Chapter 11
Ethylene and Plant Immunity

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Abstract The phytohormone ethylene is implicated in diverse biological processes including resistance against pathogens. Ethylene production in plants is enhanced in response to pathogen infection. Activated ethylene signaling contributes positively or negatively to resistance depending on enemies and environmental conditions. In some cases, reported roles of ethylene during plant immunity are controversial. Although the core ethylene signal transduction pathway from the biosynthesis, perception to transcriptional response is well characterized, it is highly interconnected with other signaling pathways such as those mediated by the phytohormones salicylic acid and jasmonates. This fact could explain the complexity and controversy of findings. Recent advances using molecular genetics, genomics and computational approaches have started untangling the role of ethylene in the complex immune signaling network. In addition, ethylene is emerging as a key modulator of plant-microbe interactions beyond plant immunity. This chapter highlights the significance of and mechanisms underlying the ethylene signaling network in plant-microbe interactions.

Keywords Botrytis-induced kinase 1 (BIK1) · Ethylene · Jasmonates (JA) · Immune signaling network · Map kinase (MAPK) · Phytohormones · Plant immunity · Salicylic acid (SA)

11.1 Introduction

Pathogen challenge of plants in many cases triggers enhanced ethylene production (Broekaert et al. 2006; Erb et al. 2012; Howe and Jander 2008; Lai and Mengiste 2013; Yang et al. 2013). The production of the ethylene precursor 1-aminocyclo-

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propane-1-carboxylic acid (ACC) by ACC synthases (ACS) is the first committed and considered as the rate-limiting step in ethylene biosynthesis (Broekaert et al. 2006). Produced ethylene binds to its receptors such as ETHYLENE RESPONSE1 (ETR1) which predominantly localize to the membrane of the endoplasmic reticulum (Merchante et al. 2013). In the absence of ethylene, the active receptors negatively regulate the key signaling component ETHYLENE INSENSITIVE2 (EIN2) through phosphorylation via the protein kinase CONSTITUTIVE TRIPLE RESPONSE1 (CTR1) (Merchante et al. 2013). Upon ethylene perception, the receptors become inactivated, which in turn facilitates EIN2 activation to mediate the ethylene signaling (Merchante et al. 2013). Upon activation, the C-terminal part of EIN2 is cleaved off and moves into the nucleus to mediate the ethylene signaling via the key transcription factors ETHYLENE INSENSITIVE3 (EIN3) and EIN3-LIKE1 (EIL1) (Ju et al. 2012; Wen et al. 2012; Qiao et al. 2012). EIN3 and EIL1 regulate expression of ethylene-responsive genes such as the transcription factor *ETHYLENE RESPONSE FACTOR1* (*ERF1*) and *OCTADECANOID-RESPONSIVE ARABIDOPSIS AP2/ERF59* (*ORA59*) (Solano et al. 1998; Zander et al. 2012). Although the core ethylene signaling pathway seems linear, recent discoveries suggest existence of a much more complex pathway with both positive and negative regulatory feedbacks (Merchante et al. 2013). Limited studies show how the ethylene signal transduction pathway eventually contributes to resistance against pathogens (Botanga et al. 2012; Lloyd et al. 2011). For instance, a quantitative metabolomics study suggests that ethylene signaling contributes to resistance against a fungal pathogen, *Botrytis cinerea* through cell wall modifications (Lloyd et al. 2011). However, further research is required to fully understand the molecular mechanisms in ethylene-mediated resistance against pathogens.

