PJSRR (2015) 1(1): 82-90

Pertanika Journal of Scholarly Research Reviews
http://www.pjsrr.edu.my/

Nitric oxide and Its Important Role in Plant Defence

Nurul Najihah, MOHD NASIR,[a] & Noor Baity, SAIDI,[b*]

[a]Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.
E-mail address: n.najihah79@gmail.com

[b]Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.
E-mail address: norbaity@upm.edu.my

[*]*norbaity@upm.edu.my*

Abstract – Nitric oxide (NO) is a signaling molecule involved in numerous physiological processes in both animals and plants. The bioactivity of NO is mainly transduced via post-translational modification of cysteine residues of proteins termed S-nitrosylation. Interestingly, a number of key regulatory components in plant defense responses have been found to be regulated by S-nitrosylation making this type of protein modification an important modulator of plant immunity. As a signaling molecule, NO intimately interact with other important molecules such as reactive oxygen species. Since the identification of NO in plants, increasing number of papers isbeing published in the area of NO biology each year. Here, a collection of papers describing the role of NO in plant immunity has been brought together to provide a bird's-eye view on the focus area.

Keywords: Nitric oxide, plant immunity, S-nitrosylation

Nitric Oxide: General Properties

Nitric oxide (NO) acts as a signaling molecule within species from every biological kingdom. Because of its unique chemistry, which permits permeability, stability and reactivity, NO and its derivatives are ideally suited to its cellular signaling function. At room temperature and at atmospheric pressure, NO is a free radical colourless diatomic gas with lipophilic property. Its small Stoke's radius and neutral charge allow rapid membrane diffusion (Kiger et al., 1993) and can play a part in cell-to-cell signaling over a brief period of time. Due to the presence of unpaired electron and the free radical nature of NO, it readily reacts with oxygen (O_2), superoxide (O_2^-), transition metals and thiols, which largely shape its cellular function within the cell (Mur et al., 2006; Neill et al., 2008; Hong et al, 2008). The reaction of NO with O_2 results in the generation of NO_x compounds (including NO_2, N_2O_3, and N_2O_4), which can either react with cellular amines and thiols, or simply hydrolyze to form end metabolites of nitrite (NO_2^-) and nitrate (NO_3) (Wendehenne et al., 2001).

Nitric Oxide Production in Animals and Plants

NO is a multifunctional effector involved in numerous mammalian physiological processes, including neurotransmission, immunological and inflammatory responses, and relaxation of vascular smooth muscle (Schmidt and Walter, 1994). However, the use of NO is not confined to the animal kingdom alone. NO is also involved in diverse physiological processes in plants, such as defense response, metabolism, cellular detoxification, transport, iron homeostasis, signaling, flowering, and lignin

biosynthesis (He et al., 2004; Bason-Bard et al., 2008; Wendehenne et al., 2014; Yu et al., 2014). Despite the importance to elucidate the biosynthesis of NO in plants, there is still much uncertainty after years of research. In animals, NO is synthesized primarily by the enzyme nitric oxide synthase (NOS), which catalyzes the NADPH-dependent oxidation of L-arginine to L-citrulline and NO (Stuehr et al., 2004). Three NOS isoforms have been identified (Nathan and Xie, 1994); neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS in macrophages (iNOS). nNOS and eNOS are considered as constitutive and both show fast and transient activation. iNOS is induced in macrophages and many other cell types in response to inflammatory agents and cytokines (Mayer and Hemmens, 1997; Beck et al., 1999). Compared to constitutive NOSs, iNOS activityis able to be sustainedlonger, ismore stable and generates more NO, thus exerting cytotoxic and antimicrobial effects on the immune systems (Beck et al., 1999).

NO synthesis in plants involves both arginine and nitrite-dependent pathways. It is well documented that potential enzymatic sources of NO in plant cells include nitrate reductase (NR) and NOS-like activity (Neill et al., 2003; Romero-Puertas et al., 2004; Wang et al., 2006; Medina-Andres et al., 2015). NR catalyzes the *in vitro* production of NO through a one-electron reduction of nitrite via the use of NAD(P)H as an electron donor (Yamasaki and Sakihama, 2000). Despite a few contradictory evidences that collectively suggest that NR is not likely to be the major generator of the NO synthesized during pathogen-triggered nitrosative burst (Hong et al., 2008; Yamasaki, 2000), it has been viewed as a candidate for NO production during plant-pathogen interaction (Neill et al., 2003). Moreover, recent genetic based approaches using NR-deficient mutants denoted that NO is mainly produced from NR that probably operates downstream of the L-arginine-dependent pathway (Corpas et al., 2009; Vitor et al., 2013)

Although there is no obvious homolog of animal NOS in the *Arabidopsis* genome, several NOS-like activities have been reported (Cueto et al., 1996; Barroso et al., 1999; Corpas et al., 2006). In addition, mammalian NOS inhibitors have been shown to effectively abrogate the pathogen-triggered NO production in plants (Delledone et al., 1998; Neill et al., 2003). Corpas et al. (2009) had elaborately compared animal and plant NOS and concluded that plants also possess L-arginine-dependent NOS activity which is different from canonical animal NOS.

