

Root Gravitropic Response of Phytochrome Mutant (*phyAB*) in *Arabidopsis*

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Phytochrome double mutant (*phyAB*) showed the delayed root gravitropic response compared to the wild type (WT) in *Arabidopsis*. After 8 hr of gravistimulation, the gravitropic response of mutant showed 48% of the WT. The delayed response started at 1.5 hr after gravistimulation. And we measured the ethylene production in the root segments of WT and mutant for 12 hr. Ethylene production of mutant decreased about 40% of the WT at 12 hr. This result suggested that the phytochrome might be linked with ethylene production in some way. Generally, ethylene inhibits the growth of plant organs including roots. We measured the root growth rate in the presence of ACC (1-aminocyclopropane-1-carboxylic acid), a precursor of ethylene. And WT showed the inhibition of root growth with ACC, but mutant did not show the inhibition as WT did. To confirm the relationship between the ethylene and gravitropic response, we measured the gravitropic response with ACC. In the presence of 10^{-6} M ACC, WT showed the 37.4% inhibition compared to the control (no ACC), whereas mutant showed the only 6.6% inhibition of control (no ACC). This research suggested the relationship between phytochrome and gravitropic response through an ethylene production.

Key words : Gravitropic response, phytochrome double mutants (*phyAB*), ethylene, *Arabidopsis*, root

Introduction

Plants perceive the stimuli from the environment including gravity and light, and they show the proper growth in response to these stimuli. Roots grow downward to absorb the water and mineral, and shoots grow upward to receive the light for the photosynthesis [11]. This response, called the gravitropism, is caused by the differential growth in root and shoot in response to the gravity. And the phototropism is caused by the differential growth in shoot by light, which grow toward the light in plants. According to the Cholodny-Went hypothesis, tropisms in plants are caused by the lateral redistribution of auxin. Now there are some findings that this redistribution of auxin might be connected the relocalization of the auxin carrier proteins such as PIN3 [10]. Further, another auxin carrier protein (PIN1) showed the relocalization in the blue light induced phototropism, which is regulated by the blue light photoreceptor, phot1 [2].

Gravitropism is a differential growth response that is in-

duced by gravity. The Cholodny-Went hypothesis has proposed that gravicurvature of roots results from growth inhibitors accumulating along the lower side of a horizontally oriented root. Evidence suggests that the growth inhibitor might be auxin [14,22,30]. It is widely accepted that auxin plays the central role in the asymmetric elongation rates observed during gravicurvature [15,27].

In hypocotyl of *Arabidopsis*, the continuous red or far-red light prevented the gravitropic response [26]. This result suggested the light could affect the gravitropic response and conversely, gravity also could regulate the phototropism. Some results reported that red and far-red light could regulate the gravitropic response in rice or maize roots, suggesting that plants regulate the root gravitropism via the photoreceptor [19,31].

The photoreceptor absorbed the red/far-red light is phytochrome in plants. Phytochromes, the light receptor, exists as two photoconvertible isomers, Pr, which absorbs maximally in red light, and Pfr, which absorbs maximally in far-red light [24]. In *Arabidopsis*, the phytochromes are encoded by a small gene family, of which there are five members (PHYA-PHYE) [4]. The expression of these genes is controlled by light and circadian oscillator [29].

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Phytochrome A shows rapid degradation in the light, and thereby accumulates in etiolated seedlings [5]. In contrast, phyB is very stable in the light and present in high level in light-grown tissue. The other phytochromes, phyC, phyD and phyE are light-stable and have complex overlapping and differential roles relative to phyA and phyB [8].

Some experiments showed that phytochrome mutants in *Arabidopsis* were involved in the gravitropic response. For example, *phyA* is the photoreceptor regulating the gravitropic response of maize roots and the elongation of *Arabidopsis* hypocotyls in far-red light controlled [31]. For R-light-regulated processes, both *phyA* and *phyB* are involved in modulating the gravitropic responses of maize roots and the gravitropic orientation and inhibition of elongation of *Arabidopsis* hypocotyls [18,19,25].

