Journal of Plant Biology, June 2001, 44(2) : 118-126

Differential Expression of ACC Synthase and ACC Oxidase Genes in Mung Bean Leaves under Saline and Oxidative Stresses

Ju-Dong Song[1], Kyung Hee Choi[2], Young-Jae Eu[1], Tae Hyong Rhew[2], Dong-Hee Lee[3], and Choon-Hwan Lee[1]*

[1]Department of Molecular Biology, Pusan National University, Pusan 609-735, Korea
[2]Department of Biology, Pusan National University, Pusan 609-735, Korea
[3]Department of Biological Science, Ewha Womans University, Seoul 120-750, Korea

We investigated the differential expression of seven ACC synthase and two ACC oxidase genes in mung bean leaves. Among these, only *ACS2*, *ACO1*, and *ACO2* were expressed in etiolated leaves. When seedlings were de-etiolated, the expression level of *ACO1* decreased by 65%, expression of *ACO2* disappeared, while that of *ACS2* was unchanged. For de-etiolated leaves treated with NaCl in the dark, the photochemical efficiency of PSII was not altered, and no genes were newly induced within the first 12 h. However, in the presence of light, transcripts of *ACS1*, *ACS3*, *ACS4*, and *ACS6* were newly accumulated, and the expression levels of *ACS2* and *ACO1* were increased. The kinetics of transcript accumulation in response to methyl viologen (MV) treatment in the light were similar to those observed in the NaCl-treated leaves in the presence of light, suggesting that changes in the latter were caused by oxidative, rather than saline, stress. However, transcripts from most of the genes began accumulating more slowly in MV-treated leaves, with those of *ACS1* and *ACS6* accumulating in much lower amounts than in NaCl-treated leaves. The reverse was true for *ACS4* transcripts.

Keywords: ACC oxidase, ACC synthase, Ethylene, Mung bean, Oxidative stress, Saline stress

Ethylene, a major, gaseous phytohormone, is one of the simplest organic molecules to possess biological activity. It regulates several physiological processes during plant growth and development (Abeles et al., 1992). This hormone is synthesized from S-adenosyl-L-methionine via ACC. In higher plants, its biosynthesis from methionine includes three enzymes: methionine adenosyltransferase, ACC synthase, and ACC oxidase. The rate-limiting step in ethylene production is ACC synthase, which is encoded by a highly divergent multigene family in a number of plant species (Kende, 1993). In contrast, ACC oxidase is encoded by a small gene family, and specific expression of those genes is also important for the regulation of ethylene biosynthesis.

Numerous ACC synthase genes have been identified in several plant species, including tomato (Rottmann et al., 1991; Yip et al., 1992; Lincoln et al., 1993; Olson et al., 1995; Oetiker et al., 1997; Shiu et al., 1998), *Arabidopsis* (van der Straeten et al., 1992; Abel et al., 1995; Liang et al., 1995, 1996; Arteca and Arteca, 1999), potato (Destefano-Beltran et al., 1995; Schlagnhaufer et al., 1997), rice (Zarembinski and Theologis, 1993; van der Straeten et al., 1997), zucchini (Huang et al., 1991), carnation (Park et al., 1992; Henskens et al., 1994), winter squash (Nakajima et al., 1990), and wheat (Subramaniam et al., 1996). In addition, three ACC oxidase genes have been isolated in tomato and melon, with each being expressed differentially in response to various environmental factors (Barry et al., 1996; Lasserre et al., 1996).

The rate of ethylene production increases in tissues undergoing biotic or abiotic environmental stresses (Yang and Hoffman, 1984). Biotic stresses may include viral, bacterial, or fungal infections as well as invasions by insects. Abiotic stresses might be caused by chilling, freezing, heat, flooding, drought, chemicals, radiation, or mechanical strains, e.g., bending, rubbing, or other physical restraints (Abeles et al., 1992). The ethylene elicited by these environmental stresses is often called "stress-ethylene".

Plants exposed to the abiotic stress of high soil salinity modify their metabolism to cope with environmental changes (Greenway and Munns, 1980). For example, when some plants are supplied with excess NaCl in their irrigation water, ethylene production may be either enhanced (Mizrahi, 1982; Botella et al., 2000) or reduced (Chrominski et al., 1986).

Studying how environmental stresses regulate gene

*Corresponding author; fax +82-51-513-9258
e-mail chlee@hywon.pusan.ac.kr

expression of ACC synthase and ACC oxidase helps researchers better understand the molecular mechanisms underlying stress-elicited ethylene production as well as the physiological roles played by "stress-ethylene" in plant responses. In mung bean, *ACS1*, *ACS2*, *ACS3* (Botella et al., 1992), *ACS4*, *ACS5* (Botella et al., 1993), *ACS6*, and *ACS7* (Kim et al., 1997; Yi et al., 1999) are members of a multigene family for ACC synthase. Likewise, *ACO1* and *ACO2* are part of a smaller multigene family for ACC oxidase (Kim and Yang, 1994). Expression of *ACS1*, *ACS6*, and *ACS7* is induced in response to auxin (Botella et al., 1992; Kim et al., 1992; Yoon et al., 1997; Yi et al., 1999). *ACS1* transcripts are also induced through mechanical strains (Botella et al., 1995). Although transcripts of ACC oxidase are constitutively present in all tissues of mung bean seedlings, its expression can be further increased by wounding or ethylene treatment (Kim and Yang, 1994; Jin et al., 1999).