A search for the enzyme(s) that catalyze(s) the pathogen-triggered NO production in *Arabidopsis* led to the cloning of the *Arabidopsis* NOS 1 (*AtNOS1*) gene, which exhibited significant sequence similarity to a snail gene that encoded a NOS-like activity, but with no homology to mammalian NOS (Guo et al., 2003). However, it has been difficult to demonstrate reproducibility of typical NOS activity through recombinant *AtNOS1* (Crawford et al., 2006). Instead, *AtNOS1* was found to serve as a chloroplast-targeted GTPase essential for proper ribosome assembly (Flores-Perez et al., 2008) and therefore renamed *Arabidopsis* nitric oxide associated 1 (*AtNOA1*). Several other mutants with altered NO levels had been found to show increased NO accumulation correlated with concentrations of putative substrates for NO biosynthesis but none of them was exclusively involved in NO production (Leitner et al., 2009). The closest evidence to the existence of a plant NOS-like enzyme is the identification of a functional NOS with 40% similarity to human NOSs in the green alga, Ostreococcustauri (Foresi et al., 2010).

On the other hand, researchers have reported that NO can also be formed non-enzymatically in a reaction between nitrogen dioxide and plant metabolites, in nitrous oxide decomposition or as a result of chemical reduction of NO_2^- at acidic pH (Wendehenne et al., 2001). This area of research remains controversial and clearly, it is imperative to determine the key NO generator in plants to improve our understanding of NO metabolism.

S-nitrosylation as a Redox-Based Signalling Factor in Plants

NO-related signaling can be attributed to various NO derivatives, collectively referred to as reactive nitrogen species (RNS), which comprise of not only the NO radical ($NO^{\cdot}$) and its nitroxyl (NO^{-}) and nitrosonium (NO^{+}) ions, but also peroxynitrite ($ONOO^{-}$), S-nitrosothiols (SNO), higher oxides of nitrogen and dinitrosyl-iron complexes (Leitner et al., 2009). NO and RNS exert their biological actions through the chemical modification of targets by reacting with different amino acids or prosthetic groups. They mostly act through the binding oftransition metals of metalloproteins (metal nitrosylation**),** the covalent modifications of cysteine (S-nitrosylation) and tyrosine (tyrosine 3-nitration). These processes emerge as specific post-translational protein modifications and the best characterized among these is S-nirosylation which involves the covalent attachment of an NO moiety to the thiol side chain of cysteine to form SNO.

S-nitrosylated proteins have been identified through a proteome-wide scale analysis in several plants including Arabidopsis, potato, pea and citrus L. (Lindermayr et al., 2005, Kato et al., 2013, Camejo et al., 2013, Tanou et al, 2009). In this framework, S-nitrosylation regulates a wide array of proteins involved in all major cellular activities and the formation of SNO may serve to stabilize and diversify NO-related signals.

As a regulatory mechanism in plants and animals, S-nitrosylation is a reversible process. Indeed, S-nitrosylated proteins can be easily de-nitrosylated as the S-NO bond is labile in a cytoplasm's reducing environment, allowing cells to flexibly and precisely modulate protein function in response to environmental signals. S-nitrosylated proteins are in dynamic equilibrium with de-nitrosylated proteins largely due to the action of glutathione (GSH) with the subsequent formation of S-nitrosoglutathione (GSNO), reconstituting the protein thiol as a consequence. GSNO has the ability to release NO or function as a transnitrosylationg agent, thus it is considered as a natural reservoir of NO (Besson-Bard et al., 2008; Leitner et al., 2009).

Two of the enzymes that are known to metabolize GSNO are S-nitrosoglutathione reductase (GSNOR) and thioredoxin (Feechan et al., 2005; Lamotte et al., 2015). The presence of GSNOR is conserved in bacteria, animals and plants (Liu et al., 2001) and due to its ubiquitous nature, this enzyme was suggested to confer protection against nitrosative stress rather than as a cell signaling factor. In contrast, thioredoxin or thioredoxin reductase denitrosylation reactions seem to be a part of a signal transduction mechanism (Lindermayr and Durner, 2009). GSNOR controls intracellular levels of GSNO and limits NO toxicity through NADH-dependent reduction of GSNO to glutathione disulfide (GSSG) and ammonia (NH_3) (Sakamoto et al., 2002). Though highly specific for GSNO, GSNOR seems to modulate the extent of total cellular SNO formation (Liu et al., 2001; Feechan et al., 2005), and thus is regarded as the key enzyme responsible for the modulation of NO-mediated signaling pathways (Cheng et al., 2015).

