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Abscisic Acid Antagonizes Ethylene Production through the ABI4-Mediated Transcriptional Repression of *ACS4* and *ACS8* in *Arabidopsis*

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ABSTRACT

Increasing evidence has revealed that abscisic acid (ABA) negatively modulates ethylene biosynthesis, although the underlying mechanism remains unclear. To identify the factors involved, we conducted a screen for ABA-insensitive mutants with altered ethylene production in *Arabidopsis*. A dominant allele of *ABI4*, *abi4-152*, which produces a putative protein with a 16-amino-acid truncation at the C-terminus of ABI4, reduces ethylene production. By contrast, two recessive knockout alleles of *ABI4*, *abi4-102* and *abi4-103*, result in increased ethylene evolution, indicating that ABI4 negatively regulates ethylene production. Further analyses showed that expression of the ethylene biosynthesis genes *ACS4*, *ACS8*, and *ACO2* was significantly decreased in *abi4-152* but increased in the knockout mutants, with partial dependence on ABA. Chromatin immunoprecipitation–quantitative PCR assays showed that ABI4 directly binds the promoters of these ethylene biosynthesis genes and that ABA enhances this interaction. A fusion protein containing the truncated ABI4-152 peptide accumulated to higher levels than its full-length counterpart in transgenic plants, suggesting that ABI4 is destabilized by its C terminus. Therefore, our results demonstrate that ABA negatively regulates ethylene production through ABI4-mediated transcriptional repression of the ethylene biosynthesis genes *ACS4* and *ACS8* in *Arabidopsis*.

Keywords: ABA, ABI4, ethylene biosynthesis, stress response, transcriptional regulation

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INTRODUCTION

Gaseous ethylene is an important phytohormone in the regulation of plant development (e.g., floral organs, fruit ripening, and senescence) and stress response (Guo and Ecker, 2004; Ji and Guo, 2013). Ethylene biosynthesis consists of three major steps. First, the ethylene precursor methionine is converted to *S*-adenosylmethionine (S-AdoMet). Then, 1-aminocyclopropane-1-carboxylic acid (ACC) is synthesized by ACC synthase (ACS) using S-AdoMet as a substrate. Finally, ethylene is released from ACC by ACC oxidase (ACO) (Yang and Hoffman, 1984). Ethylene production can be induced by floral organ development, ripening, senescence, and stress (Yamagami et al., 2003; Tsuchisaka and Theologis, 2004; Lin et al., 2009). Moreover, a variety of transcription factors regulate the transcriptional levels of the *ACS* and *ACO* genes. For example, the transcription factor hypocotyl elongation 5 (HY5), an integrator of light- and abscisic acid (ABA)-induced responses (Chen et al., 2008), participates in the regulation of ethylene biosynthesis through transcriptional modulation of the ethylene response factor (ERF) repressor AtERF11, which further suppresses the expression of *ACS2/5* and ethylene production (Li et al., 2011).

Increasing evidence has shown that the stability of ACS proteins is regulated by their C-terminal regions. MPK6-mediated phosphorylation of the C-terminal amino acid residues in ACS2/ACS6 significantly increases the levels of these proteins and ethylene production (Hernandez Sebastia et al., 2004; Liu and

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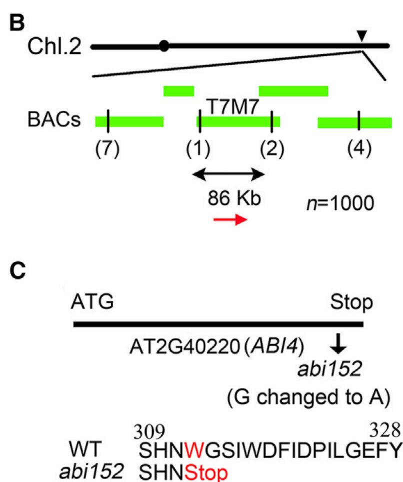
A Segregation of seed germination in response to ABA in F_2 population from the cross between *abi152* and col-0

Combination	Total number of seeds	Insensitive	Sensitive	Expected rate	χ^2
abi152/Col-0	326	251	72	3:1	0.7000



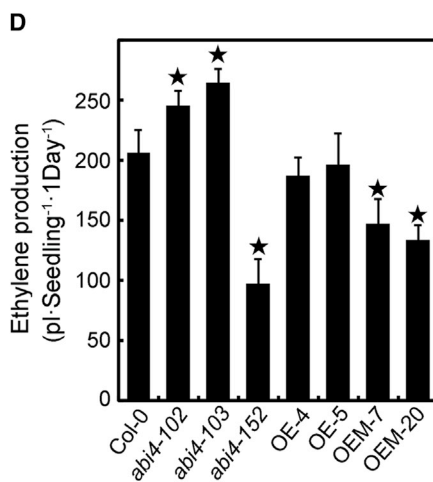


Figure 1. *abi4-152* Is a Dominant *ABI4* Mutant that Shows Decreased Ethylene Emission.

