Journal of Plant Biology, February 2007, 50(1) : 18-23

Reactive Oxygen Species Mediate IAA-Induced Ethylene Production in Mungbean (*Vigna radiata* L.) Hypocotyls

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We reported previously that ROS may function as a downstream component for signaling auxin, a hormone that induces the ethylene production. Here we demonstrated that ROS can act upstream to promote auxin-induced ethylene production in mungbean hypocotyls. When plants were treated with AVG, ethylene biosynthetic inhibitor, or ACC, ethylene precursor, we found that little effect on the ROS generation. Hypocotyls exposed to H_2O_2 showed higher ethylene accumulations compared with untreated samples. Furthermore, auxin-induced ethylene production was reduced when tissues were pre-treated with a ROS scavenger, N-acetyl-L-cystein (NAC). We attributed this stimulatory effect to the activation of ethylene biosynthetic enzymes that results from transcriptional up-regulation of *VR-ACS1* and *VR-ACS7* to encode ACC synthase, as well as of *VR-ACO1* and *VR-ACO2*, which encode ACC oxidase.

Keywords: ACC oxidase, ACC synthase, auxin-induced ethylene production, ROS

Unlike in animals, individual hormones in plants can display a broad range of pleiotropic effects on overall development. Even though the signal transduction pathways for each plant hormone are specific and independent (Yanagisawa et al., 2003; Hass et al., 2004; Dharmasiri et al., 2005), the additive/synergistic or mutual antagonistic features of their physiological effects (Yi et al., 1999; Overmyer et al., 2000) imply possible interconnections within great networks that can respond to temporal and developmental cues as well as environmental signals. During this process, numerous second signaling molecules are necessary to perform a specific event. We have previously reported that reactive oxygen species (ROS) can mediate the physiological effects of auxin. Auxin, one of the most important plant hormones, has on fundamental global cellular responses, such as tropism, apical dominance, lateral root formation, vascular differentiation, and cell elongation (Casimiro et al., 2003; Xu and Scheres, 2005). We showed that ROS production is essential for auxin-induced gravitropic responses in maize roots. Moreover, ROS has been observed in the lower cortex of the gravi-stimulated roots, leading to a differential growth rate (Joo et al., 2001). This process is blocked when the roots are pretreated with LY294002, an inhibitor of phosphatidylinositol (PtdIns) 3-kinase, implying that activation of PtdINs 3-kinase activity is involved in ROS generation through the multimeric NADPH oxidase complex (Joo et al., 2005). ROS such as a superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) is toxic byproducts produced metabolically that cause cellular injury, such as lipid peroxidation or protein and nucleic acid modification. However, they may also function as signaling molecules, at low concentrations, mediate responses to various stimuli in both plants and animal cells (Asada and Takahashi, 1987). While a high concentration of apoplastic ROS that arises from oxidative burst upon pathogen attack can result in hypersensitive, programmed cell death (Jabs et al., 1996), low levels influence cellular processes such as cell cycle progression (Reichheld et al., 1999) and the onset of secondary cell wall differentiation (Potikha et al., 1999). Abiotic or biotic stresses, which induce ROS production through the activation of NADPH oxidase, can trigger a plant defense response against different stress conditions, thus enhancing tolerance of plants to multiple stresses and pathogens (Alvarez et al., 1998; Torres et al., 2002).

Here, we investigated how auxin-induced ROS might function in ethylene production within mungbean hypocotyls by activating the expression of a subset of ethylene biosynthetic genes.

MATERIALS AND METHODS

Plant Materials and Growing Conditions

Mungbean (*Vigna radiata* L.) seeds were obtained from the market of the National Agricultural Coorperative Federation, and were imbibed in tap water for at least 6 h before being plated onto 0.5% agar media. Germination proceeded for 3 d under darkness at 27±1°C and 80% of relative humidity. Hypocotyl segments (0.8 cm-long) were excised just below the hook and incubated in various solutions indicated in figure legends. Protoplasts from 3 day-old hypocotyls were isolated with an enzyme mixture containing 1% Cellulase R-10 (Duchefa, Netherlands), 0.25% Macerozyme R-10 (Duchefa), 600 mM mannitol, 1 mM $CaCl_2$ 0.1% BSA, and 10 mM MES-KOH (pH 5.6). Purified protoplasts were re-suspended in a W5 solution containing 154 mM NaCl,

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Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; AVG, aminoethoxyvinyl glycine; IAA, indole-3-acetic acid; NAC, N-acetyl-L-cystein; ROS, reactive oxygen species.

