Chapter 7
Ethylene: Role in Plants Under Environmental Stress

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1 Introduction

When plants colonized the terrestrial ecosystems (some 475 million years ago), they had to acquire a number of organs necessary to keep erectile (i.e., root system, stem, and especially cell walls lignin enriched) (Kendrick and Crane 1997; Peter and Neale 2004; Martone et al. 2009). Likewise, terrestrial plants also had to develop a leaf system able to carry out both photobiosynthesis (i.e., carbohydrate and energy biosynthesis) and transpiration (i.e., gas exchange and generation of a force that allows the ascent and distribution of water and nutrients from the soil). However, the colonization process also caused serious problems of stress as a result of the transition from aquatic, motile ancestors into terrestrial, sessile organisms (Martone et al. 2009). Thus, the lack of mobility resulted in a complicated process of adaptation to the environment and the acquisition of defense mechanisms against diseases and predators (Ausubel 2005). In order to avoid a progressive disappearance, plants improved their physiological plasticity and developed a complicated set of signaling networks. These networks are tightly regulated by hormones that allow plants to survive by protecting them against biotic and abiotic stresses (Robert-Seilaniantz et al. 2011). Ethylene (Et), in combination with hormones such as jasmonic (JA) and salicylic (SA) acids, is one of the main players involved in the resistance and susceptibility to bacterial, fungal, and nematode pathogens (Adie et al. 2007; Kazan and Manners 2008; León-Reyes et al. 2009, 2010; Lin et al. 2009). The biosynthesis,

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transport, and accumulation of the above-mentioned hormones trigger a cascade of signaling pathways involved in plant defense. Et and JA signaling pathways are activated in response to necrotrophic plant pathogens; whereas salicylic acid (SA) play a major role during the triggering of plant defenses toward biotrophic pathogens (reviewed in Glazebrook 2005; Thaler et al. 2012). In general, SA and JA/Et defensive signaling pathways have been demonstrated to be mutually antagonistic (van Loon et al. 2006; Adie et al. 2007; Pieterse et al. 2012). Recently, it was demonstrated that both SA- and JA-dependent disease resistance is inhibited by a simultaneously reduced red:far light ratio (De Wit et al. 2013). In addition, it seems fairly clear that: (1) Et production plays a role in plant responses to flooding, salinity, drought, and several contaminant agents (e.g., ozone); and (2) plant growth-promoting rhizobacteria (PGPR) can improve plant tolerance to drought, salinity, and metal toxicity (Haas and Defago 2005; Lugtenberg and Kamilova 2009; Barreto-Figueiredo et al. 2011; Hol et al. 2013), although the role of Et in this puzzle is not fully decoded. This chapter aims to give an overview on the role of Et in the defense mechanisms of land plants against different types of environmental stresses.

2 Updated Overview of the Plant Hormone Ethylene

Et is the simplest plant hormone. Zhong and Burns (2003) showed that 7 % of the 6,000 investigated Arabidopsis genes were regulated by Et. During the plants life cycle, Et regulates key processes such as root hair development, flowering, climateric fruit ripening, seed dormancy, and germination (for review, see Czarny et al. 2006; Delseny et al. 2008; Matilla and Matilla-Vázquez 2008; García et al. 2010). In general, with the exception of lateral root initiation and fruit ripening (see flooding below), elevated levels of Et are deleterious to plant health and growth. Likewise, Et is also involved in environmental stress signaling upon wounding and during the interaction with pathogen and non-pathogen microorganisms (Pieterse et al. 2007, 2012; Verk et al. 2009). Consequently, the biosynthesis and perception of Et must be tightly controlled within the plant. The biosynthesis of Et begins with the transformation of methionine (Met), a scarce amino acid in plants, to *S-adenosylmethionine* (SAM). This conversion is catalyzed by the SAM synthase (Peleman et al. 1989). SAM synthases are not specific to the Met cycle (Yang Cycle) since SAM also serves as substrate for several reactions, including cell transmethylations. Subsequently, the 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS; *S*-adenosyl-L-Met methylthioadenosine-lyase) catalyzes the rate-limiting step in Et biosynthesis by converting SAM into ACC and 5′-methyl-thioadenosine (MTA), which regenerates Met in several steps (Bradford 2008) (Fig. 7.1, left). Besides plants, the Yang Cycle is also found in bacteria, archeae, and animals and it is well known that in higher plants it is tightly regulated (Rzewuski and Sauter 2008). The *ACS* gene was first cloned from *Cucurbita pepo* (Sato and Theologis 1989) and then significant efforts were conducted to study this key *ACS* multigene family. All *ACS* members are under strict regulatory control and the abundance of ACS proteins is

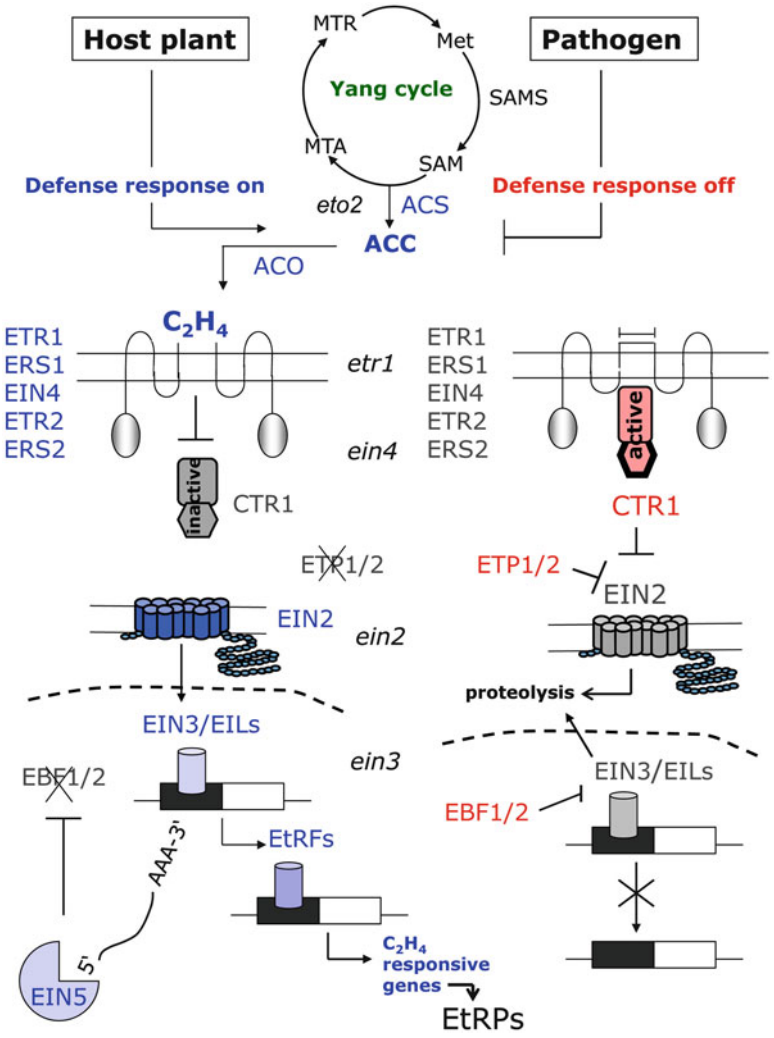

Fig. 7.1 Model explaining the involvement of the ethylene (Et) signaling in the plant defense mechanisms in the presence or absence of a pathogen. Adaptation of a scheme generously yielded by Dr. Ludwig-Müeller

closely correlated with the level of Et production in most plant tissues. Furthermore, various members of the *ACS* gene family were found to be differentially expressed in response to developmental and environmental triggers (Tsuchisaka and Theologis 2004a, b). The *ACS* family includes 6 members in rice (Rzewuski and Sauter 2008) and 12 members in Arabidopsis, of which only 9 appear to be implicated in Et biosynthesis (Yamagami et al. 2003; Vandenbussche et al. 2006; Vandenbussche and Van der Straeten 2007; Lin et al. 2009). Three types of ACS have been defined

based on their C-terminal regions, which are involved in enzyme stability. Five of the *ACS* genes and their expression patterns were described previously in deepwater rice internodes since at least two of them are sequentially induced during submergence (Rzewuski and Sauter 2008). Since *OsACS5* expression is induced within 60 min of submergence, this family member might be responsible for the early increase in ACS activity. By contrast, *OsACS1* expression is enhanced within 6 h of submergence. It has also been suggested that OsACS1 together with OsACS5 contributes to sustain Et production during long submergence (Zarembinski and Theologis 1997; van der Straeten et al. 2001). On the other hand, several MAPKs were found to regulate ACS activity (Broekaert et al. 2006; Schweighofer and Meskiene 2008). Thus, the phosphorylation of ACS2 and ACS6 by the MAPK MPK6 results in an increased production of Et (Christians et al. 2009). These phosphorylations also protect ACS2 and ACS6 from recognition and breakdown by the 26S proteasome pathway (Wang et al. 2004).