Light is one of many environmental stimuli shown to be involved in modifying ethylene production both positively and negatively, with phytochrome implicated in the control of this response in several cases [21,32].

Ethylene is involved in many plant growth and development responses and, with a few exceptions, is generally viewed as playing an inhibitory role in vegetative growth by reducing elongation of both roots and shoots [1]. Exogenous treatment of ethylene clearly reduced gravitropic responses [17,20], whereas others showed no effects [12,14]. In *Arabidopsis* roots, the effect of ethylene on root gravitropism has not been directly reported, but ethylene has been shown to alter root waving, a complex growth process mediated by gravity, nutrient conditions and thigmotropic signals [3].

Therefore, we investigated whether phytochrome could regulate the gravitropic response in *Arabidopsis* using the phytochrome mutants. And to explain the relationship between phytochrome and gravitropic response, we examined the role of ethylene production in phytochrome mutants.

Materials and Methods

Plant material and culture conditions

Seedlings of *Arabidopsis thaliana* of the Landsberg *erecta* (Ler) ecotype were used in these studies. The mutant was *phyA-201phyB-5* as described by Hennig *et al.* [13] and Devlin *et al.* [7]. Seeds were surface sterilized in 70% (v/v) ethanol for 5 min, then rinsed in 95% (v/v) ethanol for 5 min. In all experiments, seeds were sown on to a pre-steri-

lized dish that was placed on top of a growth medium containing one-half strength Murashige and Skoog salts with 1% (w/v) sucrose and 1 mM MES (pH 5.8) in 1% (w/v) agar in 10 mm Petri dishes. The dishes were sealed with Parafilm and placed on edge so that the surface of the agar was vertical. After cold treatment at 4°C for 1 to 2 day, the plates were incubated under continuous illumination with cool-white fluorescent lights (approximately 60 $\mu\text{mol}/\text{m}^2\text{s}$) at 22°C for 4 to 5 day. Seedling were used in experiments when the roots were approximately 1~2 cm in length.

Measurement of ethylene production

Root segments, including the root tip (10 mm), were incubated 23°C in the dark in 25 ml vials sealed with silicon stoppers, using a 0.2 ml MES buffer (100 mM, pH 6.8). An 1 ml gas sample was withdrawn and analyzed with a gas chromatography (Hewlett-Packard, HP5890 series II, USA) that was equipped with an aluminum column and a flame-ionization detector at 280°C.

Measurement of root growth rates and curvature

After selection, seedlings were transferred on top of a growth medium containing one-half strength MS salts with 1% (w/v) sucrose and 1 mM MES (pH 5.8) in 1% (w/v) agar in 10 mm Petri dishes. The dishes supplemented with 1-aminocyclopropan-1-carboxylic acid (ACC) of various concentrations. The seedling in dish was placed in horizontal position in the dark, and growth and curvature were analyzed with a digital imaging system described by Mullen *et al.* [23]. Roots were with infrared illumination (940 nm LED, Radio Shack) and a CCD camera interfaced to a PC with a frame grabber board (Imagination, USA).

Custom software calculated root length by an edge detection algorithm. The same software divided the root into segments based on distance from the tip and calculated the angle of these segments relative to the vertical. Analysis and adjustment of root tip orientation were performed at every 45s.

Results and Discussion

Comparison of gravitropic response between WT and *phyAB*

Phytochrome double mutants (*phyAB*) were used to test the relationship between phytochrome and the gravitropic

response of primary root of *Arabidopsis*. Figure 1 showed the gravitropic response of wild-type and *phyAB*. The seedlings with the primary root was positioned in the vertically for 1 hr in dark to adjust the darkness because the seedling was grown in white lights for 4 to 5 days. Correll and Kiss [6] also reported that *phyAB* showed the delayed gravitropic curvature compared to the WT using a different measuring system from our system. We measured the curvature using custom software at every 45 seconds as described in materials and methods. The gravitropic response of WT and mutant had a latent period of 15 min. Following the latent period, curvature reached 20 degree at 1 hr in both. The rate of gravitropic curvature in WT increased and reached 60 degree at 6 hr. The root of WT showed 52% increased at 6 hr compare to the mutant root. However, the root from *phyAB* had the slower rate than that of WT at the same period. The root curvature of *phyAB* was significantly reduced compared with WT for the time period of 2, 4 and 6 hr. There was the report that the hypocotyls of *Arabidopsis* showed inhibition of gravitropic response by red or far-red light [26]. Therefore, phytochrome may have a role in regulating root gravitropism in *Arabidopsis*.

