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ABA crosstalk with ethylene and nitric oxide in seed dormancy and germination

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Dormancy is an adaptive trait that enables seed germination to coincide with favorable environmental conditions. It has been clearly demonstrated that dormancy is induced by abscisic acid (ABA) during seed development on the mother plant. After seed dispersal, germination is preceded by a decline in ABA in imbibed seeds, which results from ABA catabolism through 8′-hydroxylation. The hormonal balance between ABA and gibberellins (GAs) has been shown to act as an integrator of environmental cues to maintain dormancy or activate germination. The interplay of ABA with other endogenous signals is however less documented. In numerous species, ethylene counteracts ABA signaling pathways and induces germination. In Brassicaceae seeds, ethylene prevents the inhibitory effects of ABA on endosperm cap weakening, thereby facilitating endosperm rupture and radicle emergence. Moreover, enhanced seed dormancy in Arabidopsis ethylene-insensitive mutants results from greater ABA sensitivity. Conversely, ABA limits ethylene action by down-regulating its biosynthesis. Nitric oxide (NO) has been proposed as a common actor in the ABA and ethylene crosstalk in seed. Indeed, convergent evidence indicates that NO is produced rapidly after seed imbibition and promotes germination by inducing the expression of the ABA 8′-hydroxylase gene, CYP707A2, and stimulating ethylene production. The role of NO and other nitrogen-containing compounds, such as nitrate, in seed dormancy breakage and germination stimulation has been reported in several species. This review will describe our current knowledge of ABA crosstalk with ethylene and NO, both volatile compounds that have been shown to counteract ABA action in seeds and to improve dormancy release and germination.

Keywords: abscisic acid, dormancy, ethylene, germination, hormone, nitric oxide, seed

INTRODUCTION

Survival of plant species mainly relies on the sexual reproduction to give birth to new individuals. In flowering plants, the seed is the main unit of dispersal and allows colonization of new geographic areas. As a consequence of the double fertilization process, a mature angiosperm seed contains a diploid embryo and protective layers comprising the tripliod endosperm, a nourishing tissue for the embryo, and the seed coat of maternal origin. During development on the mother plant, after embryogenesis completion, reserve accumulation takes place and is followed, in so-called orthodox seeds, by an intense dehydration leading to low seed water content upon dispersal. In many species, a dormant state is also induced during the maturation phase, preventing pre harvest germination and allowing seed survival until environmental conditions become suitable for germination and seedling establishment (Bentink and Koornneef, 2008; Finkelstein et al., 2004; North et al., 2010).

Dormancy has been defined as a developmental state in which a viable seed fails to germinate under favorable environmental conditions (Brewley, 1997), but different definitions and classifications have been proposed. Finch-Savage and Leubner-Metzger (2006) summarized a classification proposed by Baskin and Baskin (2004), based on the fact that dormancy results from physiological and developmental (or morphological) properties of the seed. Dormancy is therefore divided in five classes: (1) physiological dormancy (PD) can be released by different stratification (moist chilling) treatments depending on its depth, (2) morphological dormancy (MD) is due to a delay of embryo development, (3) morphophysiological dormancy (MPD) is combining both PD and MD, (4) physical dormancy (PY) is correlated with seed coat impermeability to water and needs disruption of the seed coat (scarification) to be released, and finally (5) combinational dormancy combining PD and MPD. Most species display a non-deep PD corresponding to a dormancy that can be released, depending on the species, by gibberellin (GA) treatment, stratification, scarification, or a period of dry storage (after-ripening). In this case, seeds generally combine a coat-imposed dormancy due to the covering layers of the seed (seed coat and endosperm) that prevent the radicle protrusion, and an embryo dormancy due to its incapacity to induce radicle growth.

When dormancy is released, seeds can germinate under favorable conditions, specific to each species. The germination process, that begins with seed imbibition and finishes with a developed plantlet, is divided in three distinct phases of water uptake.
Abscisic acid is formed by cleavage of C40 oxygenated carotenoids, (Arc et al., 2010b; Weisbrecht et al., 2011). Germination sensu stricto ends with radicle protrusion. It is often described has the resulting consequence of the growth potential of the embryo and the resistance of the surrounding layers. Endosperm weakening is an essential part of the modification of seed envelopes for the progress of germination and involves the activation of cell-wall modifying enzymes (Finch-Savage and Leubner-Metzger, 2006; Endo et al., 2012; Linkies and Leubner-Metzger, 2012). After dormancy release, storage/imbibition of non-dormant seeds in unfavorable conditions for germination can trigger a secondary dormancy. This is a way to protect seeds against germination too late in the year and induce a seasonal cycling of dormancy level in seeds (Cadman et al., 2006; Fouttit et al., 2011).

The regulation of seed dormancy and germination by the hormonal balance between abscisic acid (ABA) and GA, in response to environmental signals, is well documented in a number of recent reviews (Finkelstein et al., 2008; Seo et al., 2009; Nambara et al., 2010; Nonogaki et al., 2010; Weisbrecht et al., 2011; Graeber et al., 2012; Rajjou et al., 2012). The present review will describe recent knowledge about key players in the ABA metabolism and signaling pathways that control dormancy induction and maintenance and convergent evidences supporting the role of two other signaling pathways that control dormancy induction and maintenance and knowledge about key players in the ABA metabolism and signaling pathways.

ABA HOMESTASIS AND SIGNALING IN DORMANCY CONTROL

ABA SYNTHESIS

Abscisic acid is formed by cleavage of C40 oxygenated carotenoids, also called xanthophylls, which are produced in plastids from C5 precursors (Ruiz-Sola and Rodriguez-Concepcion, 2012). Key genes encoding enzymes of the ABA biosynthesis pathway have been identified through mutant selection for altered germination phenotypes, giving further evidence of the major role of ABA in the regulation of seed dormancy and germination (Figure 1). For instance, the first ABA-deficient mutant, identified in Arabidopsis thaliana, was isolated in a GA biosynthesis mutant gaf suppressor screen, on its ability to germinate in the absence of GA. It was shown to be defective in zeaxanthin epoxidase (ZEP). ABA4 is involved in the synthesis of neoxanthin, which is then cis-isomerized, together with violaxanthin, by an unknown isomerase. Carotenoid cleavage is catalyzed by a family of 9-cis-epoxy/anti-sense dioxygenases (NCED) to form xanthoxin. Xanthoxin moves to the cytosol by an unknown mechanism and is converted into abscisic aldehyde by a short-chain dehydrogenase reductase (SDR), which is then oxidized into ABA by an abscisic aldehyde oxidase (AAO3). Sulfuration of AAO3 molybdenum co-factor by ABA3 is necessary for enzyme activity. The 8′-hydroxylation by CYP707A enzymes is thought to be the predominant pathway for ABA catabolism. Hydroxylated groups of ABA and its catabolites, phasic acid (PA), neoPA, and dihydroxyphasic acid (DPN) are targets for conjugation. ABA-glucose ester is formed by ABA glucosyltransferases (UGT) and hydrolyzed by glucosidases, including BG1 and BG2.

