

## Effects of Light on the Expression of 1-Aminocyclopropane-1-Carboxylic Acid Synthase and Oxidase Genes in Mung Bean Hypocotyls

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The effects of light on the regulation of ethylene biosynthesis during development of mung bean seedlings were investigated by monitoring the differential expression of seven 1-aminocyclopropane-1-carboxylate (ACC) synthase and two ACC oxidase genes. Among them, only the expression of *VR-ACS1*, *VR-ACS6*, *VR-ACS7*, *VR-ACO1* and *VR-ACO2* was observable in etiolated mung bean hypocotyls. When the seedlings were de-etiolated for 1 d under a light/dark cycle of 16 h/8 h, the expression of *VR-ACS6*, *VR-ACS7* and *VR-ACO2* was controlled negatively by light. The expression of *VR-ACS1* showed a tendency to increase until 6 h after a dark-to-light transition and then decreased at 12 h. On the other hand, the expression of *VR-ACO1* was mostly constitutive up to 12 h after the dark-to-light transition. The opening of hypocotyl hooks during de-etiolation in the light was stimulated by the inhibition of the action of endogenous ethylene in the presence of 1-MCP. These results suggest that the negative regulation of light on the expression of ACC synthase and ACC oxidase genes eventually results in the inhibition of ethylene production with an acceleration of the opening of apical hooks.

**key words:** ethylene, 1-MCP, 1-aminocyclopropane-1-carboxylate, ACC synthase, ACC oxidase, light

### INTRODUCTION

Ethylene, a gaseous major phytohormone, is one of the simplest organic molecules with biological activity and regulates a number of physiological processes during plant growth and development [1]. Ethylene biosynthesis from methionine in higher plants is mediated by three enzymes; methionine adenosyltransferase, 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase. The rate-limiting step in the ethylene production is the conversion of S-adenosylmethionine to ACC by ACC synthase that is encoded by a highly divergent multigene family in a number of plant species [2]. Another key enzyme, ACC oxidase, is also encoded by a small gene family, and recent molecular studies have shown that the specific expression of the ACC oxidase genes plays an important role in the regulation of ethylene biosynthesis, too [3-6].

Among various environmental factors influencing ethylene production, the effect of light has been studied extensively; the light can promote [7-10] or inhibit [11-13] ethylene production depending on the tissue studied. For example, Zacarias et al. [10] reported that the light induced increase of ethylene production was due to the enhancement of ACC oxidase activity in citrus leaves. On the contrary, Gepstein and

Thimann [11] observed that white light inhibits the conversion of ACC to ethylene in tobacco and oat leaves. Furthermore, Kao and Yang [13] showed that the inhibitory effect of light on the ethylene production from ACC was relieved in the presence of CO<sub>2</sub>. Except these, little is known about relationship between the changes of endogenous ethylene and developmental events induced by light.

In mung bean, *VR-ACS1*, *VR-ACS2*, *VR-ACS3* [14], *VR-ACS4*, *VR-ACS5* [15], *VR-ACS6* and *VR-ACS7* [16-17] are presently known to be members of the multigene family of ACC synthase, and *VR-ACO1* and *VR-ACO2* are two known members of the small multigene family of ACC oxidase [4]. In the present work, we studied the temporal regulation of the in vivo expression of the whole known gene family members of ACC synthase and ACC oxidase during development of etiolated mung bean hypocotyls as a model system and the effects of light on this regulation. Based on these results, possible roles of endogenous ethylene on morphological changes of mung bean seedlings in the light will be discussed.

### MATERIALS AND METHODS

#### *Plant Material*

Mung bean (*Vigna radiata* L.) seeds were germinated in vermiculite after imbibition for 6 h in aerated tap water and then grown in darkness at 28°C. After 2 d, etiolated seedlings were illuminated for de-etiolation and kept under a light/dark cycle of

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16 h/8 h at 28°C±2°C. The intensity of light emitted from cool-white fluorescent lamps was kept at 100  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

#### Synthesis of 1-methylcyclopropene (1-MCP)

The synthesis of 1-MCP was carried out according to the method of Sisler and Serek [18]. With a syringe, 5 mL of 1.8 M solution of phenyllithium (Aldrich Chemical, USA) in solvent of 70% cyclohexane and 30% ether was injected into a 15 mL glass vial with a rubber stopper. After a 30 min interval for the relief of the excessive gas pressure in the vial, 0.3 mL of 3-chloro-2-methyl propene (Aldrich Chemical, USA) was injected, and then the 1-MCP produced remained in the solution as its lithium salt. The gaseous 1-MCP was generated from the solution by aqueous neutralization of the lithium derivative. The concentration of 1-MCP was calibrated against ethylene.

