coincided with the ethylene production in the *phyA* and the *phyB* grown in R light and FR light, respectively. These results suggest that the Pfr form of phyB in R light and the Pr form of phyA in FR light increased ethylene production via increasing ACS activity.

Key words : *Arabidopsis*, ethylene, gravitropic response, hypocotyl, phytochrome mutant

Introduction

Plants require the environmental stimuli for the growth and development. Among the stimuli, light is one of the important signals to regulate the seed germination, chloroplast development, stomata density in leaf and growth and gravitropism of root in plant survival [2, 3, 4, 6]. Both light and gravity affect the redistribution of auxin to regulate the gravitropic response in plants. Light signal regulates the gravity-induced response, and cause the development of light receptor in plants [6, 9].

Phytochrome, a photoreceptor, regulates the plant growth under R and FR conditions, and they occurs two forms such as Pr and Pfr, which absorbs R and Fr respectively. Two forms of phytochrome can convert to each other under R or FR condition [11, 21]. Phytochrome in *Arabidopsis* is encoded by five member of gene family, and is classified type I including phyA/C and type II including phyB/D/E according to the amino acid sequence similarity [5, 24]. Type I usually found in the etiolated tissue, and the plants grown in light contained both type I and type II equally. Type I was light-labile, and type II was light-stable [5].

Phytochrome A (phyA) was light-labile, and required to response to the continuous far-red light. PhyA regulated the gravitropic response in corn root, and inhibited the elongation of hypocotyl in *Arabidopsis* [13, 25]. On the other hand, phytochrome B (phyB) required to response to the R, and regulated the shade avoidance response based on the ratio of the R and FR [10, 17]. PhyA and phyB regulated the gravitropic response in *Arabidopsis* root [31], and red light-mediated root growth [7]. And Park *et al*. [22] reported that phyB regulated the root growth and gravitropism in *Arabidopsis*.

Ethylene is one of the stress hormones and participates in various plant development and differentiation including fruit ripening and senescence [27]. Ethylene synthesis begins from methionine via two major intermediates, S-adenosylmethionine (AdoMet) and 1-aminocyclopropane-1-carboxylic acid (ACC), in sequence. ACC synthase (ACS) and ACC oxidase (ACO) regulate the steps from AdoMet to ACC and

***Corresponding author**
Tel : +82-54-820-5647, Fax : +82-54-820-7705
E-mail : kimsy@anu.ac.kr

from ACC to ethylene, respectively. Several factors regulate these two enzymes, especially auxin which stimulates the ethylene production through increasing the expression level of the ACS gene [27]. Ethylene regulates the root growth and gravitropism via alteration of auxin transport [16, 23], and phytochrome might regulate ethylene biosynthesis in *Arabidopsis* root [22].

In this research, we examine the role of phytochrome in growth and gravitropic response in several light conditions such as dark, white light, R, and FR in *Arabidopsis* hypocotyl related with ethylene production.

Materials and Methods

Plant material

Seedlings of *Landsberg erecta* (Ler), *Arabidopsis thaliana*, were used as a wild-type (WT), and seedlings of *phyA-201 (PhyA)*, *phyB-1 (PhyB)*, and *phyA-201phyB-5* (*PhyAB*) were used as phytochrome mutants [8, 13].

Growth conditions

Growth conditions were as previously described [31]. *Arabidopsis* seeds were soaked in 70% ethanol for 5 min for sterilization. Seeds were grown on the solid medium containing on-half strength of Murashige and Skoog salts [20] with 1% sucrose, 1% charcoal and 1 mM MES (pH 5.8) in 1% agar in Petri dishes. The dishes containing seeds were treated with 4℃ for 1 day in vertical position. After cold treatment, the dishes were incubated under continuous illumination with cool-white fluorescent lights (approximately 60 μmol/m^2s) at 22℃ for 2 days for germination.

Light sources

For the experiment of shoot growth and gravitropic response, seedlings were grown under the cool-white fluorescent lights (approximately 60 μmol/m^2s), or R (650-680 nm, 24 μmol/m^2s), or FR (710-740 nm, 1.3 μmol/m^2s). Light source of R and FR used Led Plant Radiation System (LPRS, GFPM-1600, Good Feeling, Korea).