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Abscisic acid inactivation is a crucial mechanism to fine-tune ABA. Arc et al. ABA, ethylene, and NO crosstalk in seeds describe the implication of these processes in dormancy control.

Crosstalk in ABA conjugation or ABA-GE hydrolysis did not yet contrast, reports on functional analysis of mutant or overexpressing (Zhou et al., 2004; Okamoto et al., 2011). The conjugation of ABA reaction, and neoP A is then formed by spontaneous isomerization hydroxylation, 9.

In Arabidopsis, four homologous aldehyde oxidase (AAO) genes have been characterized, but only one of them, AAO1 encodes a protein that has proven activity on abscisic aldehyde (Seo et al., 2000). Activity of this molybdenum co-factor (Moco) by addition of a sulfur atom to the Moco center, which is catalyzed by a Moco sulfatase, which has been named ABA3 in Arabidopsis (Bittner et al., 2001; Xiong et al., 2001).

ABA CATABOLISM

Abscisic acid is synthesized from xanthoxin, by an enzyme belonging to short-chain dehydrogenase/reductase family, which is named SDR1 and is encoded by the ABA2 gene in Arabidopsis (Rosok et al., 2001; Cheng et al., 2002; Gonzalez-Guzman et al., 2002). The oxidation of the ABA-aldehyde is the final step of ABA biosynthesis, and is catalyzed by an abscisic aldehyde oxidase. In Arabidopsis, four homologous aldehyde oxidase (AAO) genes have been characterized, but only one of them, AAO1 encodes a protein that has proven activity on abscisic aldehyde (Seo et al., 2000). Activity of this molybdenum co-factor (Moco) by addition of a sulfur atom to the Moco center, which is catalyzed by a Moco sulfatase, which has been named ABA3 in Arabidopsis (Bittner et al., 2001; Xiong et al., 2001).

ABA SIGNALING PATHWAY

Genetic analyses suggest that PYR/PYL/RCAR (pyrabactin resistance1/PYR1-like/regulatory components of ABA receptor) ABA receptors, clade A type 2C protein phosphatases (PP2C) and group III sucrose non-fermenting1-related protein kinase2 SnRK2 subfamily are essential core components of the upstream signal transduction network that regulates ABA-responsive processes, including dormancy and germination (reviewed in Cutler et al., 2010). PYR/PYL/RCAR proteins constitute a 14-member family, belonging to the START-domain superfamily, also called Bet v L-fold (Ma et al., 2009; Park et al., 2009). ABA binding induces receptor conformation changes allowing the formation of a protein complex with PP2C and the inhibition of phosphatase activity (Figure 2). The clade A PP2C, including ABA INSENSITIVE1 (ABI1) and ABI2, also interact with three SnRK2s (SnRK2.2, SnRK2.3, and SnRK2.6) and, in the absence of ABA, dephosphorylate a serine residue whose phosphorylation is required for kinase activity (Soon et al., 2012). When ABA is present, PP2C binding to the receptor releases inhibition of SnRK2 activity, which can phosphorylate downstream targets.

Other types of receptors and a large number of genes, whose mutations alter ABA germination sensitivity, have been reported to participate in ABA signaling. In particular, regulatory mechanisms such as RNA processing, RNA/protein stability or chromatin remodeling have an important role. However, they will not be detailed here, since their role in ABA crosstalk with ethylene and NO in seeds still requires further investigation. In Arabidopsis seeds, extensive evidence including mutant phenotypes strongly supports a central role of the PYR/PYL, PP2C, SnRK2 complex in ABA signaling (reviewed in Cutler et al., 2010; Nambara et al., 2010). Germination of a pyr/pyl triple mutant is highly insensitive to ABA, as also observed for the snrk2.2 snrk2.3 snrk2.6 triple mutant (Fuji and Zhu, 2009; Gonzalez-Guzman et al., 2012). Moreover the snrk2 triple mutant exhibits loss of dormancy and even seed vivipary under high humidity conditions (Nakashima et al., 2009). Conversely, in accordance with PP2C being negative regulators of ABA signaling, germination in triple pp2c mutants was slower than in wild type and was inhibited by very low ABA concentrations (Rubio et al., 2009). In contrast, the gain-of-function mutations abf1-1 and abf2-1, which prevent PP2C binding to PYL/PYL/RCAR, lead to ABA insensitivity and reduced dormancy (Ma et al., 2009; Park et al., 2009).

Basic leucine zipper transcription (bZIP) factors of the ABA-RESPONSIVE ELEMENTS (ABRE) BINDING FACTOR/ABA RESPONSIVE ELEMENT BINDING FACTOR/ABA INSENSITIVE1 (ABF/AREB/ABIS) clade have been shown in different species to constitute SnRK2 downstream targets and regulate ABRE containing genes (Johnson et al., 2002; Kobayashi et al., 2005; Umezawa et al., 2009). Several family members are expressed at different seed stages and exhibit partially redundant or antagonistic functions, and ABF appears to have a predominant role in the regulation of a subset of late embryogenesis abundant (LEA) proteins during late seed development (Bensmihen et al., 2002;
FIGURE 2 | Interactions between ethylene, abscisic acid, and nitric oxide signaling pathways in the regulation of seed germination and dormancy. This scheme is based on genetic analyses, microarray data, and physiological studies on seed responsiveness to ABA, ethylene, or NO. ABA binding to PYR/PYL/RCAR receptor induces the formation of a protein complex with PP2C and the inhibition of phosphatase activity. In the absence of ABA, PP2C dephosphorylates SnRK2. When ABA is present, PP2C binding to the receptor releases inhibition of SnRK2 activity, which can phosphorylate downstream targets, including ABI3-related transcription factors. Interactions between ABI3 and ABI5 mediate transcriptional regulation of ABA-responsive genes.