Yu et al. (1998) have investigated the differential expression of all seven ACC synthase and both ACC oxidase genes in mung bean hypocotyls under various stresses. In addition, most studies of the expression of ethylene-synthetic multigene family members have used mung bean hypocotyls, and no one has presented simultaneous analysis of the stress responses for all those genes in the leaf tissue.

Here, the mung bean leaf served as the model system for studying expression of ethylene-synthetic multigene family members. NaCl was applied as an environmental stress. To better understand the molecular mechanisms responsible for regulating gene expression for ethylene synthesis, we investigated the differential expression of all the ACC synthase and ACC oxidase genes in leaves under this saline stress. Because expression patterns differ under light and dark conditions, we also compared the patterns observed in the light with those induced by treatment with an oxidative stress inducer, methyl viologen (MV).

MATERIALS AND METHODS

Seedling Development, and Light/Dark and NaCl Treatments

After imbibition for 17 to 18 h, seeds of the mung bean (*Vigna radiata* L.) were germinated for 2 d in the dark at 30°C, in 1:1:1 vermiculite:peat moss:perlite. Etiolated leaves used were collected at this stage. For de-etiolation, seedlings were grown at 26°C ± 2°C under a light/dark cycle of 16 h/8 h. The light intensity was 70 μmol m^{-2} s^{-1} from a bank of cool white fluorescent lamps. After about 39 h, seedlings of uniform size were selected, and their lower portions were excised at the epicotyl, 2 cm below the node where the primary leaves were attached. The tops of the cuttings were placed vertically in vials containing 15 mL dH_2O. These cuttings were put under lights for 1 h for acclimation; those that started to wilt were discarded. After approximately 8 h of adaptation in darkness, cuttings for use in the stress experiments were treated with 2.5 mM MV or 0.3 M NaCl for 0 - 12 h or 24 h, respectively, either in continuous darkness or in the light, following a 1-h light-adaptation period. Leaf tissues were frozen in liquid nitrogen for later use in measuring chlorophyll fluorescence or performing RT-PCR.

Measurement of Chlorophyll Fluorescence

Using the lower one-third of each leaf, we measured chlorophyll fluorescence with a portable chlorophyll fluorometer (Plant Efficiency Analyzer; Hansatech Instrument, Norfolk, UK), following 10 min of dark adaptation at room temperature. Photochemical efficiency of PSII was deduced from the maximal efficiency of PSII photochemistry, i.e., $F_v/F_m = (F_m - F_o)/F_m$ (Kitajima and Butler, 1975), where F_o is the minimal fluorescence level with all open PSII reaction centers, F_m the maximal fluorescence level with all PSII reaction centers closed, and F_v the variable fluorescence.

RT-PCR with an Internal Standard

We isolated total RNA from the upper two-thirds of each leaf, following the method of Chomezynski and Sacchi (1987). Using 1 μg of total RNA as template, we performed RT with a Reverse Transcription System (Bioneer, Korea) for 1 h at 42°C. The PCR conditions and primer sequences were the same as described by Yu et al. (1998), with 2 μL of the total 20-μL reaction mixture serving as template. 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, Austin, Texas, USA). The bands were stained with ethidium bromide and their intensities were quantified with a densitometer. Expression levels were then graphed relative to the level of the 18S rRNA.

RESULTS

Differential Expression of ACC Synthase and ACC Oxidase Genes in Etiolated and De-Etiolated Leaves

We used RT-PCR to investigate the expression patterns of all the known gene family members of ACC synthase and ACC oxidase in mung bean leaves (Fig. 1). Among these, only *ACS2*, *ACO1*, and *ACO2* were expressed in the etiolated leaves of two-day-old plants (Fig. 1A). When the seedlings were further de-etiolated for 2 d in the light, no genes were newly expressed. The level of *ACO1* decreased by 65%, expression of *ACO2* disappeared, while that of *ACS2* remained unchanged (Fig. 1, B and C).

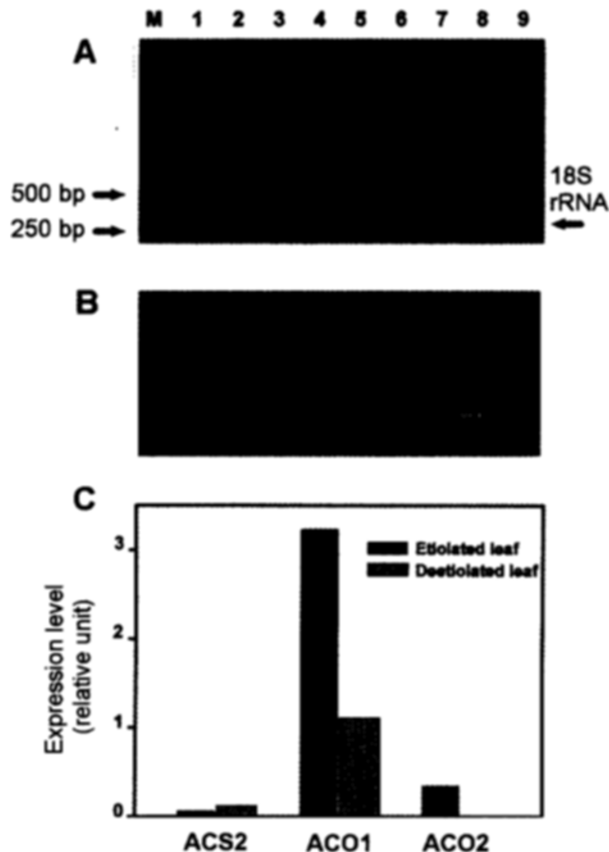

Figure 1. Differential expression of 7 ACC synthase and 2 ACC oxidase genes in etiolated and de-etiolated mung bean leaves under non-stressed conditions. **A**, RT-PCR products from etiolated leaf; **B**, RT-PCR products from de-etiolated leaf; **C**, relative intensities of RT-PCR products compared with endogenous standard 18S rRNA controls; M: marker. Lanes 1, 2, 3, 4, 5, 6, 7, 8, and 9 are *ACS1*, *ACS2*, *ACS3*, *ACS4*, *ACS5*, *ACS6*, *ACS7*, *ACO1*, and *ACO2*, respectively.

