Nitric Oxide as a Mediator for Defense Responses

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Sequential recognition of invading microbes and rapid induction of plant immune responses comprise at least two recognition systems. Early basal defenses are initiated by pathogen-associated molecular patterns and pattern recognition receptors (PRR) in the plasma membrane. Pathogens produce effectors to suppress defense but plants, in turn, can sense such effectors by dominant plant resistance (R) gene products. Plant PRR and R proteins modulate signaling networks for defense responses that rely on rapid production of reactive nitrogen species (RNS) and reactive oxygen species (ROS). Recent research has shown that nitric oxide (NO) mainly mediates biological function through chemical reactions between locally controlled accumulation of RNS and proteins leading to potential alteration of protein function. Many proteins specifically regulated by NO and participating in signaling during plant defense response have been identified, highlighting the physiological relevance of these modifications in plant immunity. ROS function independently or in cooperation with NO during defense, modulating the RNS signaling functions through the entire process. This review provides an overview of current knowledge about regulatory mechanisms for NO burst and signaling, and crosstalk with ROS in response to pathogen attack.

Nitric oxide (NO) mediates signaling during several physiological processes and stress responses in cells (Besson-Bard et al. 2008). Among them, NO has been shown to behave as a crucial component of plant immune response, and it participates, in cooperation with H2O2, in activating hypersensitive (HR) cell death during incompatible plant–pathogen interactions (Delledonne et al. 1998; Durner et al. 1998; Yoshioka et al. 2011). NO is a highly diffusible and reactive gaseous radical endogenously produced by plants. Importantly for its signaling function, its lipophilic properties allow NO to cross plant membranes easily, but how far it can really diffuse within and between cells is still unknown. Its reactivity is also of crucial importance for its signaling functions. NO derivatives are collectively called reactive nitrogen species (RNS) that comprise the radical NO-, its nitrosonium (NO+) and nitroyl ions (NO2-), and the products of reaction between the NO radical and reactive oxygen species (ROS). When both ROS and NO accumulate, such as during recognition of plant pathogens, diffusion-limited reaction of NO with superoxide (O2-) results in generation of peroxynitrite (ONOO-). NO can also react with O2- -generating NOx compounds, such as NO2, N2O5, and N2O4. All these compounds in biological systems can react with thiols or are further modified to finally produce NO2- or NO3- (Wendehenne et al. 2001). Here, we provide an overview of current knowledge about NO and the cross-reacting ROS function during defense responses in plants.

NO-generating systems.

In animals, NO is generated mainly by nitric oxide synthase (NOS). In plants, no direct homolog of any animal NOS gene has been found in any fully sequenced higher plant genome, even though a close homolog of animal NOS has been found in the unicellular green alga Ostreococcus tauri (Foresi et al. 2010). Nevertheless, NO can be produced by plant extracts incubated with NOS substrate arginine. Therefore, an NOS-like activity exists in plants (Asai and Yoshioka 2009; Chaki et al. 2009a; Corpas et al. 2006). This activity requires the same cofactors (NADPH, calcium, calmodulin, flavin adenine dinucleotide, flavin mononucleotide, and tetrahydrobiopterin BH4) as animal NOS (Asai et al. 2010a; Corpas et al. 2009) and is often referred to as the major source of NO in plants during plant–pathogen interactions; however, lack of identification of its enzymatic source and of proper genetic material has not allowed a final unequivocal clarification of its participation in plant defense (Gaupels et al. 2011a). In addition to this NOS-like activity, other oxidative enzymatic routes for NO production have been postulated, including oxidative reactions of polyamines (Tun et al. 2006) or hydroxylamines (Rumer et al. 2009), the natural occurrence of which has not been definitely proven in plants. Recently, a copper amine oxidase of Arabidopsis thaliana was also identified as a candidate for NO production from polyamines induced by abscissic acid (Wimalasekera et al. 2011). Nevertheless, the biological relevance of these findings in the context of plant defense has not been investigated.