125 mM $CaCl_2$, 5 mM KCl, 5 mM glucose, and 1.5 mM MES-KOH, (pH 5.6). Viability of the protoplasts was determined by 1% Evans Blue staining.

Assay of ROS

Intracellular ROS production in mungbean hypocotyl tissues was visualized with a fluorescence microscope (Zeiss, Germany), using the oxidation-sensitive fluorescence of dihydrorhodamine-123, as described by Joo et al. (2005). For our flow cytometric analysis, 30000 protoplasts were treated with 1 mM of ACC (1-aminicyclopropane-1-carboxylic acid; Sigma-Aldrich, USA) for various time periods, then incubated for 5 min with 5 μM 2,7-dichlorofluorescein diacetate (H_2DCF-DA) (Molecular Probes, USA). This compound was converted to 2,7-dichlorofluorescin, which was then oxidized by H_2O_2 to the highly fluorescent DCF. The fluorescence intensity was measured using a FACScan (Beckton Dickinson, USA), with excitation and emission settings of 485 nm and 535 nm, respectively. Intracellular H_2O_2 levels were quantified with a Bioxytech H_2O_2-560 assay kit (OXIS, USA), based on the oxidation of a ferrous ion (Fe^{2+}) to a ferric ion (Fe^{3+}) by hydrogen peroxide under acidic conditions.

Ethylene Production

Forty segments from mungbean hypocotyls were placed in a 12-mL glass vial with 4 mL of buffer (5 mM MES-Tris; pH 6.8). After the vial was sealed with a silicon stopper and shaken at 65 rpm and 27±1°C, a 1 mL sample was removed and analyzed by gas chromatography (GC-8A, GC-14A, Shimadzu, Japan; Flame Ionized Detector; 2 m Porapak Q column; air: 0.5 kPa, nitrogen: 100 kPa).

Enzymatic Assays of ACC Synthase and ACC Oxidase

After the treatments were completed, the hypocotyl segments at the end of treatment were thoroughly rinsed with distilled water and immersed in either an ACC synthase reaction buffer (50 mM HEPES pH 8.5, 0.5 μM pyridoxal-5′-phosphate, and 50 μM S-adenosyl-L-methione) or ACC oxidase reaction buffer (50 mM HEPES pH 8.5, 0.5 μM pyridoxal-5′-phosphate, and 1 mM ACC) in 12-mL glass vials stoppered with silicon. Following incubation at 27±1°C with gentle shaking, 1 mL of gas was withdrawn and analyzed by gas chromatography.

RT-PCR Analysis for Accumulations of ACC Synthases and ACC Oxidase

Total RNAs were prepared from hypocotyls segments with TRI reagent (Sigma, USA) and used as templates for first-strand cDNA synthesis with Superscript II RNase HP-Reverse Transcriptase (Invitrogen Life Technology, USA). An equal cDNA aliquot was subjected to PCR with gene specific primer sets, as described by Yu et al. (1998).

RESULTS

Auxin Induces ROS and Ethylene Production

Our experiments on mungbean hypocotyls treated with IAA showed that ethylene production increased in a dose-dependent manner by up to 19.5-fold at 100 uM IAA (Fig. 1A). This is consistent with the previously reported results (Peck and Kende, 1995; Arteca and Arteca, 1999). According to recent several reports, auxin might act through the ROS generated to respond to redistribution of auxin concentration (Joo et al, 2001; Joo et al., 2005). Therefore, we examined ROS production in the IAA-treated mungbean hypocotyl tissues. ROS production was elevated by about 30% and 55% in hypocotyls treated with 1 uM and 10 uM IAA compared with the control (Fig. 1B). Using FACScan