The last step of Et biosynthesis is catalyzed by ACC oxidase (ACO). In this metabolizing ACC reaction cyanoformic acid is also formed which is spontaneously degraded to cyanide (HCN) (Yip and Yang 1988). The HCN must be rapidly metabolized to keep its concentration below toxic levels. The molecular bases for HCN detoxification were recently studied in plants (Yi et al. 2012). The main HCN detoxification process described to date is catalyzed by β-cyanoalanine synthase (CAS), a pyridoxal phosphate-dependent enzyme that converts cysteine and HCN to HS and β-cyanoalanine. In Arabidopsis, the *CAS* gene family is composed of three members (Watanabe et al. 2008). The most abundant CAS protein (CYS-C1) is in the mitochondria, whereas CYS-D1 and DYS-D2 are found in the cytosol (Watanabe et al. 2008). Mitochondrial CAS is essential for formation of root hairs in Arabidopsis (García et al. 2010). HCN enhances the resistance of *N. tabacum* and Arabidopsis leaves to TMV and turnip vein clearing virus (TVCV), respectively (Wong et al. 2002). Likewise, it has also been proposed that HCN and Et are responsible for the resistance of young rice plants to blast fungus (*Magnaporthe grisea*) infection. In this fungus resistance mechanism, the induced *OsACS2* and *OsACO7* contributed specially (Iwai et al. 2006). On the other hand, plant pretreatment with KCN relieved stress induced by oxidative damage, and plainly induced the alternative oxidase (AOX) activity and Et production, proving a new fangled role of HCN against environmental stress (Xu et al. 2012).

In tomato and Arabidopsis *ACO* families are composed of four and six members, respectively (Babula et al. 2006; Lin et al. 2009). By contrast, in the rice genome six *ACO* members were found through computational analysis. Thus, in rice seedlings: (1) the highest expression of *OsACO1* was found in the very young growing internodes (i.e., *OsACO1* was induced after 4 h and at least up to 24 h of submergence; Mekhedov and Kende 1996); and (3) the expression of *OsACO2* and *OsACO3* were induced by auxin and Et, respectively, in a dose-dependent way (Chae et al. 2000). Taken together, Et biosynthesis is heavily regulated, including transcriptional and post-transcriptional control of the key enzymes (i.e., ACS and ACO). The presence of the enzyme ACC deaminase (ACCD), involved in the degradation of ACC to ammonia and α-ketobutyrate, is common in soil bacteria (Fig. 7.2), including biocontrol

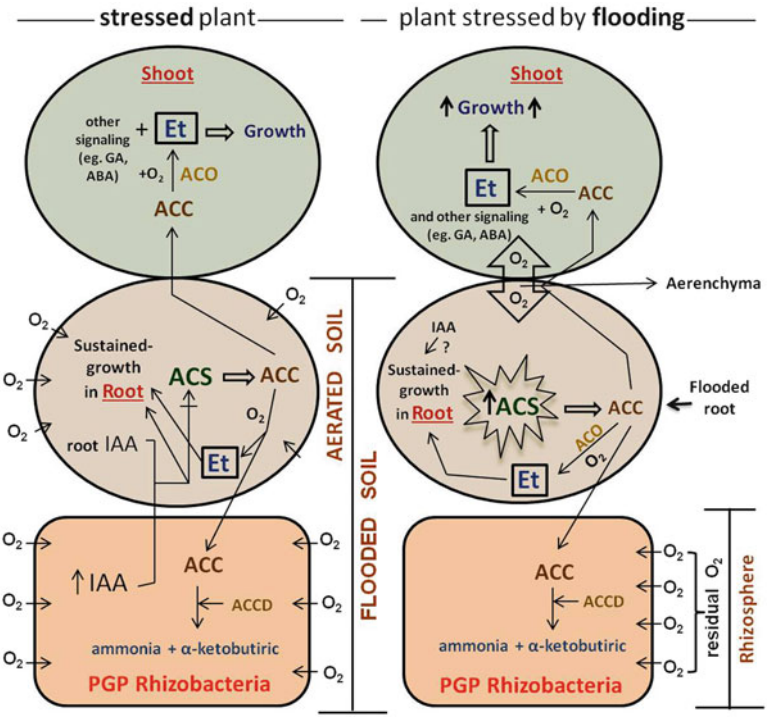

Fig. 7.2 Model explaining the role of plant growth promoting rhyzobacteria (PGPR) in generating plant growth under general stress conditions (*left*) and particularly under flooding (*right*). In the left model, PGPR synthesize and secrete IAA. Bacterial IAA, together with the IAA synthesized by the root, induce *ACS* transcription and consequently the production of ACC. A percentage of this Et precursor can be degraded by root-associated bacteria causing a notable decrease in the biosynthesis of Et. The remaining ACC is exported to the plant shoot where the ACC oxidase (ACO) catalyzes the synthesis of Et, triggering plant growth. In the right model, flooding is the environmental factor that induces ACS expression. The role of the ACC deaminase (ACCD) in both models is evident

bacterial strains (Glick et al. 2007; Chen et al. 2013; Roca et al. 2013). ACC is a frequent component of seeds, roots, and leaves exudates (Glick et al. 2007) and bacteria can act as a sink of ACC, lowering Et levels in the plant. As a consequence, plant growth can be promoted and some of the potentially deleterious consequences of high Et concentrations under environmental stresses (e.g., flooding, heavy metals, salinity, drought, and microorganisms attack) may be reduced (Glick et al. 2007; Gamalero and Glick 2012; Stearns et al. 2012). Interestingly, several plant-associated bacteria have a positive effect over the Et levels in the plants that they are colonizing. Thus, (1) some pathovars of the plant-pathogen *Pseudomonas syringae* have the ability to synthesize Et both in vitro and in vivo (Weingart and Volksch 1997; Sato et al. 1997); (2) the *Pseudomonas fluorescens* root colonization trigger an increase of ACO activity in vivo (Hase et al. 2003); (3) the expression amounts of *ACO1* and *ACO2* are up-regulated by the infection of *Botrytis cinerea*

(Adie et al. 2007); and (4) the transcriptional activation of *ACO* genes in tomato has been demonstrated in response to *P. syringae* infection (Weingart et al. 2001; Cohn and Martin 2005). Data on Et, JA, and SA production seems to conclude that a highly and tightly regulated Et biosynthesis may be used by pathogens Et producers to bypass defenses (Adie et al. 2007).

The Et signaling pathway is well established in Arabidopsis (de la Torre et al. 2006; Stepanova and Alonso 2009; Yoo et al. 2009). Thus, this gaseous hormone is sensed by receptors located in the endoplasmic reticulum. In the Arabidopsis there are five receptors (ETR1, ERS1, ETR2, ERS2, and EIN4), all of them with an active kinase domain (Stepanova and Alonso 2009; Yoo et al. 2009). The receptors operate as negative sensors of Et signaling and interact with Constitutive Triple Response 1 (CTR1), an Raf-like protein kinase (Fig. 7.1, left). In the absence of Et, CTR1 has a negative regulatory function, actively suppressing the Et signaling pathway. Upon Et-receptor binding, CTR1 is no longer capable of repressing Ethylene Insensitive 2 (EIN2) which is a transmembrane protein with homology to NRAMP metal ion transporters. EIN2 acts as a positive regulator of the Et responses. Et destabilizes the F-box proteins called ETP1 and ETP2, stabilizing EIN2 and promoting downstream effects (Qiao et al. 2009). EIN2 prevents the binding of the key Et Response Factors (EtRFs) EIN3 and its homolog EIN3-like 1 (EIL1) to EBF1 and EBF2 (EIN3 binding F-box proteins 1 and 2) which are part of an SCF E3 ligase complex (SCFEBF1/2) (An et al. 2010). Consequently, EBF1 and EBF2 are down-regulated by Et, suggesting that this gaseous hormone stabilizes EIN3/EIL1 by promoting EBF1 and EBF2 degradation by the proteasome complex. Thus, EIN3 (a short-lived transcription factor (TF) with five homologs in the Arabidopsis genome) and EIL1 are no longer degraded through the 26S proteasome pathway and induce transcription of EBF1 and EBF2 (Guo and Ecker 2003; Potuschak et al. 2003; Binder et al. 2007; Konishi and Yanagisawa 2008). When the Et levels decrease or Et is absent, EIN3 is ubiquitinated by SCFEBF1/EBF2 and degraded by the 26S proteasome. All this process is under control of EIN5, a $5' \rightarrow 3'$ exoribonuclease that acts downstream of CTR1 (Fig. 7.1, left). In the presence of Et, EIN5 promotes the *EBF1* and *EBF2*-mRNA decay, which allows the accumulation of EIN3 (Olmedo et al. 2006). In short, EIN3 is: (1) stabilized by Et; (2) phosphorylated by an MAPK cascade which can be activated by CTR1; (3) accumulated in nuclei after the increase in the Et levels with the subsequent binding to the promoter of EBF2; and finally, (4) together with EIL1, regulates the expression of target genes such as *EtRF1*, which encodes the transcription factor Et-Response Element Binding Protein (AP2/EREBP) involved in plant defense against necrotrophic pathogens (Glazebrook 2005; Verk et al. 2009; Zhao et al. 2012). EtRF1 and AtMYC2 are two notable regulators of Et–JA interactions in defense. However, AtMYC2 works in the opposite way to EtRF1 (for more information, see Adie et al. 2007). Interestingly, genes encoding group-VII EtRFs (Ethylene Response Factors) are up-regulated under anaerobic stress in several plant species (Nakano et al. 2006; Bailey-Serres et al. 2012).