Because ethylene production in most plant tissues is usually low (Yang and Hoffman, 1984), *ACS2* may have been the ACC synthase gene responsible for this low level of ethylene evolution. Likewise, under non-stressed conditions, *ACO1* might have caused the ACC oxidase gene to be expressed in the de-etiolated leaves, with *ACO2* contributing in the etiolated plants.

Differential Expression of ACC Synthase and ACC Oxidase Genes under Saline Stress

Chlorophyll fluorescence is a widely used parameter in physiological studies that monitor changes in (potential) photosynthetic activities of plants under environmental stress (Lichtenthaler, 1988; Ogren, 1990). In the current study, photochemical efficiency (Fig. 2) did not decrease when leaves were incubated in 0.3 M NaCl in darkness. However, values gradually decreased throughout the incubation period when plants were treated with NaCl and light.

When leaves were treated with 0.3 M NaCl in darkness, no genes were newly induced within the first 12 h. However, in the presence of light, transcripts of *ACS1*, *ACS3*, *ACS4*, and *ACS6* were newly accumulated, and the expression levels of *ACS2* and *ACO1* also increased (Fig. 3). However, expression of *ACS5*, *ACS7*, and *ACO2* did not noticeably increase

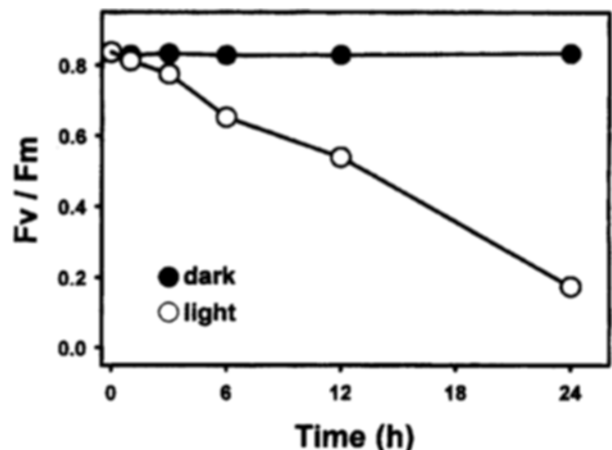

Figure 2. Effect of 0.3 M NaCl on the photochemical efficiency of PSII, or F_v/F_m. After de-etiolation of two-day-old etiolated mung bean leaves under a 16:8, L:D cycle, leaves were treated with NaCl, either in darkness or in the light after 1 h of light acclimation. Chlorophyll fluorescence was measured after 10 min of dark adaptation at 25°C. The lower one-third of each leaf was used for measuring F_v/F_m. Data are from a single, typical experiment that represented at least three independent replications.