As an alternative to oxidative routes, several reductive enzymatic systems using nitrite as a substrate for NO production were also reported for plants; however, although the described oxidative pathways are normally active under normoxia, reductive pathways usually become major NO-generating mechanisms when oxygen decreases (Gupta et al. 2011). For instance, nitrite reduction is catalyzed by a mitochondrial electron transport chain in the mitochondrial inner membrane under the condition of oxygen deprivation, probably via cytochrome c oxi-
brane-bound nitrite:NO-reductase (Ni-NOR) (Stohr et al. 2001). This enzyme, which is root specific, seems to mainly participate in sensing nitrate availability in soil and in regulating root infection by mycorrhizal fungi and, therefore, seems to not directly participate in NO production during pathogen infection (Moche et al. 2010). Another alternative candidate enzyme for NO production from nitrite is the peroxisomal xanthine oxidoreductase (XOR) that catalyzes NO production only under strict anaerobic conditions, thus strongly limiting its possible relevance for NO signaling in defense (Godber et al. 2000). By contrast, NO can clearly be produced, even in normoxic conditions, by the enzyme nitrate reductase (NR) (Yamasaki et al. 1999). NR catalyzes the reduction of nitrate to nitrite in an NAD(P)H-dependent manner. In addition, NR is capable of reducing nitrite to NO (Bright et al. 2006; Rockel et al. 2002). According to its enzymatic properties, the efficiency of this reaction in vivo could be limited by higher concentration of nitrate compared with nitrite, although the reaction efficiency could increase with an increase in nitrite concentration (Rockel et al. 2002).

In Arabidopsis, NR is encoded by genes NIA1 and NIA2, and the activity of NR proteins is tightly regulated in response to several environmental factors. Regulation occurs at the expression level and is mediated by substrate availability by binding 14-3-3 proteins and by post-translational modifications, such as phosphorylation and redox regulation (Hardin et al. 2009; Lillo et al. 2004; Wang et al. 2010). NR also undergoes sumoylation by AtSIZ1, an E3 SUMO ligase, and this modification dramatically increases its enzymatic activity, controlling nitrogen assimilation as well as NO content (Park et al. 2011). By genetic approaches using a nia1nia2 double mutant line (Wilkinson and Crawford 1993), NR has been shown to participate in NO production in several physiological contexts, such as abscissic acid–induced stomatal closure, hypoxia, and cold stress, or in nitrogen-fixing nodules (Cantrel et al. 2011; Desikan et al. 2002; Dordas et al. 2004; Horschani et al. 2011; Zhao et al. 2009). Importantly, NR participates in NO accumulation during defense responses to necrotrophic fungal pathogens (Asai et al. 2010b; Perchepied et al. 2010) and bacteria (Modolo et al. 2006; Oliveira et al. 2009), or upon perception of chemical elicitors from fungal plant pathogens or other pathogen-associated molecular patterns (Rasul et al. 2012; Shi and Li 2008; Yamamoto-Katou et al. 2006). However, by using electron paramagnetic resonance to detect NO production, Modolo and associates (2005) showed that foliar extracts of the mutant nia1nia2 still have the capacity to produce NO, like wild-type plants, upon pathogen infection when nitrite is exogenously supplied. This result suggests that additional mechanisms to reduce nitrite into NO exist, and that the reported decreased capability of NO synthesis of nia1nia2 mutant plants with defective NR activity upon pathogen infection is likely an indirect effect due to the reduced nitrite content of this line. A further argument in favor of additional, still undiscovered, NO production systems in defense was from the study of Rasul and associates (2012), in which oligogalacturonides induced NO production in Arabidopsis. This NO production was not completely suppressed using either an L-arginine-dependent NO synthesis inhibitor or genetic or pharmacological approaches to impair NR expression and activity and, therefore, further supports the possible existence of an alternative route to the NR- and arginine-dependent pathways for NO production during defense. In conclusion, differently from animals, a plethora of mechanisms have been reported for NO production in plants in addition to NOS activity (Fig. 1), although their relative participation and importance for defense against pathogens has not yet been fully clarified.