Ethylene positively regulates its own biosynthesis, by acting on ACC synthesis catalyzed by ACS and subsequent conversion to ethylene by ACO. This last step is also subject to ABA inhibition. Ethylene is perceived by receptors (among which ETR1) located in the endoplasmic reticulum. Its binding leads to the deactivation of the receptors that become unable to recruit CTR1. Release of CTR1 inhibition allows EN2 to act as a positive regulator of ethylene signaling pathway. EN2 acts upstream of nuclear transcription factors, such as EIN3, EILs, and ERFs. Ethylene de novo regulates ABA accumulation by both inhibiting its synthesis and promoting its inactivation, and also negatively regulates ABA signaling. In germinating seeds, NO enhances ABA catabolism and may also negatively regulate ABA synthesis and perception. Moreover, NO promotes both ethylene synthesis and signaling pathway. ABA, abscisic acid; ABI3, ABA insensitive3; ABI5, ABA insensitive5; ACC, 1-aminocyclopropane 1-carboxylic acid; ACO, ACC oxidase; ACS, ACC synthase; CTR1, constitutive triple response 1; CYP707A, ABA-8′-hydroxylase; EIL, EIN3-like; EIN, ethylene insensitive; EREBP, ethylene-responsive element binding protein; ERF, ethylene response factor; Et, ethylene; ETR1, ethylene receptor1; NCED, 9-cis-epoxycarotenoid dioxygenase; NO, nitric oxide; PP2C, clade A type 2C protein phosphatases; PYR/PYL/RCAR, pyrabactin resistance1/PYR1-like/regulatory components of ABA receptor; SnRK2, group III sucrose non-fermenting-1-related protein kinase 2; a dashed line is used when regulatory targets are not precisely identified.

Finkelstein et al., 2005). abi5 mutation confers ABA-insensitive germination, but it does not impair seed dormancy, suggesting that other factors might be involved in dormancy induction (Finkelstein, 1994). Nevertheless, ABI5 has been clearly proven to act as a major inhibitor of germination processes in imbibed seeds, notably through its up-regulation by stress-induced ABA accumulation (Lopez-Molina et al., 2001; Piskurewicz et al., 2008). ABI3/VIVIPAROUS1 (VP1) interacts with ABI5 for the regulation of a number of ABA-responsive genes during seed maturation and germination (Lopez-Molina et al., 2002; Piskurewicz et al., 2008, 2009). However, in contrast to abi5, abi3 mutants do not only exhibit ABA-resistant germination, but also other phenotypes including desiccation intolerance and precocious germination. They share these maturation defects with fusca3 (fus3) and leafy cotyledons2 (lec2) mutants, which, like abi3, carry mutations in B3 transcription factor family genes. These factors form a complex network regulating the expression of reserve storage and LEA genes by their binding to RT motif, and it has been suggested that the lack of dormancy induction in mutants might indirectly result from early seed developmental defects (Gutierrez et al., 2007;
Abscisic acid is produced in all seed tissues (testa, endosperm, and embryo), as suggested by the spatiotemporal expression of ABA biosynthesis genes (Lefebvre et al., 2006; Frey et al., 2012). However, ABA accumulated in seeds also originates from synthesis in vegetative tissues and transport to the seed (Frey et al., 2004; Kanno et al., 2010). Among the five Arabidopsis NCED genes, NCED6 and NCED9 exhibit the highest expression levels in developing seeds and show distinctive expression patterns. NCED6 is specifically expressed in endosperm, whereas NCED9 expression is detected in tests and embryos. Furthermore, mutant analysis indicated that ABA production in both embryo and endosperm contributes to dormancy induction (Lefebvre et al., 2006; Frey et al., 2012). In barley (Hordeum vulgare), the two HvNCED genes also exhibit differential spatiotemporal patterns of expression. In contrast to HvNCED2, HvNCED1 transcript levels vary depending on environmental conditions during grain development and modulation of ABA accumulation at late maturation stages (Chono et al., 2006). ABA inactivation by CYP707A7 during seed maturation also regulates dry seed ABA levels and dormancy depth, as deduced from cyp707a mutant analysis (Okamoto et al., 2006). Moreover, the seed dormancy increase under cold-maturation conditions is not only correlated with DOG1 up-regulation, as mentioned above, but also with CYP707A2 down-regulation (Kendall et al., 2011).

Upon Imbibition, dormancy maintenance and germination are also regulated by both ABA catabolism and neo-synthesis. A decrease in ABA levels at imbibition has been observed in both dormant and non-dormant seeds in several species; nevertheless, dormant seeds maintain higher ABA levels and in accordance exhibit lower CYP707A transcript levels, as shown in Arabidopsis and barley (Millar et al., 2006). Barley HvABA5OH transcripts were detected in coloro rhiza cells near the root apex and Arabidopsis CYP707A2 in endodermis and microplar endosperm next to the radicle (Millar et al., 2006; Okamoto et al., 2006). Moreover, it is well documented in several species that unfavorable light or temperature conditions prevent germination by coordinated regulation of NCED and CYP707A gene expression (Soo et al., 2006; Gübler et al., 2008; Toh et al., 2008; Leymarie et al., 2009, Argyris et al., 2011). Furthermore, dormancy cycling by seasonal variation of soil temperature has been recently linked to the regulation of ABA metabolism and signaling genes. Deep dormancy in winter is correlated with increased ABA levels and NCED6 expression, together with that of DOG1 and MOTHER OF FLOWERING LOCUS T (MFT). MFT encodes a phosphatidylethanolamine-binding protein, which is regulated by AB3 and AB5, and feedback regulates ABA signaling by repressing AB3 (Xie et al., 2010). In contrast, shallow dormancy in summer is correlated with a reduction in ABA levels and an up-regulation of CYP707A2 and AB2, which negatively regulates ABA signaling (Footitt et al., 2011).
In Arabidopsis, despite endosperm consists in a single cell layer in mature seeds, convergent evidence demonstrated its major role in ABA control of seed dormancy and germination. Firstly, whereas the removal of whole seed coat (endosperm and testa) releases mechanical constraints and allows development of embryos dissected from dormant seeds, the preservation of the endosperm after testa removal maintains dormancy (Bethke et al., 2007a). Secondly, using a "seed coat budding assay," Lee et al. (2010) showed that diffusion of endospermic ABA from dormant seed envelopes could prevent growth of non-dormant embryos, including those of ABA-deficient aba2 mutants. In isolated embryos, translocated ABA was able to induce ABA protein accumulation, whose level was correlated with dormancy maintenance. In addition, in a previous study, ABA transcript was detected in the embryo and the microcylindrical endosperm of imbibed seeds, suggesting a role in the inhibition of both embryo growth and endosperm rupture by ABA (Penfield et al., 2006). The tissue-specificity of ABA sensitivity is also likely regulated by the spatiotemporal expression of upstream ABA signaling components, as suggested by the differential expression of PYR/PYL genes in embryo and/or endosperm of imbibed seeds (González-Guzmán et al., 2012).