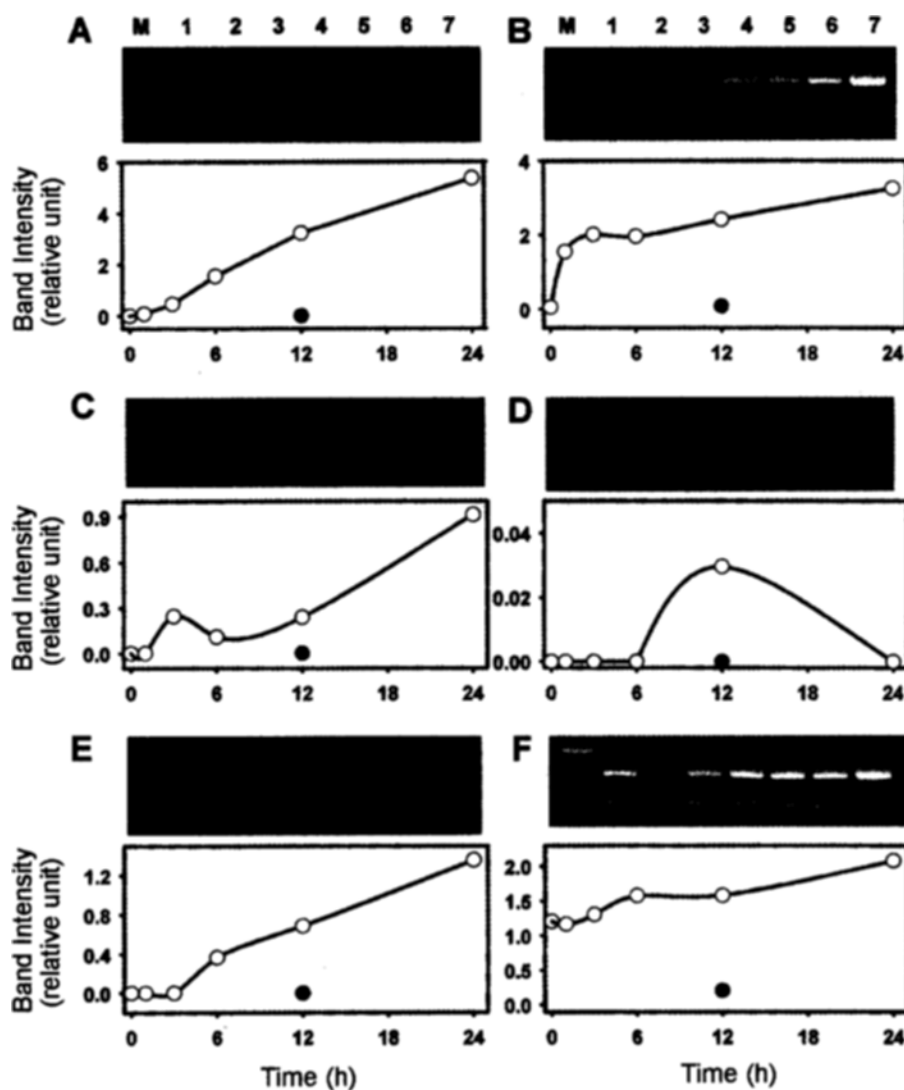

Figure 3. Differential expression of 7 ACC synthase and 2 ACC oxidase genes from the upper two-thirds of 0.3 M NaCl-treated mung bean leaves. **A**, *ACS1*; **B**, *ACS2*; **C**, *ACS3*; **D**, *ACS4*; **E**, *ACS6*; **F**, *ACO1*. M: marker, Lanes 1, 3, 4, 5, 6, and 7 are 0.3 M NaCl-treated leaves for 0, 1, 3, 6, 12, and 24 h in the light, respectively; Lane 2 represents 0.3 M NaCl-treated leaves for 12 h in darkness. Changes in intensities of RT-PCR products relative to the endogenous standard 18S rRNA controls are plotted as a graph, and the relative value for the band in Lane 2 is indicated by a black dot.

(data not shown). Based on the kinetics of transcript accumulation in response to NaCl treatment in the light, the seven members of the ACC synthase gene family could be divided into four groups: 1) *ACS2*, which is transcribed very rapidly, within the first hour; 2) *ACS1* and *ACS3*, with transcripts being accumulated after 1 h; 3) *ACS4* and *ACS6*, expressed only after 3 or 6 h, respectively; and 4) *ACS5* and *ACS7*, neither being expressed within 24 h. In considering the amount of transcript accumulated within 24 h, those from *ACS1* and *ACS2* were most abundant, *ACS4* the least abundant, and *ACS3* and *ACS6* in the middle.

ACO1 is constitutively expressed in all seedling tissues, whereas the transcript of *ACO2* is barely detectable in non-stressed seedlings (Kim and Yang, 1994). When we treated leaves with 0.3 M NaCl and light, the transcript level of *ACO1* increased gradually after 1 h and up until 24 h (Fig. 3F). However, no changes were observed in the transcript level of *ACO2* (data not shown). Likewise, when leaves were treated with 0.3 M NaCl in darkness, the transcript level of *ACO1* decreased by 80% within 12 h, while the level of *ACS2* was unchanged (Fig. 3, B and F). Therefore, we

may assume that, for seedlings treated with 0.3 M NaCl in the light, the changes in expression patterns for ACC synthase and ACC oxidase genes are due to oxidative, rather than saline, stress.

Effects of Methyl Viologen on Expression of ACC Synthase and ACC Oxidase Genes

Paraquat, also known as methyl viologen (1,1-dimethyl-4, 4-bipyridinium; the active ingredient of the non-selective herbicide Gramoxone) is widely used in weed control. MV is a model compound for studying oxidative stresses (Bowler et al., 1994). This redox-active compound acts by exacerbating the generation of a superoxide radical in a chain reaction, thereby producing other reactive oxygen species (Dodge, 1994). In the chloroplast, MV is photoreduced by Photosystem I, and is subsequently reoxidized by electron transfer to oxygen, thus forming the superoxide.

In the current study, photochemical efficiency (Fig. 4) did not decreased when leaves were incubated in 2.5 mM MV in darkness, but the value did gradually decrease throughout the incubation period when plants were treated in the presence of light. In this case, the rate of decrease in F_v/F_m was almost twice that observed in the leaves treated with NaCl and light (Fig. 2).

As with the NaCl treatments, no genes were newly induced within the first 12 h when leaves were treated with MV in darkness. However, when leaves were treated with MV and light, the same genes were

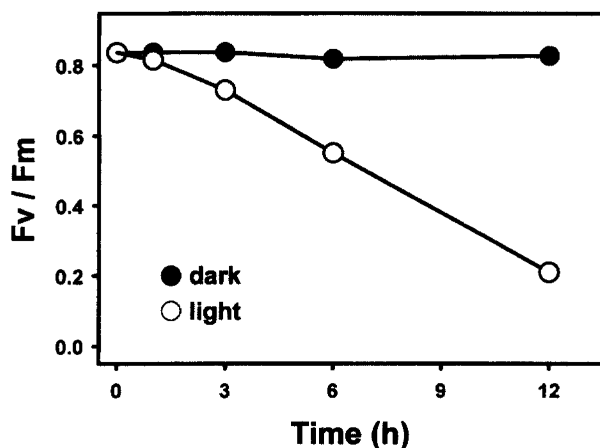

Figure 4. Effect of 2.5 mM methyl viologen (MV) on the photochemical efficiency of PSII, or F_v/F_m. Conditions for treatment with 2.5 mM MV are the same as for treatments with NaCl (see Fig. 2). Leaves were treated with MV for up to 12 h.

newly expressed as had been observed with the NaCl-treated leaves in the light (Fig. 5). Again, expression of *ACS5*, *ACS7*, and *ACO2* did not increase significantly (data not shown).