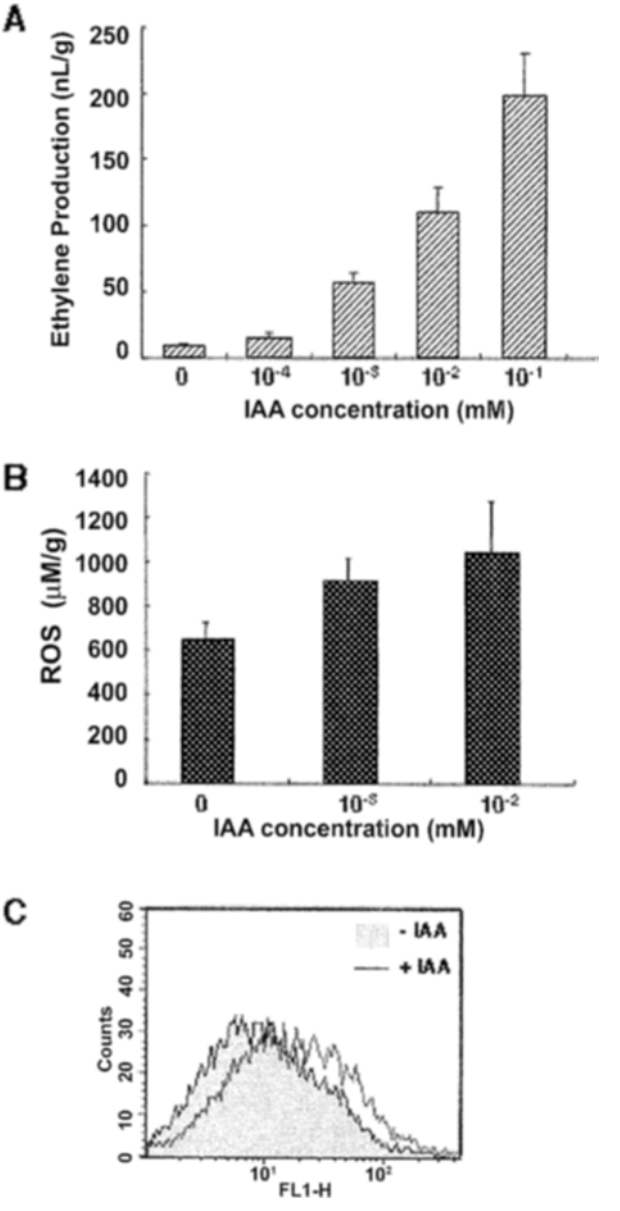

Figure 1. Effects of IAA on ethylene production and levels of intracellular ROS in mungbean hypocotyls (**A**, **B**) 40 hypocotyl segments were incubated for 9 h (**A**) or 1 h (**B**) in 5 mM Mes-Tris buffer (pH 6.8) containing different concentrations of IAA. Bars denote standard errors for 3 independent experiments. (**C**) ROS production from the protoplasts of mungbean hypocotyls pre-treated for 15 min with 10 μM of IAA, as analyzed with FACScan. Shaded area indicates control fluorescence intensity. Experiments were repeated at least 5 times with similar results.

analysis, we found that protoplast treated with 10 uM IAA displayed higher fluorescence intensities than those measured from untreated samples (Fig. 1C), indicating that ROS production is induced by IAA.

Ethylene Does Not Affect ROS Production

Because auxin induced the production of both ethylene and ROS in our mungbean hypocotyls, we investigated whether these two cellular events were either independent phenomena that occurred as pleiotropic effects of auxin, or involved in the same pathway. To monitor ROS production under the environment in which ethylene production is inhibited, we pre-treated the hypocotyls with 100 uM of AVG, an ethylene biosynthetic inhibitor. Based on the signal strength of dihydrorhodamine 123 staining, we observed that the ROS generation was not aborted by AVG treatment, thereby demonstrating that auxin induced ROS production is not ethylene-dependent (Fig. 2A). This was confirmed by applying ACC exogeneously to the hypocotyls. ACC is a biosynthetic precursor that is easily converted to ethylene, and which is often used to artificially increase ethylene concentrations in tissues. When we applied 1 mM ACC for up to 2 h before measuring ROS concentrations, we detected only minor fluctuations in the endogenous ROS level (Fig. 2B). Similar results were obtained when protoplasts were incubated in ACC-containing media (Fig. 2C). These data suggest that ethylene production is not required for auxin-induced ROS generation in mungbean hypocotyls.