Determination of hypocotyl growth and gravitropic curvature

The hypocotyl growth and gravitropic curvature was measured by the modified method of Woo *et al*. [31]. After germination as described above, seedlings were incubated for 1 day for the measurement of shoot growth and gravitropic response under the several light conditions. To eliminate the effect of light, the preparation of seedling were carried out under the green light. And the growth and gravitropic response were monitored in the dark using an infrared camera (Rexsa, DS-400 PC-camera) with the time-interval software (SupervisionCam ver. 3.2.2.4; http://supervisioncam.com). Images were recorded at 15 min intervals. The images were analyzed by UTHSCSA Image Tool Program (ver. 3.0; http://comdent.uthscsa.edu/dig/itdes.html).

Measurement of ethylene production

Ethylene production was measured by the modified method of Woo *et al.* [31]. After germination as described above, seedlings were incubated for 5 day to measure the ethylene production under the several light conditions. To eliminate the effect of light, the preparation of seedling were carried out under the green light. Ethylene production was measured in 10 mm segments including leaves excised from the tips of *Arabidopsis* hypocotyl. The segments were placed in 25 ml, silicon-capped vials containing 200 μl of MES buffer (100 mM, pH 6.8, 50 μg/ml chloramphenicol). The vials were shaken in the dark at 27℃ in an incubator. After incubation, 1-ml of gas sample was withdrawn from the vial with a syringe and injected to the gas chromatograph (HP5890 Series II; Hewlett-Packard, USA) equipped with an alumina column (80/100 Porapak-Q; 1.8-m × 2.1-mm).

Assay of *in vitro* ACC oxidase (ACO) activity

The analysis of in vitro ACO activity was performed as described by Mekhedov *et al*. [19]. Hypocotyl segments were ground with sea sand on ice in 100 mM MES buffer (pH 7.5) containing 10% glycerol, 30 mM sodium ascorbate and 2 mM DTT, and centrifuged at 15,000 rpm at 4℃ for 10 min. The supernatants (200 μl) were transferred to the 25-ml vial, and 800 μl of incubation buffer were added. The incubation buffer contained 50 mM MES buffer (pH 7.5), 10% glycerol, 30 mM sodium ascorbate, 2 mM DTT, 30 mM $NaHCO_3$, 50 μM $FeSO_4$, and 1 mM ACC. The 1-ml gas was withdrawn from the vial after 1 hr incubation at 27℃ to measure the ethylene production as an *in vitro* ACO activity.

Assay of *in vitro* ACC Synthase (ACS) activity

ACS activity was determined by the modified method of Woeste *et al.* [29]. After incubation, hypocotyl segments were ground with sea sand on ice in 250 mM potassium phosphate buffer (pH 8.0) containing 10 μM pyridoxal phosphate,

1 mM EDTA, 2 mM PMSF and 5 mM DTT. Samples were centrifuged at 15,000 rpm for 10 min. The supernatant (200 μl) was incubated for 1 hr with 5 mM AdoMet (0.1 ml) at 22℃. Ethylene production measured from this mixture was used for calculating ACS activity, using a blend of 0.1 ml of 20 mm $HgCl_2$ and 0.1 ml of NaOH/NaOCl (saturated NaOH : 5% NaOCl = 1 : 1 [v/v]) which was incubated on ice for 10 min

Statistical analysis

All experiments were conducted at least three times. To test for significance at p values of <0.05, the data mean values were calculated according to two-way ANOVA test and Duncan test.

Results and Discussion

Relationship between phytochrome and hypocotyl growth

The growth and development of *Arabidopsis* was regulated by the ratio of R:FR in environment [10]. To examine the role of phytochrome to hypocotyl growth according to the light, we used the phytochrome mutants such as *phyA*, *phyB* and *phyAB* under the several light conditions. The hypocotyl growth in the dark did not show the significant difference between WT and mutants (Fig. 1A). The hypocotyl growth of mutants was promoted compared to the WT for 8 hr under the white light (Fig. 1B). The mutant *phyAB* showed the highest growth rate among the mutants, which was about 120% of WT. The growth rate of *phyB* was higher than that of *phyA*. Therefore, the growth rate was in order as follows: *phyAB* > *phyB* > *phyA* > WT.

The results of Fig. 1A and Fig. 1B suggested that the hypocotyl growth require the light to regulate via phytochrome which is a chromophore of R and FR.

