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Wound-Induced Expression of ACC Synthase Genes in Etiolated Mung Bean Hypocotyls

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After wounding the intact tissues of developing etiolated mung bean hypocotyls, we investigated how the expression of all known members of the ACC synthase gene family was effected. Of the seven members, transcripts of only *VR-ACS1*, *VR-ACS6*, and *VR-ACS7* were detectable in the control (non-stressed) hypocotyls, and their activities were strongly correlated with growth rate. In addition, expression of both *VR-ACS1* and *VR-ACS6* was stimulated by wounding, reaching a peak after about 1 h and sustaining that effect for about 6 h. This induced response did not occur with 5-d-old seedlings, in which transcripts were not normally detected at that stage. When auxin activity and transport were blocked by co-treatment with two inhibitors, TIBA and PCIB, the expression of those two genes was significantly reduced. However, when seedlings were co-treated before being injured, the effect of wounding was not substantially altered. Our results suggest that expression of these two ACC genes in non-stressed hypocotyls is regulated by the endogenous level of auxin. Likewise, transcripts of those genes are stimulated both by wounding and by treatment with auxin, although the two signal transduction pathways are partially independent.

Keywords: ACC synthase, auxin, ethylene, wounding

When plants are subject to stresses, such as wounding, they often exhibit symptoms similar to those seen after ethylene exposure (Denny and Miller, 1935; Morgan and Drew, 1997). In higher plants, ethylene biosynthesis from methionine is mediated by three enzymes -- methionine adenosyltransferase, 1-aminocyclopropane-1-carboxylate (ACC) synthase (ACS), and ACC oxidase (ACO). The rate-limiting step is the conversion of S-adenosylmethionine to ACC by ACC synthase, which is encoded in several species by a highly divergent multigene family (Kende, 1993). Likewise, ACO is encoded by a small family of genes, whose specific expression plays an important role in regulating ethylene biosynthesis (Kim and Yang, 1994; Peck et al., 1998; Jin et al., 1999; Martinez-Madrid et al., 2002). In mung bean, members of the multigene family of ACC synthase include *VR-ACS1*, *VR-ACS2*, *VR-ACS3* (Botella et al., 1992), *VR-ACS4*, *VR-ACS5* (Botella et al., 1993), *VR-ACS6*, and *VR-ACS7* (Kim et al., 1997; Yi et al., 1999). *VR-ACO1* and *VR-ACO2* also belong to a small family of ACC oxidase multigenes (Kim and Yang, 1994).

When plant tissues are injured, ethylene production is usually promoted via differential regulation of individual transcripts from the genes that encode ACC synthase and oxidase (Callahan et al., 1992; Balague et al., 1993; Bekman et al., 2000). Using mung bean as a model system, Kim and Yang (1994) have shown that expression of ACO transcripts is increased by wounding; however, no reports have previously been published concerning the wound-induced expression of ACC synthase transcripts. In the research presented here, we used intact tissues of developing etiolated mung bean hypocotyls to examine the effect of wounding on the expression of all known members of the ACC synthase gene family. We also investigated a possible relationship between the signal transduction pathways for both wounding and auxin activity.

MATERIALS AND METHODS

Plant Material, Chemical-Stress Treatments, and Wounding

After imbibition for 6 h in aerated tap water, seeds of mung bean (*Vigna radiata* L.) were germinated in vermiculite and placed in the dark at 28°C. To examine the expression of ACC synthase genes in non-stressed hypocotyls at each developmental stage, we cut 1.5- to

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Abbreviations: ACC, 1-aminocyclopropane-1-carboxylate; ACS, ACC synthase; ACO, ACC oxidase; RT-PCR, reverse transcription-polymerase chain reaction.