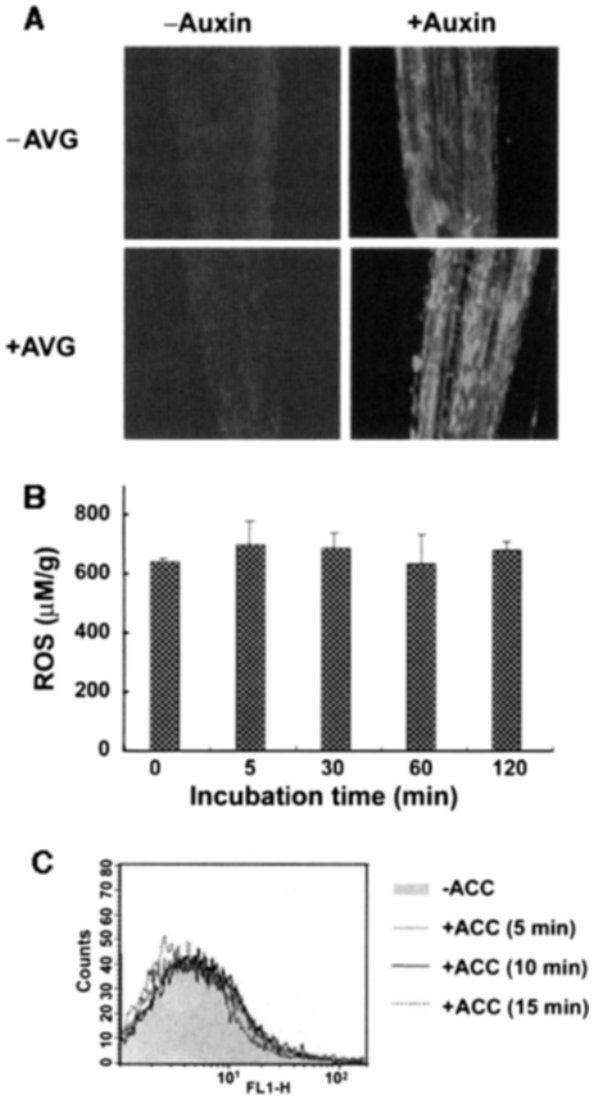

Figure 2. Ethylene did not affect ROS generation. (**A**) Mungbean hypocotyls were pretreated with 100 μM of AVG and incubated for 1 h in 5 mM Mes-Tris buffer (pH 6.8) containing 10 μM IAA. Hypocotyls were then dissected and stained with 0.003% dihydrorhodamine-123 solution for 10 min. Intensity of oxidized rhodamine was visualized by fluorescence microscopy (excitation = 485 nm and emission = 535 nm; Carl Zeiss). Experiments were repeated at least 5 times with similar results. (**B**) Hypocotyls treated with 1 mM ACC for indicated times were subjected to ROS assay (OXIS, USA). Bars denote standard errors for 3 independent experiments. (**C**) ROS production by mungbean hypocotyl protoplasts pre-treated with 1 mM ACC for indicated times were analyzed with FACScan.

Reactive Oxygen Species Are Required for Auxin-Induced Ethylene Production

We also examined whether the ROS induced by auxin might act as an upstream regulator for ethylene. Treatment