Finally, it is especially important to note that during a stress process: (1) the Et action mode is modulated by the concentration of the hormone rather than by its

presence (Pierik et al. 2006); (2) Et, SA, and JA signaling pathways, individually or in crosstalk, play significant roles in the physiology of stress in land plants (Wasternack 2007; Thaler et al. 2012); (3) during resistance to necrotrophic pathogens, Et synergistically with JA plays a key role, as demonstrated by genetic approaches (Grant and Jones 2009; Pieterse et al. 2012); and (iv) ACC-JA conjugation may be fundamental for the Et-JA crosstalk regulation (Wasternack 2007; Fonseca et al. 2009).

3 Crosstalk Between Oxygen Deficient Stress and Ethylene Biosynthesis and Signaling

O_2 is the final electron acceptor in the mitochondrial respiratory chain. In soil, and more specifically in the rhizosphere, O_2 concentrations can be limiting (hypoxia) or absent (anoxia). The decrease of O_2 diffusion capacity in the soil (e.g., compact structure, water logging, and deep flooding) limits its availability for the root (Dat et al. 2004). Thus, the O_2 shortage in the soil generates a partial pressure around radical system incapable to oxygenate in the root the machinery of respiratory ATP biosynthesis. Additionally, the consumption of O_2 by aerobic rhizosphere microorganisms can further aggravate the root stress. Indirect and direct sensing of O_2 status may be responsible for the acclimatization responses that extend survival under O_2 deprivation (Bailey-Serres and Chang 2005). For this reason, plants can adapt to this energy crisis by promoting anaerobic metabolism and thus increase substrate-level ATP production (Magneschi and Perata 2009).

Rice (*O. sativa*) is a model plant for the study of metabolic control under O_2 limiting conditions since this semiaquatic organism is well adapted to a partially flooded environment. However, abrupt flooding can cause sharp submergence by imposing, among other factors, a complex stress due to a 10^3-fold reduction in the diffusion of O_2 and CO_2. The growth of deep water rice in wetlands is adapted to gradual flooding by means of acceleration in the elongation of submerged internodes to keep aerial tissues above the air–water environment. When sudden submerged, deepwater and most lowland varieties accelerate internode and/or leaf elongation to avoid the flooding. By contrast, lowland varieties tolerant to submergence save complete submergence through a constraint in shoot elongation and carbohydrate spending, thereby conserving energy reserves to restarting development upon desubmergence (Fig. 7.3). Consequently, an immediate response must be triggered by the plant in order not to block energy biosynthesis (Geingenberger 2003; Bailey-Serres et al. 2012). Thus, almost 50 genes responding to O_2-shortage, including EtRFs, were identified in several species such as Arabidopsis, rice, cotton, and poplar (Mustroph et al. 2010). Recent reports contain excellent updates on the molecular biology of O_2-shortage response (Mustroph et al. 2010; Bailey-Serres et al. 2012; Licausi 2011, 2012).

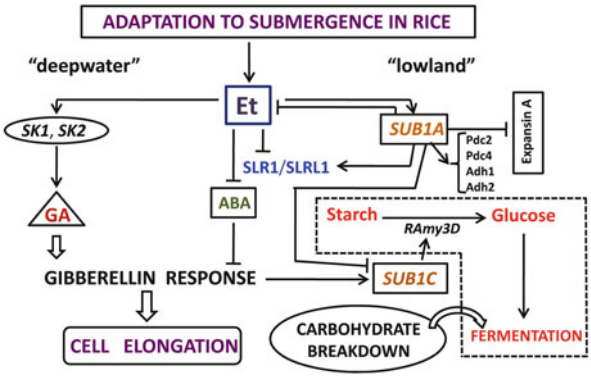

Fig. 7.3 Crosstalk between Et, ABA, and GA in submergence adaptation process of rice seedlings belonging to deepwater and lowland ecotypes

3.1 Role of Ethylene Response Factors Under Low-Oxygen Stress

A large quantity of microarray data for Arabidopsis and rice under low-O_2 stress (i.e., anoxia and hypoxia) are available, and these experiments have revealed much about plant responses to low O_2 (Licausi et al. 2010; Mustroph et al. 2010; Lee et al. 2011; Licausi 2012). For example, EtRFs are TFs unique to plants that bind specifically to TAAGAGCCGCC (GCC box) sequences found in the promoter regions of Et Response (EtR) genes (e.g., *Hookless1*). EtRFs are ubiquitous in the plant kingdom and their functional implications have been studied in a wide range of processes including response to biotic and abiotic stresses (for more information, see Pirrello et al. 2012). The EtRF family is a large gene family of TFs which is part of the *APETALA2* (*AP2*)/*EtRF* superfamily. AP2 is one of the largest families of TFs in plants, including three different sub-families which are characterized by the number of EtRF domains and by having either one or two AP2 DNA-binding domains. The EtRF, also known as the Et-Responsive Element-Binding Protein (EtREBP) family, has one AP2 domain, the RAV family has two domains (i.e., AP2 and B3), and the AP2 family has two AP2 domains (Nakano et al. 2006; Romanel et al. 2009). In Arabidopsis and rice, the EtRF family comprises about one hundred members which are categorized into ten clades. Clade VII has an MCGGAI/L highly conserved motif at its NH_2-terminal (Nakano et al. 2006). In all rice varieties studied, a sub-group VIIb exists where all members lack this NH_2-terminal motif. On the other hand, a major QTL responsible for tolerance to submergence, *Submergence1* (*SUB1*; located in chromosome 9), was identified in varieties of lowland *indica* rice (Fukao et al. 2006; Xu et al. 2012). This *SUB1* locus consists of a clade of three sub-group VIIb genes (*OsSUB1A*, *OsSUB1B*, and *OsSUB1C* genes), but the *SUB1A* is present only in *indica* and not *japonica* cultivars. *OsSUB1C* acts downstream of *OsSUB1A* (Fukao et al. 2006).

The expression of an Arabidopsis clade VII gene, *AtRAP2.2*, is induced by Et in shoots but not in roots (Hinz et al. 2010). RAP2.2 protein only affects to the induction of genes linked to sugar metabolism, fermentation, and Et biosynthesis (Hinz et al. 2010). Unlike rice, Arabidopsis possesses five genes within group VII, including *HYPOXIA-RESPONSIVE1* (*HRE1*) and *HRE2*. The plants overexpressing *HRE1* and *HRE2* showed an increased tolerance to anoxia, whereas the *hre1hre2* double mutant showed reduced tolerance (Licausi et al. 2010). A further study showed that in the presence of exogenous ACC transgenic seedlings with silenced *HRE1* displayed exaggerated apical hook curvatures. These results indicate a negative role of HRE1 in the Et responses (Yang et al. 2011). *HRE1* and *HRE2* shows a strong up-regulation under O_2 depletion, mediated by both Et-dependent and Et-independent signals (Licausi et al. 2010; Yang et al. 2011). Like *SUB1A*, *HRE1* transcript accumulation is induced by Et, which synergistically increases its rise during O_2 stress (Yang et al. 2011). Not long ago, another member of the *AP2/Etr2* family named *Octedecanoic-Responsive Arabidopsis59* (*ORA59*) was found to be as the more important integrator of the JA and Et signaling pathways. *ORA59* is induced and synergistically activated by JA and Et.

Et also induces the gene expression of alcohol dehydrogenase (*ADH1*) in Arabidopsis (Peng et al. 2001, 2005). Ethanolic fermentation through ADH1 activity contributes substantially to low-O_2 stress adaptation. For this reason, an *adh1* null mutant showed lower survival when exposed to low-O_2 pressure (Ellis et al. 1999). Likewise, the pyruvate decarboxylases (*PDC1* and *PDC2*) overexpression in Arabidopsis results in improved survival under low-O_2 conditions (Ismond et al. 2003). EtRFs are also involved in several developmental processes such as zygotic embryogenesis (Riechmann and Meyerowitz 1998) and abiotic and biotic stress responses (Fujimoto et al. 2000; Sakuma et al. 2002).