The phytohormones ethylene, jasmonates (JA) and salicylic acid (SA) are immune-related hormones. In general, ethylene and JA-mediated signaling play important roles in resistance against necrotrophic pathogens which actively kill plants to get nutrients whereas SA signaling is a major contributor of resistance against biotrophic pathogens which require living hosts for multiplication (Glazebrook 2005). Roles of ethylene signaling in resistance against these different types of pathogens are sometimes controversial. For instance, upon recognition of conserved bacterial signatures such as flagellin, ethylene production is enhanced as a plant immune response (Liu and Zhang 2004; Broekaert et al. 2006). On the other hand, bacterial virulence factors actively trigger ethylene production for virulence (Kenyon and Turner 1992; Xiao et al. 2007). These results raise a question whether ethylene signaling is a positive or negative regulator in immunity against bacterial pathogens. This controversy could be explained by the fact that ethylene signaling is highly interconnected with many signaling pathways including those mediated by JA and SA, which results in the complex ethylene signaling network (Fig. 11.1) (Glazebrook 2005; Pieterse et al. 2012). Furthermore, plant immune responses are affected by many abiotic environmental factors such as temperature and light (Hua 2013). Thus, the outcome of ethylene signaling is influenced by many factors and interlinked signaling pathways. Therefore, the contribution of ethylene signaling in plant immunity is not easy to determine and sometimes controversial. This book

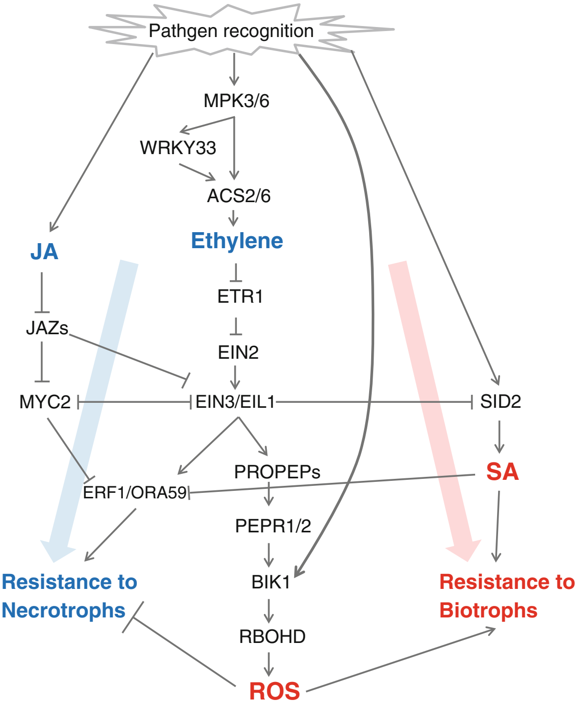

Fig. 11.1 A simplified schematic representation of the ethylene signaling network in plant immunity. *Arrows* and *end-blocked lines* indicate positive and negative regulation, respectively. In general, ethylene and JA signaling contribute to resistance against necrotrophs while SA signaling against biotrophs. MAPK signaling contributes to enhanced ethylene production in plant immunity. BIK1 is important for ROS production through RBOHD. Ethylene-activated PEPR signaling is involved in immune signal amplification through BIK1

chapter focuses on recent discoveries in ethylene and plant immunity, with a special emphasis made on studies that impact our mechanistic understanding of how ethylene signaling is integrated in the complex signaling network in plant immunity against microbial pathogens. Ethylene signaling is also a major signaling cassette for resistance against insect herbivores and rhizobacteria-mediated induced systemic resistance (ISR), which are, however, extensively reviewed elsewhere (Howe and Jander 2008; Erb et al. 2012; Van Wees et al. 2008) and are not discussed here.

11.2 Ethylene and MAP Kinases

Mitogen-activated protein kinase (MAPK) cascades are important signaling pathways that link extracellular stimuli to intracellular responses in eukaryotes through phosphorylation of substrates, and many studies show their implications in plant immunity