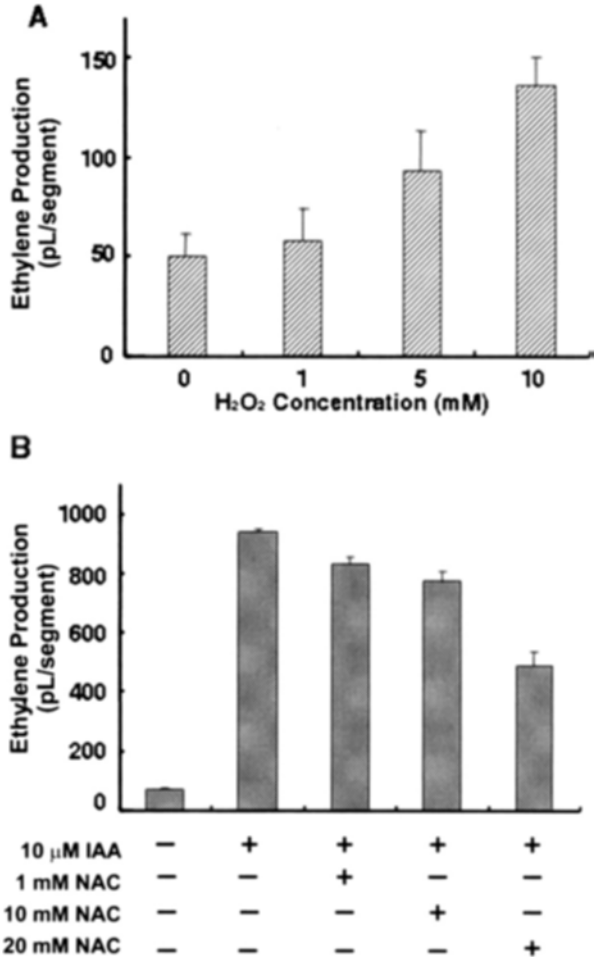

Figure 3. Effect of ROS on ethylene production, measured from hypocotyl segments incubated in 5 mM Mes-Tris buffer (pH 6.8) containing indicated concentrations of H_2O_2 for 9 h (**A**), or containing IAA and different concentrations of NAC (**B**). Bars denote standard errors for 3 independent experiments.

with H_2O_2 led to a dose-dependent increase, with levels being 2.5-fold higher at 10 mM H_2O_2 compared with the buffer-treated controls (Fig. 3A). Interestingly, when endogenous ROS was scavenged by NAC (N-acetyl-L-cystein; Sigma-Aldrich, USA), auxin-induced ethylene production was inhibited leading to a 48% of reduction in samples treated with 20 mM NAC (Fig. 3B). This indicates that ROS are necessary for auxin-induced ethylene production. Nevertheless, the mechanism by which this is accomplished should be further studied. To investigate which steps for ethylene production are influenced by ROS, we measured the activity of ethylene biosynthetic enzymes in the presence of H_2O_2. Within the first hour of incubation, ACC synthase activity began to rise 25% to 55% with 10 nM to 1 mM of H_2O_2, respectively. Over longer incubation periods, the degree of increase in ACC synthase activity was more dramatic. For examples, after 6 h activity was 3-fold higher at 1 mM of H_2O_2 compared with tissues incubated in the buffer alone (Fig. 4A). The pattern of stimulation was similar for ACC oxidase activity at 6 h, but shorter treatment with H_2O_2 did not lead to any change in ACC oxidase activity (Fig. 4B). Because different kinetics were involved in the increased activity of these two rate-limiting ethylene-biosynthetic enzymes, especially in thrie initial responses to H_2O_2, we suggest that its direct target is ACC synthase. Moreover, accumulation of ACC (the product of ACC synthase) is required to trigger subsequent activation of ACC oxidase.

The genes that encode ACC synthase and ACC oxidase belong to multigene families, with transcriptional expression of each being regulated differentially by various developmental and/or environmental factors (Theologis, 1992; Kende, 1993). Therefore, we examined whether H_2O_2 could change the levels of transcripts for ACC synthase and ACC oxidase induced by auxin. Compared with the buffer-treated control, exposure to IAA treatment caused higher expression of *VR-ACS1*, *VR-ACS6*, and *VR-ACS7*, with the greatest increase measured from *VR-ACS1* (Fig. 5A, Lane 2). Interestingly, H_2O_2 treatment also resulted in higher accumulations of *VR-ACS1* and *VR-ACS7* than has been observed with auxin, compared with their corresponding controls. *VR-ACS6* expression, however, was not affected by H_2O_2 treatment (Fig. 5A, Lane 3). These results indicate that ROS also can induce the expression of a subset of ACC synthase, which might explain the increase in enzyme activity and subsequent ethylene production. We also examined whether NAC could abolish the expression of ACC synthase that had been induced by IAA. When hypocotyls were pretreated with NAC before the IAA application, no expression was detected for *VR-ACS1* and *VR-ACS7* (Fig. 5A, Lane 4). Furthermore, genes for two ACC oxidases, *VR-ACO1* and *VR-ACO2*, that are known to be induced by IAA, showed increased accumulations when treated with H_2O_2, but were inhibited by NAC pretreatment (Fig. 5B). Therefore, these results strongly suggest that H_2O_2 is one of the second messengers that mediate auxin-induced ethylene production through the activation of gene expression for ethylene bio-