ETHELYNE BIOSYNTHESIS, SIGNALING, AND ABA CROSS-TALK IN SEED GERMINATION

ETHELYNE BIOSYNTHESIS AND SIGNALING

Ethylene biosynthesis pathway in germinating seeds is the same as that described in other plant organs (Figure 3); in which S-adenosyl-methionine (SAM) and ACC, the by-product of S-adenosyl-L-methionine methylthioadenosine-lyase (ACS), the by-product being S-methylthioadenosine (MTA) is recycled back to methionine through the Yang cycle (Yang and Hoffman, 1984; Wang et al., 2002; Rzewuski and Sauter, 2008). The first step of ethylene biosynthesis is the conversion of S-AdoMet to ACC catalyzed by ACC synthase (S-adenosyl-L-methionine methylthioadenosine-lyase, ACS), the by-product being S-methylthioadenosine (MTA), which is recycled back to methionine through the Yang cycle (Yang and Hoffman, 1984; Kende, 1993). The second step corresponds to the oxidation of ACC by ACC oxidase (ACO) to form ethylene, CO2, and hydrogen cyanide (HNC). Cyanide produced during this final step of ethylene synthesis is detoxified to β-cyanoalanine by β-cyanoalanine synthase (β-CAS). Both ACS and ACO are encoded by a multigene family. In Arabidopsis, nine active ACS genes have been characterized (Yamagami et al., 2003; Wang et al., 2005; Dong et al., 2007a). Most of them can be induced by cycloheximide (ACS2, ACS4, ACS6), wounding (ACS2, ACS4), and ethylene treatment (ACS2, ACS6; reviewed in Wang et al., 2002). In addition, ACS6 can also be induced by cyanide (Smith and Arntzen, 2000) or ozone treatment (Vahala et al., 1998). ACS activity controls in vivo ethylene production and has fundamental contribution during seed germination (Martí and Martínez-Vázquez, 2008; Linkies and Leubner-Metzger, 2012).

In Arabidopsis, five membrane-localized receptors have been identified: ethylene resistant 1 (ETR1), ETR2, ethylene response sensor 1 (ERS1), ERS2, and ethylene insensitive 4 (EIN4; Figure 2). Among them, ETR1 and ERS1 contain three transmembrane domains in the N-terminus and a histidine kinase domain in the C-terminus (Kendrick and Chang, 2008). Binding of C2H4 to the receptors occurs in the hydrophobic N-terminal part of the receptor dimer and requires a copper co-factor (Hall et al., 2007). The signaling pathway of C2H4 is controlled by CTR1 (constitutive triple response 1), a serine-threonine protein kinase that acts as a negative regulator, downstream of the receptor and upstream of EIN2. C2H4 binding results in the inactivation of the receptor–CTR1 complex, and in turn allows activation of a kinase cascade controlling EIN2 and its transcription factors in the nucleus such as EIN3, EIL1, ethylene-responsive element binding proteins (EREBPs)/ethylene-responsive factors (ERFs), which activate the transcription of ethylene-responsive genes (Wang et al., 2002; Liu et al., 2004; Rzewuski and Sauter, 2008; Yoo et al., 2008; Stepanova and Alonso, 2009). EIN2 works downstream of CTR1 and upstream of EIN3 (Alonso et al., 1999). Recently, Qiao et al. (2009) demonstrated that EIN2 protein level is regulated through its degradation by the proteasome in the presence of the hormone via 2 F-box proteins ETP1 and ETP2, in the presence of C2H4, ETP1 and ETP2 levels are low, thus increasing EIN2 protein level.

SEED RESPONSIVENESS TO EXOGENOUS ETHYLENE

The influence of ethylene on seed germination is well documented (Corbineau and Côme, 1995; Kepczynski and Kepczynska, 1997; Matilla, 2000; Matilla and Martínez-Vázquez, 2008). Ethylene, ethylphor (an ethylene-releasing compound), or ACC (the precursor of ethylene) stimulate seed germination in numerous
species, among which several parasitic plants such as Orobanche ramosa (Chan et al., 1979) and some Striga species (Eigley and Dale, 1970; Behavvi and Epler, 1986). Application of ethylene promotes germination of either primary dormant or secondary dormant seeds (Table 1). It breaks seed coat-imposed dormancy in cocklebur (Xanthium pennsylvanicum; Katoh and Esashi, 1975; Esashi et al., 1979), subterranean clover (Trifolium subterraneum; Esashi and Leopold, 1969), Rumex crispus (Taylorson, 1979) and Arabidopsis (Srivastava et al., 2003), and embryo dormancy in apple (Malus domestica; Kepczynski et al., 1977; Simka and Gladon, 1984), sunflower (Helianthus annuus; Corbineau et al., 1997, Matilla, 2000), and beechnut (Fagus sylvatica; Calvo et al., 2004a). It can also overcome thermodynamic dormancy in lettuce (Abelos, 1986) or secondary dormancy in sunflower (Corbineau et al., 1988), Amaranthus caudatus (Kepczynski et al., 1996a), and Amaranthus paniculatus (Kepczynski and Kepczynska, 1993). Likewise, it stimulates germination of non-dormant seeds placed in non-optimal conditions (Kepczynski and Kepczynska, 1997, Matilla, 2000).

For example, it can overcome the inhibition of germination imposed by high temperatures (Abelos, 1986; Gallardo et al., 1991) or osmotic agents (Negm and Smith, 1978; Kepczynski and Karssen, 1985), and alleviates the salinity effect in numerous halophytes (Khan et al., 2009).

The stimulatory effect of exogenous ethylene increases with hormone concentration, and the efficient concentrations range from 0.1 to 200 \( \mu \text{L} \cdot \text{L}^{-1} \), depending on species and depth of their dormancy. Ethylene at 1.25 \( \mu \text{L} \cdot \text{L}^{-1} \) allows 100% germination of dormant sunflower seeds required 12.5 \( \mu \text{L} \cdot \text{L}^{-1} \) to fully germinate at 15\(^\circ\)C. Breaking of dormancy during chilling of apple seeds, or during dry storage of sunflower achenes, results in an increasing sensitivity to ethylene (Simka, 1989; Corbineau and Côme, 2003).

In Xylostea subserrata, non-dormant seeds are at least 50-fold more sensitive to ethylene than freshly harvested dormant ones (Ribeiro and Barros, 2006). Improvement of dormant seed germination does not require a continuous application of ethylene, a short treatment in the presence of this compound is sufficient to improve germination of dormant seeds in various species (Schönbeck and Eigley, 1981; Corbineau and Côme, 2003; Kepczynski et al., 2003). Seed responsiveness to ethylene decreases during prolonged pre-incubation under conditions favoring the maintenance of dormancy, probably due to an induction of a secondary dormancy (Speir et al., 1974, Esashi et al., 1979; Jones and Hall, 1984; Corbineau and Côme, 2003).

