Ethylene and the Regulation of Physiological and Morphological Responses to Nutrient Deficiencies

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To cope with nutrient deficiencies, plants develop both morphological and physiological responses. The regulation of these responses is not totally understood, but some hormones and signaling substances have been implicated. It was suggested several years ago that ethylene participates in the regulation of responses to iron and phosphorous deficiency. More recently, its role has been extended to other deficiencies, such as potassium, sulfur, and others. The role of ethylene in so many deficiencies suggests that, to confer specificity to the different responses, it should act through different transduction pathways and/or in conjunction with other signals. In this update, the data supporting a role for ethylene in the regulation of responses to different nutrient deficiencies will be reviewed. In addition, the results suggesting the action of ethylene through different transduction pathways and its interaction with other hormones and signaling substances will be discussed.

When plants suffer from a mineral nutrient deficiency, they develop morphological and physiological responses (mainly in their roots) aimed to facilitate the uptake and mobilization of the limiting nutrient. After the nutrient has been acquired in enough quantity, these responses need to be switched off to avoid toxicity and conserve energy. In recent years, different plant hormones (e.g. ethylene, auxin, cytokinins, jasmonic acid, abscisic acid, brassinosteroids, GAs, and strigolactones) have been implicated in the regulation of these responses (Romera et al., 2007, 2011, 2015; Liu et al., 2009; Rubio et al., 2009; Kapulnik et al., 2011; Kiba et al., 2011; Iqbal et al., 2013; Zhang et al., 2014).

Before the 1990s, there were several publications relating ethylene and nutrient deficiencies (cited in Lynch and Brown [1997] and Romera et al. [1999]) without establishing a direct implication of ethylene in the regulation of nutrient deficiency responses. In 1994, Romera and Alcántara (1994) published an article in *Plant Physiology* suggesting a role for ethylene in the regulation of Fe deficiency responses. In 1999, Borch et al. (1999) showed the participation of ethylene in the regulation of P deficiency responses. Since then, evidence has been accumulating in support of a role for ethylene in the regulation of both Fe (Romera et al., 1999, 2015; Waters and Blevins, 2000; Lucena et al., 2006; Waters et al., 2007; García et al., 2010, 2011, 2013, 2014; Yang et al., 2014) and P deficiency responses (Kim et al., 2008; Lei et al., 2011; Li et al., 2011; Nagarajan and Smith, 2012; Wang et al., 2012, 2014c). Both Fe and P may be poorly available in most soils, and plants develop similar responses under their deficiencies (Romera and Alcántara, 2004; Zhang et al., 2014). More recently, a role for ethylene has been extended to other deficiencies, such as K (Shin and Schachtman, 2004; Jung et al., 2009; Kim et al., 2012), S (Maruyama-Nakashita et al., 2006; Wawrzyńska et al., 2010; Moniuszko et al., 2013), and B (Martín-Rejano et al., 2011). Ethylene has also been implicated in both N deficiency and excess (Tian et al., 2009; Mohd-Radzman et al., 2013; Zheng et al., 2013), and its participation in Mg deficiency has been suggested (Hermans et al., 2010).

In this update, we will review the information supporting a role for ethylene in the regulation of different nutrient deficiency responses. For information relating ethylene to other aspects of plant mineral nutrition, such as N_2 fixation and responses to excess of nitrate or essential heavy metals, the reader is referred to other reviews (for review, see Maksymiec, 2007; Mohd-Radzman et al., 2013; Steffens, 2014).

ETHYLENE SYNTHESIS AND SIGNALING UNDER NUTRIENT DEFICIENCIES

Nutrient deficiencies can influence both ethylene synthesis and signaling. In general, ethylene production increases under different nutrient deficiencies. Additionally, ethylene production can increase upon excess of some nutrients, like nitrate (Tian et al., 2009; Mohd-Radzman et al., 2013) or essential heavy metals (Maksymiec, 2007). In 1999, Romera et al. (1999) showed that Fe-deficient roots of several dicots produced more ethylene than the Fe-sufficient ones, even before the plants showed any other symptom of deficiency (which could lead to tissue necrosis and thereby, stimulation of wound ethylene; Lynch and Brown, 1997). At the same time, Borch et al. (1999) and Gilbert et al. (2000) showed that P-deficient

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roots produced more ethylene than the P-sufficient ones. After these reports, the higher ethylene production by Fe-deficient roots has been confirmed by other authors (cited in García et al. [2010] and Romera et al. [2015]). In relation to P, there has been research confirming higher ethylene production by P-deficient roots (Li et al., 2009) and showing higher ethylene production by P-deficient shoots (Kim et al., 2008).

In the last 10 years, increased ethylene production by roots and/or shoots has been described for other nutrient deficiencies, such as K (Shin and Schachtman, 2004; Benlloch-González et al., 2010), S (Zuchi et al., 2009; Moniuszko et al., 2013), N (Zheng et al., 2013), and Mg (Hermans et al., 2010).