The kinetics of transcript accumulation in response to MV treatment in the light were similar to those from the NaCl treatments (compare with Fig. 3). However, transcripts for most of the genes had slower initial accumulations. In fact, the amount of transcript for *ACS1* and *ACS6* was only 20% and 10%, respectively, of that measured in the NaCl-treated leaves. In contrast, the amount of *ACS4* transcript accumulation was about four times greater than that found with the NaCl-treated leaves.

When leaves were treated with MV and light, the transcript level of *ACO1* increased gradually after 1 h and continued up to 12 h (Fig. 5F). However, we observed no changes in the transcript level for *ACO2* (data not shown). As with the NaCl-treated leaves, the level of *ACO1* in the dark decreased by 60% within 9 h, while the level of *ACS2* was not changed significantly (Fig. 5, B and F).

DISCUSSION

This is the first report on differential expression of all the gene family members of ACC synthase and ACC oxidase known to exist in mung bean leaves. Only some of the members had been investigated previously. For example, we found that, in non-stressed leaves, only transcripts of de-etiolated *ACS2* and *ACO1* accumulated (Figs. 1, 3, and 5). Similar results have been reported in studies with *ACS1* (Botella et al., 1995); *ACS1*, *ACS6*, and *ACS7* (Yi et al., 1999); *ACO1* (Kim and Yang, 1994; Jin et al., 1999); and *ACO2* (Kim and Yang, 1994).

Changes in the expression patterns of ACC synthase and ACC oxidase genes had not been previously reported for mung bean leaves treated with NaCl or MV. However, Botella et al. (1995) did find that *ACS1* transcripts increased as a result of mechanical strain, and Yi et al. (1999) showed that transcripts of *ACS1*, *ACS6*, and *ACS7* were not induced by treatments with such plant hormones as IAA and brassinosteroids. Likewise, the expression of ACC oxidase gene(s) in mung bean leaves has been known to change because of ethylene (Kim and Yang, 1994; Jin et al., 1999) or wounding (Kim and Yang, 1994).

In our etiolated mung bean leaves, *ACO2* was expressed along with *ACS2* and *ACO1*, thereby demonstrating for the first time the differential expression