#### Treatments of ethylene and 1-MCP

For the treatment of gases, about 10 etiolated mung bean seedlings grown for 2 d after planting were transferred in a 2 L glass jar sealed with a rubber stopper, and the seedlings were treated with gases (1  $\mu\text{L/L}$  1-MCP or 100  $\mu\text{L/L}$  ethylene) by injection through the rubber stopper and the seedlings were kept in the jar for 24 h under the same condition for de-etiolation before taking pictures.

#### Reverse transcription-polymerase chain reaction (RT-PCR) with an internal standard

Total RNA was extracted from mung bean hypocotyls according to the method of Chomezynski and Sacchi [19]. Using 1  $\mu\text{g}$  of the total RNA as a template, the first strand cDNA was synthesized using Reverse Transcription System (Bioneer, Korea) at 42°C. PCR conditions and the primer sequences were the same as described in Yu *et al.* [20].

Oligonucleotide primers for PCR were designed as follows; *VR-ACS1*, 5'-AAAACGCGTTTCATCCCACA-3' and 5'-CATGCATGGTGGATAACGCTT-3'; *VR-ACS2*, 5'-AGTGGT-GATCAATGGGAGGA-3' and 5'-ACAGATCTAGGACAA-CGTCA-3'; *ACS3*, 5'-TCGCAAACCTCAACACATGCT-3' and 5'-CTCGAACAAGAGGTGATGTA-3'; *VR-ACS4*, 5'-AACCAGTCAACGCTCGATAT-3' and 5'-CAAGAAGAC-CCCGGAGAGAT-3'; *VR-ACS5*, 5'-ACGCTTTACTAGTT-CCTACT-3' and 5'-GTTTCTGTATTGGCGACCCT-3'; *VR-ACS6*, 5'-CATTTGATCAGCGACGAGAT-3' and 5'-CCGT-TCCATGGCTAGGTTTA-3'; *VR-ACS7*, 5'-CATTTGATC-AGCGACGAGAT-3' and 5'-CGCGAGACGATAACCGG-AAA-3'; *VR-ACO1*, 5'-ATCAATGACTTGGACTGGGA-3' and 5'-ACCTTGTTCTTTAGCCTGA-3'; *VR-ACO2*, 5'-ATCAATGACTTGGACTGGGA-3' and 5'-CTGGATTGT-AAAACGACGCT-3'.

For the internal standard, a 315-bp fragment from 18S ribosomal RNA was amplified in the same reaction mixture, as specified by the manufacturer (QUANTUM-RNA 18S Internal Standards, Ambion, USA). A 2(10 pmol):8(40 pmol)

ratio of 18S primers to competimers was used.

## RESULTS

#### Temporal regulation of the expression of ACC synthase and ACC oxidase genes in hypocotyls of etiolated mung bean seedlings

The growth of etiolated mung bean hypocotyls was shown in Figure 1A. The hypocotyls of etiolated mung bean seedlings grew very rapidly at 28°C until 4 d after planting. At 4 d, epicotyls began to elongate (data not shown), and the growth rate of the hypocotyls decreased to the contrary. Using hypocotyls at two different growth stages, the expression patterns of all the known gene family members of ACC synthase and ACC oxidase were examined. Among all the 9 members, the expression of *VR-ACS1*, *VR-ACS6*, *VR-ACS7*, *VR-ACO1* and *VR-ACO2* was detectable. Except for the *VR-ACO1*, the expression levels of the ACC synthase and ACC oxidase genes became low at 5 d (Figure 1B). At this time, the