To examine the effect of R and FR, we measured the hypocotyl growth under the R (Fig. 1C). The growth of *phyB* and *phyAB* in R was stimulated about 120% of WT and *phyA* at 4 hr. These stimulations continued for 8 hr. However, the growth rate of *phyA* was similar to that of WT for 8 hr. These results suggested that Pfr form of phyB among the phytochrome might be important to regulate the hypocotyl growth under the red light condition. Recently, active phyB (Pfr) reduces IAA levels by the repression of *TTA1* transcript levels [26]. Therefore, the result of Fig. 1C suggested that mutant of phyB such as *phyB* and *phyAB* increase the shoot growth because the auxin level in shoot might increase.

The illumination of FR caused the stimulation of *phyA* and *phyAB* for 8 hr (Fig. 1D). Unlike the results of R (Fig. 1C), *phyB* did not show the stimulation of growth for 8 hr. Therefore, Pr form of phyA could be a role in regulation of hypocotyl growth under the FR condition.

In conclusion, all the phytochrome mutants showed the stimulation of hypocotyl growth in several light illumination compared to the WT. And we suggested that R and FR could regulate the hypocotyl growth via phyB and phyA respectively.

Relationship between phytochrome and gravitropic response

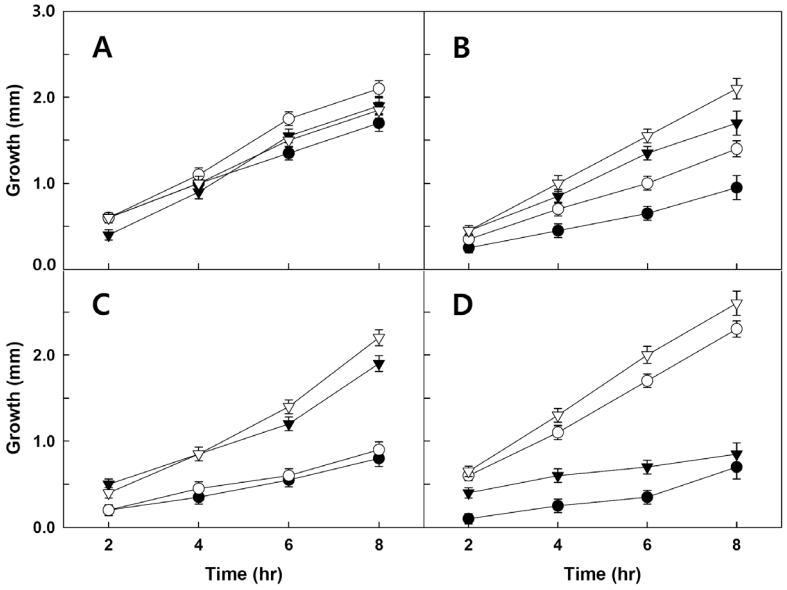

Fig. 1. Hypocotyl growth of phytochrome mutants of *Arabidopsis* grown in several light conditions. After a vernalization for 1 day at 4℃, seeds were germinated under the white light (60 μmol/m^2s) for 2 days. The germinated seeds were grown in the dark (A), white light (B), red light (C), and far-red light (D) for 1 day. The proper number of seedling was placed in petri dish with agar plate under the green light, and the petri dish was placed in vertical position in the dark to measure the growth for 8 hr. The growth was monitored in the dark using an infrared camera as described in Material and Methods. Symbols are mean values ± SE from 5 independent experiments. ●: wild type, ○: *phyA*, ▼: *phyB*, ▽: *phyAB*.

There was an evidence that phytochrome is required to regulated the physiological response such as gravitropism. Bouccalandro *et al*. [2] reported that light promotes the expression of *PHYTOCHROME KINASE SUBSTRATE1* (*PKS1*) in root of *Arabidopsis*. And the negative phototropism and the enhanced PKS1 expression in response to blue light required phytochrome A.

Based on the results of Fig. 1, we detected the hypocotyl gravitropic response of mutants to explain the role of phytochrome in shoot placed in horizontal position under the several light conditions.

Gravitropic response was not affected in the dark the same as the growth of hypocotyl (Fig. 2A). In the white light, gravitropic response of *phyAB* was stimulated about 200% of WT for 8 hr. *PhyA* and *phyB* also exhibit the stimulation of gravitropic response compared to the WT (Fig. 2B). There results were similar to the pattern of hypocotyl growth in the white light (Fig. 1B). These results suggested that shoot growth and gravitropic response might be regulated by the light via phytochrome.

Under the R condition, gravitropic response was stimulated compared to the WT the same as the hypocotyl growth (Fig. 2C). The curvature of *phyAB* and *phyB* was about 55°, and WT and *phyA* was about 20° at 8 hr. According to the Fig 2D, gravitropic response of *phyAB* and *phyA* was stimulated compared to that of *phyB* and WT in the FR. Both curvature of *phyAB* and *phyA* was about 60° when WT and *phyB* was about 20° at 8 hr.