(Meng and Zhang 2013). Activation of MAPKs is carried out by their upstream kinases, MAPK kinases (MAPKKs) which are regulated by their upstream kinases, MAPKK kinases (MAPKKKs) (Meng and Zhang 2013). The MAPKs MPK3 and MPK6 are activated and are major regulators during plant immunity (Meng and Zhang 2013). *Arabidopsis* mutants deficient in both *MPK3* and *MPK6* are lethal, suggesting a functional overlap between MPK3 and MPK6 (Wang et al. 2007). Ethylene production is enhanced upon perception of microbe-associated molecular patterns (MAMPs) such as flg22, a part of the bacterial flagellin (Liu and Zhang 2004; Avni et al. 1994; Bar and Avni 2009). MPK6 phosphorylates selected isoforms of ACS, the rate-limiting enzyme of ethylene biosynthesis (Liu and Zhang 2004). Phosphorylation of type I isoforms ACS2 and ACS6 by MPK6 leads to the stabilization of the ACS proteins, resulting in elevated levels of ACS activity and ethylene production (Liu and Zhang 2004). Unphosphorylated ACS6 protein is rapidly degraded by the 26S proteasome pathway, which is blocked through its phosphorylation by MPK6 (Joo et al. 2008). Overexpression of a phospho-mimic ACS6 is sufficient for enhanced ethylene production (Liu and Zhang 2004). MPK3 plays also an important role in phosphorylation of ACS2 and ACS6 proteins. Upon infection with the necrotrophic fungal pathogen *B. cinerea*, ethylene production is greatly compromised in a conditional *mpk3 mpk6* double mutant but no single mutants, indicating overlapping roles for these MAPKs in *Botrytis*-induced ethylene production (Han et al. 2010). Double mutation in *ACS2* and *ACS6* genes greatly reduces *B. cinerea*-induced ethylene production (Han et al. 2010). Involvement of MPK3 and MPK6 for ethylene production is also supported by another study (Xu et al. 2008). Thus, the stabilization of ACS2 and ACS6 proteins through phosphorylation by MPK3 and MPK6 is the critical step for enhanced ethylene production in plant immunity.

Regulation of ACS activity seems more complex. A tomato type I ACS is phosphorylated by a calcium-dependent protein kinase (CDPK) and its phosphorylation stabilizes the enzyme, leading to increased ACS activity and ACC content (Kamiyoshihara et al. 2010; Tatsuki and Mori 2001). ACS6 protein is dephosphorylated by the protein phosphatase 2A ROOTS CURL IN 1-N-NAPHTHYL-PHATHALAMIC ACID1 (RCN1). Mutation in *RCN1* leads to ethylene overproduction dependent on *ACS2* and *ACS6* (Skottke et al. 2011) although the role of RCN1 in plant immunity remains to be investigated. A subset of *ACS* genes is transcriptionally induced upon pathogen attack. Recently, it was shown that expression of *ACS* genes is regulated by MPK3 and MPK6. The transcription factor WRKY33 is a substrate of MPK3 and MPK6 (Mao et al. 2011). When MPK3 and MPK6 are activated by expression of the constitutive active form of the upstream MAPKK or *B. cinerea* infection, mutants deficient in *WRKY33* are partially compromised in expression of *ACS2* and *ACS6* and ethylene production (Li et al. 2012). Furthermore, WRKY33 directly binds to the promoters of *ACS2* and *ACS6* genes (Li et al. 2012). These results suggest that WRKY33 is activated by MPK3 and MPK6 and activated WRKY33 directly regulates expression of *ACS2* and *ACS6*, resulting in enhanced ethylene production. Taken together, regulation of ACS activity involves multiple steps with positive and negative outcomes and is the critical step for increased ethylene production during plant immunity.

The controversial involvement of a MAPK cascade in signaling downstream of ethylene perception has been discussed (Yoo et al. 2009; Ji and Guo 2013; Merchante et al. 2013; An et al. 2010; Yoo et al. 2008; Ouaked et al. 2003). For instance, MPK6 is activated by the treatment with ACC. This activation does not occur in *etr1* but does in *ein2* and *ein3*, suggesting that MPK6 is a signaling component between the ethylene receptor ETR1 and signaling component EIN2 (Ouaked et al. 2003). In addition, both MPK3 and MPK6 are activated by ACC through the MAPKK MKK9. The *mkk9* mutant exhibits a wide range of ethylene-insensitive phenotypes (Yoo et al. 2008). Furthermore, MPK3 and MPK6 activated by MKK9 directly phosphorylate EIN3, which contributes to its stabilization and activation of downstream transcriptional reprogramming (Yoo et al. 2008). However, another study shows that EIN2 is absolutely required for ethylene-induced EIN3/EIL1 stabilization whereas MKK9 is not (An et al. 2010). Furthermore, ethylene response phenotypes and gene expression downstream of EIN3 are not compromised in the *mkk9* mutant (An et al. 2010). Thus, the involvement of the MAPK cascade in ethylene signaling after ethylene perception is clearly controversial and requires further studies to clarify the point.