The higher ethylene production described for nutrient deficiencies has been further supported by results showing up-regulation of genes implicated in ethylene synthesis. Ethylene is synthesized from Met through a pathway that requires the enzymes *S*-adenosyl methionine synthetases (SAMS), 1-aminocyclopropane-1-carboxylic acid synthase (ACS), and 1-aminocyclopropane-1-carboxylic acid oxidade (ACO; Sauter et al., 2013). *SAMS*, *ACS*, and *ACO* genes (Table I shows gene names and functions) were up-regulated under Fe deficiency (for review, see Romera et al., 2015) and also, P deficiency (Hernández et al., 2007; Lei et al., 2011; O'Rourke et al., 2013; Wang et al., 2014b). Shin and Schachtman (2004) have found up-regulation of two Arabidopsis (*Arabidopsis thaliana*) *ACO*s under K deficiency, Nikiforova et al. (2003) have found up-regulation of *AtSAMS* under S deficiency, Zhao et al. (2015) have found up-regulation of an *AtACO* under N deficiency, and Hermans et al. (2010) have found up-regulation of several *AtACS* under Mg deficiency.

Other than ethylene synthesis, nutrient deficiencies can also affect ethylene responsiveness. He et al. (1992) showed increased sensitivity to ethylene in N- and P-deficient roots, which has been further supported in more recent publications (Ma et al., 2003; Kim et al., 2008). Although ethylene's mode of action is not fully understood, a linear signaling pathway has been proposed in Arabidopsis (Shakeel et al., 2013; Wang et al., 2013):

$$\text{ET} \dashv \text{ET receptors} \rightarrow \text{CTR1} \dashv \text{EIN2} \rightarrow \text{EIN3/EILs} \rightarrow \text{ERFs} \rightarrow \text{ET responses}$$

where ET indicates ethylene, $\dashv$ indicates negative effect, $\rightarrow$ indicates positive effect, CTR1 indicates Constitutive Triple Response1, EIN2 indicates Ethylene Insensitive2,

Table I. *Genes related to nutrient deficiency responses used in this update*

ER, endoplasmic reticulum; TF, transcription factor.

Name	Function
Ethylene synthesis genes	
SAMS	SAMS
ACS	ACS
ACO	ACO
Ethylene signaling genes	
*ETR*s and *ERS*s	Ethylene receptors
CTR1[a]	Kinase
EIN2[b]	Protein acting downstream of CTR1 (localized to ER membrane)
EIN3[c]	TF acting downstream of EIN2
EILs[c]	TFs acting downstream of EIN2
SLIM1 (*EIL3*)	*SLIM1* is an allele of *EIL3*
*ERF*s	TFs acting downstream of EIN3
RAP2.11	Is an ERF
Fe-related genes	
FIT (*FER* homolog)	TF (master regulator of most Fe acquisition genes)
MED16	Mediator (interacts with EIN3/EIL1 for *FIT* transcription)
P-related genes	
PT1, *PT2*, and *PT5*	Phosphate transporters
ACP5 and *PAP1*	Acid phosphatases
PHO2	E2 conjugase (negative regulator of P deficiency responses)
S-related genes	
*SULTR*s	Sulfate transporters
APR	Adenosine 5′-phosphosulfate reductase
APS4	ATP sulfurylase (negative regulator of S deficiency responses)
K-related genes	
HAK5	K^+ transporter
N-related genes	
*NRT*s	Nitrate transporters

[a]Its mutation leads to a constitutive triple-response phenotype, similar to the one of wild-type plants treated with ethylene. [b]Its mutation leads to an ethylene-insensitive phenotype. [c]Their mutations lead to ethylene-insensitive phenotypes.

EIL indicates Ethylene Insensitive-Like, and ERF indicates Ethylene Response Factor. In the absence of ethylene, the kinase CTR1 phosphorylates EIN2 (which is localized to the endoplasmic reticulum membrane), preventing the cleavage and translocation of the EIN2 C-terminal fragment into the nucleus. In the presence of ethylene, this is bound to its receptors, and CTR1 is inactivated, resulting in dephosphorylation of EIN2 and its cleavage. The EIN2 C-terminal fragment is then translocated into the nucleus, where it participates in stabilization of the transcription factor EIN3 and downstream gene activation (Shakeel et al., 2013; Wang et al., 2013). EIN3 belongs to a small family of transcription factors that also includes various EIL proteins: EIL1, EIL2, and EIL3 (Wang et al., 2013). Mutants of CTR1 present constitutive activation of ethylene signaling, whereas mutants of EIN2 and EIN3/EILs display reduced sensitivity to ethylene (Shakeel et al., 2013; Wang et al., 2013). The ERF transcription factors act downstream of EIN3 to activate or repress ethylene-responsive genes, although some ERFs can be activated by ethylene-independent transcription factors not related to EIN3 (Wang et al., 2013; Thirugnanasambantham et al., 2015).

Ethylene responsiveness could be related to changes in the expression of genes implicated in ethylene signaling. Several of these genes were up-regulated under Fe deficiency, like *Ethylene Triple Responses* (*ETR*s; coding for ethylene receptors), *Ethylene Response Sensors* (*ERS*s; coding for ethylene receptors), *EIN2*, *EIN3*, *EIL*s, and *ERF*s (O'Rourke et al., 2007; García et al., 2010, 2014; Wang et al., 2014a). Similarly, Shin and Schachtman (2004) found increased expression of *AtETR2* (encoding an ethylene receptor) and Kim et al. (2012) found increased expression of Arabidopsis *Rhoptry-Associated Protein2.11* (*AtRAP2.11*; encoding an ERF) under K deficiency. In relation to N, Zheng et al. (2013) have shown increased expression of *AtEIN3* and *AtEIL1*, and Zhao et al. (2015) have shown increased expression of several *AtERF*s under this deficiency. Very recently, Ramaiah et al. (2014) have described the up-regulation of *AtERF070* (encoding an ERF) in P-deprived roots and shoots.