Both hypocotyl growth and gravitropic response was stimulated as the same manner under the R and FR, and even in the dark condition. These data suggested that hypocotyl growth is closely related to the gravitropic response via the conversion from Pr to Pfr of phyA and/or phyB by the R and FR.

Kim, et al. [15] suggested that epidermal phyB inhibits hypocotyl gravitropic response in *Arabidopsis*. They explained phyB promote the degradation of phytochrome interacting factors (PIFs) that negatively regulate various light responses [9]. And the inhibition of hypocotyl negative gravitropism regulated by converting starch-filled gravity-sensing endodermal amyloplasts to other plastids with in red or far-red light. PIFs inhibited the conversion of amyloplast to etioplast in the dark [14]. In this experiment, *phyB* has mutations in the genes for phyB, so *phyB* could not show the inhibition of the negative gravitropism anymore.

Ethylene production in phytochrome mutants in the various light conditions

It has been reported that light and ethylene coordinated the regulation of hypocotyl elongation through microtubule destabilizing protein via PIF3 [18]. And Yu *et al*. [32] reviewed the effect of integration of ethylene and light on the hypocotyl growth in *Arabidopsis*. In this experiment, we measured the ethylene production to investigate the coordination of light and ethylene to understand the role of phytochrome in hypocotyl growth and gravitropism.

Under the white light, ethylene production reduced in the phytochrome mutants compared to the WT (Fig. 3). The reduction of ethylene production of *phyA* and *phyB* was about 13%~30%, and *phyAB* was about 50% of WT. These data sug-

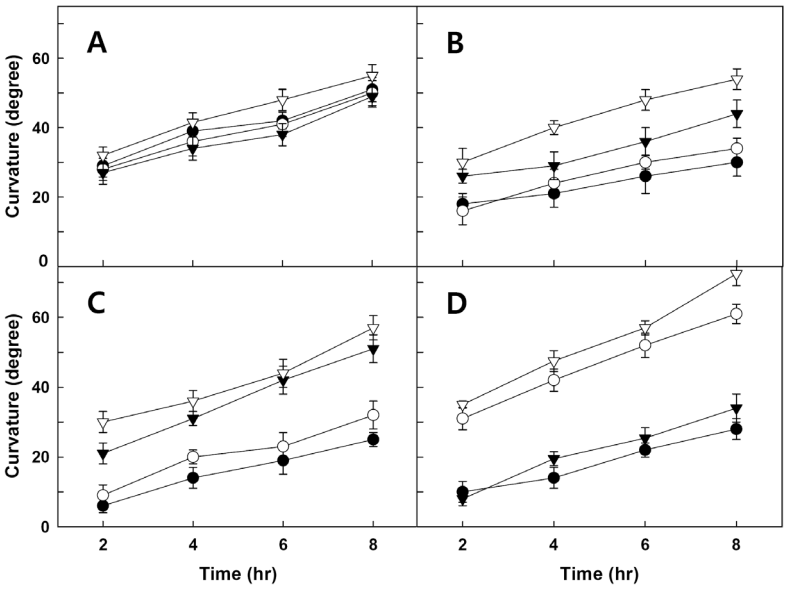

Fig. 2. Hypocotyl gravitropic response of phytochrome mutants of *Arabidopsis* grown in several light conditions. After a vernalization for 1 day at 4℃, seed were germinated under the white light (60 μmol/m^2s) for 2 days. The germinated seeds were grown in the dark (A), white light (B), red light (C), and far-red light (D) for 1 day. The proper number of seedling was placed in petri dish with agar plate under the green light, and the petri dish was placed in horizontal position in the dark to measure the growth for 8 hr. The growth was monitored in the dark using an infrared camera as described in Material and Methods. Symbols are mean values ± SE from 7 independent experiments. ●: wild type, ○: *phyA*, ▼: *phyB*, ▽: *phyAB*.