(A) Genetic segregation assay in response to ABA. Insensitive and sensitive indicate that the seeds were germinated or not germinated in 0.5 μM ABA containing MS medium, respectively.

(B) Physical map of the *abi152* locus. The numbers of recombinants are shown in parentheses.

(C) Mutation site of *ABI4* in *abi152*.

(D) Ethylene emission in 4-day-old seedlings. The values are the means ± SE (n = 3). P values (★: mutant versus Col-0) were determined with a two-tailed Student's t-test at $P < 0.05$.

Zhang, 2004). Similarly, *Arabidopsis* ethylene overproduction in *eto2* and *eto3* mutants was caused by disrupted C-terminal extensions in ACS5 and ACS9, respectively. These mutant proteins are more stable because they are more resistant to degradation by the 26S proteasome (Vogel et al., 1998a; Chae et al., 2003; Joo et al., 2008). ETO1, a component of the E3-ligase complex, directly interacts with ACS5, and mutated forms of ETO1 lead to increased stability of the ACS5 protein and the overproduction of ethylene (Guzman and Ecker, 1990; Wang et al., 2004). ABA treatment prevents the induction of ethylene biosynthesis (Wright, 1980; Li et al., 2011), whereas ethylene production is increased in the ABA-deficient mutant *aba2-1* compared to the wild type (LeNoble et al., 2004). Moreover, the ABA-activated CDPK protein kinases CPK4 and CPK11 can stabilize ACS6 by phosphorylating its C-terminus, promoting ethylene biosynthesis (Luo et al., 2014), and ABI1 regulates ozone-induced ethylene biosynthesis by affecting the ACS6 phosphorylation level, which is controlled by MPK6 (Ludwikow et al., 2014). These findings reveal an antagonistic interaction between ABA and ethylene biosynthesis that is regulated by protein phosphorylation. However, the mechanism of how ABA represses ethylene biosynthesis at the transcriptional level remains to be investigated.

ABI4 is a transcription factor involved in many aspects of plant development and stress responses (Finkelstein et al., 1998; Söderman et al., 2000; Penfield et al., 2006; Kerchev et al., 2011; Lee et al., 2015). This protein functions as both a repressor and an activator (Wind et al., 2013), and is partially regulated by the 26S proteasomal pathway (Finkelstein et al., 2011). In the present study, we identified *abi4-152*, a dominant mutation that converts Trp 313 to a premature termination codon in ABI4, resulting in a 16-amino-acid truncation of the C terminus. The fusion of ABI4-152 peptide (a truncated version of ABI4 in *abi4-152*) accumulated to higher levels than full-length ABI4 in plant cells, indicating that these 16 amino acids affect the protein's stability. The *abi4-152* mutant was insensitive to low concentrations of ABA and produced less ethylene, revealing both the repressor and activator biochemical function of ABI4. Thus, our results demonstrate that ABA negatively regulates ethylene production in *Arabidopsis* by repressing the expression of the ethylene biosynthesis genes *ACS4* and *ACS8*.

RESULTS

ABI4 Is a Negative Regulator in Ethylene Production

To identify factors in ABA-regulated ethylene biosynthesis, we screened ethyl methanesulfonate (EMS)-mutagenized seeds for mutants that could germinate on 0.5 μM ABA, which is inhibitory to the wild-type seeds (Supplemental Figure 1A). Four independent ABA-insensitive mutants were selected for further investigation. Relative to the wild type, the *abi227* and *abi304* mutants produced significantly more ethylene, whereas *abi81* and *abi152* produced only half that of wild type (Supplemental Figure 1B).

Although the *Arabidopsis* mutants *eto1*, *eto2*, *eto3*, *hy5*, and *xbat32* produce elevated levels of ethylene (Guzman and Ecker, 1990; Woeste et al., 1999; Chae et al., 2003; Li et al., 2011; Lyzenga et al., 2012), no single mutant with reduced ethylene production has been previously reported. Therefore, we focused our experiments on the mutant *abi152*, which showed reduced ethylene production. Phenotypic segregation experiments indicated that *abi152* is a dominant allele (Figure 1A), and genetic mapping revealed that the mutation is between the single nucleotide polymorphism markers S76 and S84 on the long arm of chromosome 2 (Figure 1B). DNA sequencing analysis of the genomic DNA in this interval revealed a single nucleotide change of G to A in the *ABI4* gene in *abi152*, which was identical to that of *abi81*, as noted above. This mutation converts Trp 313 to a premature termination codon, resulting in a 16-amino-acid truncation of the ABI4 C terminus (Figure 1C). ABI4 is a member of the ERF family and is an important element in the ABA signaling pathway (Finkelstein et al., 1998), and several recessive loss-of-function

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