However, recent findings in plant NO research field provide some important information about upstream signaling cascades from pathogen perception to induction of NO production, as well as of production of ROS, acting together and modulating NO activity. In Nicotiana benthamiana, gain-of-function and loss-of-function analyses showed that mitogen-activated protein kinase (MAPK) cascade MEK2-SIPK/NTF4 controls NO and ROS production induced by elicitor INIF1, with the latter modulated also by MEK1-NTF6 cascade. Similarly, NO accumulation induced by fungal elicitor Nep1Mo was mediated by the same MAPK-signaling cascade. By using NOS or NR inhibitors, NOS-like activity and NR activity participated in NO burst induced by elicitor INF1 or by constitutively active MEK2. By silencing NbrRBOHB, ROS production induced by INF1 depended on plasma membrane NADPH oxidase (Asai and Yoshioka 2009; Asai et al. 2008; Yoshioka et al. 2011; Zhang et al. 2012). Finally, by using virus-induced gene silencing (VIGS), flavin biosynthesis enzyme RibA participated in regulating NO and ROS production induced by constitutively active MEK2, probably because of its participation in biosynthesis of prosthetic groups important for NOS, NR, and RBOH activities (Asai et al. 2010a). Interestingly, MPK6, the Arabidopsis ortholog of SIPK, interacts in vitro and in vivo with NIA2 and phosphorylates NIA2 at a specific serine residue, leading to an increase in NR activity and NO biosynthesis in response to H2O2 during Arabidopsis root development (Wang et al. 2010). According to these results, and given the high conservation of docking sequence and phosphorylation sites in NR proteins, NR may share with mammalian NOS a regulatory mechanism based on phosphorylation at a specific serine residue (Wang et al. 2011). Nevertheless, the possible relevance of this specific regulation for NO production activity in the context of plant defense has not been investigated.

In addition to MAPK, other signaling molecules participating in the signaling pathway leading to NO production during defense are currently emerging. For example, an exogenous treatment with extracellular adenosine triphosphate (eATP), a regulator of pathogen defense in plants, induces phosphatidic acid accumulation and NO synthesis in tomato cultured cells, and this NO synthesis is dependent on phosphatidic acid and Ca2+.

Fig. 1. Oxidative and reductive routes for nitric oxide (NO) synthesis in plants. NO can be produced from nitrite via reductive enzymatic routes, catalyzed by nitrate reductase (NR) or nitrite-NO reductase (Ni-NOR) in roots. Additional reductive enzymatic routes for NO synthesis from NO2 mediated by the mitochondrial electron transport chain or peroxysomal xanthine oxidoreductase (XOR) exist but typically occur under anoxic conditions (white arrows). Oxidative NO biosynthetic pathways include the unidentified nitric oxide synthase (NOS)-like enzyme, and NO production from polyamines (PAs) mediated by enzymes, such as copper amine oxidase (CuAO).
influx (Sueldo et al. 2010). Nevertheless, more recent data question the participation of NO, at least in the eATP-induced cell death process (Sun et al. 2012). Cytosolic calcium increase is well known as an essential early event in pathogen-induced immune signaling acting upstream of NO but the molecular events mediating it and linking it to NO increase are not fully understood. Recent data indicate that the cyclic nucleotide-gated channel 2 (CNGC2), a nonselective cation-conducting channel activated by cyclic nucleotides, is a possible candidate mediating this event (Ali et al. 2007). By using inhibitors of adenylyl cyclase or phosphodiesterase, the enzymes responsible for cAMP synthesis and degradation, Mα and associates (2009) showed participation of this cyclic nucleotide in NO generation through CNGC2 and Ca\(^{2+}\) influx regulation. Recently, Qi and associates (2010) identified receptor AtPepR1 participating in pathogen defense that includes a cyclase domain. They showed that the specific ligand AtPep3 induces a cytosolic calcium increase dependent on the receptor and on CNGC2. Even if the receptor cyclase activity has been shown only in vitro and is substantially lower compared with cyclase activity reported for animal cells, they speculated that the receptor could activate CNGC2 through its cyclase activity. These data seems to suggest that cyclic nucleotide signaling, which is traditionally regarded as acting downstream of NO, could also participate in regulation of NO biosynthesis during defense.

**NO signaling.** 

The best-characterized signaling pathway through which NO exerts its action in animals is mediated by second messenger cGMP. NO interacts with the heme ferrous iron of the enzyme soluble guanylate cyclase (sGC) to activate it and, thus, lead to cGMP production. In plants, pathogen infections and NO accumulation can also lead to cGMP production, but plant enzyme(s) responsible for the production have not been identified (Isner and Maathuis 2011; Meier et al. 2009). Only recently, by using a GC- and NO-binding domain search, an NO-sensitive GC enzyme has been identified in *A. thaliana*. Nevertheless, its predicted GC activity has been confirmed only in vitro, and its possible relevance for NO signaling in plants still awaits demonstration (Mulaudzi et al. 2011). NO signaling acts through MAPK. For example, different hormone signaling in different physiological contexts, as well as in cell death (Clarke et al. 2000; Pagnussat et al. 2004). NO signaling can directly regulate MAPK activity, as shown for the animal system, or act through interaction of MAPK with other proteins that are directly modified by NO (Wawer et al. 2010). Finally, reports exist providing evidence of a role for other second messengers, such as calcium and phosphatidic acid, that were also involved in NO biosynthesis in mediating NO downstream signaling in defense (Laxalt et al. 2007; Raho et al. 2011). However, the best-characterized signaling mechanism through which NO exerts its function in plants during defense is based on its chemical reaction with proteins (Leitner et al. 2009).