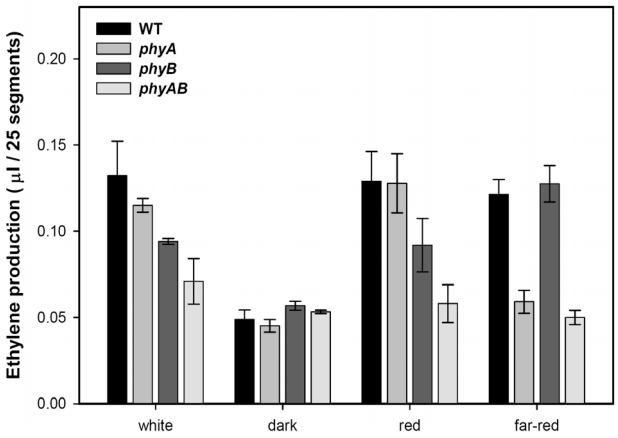

Fig. 3. Ethylene production in phytochrome mutants of *Arabidopsis* hypocotyl grown in several light conditions. After a vernalization for 1 day at 4℃, seeds were germinated under the white light (60 μmol/m^2s) for 2 days. The germinated seeds were grown vertically in several light conditions for 5 day. Hypocotyl segments (10 mm) were collected under the green light to avoid the light effect. The ethylene production was measured at 4 hr incubation as described in Material and Methods. Bars are mean values ± SE from 4 independent experiments.

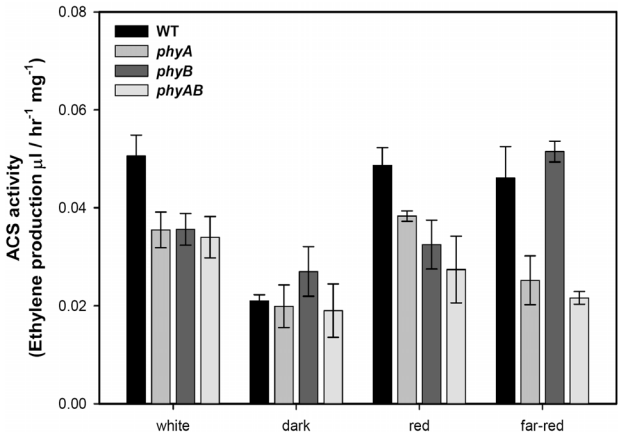

Fig. 4. Measurement of *in vitro* ACS activity in phytochrome mutants of *Arabidopsis* hypocotyl grown in several light conditions. After a vernalization for 1 day at 4℃, seeds were germinated under the white light (60 μmol/m^2s) for 2 days. The germinated seeds were grown vertically in several light conditions for 5 day. Hypocotyl segments (10 mm) were collected under the green light to avoid the light effect. *In vitro* ACS activity was measured as described in Material and Methods. Bars are mean values ± SE from 4 independent experiments.

gested that the phytochrome could affect the ethylene production in hypocotyl of *Arabidopsis*.

To identify the interaction between phytochrome and the ethylene production, we measured ethylene biosynthesis in the hypocotyl grown in the dark. It seems that there is no significant difference in ethylene production between WT and mutants (Fig. 3), suggesting that phytochrome might be involved in the ethylene production.

On the other hand, the hypocotyl grown in the R showed the reduction of ethylene production in three mutants compared to the WT (Fig. 3). Among the mutants, *phyAB* exhibited the lowest ethylene production such as 55% of WT. However, ethylene production of *phyA* did not inhibit compared to other mutants. These results suggested phyB might play a role in the regulation of the ethylene production rather than phyA in the R.

The ethylene production in the hypocotyl grown in the FR was reduced in mutants except *phyB* (Fig. 3). This result indicated that the ethylene production in *phyB* increased unlike in the hypocotyl grown in the R. Therefore, ethylene production was regulated by Pr form of phyA in the FR.

In conclusion, ethylene production was regulated by Pfr form of phyB in the R, and Pr form of phyA in the FR. This regulation of phytochrome in ethylene production affected the growth and gravitropic response in hypocotyl of *Arabidopsis*.

To confirm of these results, we measured the *in vitro* ACS activity in phytochrome mutant grown in various light conditions (Fig. 4). The ACS activity did not exhibit the significant difference between WT and mutants in the hypocotyl grown in the dark. However, the ACS activities of all mutants were reduced by 30% of WT in hypocotyl grown in the white light. These results suggested that the ACS activity referred to patterns similar to ethylene production. In mutants grown in the R, the ACS activities were reduced by 20~45% of wild type, and *phyAB* showed the lowest activity among the mutants. In the hypocotyl grown in the FR, *phyA* and *phyAB* exhibited the reduced ACS activity about 50% of WT, even though *phyB* did not reduced the ACS activity. To understand the step regulated by phytochrome, we measured the ACO activities in mutants and WT in several light conditions. And there was no difference in the ACO activity between WT and mutants (Fig. 5). These data suggested that the ethylene production related to phytochrome was regulated via the ACS activity in ethylene synthesis pathway.