There is another layer of interactions between ethylene and MAPK signaling. MPK6 interacts with and phosphorylates the transcription factor ETHYLENE RESPONSE FACTOR104 (ERF104), and the complex is dissociated in response to flg22 (Bethke et al. 2009). This complex dissociation requires MPK6 activity and ethylene signaling as inactive variants of MPK6 fails to release ERF104 in response to flg22 and the complex dissociation is compromised in the ethylene-insensitive *ein2* and *ein3 eil1* mutants (Bethke et al. 2009). These results suggest that ethylene signaling acts on MPK6 to allow ERF104 to access target genes.

11.3 Ethylene and Pattern-Triggered Immunity

Plants recognize MAMPs derived from microbes through plasma membrane-localized pattern recognition receptors (PRRs) and trigger pattern-triggered immunity (PTI) (Monaghan and Zipfel 2012; Tsuda and Katagiri 2010). The best characterized PRRs include *Arabidopsis* FLAGELLIN SENSING2 (FLS2) for flg22 and ELONGATION FACTOR-TU (EF-Tu) RECEPTOR (EFR) for elf18 (a part of the bacterial EF-Tu) (Zipfel et al. 2004, 2006). BRI1-ASSOCIATED KINASE1 (BAK1) is a part of the receptor complexes for both the MAMPs flg22 and elf18 (Chinchilla et al. 2007; Sun et al. 2013). The receptor complexes interact with and phosphorylate BOTRYTIS-INDUCED KINASE1 (BIK1) for downstream immune responses (Cui et al. 2010; Lu et al. 2010; Kadota et al. 2014; Li et al. 2014; Lin et al. 2014). Ethylene signaling is important for accumulation of the PRR FLS2. Expression of *FLS2* is directly controlled by EIN3 and EIL1 through their bindings on the *FLS2* promoter and is compromised in ethylene signaling mutants (Mersmann et al. 2010; Boutrot et al. 2010). Although ethylene signaling plays a critical role in the basal accumulation of FLS2 before infection, it does not seem to be a major contributor of later transcriptional induction of *FLS2* during PTI (Tsuda et al. 2009; Mersmann et al. 2010). Nevertheless, the basal FLS2 accumulation controlled by ethylene signaling contributes to early immune responses during flg22-triggered PTI (Boutrot et al. 2010; Mersmann et al. 2010).

The *Arabidopsis* PRRs PEP1 RECEPTOR1 (PEPR1) and PEPR2 recognize the endogenous elicitor-active epitopes conserved in ELICITOR PEPTIDE PRECURSORs (PROPEPs) such as Pep1 and trigger immune responses somewhat similar to those in PTI (Huffaker et al. 2006; Yamaguchi et al. 2006; Huffaker and Ryan 2007; Yamaguchi et al. 2010; Ma et al. 2012). Immune responses such as production of reactive oxygen species (ROS), callose deposition and transcriptional reprogramming including *PROPEP2* induction in response to flg22 and elf18 are compromised in the ethylene-insensitive *ein2* mutants (Tintor et al. 2013). Resistance triggered by elf18 against the hemi-biotrophic bacterial pathogen *Pseudomonas syringae* is compromised in *ein2* and *pepr1 pepr2* mutants (Tintor et al. 2013). As indicated in the name, *BIK1* was isolated as a gene whose mutants showed susceptibility to *B. cinerea* and is implicated in ethylene signaling (Veronese et al. 2006; Laluk et al. 2011). PEPR1 specifically interacts with BIK1 and the related protein PBS1-LIKE1 (PBL1) to trigger Pep1-induced immunity (Liu et al. 2013). PEPR1 directly phosphorylates BIK1 in response to Pep1 as analogous to FLS2-BIK1 in response to flg22 (Liu et al. 2013). Interestingly, mutants deficient in *PEPR1* and *PEPR2* or *BIK1* show reduced sensitivity to ethylene and are compromised in ethylene-induced resistance to *B. cinerea* (Liu et al. 2013). Ethylene treatment induces BIK1 phosphorylation in a PEPR-dependent manner but Pep1-triggered BIK1 phosphorylation is independent of *EIN3* and *EIL1* (Liu et al. 2013). These results illustrate a signaling mechanism by which ethylene and PEPR signaling pathways are coordinated to amplify PTI responses: MAMP recognition triggers BIK1 phosphorylation and ethylene production, enhanced ethylene production is transduced through the core ethylene signal transduction pathway (receptors-EIN2-EIN3/EIL1) to induce expression of *PROPEP* genes, and Pep peptides presumably processed from PROPEP proteins are perceived by PEPRs to further activate BIK1 through phosphorylation.