Whether the expression of these genes enhances or decreases the sensitivity to ethylene deserves a deeper investigation. Because ethylene receptors act as negative regulators of ethylene signaling, their increase would decrease sensitivity to ethylene (Wang et al., 2013). Possibly, the induction of ethylene receptor genes may function as a dampening mechanism, slowing down an ethylene response after it has been initiated.

ETHYLENE PARTICIPATION IN NUTRIENT DEFICIENCY RESPONSES

In addition to the higher ethylene production of nutrient deficient plants (see above), other results also support a role for ethylene in the regulation of nutrient deficiency responses. These other results are mainly based on the use of ethylene inhibitors, like cobalt or silver thiosulfate (STS), the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), the ethylene-releasing substance ethephon, ethylene itself, ethylene mutants (ethylene insensitive, ethylene constitutive, or ethylene overproducers), and molecular biology techniques, such as transgenic lines, transcriptomics, fluorescence imaging, luciferase imaging, GUS assay, and yeast (*Saccharomyces cerevisiae*) two-hybrid assay (Romera and Alcántara, 2004; Maruyama-Nakashita et al., 2006; Jung et al., 2009; García et al., 2010; Lei et al., 2011; Kim et al., 2012; Yang et al., 2014). In most cases, ethylene, with production that increases under the nutrient deficiency, acts as activator of the responses. Consequently, ethylene inhibitors block the responses (Fig. 1), whereas ethylene itself or ethylene precursors (ACC and ethephon) promote them (Romera and Alcántara, 1994, 2004; Jung et al., 2009; Tian et al., 2009; Lei et al., 2011; Li et al., 2011; Wang et al., 2012).

Ethylene has been implicated in the regulation of both morphological and physiological responses to nutrient deficiencies (Romera and Alcántara, 1994, 2004; Jung et al., 2009; Lei et al., 2011; Wang et al., 2012, 2014c). Morphological responses include responses like changes in root system architecture (RSA), development of root hairs, development of cluster or proteoid roots (clusters of closely spaced short lateral rootlets formed in some plant species adapted to poor soils; Wang et al., 2014b), and development of root transfer cells (cells with increased surface area because of invaginations of the plasma membrane; Kramer et al., 1980). Most of these root modifications enhance nutrient uptake by increasing the surface of contact of roots with soil and chemically modifying the soil environment (Wang et al., 2014b). Physiological responses are changes in processes aimed to facilitate the mobilization and uptake of nutrients. Some physiological responses are the acidification of the

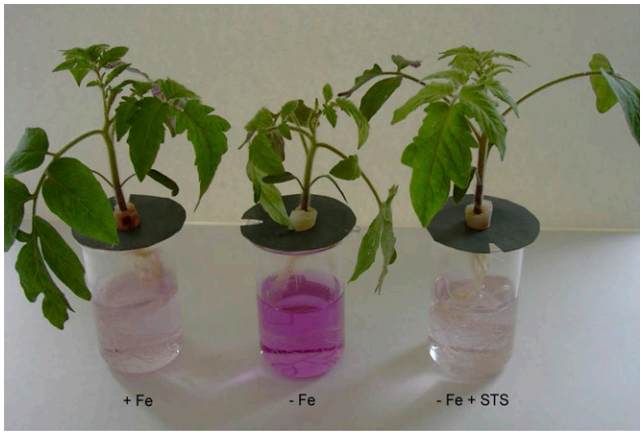

Figure 1. Ethylene generally acts as an activator of responses to nutrient deficiencies. Consequently, ethylene inhibitors block these responses. As an example, ferric reductase activity is enhanced under Fe deficiency (denoted by the purple color of the assay solution) but blocked upon application of the ethylene inhibitor STS. Tomato plants grown in complete nutrient solution (+Fe) were transferred for the last 3 d to nutrient solution either without Fe (−Fe) or without Fe plus 400 μM STS (−Fe + STS). Ferric reductase activity was determined as described by Romera and Alcántara (1994).

rhizosphere, the release of chelating agents into the medium, the increased amount of specific transporters in root epidermal cells, the increase of internal transporters and chelating agents (to improve mobilization of nutrients inside the plant), the enhancement of root ferric reductase activity, and the enhancement of root acid phosphatase activity.

Morphological Responses

Ethylene has been implicated in the development of subapical root hairs, root transfer cells, and cluster roots induced under Fe or P deficiency (Kramer et al., 1980; Romera and Alcántara, 1994, 2004; Schmidt et al., 2000a; Waters and Blevins, 2000; Schmidt and Schikora, 2001; Schikora and Schmidt, 2002; Zaid et al., 2003; Zhang et al., 2003, 2014; Wang et al., 2014b). Other than these deficiencies, ethylene has also been implicated in the development of root hairs caused by K (Jung et al., 2009) or B deficiency (Martín-Rejano et al., 2011).