It has been reported that the level of ethylene could not regulate the hypocotyl negative gravitropism in tomato [12]. They suggested that ethylene is not a mediator of the negative gravitropic response of tomato shoots. Further, these

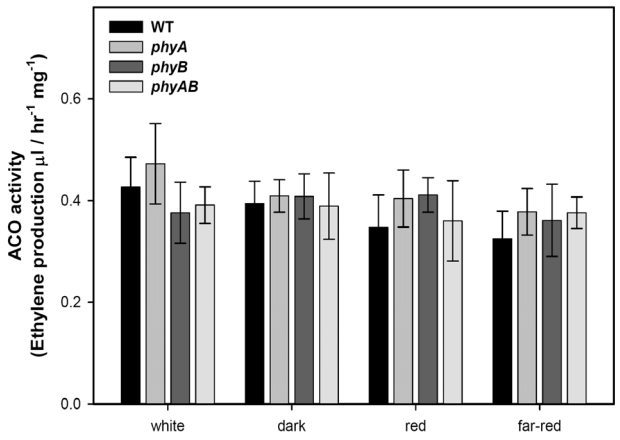

Fig. 5. Measurement of *in vitro* ACO activity in phytochrome mutants of *Arabidopsis* hypocotyl grown in several light conditions. After a vernalization for 1 day at 4℃, seeds were germinated under the white light (60 μmol/m^2s) for 2 days. The germinated seeds were grown vertically in several light conditions for 5 day. Hypocotyl segments (10 mm) were collected under the green light to avoid the light effect. *In vitro* ACS activity was measured as described in Material and Methods. Bars are mean values±SE from 4 independent experiments.

data could not rule out the possibility that low ethylene levels are necessary for full gravitropic response. And some evidences suggested that ethylene may regulate gravitropism in shoot [28]. They reported that the treatment of ethylene inhibitors reduced the initial gravitropic curvature. Woltering *et al*. [30] suggested that one of the ACS genes (*Am-ACS3*) was abundantly expressed in the bending zone cortex at the lower side of the stems within 2 hr of gravistimulation. This implies that ethylene biosynthetic pathway respond to gravistimulation.

Further experiments need to explain that the phytochrome regulates the hypocotyl growth and gravitropic curvature via ethylene production in *Arabidopsis* shoots according to the internal IAA level and the cortical microtubule arrangement, which control the direction of elongation [1].

In these studies, phytochrome mutants such as *phyA*, *phyB*, *phyAB* exhibited the various responses according to the type of light. Under the dark condition, hypocotyl growth and shoot gravitropic curvature did not show any difference between WT and phytochrome mutants. And the phytochrome mutants showed the promotion of growth and gravitropic curvature compared to the WT in the white light. On the other hand, the growth and gravitropic curvature of *phyA* inhibited compared to other mutants in the R. In the FR, *phyB* exhibited the lowest rate of growth and curvature among the mutants. Both in the R and FR, mutants exhibited the promotion of the growth and gravitropic curvature compared to the WT. The ethylene production did not significantly different in mutants and WT grown in the dark. In the hypocotyl grown in the white light, ethylene production of all mutants had reduced over the WT. The ethylene production of *phyA* grown in the R and *phyB* grown in the FR had increased over the other mutants, and the amounts of ethylene production were about the same to WT in the hypocotyl grown in the R or FR. The activity of ACS, converting AdoMet to ACC in ethylene synthesis, coincided with the ethylene production in the *phyA* and *phyB* grown in the R and FR respectively. These results suggested that Pfr form of phyB in the R and Pr form of phyA in the FR increased the ethylene production via increasing ACS activity in the R. This increased ethylene production regulated the hypocotyl growth and gravitropic curvature in *Arabidopsis* shoots.

Acknowledgement

This research was supported by a grant from 2015 Research Funds of Andong National University.