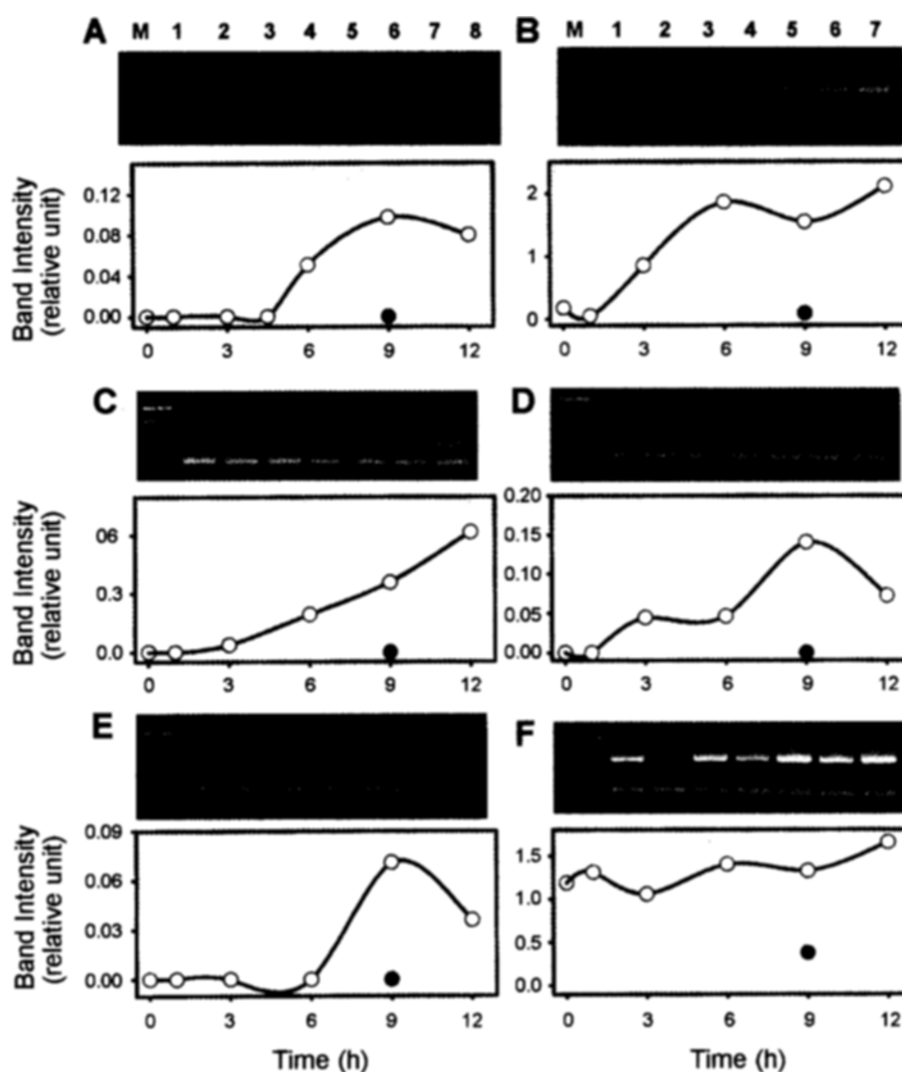

Figure 5. Differential expression of 7 ACC synthase and 2 ACC oxidase genes in 2.5 mM methyl viologen-treated mung bean leaves. The upper two-thirds of each leaf were used for the isolation of total RNA and for RT-PCR. **A**, *ACS1*; **B**, *ACS2*; **C**, *ACS3*; **D**, *ACS4*; **E**, *ACS6*; **F**, *ACO1*. M: marker. For **A**, Lanes 1, 3, 4, 5, 6, 7, and 8 are 2.5 mM MV-treated leaves for 0, 1, 3, 4.5, 6, 9, and 12 h in the light, respectively; Lane 2 represents 2.5 mM MV-treated leaves for 9 h in darkness. For **B** to **F**, Lanes 1, 3, 4, 5, 6, and 7 are 2.5 mM MV-treated leaves for 0, 1, 3, 6, 9, and 12 h in the light, respectively; Lane 2 represents 2.5 mM MV-treated leaves for 9 h in darkness. Changes in intensities of RT-PCR products relative to the endogenous standard 18S rRNA controls are plotted as a graph, and the relative value for the band in Lane 2 is indicated by a black dot.

of all the known gene family members of ACC synthase and ACC oxidase in the mung bean leaf; differential expression had already been reported in mung bean etiolated hypocotyls (Yu et al., 1998).

Expression patterns for each gene differ according to tissue type, developmental stage, and environmental conditions. For example, those for intact, etiolated hypocotyls (Yu et al., 1998) are not the same as the patterns observed in etiolated (Fig. 1A) and de-etiolated leaves (Fig. 1B). *ACS2* is not expressed in hypocotyls, and the expression level of *ACO2* is considerably high similar to that of *ACO1*. However, Kim and Yang (1994) reported that *ACO2* transcripts are negligible in mung bean hypocotyls. Yu et al. (1998) have also found that *ACO2* transcript levels decrease when hypocotyls are incubated in a similar manner. In the current study, transcription of *ACO2* was not observed, and that of *ACO1* decreased significantly in the de-etiolated leaves (Fig. 1, B and C).In addition, Yu et al. (1998) reported the differential expression of the

whole genes in mung bean hypocotyls under abiotic stresses and in hypocotyls treated with various chemicals.

In our etiolated leaves, treatment with light apparently inhibited ACC oxidase gene expression, as we inferred from the reduced transcript levels of both *ACO1* and *ACO2* (Fig. 1). However, when the de-etiolated leaves were returned to darkness, the level of the *ACO1* transcripts decreased further (Figs. 3 and 5). This suggests that the light-dependent regulation mechanism for *ACO1* in etiolated mung bean plants may differ from that present in de-etiolated leaves. This possibility should be investigated further.

Several factors contribute to the plant damage caused by salt, including osmotic stress and the accumulation of toxic ions. Both the amounts and the activities of enzymes involved in scavenging oxygen radicals are altered by saline stress (Yardena et al., 1997). Ethylene also induces a variety of plant defense genes (Ecker and Davis, 1987), as well as increasing peroxidase activity (Abeles et al., 1988). In experiments with H_2O_2 and paraquat, Mehlhorn (1990) suggested that ethylene production was induced in plants exposed to oxidative stress in order to enhance ascorbate peroxidase activity. Therefore, a plant's susceptibility to oxidative stress could be reduced because of its increased ability to detoxify H_2O_2.

Methyl viologen is a strong autooxidable electron acceptor in PSI, and will accept electrons from the iron-sulfur cluster Fe-SA/ Fe-SB of PSI. This results in depletion of NADPH and inhibition of CO_2 fixation (Preston, 1994). Newly formed MV radicals then react directly with O_2 to produce superoxide. The toxic reactive oxygen species produced as a result of primary MV action rapidly damages the targeted plants (Dodge, 1994).

We observed differential expression of ACC synthase and ACC oxidase family members in NaCl-treated leaves in the light but not in the dark (Fig. 3). This suggests that expression may be induced, at least partially, by oxidative stress. When expression patterns for MV-treated leaves (Fig. 5) are compared with those from the NaCl treatments (Fig. 3), we could conclude that: (1) the same genes are induced in both cases; (2) induction occurs only in the light; and (3) the kinetics of transcript accumulation are very similar between chemical treatments.

However, some of our study responses were not alike. For example, transcripts for most of the genes initially accumulated more slowly in the MV-treated leaves. This slow response may have been due to the more rapid influence of MV on photosynthetic efficiency compared with NaCl-indeed, MV affects the photosynthetic electron transport chain more directly. In addition, the amounts of the accumulated transcripts for *ACS1* and *ACS6* in MV-treated leaves were much less than in the NaCl-treated leaves; the reverse was true for *ACS4* transcripts. These two major differences should be investigated in future studies.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Korea Research Foundation (1998-001-D00792).

Received May 28, 2001; accepted June 27, 2001.