Recently, two reports showed that BIK1 directly interacts with and phosphorylates the NADPH oxidase RESPIRATORY BURST OXIDASE HOMOLOG D (RBOHD) to regulate ROS production (Kadota et al. 2014; Li et al. 2014), suggesting regulation of ROS production by ethylene signaling through BIK1. Consistently, biphasic production of ROS is dependent on ethylene signaling during the hemi-biotrophic oomycete pathogen *Phytophthora parasitica* infection in tobacco (Wi et al. 2012). Several studies also support the idea that ethylene signaling enhances ROS production (Desikan et al. 2006; Pogany et al. 2009). Thus, ethylene signaling can contribute to resistance against pathogens through ROS production regulated by BIK1. However, involvement of ethylene signaling on ROS accumulation is more complicated as ethylene-induced flavonols are shown to scavenge ROS (Watkins et al. 2014). ROS positively or negatively regulates ethylene production depending on context (Pogany et al. 2009; Wi et al. 2012), which could be explained by the fact that ROS signaling is highly interconnected with other signaling pathways such as MAPK, JA and SA (Scheler et al. 2013; Torres et al. 2006).

11.4 Ethylene and Other Hormone Signaling Pathways

Roles of ethylene signaling during plant immunity are dependent on infectious agents and sometimes controversial, which could be explained by its intimate interactions with JA and SA signaling (Fig. 11.1). This section highlights studies deciphering molecular mechanisms of interactions between ethylene and JA or SA signaling.

Synergy between ethylene and JA signaling in plant immune responses such as transcriptional reprogramming has been observed in many cases (Lorenzo et al. 2003; Xu et al. 1994; Penninckx et al. 1996, 1998; Thomma et al. 1998, 1999). For instance, expression of *ERF1* is induced by ethylene, JA or synergistically by both hormones and requires both the JA co-receptor CORONATIVE INSENSITIVE1 (COI1) and the ethylene signaling component EIN2 (Lorenzo et al. 2003). Overexpression of *ERF1* rescues immune response defects in *coi1* and *ein2* mutants (Lorenzo et al. 2003). Similar observations were made for another transcription factor, ORA59 (Pre et al. 2008). These results indicate that induced expression of *ERF1* and *ORA59* is the consequence for synergy between ethylene and JA signaling. Perception of JA (JA-isoleucine) by COI1 triggers degradation of negative regulators of JA signaling JASMONATE ZIM-DOMAIN (JAZ) proteins which interact with for instance the key transcription factor of JA signaling MYC2 and its homologs, resulting in repressing their functions (Thines et al. 2007; Chini et al. 2007; Yan et al. 2009; Fernandez-Calvo et al. 2011). A study uncovered that activity of the key transcription factors of ethylene signaling EIN3 and EIL1 are also suppressed by JAZ proteins through the co-repressor HISTONE DEACETYLASE6 (HDA6) (Zhu et al. 2011). Thus, EIN3 and EIL1 are interaction sites for synergy between ethylene and JA signaling; ethylene stabilizes and JA de-represses EIN3 and EIL1, resulting in high expression of *ERF1* and *ORA59*. Ethylene and JA signaling also act antagonistically (Lorenzo et al. 2004). Recently, two reports revealed the molecular mechanism of this antagonism. MYC2 physically interacts with EIN3 to inhibit its DNA binding activity and conversely, EIN3 represses MYC2 function (Song et al. 2014; Zhang et al. 2014). MYC2 also directly binds the promoter of *ORA59* to suppress its expression and *myc2* mutants are resistant to *B. cinerea* infection (Zhai et al. 2013). Thus, coordinated expression of the transcription factors such as *ERF1* and *ORA59* by ethylene and JA is important for resistance against necrotrophic pathogens such as *B. cinerea* and ethylene and JA signaling interlink at the key transcription regulators such as JAZs, MYC2 and EIN3/EIL1 with synergy and antagonism.