Important Role of NO and SNO in Plant Disease Resistance

The function of NO in signaling defense responses during plant-pathogen interactions has been well documented in many experiments conducted years ago. A widespread feature of plant disease resistance is the programmed hypersensitive response (HR), a programmed execution of plant cells at sites of attempted infection that serve to limit the pathogen spread (Delledonne et al., 2001; Mur et al., 2006). NO is suggested to play a key signaling role during HR, next to the accumulation of ROS and salicylic acid (Agurla et al., 2014). In animals, many biological effects of NO including apoptosis are mediated by the highly toxic molecule, $ONOO^{-}$, which is relatively non-toxic in plants (Bonfoco et al., 1995). On the contrary, HR-associated cell death in plants is proposed to be mediated by the relative levels of NO and H_2O_2 that areformed by the dismutation of O_2^{-} by SOD. In many cases, impairment of NO production via genetic mutation or treatment with NO inhibitor will negatively affect H_2O_2 accumulation, leading to suppressed HR advancement (Rasul et al., 2012; Vitor et al., 2013; Kulik et al., 2014; Trapet et al., 2014; Qiao et al., 2015). In plants, $ONOO^{-}$ is continuously produced in healthy cells, exposing them to an environment rich in $ONOO^{-}$. Therefore, plants have developed some

detoxification mechanisms, for example, through the action of peroxiredoxin II E (PrxIIE), a member of the peroxiredoxin family of antioxidant enzymes responsible for lipidoxidation and tyrosine nitration (Romero-Puertas et al., 2007). Interestingly, PrxIIE has been found to be S-nitrosylated during the HR resulting in inhibition of its hydroperoxide-reducing peroxidise activity together with its ability to detoxify $ONOO^-$ and also increasing the amount of tyrosine nitration (Romero-Puertas et al., 2007). In conclusion, NO regulates the effect of its own reactive species through S-nitrosylation of crucial components of the antioxidant defence system. NO also controls cell death in plants through S-nitrosylation of *Arabidopsis* metacaspase 9 and cytosolic glyceraldehyde 3-phosphate dehydrogenase, both of which can act as potential executioners of programmed cell death (Belenghi et al., 2007). The involvement of NO in controlling cell death is further unearthed by the study of one of the mammalian NADPH oxidase homologs in plants, respiratory burst oxidase homolog (RBOH) D (Yun et al., 2011). RBOH is involved in the production of reactive oxygen species (ROS), particularly hydrogen peroxide, which is produced in response to pathogen recognition (Torres et al., 2002). NO was shown to inhibit AtRBOHD activity through S-nitrosylation at Cys-890, thus influencing the inherent effect of ROS during HR-associated cell death in plants. Based on the fact that Cys-890 is evolutionary conserved, it was suggested that S-nitrosylation of this specific residue might regulate the activity of NADPH oxidase in many other eukaryotes.

NO is not only thought to function during the development of hypersensitive cell death but also in the establishment of plant disease resistance complementary to and independent of ROS (Wang et al., 2013; Trapet et al., 2014). Administration of NO donors induced the expression of defense-related genes encoding phenylalanine ammonia lyase (PAL), the first enzyme of phenylpropanoid biosynthesis pathway and pathogenesis-related protein 1 (PR-1) (Durner et al., 1998; Vitor et al, 2013). NO action in plants, at least partially, is mediated through the SA-dependent signaling pathway. NO treatment induces GSH accumulation which is required to elevate endogenous SA accumulation (Kovac et al., 2015) that results in induction of *PR* genes (Durner et al., 1998; Kovac et al., 2015). *NPR1*, a master regulator of SA-mediated defense genes and a crucial component of disease resistance and signal cross-talk is known to be redox-regulated (Tada et al., 2008), adding an important clue towards understanding NO's signaling functions (Figure 1). Treatment with GSNO induces *NPR1*-dependent defense response in Arabidopsis (Kovac et al., 2015). S-nitrosylation of *NPR1* controls its subcellular localization through oligomer-monomer exchange and thus its transcription co-factor activity. Mutations at critical cysteine residues in NPR1 increased monomer accumulation, constitutive nuclear localization and *NPR1*-mediated gene expression in the absence of a pathogen (Mou et al., 2003; Tada et al., 2008). Another very interesting example for the regulatory function of NO is S-nitrosylation of *AtSABP3*, which may interfere with the signal cross-talk as both carbonic anhydrase and SA-binding activities of the protein are inhibited (Wang et al., 2009).