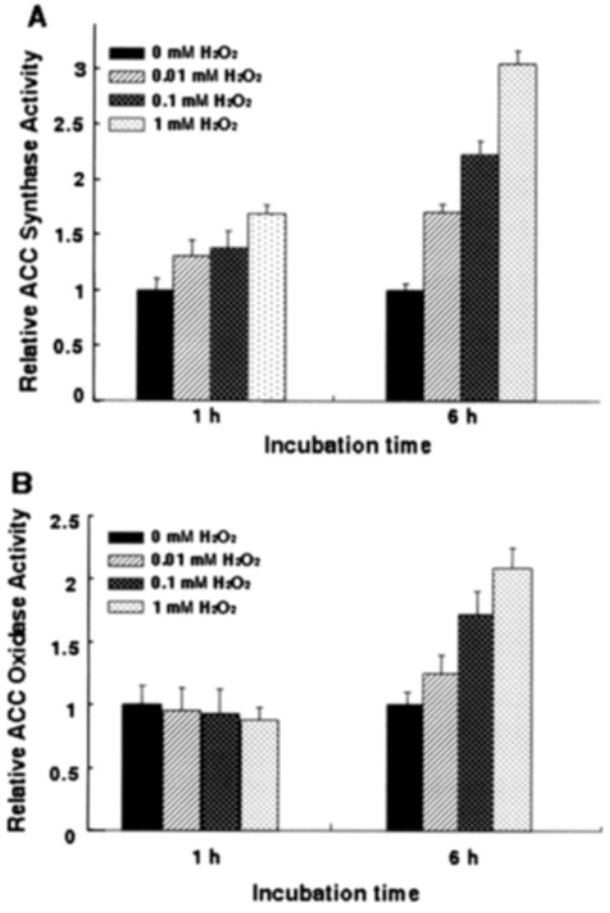

Figure 4. ROS affect activities of ethylene biosynthetic enzymes. Hypocotyl segments were incubated in 5 mM Mes-Tris buffer (pH 6.8) with indicated concentrations of H_2O_2. Enzyme activities of ACC synthase (**A**) or ACC oxidase (**B**) are shown. Bars represent standard errors for 5 independent experiments.

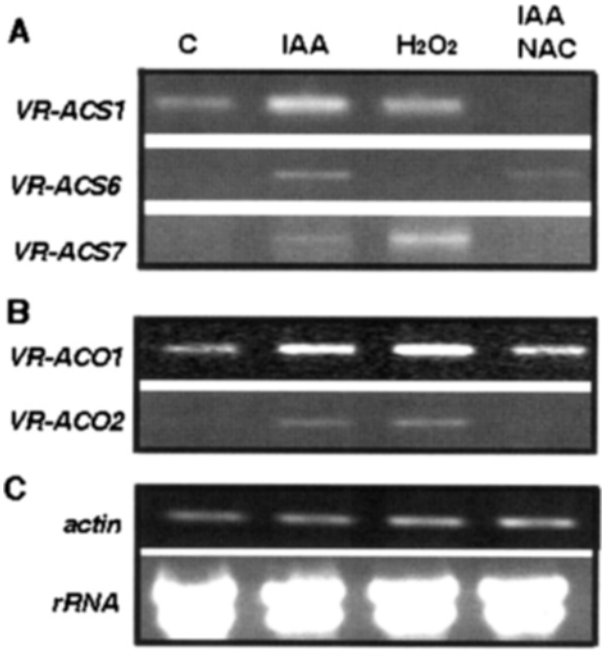

Figure 5. ROS affect expression of ethylene biosynthetic genes. RT-PCR analyses for ACC synthases (**A**) and ACC oxidases (**B**) were performed with RNAs from hypocotyl segments pre-incubated in 5 mM Mes-Tris buffer (pH 6.8) with or without 20 mM NAC for 5 h, then treated for 1 h with 10 μM of IAA or 10 mM H_2O_2. The same amounts of RNAs are shown in (**C**) by expression of actin and RNA gel.

synthetic enzymes in mungbean hypocotyls.