Table 1. Species whose seed dormancy is broken by ethylene or ethaphon, an ethylene-releasing compound, or 1-aminoacyclopropane-1-carboxylic acid (ACC).
Ethylene production depends on both ACS activity that modulates ACC content, and the activity of ACO, the key enzyme that converts ACC into ethylene. Evolution of ethylene production during germination is associated with an increase in ACO activity, as well as a progressive accumulation of ACS and ACO transcripts, with generally a sharp increase during endosperm rupture or/and radicle protrusion (Gomez-Jimenez et al., 1998, Matilla and Matilla-Vazquez, 2008; Linkies et al., 2009; Iglesias-Fernandez and Matilla, 2010; Linkies and Leubner-Metzger, 2012). In *Sinapis officinalis*, SoACS7 transcript accumulation is very low during seed imbition, a more notable expression being detected when endosperm rupture reached 50–100%, whereas SoACO2 expression is detected at early stages during seed imbition, and then rises during the germination process (Iglesias-Fernandez and Matilla, 2010). Similarly, expression of *PaACO1* in *Pisum sativum* (Petruszelli et al., 2003) and *BrACO1* in turnip (*Brassica rapa*; Rodriguez-Gacio et al., 2004) is maximal at radicle emergence. In two Brassicaceae species, *Arabidopsis* and *Lepidium sativum*, *ACO* and *ACO2* have been demonstrated to be the major ACOs involved in ethylene synthesis in seeds (Linkies et al., 2009; Linkies and Leubner-Metzger, 2012). In *Lepidium sativum*, the correlation between *ACO1* and *ACO2* transcript accumulation with in vivo ACO activity enzyme suggests that *ACO* is regulated at the transcriptional level during germination.

Ethylene has been shown to regulate its own synthesis by inducing ACO transcription (Lin et al., 2009). It is required for the stimulation of *ACO* gene expression in pea (Petruszelli et al., 2000, 2003), beeschnut (Calvo et al., 2004b), and turnip (Puga-Hermida et al., 2003). In contrast, expression of *SoACS7* in *Sinapis officinalis* and *PaACO1* in pea is not affected (Petruszelli et al., 2000, 2003; Iglesias-Fernandez and Matilla, 2010).

Induction of thermodynamics is often associated with a reduced ethylene production, which may result in chickpea (*Cicer arietinum*) from a greater ACC-malonate transferase activity and an S-AdoMet channeling toward the polyamine pathway, thus reducing ethylene precursor availability (Martínez-Reina et al., 1996), or from ACO activity inhibition, as observed in chickpea and sunflower (Corbineau et al., 1988; Gallardo et al., 1991). Incubation at high temperature (35°C) of lettuce seeds induces a reduction in ethylene production (Prusinski and Khan, 1990), associated with a complete repression of *ACO* transcripts, with generally a sharp increase during germination and dormancy (reviewed in Kepczynski and Kepczynska, 1997; Matilla and Matilla-Vazquez, 2008). Cyanide treatment, which breaks embryo dormancy in apple and sunflower, stimulates ethylene production (Oracz et al., 2008; Gniazdowska et al., 2010). In apple 5-day-old seedlings, it increases ACS and ACO activities (Bogatek et al., 2004), whereas in sunflower it reduces in vivo ACC-dependent ethylene production (i.e., in vivo ACO activity) and HaACO and HaACO expression (Oracz et al., 2008). However, in *Arabidopsis*, cold stratification down-regulates the expression of *ACO* genes in dormant seeds. Analysis of mutant lines altered in ethylene biosynthesis and signaling pathway demonstrated the involvement of ethylene in regulating seed germination. Mutations in **ETHYLENE RESISTANT1 (ETR1)** and **ETHYLENE INSENSITIVE2 (EIN2)** genes result in poor germination and deeper dormancy compared to wild type, in contrast **constitutive triple response1 (ctr1)** seeds germinate slightly faster (Bleecker et al., 1988; Leubner-Metzger et al., 1996; Beaudoin et al., 2000; Dohle and Reddy, 2010). ERF genes might also play a key (pivotal) role in ethylene responsiveness and germination regulation (Leubner-Metzger et al., 1996; Pirrello et al., 2006). In beeschnut, Jimenez et al. (2005) demonstrated that the expression of *FaERF1*, a transcription factor involved in C2H4 signaling and sharing high homology with *Arabidopsis* *ERF* genes, increases during dormancy release in the presence of ethephon or after chilling. In sunflower, *ERF1* expression is fivefold higher in non-dormant than in dormant embryos, and also markedly stimulated by gaseous HCN, which breaks dormancy (Oracz et al., 2008). Beeschnut *FaERF1* is almost undetectable in dormant seeds incubated under high temperature conditions that maintain dormancy, or in the presence of germination inhibitors, either ABA or AOA, an inhibitor of ethylene biosynthesis, but increases during moist chilling that progressively breaks dormancy (Mortensen et al., 2004; Jimenez et al., 2005). In tomato (*Solanum lycopersicon*), *SERF2* transcript accumulation is higher in germinating seeds than in non-germinating ones, and its overexpression in transgenic lines results in premature seed germination (Pirrello et al., 2006). Interestingly, in lettuce seeds, expression of genes involved in ethylene signaling (*CTB*, *EIN2*, and *ETR1*) is less affected by high temperature than that of biosynthesis genes (**ACS** and **ACO**; Argyris et al., 2008).

**CROSSTALK BETWEEN ETHYLENE AND ABA**

The antagonistic interaction between ABA and C2H4 during germination was demonstrated in numerous species (Leubner-Metzger et al., 1996; Beaudoin et al., 2000; Ghassemi et al., 2001; Kucera et al., 2005; Matilla and Matilla-Vazquez, 2008; Studies using inhibitors of ACS activity (AVG: aminoethoxyvinylglycine; AOA: amino-oxyacetic acid), ACO activity (CoC8; α-ABO: α-aminoisobutyric acid), or ethylene action (2,5-NBD: 2,5-norbornadiene; STS: silver thiosulfate) demonstrated that ethylene evolved by seeds plays a promotive role in germination and dormancy breakage (Kepczynski et al., 1977, 2003; Sinula and Gladon, 1989; Corbineau et al., 1990; Esashi, 1991; Longan and Stewart, 1992; Gallardo et al., 1994; Hermann et al., 2007). Conversely, application of exogenous ACC stimulates germination of various ethylene-sensitive seeds such as lettuce (Fu and Yang, 1983), sunflower (Corbineau et al., 1990), cocklebur (Sato et al., 1984), *Amaranthus caudatus* (Kepczynski, 1986) and *Amaranthus retroflexus* (Kepczynski et al., 1996b), chickpea (Gallardo et al., 1994), sugar beet (*Beta vulgaris *, Hermann et al., 2007). Thermodynamics in lettuce, *Amaranthus caudatus* and chickpea is also reversed by exogenous ACC (Gallardo et al., 1996; Kepczynski et al., 2003). This stimulatory effect of ACC suggests that dormancy might be related to low C2H4 production due to insufficient levels of endogenous ACC, i.e., low ABA activity.