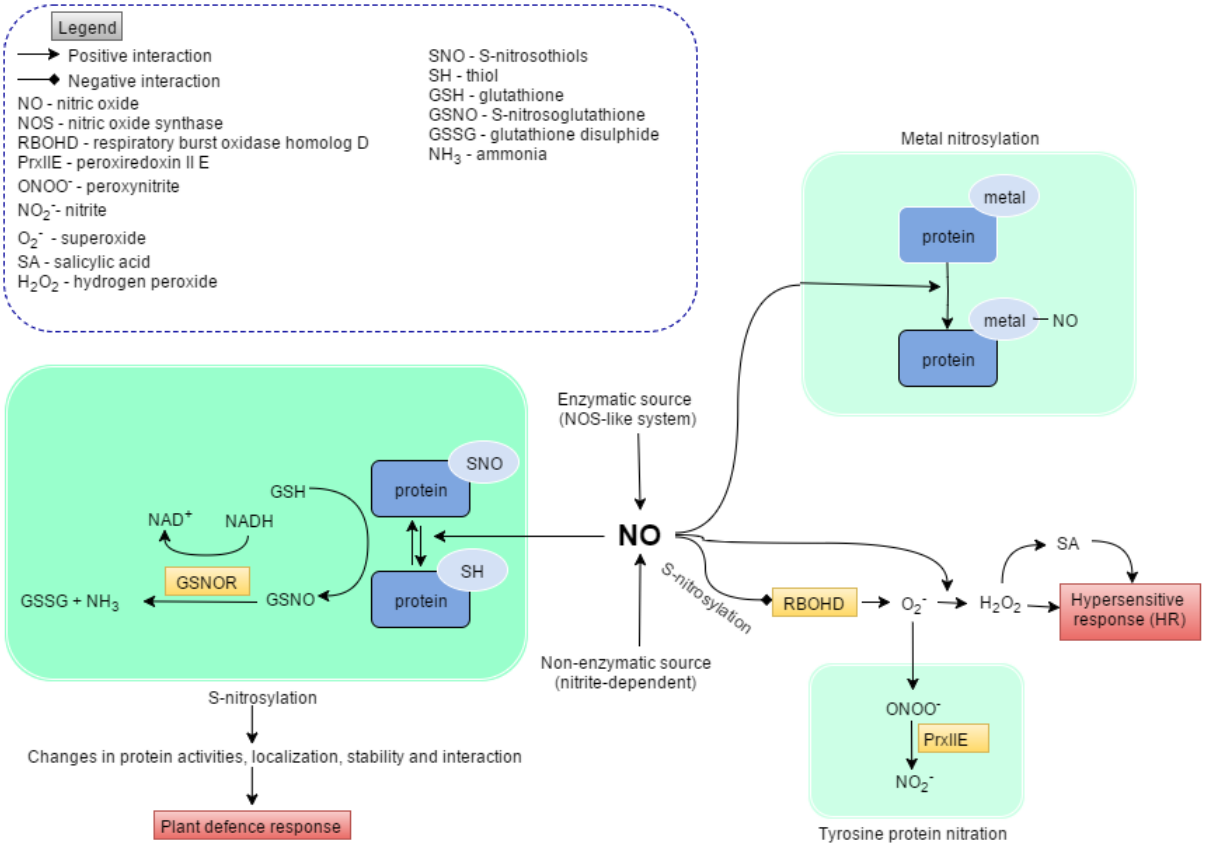

Figure 1: Cross-talk of nitric oxide (NO) in plant cells

In addition to the data presented above, the importance of NO and SNO in plant disease resistance were presented through the analysis of a GSNOR knock-out mutant. Loss-of-function mutation in *Arabidopsis GSNO reductase 1* (*AtGSNOR1*) resulted in increased cellular levels of SNOs and compromised all modes of disease resistance (Feechan et al., 2005). GSNO accumulation in the mutant plant is accompanied by a marked decrease in SA content and increased susceptibility to various pathogenic microbes. Interestingly, the mutant showed an increase in HR even in the absence SA and ROS, suggesting that plant cell death mediated by an increase in SNO alone is sufficient to convey resistance in the absence of associated defence responses (Yun et al., 2011). Conversely, enhanced *AtGSNOR1* activity results in increased protection against ordinarily virulent microbial pathogens. *AtGSNOR1* also positively regulates the signaling network controlled by the plant immune system activator, SA (Feechan et al., 2005). Subsequently, similar results were obtained by Tada et al. (2008) through *NPR1* studies. Surprisingly, when using antisense strategy, basal resistance has been reported to increase in *atgsnor1* antisense plants, correlating with higher levels of intracellular SNOs and constitutive activation of *PR-1* (Rusterrucci et al., 2007), which is the opposite result to that obtained by Feechan et al. (2005). Probably the contradictory reactions of the GSNOR modified plants might be a result of different cellular levels of SNO that change dramatically in *atgsnor1* mutants (Feechan et al., 2005) compared to minor changes in the antisense plants (Rusterucci et al., 2007). Nevertheless, both studies underline the physiological importance of SNO formation and turnover in regulating multiple modes of plant disease resistance. The work of Feechan et al. (2005) is supported by a study on the levels of SNO and NO in *Arabidopsisthaliana* cell suspension cultures (Frungillo et al., 2014). According to the study, antisense GSNOR transgenic lines displayed higher levels of SNO and NO under optimal growth conditions; in accordance with the designated role of GSNOR as a modulator for GSNO. A similar observation was reported in a study with different organs of healthy pepper plants where tissues with the lowest GSNOR activity presented higher GSNO and NO contents (Airaki et al., 2011). In the involvement of GSNO, the postulated mobile

signal modulation by GSNOR in the systemic defense response was also investigated and GSNO was shown to act synergistically with classical defense hormones such as SA and JA (Espunya et al., 2012; Freschi, 2013; Zhou et al., 2015). Collectively, GSNOR would serve as an ideal target for crop improvement through genetic engineering.