LITERATURE CITED

Abel S, Nguyen MD, Chow W, Theologis A (1995) ACS4, a primary indoleacetic acid-responsive gene encoding 1-aminocyclopropane-1-carboxylate synthase in Arabidopsis thaliana. Structural characterization, expression in Escherichia coli, and expression characteristics in response to auxin. J Biol Chem 270: 19093-19099

Abeles FB, Dunn LJ, Callahan A, Dinterman RE, Schmidt J (1988) Induction of 33-kD and 60-kD peroxidases during ethylene-induced senescence of cucumber cotyledons. Plant Physiol 87: 609-615

Abeles FB, Morgan PW, Saltveit ME Jr (1992) Ethylene in Plant Biology, Ed 2, Academic Press, New York

Arteca JM, Arteca RN (1999) A multi-responsive gene encoding 1-aminocyclopropane-1-carboxylate synthase (*ACS6*) in mature *Arabidopsis* leaves Plant Mol Biol 39: 209-219

Barry CS, Blume B, Bouzayen M, Cooper W, Hamilton AJ, Grierson D (1996) Differential expression of 1-aminocyclopropane-1-carboxylate oxidase gene family of tomato. Plant J 9: 525-535

Botella JR, Arteca JM, Schlagnhaufer CD, Arteca JM, Arteca RN, Philips AT (1993) Identification of two new members of the 1-aminocyclopropane -1-carboxylate synthase encoding multigene family in mung bean. Gene 123: 249-253

Botella JR, Arteca JM, Schlagnhaufer CD, Arteca RN, Philips AT (1992) Identification and characterization of a full length cDNA encoding for an auxin-induced 1-aminocyclopropane-1-carboxylate synthase from etiolated mung bean hypocotyls segments and expression of its mRNA in response to indole-3-acetic acid. Plant Mol Biol 20: 425-436

Botella JR, Arteca RN, Frangos JA (1995) A mechanical strain-induced 1-aminocyclopropane-1-carboxylic acid synthase gene. Proc Natl Acad Sci USA 92: 1595-1598

Botella MA, Amor FD, Amoros A, Serrano M, Martinez V, Cerda A (2000) Polyamine, ethylene and other physico-

chemical parameters in tomato (*Lycopersicon esculentum*) fruits as affected by salinity. Physiol Plant 109: 428-434

Bowler C, van Camp W, van Montagu M, Inze D (1994) Superoxide dismutase in plants. Crit Rev Plant Sci 13: 199-218

Chomezynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol chloroform extraction. Anal Biochem 162: 156-159

Chrominski A, Weber DJ, Smith BN (1986) NaCl-salinity-dependent conversion of ACC to ethylene in the halophyte *Allenrolfea occidentalis*. Naturwissenschaften 73: 274-275

Destefano-Beltran LJ, van Caeneghem W, Gielen J, Richard L, van Montagu M, van der Straeten D (1995) Characterization of three members of the ACC synthase gene family in *Solanum tuberosum L*. Mol Gen Genet 246: 496-508

Dodge AD (1994) Herbicide action and effects on detoxification processes, *In* CH Foyer, PM Mullineaux, eds, Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants, CRC Press, Boca Raton, Florida, USA, pp 219-236

Ecker JR, Davis RW (1987) Plant defense genes are regulated by ethylene. Proc Natl Acad Sci USA 84: 5202-5206

Greenway H, Munns R (1980) Mechanisms of salt tolerance in nonhalophytes. Annu Rev Plant Physiol 31: 149-190

Henskens JAM, Rouwendal GJ, ten Have A, Woltering EJ (1994) Molecular cloning of two different ACC synthase PCR fragments in carnation flowers and organ-specific expression of the corresponding genes. Plant Mol Biol 26: 453-458

Huang P, Parks JE, Rottmann WH, Theologis A (1991) Two genes encoding 1-aminocyclopropane-1-carboxylate synthase in zucchini (*Cucurbita pepo*) are clustered and similar but differentially regulated. Proc Natl Acad Sci USA 88: 7021-7025

Jin ES, Lee J-H, Park J-A, Kim WT (1999) Temporal and spatial regulation of the expression of 1-aminocyclopropane-1-carboxylate oxidase by ethylene in mung bean (*Vigna radiata*). Physiol Plant 105: 132-140

Kende H (1993) Ethylene biosynthesis. Annu Rev Plant Physiol Plant Mol Biol 44: 283-307

Kim WT, Campbell A, Moriguchi T, Yi HC, Yang SF (1997) Auxin induces three genes encoding 1-aminocyclopropane-1-carboxylate synthase in mung bean hypocotyls. J Plant Physiol 150: 77-84

Kim WT, Silverstone A, Yip WK, Dong JG, Yang SF (1992) Induction of 1-aminocyclopropane-1-carboxylate synthase mRNA by auxin in mung bean hypocotyls and cultured apple shoots. Plant Physiol 98: 465-471

Kim WT, Yang SF (1994) Structure and expression of cDNAs encoding 1-aminocyclopropane-1-carboxylate oxidase homologs isolated from excised mung bean hypocotyls. Planta 194: 223-229

Kitajima M, Butler WL (1975) Quenching of chlorophyll florescence and primary photochemistry in the chloroplasts by dibromothymoquinone. Biochim Biophys Acta 376: 105-115

Lassere E, Bouquin T, Hernandez JA, Bull J, Pech J-C, Balague C (1996) Structure and expression of three genes encoding ACC oxidase homologs from melon (*Cucumis melo* L.). Mol Gen Genet 251: 81-90

Liang X, Oono Y, Shen NF, Kohler C, Li K, Scolnik PA, Theologis A (1995) Characterization of two members (*ACS1* and *ACS3*) of the 1-aminocyclopropane-1-carboxylate synthase gene family of *Arabidopsis thaliana*. Gene 167: 17-24

Liang X, Shen NF, Theologis A (1996) Li^+-regulated 1-aminocyclopropane-1-carboxylate acid synthase gene expression in *Arabidopsis thaliana*. Plant J 10: 1027-1036

Lichtenthaler HK (1988) Application of Chlorophyll Fluorescence in Photosynthesis Research: Stress Physiology, Hydrobiology and Remote Sensing. Kluwer Academic Publishers, Dordrecht, The Netherlands