2-, 10- to 12- and 18- to 20-cm-long whole hypocotyls from 1, 3 and 5-d-old seedlings, respectively. To initiate our chemical-stress co-treatments, we sprayed the entire surface of shoots from 1.5-d-old seedlings with a solution containing 100 μM of 2-(4-Chlorophenoxy)-2-methyl-propionic acid (PCIB), an auxin antagonist, and 50 μM of 2,3,5-tri-iodobenzoic acid (TIBA), an auxin-transport inhibitor. For the wounding experiments, we removed 6- to 7-cm-long whole hypocotyls from 2.5-d-old seedlings or 3-cm-long hypocotyls from near the root-hypocotyl junction on 5-d-old seedlings. After cutting the samples into <1-cm pieces, we held these wounded segments for 0.5, 1.0, 3.0, or 6.0 h at room temperature on wet filter paper before proceeding with the analysis.

Reverse Transcription Polymerase Chain Reaction (PCR) with an Internal Standard

Total RNA was extracted from the untreated and previously treated tissues according to the method of Chomezynski and Sacchi (1987). Using 1 μg of total RNA as a template, first-strand cDNA was synthesized by a Reverse Transcription System (Bioneer, Korea) for 1 h at 42°C. The PCR conditions and primer sequences were previously described by Yu et al. (1998). As an 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). The bands were then stained with ethidium bromide. A 2:8 (10:40 pmol) ratio and a 3:7 (15:35 pmol) ratio of 18S primers to Competimers (Ambion) were used for 35 and 25 PCR cycles, respectively.

RESULTS AND DISCUSSION

Expression of ACC Synthase Genes in Intact, Etiolated Mung Bean Hypocotyls

Before applying the wounding stress, we examined the time-dependent expression of all known members of the ACC synthase gene family in etiolated mung bean hypocotyls at three different germination stages after planting (Fig. 1). Of the seven members, expression was detected for only *VR-ACS1*, *VR-ACS6*, and *VR-ACS7*. Transcript levels for these ACC synthase genes gradually decreased in the first 5 d of germination, before completely disappearing for *VR-ACS1* and *VR-ACS6* and dropping significantly for *VR-ACS7*. Expression was no longer detectable for any of the three once hypocotyl growth was nearly finished after 6 d (data not shown).

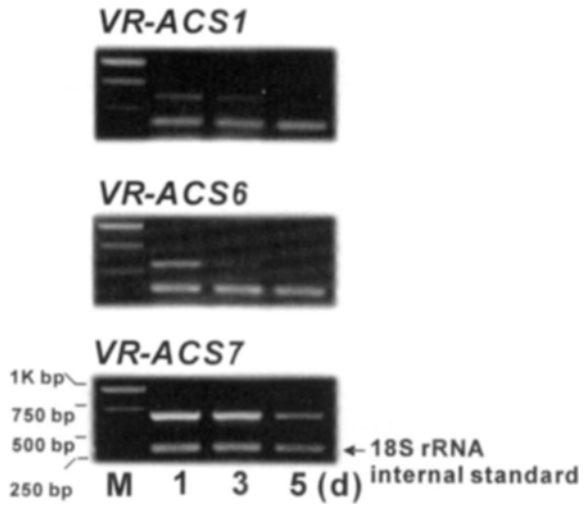

Figure 1. Time-dependent expression of ACC synthase genes in hypocotyls of etiolated mung bean seedlings at 1, 3, and 5 d after planting. M, size marker. In each sample lane of the electrogram, a band for the 315-bp internal standard is indicated by an arrow, as in the case of *VR-ACS7*.

Therefore, we assumed that the transcriptional activities of these genes were strongly correlated with hypocotyl growth rates.

Effects of Wounding on Expression of ACC Synthase Genes in Etiolated Mung Bean Hypocotyls

Expression levels for *VR-ACS1* and *VR-ACS6* decreased after 1 d, and were relatively low by Day 3 (Fig. 1). Wounding stress was applied at 2.5 d, in the middle of this declining period. Afterward, both their expressions increased to maximum levels at about 1 h, and decreased again to faint detection by 6 h (Fig. 2A). In contrast, the expression level of *VR-ACS7*, which was relatively high before stress was induced, was not altered significantly by wounding (Fig. 2A). This was further demonstrated via PCR amplification with fewer cycles (Fig. 2B). Furthermore, no wound-induced alterations were detected in the expression of *VR-ACS2*, *VR-ACS3*, *VR-ACS4*, and *VR-ACS5* (data not shown).