Conclusion and Perspective

Fundamental studies to understand the complexity of S-nitrosylation in the plant biological system especially during the execution of plant stress responses is undeniably crucial since S-nitrosylation provides a distinctive platform for NO to exert its impact on various proteins associated with pathophysiological mechanisms. To date, the number of candidates for S-nitrosylation is increasing and the physiological relevance of the S-nitrosylation process is becoming more evident. Notwithstanding the infancy of S-nitrosylation research, we may want to consider the wider perspective of its application in the real world. Exploring S-nitrosylation in crop plants would shed light on how S-nitrosylation controls the expression of plant disease resistance. The associated components could be the target of genetic modifications for the development of durable disease resistant crops.

References

Agurla, S., Gayatri, G., Raghavendra, A. S., (2014).Nitric oxide as a secondary messenger during stomatal closure as a part of plant immunity response against pathogens.*Nitric Oxide*, 43, 89-96.

Airaki, M., Sanchez-Moreno, L., Leterrier, M., Barrosso, J. B., Palma, J. M., Corpas, F. J., (2011). Detection and quantification of S-nitrosoglutathione (GSNO) in Pepper (Capsicum annuum L.) plant organs by LC-ES/MS. *Plant and Cell Physiology*, 52(11), 2006-2015.

Barroso, J. B., Corpas, F. J., Carreras, A., Sandalio, L. M., Valderrama, R., Palma, J. M., (1999). Localization of nitric-oxide synthase in plant peroxisomes. *The Journal of Biological Chemistry*, *274*(51), 36729-36733.

Beck, K. F., Eberhardt, W., Frank, S., Huwiler, A., Messmer, U. K., Muhl, H., (1999). Inducible NO synthase: Role in cellular signalling. *The Journal of Experimental Biology*, *202*(Pt 6), 645-653.

Belenghi, B., Romero-Puertas, M. C., Vercammen, D., Brackenier, A., Inze, D., Delledonne, M., (2007). Metacaspase activity of arabidopsis thaliana is regulated by S-nitrosylation of a critical cysteine residue. *The Journal of Biological Chemistry*, *282*(2), 1352-1358.

Besson-Bard, A., Pugin, A., &Wendehenne, D. (2008).New insights into nitric oxide signaling in plants. *Annu.Rev.Plant Biol.*, *59*, 21-39.

Camejo, D., del Carmen Romero-Puertas, M., Rodríguez-Serrano, M., Sandalio, L. M., Lázaro, J. J., Jiménez, A., (2013). Salinity-induced changes in S-nitrosylation of pea mitochondrial proteins. *Journal of Proteomics*, *79*, 87-99.

Cao, H., Bowling, S. A., Gordon, A. S., & Dong, X. (1994). Characterization of an arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. *The Plant Cell*, *6*(11), 1583-1592.

Chaki, M., Shekariesfahlan, A., Ageeva, A., Mengel, A., von Toerne, C., Durner, J., (2015). Identification of nuclear target proteins for S-nitrosylation in pathogen-treated arabidopsis thaliana cell cultures. *Plant Science*, *238*, 115-126.

