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Spotlight

Is losing ethylene a losing game?

It is well established that the gaseous plant hormone ethylene modulates growth and development and mediates responses to biotic and abiotic stresses. Seed plants produce ethylene from S-adenosyl-L-methionine, which is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS); ACC is then converted to ethylene by a dedicated enzyme, ACC oxidase (ACO) (Figure 1A). Many seed plant genomes carry multiple *ACO* homologs, allowing for spatiotemporal fine-tuning of ethylene biosynthesis. Non-seed plants (e.g., ferns, bryophytes) lack ACO homologs (Li et al., 2018) and produce ethylene presumably by an unknown non-ACC-dependent pathway. Numerous responses to ethylene have been identified by treating plants with exogenous ethylene. However, the removal of internally synthesized ethylene is required to determine the roles of endogenous ethylene.

Li et al. (2022a) recently reported in *Molecular Plant* the creation of ‘‘ethylene-free’’ *Arabidopsis* mutants that are unable to produce detectable levels of ethylene, as a result of knocking out all five *ACO* genes using CRISPR/Cas9 technology. In the same issue, Heydlauff et al. (2022) genetically crossed *Arabidopsis aco* T-DNA insertion lines to generate an *aco* quadruple mutant that is heterozygous for a fifth *aco* mutation. Both papers investigated the role of ethylene in aspects of reproduction; Li et al. (2022a) focused on programmed cell death/degeneration of the synergid cells, while Heydlauff et al. (2022) focused on the roles of the ethylene transcription factor ETHYLENE-INSENSITIVE3 (EIN3) in endosperm and seed development. It was previously reported that ethylene is required to initiate programmed cell death of the synergid cells, and can even bypass the fertilization requirement (Völz et al., 2013). However, surprisingly, Li et al. (2022a) showed that the *aco* quintuple knockout mutants have normal synergid cell death, indicating that ethylene is not necessarily required in this process (Figure 1B). Even so, they found that ethylene-independent cell death of the persistent synergid cell requires EIN3 and its homolog EIN3-LIKE1 (EIL1) (Li et al., 2022a).

Heydlauff et al. (2022) similarly showed that EIN3, but not ethylene gas, is responsible for proper cell death of the persistent synergid cell, which promotes endosperm development. Moreover, they demonstrated that, post fertilization, EIN3 has an opposing role that represses endosperm and seed development, but in this role EIN3 is dependent on a sporophytic ethylene signal (Figure 1B). Collectively, these novel findings place EIN3 at the crossroads of early seed development, with opposing signals controlled by the maternal sporophytic tissue versus the fertilized endosperm. Both papers conclude that losing the ability to synthesize ethylene does not necessarily compromise successful sexual reproduction, and both raise the idea of an unknown mechanism that controls EIN3 action independent of ethylene.

What about vegetative growth? The *Arabidopsis* ethylene-free mutants display phenotypes typically observed in plants that have reduced ethylene production or sensitivity (Li et al., 2022a). Dark-grown ethylene-free seedlings have slightly longer hypocotyls than the wild type, while light-grown ethylene-free plants produce more lateral roots and larger petals (Li et al., 2022a). Notably, none of these phenotypes are dramatic, consistent with the subtle phenotypes observed in *acs* higher-order mutants (Tsuchisaka et al., 2009) and in ethylene-insensitive mutants (Bleecker et al., 1988; Guzman and Ecker, 1990). Ethylene-insensitive mutants also have a larger rosette and delayed senescence (e.g., Bleecker et al., 1988), which have yet to be examined in the *aco* quintuple mutants. When ethylene levels are highly induced in *Arabidopsis* (e.g., in *ethylene overproducer* mutants [*eto*]), vegetative growth is significantly diminished (Guzman and Ecker, 1990). This suggests that a lack of ethylene production or reduced ethylene sensitivity enhances growth, whereas basal levels of ethylene mildly repress growth (Figure 1C). This is corroborated by ethylene's downstream action that operates via ETHYLENE RESPONSE FACTORS (ERFs), which are transcription factors involved in growth repression of developing leaves (Dubois et al., 2018). In essence, losing ethylene could be somewhat beneficial for vegetative growth.

Interestingly, ethylene might be dispensable for growth and development in some aquatic plants that have a fully submerged lifestyle. The genomes of two marine seagrasses, *Zostera muelleri* (Golicz et al., 2015) and *Zostera marina* (Olsen et al., 2016), lack the genes for both of the ethylene biosynthesis enzymes (ACS and ACO), as well as genes for core ethylene signaling components, suggesting that these two marine monocots are incapable of producing and responding to ethylene. Most likely, adaptation to a marine lifestyle ~64–72 million years ago resulted in the loss of ethylene biosynthesis and signaling. The fresh-water monocot *Potamogeton pectinatus*, which also has a fully submerged habitat, lacks detectable ACO activity and is incapable of generating ethylene despite producing ACC, but still responds to exogenous ethylene (Voesenek et al., 2015). Given that ethylene diffuses roughly 10 000 times slower in water than in air, losing the ability to synthesize ethylene could prevent unwanted ethylene entrapment in fully submerged angiosperms. Such accumulation of high levels of cellular ethylene could lead to severe impairments in growth, and thus losing ethylene may have been beneficial in these aquatic species (Voesenek et al., 2015).