The changes in expression levels for *VR-ACS1* and *VR-ACS6* due to wounding are very similar to the induction kinetics of ethylene reported by Saltveit and Dilley (1978). By cutting etiolated pea seedlings to damage the epicotyl segments, they were able to promote an increase in ethylene production, which was manifested by an initial lag of 26 min, followed by a maximum level of production at 56 min and a decline to a minimum at 90 min.

Because we observed wound-inducible expression

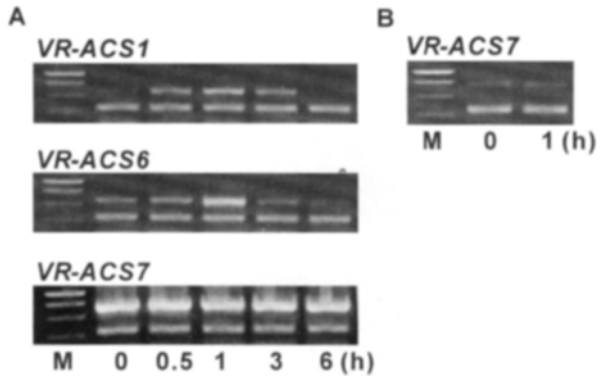

Figure 2. Effects of wounding on expression of ACC synthase genes in hypocotyls of 2.5-d-old etiolated mung bean seedlings. **A,** Differential expression at 5 different time points. **B,** Expression of *VR-ACS7* after PCR amplification with 25 cycles rather than the normal 35 cycles. M, size marker. In each sample lane of the electrogram, a band for the 315-bp internal standard is shown, as described in Figure 1.

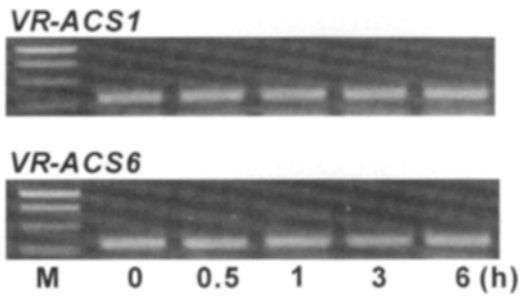

Figure 3. Effects of wounding on expression of *VR-ACS1* and *VR-ACS6* in hypocotyls of 5-d-old etiolated mung bean seedlings. Gene expression was measured at 5 different time points after wounding stress was applied. M, size marker.

of *VR-ACS1* and *VR-ACS6* in the hypocotyls at a stage when expression usually occurred, we decided to examine gene response when transcripts normally were not detectable. We selected the lower portions of 5-d-old hypocotyls because expression at this stage (5 d after planting) was generally barely detectable (Fig. 1), and because their levels were even lower than those measured in the upper portions (data not shown). As shown in Figure 3, wound-induced expression of these genes was not detected or was very weak. Therefore, it was impossible to induce expression of *VR-ACS1* and *VR-ACS6* by wounding in hypocotyls where their expression levels were not detectable.

Relationship between Auxin and Wounding Effects on the Expression of *VR-ACS1* and *VR-ACS6* in Etiolated Mung Bean Seedlings

Auxin acts as an internal stimulus to dramatically

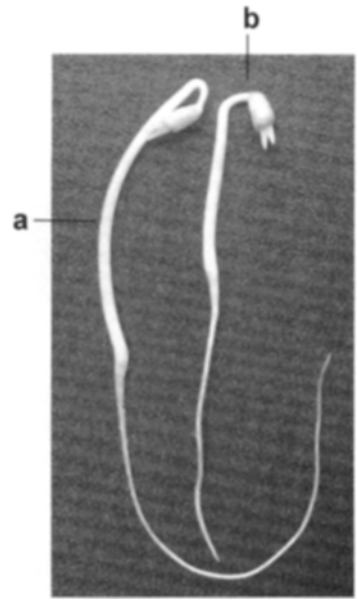

Figure 4. Inhibitory effects of co-treatments with PCIB and TIBA on gravitropism in 2.5-d-old etiolated mung beans. Seedlings were gravistimulated for 2 h. a, control; b, co-treated with PCIB and TIBA for 24 h.