Cheng, T., Chen, J., Abd_Allah, E. F., Wang, P., Wang, G., Hu, X., Shi, J. (2015). Quantitative proteomics analysis reveals that S-nitrosoglutathione reductase (GSNOR) and nitric oxide signalling enhance poplar defense against chilling stress. *Planta*, DOI 10.1007/s00425-015-2374-5.

Corpas, F. J., Barroso, J. B., Carreras, A., Valderrama, R., Palma, J. M., León, A. M., (2006). Constitutive arginine-dependent nitric oxide synthase activity in different organs of pea seedlings during plant development. *Planta*, *224*(2), 246-254.

Corpas, F. J., Palma, J. M., DelRío, L. A., Barroso, J. B. (2009). Evidence supporting the existence of L-arginine-dependent nitric oxide synthase activity in plants.*NewPhytol*, 184, 9–14.

Corpas, F. J., Chaki, M., Leterrier, M., & Barroso, J. B. (2009). Protein tyrosine nitration: A new challenge in plants. *Plant Signaling & Behavior*, *4*(10), 920-923.

Crawford, N. M., Galli, M., Tischner, R., Heimer, Y. M., Okamoto, M., & Mack, A. (2006). Response to zemojtel et al.: Plant nitric oxide synthase: Back to square one. *Trends in Plant Science*, *11*.

Cueto, M., Hernández-Perera, O., Martín, R., Bentura, M. L., Rodrigo, J., Lamas, S., (1996). Presence of nitric oxide synthase activity in roots and nodules of lupinusalbus. *FEBS Letters*, *398*(2), 159-164.

Delledonne, M., Xia, Y., Dixon, R. A., & Lamb, C. (1998). Nitric oxide functions as a signal in plant disease resistance. *Nature*, *394*(6693), 585-588.

Delledonne, M., Zeier, J., Marocco, A., Lamb, C. (2001). Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. *Proceedings of the National Academy of Science USA*, 98, 13454–13459.

Domingos, P., Prado, A. M., Wong, A., Gehring, C., & Feijo, J. A. (2015). Nitric oxide: A multitasked signaling gas in plants. *Molecular Plant, 8*(4), 506-520.

Durner, J., Wendehence, D., Klessig, D. F. (1998).Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose.*Proceedings of the National Academy of Sciences of the United States of America*, 95, 10328-10333.

Espunya M. C., De Michele R., Gomez-Cadenas A., Martinez M. C. (2012). S-nitrosoglutathione is a component of wound- and salicylic acid-induced systemic responses in Arabidopsis thaliana. *Journal of Experimental. Botany*, 63, 3219–3227.

Feechan, A., Kwon, E., Yun, B. W., Wang, Y., Pallas, J. A., & Loake, G. J. (2005). A central role for S-nitrosothiols in plant disease resistance. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(22), 8054-8059.

Flores-Perez, U., Sauret-Gueto, S., Gas, E., Jarvis, P., & Rodriguez-Concepcion, M. (2008). A mutant impaired in the production of plastome-encoded proteins uncovers a mechanism for the homeostasis of isoprenoid biosynthetic enzymes in arabidopsis plastids. *The Plant Cell*, *20*(5), 1303-1315.

Foresi, N., Correa-Aragunde, N., Parisi, G., Calo, G., Salerno, G., & Lamattina, L. (2010). Characterization of a nitric oxide synthase from the plant kingdom: NO generation from the green alga ostreococcustauri is light irradiance and growth phase dependent. *The Plant Cell*, *22*(11), 3816-3830.

Freschi, L. (2013). Nitric oxide and phytohormone interactions: Current status and perspectives. *Frontiers in Plant Science*, *4.*

Frungillo, L., Skelly, M. J., Loake, G. J., Spoel, S. H., Salgado, I. (2014). S-nitrosothiols regulate nitric oxide production and storage in plants through the nitrogen assimilation pathway. *Nature Communications*, 5, 5401.

Guo, F. Q., Okamoto, M., & Crawford, N. M. (2003). Identification of a plant nitric oxide synthase gene involved in hormonal signaling. *Science (New York, N.Y.)*, *302*(5642), 100-103.

He, S. Y., Nomura, K., & Whittam, T. S. (2004). Type III protein secretion mechanism in mammalian and plant pathogens. *BiochimicaEtBiophysicaActa (BBA)-Molecular Cell Research*, *1694*(1), 181-206.

