Ethylene in mutualistic symbioses

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Ethylene (ET) is a gaseous phytohormone that participates in various plant physiological processes and essentially contributes to plant immunity. ET conducts its functions by regulating the expression of ET-responsive genes or in crosstalk with other hormones. Several recent studies have shown the significance of ET in the establishment and development of plant-microbe interactions. Therefore, it is not surprising that pathogens and mutualistic symbionts target ET synthesis or signaling to colonize plants. This review introduces the significance of ET metabolism in plant-microbe interactions, with an emphasis on its role in mutualistic symbioses.

Introduction

Various microorganisms frequently attack plants with the purpose to acquire nutrients. The outcome of these interactions can be neutral (commercialism), harmful (parasitism), or beneficial (mutualism) to the host. In mutualistic associations, the interaction between plant and microbe is thought to be highly balanced and plants apparently control the degree of colonization. Under certain environmental conditions, plants might lose this control and mutualists can even cause diseases.[1] This raises the question whether plants distinguish between pathogenic and mutualistic invaders in all cases. Various studies have shown that like pathogens mutualistic symbionts activate immune signaling in plants upon perception.[2,3] Thus, both, mutualistic symbionts and pathogens must obviously overcome the host's surveillance system to colonize plants. While strategies of mutualists are less well understood,[4] pathogens release so-called effector proteins to disturb plant immune signaling or to reprogram host metabolism including modifications of the host hormone homeostasis.[5] Plant immunity strongly relies on plant hormones to orchestrate a complex and interactive network of defense pathways to ward off attackers.[6-8] Among plant hormones, ethylene (ET) essentially participates in the activation and regulation of plant immunity.[9] ET is formed from methionine via the activity of the enzyme S-adenosyl-l-methionine (SAM) synthetase, which converts methionine to SAM.[10] 1-aminocyclopropane-1-carboxylic acid (ACC) synthases (ACS) use SAM to generate ACC. ACC is converted to ET by the enzyme ACC oxidase. ACSs are involved in ET biosynthesis.[11] The abundance of ACSs closely correlates with the level of ET production in most plant tissues and various *ACS* gene family members are expressed differentially in response to developmental and environmental triggers.[11,12]

ET perception and signaling is highly regulated at transcriptional and post-transcriptional levels, and is well defined in the model plant Arabidopsis.[13] ET is recognized by endoplasmic reticulum (ER) membrane-bound receptors (e.g., ETHYLENE RECEPTOR1, ETR1). In the absence of ET, the receptors activate the Raf-like protein kinase CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) to block the ET response pathway.[14] In contrast, binding of ET inactivates the receptors and deactivates CTR1, which allows downstream effectors like ETHYLENE INSENSITIVE 2 (EIN2) to translocate from the ER membrane to the nucleus and to function as a positive regulator of ET signaling.[13,15,16] ET signaling downstream of EIN2 is mediated by EIN3 and EIN3-like (EIL) proteins, the key transcription factors regulating ET responses.[17] ET can stabilize EIN3 by preventing its degradation by EIN3-binding F-box proteins 1 and 2 (EBF1/2). In the absence of ET, EBF1/2 negatively regulate ET signal transduction by subjecting EIN3 and possibly the related EIL proteins to an ubiquitin/26S proteasome pathway.[18-20] Upon ET treatment, EIN3 quickly stabilize and accumulates in the nucleus, where it functions as transcription factor. EIN3 and EIL1 regulate ET-responsive genes by binding to the primary ET response element in promoter regions. ETHYLENE RESPONSE FACTOR 1 (ERF1) is another transcriptional regulator of another set of ET-responsive genes. EIN3 and ERF1 act sequentially in a cascade of transcriptional regulation initiated by ET.[21] The impact of ET and other plant hormones on plant immunity has been introduced in various excellent reviews, which, however, provide limited information on its role in mutualistic interactions.[9,22,23] The present review aims to highlight similarities and differences of ET functions in pathogenic and mutualistic symbioses.

Function of ET in Plant Immunity

Plant immunity is immediately activated after microbial recognition and, if microbes lack effective counterstrategies, usually stops the establishment of pathogenic and presumably mutualistic plant-microbe interactions. Like pathogens, mutualistic symbionts trigger plant immunity, as the underlying perception system does not discern life styles but recognizes microbe-derived molecules. So-called pattern recognition receptors (PRRs) perceive highly conserved microbial molecules termed microbe-associated molecular patterns (MAMPs). Well described MAMPs are fungal chitin or bacterial flagellin (active epitope flg22), which are detected in Arabidopsis by the PRRs CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) and FLAGELLIN-SENSING