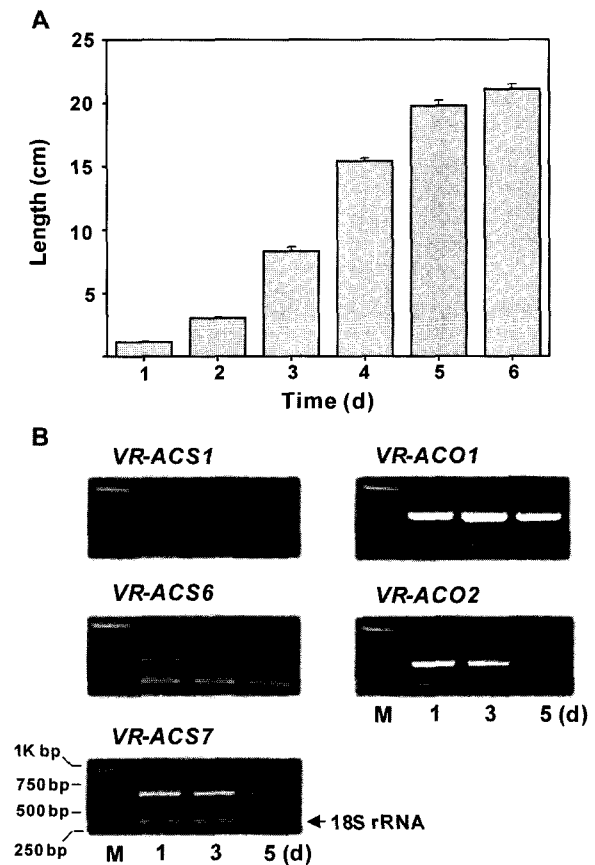


Figure 1. Growth and differential expression of ACC synthase and oxidase genes in the hypocotyls of etiolated mung bean seedlings. A, Growth of etiolated mung bean hypocotyls for 6 d. B, Time-dependent expression of ACC synthase and oxidase genes in the hypocotyls of etiolated mung bean seedlings at 1, 3 and 5 d after planting. M, size marker. In each sample lane of the electrogram, a band for the internal standard of 315 bp is shown as indicated by an arrow in the case of *VR-ACS7*.

expression of *VR-ACS1* and *VR-ACS6* was no longer detectable and that of *VR-ACS7* and *VR-ACO2* dropped significantly. In particular, the expressions of these four genes were disappeared after 6 d coincidentally when the growth of hypocotyl almost stopped (data not shown). It was remarkable that the expression of *VR-ACO1* was relatively constitutive throughout the whole germination period.

*Differential expression of ACC synthase and oxidase genes in hypocotyls of de-etiolated mung bean seedlings*

When seedlings grown for 2 d under dark condition were de-etiolated for 1 d under a light/dark cycle of 16 h/8 h, the hypocotyl growth was inhibited (data not shown), and the expressions of *VR-ACS1* and *VR-ACS6* were disappeared, and those of *VR-ACS7* and *VR-ACO2* were decreased (Figure 2), which was quite different from their expression patterns in etiolated plants in a comparable stage (namely, 3d after planting). Nevertheless, the expression of *VR-ACO1* was



Figure 2. Differential expression of ACC synthase and oxidase genes in de-etiolated mung bean hypocotyls. Seedlings grown for 2 d under dark condition were de-etiolated for 1 d under a light/dark cycle of 16 h/8 h. M, size marker. Lanes 1, 2, 3, 4 and 5 represent *VR-ACS1*, *VR-ACS6*, *VR-ACS7*, *VR-ACO1* and *VR-ACO2*, respectively. In each sample lane of the electrogram, a band for the internal standard of 315 bp is shown as described in Figure 1.

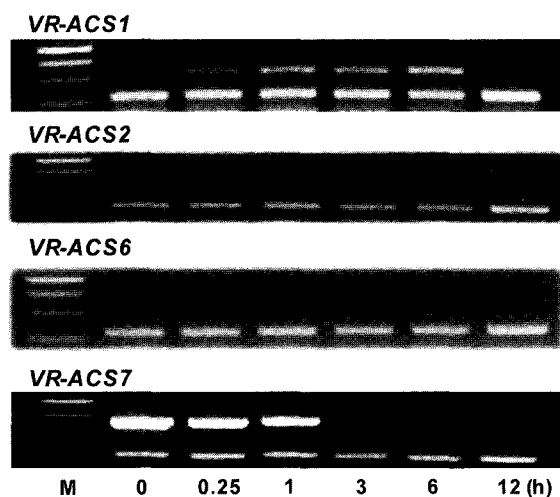


Figure 3. Time-dependent expression of ACC synthase genes in the etiolated hypocotyls of 2 d-old seedlings after dark/light transition for de-etiolation for the designated period of time. Seedlings grown for 2 d under dark condition were de-etiolated for 12 h under a light/dark cycle of 16 h/8 h. M, size marker. In each sample lane of the electrogram, a band for the internal standard of 315 bp is shown as described in Figure 1.

almost constitutive (Figure 2).