**S-nitrosylation of NO sensor proteins.**

S-nitrosylation consists of the covalent addition of an NO moiety to the sulfhydryl group of cysteine residues in target proteins, forming S-nitrosothiol (SNO) (Lindermayr and Durner 2009). This modification does not generically target all cysteine residues of proteins but specifically affects a subset of cysteine sensor residues that, given their molecular environment, shows higher susceptibility to oxidation. These particularly reactive cysteine residues can undergo different redox-based post-translational modifications, including S-nitrosylation, to sense redox changes such as those that typically occur during biotic stresses. Modification of key regulatory proteins affect their activities, localization, interaction with other proteins, and stability (Yun et al. 2012). Motif-based computational identification of cysteines that can undergo S-nitrosylation is not straightforward but different predictive methods have been developed that can be used with some success to identify putative targets also in plants (different computational strategies are discussed by Fares and associates [2011] and Weerapana and associates [2010]).

The level of SNO in plant cells is controlled by both NO content and turnover of S-nitrosoglutathione (GSNO) that performs protein trans-nitrosylation but also acts as an NO-bioreservoir (Zhang and Hogg 2004). This turnover is regulated by GSNO reductase (GSNOR), an evolutionarily conserved enzyme that controls intracellular levels of GSNO and, indirectly, S-nitrosylated proteins in many eukaryotes (Liu et al. 2001). In animals, in addition to GSNOR, thioredoxins or thioredoxin reductases can also directly degrade SNO to modulate the SNO content (Benhar et al. 2008), and evidence of their participation in denitrosylation reactions during defense response has emerged recently for plants as well (Spoel and Loake 2011; Tada et al. 2008). Plants with impaired GSNO metabolism are compromised in several physiological processes (Kwon et al. 2012; Lee et al. 2008), indicating that fine-tuned control of SNO homeostasis is important for growth, development, and resistance to abiotic and biotic stresses. Interestingly, some contradictory results have shown correlation between GSNO content and plant disease resistance or cell death (Chen et al. 2009; Espunya et al. 2012; Feechan et al. 2005; Holzmeister et al. 2011; Rusterucci et al. 2007; Yun et al. 2011). In any case, the levels of GSNO increase during the establishment of plant disease resistance and alterations in the GSNO content, by either genetic or pharmacological approaches, strongly affect defense responses and cell death, as well as salicylic acid (SA) signaling and cross-talk with other hormones participating in defense.

Several proteomic studies have identified numerous candidate proteins that undergo S-nitrosylation in plants. The physiological relevance of this modification for many of these candidates has been investigated (Astier et al. 2011, 2012; Terrile et al. 2012; van der Linde et al. 2011; Yun et al. 2011). Defense-related proteins regulated by S-nitrosylation are either enzymes or transcriptional activators or co-activators participating in plant immunity or HR cell death.

Among them, the nonexpressor of NPR1 (pathogenesis-related (PR)1) has long been known to act as a master positive regulator in plant immunity, co-activating defense gene expression and key components of SA-mediated signal transduction. Recently, Wu and associates (2012) showed this protein to directly bind SA, working as an SA receptor. NPR1 undergoes redox regulation and S-nitrosylation that control its activity (Mou et al. 2003; Tada et al. 2008). In unchallenged cells, NPR1 oligomerizes through intermolecular disulfide bridges sequesters NPR1 in the cytoplasm. As a consequence of redox changes induced by pathogen inoculation and of SA accumulation, the protein is reduced, monomerized, and translocated to the nucleus, where it upregulates a set of disease resistance genes. S-nitrosylation was first shown to promote disulfide bond formation and oligomerization (Tada et al. 2008). In addition, Lindermayr and colleagues (2010) showed that treatment with GSNO promotes translocation of NPR1 into the nucleus. Novel research is expected to clarify cross-talk between NO and SA signaling and these apparently contradictory data. In the nucleus, NPR1 interacts with the TGACG-motif binding factor (TGA) that binds to elements of the PR1 promoter, promoting PR gene expression and defense. TGA is also redox regulated and S-nitrosylated. Its oxidized form carries disulfide bonds, precluding its interaction with NPR1. GSNO-mediated S-nitrosylation of cysteine residues partici-
pating in disulfide bond formation protects TGA from oxidative modification, resulting in increased DNA-binding activity and stability of TGA when NPR1 is translocated into the nucleus (Lindermayr et al. 2010).