DISCUSSION

Ethylene is a simple gaseous phytohormone that affects numerous physiological aspects of plant growth and development (Theologis, 1992; Hass, et al., 2004). Its endogenous concentration is normally in low in tissues, but its production can be dramatically induced by seed germination, fruit ripening, leaf and flower senescence, or biotic and abiotic stresses (Kende, 1993; Yoon et al., 1997). Auxin is one inducer of this production. When we incubated mungbean hypocotyls in a low concentration of IAA, ethylene levels increased by 8 to 19.5-fold (Fig. 1). Previously, we reported that auxin treatment on the one side of a maize root tip stimulates ROS generation, resulting in gravitropic curvature (Joo et al., 2001). To examine the inter-dependency between ethylene and ROS, both of which are generated by auxin, we tried to identify which one functioned as an upstream regulator of the other. In winter squash (*Cucurbita maxima*), wounding and jasmonic acid induce the ethylene production through increased expression of *CM-ACS1*. This elevation in *CM-ACS1* accumulation is inhibited by diphenylene iodonium (DPI), which blocks the superoxide-generating enzyme NADPH oxidase (Watanabe and Sakai, 1998; Watanabe et al., 2001). Based on this, we theorized that ethylene production could be affected by ROS generated through various metabolic routes. *Rcd1* mutants develop HR-like lesions in response to ozone (O_3) because of the over-accumulation of superoxide radicals. Therefore, treatment with norbornadiene or the introduction of ethylene-insensitive allele (*ein2*) to impair this ethylene perception then blocked superoxide production (Overmyer et al., 2000). This indicates that ethylene is required for ozone-induced superoxide accumulation and lesion propagation, acting as an upstream promoting factor.

In our experimental systems, neither the ethylene biosynthetic inhibitor AVG nor the biosynthetic precursor ACC had any noticible influence on auxin-induced ROS production (Fig. 2). Instead, exogenous treatment with H_2O_2 or the ROS scavenger NAC resulted in respective increases or reductions of ethylene contents (Fig. 3). We believed, therefore, that ROS might be a mediator of auxin-induced ethylene production in mungbean hypocotyl segments. The ROS likely affected this production by increasing the activity of ethylene biosynthetic enzymes through the accumulation of transcripts, especially for a subset of ACC synthase genes (Fig. 4, 5). Those biosynthetic genes are present in large multigene families, in which members respond differently to various developmental and environmental cues. At least seven ACC synthase genes have been identified in mungbean plants (Botella et al., 1993; Kim and Yang, 1994). Among them, transcripts for *VR-ACS1*, *VR-ACS6*, and *VR-ACS7* are induced in the hypocotyls in response to auxin (Kim et al., 1997; Yoon et al., 1997). When we examined their transcript levels, we found that H_2O_2 induced the expression of *VR-ACS1* and *VR-ACS7* for ACC synthase, and *VR-ACO1* and *VR-ACO2* for ACC oxidase, respectively. Those latter two biosynthetic genes that encode for ACC oxidase also are believed to increase their transcript levels in response to auxin in mungbean hypocotyls (Yu et al., 1998). Therefore, we propose that ROS is required for the transcriptional activation of a different subset of ethylene biosynthetic genes that lead to the auxin-induced ethylene production in mungbean hypocotyls.

ACKNOWLEDGEMENTS

This work was supported by Grant R01-2005-10613-0 (to K.H.N.) from the Basic Research Program of the Korea Science and Engineering Foundation (KOSEF), and by Grant R15-2003-012-01003-0 (to K.H.N.) from KOSEF to the Environmental Biotechnology Research Center. This work was also funded by the KOSEF through the Center for Cell Signaling Research at Ewha Womans University and the Crop Functional Genomics Center (Grant CG133 to Y.S.B.).