*Light-dependent regulation of the expression of ACC synthase and ACC oxidase genes*

Time-dependent expression of ACC synthase genes in the etiolated hypocotyls of 2 d-old seedlings after dark/light transition for de-etiolation was monitored for 12 h (Figure 3). The transcripts accumulation of *VR-ACS6* and *VR-ACS7* in the hypocotyls of etiolated mung bean seedlings is negatively regulated by light. Interestingly, after the start of dark-to-light transition, the expression of *VR-ACS6* dropped rapidly within 15 min, and that of *VR-ACS7* decreased gradually and then disappeared at 12 h. On the other hand, the expression of *VR-ACS1* showed a tendency to increase until 6 h and then decrease at 12 h after the dark-to-light transition, although the expression of *VR-ACS1* was fluctuating. In addition, we could observe a weak expression of *VR-ACS2* at 6 h after the dark-to-light transition (Figure 3).

Figure 4 shows the effect of light on the regulation of *VR-ACO1* and *VR-ACO2* expression. Light inhibited the expression of *VR-ACO2* to a lesser extent when compared with those of *VR-ACS6* and *VR-ACS7*. In contrast to *VR-ACO2*, *VR-ACO1* was almost constitutively expressed for 12 h following the dark-to-light transition.

*Effect of ethylene on the morphology of mung bean seedling during de-etiolation in the light*

To examine the effect of endogenous ethylene on photomorphogenic development, etiolated mung bean seedlings grown for 2 d were treated with ethylene or 1-MCP, ethylene action inhibitor, during de-etiolation. As shown in Figs. 5Aa and 5B, the opening of hypocotyl hooks was stimulated when the action of endogenous ethylene was blocked in the presence of 1-MCP during de-etiolation. During de-etiolation in the light, a typical triple response to exogenous ethylene

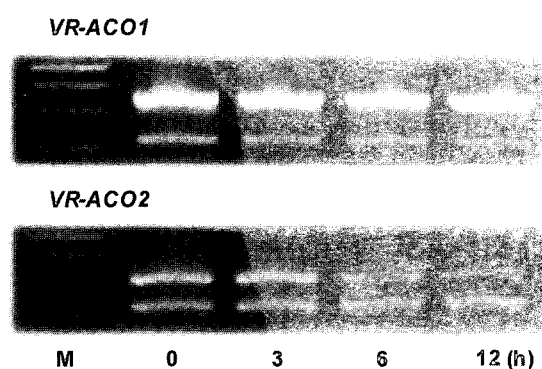


Figure 4. Time-dependent expression of ACC oxidase genes in the etiolated hypocotyls of 2 d-old seedlings after dark/light transition for de-etiolation for the designated period of time. Seedlings grown for 2 d under dark condition were de-etiolated for 12 h under a light/dark cycle of 16 h/8 h. M, size marker. In each sample lane of the electrogram, a band for the internal standard of 315 bp is shown as described in Figure 1.

