Analysis of an ACC hypersensitive *A. thaliana* mutant

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Question

Does the *ahh1* phenotype follow the enhanced ethylene response pattern or the canonical hypersensitive pattern?

Background

Ethylene is a gaseous hormone that plays an important role in many cellular processes in plants, including the promotion of fruit ripening and the inhibition of cell elongation. We have identified an *Arabidopsis thaliana* mutant that is hypersensitive to ACC, an ethylene precursor.

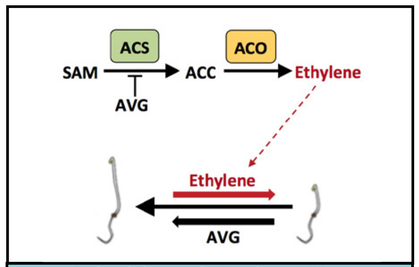

Figure 1. In the ethylene synthesis pathway, ACS converts ACC to ethylene, which inhibits hypocotyl elongation. AVG, a competitive inhibitor of ACS, rescues hypocotyl elongation.

This ACC Hypersensitive Hypocotyl 1 (*ahh1*) mutant has short hypocotyls on untreated MS1 media and is rescued by AVG treatment. Moreover, it shows an exaggerated response to exogenous ACC compared to the wild-type, Col-0.

Hypothesis

The *ahh1* mutant contains a T-DNA insertion in the B8 subunit of protein phosphatase 2A. However, since the *ahh1* phenotype and the T-DNA insertion do not co-segregate, a secondary mutation is likely causing the ACC sensitivity. Our goal is to characterize this secondary mutation by determining whether *ahh1* follows the "eer" ethylene induction pattern (hypothesis 1) or the canonical hypersensitive pattern (hypothesis 2).

	ACO2	ETR2	ERF1	EBP1
Col-0 (wild-type)	↑	↑	↑	↑
ein2 (ethylene insensitive)	–	–	–	–
eer5 (enhanced eth. response)	↑	↑	↑	–
Hypothesis 1: ahh1 feedback	↑	↑	↑	–
Hypothesis 2: ahh1 canonical	↑	↑	↑	↑

Figure 2. Transcript abundance of four "classic" ethylene-inducible genes with ethylene treatment (Christians 2008, *Plant Journal* **55**: 3). The expected response of *ahh1* under the two alternative hypotheses is shown in the bottom two rows.

The first hypothesis predicts that *ahh1* follows the "eer" induction pattern. In this feedback model, transcript abundance of ACO2, ETR2, and ERF1 will increase with ACC treatment, but EBP1 levels will remain the same. The second hypothesis predicts that *ahh1* follows the canonical induction pattern. In this model, transcript abundance of all four genes will increase with ACC treatment, but to an even greater extent than they do in Col-0.

Results

Col-0 shows induction of all 4 ethylene-inducible genes with ACC treatment

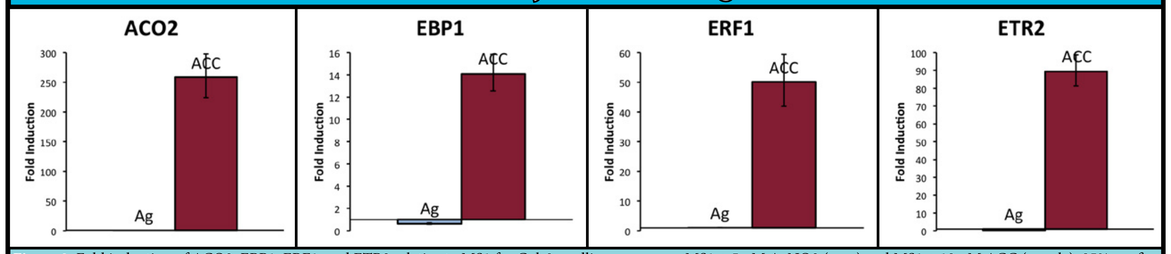

Figure 3. Fold induction of ACO2, EBP1, ERF1, and ETR2 relative to MS1 for Col-0 seedlings grown on MS1 + 5 uM AgNO3 (gray) and MS1 + 10 uM ACC (purple). 95% confidence intervals of the mean shown with error bars.