Interestingly, AtSABP3 (A. thaliana SA binding protein 3) shows SA binding properties, and carbonic anhydrase activity required to express resistance against pathogens undergoes S-nitrosylation during HR (Wang et al. 2009). S-nitrosylation suppresses both binding of SA and its carbonic anhydrase activity. Inhibition of AtSABP3 CA functioning by S-nitrosylation could contribute to a negative feedback loop that modulates plant defense response and cell death (Wang et al. 2009). In any case, this finding further reinforces the connection between NO and SA in plant defense.

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is an important glycolytic enzyme. Like NPR1, this enzyme undergoes a complex redox regulation and its activity is inhibited by oxidation, S-nitrosylation, and nitration. In animals, GAPDH binds to and stabilizes Siah1 (an E3 ubiquitin ligase) upon S-nitrosylation, promoting its translocation to the nucleus, where it degrades nuclear proteins, finally promoting apoptosis. New studies are proposing now that GAPDH could act as a nuclear nitrosylase, propagating by trans-nitrosylation NO signaling within defined cellular pathways (Kornberg et al. 2010; Stamler and Hess 2010). Accordingly, trans-nitrosylation by GAPDH of nuclear substrates histone deacetylase 2 or class III deacetylase sirtuin-1 modulate transcription (Hess and Stamler 2012; Sen and Snyder 2011). In plants, isoforms NtGAPCa and NtGAPCb in tobacco cells constitutively interact with salt-stress-activated sirtuin-1 and stability of TGA when NPR1 is translocated into the nucleus (Lindermayr et al. 2010).

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Pro-metacaspase 9 (pMC9) is a relative of animal caspases acting as programmed cell death executioners, which are S-nitrosylated cysteines located at the active site. These modifications keep pMC9 inactive under normal conditions, inhibiting its autoprocessing activity, similar to mammalian caspases that are also S-nitrosylated in inactive resting cells. In this case, differently from previously discussed S-nitrosylated proteins, signaling promotes denitrosylation that actively triggers cell death (Belenghi et al. 2007).

NO directly modulates its reactivity by S-nitrosylation of ROS producers and ROS or RNS scavengers. NADPH oxidase AtRBOHD is a major participant in ROS production induced by pathogens in Arabidopsis (Torres et al. 2005). Yun and associates (2012) showed S-nitrosylation of Arabidopsis AtRBOHD and the consequences for its ROS production activity; cysteine 890 S-nitrosylation of AtRBOHD during defense decreases NADPH oxidase activity and then reduces ROS accumulation, limiting the later stages of HR cell death. However, peroxiredoxin II E (PrxII E) detoxifies H2O2 and ONOO· under normal conditions, and its activity is inhibited by S-nitrosylation. During HR, S-nitrosylation of PrxII E would increase ONOO· availability and related signaling by inhibiting the activity of its detoxifying enzyme (Romero-Puertas et al. 2007).

In conclusion, NO signaling during defense response mediated by cysteine S-nitrosylation includes modification of different target regulatory proteins participating in plant defense, leading to either protein relocalization or activity modulation in which all proteins finally contribute, even if in different ways, to the development and control of defense in plants (Fig. 2).

**Protein tyrosine nitration by ONOO·**

ONOO· is formed in a diffusion-controlled reaction between stoichiometric amounts of NO and superoxide, both of which accumulate during HR. Accumulation of ONOO· during biotic stress has been confirmed by using specific fluorescent dye.