Finally, the degradation of clade VII-EtRF proteins is carried out by the N-end rule pathway (i.e., N-erp; Hinz et al. 2010; Gibbs et al. 2011; Bailey-Serres et al. 2012). More specifically, all five Arabidopsis VII-EtRFs proteins are N-end rule substrates. N-erp is a pathway to degrade proteins that relates the in vivo stability of a specific protein to the nature of its N-terminal. These N-terminal destabilizing residues are known as N-degrons (Varshavsky 2011). In eukaryotes, N-erp is a part of the ubiquitine (Ub) system (Graciet and Wellmer 2010).

3.2 Crosstalk Between Low-O_2 and Ethylene Under Submergence

Many investigations have demonstrated the involvement of Et in O_2-shortage responses (i.e., flooding and submergence). In contrast to flooding avoidance, which involves increased Et and enhanced stem elongation rates to permit the plant to have access to atmospheric O_2 (Kende et al. 1998), submergence tolerance is the result of an efficient reduction in the consumption of carbohydrates and an ethanolic fermentation-dependent metabolism, together with a reduced production of Et and

restricted cell elongation (Jackson and Ram 2003). Careful research in rice and a wetland dicot, marsh dock (*Rumex palustris*), pointed out that Et accumulation in submerged organs triggers a hormonal signaling pathway that cause the reduction of the antagonism between gibberellins (GA) and abscisic acid (ABA) which is usually responsible for the restriction of the internodal cell elongation. In submerged parts, the restriction of internodal elongation is achieved via a decreased responsiveness to GA arising from elevated levels of DELLA proteins that repress GA-induced growth (Fukao and Bailey-Serres 2008). *SNORKEL* (*SK*) *1* and *2* and *SUB1A* (EtRFs that confers prolonged tolerance to submergence in deepwater rice) genes are involved in the above signaling cascade (Hattori et al. 2009; Bailey-Serres and Voesenek 2010). The deepwater rice adaptation to flooding is the result of its ability to elongate the cell internodes. These internodes possess hollow structures which prevent plant drowning allowing gas exchange with the atmosphere. The internode elongation response in deepwater rice is regulated by Et (Hattori et al. 2009). Many physiological and molecular studies have shown that Et, GA, and ABA signaling are implicated in the elongation response. However, most of the gene(s) involved in this trait needs to be identified. Thus, the Hattori´s group found for the first time that the EtRFs-encoding genes *SK1* and *SK2* trigger deepwater response. Consequently, the deepwater rice requires *SK1* and *SK2* to extend the hollow stem to the water surface through the elongation of its stem internodes (Hattori et al. 2009). Therefore, under these deepwater conditions, Et accumulates and induces expression of *SK1* and *SK2* whose products triggers notable internode elongation via GA (Hattori et al. 2009).

As indicated above (section "Role of Ethylene Response Factors Under Low-Oxygen Stress"), several EtRF proteins from the major QTL SUB1 were demonstrated to have a main role in submergence tolerance in rice (Xu et al. 2006). Both flooding and submergence are controlled by *SUB1A*, *SUB1B*, and *SUB1C*. However, since the expression of *SUB1A-1* confers submergence tolerance to submergence intolerant rice plants, *SUB1A* is thought to be the key gene in this *SUB1* gene cluster (Xu et al. 2006). Some key features of *SUB1A-1* are described below. *SUB1A-1* overexpression in *japonica* rice, a flooding-sensitive cultivar, resulted in an enhanced *ADH1* expression and tolerance to flooding (Fukao et al. 2006; Xu et al. 2006). Several authors have proposed that the conferred submergence tolerance is the result of a complex signaling pathway that reduces carbohydrate consumption and growth elongation (Fukao et al. 2006; Xu et al. 2006; Perata and Voesenek 2007; Jung et al. 2010). *SUB1A-1* transcripts, as with *SK1* and *SK2*, are Et-induced. Additionally, (1) *SUB1A*-1 boosts the accumulation of *SLENDER RICE 1* (*SLR1*) and *SLENDER RICE-LIKE 1* (*SLRL1*), two negative regulators of GA responses; and (2) *SUB1A-1* protein ultimately limits Et biosynthesis (Fukao et al. 2006; Fukao and Bailey-Serres 2008). Other effects induced by SUB1A-1 were described by Bailey-Serres et al. (2012). All together, *SUB1A-1* seems to be included in an appropriate point in the signaling pathway belonging to submergence response. Thus, SUB1A-1 maintains cell viability and prevents plant growth during submergence stress. Furthermore, during a subsequent recovery period (i.e., reoxygenation), SUB1A-1 is also involved in homeostasis restoration. The reduced elongation response is only beneficial when the submergence is deep and/or relatively short

lasting. However, when the submergence is prolonged but relatively shallow floods, several plant species have been shown to elongate their stems in a hormonal-dependent manner. Thus, the accumulated Et inhibits ABA biosynthesis and increases its degradation resulting in reduced levels of ABA (Benschop et al. 2005, 2006; Saika et al. 2007). The decline of ABA levels results in the release of the repression of GA biosynthesis promoting the increase of the concentration of bioactive GA in the submerged tissues. Additionally, in response to Et and submergence, the sensitivity to GA is also enhanced, through yet unknown mechanisms. *SK1* and *SK2* genes, belonging to the same APETALA2/EtRF subfamily as the *SUB1A-1* gene, play a role in rice elongation when submerged (Hattori et al. 2009). Although it is not known whether the *SK* genes interfere with GA biosynthesis or action, it has been demonstrated that they act upstream of GA. A rapid underwater elongation requires carbon and energy, and, therefore, depends on the accessibility to nonstructural carbohydrates. Chen et al. (2010) shown that the translocation of newly fixed carbon to the elongation tissues and the mobilization of starch can both be induced under submergence conditions (Chen et al. 2010). Model explaining the relationship between Et, ABA, and GA in submergence adaptation process of rice is indicated in Fig. 7.3.

SUB1A perhaps can represses cell elongation though an involving expansin-A, increase in ethanolic fermentation via control of ADH gene expression, and a decrease in carbohydrate consumption, among other metabolic factors (Bailey-Serres and Voesenek 2010). Strikingly, SUB1A represses SUB1C which acts in an antagonistic way by promoting GA-induced carbohydrate breakdown and cell elongation. Both SUB1A and SUB1C are induced by Et. However, since SUB1A responds to Et at concentrations two orders the magnitude lower than SUB1C, is expected to be induced earlier. Therefore, in the presence of SUB1A, a delay in the induction of the expression of SUB1C during submergence is observed (for more information see Rzewuski and Sauter 2008). Although it is clear that several hormones, cell wall loosening proteins and carbohydrates are required for the elongation response, nowadays is poorly understood which part of the signal transduction pathway may cause the differences within and among naturally occurring species. In contrast to wild species, more research has been done in cultivated rice varieties to explain the variation in underwater elongation.

Recently, Chen et al. (2010) suggested that, under submergence conditions, the variation in the elongation rate of the petioles of the wetland plant *Rumex palustris* is controlled by an Et-regulated pathway that alters the dynamics of endogenous ABA levels in the petioles. This variation in the endogenous ABA concentration affects the responsiveness to GA and consequently the underwater petiole elongation rate. In this wetland species, the stimulation or inhibition of the underwater elongation is controlled by the *AP2/EtRF* genes (Voesenek and Bailey-Serres 2009). The slow elongating varieties maintain relatively high levels of ABA, which then results in a limited GA responsiveness and thus reduced growth rate. The effect of ABA on GA in the model species *R. palustris* suggests a novel role of ABA regulating GA. Notoriously, if we compare this study with previous research investigating the role of Et and ABA under submergence conditions in the fast and slow elongating species *R. palustris* and *R. acetosa*, respectively, the results strongly indicate

that differences between and within species in petiole elongation induced by flooding are controlled by the same switch point(s) and pathway(s), i.e., by regulating the levels of ABA and the subsequent GA responses (Benschop et al. 2005; Chen et al. 2010). It may be hypothesized that the inter- and intra-species genotypic variation in wetland plant species is the result of the strong selective force exerted by flooding stress.