As in the interaction between ethylene and JA, ethylene and SA signaling also interact positively and negatively. EIN3 directly binds the promoter of the key SA biosynthesis gene *SALICYLIC ACID INDUCTION-DEFICIENT2* (*SID2*) to repress its expression (Chen et al. 2009). Consistently, the *ein3 eil1* double mutant shows constitutive SA accumulation (Chen et al. 2009). On the other hand, SA signaling suppresses ORA59 protein accumulation (Van der Does et al. 2013). Thus, ethylene and SA signaling can be mutually inhibitory. Positive interactions between ethylene and SA signaling are also proposed although the molecular mechanisms are not clear (Lawton et al. 1994; Mur et al. 2008).

JA and SA signaling are generally inhibitory to each other (Glazebrook 2005; Spoel et al. 2003), and ethylene signaling modulates this cross talk (Leon-Reyes et al. 2009, 2010). Thus, interactions of ethylene signaling with JA and SA signaling are very complex. A study tackled this complexity through quantitative measurements of immunity levels of a quadruple mutant in which the ethylene, JA, SA and PHYTO-ALEXIN DEFICIENT4 (PAD4, an important regulator of SA accumulation) (Zhou et al. 1998; Jirage et al. 1999) signaling sectors are all disrupted as well as all combinatorial mutants (single, double and triple mutants) (Tsuda et al. 2009). The signaling allocation analysis was used to estimate contributions of each single signaling sector and interactions among the signaling sectors. The analysis revealed that on the contrary to previous ideas, the ethylene signaling sector as well as the JA and SA signaling sectors can contribute positively to both the biotrophic pathogen *P. syringae* and the necrotrophic fungal pathogen *Alternaria brassicicola* as the single signaling sectors (Tsuda et al. 2009). Recently, a dynamic signaling network model using the same mutant set was built to describe signal flows in the network during PTI triggered by different MAMPs against *P. syringae* strains (Kim et al. 2014). The model predicted that the ethylene signaling sector inhibits the JA and PAD4 signaling sectors and is the sole inhibitory sector in the PTI signaling network (Kim et al. 2014). These results clearly point to the importance of multiple and combinatorial mutant analysis to elucidate true functions of signaling sectors in highly interconnected networks. Taken together, ethylene signaling is a critical component for immunity against different types of pathogens as an individual signaling sector and an important modulator of immune responses by influencing JA and SA signaling.

11.5 Emerging Roles of Ethylene

Plants are associated with numerous microbes in natural environments (Bulgarelli et al. 2013). Most associated microbes are non-pathogenic and sometimes beneficial for plant fitness (Bulgarelli et al. 2013). Using a synthetic bacterial community representing the most abundant phyla in the phyllosphere, a recent study found that the *ein2* mutation strongly affects bacterial community composition but mutations in JA and SA biosynthesis had little effect (Bodenhausen et al. 2014). Similarly, a lower bacterial density and an altered community were observed in ethylene-insensitive tobacco plants compared to wild-type plants (Long et al. 2010). Interestingly, some bacteria and fungi produce ethylene to interfere with plant responses (Fukuda et al. 1993; Volksch and Weingart 1998). Some plant root-associated bacteria produce the ACC degradation enzyme ACC deaminase (ACCD) to promote plant root growth (Saleem et al. 2007). Thus, ethylene signaling is a crucial component of plant-microbe interactions beyond plant immunity.