Nutrient deficiencies can also change RSA by altering the number, length, and diameter of roots (Gruber et al., 2013). The RSA modifications depend on each specific nutrient and the extent of the deficiency (mild, moderate, or severe). Generally, nutrient-deficient plants exhibit a shallower architecture that results from inhibition of primary root elongation (Kramer et al., 1980; López-Bucio et al., 2003; Ma et al., 2003; Jung et al., 2009; Martín-Rejano et al., 2011; Nagarajan and Smith, 2012, Wang et al., 2012; Gruber et al., 2013; Zhang et al., 2014). Some exceptions are S deficiency, with relatively little influence on the morphology of roots, and N deficiency, which stimulates primary root elongation and particularly, lateral root elongation (Gruber et al., 2013). Several deficiencies, like B, Fe, or P deficiency, can cause an increase in lateral root density (Kramer et al., 1980; López-Bucio et al., 2003; Miura et al., 2011; Gruber et al., 2013; Zhang et al., 2014). Ethylene has been implicated in most of these RSA changes (Borch et al., 1999; Gilbert et al., 2000; Ma et al., 2003; Zhang et al., 2003, 2014; Jung et al., 2009; Martín-Rejano et al., 2011; Miura et al., 2011; Chérel et al., 2014) along with auxin and other hormones and signaling substances ("Ethylene Interacts with Other Signals for the Regulation of Nutrient Deficiency Responses"). Very recently, Ramaiah et al. (2014) have found that *AtERF070* (encoding an ERF) was greatly induced under P deprivation and can affect RSA. This indicates that ethylene, through an ERF transcription factor that participates in ethylene signaling (see above), can modify the architecture of roots.

The participation of ethylene in the regulation of the morphological responses described above is supported by results showing that ethylene inhibitors negatively affect these changes in nutrient-deficient plants, whereas ethylene precursors promote them in nutrient-sufficient plants (Romera and Alcántara, 1994; Borch et al., 1999; Schmidt et al., 2000a; Ma et al., 2003; Zaid et al., 2003; Zhang et al., 2003, 2014; Romera et al., 2007; Wang et al., 2012, 2014b). As an example, ethylene inhibitors almost totally block the development of subapical root hairs in Fe-, K-, or P-deficient plants (Zhang et al., 2003; Romera and Alcántara, 2004; Jung et al., 2009). Additionally, results obtained with ethylene mutants support this participation (Jung et al., 2009; Wang et al., 2012; see below).

Physiological Responses

In addition to a role for ethylene in the regulation of morphological responses, many data support its role in the regulation of physiological responses. In relation to Fe nutrition, ethylene participates in the up-regulation of many genes implicated in Fe acquisition and homeostasis (Lucena et al., 2006; Waters et al., 2007; García et al., 2010; Romera et al., 2015). Some of the genes up-regulated by ethylene encode transcription factors that are key regulators of most of the responses to Fe deficiency, such as *Arabidopsis Fer-like Fe Deficiency Transcription Factor* (*AtFIT*; Colangelo and Guerinot, 2004) or its tomato (*Solanum lycopersicum*) homolog tomato *Fe Efficiency Response* (*SlFER*; Brumbarova and Bauer, 2005). Very recently, it has been shown that AtEIN3 and AtEIL1, both related to ethylene signaling (see above), interact with Arabidopsis Mediator16 (AtMED16) to form a complex implicated in the transcription of *AtFIT* (Yang et al., 2014).

In the last 10 years, the role of ethylene in the regulation of physiological responses has been extended to other nutrient deficiencies. In K nutrition, ethylene has been shown to be implicated in the up-regulation of the K^+ transporter Arabidopsis High Affinity K^+ Transporter5 (AtHAK5; Jung et al., 2009), possibly through RAP2.11 (an ERF; Kim et al., 2012). In S nutrition, the up-regulation of several sulfate transporter genes (Arabidopsis *Sulfate Transporter1;1* [*AtSULTR1;1*], *AtSULTR1;2*, *AtSULTR3;4*, and *AtSULTR4;2*) as well as other S-responsive genes is greatly diminished in *sulfur limitation1* (*slim1*; *eil3*) mutants (Maruyama-Nakashita et al., 2006). The similarity of AtEIL3 with AtEIN3 suggests that it could be a positive regulator of ethylene signaling (see above). The participation of ethylene in S deficiency responses has also been supported by other experimental results (Koprivova et al., 2008; Wawrzyńska et al., 2010; Iqbal et al., 2012; Moniuszko et al., 2013). Koprivova et al. (2008) found up-regulation of several Arabidopsis *Adenosine 5′-Phosphosulfate Reductases* (*AtAPR*s; encoding adenosine 5′-phosphosulfate reductase, a key enzyme of sulfate assimilation) upon ACC treatment, and Iqbal et al. (2012) found increased ATP-sulfurylase activity (also implicated in sulfate assimilation) upon ethephon application. However, Wawrzyńska et al. (2010) showed that tobacco (*Nicotiana tabacum*) *Upregulated by Sulfur Deficit 9C*, a tobacco gene strongly induced by S deficiency, is activated by the NtEIL2 transcription factor related to ethylene signaling (see above). In P nutrition, Lei et al. (2011) in Arabidopsis and Li et al. (2011) in *Medicago falcata* have implicated ethylene in up-regulation of phosphate transporter genes (Arabidopsis *Phosphate Transporter1* [*AtPT1*], *AtPT2*, *MfPT1*, and *MfPT5*) and enhanced phosphatase activity (through higher expression of Arabidopsis *Acid Phosphatase5* (*AtACP5*) and

M. falcata Purple Acid Phosphatase1 (*MfPAP1*) encoding phosphatases) of P-deficient roots. In N nutrition, ethylene has been shown to up-regulate Arabidopsis *Nitrate Transporter1.1* (*AtNRT1.1*), whereas it down-regulates *AtNRT2.1* (both encoding nitrate transporters; Tian et al., 2009; Zheng et al., 2013). The negative effect of ethylene on the regulation of some nutrient deficiency responses has also been shown in P starvation-induced anthocyanin (Lei et al., 2011; Wang et al., 2012) and N starvation-induced anthocyanin (Wang et al., 2015), both of them inhibited by ethylene.