Received November 9, 2006; accepted January 16, 2007.

LITERATURE CITED

Alvarez ME, Pennel IRI, Meijer PJ, Ishikawa A, Dixon RA, Lamb C (1998) Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell 92: 773-784

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

Asada K, Takahashi M (1987) Production and scavenging of active oxygen in photosynthesis, *In* DJ Kyle, ed, Photoinhibition (Topics in Photosynthesis), Vol 9. Elsevier, pp 227-287

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

Casimiro I, Beeckman T, Graham N, Bhalerao R, Zhang H, Casero P, Sandberg G., Malcolm JB (2003) Dissecting *Arabidopsis* lateral root development. Trends Plant Sci 8: 165-171

Dharmasiri N, Dharmasiri S, Estelle M (2005) The F-box protein TIR1 is an auxin receptor. Nature 435: 441-445

Hass C, Lohrmann J, Albrecht V, Sweere U, Hummel F, Yoo SD, Hwang I, Zhu T, Schafer E, Kudla J, Harter K (2004) The response regulator 2 mediates ethylene signaling and hormone signal integration in *Arabidopsis*. EMBO J 23: 3290-3302

Jabs T, Dietrich RA, Dangle JL (1996) Initiation of runaway cell death in an *Arabidopsis* mutant by extracellular superoxide. Science 273: 1853-1856

Joo JH, Bae YS, Lee JS (2001) Role of auxin-induced reactive oxygen species in root gravitropism. Plant Physiol 126: 1055-1060

Joo JH, Yoo HJ, Hwang I, Lee JS, Nam KH, Bae YS (2005) Auxin-induced reactive oxygen species production requires the activation of phosphatidylinositol 3-kinase. FEBS Lett 579: 1243-1248

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

Kim JH, Kim WT, Kang BG, Yang SF (1997) Induction of 1- aminocyclopropane-1-carboxylate oxidase mRNA by ethylene in mungbean hypocotyls: Involvement of both protein phosphorylation and dephosphorylation in ethylene signaling. Plant J 11: 399-405

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

Overmyer K, Tuominen H, Kettunen R, Betz C, Langebartels C, Sandermann H, Kangasjärvi J (2000) Ozone-sensitive *Arabidopsis rcd1* mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. Plant Cell 12: 1849-1862

Peck SC, Kende H (1995) Sequential induction of the ethylene biosynthetic enzymes by indole-3 acetic acid in etiolated mungbean. Plant Mol Biol 28: 243-255

Potikha TS, Collins CC, Johnson DI, Delmer DP, Levine A (1999) The involvement of hydrogen peroxide in the differentiation of secondary walls in cotton fibers. Plant Physiol 119: 849-858

Reichheld JP, Vernoux T, Lardon F, van Montagu M, Inze D (1999) Specific checkpoints regulate plant cell cycle progression in response to oxidative stress. Plant J 17: 647-656

Theologis A (1992) One rotten apple spoils the whole bushel: The roles of ethylene in fruit ripening. Cell 70: 181-814

Torres MA, Dangle JL, Jones JDG (2002) *Arabidopsis* gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. Proc Natl Acad Sci USA 99: 517-522

Watanabe T, Sakai S (1998) Effects of active oxygen species and methyl jasmonate on expression of the gene for a wound-inducible 1- aminocyclopropane-1-carboxylate synthase in winter squash (*Cucurbita maxia*). Planta 206: 570-576

Watanabe T, Seo S, Sakai S (2001) Wound-induced expression of a gene for 1- aminocyclopropane-1-carboxylate synthase and ethylene production are regulated by both reactive oxygen species and jasmonic acid in *Cucurbita maxima*. Plant Physiol Biochem 39: 121-127

Xu J, Scheres B (2005) Cell polarity: ROPing the ends together. Curr Opin Plant Biol 8: 613-618

Yanagisawa S, Yoo SD, Sheen J (2003) Differential regulation of EIN3 stability by glucose and ethylene signaling in plants. Nature 425: 521-525

Yi HC, Joo JS, 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

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

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