3.3 Ethylene and Flooding

To survive flooding, many plant species have evolved by developing new adaptive traits (Bailey-Serres and Voesenek 2010; Bailey-Serres et al. 2012). The privatization of O_2 to the roots is the main consequence of flooding. Flooding together with salinity, dryness, and temperature is an important generator of abiotic stress and significantly affects distribution of plants in terrestrial environments. Shortage (i.e., hypoxia; $[O_2] < 50$ mmol m^{-3}) or absence (i.e., anoxia) of O_2 in waterlogged environments generates different responses in root systems (Matilla and Rodríguez-Gacio 2013). Under flooding, gases diffuse 10^4-fold slower. Thus, within the first 60 min of flooding, a decline from 20.8 to 7.9 kPa in the partial pressure of O_2 was observed, which continues to decrease to 1 kPa after 24 h. Under low O_2 conditions, soil microorganisms are the main consumers of the available O_2 and several toxic compounds may accumulate in the rhizosphere. The O_2 consumption by soil microorganisms generates a strong stress around the roots. Some plants (e.g., rice) may remain temporarily in soils with low O_2 levels and show a positive response to Et and enhanced tissue sensitivity to GAs (Knaap et al. 1996). Therefore, survival of rice upon a great increase of the water level depends on the fast elongation of the stem, which is Et-regulated. *OsACS1* alone, or in combination with *OsACS5*, maintains Et production during submergence (van der Straeten et al. 2001; Rzewuski and Sauter 2008). It was hypothesized the increase in the *OsACS* expression, together with the increase in the activity of OsACS due to the escape of OsACS1 from OsEOL-mediated degradation, result in a rising of Et production within the first hours of submergence (Yoshida et al. 2006). The appearance of aerenchyma vessels (i.e., soft tissues), which allow O_2 exchange from the aerial parts to the root tissues and adventitious roots, was an evolutionary key for the flooding adaptation (Vartapetian and Jackson 1997; Watkin et al. 1998; Bacanammwo and Purcell 1999; Gunawardena et al. 2001; Aschi-Smiti et al. 2004). Aerenchyma formation occurs through two different processes: schizogeny and lysigeny. Schizogenous aerenchyma is characteristic of *Rumex* spp. and involves reorganization of the cell wall (CW) and cell separation. However, in plant such as Arabidopsis or rice, programmed cell death (PCD) is responsible for the formation of lysigenous aerenchyma. Many of the adaptive growth responses occurring in roots under hypoxic conditions, including aerenchyma formation, occur in response to Et which is stored by physical trapping in flooding soil solution and submerged parts of plants at concentrations of 10^3 mm^3 dm^{-3} (Voesenek et al. 2006). Therefore the application of Et

induces the aerenchyma formation in hypoxic maize roots, while the presence of Et inhibitors repress its appearance (Dat et al. 2004).

The expression level of genes responsible for Et biosynthesis is up-regulated under flooding conditions (van der Straeten et al. 1997; Peng et al. 2005). Thus, in root tissues ACO activity is inhibited (Voesenek et al. 1993) and ACS activated (Van Der Straeten et al. 2001; Rieu et al. 2005), generating high levels of ACC (Geisler-Lee et al. 2010). Notably, since ACC is a mobile molecule does not necessarily require to be produced at sites where Et acts. Under flooding, ACC synthesized in plant roots is transported via the xylem to enable the biosynthesis of Et in the distant tissues (Finlayson et al. 1991). Therefore, the ACC must be transported to the next aerobic zones (i.e., shoots) for its conversion into Et. In tomato plants, English et al. (1995) showed that ACO activity regulates the Et production in response to flooding. On the other hand, during flooding of *Rumex palustris*, Et biosynthesis seems to be limited at the level of ACO activity rather than by ACS (Voesenek et al. 1993). However a portion of ACC biosynthesized by the roots is translocated to the rhizosphere and become available to bacteria possessing ACCD (see above, section "Updated Overview of the Plant Hormone Ethylene") (Fig. 7.2). If bacteria with ACCD are not abundant in the rhizosphere, the ACC is mostly translocated to the oxygenated upper parts of the plant for subsequent transformation to Et (Grichko and Glick 2001). Interestingly, aerenchyma formation does not always require Et. In some species such as Arabidopsis, constitutive lysigenous aerenchyma is formed in response to Et and H_2O_2 signaling (Mühlenbock et al. 2007). In support of the latter, the involvement of ROS, Ca^{2+} signaling, and CW metabolism in aerenchyma formation was recently demonstrated under waterlogged conditions (Rajhi et al. 2011).

Finally, to summarize rice adaptation to flooding: (1) the triggered Et biosynthesis and accumulation leads to an increase in bioactive GA and appearance of PCD; (2) PCD of epidermal cells facilitates emergence of adventitious roots at the nodes of the submerged stems, while GA induces the internodal growth; (3) Et prevents ABA biosynthesis and consequently the GA action on growth and PCD.

3.4 Crosstalk Between Low-O_2 and Ethylene in Seeds

The production of seeds is crucial and represents the main strategy that allows most plants species to maintain their genetic diversity, survive, and spread. Before germination is triggered, viable seeds can overcome long periods of severe desiccation and dormancy (Iglesias-Fernández et al. 2011; Graeber et al. 2012). Indeed, one of the key milestones during plant evolution has been the acquisition of desiccation tolerance (Linkies et al. 2010). Under desiccation conditions, the seed undergoes strong metabolic and hormonal readjustments, such as an increase in dehydrin and ABA levels (Rodríguez-Gacio et al. 2009; Leprince and Buitink 2010). During seed development and early imbibition, the internal high metabolic activity and the outer seed layers (i.e., seeds coats) prevent O_2 diffusion. Likewise, this hypoxic environment inside the seed causes an ATP deficiency (Borisjuk and Rolletschek 2009).

Hence, the seed needs to develop strategies to reduce or prevent O_2 restriction besides an ability to adjust its endogenous levels of O_2 as well as O_2 demands. For this, the seed requires mechanisms for O_2-sensing and O_2-dependent regulatory systems (Bailey-Serres and Chang 2005; Borisjuk and Rolletschek 2009). Although the O_2 sensors have not been definitely identified, in Arabidopsis seedlings, two independent research groups have recently demonstrated that one branch of the Ub-dependent N-end rule pathway functions as a mechanism for sensing O_2 (Gibbs et al. 2011; Licausi 2011; Licausi et al. 2011). Additionally, an increasing amount of data supports the leading role for the non-symbiotic hemoglobins/NO (nsHbs/NO) cycle in O_2-sensing (Sairam et al. 2009; Siddiqui et al. 2010; Matilla and Rodríguez-Gacio 2013). However, it has not yet been successfully demonstrated whether Et biosynthesis and signaling are involved in triggering processes of hypoxia in seeds. The down-regulation of the nsHbs1 biosynthesis in *Hordeum vulgare* (barley) enhanced the production of Et in *Zea mays* (maize) suspension cells during hypoxia (Manach-Little et al. 2005). On the other hand, studies in *Gossypium hirsutum* showed that *GhnsHb1* expression is up-regulated by Et, SA, and JA, suggesting that GhnsHb1 may be involved in defensive mechanisms (Qu et al. 2006).

4 Ethylene and Plant Defense Against Microorganisms

Land plants are anchored to the soil and therefore the root system is in close contact with the neighboring soil environment (Darrah and Roose 2007). The release of nutrients in the form of root exudates to the rhizosphere (Loyola-Vargas et al. 2007; Newman and Römheld 2007; Uren 2007; Badri and Vivanco 2009) results in a highly active and dense population of microorganisms. In fact, bacterial population densities in the rhizosphere can reach 1–2 orders of magnitude higher than in the bulk soil (Molina et al. 2000; Morgan et al. 2005). The root exudation occurs through root hairs and both the apex and young parts of roots (Newman and Römheld 2007; Uren 2007) and influences microbial root colonization (Lugtenberg and Bloemberg 2004; Gamalero et al. 2005; Watt et al. 2006). At the same time, rhizosphere colonizing microorganisms can directly alter the metabolism and development of the root system (Ahemad and Khan 2011; Berendsen et al. 2012).

The presence of rhizosphere microorganisms can affect the root exudate properties due to an active degradation of its components (Jones et al. 2003). Furthermore, rhizosphere microorganisms can also increase the exudation levels and alter the root exudates composition, facilitate the availability of some soil nutrients and promote the plant growth (Phillips et al. 2004; Rosas et al. 2006; van Loon 2007; Lugtenberg and Kamilova 2009; Matilla et al. 2010). Additionally, plant-associated microorganisms can synthesize plant hormones such as cytokinins, GA, and auxins (Preston 2004; Vessey 2003; Ahemad and Khan 2011; Roca et al. 2013) besides releasing Et (Freebairn and Buddenhagen 1964; Weingart and Volksch 1997; Sato et al. 1997). Microorganisms use two different Et biosynthetic pathways, both different from

that of higher plants (see above). Thus, most of these microorganisms produce small traces of the hormone via the 2-keto-4-methylthiobutyric acid (KMBA) pathway, in which the NADH:Fe(III)EDTA oxidoreductase generates hydroxyl radicals from molecular O_2 (Fukuda et al. 1989; Nagahama et al. 1992). However, several microorganisms can synthesize Et using 2-oxoglutarate as precursor via an Et-forming enzyme (Weingart and Volksch 1997).