ARE THE DIFFERENT NUTRIENT DEFICIENCY RESPONSES REGULATED SIMILARLY BY ETHYLENE?

Because ethylene has been implicated in the regulation of many nutrient deficiency responses, the question arises as to whether ethylene regulates all of them through the same transduction pathway. The answer to this question is clearly that different responses can be regulated by ethylene through different transduction pathways based on results with ethylene mutants. However, many results suggest that ethylene does not act alone but acts in conjunction with other hormones and signaling substances to regulate the responses.

Results from ethylene-insensitive mutants suggest that, even within a deficiency, different responses can be regulated through different transduction pathways. The tomato ethylene-insensitive mutant *Never ripe* does not increase adventitious root formation under P deficiency but is normal in other morphological responses (Kim et al., 2008). Similarly, the development of subapical root hairs is impaired in the Arabidopsis ethylene-insensitive mutant *ein2* under Fe deficiency (Schmidt and Schikora, 2001), whereas the enhanced ferric reductase activity (Fig. 2) and the expression of Fe acquisition genes are not impaired (García et al., 2010).

The idea of multiple transduction pathways for regulating nutrient deficiency responses by ethylene is further reinforced by comparing different deficiencies and looking at the results from ethylene overproducer and ethylene-constitutive mutants. The up-regulation of the *AtPT2* gene (induced under P deficiency; Fig. 2) and the *AtHAK5* gene (induced under K deficiency) is impaired in the Arabidopsis *ein2* mutant (Jung et al., 2009; Lei et al., 2011), whereas increased ferric reductase activity under Fe deficiency is not impaired (Fig. 2). Both the Arabidopsis ethylene-constitutive mutant *ctr1* and the Arabidopsis ethylene overproducer mutant *eto* have constitutive subapical root hairs (a nutrient deficiency symptom) in complete nutrient solution; however, neither of these mutants has full constitutive activation of P, Fe, or K physiological responses (Fig. 2; Schmidt et al., 2000b; Romera and Alcántara, 2004; García et al., 2007, 2014; Jung et al., 2009; Lei et al., 2011; Wang et al., 2012). In the same way, root hairs, transfer cells, and cluster roots are almost fully induced by ACC or ethephon in plants grown with high levels of P, Fe, or K,

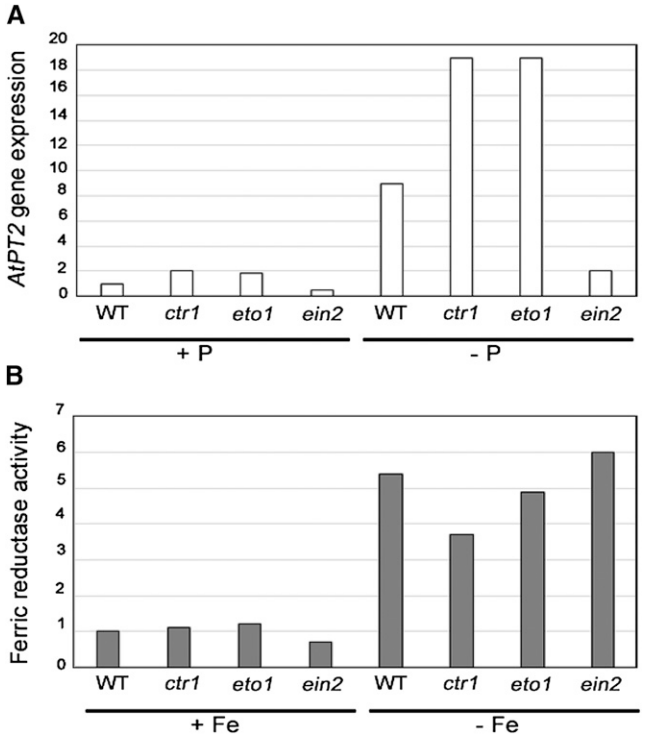

Figure 2. Effect of P deficiency on *AtPT2* gene expression (A) and Fe deficiency on ferric reductase activity (B) in Arabidopsis wild-type (WT) Columbia-0 plants and ethylene mutants (*ctr1*, ethylene-constitutive mutant; *ein2*, ethylene-insensitive mutant; *eto1*, ethylene overproducer mutant). Fold changes were normalized to transcript levels of the wild type on P sufficiency (A) and ferric reductase activity of the wild type on Fe sufficiency (B). Data for P treatments were redrawn from Lei et al. (2011) with permission, and data for Fe were from García et al. (2010, 2014) and M.J. García, F.J. Romera, C. Lucena, E. Alcántara, and R. Pérez-Vicente (unpublished data).

whereas physiological responses are activated to a lesser degree than when applied to plants grown with low levels or in absence of these nutrients (Romera and Alcántara, 1994; Schmidt et al., 2000a; Zaid et al., 2003; Zhang et al., 2003; Jung et al., 2009; Lucena et al., 2006; Lei et al., 2011; Li et al., 2011; García et al., 2013).