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ABA sensitivity, while increased ethylene sensitivity in *ctr1* (Wang et al., 2007). ABA-GE levels are reduced in *Arc* et al. ABA, ethylene, and NO crosstalk in seeds (Kucera et al., 2005; Linkies et al., 2009; Subbiah and Reddy, 2010). Mutations in *eto1*, for example, enhance the ABA insensitivity and expressivity of *eto1* decrease it (Beaudoin et al., 2000). No significant difference in ABA sensitivity is observed in *ein1*, *ein2*, *ein5*, and *ein7* (Subbiah and Reddy, 2010).

In Arabidopsis and Lepidium sativum, ethylene counteracts the inhibitory effects of ABA on endosperm cap weakening and endosperm rupture (Linkies et al., 2009). ABA also increases the ethylene requirement to release primary and secondary dorman-
cies (Kepczynski and Kepczynska, 1997; Corbíneu and Côme, 2003; Kepczynski et al., 2003). Inhibition of germination by ABA is associated with a reduction in ethylene production (Kepczynski and Kepczynska, 1997; Matilla, 2000). ABA clearly inhibits in vivo ACO activity, and this inhibition correlates with a decreased accumulation of ACO transcripts (Bailly et al., 1992; Petruzelli et al., 2008; Linkies et al., 2009). In Arabidopsis, the accumu-
lation of ACO1 transcripts in both the embryo and endosperm during germination is inhibited by ABA, and the high levels of ACO transcripts in ABA-insensitive mutants suggest the regu-
lation of ACO expression by ABA (Penfold et al., 2006; Carrera et al., 2008; Linkies et al., 2009). In the embryo, ACO2 transcript accumulation is also inhibited by ABA (Penfold et al., 2006). In Lepidium sativum, inhibition of both ACO1 and ACO2 by ABA is restricted to the endosperm cap (Linkies et al., 2009). In accord-
dance, microarray analysis in Arabidopsis aba2 mutant detected an up-regulation of ACO transcript accumulation (Cheng et al., 2009). Moreover, inhibition of shoot growth in tomato ABA-
deficient mutants, *flaca* and *notabilis*, and in Arabidopsis aba2 results from increased ethylene production (Sharp et al., 2000; Le Noble et al., 2004). In contrast to pea, chick pea, Lepidium sativum, and Arabidopsis, there is an ABA-mediated up-regulation of ACC accumulation and ACO expression in sugar beet seeds (Hermann et al., 2007).

**Effect of ethylene on ABA metabolism and signaling**

Treatment with exogenous ethylene or ACC does not affect ABA content nor expression of genes involved in ABA biosynthesis in Lepidium sativum (Linkies et al., 2009) and sugar beet (Hermann et al., 2007). Nevertheless, seeds of Arabidopsis ethylene-insensitive mutants, *etr1* and *ein2*, exhibit higher ABA content than wild type and consistently germinate more slowly (Kende et al., 1998; Beaudoin et al., 2000; Ghaseeman et al., 2008; Chiwocha et al., 2005; Wang et al., 2007). ABA-GE levels are reduced in *etr1-2* seeds; increased ABA accumulation might therefore be attributed to a decrease in ABA conjugation (Chiwocha et al., 2005). However, ethylene may also regulate other enzymatic steps, since a microar-
ray analysis reported NCE30 up-regulation in *ein2* and CYP707A2 down-regulation in *etr1-2* (Cheng et al., 2009). High ABA levels in *ein2* were also associated with an up-regulation of ABA1 (Wang et al., 2007), which was, however, not detected on microarrays (Cheng et al., 2009).

Several reports suggest that, during germination, ethylene not only acts on ABA metabolism to reduce ABA levels, but also negatively regulates ABA signaling (Gazzarrini and McCourt, 2001; Kucera et al., 2005). Indeed, mutations that reduce ethy-
lene sensitivity (e.g., *etr1*, *ein2*, and *ein6*) result in an increase in ABA sensitivity, while increased ethylene sensitivity in *ctr1* and *cto1* reduces ABA sensitivity (Beaudoin et al., 2000; Ghaseeman et al., 2008; Brady and McCourt, 2003; Chiwocha et al., 2005; Kucera et al., 2005; Linkies et al., 2009; Subbiah and Reddy, 2010). Mutations in *CTR1*, for example, enhance the ABA insensitivity of ab1-1 seeds, when C2H4-insensitive mutants like *ein2* reduce it

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Nitric oxide synthase-like activity

In animals, NO biosynthesis is mainly catalyzed by three isoforms of NO synthase (NOS, Alexander et al., 2001). These enzymes metabolize l-arginine (L-Arg) into l-citrulline and NO via the following reaction:

\[ \text{L-Arg} + \text{NAD(P)}\text{H} + \text{O}_2 \rightarrow \text{l-citrulline} + \text{NAD(P)}^+ + \text{H}_2\text{O} + \text{NO} \]

To date, despite the identification of a green alga NOS (Forrester et al., 2000), the search for a NOS homolog enzyme in higher plants only encountered failure, although biochemical assays highlight the existence of a NOS-like activity in several plant tissues and organs (Frohlich and Durner, 2011). Moreover, exogenous application of NOS inhibitors (structural analogs of L-Arg such as L-NAME, N-nitro-l-arginine methyl ester) significantly reduced NO release under diverse conditions in several plant species (Crawford, 2006). Using these approaches, a NOS-like activity was detected in sorghum (Sorghum bicolor) and soybean (Glycine max) imbibed seeds (Simontacchi et al., 2007). Nonetheless, the liability of such proofs is now debated in light of the discovery of other L-Arg-dependent NO synthesis pathways (Tun et al., 2006). Moreover, the recent finding that L-NAME can account for the nitric oxide synthase-like (NOS-like) activity in plants only encountered failure, although biochemical assay highlights NO release under diverse conditions in several plant species (Crawford et al., 2007).