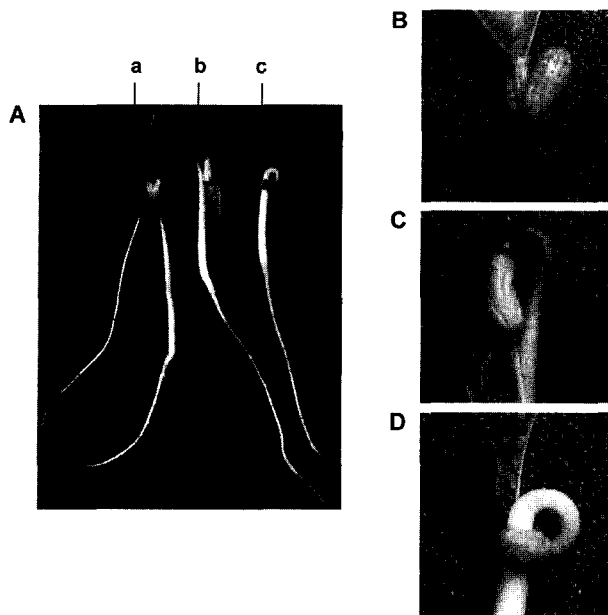


Figure 5. Effect of ethylene on the morphology of mung bean seedlings after dark/light transition for de-etiolation for 24 h. A, Etiolated seedlings grown for 2 d under dark condition were de-etiolated for 24 h under a light/dark cycle of 16 h/8 h along with the treatment of 1-MCP or exogenous ethylene. a and B, treated with 1-MCP for 24 h; b and C, control; c and D, treated with ethylene for 24 h.

was also observed similar to the one observed in darkness (Figs. 5Ac and 5D). The triple response is generally observed in etiolated seedlings and characterized by an inhibition of elongation of the roots and hypocotyls of seedlings, radial expansion of hypocotyls and exaggeration of the curvature of the hooks [21].

## DISCUSSION

In the present work, we showed that the expression of ACC synthase and oxidase genes responsible for the synthesis of endogenous ethylene during development of non-stressed (or intact) mung bean hypocotyls in the light was quite different from their expressions in etiolated plants.

Most of the studies on the differential expression of ACC synthase and oxidase genes in mung bean plants have been carried out using excised mung bean tissues that has been subjected to severe agitation and incubation in a buffered solution for several hours [3-4,14,17,22-23]. Moreover, these studies have been restricted to only some of the reported isogenes. To overcome these limitations, we studied the temporal regulation of the expression of all known members of ACC synthase and ACC oxidase gene family using intact tissues of etiolated mung bean seedlings under development.

Our results demonstrated that expression of *VR-ACS1*, *VR-ACS6*, *VR-ACS7*, *VR-ACO1*, and *VR-ACO2* was detectable in the hypocotyls of etiolated mung bean seedlings. The transcriptional activities of these genes seemed to be strongly

related with the growth period of the hypocotyls. Because the growth rate of hypocotyls was reduced in the light, we determined the effect of light on the expression of ACC synthase and oxidase genes during greening (or de-etiolation) of the etiolated seedlings. Although the effect of light on ethylene production has been studied extensively, most of the published reports focused on the stimulation or inhibition of the conversion process of ACC to ethylene by light. Grodzinski *et al.* [8, 12] and Kao and Yang [13] showed that light inhibition of ethylene production might result from a decrease in CO<sub>2</sub> concentration in the closed flasks. Therefore, the addition of CO<sub>2</sub> was reported to stimulate ACC oxidase activity [24-26] or ACC oxidase synthesis [25]. In addition, light and CO<sub>2</sub> markedly influenced the production of ethylene without significantly changing the endogenous level of ACC. However, in most plant tissues, the level of active ACC synthase governs the rate of ethylene production, and the level of ACC synthase in the cell is regulated primarily at the transcriptional level.

Our results showed that dark-light transitions dramatically affected the expression of both ACC synthase and oxidase genes. The expression of ACC synthase and ACC oxidase genes was negatively regulated by light, except for weak induction of *VR-ACS2* and constitutive expression of *VR-ACO1*. Generally, light is known to accelerate the opening of apical hooks [27], and one of the most possible regulatory factors for this process is ethylene [28]. When the mung bean seedlings were grown in the light, hypocotyl hooks could be opened more rapidly than they were grown in darkness (data not shown). The current data showed that the negative regulation of light on the expression of ACC synthase and ACC oxidase genes eventually resulted in the inhibition of ethylene production with an acceleration of the opening of apical hooks. This concept was supported by the fact that the opening of hypocotyl hooks during de-etiolation in the light was stimulated when the action of endogenous ethylene was blocked by the treatment of 1-MCP.

The high expression of *VR-ACS7* at 24 h after the dark-to-light transition (Figure 2) compared with that observed at 12 h (Figure 3) is probably due to the increase of its expression in darkness, because the sample at 24 h after the dark-to-light transition was experienced dark-period for 8 h after the 16 h light period was ended under a 16 h/8 h light/dark cycle.

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