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2 (FLS2), respectively.[24,25] The immune response activated by PRRs is defined as MAMP-triggered immunity (MTI). Certain signal transduction pathways associated with pathogen recognition are linked with increased ET production.[26] Moreover, *FLS2* transcription is controlled by binding of EIN3 and EIL1 to the FLS2 promoter.[27] flg22 treatment induces ET production due to the phosphorylation of the rate-limiting ET biosynthetic enzymes ACS2 and ACS6 by the MAP kinases 3 and 6. These MPKs further phosphorylate EIN3 resulting in its stabilization.[17,28] However, not only EIN3 and EIL1 define ET as an integral part of the plant immune system. ET activates various transcription factors (e.g., ERF1) that are involved in the regulation of immunity-associated genes. By this means and via its complex interactions with other hormones such as salicylic acid (SA) or jasmonate (JA), ET contributes to the robustness and effectiveness of plant immunity.[23,29] ET-associated immunity was shown to affect the virulence of different pathogens with necrotrophic and hemibiotrophic lifestyles.[30-32] Plants disturbed in translating ET production to signaling such as the *ein2* mutant are impaired in MTI, resulting in enhanced susceptibility to the bacterial pathogen *Pseudomonas syringae*.[26] ET application or constitutive expression of *ERF1* protects plants against the necrotrophic fungus *Botrytis cinerea*, while ET-insensitive mutants (e.g., *ein2*) show increased susceptibility to *B. cinerea*.[33,34] In addition to its impact on local resistance, ET is further required for induced systemic resistance (ISR) activated in roots by rhizobacteria and resulting in the systemic protection of leaves against microbial pathogens.[35]

ET in Mutualistic Plant-Microbe Interactions

Despite its impact on mutualistic plant-microbe symbioses, little attention has been paid to the function of ET in the establishment of these associations.[36-41] For instance, ET application was shown to negatively influence nodulation in legume-rhizobia symbioses as ET prevented Nod factor signaling in *Medicago truncatula*.[36] In addition, ET significantly restricted the spread of the arbuscular mycorrhizal (AM) fungus *Glomus aggregatum* in pea (*Pisum sativum*). Interestingly, the number of fungal appressoria formed was not affected by ET, but the appressoria showed abnormal morphology (swollen and highly branched). This resulted in a reduction of AM fungal entry into the root tissue, slower hyphal growth, and therefore reduced colonization. Colonization generally proceeded, although the extension of colonization units was restricted.[37] ET has been further suggested as a negative regulator in early phases of the symbiotic interaction of *M. truncatula* with the mycorrhizal fungi *Endogone versiformis* (syn. *G. versiforme*) and *Rhizophagus intraradices* (syn. *G. intraradices*).[40] It is most likely a key regulator of *Rhizophagus clarus* (syn. *G. clarum*) root colonization in tomato mutants *epinastic* (*epi*)[39] and *never ripe*,[41] which show an ET overproduction and low ET sensitivity phenotype, respectively.[42] The inhibitory effects of ET on root colonization of these mutants was mainly explained by a reduced intraradical fungal growth.[42] Finally, ET affected colonization of *Nicotiana attenuata* by *R. intraradices*.[38] Although all these studies underpin a negative impact of ET on root colonization by mycorrhizal fungi, this effect of ET is apparently

concentration-dependent.[43] While higher ET levels are considered as being inhibitory to the formation of AM, very low concentrations may promote arbuscule formation.[38,42] Therefore, the amount of ET synthesized at a particular time is apparently essential for AM establishment.[38,44] These results suggest an immune function of high ET levels that block the establishment of mutualistic symbioses. This is unexpected, considering the general biotrophic life style of mutualists. Although ET is part of MTI and thought to substantiate MTI,[26,27,29] ET mostly wards off necrotrophic rather than biotrophic pathogens.[8,23] Since ET accumulation in beneficial root interactions, including the ectomycorrhizal interactions of *Laccaria laccata* and *Pisolithus tinctorius* with roots of *Pinus mugo*,[45] is obviously not part of the establishment of the symbiosis, it might represent a defense response upon perception of the mutualists by the root immune system. In fact, MAMPs elicit immune responses in roots that are at least partially similar to those in leaves.[3,46] In addition to root oxidative burst, MAMPs induce MPK3 and 6 phosphorylation and expression of canonical marker genes in roots.[3,47] Moreover, mutualistic fungi such as *Piriformospora indica* release immunity-activating MAMPs, which can impair the establishment of the mutualistic root symbioses.[3] Consistent with this, flg22-triggered defense responses in roots of *Lotus japonicas* negatively influenced nodulation by inhibiting rhizobial infections and delaying nodule organogenesis[48] or disturbed Arabidopsis root colonization by *P. indica*.[3] Therefore, mutualists might have developed strategies to prevent the activation of root immunity as reported for plant-rhizobia symbioses. Despite the presence of FLS2 in both plants, flagellin from *Mesorhizobium loti* or *Sinorhizobium meliloti* does not elicit immune responses in *L. japonicus* and tomato; most probably due to the differences in the N termini of these flagellins, which prevent recognition by FLS2.[48] Apparently, this represents a co-evolutionary adaptation between rhizobia and its hosts to support the establishment of the mutualistic symbioses. Mutualistic microbes have developed further strategies to disturb immune activation. Like pathogens, mutualists secrete so-called effectors in order to disturb host immune signaling. Considering the role of ET in plant immunity and its negative impact on the establishment of mutualistic fungal symbioses, mutualists might use effectors or have developed yet unknown strategies to block ET signaling. The mycorrhizal fungus *R. intraradices* was shown to release the effector Sp7 in order to suppress plant defense responses.[49] Sp7 has a nuclear localization signal, as well as several repeat domains of unknown function. The effector interacts with the host transcription factor ERF19, suggesting a biological function of Sp7 in regulating the expression of several ET responsive defense genes. Interestingly, fungal extracts from *R. intraradices* induced expression of *ERF19* in *M. truncatula* roots while the infection of *M. truncatula* roots *by R. intraradices* only transiently induced *ERF19* gene expression.[49] Similarly, the plant growth-promoting bacterium *Pseudomonas fluorescens* is apparently able to suppress the expression of genes encoding certain ET transcription factors during bacterial root colonization.[35] Other plant growth-promoting bacteria (PGPBs), such as *Rhizobacteria* spp., possess the enzyme ACC deaminase, which degrades the ET precursor ACC and thus facilitates plant growth and development