References

1. Bashline, L., Lei, L., Li, S. and Gu, Y. 2014. Cell wall, cytoskeleton, and cell expansion in higher plants. *Mol. Plant* **7,** 586-600.
2. Boccalandro, H. E., De Simone, S. N., Bermann-Honsberger, A., Schepens, I., Fankhause, C. and Casal, J. J. 2008. *PHYTOCHROME KINASE SUBSTRATE1* regulates root phototropism and gravitropism. *Plant Physiol*. **146,** 108-115.
3. Boccalandro, H. E., Rugnone, M. L., Moreno, J. E., Ploschuk, E. L., Serna, L., Yanovsky, M. J. and Casal, J. J. 2009. Phytochrome B enhances photosynthesis at the expense of water-use efficiency in Arabidopsis. *Plant Physiol*. **150,** 1083-1092.
4. Chen, M., Chory, J. and Fankhauser, C. 2004. Light signal transduction in higher plants. *Annu. Rev. Genet*. **38,** 87-117.
5. Clack, T., Mathews, S. and Sharrock, R. A. 1994. The phytochrome apoprotein family in Arabidopsis is encoded by five genes: the sequences and expression of *PHYD* and *PHYE*. *Plant Mol. Biol*. **25,** 413-427.
6. Correll, M. J. and Kiss, J. Z. 2002. Interactions between gravitropism and phototropism in plants. *J. Plant Growth Regul*. **21,** 89-101.
7. Correll, M. J. and Kiss, J. Z. 2005. The roles of phytochromes in elongation and gravitropism of roots. *Plant Cell Physiol*. **46,** 317-323.
8. Devlin, P. F., Robson, R. H., Patel, S. R., Goosey, L., Sharrock,

R. A. and Whitelam, G. C. 1999. Phytochrome D acts in the shade-avoidance syndrome in Arabidopsis by controlling elongation growth and flowering time. *Plant Physiol*. **119,** 909-915.
9. Franklin, K. A. and Quail, P. H. 2009. Phytochrome functions in Arabidopsis development. *J. Exp. Bot.* **61,** 11-24.
10. Franklin, K. A., Praekelt, U., Stoddart, W. M., Billingham, O. E., Halliday, K. J. and Whitelam, G. C. 2003. Phytochromes B, D, and E act redundantly to control multiple physiological responses in Arabidopsis. *Plant Physiol.* **131,** 1340-1346.
11. Franklin, K. A. and Whitelam, G. C. 2004. Light signals, phytochromes and cross-talk with other environmental cues. *J. Exp. Bot*. **55,** 271-276.
12. Harrison, M. and Pickard, B. G. 1986. Evaluation of ethylene as a mediator of gravitropism by tomato hypocotyls. *Plant Physiol*. **80**, 592-595.
13. Hennig, L., Stoddart, W. M., Dieterle, M., Whitelam, G. C. and Schäfer, E. 2002. Phytochrome E controls light-induced germination of Arabidopsis. *Plant Physiol.* **128,** 194-200.
14. Kim, K., Shin, J., Lee, S. H., Kweon, H. S., Maloof, J. N. and Choi, G. 2011. Phytochromes inhibit hypocotyl negative gravitropism by regulating the development of endodermal amyloplast through phytochrome-interacting factors. *Proc. Natl. Acad. Sci. USA* **108**, 1729-1734.
15. Kim, J., Song, K., Park, E., Kim, K., Bae, G. and Choi, G. 2016. Epidermal phytochrome B inhibits hypocotyl negative gravitropism non-cell autonomously. *Plant Cell* **28**, 2270-2785.
16. Kim, S. Y., Kim, Y. K., Kwon, K. S. and Kim, K. W. 2000. Action of malformin A_1 on gravitropic curvature in primary roots of maize (*Zea mays* L.). *J. Plant Biol.* **43**, 183-188.
17. Liscum, E. and Hangarter, R. P. 1993. Genetic evidence that the red-absorbing form of phytochrome B modulates gravitropism in *Arabidopsis thaliana*. *Plant Physiol*. **103**, 15-19.
18. Ma, Q., Wang, X., Sun J. and Mao, T. 2017. Coordinated regulation of hypocotyl cell elongation by light and ethylene through a microtubule destabilizing protein. *Plant Physiol.* **176**, 678-690.
19. Mekhedov, S. L. and Kende, H. 1996. Submergence enhances expression of a gene encoding 1-aminocyclopropane-1-carboxylate oxidase in deepwater rice. *Plant Cell Physiol*. **37,** 531-537.
20. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15,** 473-497.
21. Nagatani, A. 2004. Light-regulated nuclear localization of phytochromes. *Curr. Opin. In Plant Biol*. **7,** 708-711.
22. Park, J. H., Lee, S. S., Woo, S. H. and Kim, S. Y. 2012. Effect of light on root growth and gravitropism response of phytochrome mutants of Arabidopsis. *J. Life Sci*. **22,** 681-686.
23. Ruzicka, K., Ljung, K., Vanneste, S., Podhorska, R., Beeckman, T., Friml, J. and Benkova, E. 2007. Ethylene regulates root growth through effects on auxin biosynthesis and transport-dependent auxin distribution. *Plant Cell* **19**, 2197-2212.
24. Sharrock, R. A. and Quail, P. H. 1989. Novel phytochrome sequences in *Arabidopsis thaliana*: structure, evolution, and differential expression of a plant regulatory photoreceptor family. *Gene Dev.* **3,** 1745-1757.
25. Takano, M., Kanegae, H., Shinomura, T., Miyao, A., Hirochika, H. and Furuya, M. 2001. Isolation and characterization of rice phytochrome A mutants. *Plant Cell*. **13,** 521-534.
26. Tao, Y., Ferrer, J. L., Ljung, K., Pojer, F., Hong, F., Long, J. A. and Li, L. 2008. Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. *Cell* **133,** 164-176.
27. Van de Poel, B., Smet, D. and Van Der Straete, D. 2015. Ethylene and hormonal cross talk in vegetative growth and development. *Plant Physiol*. **169,** 61-72.
28. Wheeler, R. M., White, R. G. and Salisbury, F. B. 1986. Gravitropism in higher plant shoot. IV. Further studies on participation of ethylene. *Plant Physiol*. **82,** 534-542.
29. Woeste, K. E., Ye, C. and Kieber, J. J. 1999. Two Arabidopsis mutants that overproduce ethylene are affected in the post-transcriptional regulation of 1-aminocyclopropane-1-carboxylic acid synthase. *Plant Physiol.* **119,** 521-529.
30. Woltering, E. J., Balk, P. A., Nijenhuis-deVries, M. A., Faivre, M., Ruys, G., Somhorst, D. Philosoph-Hadas, S. and Friedman, H. 2005. An auxin-responsive 1-aminocyclopropane- 1-carboxylate synthase is responsible for differential ethylene production in gravistimulated *Antirrhinum majus* L. flower stems. *Planta* **220,** 403-413.
31. Woo, S. H., Oh, S. E., Kim, J. S., Mullen, J. L., Hangarter, R. P. and Kim, S. Y. 2008. Root gravitropic response of phytochrome mutant (*phyAB*) in *Arabidopsis*. *J. Life Sci.* **18,** 148-153.
32. Yu, Y. and Huang, R. 2017. Integration of ethylene and light signaling affects hypocotyl growth in Arabidopsis. *Front Plant Sci*. **8,** 57. doi: 10.3389/fpls.2017.00057.