Measurement of ethylene production in WT and mutant plant

Ethylene, a gaseous plant hormone, is another important mediator of root gravitropism. It inhibits stem and root elongation [1,30]. Altering its production may, in turn, control the elongation of root in maize [14]. Based on these

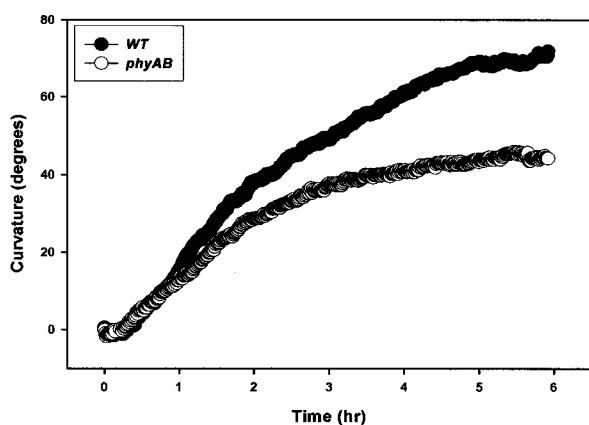


Fig. 1. Gravitropic response of WT and phytochrome mutant in *Arabidopsis* roots for 6 hr. The seedlings were placed in horizontal position in the dark to measure the curvature for 6 hr described in the materials and methods.

facts, we measured the ethylene production in the root segments of WT and *phyAB* for 12 hr (Fig. 2). The difference of ethylene production between WT and *phyAB* started at 2 hr and increased significantly for 12 hr. Ethylene production of *phyAB* decreased compare to the WT, and ethylene production of mutant showed about 60% of the WT at 12 hr. This result suggested that the mutation of phytochrome A and phytochrome B caused the inhibition of ethylene production in root segments of *Arabidopsis*. Foo et al. [9] reported that the *phyAB* mutant developed severe defects in development, including short, thick, distorted internodes and reduced leaf expansion in pea seedlings. They suggested that many defects in *phyAB* mutant plants might be due to elevated ethylene production in pea seedling. Recently, there was evidence that phytochrome may play an important role as coordinator of shoot and root development in *Arabidopsis* [28]. Based on these previous studies, it is possible to explain that the inhibition of gravitropic response of mutant plant might be linked to the reduction of the ethylene production.

Measurement of growth rate

According to our results, we need to measure the root growth rate in vertical position (Fig. 3). The root growth rate of mutant showed 22% inhibition of the WT growth rate. To test a role of ethylene in mutant plant, we applied the 1-aminocycloprpane-1-carboxylic acid (ACC), a precursor of ethylene, to agar plate and compare the root growth rate. The mutant plant showed 25% and 19% inhibition of the

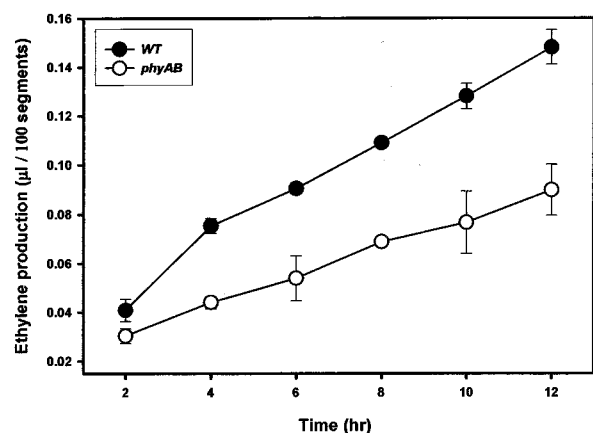


Fig. 2. Ethylene production in WT and phytochrome mutant of *Arabidopsis* for 12 hr. The ethylene production was measured for 12 hr in root segments (10 mm) of phytochrome mutant (*phyAB*) and WT. Bars represent the mean \pm SE. (n=4)