From all of the results above, several conclusions can be drawn. First, different nutrient deficiency responses can be regulated by ethylene through distinct transduction pathways. Second, for some responses, like Fe physiological responses, ethylene could act through a pathway where EIN2 and possibly, CTR1 are not strictly required (Fig. 3). Third, morphological and physiological responses can be differently regulated by ethylene. Fourth, for the regulation of physiological responses, ethylene could act in conjunction with nutrient-related repressive signals. The existence of an alternate route for ethylene signaling, other than the conventional one including CTR1 and EIN2 (Fig. 3; Shakeel et al., 2013), is further supported by results showing that the Arabidopsis *ctr1* and *ein2* mutants respond to both ACC (García et al., 2010, 2014) and ethylene inhibitors (García et al., 2007; Jung et al., 2009) for some physiological

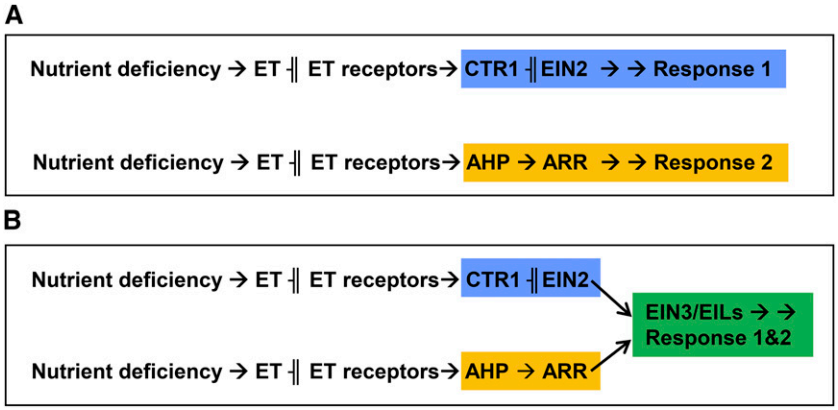

Figure 3. Ethylene (ET) could regulate different nutrient deficiency responses through two distinct signal transduction pathways. One pathway is CTR1-EIN2 dependent, and the other is CTR1-EIN2 independent (instead, using Arabidopsis His Phosphotransfer [AHP] proteins and Arabidopsis Response Regulators [ARRs]; Shakeel et al., 2013). This model is supported by data showing that *ctr1* and *ein2* mutants respond to both ACC and ET inhibitors for some physiological responses (García et al., 2007, 2010, 2014; Jung et al., 2009). It is possible that both pathways can act independently (A) or that both can interact and converge downstream through EIN3/EILs (B) depending on the responses (Table I). ⊣, Inhibition; →, promotion.

responses. In relation to the last two conclusions, we can speculate that, because physiological responses are not fully activated in the *ctr1* and *eto* mutants (Fig. 2), whereas morphological responses (at least in root hairs) are, some nutrient-related signals act negatively to block physiological responses. These signals probably act downstream of CTR1 in the ethylene signaling pathway. This does not preclude that these signals can also affect ethylene synthesis.

ETHYLENE INTERACTS WITH OTHER SIGNALS FOR THE REGULATION OF NUTRIENT DEFICIENCY RESPONSES

Morphological and physiological responses work together to effectively increase nutrient uptake (Lucena et al., 2006; Jung et al., 2009; Wang et al., 2014b). Consequently, their regulation is coordinated through the participation of similar signals for both kind of responses, like hormones (e.g. auxin, ethylene, cytokinins, jasmonic acid, brassinosteroids, gibberellins, abscisic acid, and strigolactones) and other signaling substances, such as nitric oxide (NO), reactive oxygen species (ROS), and sugars (Romera et al., 2007, 2011, 2015; Rubio et al., 2009; Hammond and White, 2011; Kapulnik et al., 2011; Lei and Liu, 2011; Iqbal et al., 2013; Zhang et al., 2014). Other than these common signals, there are other more nutrient-specific signals, such as mineral ions, microRNAs, reduced glutathione (GSH), and peptides (Lappartient et al., 1999; Liu et al., 2009; Buhtz et al., 2010; García et al., 2013; Zeng et al., 2014; Zhang et al., 2014), that could confer specificity (at least a certain degree of specificity) to the different nutrient deficiency responses. Despite this, cross talk in the activation of physiological responses under different nutrient deficiencies (e.g. a K physiological response can be activated under P deficiency) has been described (Shin et al., 2005; Waters et al., 2012; Wang et al., 2014b), probably because of the common implication of ethylene and other signals in their regulation.

Different results suggest that some nutrient-related repressive signals (e.g. mineral ions, peptides, and GSH) can move from shoots to roots through the phloem (Dong et al., 1998; Lappartient et al., 1999; García et al., 2013; Zhang et al., 2014). Additionally, other signals, like auxin, sugars, and microRNAs, can also move through the phloem (Romera et al., 2007, 2011; Buhtz et al., 2010; Lei and Liu, 2011). This would provide a way for shoots to inform roots of their nutrient status and could serve to integrate the role of both shoots and roots in the regulation of nutrient deficiency responses.