Nitric oxide synthase

In Arabidopsis, NR involvement in NO-mediated signal transduction pathways (Bright et al., 2006; Neill et al., 2008; Gupta et al., 2011). In seeds, the NO-mediated positive effect of NR (NR-NIR) can drastically increase under certain conditions such as oxygen deprivation (Rockel et al., 2002). Overall, conditions leading to NR-mediated nitrite production exceeding the rate of nitrite removal can lead to a substantial increase in NO production by NR. Both the nitrate and nitrite reductase activities of NR are tightly controlled by post-translational modifications (PTM; Lillo et al., 2004; Park et al., 2011; Wang et al., 2011). In Arabidopsis, NR and NR-NIR activities are stimulated by sumoylation mediated by the E3 SUMO ligase ASIZ1 (Park et al., 2011). Furthermore, the H$_2$O$_2$-induction of NO biosynthesis in Arabidopsis roots was recently proposed to depend on mitogen-activated protein kinase 6 (MPK6)-mediated phosphorylation of one of the NR isoforms (Sei 627 in Arabidopsis NIA2; Wang et al., 2010, 2011). Moreover, NO was reported to inhibit NR activity in wheat leaves (Rosales et al., 2011). In Arabidopsis seedlings, GA may also negatively regulate light-induced NR activity at post-translational level (Zhang et al., 2011).

Distinct studies reported an implication of NR in NO-mediated signal transduction pathways (Bright et al., 2006; Neill et al., 2008; Gupta et al., 2011). In seeds, the NO-mediated positive effect of NO$_3^-$ and NO$_2^-$ on dormancy release supports an involvement of nitrite-dependent reductive pathways in NO biosynthesis, possibly via NR-Nir activity or at least depending on NR activity in the case of exogenous NO$_2^-$ (Bethke et al., 2006a). Accordingly, NR activity was detected concomitantly with a NOS-like activity in soybean and sorghum embryonic axes, both enzymatic activities appeared to parallel the accumulation of NO upon seed imbibition (Simontacchi et al., 2007).

In Arabidopsis, NR is encoded by two homologous genes, NIA1 and NIA2 (Wilkinson and Crawford, 1991). The relative contribution of these two isoforms to NO production was suggested to differ with a possible predominant involvement of NIA1 in NO production (Bauböck, 2004). Despite NO has been demonstrated to break seed dormancy (Bethke et al., 2006b; Liu et al., 2009), NR involvement in Arabidopsis seed germination remains unclear. Two distinct research groups assessed the germination characteristics of the nia1 nia2 double mutant (also named G4-3), obtained by Wilkinson and Crawford (1993). In the first study, G4-3 seeds...
were found to be less dormant than wild type seeds (Alboresi et al., 2005), but more dormant in the second (Lozano-Juste and Leon, 2010). Differences in culture environments of mother plants, germination conditions or duration of seed storage may explain these contrasted results (Clerks et al., 2004; Matakiadis et al., 2009).

**Polymamines and hydroxylamines**

Upon exogenous application of the polyamines, spermine (spm) and spermidine (spd), a rapid NO production from Arabidopsis seedlings has been observed under aerobic conditions (Tom et al., 2006). In plants, the tri-amine Spd and tetra-amine Spm are formed by successive additions of aminopropyl groups (resulting from S-AdoMet decarboxylation) to the diamine putrescine (Put, reviewed in Wimallasinha et al., 2011a). Put can be synthesized either from L-Arg (by L-Arg decarboxylase) or from L-ornithine (by ornithine decarboxylase). However, as Arabidopsis lacks ornithine decarboxylase activity, polyamines are exclusively produced from L-Arg (Hanfrey et al., 2001). Thus, NO biosynthesis from polyamines can be considered as a L-Arg-dependent pathway in Arabidopsis.

Plant cells are also able to produce NO through hydroxylamine oxidation and this reaction is promoted by reactive oxygen species (ROS) accumulation (Rümer et al., 2009). Thus, NO might be oxidized and this reaction is promoted by reactive oxygen species. Its implication has been proposed in several physiological processes in roots (Stöhr and Stremlau, 2006), but has not been so far investigated in seeds.

**Mitochondrial respiration**

Depending on the oxygen availability, several heme proteins can either act as NO scavengers or NO producers. In hypoxic mitochondria, deoxyhemeproteins can catalyze a NR-independent nitrite reduction into NO using electrons from the electron transport chain (Plantich et al., 2005). The re-oxidation of NO into nitrite can then occur either non-enzymatically inside the mitochondria, or in the cytosol, through the nicotinamide adenine dinucleotide phosphate (NADPH)-dependent dioxygenase activity of class-I non-symbiotic hemoglobin (nsHb1) that metabolizes NO into nitrate, which is subsequently reduced into nitrite by NR (Igamberdiev and Hill, 2004; Perazzolli et al., 2004). These reactions constitute the so-called hemogloblin–NO cycle (displayed in red in Figure 4, (Igamberdiev et al., 2003). nsHb1 proteins participate in NO scavenging, thereby playing an essential role in NO homeostasis. Accordingly, modulation of nsHb1 expression in plants was shown to directly impact NO levels at distinct developmental stages including in seeds (Hebelstrup and Jensen, 2008; Thiel et al., 2011) and in diverse environmental conditions (Dordas, 2009; Cantrel et al., 2011).

The very active mitochondrial respiration upon seed imbibition may result in an oxygen consumption exceeding the atmospheric diffusion, thus leading to localized hypoxia in germinating seeds (Benamar et al., 2008). In such conditions, nitrite-dependent NO production may occur in mitochondria and modulate respiration through reversible NO-mediated inhibition of cytochrome c oxidase (COX), thereby regulating oxygen consumption to avoid anoxia (Benamar et al., 2008). Therefore, this nitrite-dependent NO biosynthesis in mitochondria may be of significant importance in germinating seeds. However, its possible role in NO-mediated dormancy release has not yet been established.

Overall, current evidence supports the co-existence of several distinct NO biosynthesis pathways in seeds. Their relative contribution is probably highly dependent on both oxygen and ROS levels that may change along the time-course of imbibition. Further investigations will be required to elucidate the regulation of NO accumulation during seed imbibition.

**S-nitrosoglutathione: a reversible “storage” pool of nitric oxide?**

As for plant hormones, any mechanism directly influencing NO levels besides biosynthesis pathways may have a pivotal role in the regulation of NO signaling. In particular, since NO can react with reduced glutathione (GSH) to form S-nitroso glutathione (GSNO), GSNO has been proposed to constitute a storage and transport form for NO in plants and seeds (Sakamoto et al., 2002). Such modulation of NO storage pool would have a significant impact on NO levels. GSNO can further be metabolized by the GSNO reductase (GSNOR). Accordingly, gnam mutants have multiple phenotypes suggesting GSNOR involvement in several growth and developmental processes including seed germination (Lee et al., 2008; Holzmeister et al., 2011; Kwon et al., 2012).

**Molecular Targets of Nitric Oxide in Seeds**

Due to its chemical nature, NO is highly reactive and can interact with diverse molecules in plant cells. A number of NO-regulated genes have been identified in plants (Besson-Bard et al., 2009). These genes encode proteins involved in a wide range of functions from signal transduction to stress responses. However, the main challenge remains to pinpoint the direct molecular targets of NO, which are still poorly documented in plants. However, it is generally assumed that proteins constitute direct relevant NO targets.
Besides its capacity to bind to transition metals of metalloproteins, NO can cause protein PTM, such as cysteine S-nitrosylation or tyrosine nitration (Moreau et al., 2010). These modifications remain poorly characterized in plants and particularly in seeds. However, as discussed below, there is strong experimental evidence indicating that NO signaling in seeds could principally rely on PTM of specific proteins (Delledonne, 2005).