If the loss of ethylene does not jeopardize normal growth or reproduction, are there any situations in which losing ethylene is

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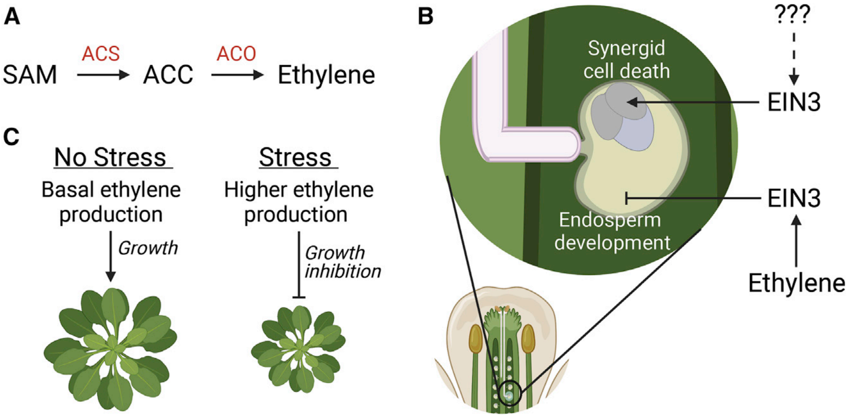

Figure 1. The involvement of ethylene in plant growth, reproduction, and stress resilience.
(A) The ethylene biosynthesis pathway in seed plants starts with the formation of 1-aminocyclopropane-1-carboxylic acid (ACC) from S-adenosyl-L-methionine (SAM) by ACC synthase (ACS). ACC is subsequently converted into ethylene gas by ACC oxidase (ACO).
(B) The ethylene master transcription factor, EIN3, promotes endosperm expansion by mediating cell death of the persistent synergid cell independent of ethylene. Separately, EIN3 represses endosperm expansion and seed growth via a sporophytic ethylene signal.
(C) Under ideal growth conditions (no stress), basal levels of ethylene production allow for normal growth, whereas enhanced ethylene production induced by stress results in growth inhibition. Figure created with Biorender.com.

disadvantageous? Given that it is well known that biotic and abiotic stresses trigger ethylene production and responses (e.g., Tsuchisaka et al., 2009; Voesenek et al., 2015; Chen et al., 2021), it is likely that ethylene is crucial for stress resilience. For example, it is possible that a stress-triggered boost in ethylene levels represses growth, saving crucial energy and tipping the plant into survival mode (Figure 1C). We thus expect the *aco* quintuple mutants to be less effective in responding to stressful conditions, although this remains to be investigated. Perhaps losing ethylene is not a losing game for normal growth and reproduction in *Arabidopsis*, but losing ethylene might impose a survival penalty in the face of stress. In fact, the findings of Li et al. (2022a) and Heydlauff et al. (2022) could be different for plants grown in their natural habitat where they would encounter a variety of biotic and abiotic stresses that are not present under laboratory growth conditions. Alternatively, the *aco* quintuple mutants could conceivably contain a low level of internal ethylene (perhaps produced by an alternative mechanism) that is below the limit of detection. We predict species that produce far higher levels of ethylene compared with *Arabidopsis*, such as tomato, could be profoundly affected if ethylene is lost. For instance, it is well known that ethylene plays a key role in fruit ripening.

The development of *aco* quintuple mutants provides a breakthrough genetic tool in ethylene biology that brings new opportunities and raises new questions. The ethylene-free mutants can now be used to examine the roles of ethylene biosynthesis during stress. Given recent discoveries regarding ethylene-independent ACC signaling (Li et al., 2022b), the ethylene-free plants can help to uncover novel ACC signaling functions and aid in elucidating the unknown ACC signaling pathway. It will be interesting to determine what happens to ACC levels in the *aco* mutants; is there a negative feedback mechanism that lowers ACC levels when not consumed to produce ethylene, or does the lack of ethylene production result in ACC accumulation? Such feedback has been observed in ethylene-insensitive mutants (e.g., *etr1-1* and *ein2-1*), which have a higher ethylene production rate compared with wild-type plants (Bleecker et al., 1988; Guzman and Ecker, 1990). Alternatively, the *aco* quintuple mutants could compensate for ACC accumulation by generating higher levels of ACC conjugates. It is currently unknown whether these ACC derivatives are biologically active. The field can now take advantage of the *aco* mutants, combined with metabolic and biochemical analyses of ACC homeostasis, to fully understand the distinct roles of ethylene and ACC in driving plant growth, reproduction, and stress responses.

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