초록 : 애기장대의 하배축에서 피토크롬이 생장과 굴중성 반응에 미치는 영향

이상승 · 김순영*
(안동대학교 생명과학과)

피토크롬은 빛을 인지하여 식물의 생장과 발달에 영향을 미치고, 식물호르몬인 에틸렌은 식물의 줄기 뿌리의 생장을 조절한다. 본 연구는 *phyA*, *phyB* and *phyAB*와 같은 애기장대의 피토크롬 돌연변이체를 이용하여 다양한 빛 조건(암소, white light, red light, far red light)에서 하배축의 생장과 굴중성 반응을 측정하였다. 모든 빛 조건에서 돌연변이체 *phyAB*는 다른 돌연변이체와 wild type (WT)보다 생장과 굴중성 반응이 가장 촉진되었다. Red light (R)에서 *phyB*가 *phyA*보다 굴중성 반응이 촉진되었으나 far red light (FR)에서는 *phyB*가 *phyA*보다 굴중성 반응이 억제되었다. 하배축의 생장도 굴중성 반응과 같은 양상으로 조절되었다. 피토크롬의 작용을 설명하기 위하여 에틸렌 생성과 *in vitro* ACS, ACO 활성을 측정하였다. White light에서 돌연변이체보다 WT에서 에틸렌 생성이 촉진되었다. 그러나 R에서 키운 *phyA*와 FR에서 키운 *phyB*에서 에틸렌 생성이 촉진되어 WT와 비슷한 생성량을 보였다. ACS 활성도 에틸렌 생성량의 양상과 일치하였다. 이 결과는 R에서는 phyB의 Pr 형태가, 그리고 FR에서는 phyA의 Pfr 형태가 에틸렌 생성을 조절하여 하배축의 생장과 굴중성 반응을 조절한다는 가능성을 제시한다.