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**Fig. 2.** Cysteine S-nitrosylation controls the activity of regulatory proteins participating in plant defense by either promoting (green) or inhibiting (red) their activity. Under physiological conditions, S-nitrosylation inhibits pro-metacaspase 9 (pMC9), a cysteine protease participating in hypersensitive response (HR) cell death (left panel). Induced nitric oxide (NO) production during defense (right panel) leads instead to S-nitrosylation of non-expressor of NPR1 monomer, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), salicylic acid binding protein 3 (SABP3), peroxiredoxin II E (PrxII E), respiratory burst oxidase homolog D (RBOHD), and glycine decarboxylase (GDC). S-nitrosylation of the transcriptional co-activator NPR1 promotes NPR1 nuclear translocation and expression of defense genes. Similar to NPR1, GAPDH S-nitrosylation also promotes nuclear translocation. Normal GAPDH activity is blocked upon S-nitrosylation, as well as SABP3, PrxII E, RBOHD, and GDC activities. NPR1 and SABP NO sensor proteins participate more in defense establishment and immune signaling, and the other indicated sensor proteins, whose activity is regulated by NO, directly modulate HR cell death.
HKGreen-2 in Arabidopsis leaf discs challenged with the avirulent pathogen Pseudomonas syringae pv. tomato carrying the AvrB gene (Gaupels et al. 2011b), which agreed with previous results obtained with a less specific APF dye (Saito et al. 2006). ONOO− is a strong oxidant agent that leads to cell death in mammals but not in plants (Delledonne et al. 2001), and it is now widely recognized to exert important signaling functions by nitrating proteins (Arasimowicz-Jelonek and Floryszak-Wieczorek 2011; Vandelle and Delledonne 2011).

Tyrosine nitration consists of adding a nitro group to the ortho-position of the aromatic ring of tyrosine residues, forming 3-nitrotyrosine. This kind of protein modification appears to occur selectively, because only a limited number of proteins and tyrosines are targeted by nitration. Despite this selectivity, a consensus primary sequence for tyrosine nitration has not been defined. Thus, nitration may depend on a secondary or higher-order structure and is promoted by exposure of the aromatic ring on the surface of the protein, proximity to negatively charged residues, and the absence of nearby cysteine residues, although this last criterion is still debatable (Bayden et al. 2011). Nitration can alter protein conformation and impose steric restriction, thus influencing protein structure and activity. In animals, depending on the protein, tyrosine nitration has either a stimulatory or an inhibitory effect (Radi 2004) on denitrase activity, which would account for reversibility of this modification; however, the corresponding enzyme has not been isolated (Gorg et al. 2007).

In plants, investigation of the functional impact of tyrosine nitration is just at the beginning. For a long time, protein tyrosine nitration has been considered to be a marker for nitrosative stress. However, the observed correlation between ONOO− accumulation and the transient increase in tyrosine-nitrated proteins during HR (Romero-Puertas et al. 2007), the selectivity and putative reversibility of this post-translational modification (Alvarez et al. 2011; Melo et al. 2011), and the identification of several putative target proteins undergoing tyrosine nitration under physiological conditions (Chaki et al. 2009b; Lozano-Juste et al. 2011) suggest a signaling role.

By using a proteomic approach, eight target proteins participating in photosynthesis, ATP synthesis, the Calvin cycle, glycolysis, and nitrate assimilation differentially nitrate in A. thaliana plants challenged with avirulent P. syringae pv. tomato during HR (Cecconi et al. 2009). Lozano-Juste and associates (2011) discuss particular aspects and challenges in proteomic approaches related to the identification of tyrosine residues undergoing nitration in their most complete study on the nitro-proteome, identifying 127 proteins putatively nitrated in plants. However, only a few reports are available on the identification of in vivo nitration sites in plants, probably because of the low abundance and limited stability of this modification (Galeský et al. 2011). The functional consequences of nitration have been studied for only two proteins in plants but, unfortunately, neither protein seems to participate in resistance (Alvarez et al. 2011; Melo et al. 2011). Both studies report inhibition of activity: inhibition by nitration of a specific tyrosine residue of A. thaliana O-acetylserine(thiol)lyase A1 (OASA1), which catalyzes the last step of sulfur assimilation (Álvarez et al. 2011), and the nodule glutamine synthase isoenzyme is inhibited by nitration of a specific tyrosine in relation to reduced nitrogen fixation rates (Melo et al. 2011).

Conclusions.

We described here the pivotal role of NO as a mediator for plant immunity and current knowledge about its production and signaling. Our understanding of the NO role in defense signaling is still fragmentary, and a strong limitation especially arises because the enzymatic sources of NO in plant–pathogen interactions still remain elusive. Therefore, identification and characterization of NO-producing enzymes in plants during microbe interaction, as well as of genes participating in NO signaling during HR, remain mandatory tasks. To this aim, genetic screenings are still attractive approaches. Screening Arabidopsis mutant collections to identify genes participating in NO signaling during cell death, as well as proteomic approaches or gene hunting by using VIGS to find novel NO-producing enzymes in plants, are promising approaches that should be considered to further improve our knowledge about NO synthesis and signaling during plant defense.

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LITERATURE CITED