Relative abundance of ACO2, EBP1, ERF1, and ETR2 transcripts in Col-0 and *ahh1*

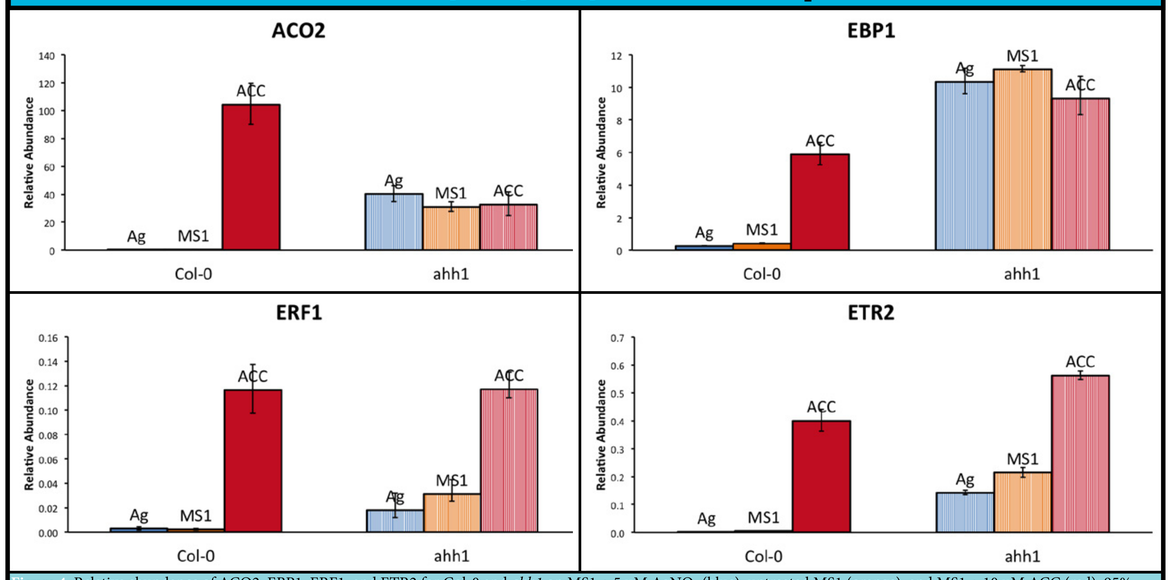

Figure 4. Relative abundance of ACO2, EBP1, ERF1, and ETR2 for Col-0 and *ahh1* on MS1 + 5 uM $AgNO_3$ (blue), untreated MS1 (orange), and MS1 + 10 uM ACC (red). 95% confidence intervals of the mean shown with error bars. Cycle times normalized to GENE1.

Baseline in *ahh1* elevated compared to Col-0

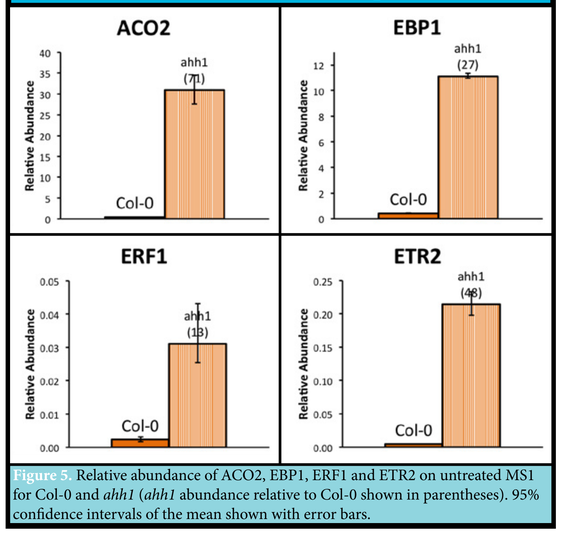

Figure 5. Relative abundance of ACO2, EBP1, ERF1 and ETR2 on untreated MS1 for Col-0 and *ahh1* (*ahh1* abundance relative to Col-0 shown in parentheses). 95% confidence intervals of the mean shown with error bars.