Auxin, sugars, NO, and ROS generally accumulate in roots under nutrient deficiencies (Shin et al., 2005; Tewari et al., 2006; Graziano and Lamattina, 2007; Romera et al., 2007; Jung et al., 2009; Wang et al., 2010, 2014b; Kiba et al., 2011; Iqbal et al., 2013) and can positively interact with ethylene to regulate nutrient deficiency responses. Auxin can stimulate ethylene production, and ethylene can affect auxin transport and accumulation (Romera et al., 2007, 2011; Muday et al., 2012). The exact relationship of sugars and ethylene is not totally known, but hexokinases are a critical node in mediating plant Glc and ethylene responses (Karve et al., 2012). Ethylene can stimulate both ROS and NO accumulation (Shin et al., 2005; Jung et al., 2009; García et al., 2011; Steffens, 2014), whereas both ROS and NO can stimulate ethylene production (Ahlfors et al., 2009; García et al., 2011; Iqbal et al., 2013). Furthermore, ROS can stimulate ethylene production through NO accumulation (Ahlfors et al., 2009). Probably, ROS and NO influence the production of ethylene in a positive feedback loop, leading to enhancement of the nutrient deficiency signal as described for ethylene and NO (Fig. 4; García et al., 2011).

Signals Interacting with Ethylene for the Regulation of Morphological Responses

One of the signals most closely related to ethylene for the regulation of morphological responses is auxin. In supporting this view, it should be noted that subapical root hairs (Schmidt and Schikora, 2001; Zhang et al., 2003; Romera et al., 2007; Martín-Rejano et al., 2011), transfer cells (Schmidt et al., 2000a; Schikora and Schmidt, 2002), and cluster roots (Zaid et al., 2003; Wang et al., 2014b) are similarly affected by either ethylene or auxin treatments. In the same way, lateral root formation and inhibition of root elongation under different deficiencies ("Ethylene Participation in Nutrient Deficiency Responses") have been associated with auxin and ethylene (Zhang et al., 2003; López-Bucio et al., 2003; Miura et al., 2011; Muday et al., 2012; Chérel et al., 2014). Both hormones synergistically inhibit root elongation and play an antagonistic role on lateral root formation, where auxin stimulates while ethylene inhibits it (López-Bucio et al., 2003; Miura et al., 2011; Muday et al., 2012; Chérel et al., 2014; Wang et al., 2014b). Ethylene increases rootward auxin transport by up-regulating PIN-FORMED3 (PIN3) and PIN7 (auxin efflux carriers) in the central cylinder, which may deplete the lateral root-forming zone of auxin while increasing auxin accumulation in the root apex. This effect may be responsible for the negative regulation of lateral root formation by ethylene (Muday et al., 2012; Chérel et al., 2014). At the root tip, up-regulation of AUXIN RESISTANT1 (AUX1; auxin influx carrier) and PIN2 (auxin efflux carrier) enhances shootward auxin transport into the elongation zone, thereby reducing primary root elongation and promoting the development of subapical root hairs by up-regulating root hair-specific genes (Muday et al., 2012; Lee and Cho, 2013).

Strigolactones are other hormones that participate in the regulation of root hair elongation, and their role has been related to interactions with auxin and ethylene (Kapulnik et al., 2011).

NO has also been implicated in the development of subapical root hairs (Graziano and Lamattina, 2007) and cluster roots (Wang et al., 2010) under Fe or P deficiency. Similarly, ROS has also been implicated in the development of subapical root hairs under K, N, and P deficiency (Shin et al., 2005; Jung et al., 2009). As previously described, both NO and ROS can influence the production of ethylene and vice versa. Moreover, ACC and ethephon induce NO and ROS accumulation in the subapical region of the roots, where subapical root hairs develop (Jung et al., 2009; García et al., 2011).

Signals Interacting with Ethylene for the Regulation of Physiological Responses

Different signals could interact with ethylene to activate or suppress physiological responses depending on the nutrient status of the plants. Under nutrient sufficiency, several nutrient-related repressive signals, some of them moving through the phloem (e.g. mineral ions, peptides, and GSH), could negatively interact with ethylene to inactivate nutrient responses (Fig. 4; Lappartient et al., 1999; García et al., 2013; Zhang et al., 2014). It has been proposed that some Fe compound moves through the phloem (probably an Fe peptide) and could negatively interact with ethylene signaling to regulate Fe physiological responses (García et al., 2013; Romera et al., 2015). Similarly, GSH moving in the phloem has been described as a suppressor of some S physiological responses (Lappartient et al., 1999), and GSH could negatively interact with ethylene signaling (Chen et al., 2013).