Hong, J. K., Yun, B. W., Kang, J. G., Raja, M. U., Kwon, E., Sorhagen, K., (2008). Nitric oxide function and signalling in plant disease resistance. *Journal of Experimental Botany*, *59*(2), 147-154.

Kato, H., Takemoto, D., & Kawakita, K. (2013).Proteomic analysis of S-nitrosylated proteins in potato plant. *PhysiologiaPlantarum*, *148*(3), 371-386.

Kiger, L., Poyart, C., & Marden, M. C. (1993). Oxygen and CO binding to triply NO and asymmetric NO/CO hemoglobin hybrids. *Biophysical Journal*, *65*(3), 1050-1058.

Kovacs, I., Durner, J., & Lindermayr, C. (2015). Crosstalk between nitric oxide and glutathione is required for NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1)-dependent defense signaling in Arabidopsis thaliana. *New Phytologist*, *208*(3), 860-872.

Kulik, A., Noirot, E., Grandperret, V., Bourque, S., Fromentin, J., Salloignon, P., (2014). Interplays between nitric oxide and reactive oxygen species in cryptogeinsignalling. *Plant, Cell & Environment, 38*(2), 331-348.

Lai, T., Chen, Y., Li, B., Qin, G., & Tian, S. (2014). Mechanism of penicilliumexpansum in response to exogenous nitric oxide based on proteomics analysis. *Journal of Proteomics, 103*, 47-56.

Lamotte, O., Bertoldo, J. B., Bensson-Bard, A., Rosnoblet, C., Aime, S., Hichami, S., Terenzi, H., Wendehenne, D. (2015). Protein S-nitrosylation: specificity and identification strategies in plants. *Frontiers in Chemistry*, 2 (114), 1-8.

Leitner, M., Vandelle, E., Gaupels, F., Bellin, D., &Delledonne, M. (2009). NO signals in the haze: Nitric oxide signalling in plant defence. *Current Opinion in Plant Biology, 12*(4), 451-458.

Lindermayr, C., Saalbach, G., &Durner, J. (2005).Proteomic identification of S-nitrosylated proteins in arabidopsis. *Plant Physiology, 137*(3), 921-930.

Lindermayr, C. and Durner, J. (2009). S-nitosylation in plant: pattern and function. *Journal of Proteomics*, 73, 1-9.

Liu, L., Hausladen, A., Zeng, M., Que, L., Heitman, J., &Stamler, J. S. (2001). A metabolic enzyme for S-nitrosothiol conserved from bacteria to humans. *Nature, 410*(6827), 490-494.

Mayer, B., &Hemmens, B. (1997).Biosynthesis and action of nitric oxide in mammalian cells. *Trends in Biochemical Sciences, 22*(12), 477-481.

Medina-Andres, R., Solano-Peralta, A., Saucedo-Vazquez, J. P., Napsucialy-Mendivil, S., Pimentel-Cabrera, J. A., Sosa-Torres, M. E., (2015). The nitric oxide production in the moss physcomitrella patens is mediated by nitrate reductase. *PloS One, 10*(3), e0119400.

Mikula, I., Wyrwicz, L. S., Wanker, E. E., Mundlos, S., Vingron, M., Martasek, P., (2006). Plant nitric oxide synthase: A never-ending story? *Trends Plant Sci, 11*, 526-527.

Mou, Z., Fan, W., Dong, X. (2003). Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. *Cell*, 113, 935–944

Mur, L. A., Carver, T. L., & Prats, E. (2006). NO way to live; the various roles of nitric oxide in plant-pathogen interactions. *Journal of Experimental Botany, 57*(3), 489-505.

Nathan, C., & Xie, Q. (1994). Nitric oxide synthases: Roles, tolls, and controls. *Cell, 78*(6), 915-918.

Neill, S. J., Desikan, R., & Hancock, J. T. (2003).Nitric oxide signalling in plants. *New Phytologist, 159*(1), 11-35.

Neill, S., Bright, J., Desikan, R., Hancock, J., Harrison, J., & Wilson, I. (2008).Nitric oxide evolution and perception. *Journal of Experimental Botany, 59*(1), 25-35.

Qiao, M., Sun, J., Liu, N., Sun, T., Liu, G., Han, S., (2015).Changes of nitric oxide and its relationship with H 2 O 2 and ca 2 in defense interactions between wheat and puccinia triticina. *PloS One, 10*(7), e0132265.

Rasul, S., Dubreuil- maurizi, C., Lamotte, O., Koen, E., Poinssot, B., Alcaraz, G., (2012). Nitric oxide production mediates oligogalacturonide- triggered immunity and resistance to botrytis cinerea in arabidopsis thaliana. *Plant, Cell & Environment, 35*(8), 1483-1499.