increase the rate of ethylene production in a concentration-dependent manner. This results from an elevation in endogenous ACC synthase activity that is prompted by the activation of transcription of a specific ACC synthase isogene (Nakagawa et al., 1991; Peck and Kende, 1997; Yoon et al., 1997). In mung bean, expression of *VR-ACS1* and *VR-ACS6* is induced by treatment with auxin (Botella et al., 1992; Kim et al., 1992; Yoon et al., 1997). Therefore, to determine whether endogenous auxin played a role in this increase, we co-treated 1.5-d-old etiolated seedlings with PCIB, an antagonist of auxin activity, and TIBA, an auxin-transport inhibitor. This chemical treatment inhibited the development of gravicurvature 2 h after gravistimulation was initiated in the hypocotyls (Fig. 4b). In doing so, we were able to demonstrate an in-vivo means for blocking the endogenous effect of auxin.

Following co-treatment with PCIB and TIBA for 24 h, expression of *VR-ACS1* and *VR-ACS6* was significantly reduced, being nearly undetectable for the former, and very weak for the latter (Fig. 5). These results suggest that their activity in the non-stressed hypocotyls of etiolated mung bean seedlings must be regulated by the endogenous level of auxin. In contrast, when the co-treatment was applied before the tissues were injured, the effect of wounding on the expression of *VR-ACS1* and *VR-ACS6* was not substantially altered, although some minor changes were observed at 3 h (Fig. 6).

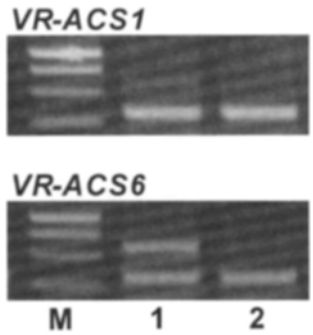

Figure 5. Effects of co-treatment with PCIB and TIBA on expression of *VR-ACS1* and *VR-ACS6* in 2.5-d-old etiolated seedlings. M, size marker. Lanes 1 and 2 are 1.5-d-old controls and plants co-treated for 24 h, respectively.

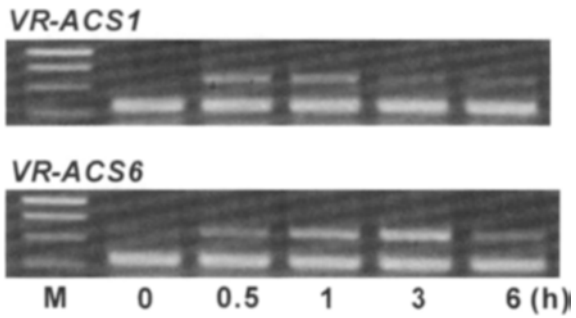

Figure 6. Differential expression patterns of *VR-ACS1* and *VR-ACS6* in wounded hypocotyls of 2.5-d-old etiolated seedlings after co-treatment with PCIB and TIBA for 24 h. Gene expression was measured at 5 different time points after wounding. M, size marker.

Treating undamaged tissues with auxin also results in the accumulation of transcripts in a pattern similar to that seen with wounded tissues (Ebener et al., 1993; Bagyan et al., 1995). However, Cheong et al. (2002) have reported that auxin-responsive genes can also be negatively regulated by wounding. Nevertheless, we propose that the transcripts of our two ACS genes are stimulated both by wounding and by the auxin treatment, and their signal transduction pathways are partly independent.

In conclusion, we have shown here that wounding stimulates the expression of *VR-ACS1* and *VR-ACS6*, reaching a peak after about 1 h, and maintaining that effect for about 6 h. We also suggest that auxin is one of the regulatory factors for this pattern of expression in non-stressed hypocotyls of etiolated mung bean seedlings, and that the signal transduction pathway for the wounding response is, at least partially, independent of that induced by treatment with auxin.

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