During evolution, plants have acquired a complex system of defense mechanisms that protect them against plant-pathogenic fungi, oomycetes, and bacteria, besides viruses and nematodes (Bari and Jones 2009). Successful plant pathogens can interfere or block the plant immune system whereas beneficial plant–microorganisms associations can promote plant growth and help to overcome different environmental stresses. However, beneficial microorganisms are firstly recognized as potential pathogens and the plants can react to their presence by activating an immune response (Pieterse et al. 2012). Thus, the recognition of pathogen- or microbe-associated molecular patterns (PAMP/MAPS) by the plant can also trigger the so-called effector-triggered immunity (De Vleesschauwer and Höfte 2009). Found mostly in plant-associated bacteria, PAMP/MAPS are bacterial determinants such as flagella, lipopolysaccharides, siderophores, and antibiotics, amongst others (reviewed by Bakker et al. 2007; De Vleesschauwer and Höfte 2009; Vlot et al. 2009). Recently, it was shown that microbial elicitors and JA differentially modulates the plant's innate immune response (Flury et al. 2013). Plant pathogen infection may result in the induction of systemic acquired resistance (SAR), a broad spectrum, and long-lasting disease resistance. SAR is generally involved in the protection against (hemi-)biotrophic pathogens (Glazebrook 2005) and its induction requires the accumulation of SA. Moreover, SAR-induced plants show increased expression of pathogenesis-related (PR) genes (Durrant and Dong 2004; Vlot et al. 2009; Fu and Dong 2013). On the other hand, the plant root colonization by certain non-pathogenic PGPRs can suppress disease by triggering systemic induced resistance (ISR). ISR is phenotypically similar to SAR but it is dependent of the Et and JA signaling pathways (van Loon and Bakker 2005; De Vleesschauwer and Höfte 2009) (Fig. 7.4). In general, ISR is associated with defense against necrotrophic pathogens and herbivorous (Glazebrook 2005; Pieterse et al. 2012) and is not associated with an enhanced expression of PR genes (van Loon and Bakker 2005; De Vleesschauwer and Höfte 2009). Interestingly, the ISR induced by the rhizobacteria *Pseudomonas fluorescens* WCS417r is not associated with the endogenous increase of the JA and Et, suggesting that enhanced hormonal sensitivity causes this improved defense (Pieterse et al. 2000; De Vleesschauwer and Höfte 2009 and references therein). PGPR-mediated ISR has been shown to be efficient against a broad range of plant pathogens on both monocotyledonous and dicotyledonous species (reviewed by Bakker et al. 2007; De Vleesschauwer and Höfte 2009) and it is well known that for its induction an effective colonization of the rhizosphere is required (Raaijmakers et al. 1995). Both SAR and ISR signaling pathways have been shown to be dependent on the transcriptional activator NPR1 (Non-expresser of Pathogenesis-Related; Pieterse et al. 1998, 2007; Niu et al. 2011; Zhang et al. 2012) (Fig. 7.4).

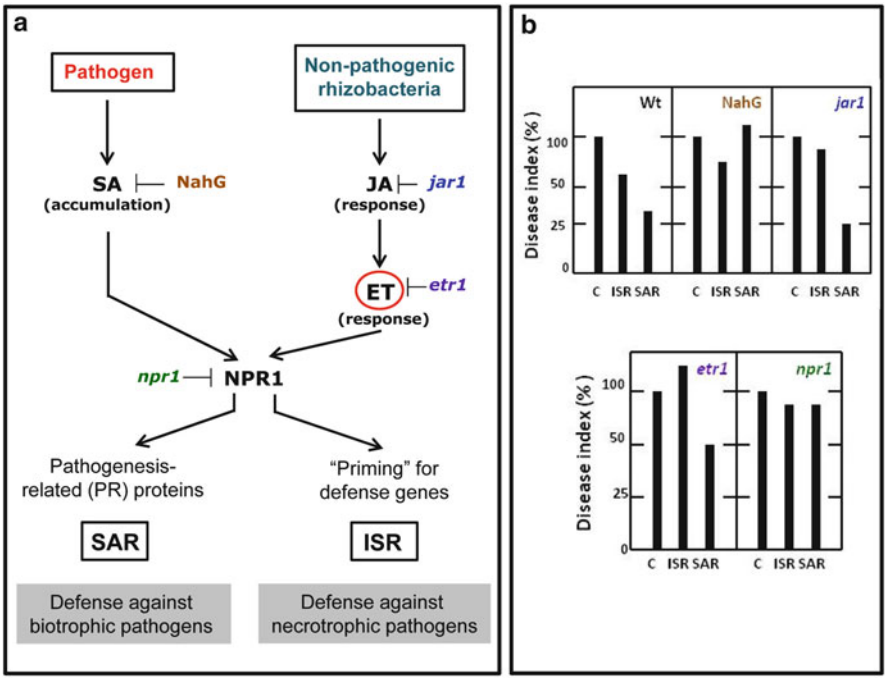

Fig. 7.4 Elicitation of induced systemic resistance (ISR) and systemic acquired resistance (SAR) transduction pathways in *Arabidopsis thaliana*. (**a**) Simplified model for triggering of SAR and ISR. *etr1* (ET receptor mutant 1 plants); *jar1* (JA response 1 mutant); NahG (SA non-accumulating transgenic plants); *npr1* (non-expressor of PR genes 1 mutant plants). (**b**) Quantification of ISR and SAR in Arabidopsis plants infected with *P. syringae* pv. tomato DC3000. ISR was induced by inoculating plant roots with the rhizobacterium *P. fluorescens* WCS417r. SAR was triggered by infiltrating plant leaves with an avirulent variant of *P. syringae* pv. *tomato*. Disease index represents the percentage of leaves showing symptoms relative to the control plants. Wt: wild type; C: non-treated plants. Adapted from Pieterse et al. (1998) with permission of Dr. Pieterse

4.1 Involvement of Ethylene in Pathogenic Infections

In ISR-triggered plants no defense mechanism is activated before the recognition of a pathogen. However, the plant tissues are sensitized to react faster and strongly in response to the pathogen, a phenomenon known as "priming" (Verhagen et al. 2004; Conrath 2009). For example, experiments with endophytic biocontrol strain *Enterobacter radicincitans* DSM 16656 demonstrated that this bacterium is capable of inducing priming via SA or JA/Et signaling pathways to protect plants against potential pathogen attack (Brock et al. 2012) (Fig. 7.4). Importantly, primed plants show a wide spectrum of resistance with low impact on the plant fitness (i.e., plant growth and seed production) (Van Hulten et al. 2006). A number of studies show that priming: (1) often depends on the induced disease resistance key regulator Non-expresser of Pathogenesis-Related genes (NPR1) (León-Reyes et al. 2009);

and (2) is an evolutionary advantage over constitutive activation of defense response (Van Hulten et al. 2006; Conrath 2009).

A hypothesis on the involvement of the Et signaling in the plant defense mechanisms in the presence or absence of a pathogen is shown in Fig. 7.1. It has long been known that Et can act positively and negatively on plant immunity (van Loon et al. 2006). Thus, pathogen attack activates Et production in many plants (Broekaert et al. 2006; van Loon et al. 2006 and references therein) and rhizobacteria-mediated ISR requires responsiveness to Et and JA (van Wees et al. 2008; Pieterse et al. 2007). Unfortunately, the role of Et during the plant–pathogen interaction has remained secondary and deserves more attention. Thus, after the infection, plants often respond with a rapid rate of Et biosynthesis (Iwai et al. 2006; van Loon et al. 2006 and references therein). Pathogenic infection triggers a rapid and low Et biosynthesis from pre-existing ACC in affected tissues. This first Et wave may be a protective response by the plant (van Loon et al. 2006). Subsequently, the activation of the transcription of the ACS genes to generate a net biosynthesis of Et immediate precursor and then a highly elevated ACO activity provokes a second wave of hormone (Iwai et al. 2006; van Loon et al. 2006 and references therein). If the pathogenic attack is ongoing, autocatalytic biosynthesis of Et takes place. This remarkable process is highly damaging for the infected plant. Therefore, it is logical to suppose that (1) the inhibition of the biosynthesis of Et decreases the severity of infection; and (2) transgenic plants with high expression of ACCD are strongly protected against some pathogenic attacks (Czarny et al. 2006; Glick et al. 2007).