ahh1 seedlings show weak ctr-like phenotype

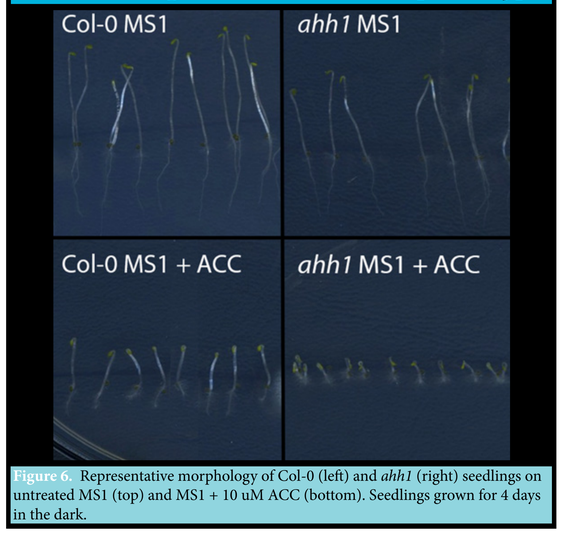

Figure 6. Representative morphology of Col-0 (left) and *ahh1* (right) seedlings on untreated MS1 (top) and MS1 + 10 uM ACC (bottom). Seedlings grown for 4 days in the dark.

Conclusions

In *ahh1*, ETR2 and ERF1 are induced with ACC, but ACO2 and EBP1 are not induced. Therefore, *ahh1* does not show canonical induction behavior. Moreover, its induction pattern is also not similar to the enhanced ethylene response mutants. From this information, we conclude that we are characterizing an unmapped ethylene lesion.

	ACO2	ETR2	ERF1	EBP1
ahh1	–	↑	↑	–

Figure 7. Induction pattern of *ahh1* with ACC treatment.

Since $AgNO_3$ treatment does not completely inhibit ACC induction in *ahh1*, the *ahh1* mutation likely affects a component after ETR1 in the ethylene reception pathway.

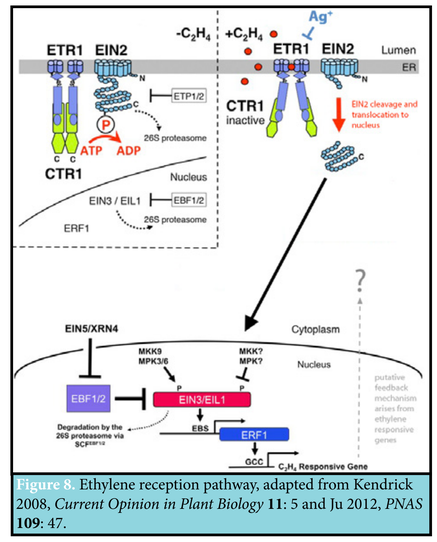

Figure 8. Ethylene reception pathway, adapted from Kendrick 2008, *Current Opinion in Plant Biology* **11**: 5 and Ju 2012, *PNAS* **109**: 47.

The baseline expression levels of all four genes in *ahh1* show a 10- to 100-fold increase compared to Col-0. The *ctr1-1* mutant has also been documented to have a higher baseline expression than Col-0 for ETR2 (Street 2013, *Plant Physiology* **168**: 3), ERF1 and EBP1 (Bie 2014, *Int. J. Mol. Sci.* **15**: 9). Moreover, *ahh1* seedlings show a weak ctr-like phenotype. These data suggest that CTR1 may be affected in the *ahh1* mutant.

Future Directions

Repeating gene expression analysis of *ahh1* seedlings that do not have the B8 T-DNA insertion will remove possible combination effects from the current data. Next, determining if there is linkage between the *ahh1* mutation and the B8 T-DNA insertion can help identify the possible location of the mutation. Finally, analyzing ACC induction patterns in *ctr1-1* could inform *ahh1*'s similarity to *ctr1-1*.

Acknowledgments

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