Many S-nitrosylated proteins identified in plants are implicated in various metabolic processes (Lindermayr et al., 2005; Abat et al., 2008; Romero-Puertas et al., 2008; Tanou et al., 2009; Palmieri et al., 2010). In dry Arabidopsis seeds, a β-subunit of the mitochondrial ATP synthase complex was found to be S-nitrosylated, suggesting that NO could participate in the regulation of the seed energy status (Arc et al., 2011). In wheat seeds, a parallel increase in NO and protein S-nitrosylation was reported during seminiferous germination (Sen, 2010). At least 13 modified proteins were detected, but not identified. In recalcitrant Antiaris toxicaria seeds, desiccation impedes subsequent germination by enhancing H2O2 accumulation (Bai et al., 2011). This stress is associated with an increased carbonylation and a reduced S-nitrosylation of the antioxidant enzymes of the ascorbate-GSH pathway. Conversely, NO pre-treatments promote germination of desiccated seeds through PTM pattern reversion that enhances antioxidant enzyme activities (Bai et al., 2011). The balance between carbonylation and S-nitrosylation of these proteins was proposed to act as molecular switch tuning their activity according to the redox environment (Lounish et al., 2013).

**CROSSTALK BETWEEN NO, ETHYLENE, AND ABA**

In stomatic guard cells, ABA-induced stomatal closure is mediated by the successive accumulation of ROS and NO, acting as secondary messengers in ABA signaling (Neill et al., 2008). Even though similar actors are present in seeds, the picture is quite different, as both ROS and NO counteract ABA-inhibition of seed dormancy release and germination (Bethke et al., 2006b; Liu et al., 2009). Indeed, during the first stage of seed imbibition, a rapid accumulation of NO, possibly at the endosperm layer, was shown to reduce ethylene production in apple seeds (Siska and Lewandowska, 1991). Accordingly, an antagonism may exist between a positive polyamine effect mediated by NO and a negative effect due to a competition with ethylene biosynthesis for S-AdoMet pool (Lindermayr et al., 2006). S-AdoMet is the precursor of ethylene and polyamines, thus the polyamine-dependent NO biosynthesis. Consistently, NO and ethylene accumulation are negatively correlated in ripe fruits (Manjunatha et al., 2012). In addition, exogenous Spm was shown to reduce ethylene production in apple seeds (Siniska and Lewandowska, 1991). Accordingly, an antagonism may exist between a positive polyamine effect mediated by NO and a negative effect due to a competition with ethylene biosynthesis for S-AdoMet. Furthermore, a copper amine oxidase (CuAO1) involved in polyamine catabolism has also been shown to regulate NO biosynthesis and participate to ABA signaling (Wimalasekera et al., 2011b).

As mentioned above, in Brassicaceae species, ethylene positively regulates seed germination by stimulating the weakening and rupture of seed testa and endosperm by countenacting the inhibitory action of ABA on radicle protrusion (Linkies et al., 2009). In apple embryos, inhibition of ethylene biosynthesis prevents the growth of isolated embryos unaffected by NO donors or scavengers, the endosperm layer might be the primary site of NO synthesis and action in seeds, and in accordance was shown to perceive and respond to NO (Bethke et al., 2007a). Besides its effect on the hormonal balance, it has been speculated that NO may accelerate flux through the pentose phosphate pathway by indirectly increasing the oxidation of NADPH (Hendricks and Taylorson, 1974; Bethke et al., 2007b). An increase in glucose catabolism via this pathway may in turn promote dormancy release (Roberts and Smith, 1977).

Several lines of evidence suggest that NO crosstalk with ABA and ethylene may involve protein modifications. Among the proteins recently identified as candidates for a regulation by tyrosine nitration in Arabidopsis seedlings (Lozano-Jusfe et al., 2011), at least two may be involved in the interplay between ABA and NO in seeds. The first one is the Moco sulfatase ABA3 that catalyzes the conversion from the de-sulf to the sulfo form of the Moco (Wollers et al., 2008). The de-sulf form of Moco (also called the “oxo” form) is the co-factor of NR, involved in nitrite and NO generation in plants while the sulfo form is the co-factor of the aldehyde oxidase required for the last step of ABA synthesis (Mendel, 2007). If proven, modulation of ABA3 activity by nitration could affect the equilibrium between ABA and NO production in plants. The second protein is the E3 SUMO ligase ASIZ1 recently demonstrated to stimulate NR and NR- NR activities, and negatively regulate ABA signaling by AB15 sumoylation (Mitra et al., 2009; Park et al., 2011). Thus, such modifications could have an important impact in seeds. Similarly, PTM contribution in the NO regulation of ethylene action has been also reported. In Arabidopsis, the up-accumulation of NO under hypoxia stimulates ethylene biosynthesis, possibly through PTM of key enzymes such as ACS and ACO by S-nitrosylation (Hebelstrup et al., 2012). In contrast, ethylene biosynthesis can be reversibly inhibited by NO through S-nitrosylation of methionine adenosyltransferase (MAT), leading to the reduction of the S-AdoMet pool (Lozano-Jusfe et al., 2006).

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promotion of dormancy release and germination by NO donors (Giniaczewska et al., 2007). Dormancy breaking of apple seeds by NO induces a transient production of ROS, stimulating ethylene accumulation thanks to an increase in both ACS and ACO activity (Giniaczewska et al., 2010). NO may also act on ethylene signal- ing since EREBP was described as a class of transcription factors induced by NO (Peroni et al., 2004). Moreover during tobacco seed germination, EREBP-3 that is transiently induced just before endosperm rupture is stimulated by ethylene and inhibited by ABA (Leubner-Metzger et al., 1999). Therefore, a synergic link seems to exist, at different levels, between NO and ethylene during seed germination, that counteracts ABA action.

CONCLUSION

Significant advances have been recently obtained in the under- standing of the ABA and ethylene metabolism and signaling pathways. In contrast, current knowledge on NO biosynthe- sis, signaling and action is far too incomplete, especially in seeds, and would require further investigation. Future research efforts should also lead to the identification of downstream target- genes of signaling components, in order to fully understand how ABA is able to induce and maintain dormancy, or ethylene to stimulate germination. Moreover unraveling the role of post- translational mechanisms will be particularly crucial to developing a deeper understanding of hormonal pathways and deciphering NO regulatory network.

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