The ISR model system Arabidopsis-*Pseudomonas fluorescens* WCS417r is one of the best characterized (Pieterse et al. 2007; De Vleesschauwer and Höfte 2009 and references therein). In this model, the Arabidopsis mutants *etr1* (ET-response) and *jar1* (JA-response) were unable to trigger resistance against the pathogen bacteria *P. syringae* after colonization with *P. fluorescens* WCS417r (Pieterse et al. 1998). Investigation with other mutants in Et signaling concluded that the establishment of ISR requires an intact Et signaling pathway (Ton et al. 2002a). Particularly interesting results emerged from the study of the *eir1* mutant, insensitive to Et in the roots but not in the shoots. Arabidopsis *eir1* plants were unable to show ISR after root colonization by the rhizobacteria WCS417r. However, *eir1* mutants exhibited ISR when the strain WCS417r was infiltrated into the leaves, suggesting the importance of responsiveness to Et at the site of application (Knoester et al. 1999). Interestingly, in Arabidopsis, *etr1* plants failed to exhibit ISR after treatment with ACC or JA. However, *jar1* plants were able to response to JA but not to ACC suggesting that JA pathway acts upstream of Et pathway in the signaling cascade (Pieterse et al. 1998).

It is interesting to point that the locus *ISR1*, encoding a key component of the Et signal transduction pathway, is required for both ISR and basal resistance in Arabidopsis (Ton et al. 1999, 2001, 2002b). Likewise, the endogenous Et levels are crucial for the development and fine-tuning of appropriate defense responses (Zhao et al. 2012, and references therein). The importance of Et content in plant defense responses may have led to the development of Et-producing pathogens. These evolved pathogens might interfere with the Et plant status altering or preventing the defense response to their benefit.

As described previously, Et alone or in combination with other hormones is involved in determining the most appropriate defensive response. However, the function of Et in plant defense is complex and highly regulated. This is reflected in the enumeration of Et-associated mutants and their susceptibility to phytopathogens (van Loon et al. 2006). For example, although *ACS* expression is poorly understood during pathogenesis, recent results indicate that the rice OsEDR1 (Enhanced Disease Resistance 1; ortholog of Arabidopsis EDR1) is a positive regulator of Et biosynthesis. Thus, the expression of the ACS gene family was suppressed in OsEDR1-defective mutants resulting in rice plants more resistant against the biotrophic pathogen *Xanthomonas oryzae* pv. *oryzae* (Shen et al. 2011). The TFs EIL1 and EIN3 regulate the expression of the Et transcriptional activator ERF1. Likewise, ERF1 regulates EtR and Et defense-related genes (e.g., Pathogenesis-Related gene 3 (PR-3) and Plant Defensin 1.2) playing a role in the defense against necrotrophic pathogens (Berrocal-Lobo and Molina 2004; Adie et al. 2007). In Arabidopsis, Et appears to act antagonistically in SA signaling. Thus, it was demonstrated that EIL1 and EIN3 repress SA biosynthesis by binding to the *isochorismate synthase 1* promoter, a well-known SA biosynthetic gene (Robert-Seilaniantz et al. 2011; Pieterse et al. 2012). Conversely, Et potentiated the response of Arabidopsis plants to SA, resulting in a increased expression of *PR-1*, an SA-responsive gene (De Vos et al. 2006). Moreover, in tobacco (*Nicotiana tabacum*) Et was shown to be key player for the establishment of SA-dependent SAR against TMV (León-Reyes et al. 2009 and references therein).

Considerable research in recent years has demonstrated that Et regulates the expression of defensive genes such as *PR-2* (β-1, 3-glucanases), *PR-3* (chitinases), and *PR-12* (plant defensin factors) (van Loon et al. 2006). However, Et works as a component of a tangled network of signaling compounds including SA, JA, and ABA. Likewise, in different plant species the presence of the GCC box (see section "Role of Ethylene Response Factors Under Low-Oxygen Stress") was demonstrated to be essential, and sometimes sufficient, for the regulation of the expression *PR* genes by EtRFs (Adie et al. 2007). The EtRFs–GCC binding can also take place in promoters of *EtR* genes not involved in pathogenesis (e.g., *Hookless1*), evidencing a wider role for GCC box in the transcriptional regulation by Et. On the other hand, EtRF family members can activate or repress concrete defense pathways, often with opposite effects, resulting in susceptibility or resistance to the attacking pathogens (Berrocal-Lobo and Molina 2004; McGrath et al. 2005; Ham et al. 2006). Other examples of the involvement of Et in plant defense are listed below. In Arabidopsis, Et has also been involved in both local and systemic defensive responses against the necrotrophic fungus *Alternaria brassicicola*. Et, but not SA or JA, was capable of inducing the expression of the Arabidopsis secreted lipase GLIP1, which shows antifungal activity against *A. brassisicola* (Oh et al. 2005). More recently, the elicitation of systemic resistance was shown to not significantly alter the structure community of rhizosphere bacteria (Doornbos et al. 2011). Referring to aggressive pathogens, the necrotrophic fungus *Botrytis cinerea* is one of the most stressful and destructive (Williamson et al. 2007). Et, synergistically with JA, plays a key role during resistance to necrotrophic pathogens (van Loon et al. 2006; Grant and Jones 2009). In a recent study, Zhang et al. (2012) found that the mutation of the Arabidopsis

mediator complex subunit 16 (MED16) blocks the expression of several Et and JA response genes compromising, consequently, the plant defenses against necrotrophic pathogens such as *B. cinerea* and *A. brassicicola*. Furthermore, studies with Arabidopsis have shown that the *ein2* and the *ein3eil1* double mutant, both Et-insensitive, are more susceptible to *B. cinerea* (Alonso et al. 2003). Several EtRFs (e.g., ORA59, RAP2.2, and EtRF1) have been also recognized as remarkable regulators in the *Botrytis* resistance (Nakano et al. 2006; Wehner et al. 2011; Zhao et al. 2012). Moreover, ectopic expression of EtRF1 and ORA59 enhanced resistance of *Arabidopsis* to *B. cinerea*, *Fusarium oxysporum*, and *Plectosphaerella cucumerina* (Berrocal-Lobo and Molina 2004; Pré et al. 2008). Taken together with the RAP2.2 function in low-O_2 tolerance (see section "Role of Ethylene Response Factors Under Low-Oxygen Stress"), the Zhao group's data suggested that RAP2.2 (1) may act as a global regulatory protein in the Et signaling pathway and could play a dual role in the low-O_2 tolerance and *Botrytis* resistance; and (2) might serve as a global TF involved in the regulation of the Et signaling pathway and as node in the crosstalk signaling between biotic and abiotic stress responses (Zhao et al. 2012). Recently, it has been shown that EtRF6 is a notable regulator of biotic stress defense. Thus, EtRF6 controls the ROS-responsive genes expression after activation by MPK3/MPK6 (Wang et al. 2013). Likewise, ERF6 plays a dual role under stress as it activates both stress tolerance and growth inhibition, and both roles take play independently from each other (Dubois et al. 2013).

4.2 Non-pathogenic Infections and Induced Ethylene Production

As described above, different biotic and abiotic stresses can cause an imbalance in the Et production of land plants and the increased level of gaseous phytohormone can inhibit the overall plant growth or the length of specific organs including roots (Bleecker and Kende 2000; Mayak et al. 2004; De la Torre et al. 2006; Matilla and Matilla-Vázquez 2008). Et and JA have been shown to be required for the establishment of a broad-spectrum ISR response, stressing the crucial modulating role of Et in plant defense (van Wees et al. 2008). Thus, Et and JA are indispensable for the development of ISR in leaves after root colonization by beneficial microorganisms such as *Piriformospora indica* (Verma et al. 1998) and *P. fluorescens* (van der Ent et al. 2009). The fungus *P. indica* colonizes plant roots and promotes Arabidopsis growth and seed production. Interestingly, the growth of Arabidopsis Et-related mutants *etr1*, *ein2*, and *ein3eil1* was not promoted by the *P. indica*, although the roots were more colonized by the fungus (Camehl et al. 2010). Conversely, the overexpression of EtRF1 reduced *P. indica* colonization and constitutively activated plant defense. Camehl et al. (2010) suggested that the Et homeostasis is required to balance fungal colonization and defense responses. Recent studies have also demonstrated that *P. indica* induces ACC biosynthesis (Khatabi et al. 2012). The ability to inhibit the Et biosynthesis without the necessity of applying exogenous inhibitors has allowed the study of the accurate role of Et in multiple stress and developmental-related phenomena. Thus, the heterologous expression of the *Pseudomonas ACCD*