Lincoln JE, Campbell AD, Oetiker J, Rottmann WH, Oeller PW, Shen NF, Theologis A (1993) LE-*ACS4*, a fruit ripening and wound induced 1-aminocyclopropane-1-carboxylate synthase gene of tomato (*Lycopersicon esculentum*). Expression in *Escherichia coli*, structural characterization, structural characterization, expression characteristics, and phylogenetic analysis. J Biol Chem 268: 19422-19430

Mehlhorn H (1990) Ethylene-promoted ascorbate peroxidase activity protects plants against hydrogen peroxide, ozone and paraquat. Plant Cell Environ 13: 971-976

Mizrahi Y (1982) Effect of salinity on tomato fruit ripening. Plant Physiol 69: 966-970

Nakajima N, Mori H, Yamazaki K, Imaseki H (1990) Molecular cloning and sequence of a complementary DNA encoding 1-aminocyclopropane -1-carboxylate synthase induced by tissue wounding. Plant Cell Physiol 31: 1021-1029

Oetiker JH, Olson DC, Shiu OY, Yang SF (1997) Differential induction of seven 1-aminocyclopropane-1-carboxylate synthase genes by elicitor in suspension cultures of the tomato (*Lycopersicon esculentum*). Plant Mol Biol 34: 275-286

Ogren E (1990) Evaluation of chlorophyll fluorescence as a probe for drought stress in willow leaves. Plant Physiol 93: 1280-1285

Olson DC, Oetiker JH, Yang SF (1995) Analysis of LE-*ACS3*, an ACC synthase gene expressed during flooding in roots of tomato plants. J Biol Chem 270: 14056-14061

Park KY, Drory A, Woodson WR (1992) Molecular cloning of a 1-aminocyclopropane-1-carboxylate synthase from senescing carnation flower petals. Plant Mol Biol 18: 377-386

Preston C (1994) Resistance to photosystem I disrupting herbicides, *In* SB Powles, JAM Holtum, eds, Herbicide Resistance in Plants: Biology and Biochemistry, Lewis Publishers, Boca Raton, Florida, USA pp 61-82

Rottmann WE, Petre GF, Oeller PW, Keller JA, Shen NF,

Nagy BP, Tayler LP, Campbell AD, Theologis A (1991) 1-aminocyclopropane-1-carboxylate acid synthase in tomato is encoded by a multigene family whose transcription is induced during fruit and floral senescence. J Mol Biol 222: 937-961

Schlagnhaufer CD, Arteca RN, Pell EJ (1997) Sequential expression of two 1-aminocyclopropane-1-carboxylase synthase genes in response to biotic and abiotic stresses in potato (*Solanum tuberosum* L.) leaves. Plant Mol Biol 35: 683-688

Shiu OY, Oetiker JH, Yip WK, Yang SF (1998) The promoter of *LE-ACS7*, an early flooding-induced-1-aminocyclopropane-1-carboxylate synthase gene of tomato, is tagged by a *Sol3* transposon. Proc Natl Acad Sci USA 95: 10334-10339

Subramaniam K, Abbo S, Ueng PP (1996) Isolation of two differentially expressed wheat ACC synthase cDNAs and the characterization of one of their genes with root-predominant expression. Plant Mol Biol 31: 1009-1020

van der Straeten D, Anuntalabhochai S, Caeneghem WV, Zhou ZY, Gielen J, van Montagu M (1997) Expression of three members of the ACC synthase gene family in deepwater rice by submergence, wounding and hormonal treatments. Plant Sci 124: 79-87

van der Straeten D, Rodrigues-Pousada RA, Villarroel R, Hanley S, Goodman HM, van Montagu M (1992) Cloning, genetic mapping, and expression analysis of an *Arabidopsis thaliana* gene that encodes 1-aminocyclopropane -1-carboxylate synthase. Proc Natl Acad Sci USA 89: 9969-9973

Yang SF, Hoffman NE (1984) Ethylene biosynthesis and its regulation in higher plants. Annu Rev Plant Physiol 35: 155-189

Yardena GD, Zohara Y, Barbara AZ, Gozal B-H (1997) Salt and oxidative stress: similar and specific responses and their relation to salt tolerance in citrus. Planta 203: 460-469

Yi HC, Joo SJ, Nam KH, Lee JS, Kang BG, Kim WT (1999) Auxin and brassinosteroid differentially regulate the expression of three members of the 1-aminocyclopropane-1-carboxylate synthase gene family in mung bean (*Vigna radiata* L.). Plant Mol Biol 41: 443-454

Yip WK, Moore T, Yang SF (1992) Differential accumulation of transcripts for four tomato 1-aminocyclopropane-1-carboxylate synthase homologs under various conditions. Proc Natl Acad Sci USA 89: 2475-2479

Yoon IS, Mori H, Kim JH, Kang BG, Imaseki H (1997) VR-*ACS6* is an auxin-inducible 1-aminocyclopropane-1-carboxylate synthase gene in mung bean (*Vigna radiata*). Plant Cell Physiol 38: 217-224

Yu SJ, Kim SY, Lee JS, Lee DH (1998) Differential accumulation of transcripts for ACC synthase and ACC oxidase homologs in etiolated mung bean hypocotyls in response to various stimuli. Mol Cells 8: 350-358

Zarembinski TI, Theologis A (1993) Anaerobiosis and plant growth hormones induce two genes encoding 1-aminocyclopropane-1-carboxylate synthase in rice (*Oryza sativa* L.) Mol Biol Cell 4: 363-373