by decreasing plant ET levels.[50] However, PGPBs might rely on ACC deaminase activity not only to block ET immunity. Many PGPBs depend on ACC degradation products as nitrogen source.[51,52] Thus, the positive correlation between ACC deaminase activity and PGPB growth might have nutritional and/or immunity-related reasons.

ET does not per se counteract the establishment of mutualistic symbioses. For instance, nodule initiation and associated cell division at the base of lateral roots in the semiaquatic legume *Sesbania rostrata* requires ET.[53] In the absence of ET, *S. rostrata* colonization and nodulation did not occur.[53] ET effectively supports fungal root colonization of the mutualistic fungus *P. indica* in the monocot barley and dicot Arabidopsis. In barley, the free ACC content is elevated during early colonization stages. Further, while treatment with the ET precursor ACC supported barley root colonization, application of the ET inhibitor 1-methylcyclopropene (MCP) reduced it. In Arabidopsis, the effect of ET on *P. indica* colonization was studied using ET mutants.[54,55] Similar to barley, the Arabidopsis ET insensitive mutant *ein2-1* showed reduced root colonization while Arabidopsis mutants *ctr1* and *eto1-1*, which display constitutive ET signaling or ET biosynthesis, respectively, exhibited significantly increased root colonization.[55] This study further showed that ET-induced immunity might not disturb but support root colonization by *P. indica*. Arabidopsis plants overexpressing *ERF1* (35::*ERF1*), and thus displaying constitutive ET-associated defense, showed improved root colonization by *P. indica*.[55] These findings may be explained by the antagonism of ET and SA-mediated immune signaling. Chen et al. (2009) demonstrated the repression of SA immunity by EIN3/EIL1.[56] EIN3/EIL1 are further activated by the JA pathway[23] and *P. indica* was dependent on JA for suppression of SA-mediated immunity and for successful fungal root colonization.[3] Thus, *P. indica* may recruit the ET and JA pathway to block SA mediated immunity, which would otherwise effectively stop root colonization.[3] Interestingly, ET signaling even supported later cell death-dependent colonization of *P. indica*, which is in contrast to the effectiveness of ET signaling in stopping necrotrophic pathogens such as *B. cinerea*. This is most probably explained by different types of cell death activated by *P. indica* and necrotrophic pathogens. *P. indica*-induced cell death is molecularly and biochemically different from necrotrophic

cell death as already implicated by the absence of root necrotization in *P. indica*-colonized roots.[57-59] *P. indica* induces a vacuolar programmed cell death by impairing ER function. The resulting ER stress activates a vacuolar processing enzyme (γVPE), whose caspase 1-like activity is required for the execution of cell death.[58] Considering the improved root colonization in *ctr1* and *eto1* mutants at late interaction stages, *P. indica* might even use the pro cell death activities of ET in favor of its cell death-dependent life style.

Conclusions

In general, ET is apparently a negative regulator of mutualistic root symbioses, although exceptions occur (e.g., *P. indica*). Considering the biotrophic life style of these root microbes, these findings are in clear contrast to the mostly supportive effect of ET on leaf colonization by biotrophic pathogens. To understand this discrepancy, further analyses of the function of ET in root symbioses is required. This includes basic experiments to answer whether ET impairs root symbioses by affecting immunity or physiological processes (e.g., root hair development). It is furthermore almost unknown, which role ET has as part of hormonal regulatory networks in roots. Studies in tomato showed the significance of hormonal crosstalk, as abscisic acid supported mycorrhization by antagonising ET.[60,61] These analyses will reveal hormonal networks that might significantly differ from leaf tissue. The apparent absence of an ET-JA synergism in roots to support biotrophic mycorrhization as reported for biotrophic plant-pathogen interactions in leaves might be a first indication. In contrast to ET, JA seems to support nodulation and mycorrhization of roots.[62]

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