gene in tomato plants showed to greatly decrease the production of Et (Klee et al. 1991). No apparent vegetative phenotypic abnormalities were detected in these tomato transgenic plants. However, there were notable alterations in the reproductive phase (i.e., several weeks delayed fruit ripening). After these early results, the ACCD was considered as a marker for the Et role in many stress and developmental processes. Interestingly, degradation of ACC in tomato inhibits Et biosynthesis but does not prevent the ability of fruits to sense Et and no ripening defects were observed in transgenic fruits exposed to Et (Klee et al. 1991). On the other hand, during the symbiotic association between rhizobia and legumes, the exogenous application of Et inhibits the formation and functioning of radical nodules. As an example, a *Medicago truncatula* Et-insensitive mutant showed increased nodulation by its symbiont *Sinorhizobium meliloti* (Penmetsa and Cook 1997). Additionally, the results of Stearns et al. (2012) support the possibility of a direct connexion between Et and auxin response, and evidenced the stress-reducing benefits of ACCD-expressing PGPRs (Fig. 7.2). Thus, some ACCD-encoding rhizobial strains can decrease Et production in the plant and therefore enhance the formation of nodules. This increased nodulation was enhanced when ACCD-containing PGPRs and rhizobial strains were co-inoculated (Baby et al. 2011). Soil bacteria expressing ACCD reduce the level of Et and confer resistance and growth of plant under various stresses (Glick et al. 1998, 2007) including flooding and pathogen attack (Wang et al. 2000; Farwell et al. 2007; see section "Updated Overview of the Plant Hormone Ethylene"). It has been hypothesized that under conditions of stress, the root excretes the majority of ACC to the rhizosphere where it is degraded by the ACCD of appropriate bacteria (e.g., *Pseudomonas* sp.; Zahir et al. 2009). Therefore, rhizobacteria with ACCD activity have the ability to reduce Et production in roots and promote plant growth (e.g., root elongation) under several stress conditions (Siddikee et al. 2011; Chen et al. 2013) (Fig. 7.2). For example, in vitro experiments showed that ACCD-producing PGPRs enhanced the salt tolerance of important crops such as canola (Cheng et al. 2007), tomato (Mayak et al. 2004), and wheat (Zahir et al. 2009). Much work is still required to transfer these results to field conditions in order to gain insight on how microorganisms induce ACC biosynthesis in plant roots. However, some progress has already been made in this regard (Ma et al. 2004; Gamalero et al. 2008; Gamalero and Glick 2012).

5 The Relationship Between Ethylene and Other Environmental Stress-Inducing Factors

5.1 Ozone

Ozone (O_3) is a highly unstable and reactive allotrope of O_2. O_3 is a common constituent of troposphere, with powerful oxidizing properties and the most phytotoxic air pollutant affecting plants, causing damage to the photosynthetic apparatus

(Ashmore 2005; Wittig et al. 2009). Surface O_3 concentrations (i.e., >60 nL L^{-1}) have been shown to negatively affect the yields of crops (Fiscus et al. 2005). Et production is (1) the quickest and most commonly observed response to O_3 (Kangasjärvi et al. 2005), including in many important crop plants (Wilkinson and Davies 2009); (2) highly correlated with O_3 injury (Tamaoki et al. 2003); and (3) clearly associated with the induction of Hypersensitive Response (HR) and PCD (Kangasjärvi et al. 2005; Overmyer et al. 2003, 2005). On the other hand, in some species it was demonstrated the prominent role of JA in the O_3–Et signaling pathway (Tamaoki et al. 2003; Grantz et al. 2010).

Rice, a moderately O_3-sensitive crop species, has significant reductions in its yields (~15–20 %) due to elevated O_3 levels (Shi et al. 2009). Moreover, O_3 also induces a quick stomatal closure response (Wittig et al. 2007; Wilkinson and Davies 2009). ABA is considered the main regulator of stomatal functioning in plants and induces stomatal closure via a network of chemical messengers (Acharya and Assmann 2008) and Et has been shown to antagonize the stomatal response to ABA (Tanaka et al. 2006). Thus, plants pretreated with 1-methylcyclopropene (1-MCP), an Et perception antagonist, were able to close the stomata normally in response to ABA (Wilkinson and Davies 2009). On the other hand, when O_3 penetrates the plant leaf through the stomata, it is quickly transformed to ROS (e.g., O_2^- anion and H_2O_2) in the apoplast (Baier et al. 2005). Subsequently, in Arabidopsis, the H_2O_2 production in guard cells as a consequence of oxidative stress of O_3 causes stomatal closure in an Et-dependent manner (Matilla-Vázquez and Matilla 2012; and references therein). In this process, Et also induces the stomatal closure stimulating the production of H_2O_2 by the NADPH-oxidase AtRbohF (Matilla-Vázquez and Matilla 2012). For more detailed information about the O_3 harmful effects on stomata movements, see Wilkinson and Davies (2010).

As indicated above (section "Cross-Talk Between Oxygen Deficient Stress and Ethylene Biosynthesis and Signaling"), when the root system is subject to stresses like flooding, the ACC is transported from there to the oxygenated parts (e.g., shoots) and transformed in Et by ACO. However, to our knowledge, studies on spatial alterations of ACC content and Et production in response to O_3 still remain to be performed. Several mutants and accessions of Arabidopsis described as O_3-sensitive have now been demonstrated that overproduce Et (Kangasjärvi et al. 2005), and Arabidopsis mutants insensitive to Et are O_3-tolerant. Recently, (1) an essential JA–Et interaction was found to be mediated by JA-Zim domain (JAZ). These JAZ proteins repress the transcription of JA-responsive genes and interact with TFs involved in mediating responses to Et (Wager and Browse 2012); and (2) O_3 surface levels induce plant physiology responses in *Gossypium barbadense* with no increase in the production of Et (Grantz et al. 2010; Grantz and Vu 2012). However, when the plants were exposed to high O_3 levels, Et biosynthesis was induced and further enhanced in MeJA-treated plants (Grantz and Vu 2012). In *G. barbadense*, the application of MeJA as an anti-ozonant has been proposed.

5.2 Freezing

Although Et regulates several specific aspects of plant responses against biotic and abiotic stress (sections "Cross-Talk Between Oxygen Deficient Stress and Ethylene Biosynthesis and Signaling" and "Ethylene and Plant Defense Against Microorganisms"), their definite role in freezing stress remains unclear (Zhang and Huang 2010 and references therein). In general, high levels of Et production are associated with chilling sensitivity (see Morgan and Drew (1997) for review of earlier literature). Nevertheless, the TFs known as C-repeat Binding Factor (CBF), belonging to the AP2/ERF superfamily, are involved in the well-understood cold signaling pathway (CBF/DREB) transcriptional regulatory cascade. Recent results in Arabidopsis demonstrated the negative effect of Et biosynthesis and signaling over the plant freezing tolerance by repressing type-A *Arabidopsis Response Regulators* (ARR) genes and the cold-inducible CBFs (Shi et al. 2012). Namely, ETR1 and EIN4, in contrast to EIN2 and EIN3/EIL1, have positive roles during the modulation of the plant adaptations to freezing. Diverse and contradictory implications of Et biosynthesis in chilling sensitivity were previously shown in maize, mung bean, tomato, cucumber, and tobacco plants (more information in Shi et al. 2012).

6 Conclusions and Future Perspective

At present, there is no doubt about the critical role of Et in plant defense strategies against biotic and abiotic stresses. Et participates in a highly complex and tightly regulated signaling network that also includes crosstalk with JA, SA, GA, and ABA signaling pathways. In order to obtain goods and services orientated to the development of modern agriculture, the knowledge of all these plant signaling networks has undergone a strong progress during the last decade. As a result, the number of biocontrol and biotechnological strategies designed to improve plant responses to stressful environmental cues, such as low O_2, freezing, and pathogens, is growing exponentially. It seems beyond doubt that the level of endogenous Et is critical for the establishment and adjustment of appropriate plant responses, and that these processes require tight spatial and temporal regulation of Et biosynthesis. A major research priority to improve the understanding of the Et signaling at molecular level was the identification of transcriptional networks that regulate the synthesis of developmental modulators. Thereby, functional analysis of the large ERF family is helping to characterize how Et coordinates plant adaptive responses to stress. Ultimately, unscrambling how plants alter their microbiome and the mechanisms by which plant-associated microorganisms control plant health will provide an excellent opportunity to enhance crop productivity and quality. However, the molecular mechanisms by which rhizosphere microorganisms are recognized to subsequently activate Et-mediated responses are still poorly understood.

Due to ET action is included in a plant hormone network, it is indispensable to unravel the ET crosstalk with SA-, JA-, and ABA-depeudent signaling pathways.

The result of this extensive study is to understand the plant response to a particular type of stress. This biotechnology challenge will require the characterization and contribution of the molecular components involved in this tangled network. To fill this complicated puzzle, molecular platforms as microarrays, protein–protein interactions, knock-out gene collection, or RNA-seq facilities must be utilized to this aim without ruling out new -omics technologies.

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