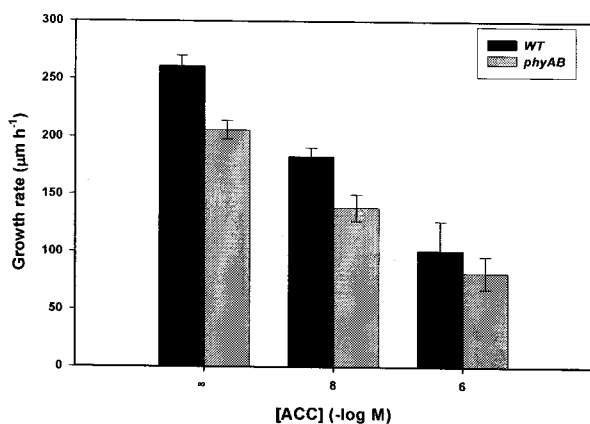


Fig. 3. Effect of exogenous ACC on root elongation in WT and phytochrome mutant seedling. The dishes supplemented with various concentrations of 1-aminocyclopropan-1-carboxylic acid (ACC). The seedling in dish was placed in vertical position in the dark, and growth was analyzed with a digital imaging system described by Mullen *et al.* [23]. Bars represent the mean \pm SE, n=10

WT with 10^{-8} M and 10^{-6} M ACC, respectively (Fig. 3). In general, ethylene affects the growth pattern of plant by reducing the rate of elongation and increasing lateral expansion, leading to swelling of the tissue. These effects of ethylene are common to growing shoots of most dicots, forming part of the triple response [1]. In our experiment with ACC, a precursor of ethylene (Fig. 3), data suggested that the difference of growth rate between mutant and WT reduced in the presence of higher concentration of ACC such as 10^{-6} M ACC. This data means that the reduced inhibition of root growth in mutant with high concentration of ACC might be due to the reduced sensitivity of ethylene compare to the WT.

Measurement of gravitropic response in the presence of ACC

To confirm the result of the role of ethylene in mutant, we measured the root gravitropic response in the presence of ACC. Without ACC application, gravicurvature of mutant showed 26.4% inhibition of the WT, and the inhibition rate of gravicurvature in mutant was 52.8% and 44.2% in the presence of 10^{-8} M and 10^{-6} M ACC, respectively at 2 hr (Fig. 4). That is, the inhibition rate was increased when ACC was applied. When roots were received the gravistimulation for 4 hr, mutant showed 27.4% inhibition of the WT without ACC. However, the mutant exhibited the 37.4% and 6.6% inhibition of the WT with 10^{-8} M and 10^{-6} M ACC, respectively at 4 hr (Fig. 4). This response also could be observed at 6 hr. This data means that the inhibition of gravicurvature in mutant plant could be restore to the gravicurvature of WT in the presence of ACC, a precursor of ethylene. That is, we could not observe any difference between WT and mutant with 10^{-6} M ACC at 4 hr and 6 hr (Fig. 4).

It has been known that ethylene could modify positive curvature in the primary roots of maize by affecting their gravity-induced lateral auxin transport [16]. Kim *et al.* suggested that the increased ethylene reduced the gravitropic response resulted from the lateral auxin transport by ethylene in roots of maize, and proper concentration of ethylene might be required for the regulation of gravitropism in maize root [16]. From these results, we suggested that mutant showed delayed gravicurvature because of lower production of ethylene compared to the WT. Another possibility is that mutant might have lower sensitivity of ethyl-