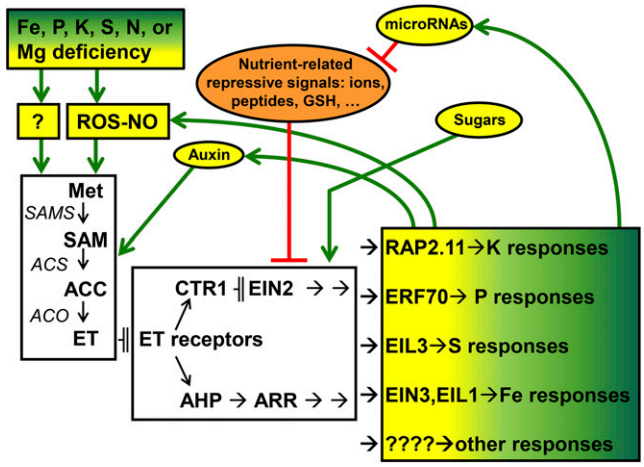

Figure 4. Working model to explain the role of ethylene (ET) on the regulation of responses to different nutrient deficiencies in plants. Nutrient deficiencies can enhance ET production by up-regulating *SAMS*, *ACS*, and *ACO* genes (Shin and Schachtman, 2004; García et al., 2010; Hermans et al., 2010), although the steps leading to this up-regulation are not yet clear. A possibility is that, at first, nutrient deficiencies cause oxidative stress and consequently, ROS accumulation and possibly, NO accumulation (Shin et al., 2005; Tewari et al., 2006; Graziano and Lamattina, 2007; Ahlfors et al., 2009; Jung et al., 2009; García et al., 2011; Iqbal et al., 2013; Steffens, 2014). This ROS-NO accumulation would stimulate ET production, which in turn, could increase ROS-NO production (Ahlfors et al., 2009; Jung et al., 2009; García et al., 2011; Iqbal et al., 2013) in a positive feedback loop, leading to enhancement of the nutrient deficiency signal (García et al., 2011). When ET is perceived by the receptors, it could act through a CTR1-EIN2-dependent pathway for the regulation of some responses or a CTR1-EIN2-independent pathway for the regulation of other responses (Fig. 3). At the end of these transduction pathways, different ET-related transcription factors, such as RAP2.11, ERF070, EIL3, EIN3/EIL1, or others, would activate different nutrient responses (Maruyama-Nakashita et al., 2006; Kim et al., 2012; Ramaiah et al., 2014; Yang et al., 2014). Under nutrient sufficiency, several signals that can move through the phloem (mineral ions, peptides, GSH, etc.; Lappartient et al., 1999; García et al., 2013; Zhang et al., 2014) could negatively interact with ET to inactivate nutrient responses. However, under nutrient deficiency, other signals that can move through the phloem (microRNAs, auxin, sugars, etc.; Kasajima et al., 2007; Lejay et al., 2008; Hammond and White, 2011; Lei and Liu, 2011; Hu et al., 2015) could positively interact with ET to activate the responses. Additionally, ET can influence auxin accumulation and distribution and the expression of some microRNAs (see text for details). Yellow background indicates signals that activate responses, orange background indicates signals that repress responses. AHP, Arabidopsis His Phosphotransfer; ARR, Arabidopsis Response Regulators; ⫫, inhibition; →, promotion.

Cytokinins have also been described as negative regulators of several physiological responses to nutrient deficiencies (Rubio et al., 2009; Hammond and White, 2011), although their exact relationship with ethylene will require additional research.

Under nutrient deficiency, some signals, such as auxin, NO, ROS, and sugars, have been described as activators of physiological responses to different deficiencies (Graziano and Lamattina, 2007; Kasajima et al., 2007; Lejay et al., 2008; Jung et al., 2009; García et al., 2010, 2011; Wang et al., 2010; Hammond and White, 2011; Kiba et al., 2011; Lei and Liu, 2011; Zhang et al., 2014). Because these signals can interact with ethylene in several ways (see above), it is possible that the roles of all of them on the activation of the responses could be tightly interrelated (Fig. 4; Jung et al., 2009; Romera et al., 2011, 2015).

Under nutrient deficiency, some nutrient-related signals that can move through the phloem, like microRNAs (Buhtz et al., 2010), have also been described as activators of many physiological responses (Zeng et al., 2014; Hu et al., 2015). Many microRNAs increase their expression under nutrient deficiencies: as examples, microRNA399 (miRNA399) is strongly induced under P, Fe, or K deficiency; miR827 is strongly induced under P deficiency; miR395 is strongly induced under S deficiency; miR158 is strongly induced under Fe deficiency; and miR397, miR398, and miR857 are strongly induced under Cu deficiency (Buhtz et al., 2010; Kawashima et al., 2011; Waters et al., 2012; Lin et al., 2013; Zeng et al., 2014; Hu et al., 2015). They usually down-regulate the expression of target genes by posttranscriptional cleavage (Kawashima et al., 2011; Zeng et al., 2014; Hu et al., 2015). The down-regulation of some target genes, such as Arabidopsis *Phosphate2* (*AtPHO2*; encoding an E2 ubiquitin conjugase) or Arabidopsis *ATP Sulfurylase4* (*AtAPS4*; encoding an ATP sulfurylase), caused by miR399 or miR395, respectively, leads to the accumulation of P or S in shoots and the prevention of their transport in the phloem from shoots to roots (Dong et al., 1998; Liang et al., 2010; Kawashima et al., 2011; Zhang et al., 2014). This suggests that some microRNAs could restrict the movement of nutrient-related repressive signals from shoots to roots, thereby positively interacting with ethylene in the activation of the responses (Fig. 4). Moreover, ethylene could potentiate the effects of some microRNAs. As an example, miR395 is induced by S deficiency in a SLIM1(EIL3)-ethylene-dependent manner (Fig. 4; Kawashima et al., 2011).

CONCLUSION

In addition to the participation of ethylene in the regulation of Fe and P deficiency responses, its role has been extended to other deficiencies. This implies that it acts as a general coordinator of many nutrient deficiency responses. Its participation in so many deficiencies suggests that, to confer specificity to the different responses, ethylene should act through different transduction pathways and/or in conjunction with other signals. Its interaction with some of these signals, such as auxin and NO, is partly known. However, its interaction with other signals, such as peptides, microRNAs, and GSH-related compounds moving in the phloem, is practically unknown. Deeper research is required in the near future to clarify the shared steps related to ethylene signaling for the different responses and the signaling steps specific for each response. In addition, it would be necessary to extend research on the role of ethylene to other nutrient deficiency responses not studied yet.

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