11.6 Ethylene and Practical Applications

As pointed out, ethylene signaling is implicated in many aspects of plant-microbe interactions, which provides great potential for agricultural improvements against diseases. Although manipulation of ethylene levels for regulating fruit ripening is popular, ethylene-mediated strategies to improve disease resistance are in infancy stages. It is speculated based on the analyses of transgenic rice plants defective in ethylene, the phytohormone abscisic acid (ABA) and MAPK pathways that ethylene improves resistance to rice (*Oryza sativa*) blast caused by the hemi-biotrophic fungal pathogen *Magnaporthe oryzae* through its antagonistic interactions with ABA and OsMAPK5 (Bailey et al. 2009). Consistently, a transgenic rice line, which expresses the rice *ACS2* under a pathogen-inducible promoter to overproduce ethylene upon infection, shows increased resistance to both *M. oryzae* and the necrotrophic fungal pathogen *Rhizoctonia solani* that causes sheath blight (Helliwell et al. 2013). Since ethylene production is pathogen-inducible in these plants, it has low or no effect on crop yield under normal circumstances. *Resistance* (*R*)-gene mediated immunity which provides race-specific resistance has been used for rice improvement against *M. oryzae* but is often overcome by emerging virulent strains (Bonman 1992; Dai et al. 2010). Therefore, manipulation of ethylene signaling can be a viable alternative strategy for rice disease management in the field.

In addition to induced ethylene production, reduction of ethylene levels has been also tried. For instance, plant-produced ethylene suppresses *Agrobacterium tumefaciens*-mediated transformation which has been used to generate transgenic plants (Hao et al. 2010; Someya et al. 2013). Low transformation efficiency has been an issue for generation of transgenic plants in some species and the transformation efficiency is increased by expressing the ACC degradation enzyme ACCD, pointing to its great potential for more efficient generation of transgenic plants (Nonaka et al. 2008). In addition, reducing ethylene levels in plants by generating transgenic plants or inoculation of roots with bacteria that produce ACCD improves disease resistance or tolerance against certain pathogens (Gontia-Mishra et al. 2014). For instance in tomato, reduction of pathogen-induced ethylene production by expressing an ACCD improves disease tolerance for *Verticillium* wilt caused by *Verticillium dahlia* (Robison et al. 2001). Similarly, the treatment of tomato plants with the ACCD-producing bacteria reduces the severity of *A. tumefaciens* or *Agrobacterium vitis*-mediated crown gall disease (Toklikishvili et al. 2010). These examples illustrate potential applications in agriculture through manipulation of ethylene effects.

11.7 Conclusions and Perspectives

As described, ethylene signaling is highly interconnected with other signaling with synergism and antagonism. An important question is under what conditions these complex interactions occur. Recently, the dynamic ethylene-induced transcriptional reprogramming was characterized using genome-wide chromatin immunoprecipitation sequencing and transcript sequencing with samples taken at multiple time points after ethylene treatment (Chang et al. 2013). This study revealed that ethylene-induced transcription occurs in temporal waves controlled by EIN3 which modulate numerous downstream transcriptional cascades, pointing out the importance of time-course experiments to understand the role of ethylene signaling in the dynamic and complex transcriptional network. Moreover, considering the high interconnectivity of ethylene signaling with other signaling pathways, simple genetics using single mutants may not be enough to fully understand the ethylene signaling network and may lead to misinterpretations. Multiple combinatorial mutant analyses were proved to be useful and therefore, should be considered in future studies to understand how ethylene signaling is integrated in the immune signaling network and contributes to plant immunity. In nature, plants are associated with a large number of microbes including pathogens, commensals and mutualists that affect plant fitness. In addition to its significant roles in plant immunity, ethylene signaling is also a critical component for interactions with non-pathogenic microbial communities. It is poorly understood how ethylene signaling affects the outcome of plant-microbe interactions. Metabolomics and proteomics approaches combined with genetics would be helpful to answer this question. Finally, accumulation of knowledge on the ethylene-mediated immune signaling network at the molecular level will certainly contribute to development of agricultural disease management.

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