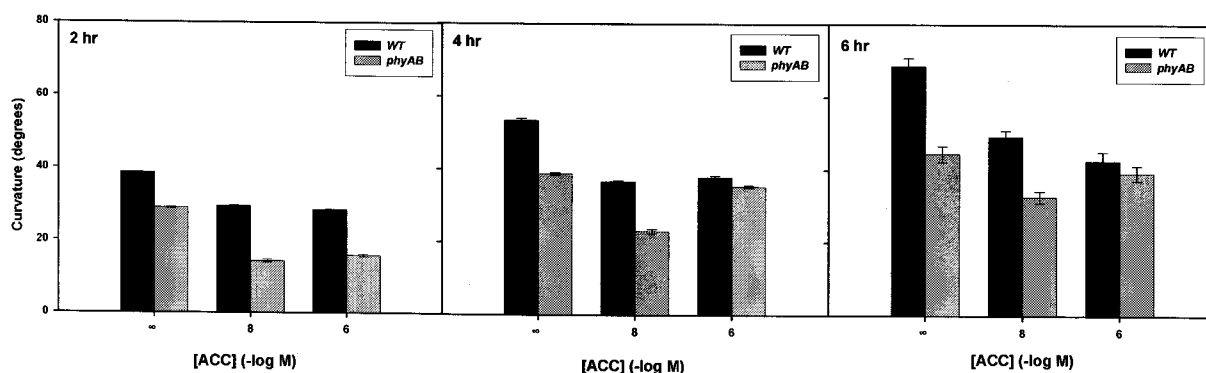


Fig. 4. Effect of exogenous ACC on gravitropic response in WT and phytochrome mutant seedling. The dishes supplemented with various concentrations of 1-aminocyclopropan-1-carboxylic acid (ACC). The seedling in dish was placed in horizontal position in the dark, and curvature was analyzed with a digital imaging system described by Mullen *et al.* [23]. Bars represent the mean \pm SE, n=3

ene compared the WT because we could not observe the any inhibition of gravicurvature with 10^{-6} M ACC compared to the WT.

In conclusion, *phyAB* mutant showed delayed gravitropic response because of lower production ethylene compared to the WT. And these data suggested that the phytochrome, a light receptor in shoot, might play a role to regulate the root gravitropic response through an ethylene production. Further experiments need to do with the inhibitors of ethylene production or action to confirm our suggestions.

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초록 : *Arabidopsis* 피토크롬 돌연변이체 (*phyAB*)의 뿌리 굴중성 반응

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*Arabidopsis*의 피토크롬 2중 돌연변이형 (*phyAB*)은 야생형 (WT)과 비교하여 뿌리의 굴중성 반응이 지연되었다. 중력 자극을 받은 지 8시간 후에 돌연변이체의 굴중성 반응은 야생형의 48%를 나타내었다. 지연된 반응은 중력 자극을 준 후 1.5 시간 뒤에 나타났다. 12시간 동안 야생형과 돌연변이형의 뿌리 절편에서 에틸렌 생성을 측정하였다. 돌연변이형의 에틸렌 생성은 12시간이 경과한 후에 야생형의 40% 정도로 감소되었다. 이러한 결과는 피토크롬이 에틸렌 생성과 연관되어 있음을 제시하고 있다. 일반적으로 에틸렌은 식물의 뿌리나 줄기를 억제한다. 본 연구에서는 에틸렌 전구체인 1-aminocyclopropane-1-carboxylic acid (ACC)를 처리하여 뿌리의 성장을 측정하였다. 야생형은 ACC 존재하에 뿌리 생장이 억제되었으나, 돌연변이형은 야생형만큼 억제를 나타내지 않았다. 이 결과를 확인하기 위하여 ACC 존재 하에 굴중성 반응을 측정한 결과, 야생형은 ACC가 없는 경우와 비교하여 37.4%의 억제를 나타냈으나, 돌연변이형은 ACC가 없는 경우와 비교하여 6.6%만을 억제하였다. 이 결과는 피토크롬이 에틸렌 생성을 통하여 뿌리 굴중성 반응을 조절한다는 것을 제시한다.