Romero-Puertas, M. C., Perazzolli, M., Zago, E. D., & Delledonne, M. (2004). Nitric oxide signalling functions in plant–pathogen interactions. *Cellular Microbiology, 6*(9), 795-803.

Romero-Puertas, M. C., Laxa, M., Matte, A., Zaninotto, F., Finkemeier, I., Jones, A. M., (2007). S-nitrosylation of peroxiredoxin II E promotes peroxynitrite-mediated tyrosine nitration. *The Plant Cell, 19*(12), 4120-4130.

Rusterucci, C., Espunya, M. C., Diaz, M., Chabannes, M., Martinez, M. C. (2007). *S*-nitrosoglutathione reductase affords protection against pathogens in Arabidopsis, both locally and systemically. *Plant Physiology*, 143, 1282-1292.

Sakamoto, A., Ueda, M., Morikawa, H. (2002).Arabidopsis glutathione dependent formaldehyde dehydrogenase is an S-nitrosoglutathione reductase. *FEBS Lett*, 515, 20–24.

Schmidt, H. H., & Walter, U. (1994).NO at work. *Cell, 78*(6), 919-925.

Stuehr, D. J., Santolini, J., Wang, Z. Q., Wei, C. C., & Adak, S. (2004). Update on mechanism and catalytic regulation in the NO synthases. *The Journal of Biological Chemistry, 279*(35), 36167-36170.

Tada, Y., Spoel, H. S., Pajerowska-Mukhtar, K., Mou, Z., Song, J., Zuo, J., Dong, X. (2008). Plant immunity requires conformational charges of NPR1 via S-nitrosylation and Thioredoxins. *Science*, 321, 952-956.

Tanou, G., Job, C., Rajjou, L., Arc, E., Belghazi, M., Diamantidis, G., (2009). Proteomics reveals the overlapping roles of hydrogen peroxide and nitric oxide in the acclimation of citrus plants to salinity. *The Plant Journal*, *60*(5), 795-804.

Torres, M. A., Dangl, J. L., Jones, J. D. (2002). Arabidopsis gp91phox homologues *AtrbohD* and *AtrbohF* are required for accumulation of reactive oxygen intermediates in the plant defense response. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 517–522.

Trapet, P., Kulik, A., Lamotte, O., Jeandroz, S., Bourque, S., Nicolas-Francès, V., (2014). NO signaling in plant immunity: A tale of messengers. *Phytochemistry*, *112*, 72-79.

Vitor, S. C., Duarte, G. T., Saviani, E. E., Vincentz, M. G., Oliveira, H. C., & Salgado, I. (2013). Nitrate reductase is required for the transcriptional modulation and bactericidal activity of nitric oxide during the defense response of arabidopsis thaliana against pseudomonas syringae. *Planta*, *238*(3), 475-486.

Wang, D., Amornsiripanitch, N., & Dong, X. (2006).A genomic approach to identify regulatory nodes in the transcriptional network of systemic acquired resistance in plants.*Public Library of Science Pathogen*, *2*, e213.

Wang, L., Tsuda, K., Sato, M., Cohen, J. D., Katagiri, F., Glazebrook, J., (2009). Arabidopsis CaM binding protein CBP60g contributes to MAMP-induced SA accumulation and is involved in disease resistance against *Pseudomonas syringae*. *Public Library of Science Pathogen*, 5, e1000301.

Wang, Y., Loake, G. J., Chu, C. (2013). Cross-talk of nitric oxide and reactive oxygen species in plant programmed cell death. *Front. Plant Science*, 4,314.

Wendehenne, D., Pugin, A., Klessig, D. F., & Durner, J. (2001). Nitric oxide: Comparative synthesis and signaling in animal and plant cells. *Trends in Plant Science*, *6*(4), 177-183.

Wendehenne,D., Gao,Q.M., Kachroo,A., & Kachroo,P. (2014). Free radical mediated systemic immunity in plants. *Curr.Opin.PlantBiol*, 20C, 127–134.

Yamasaki, H., &Sakihama, Y. (2000). Simultaneous production of nitric oxide and peroxynitrite by plant nitrate reductase: In vitro evidence for the NR-dependent formation of active nitrogen species. *FEBS Letters*, *468*(1), 89-92.

Yamasaki, H. (2000). Nitrite-dependent nitric oxide production pathway: Implications for involvement of active nitrogen species in photoinhibition in vivo. *Philosophical Transactions of the Royal Society of London.Series B, Biological Sciences*, *355*(1402), 1477-1488.

Yu, M., Lamattina,L.,Spoel,S.H.,andLoake,G.J. (2014). Nitric oxide function in plant biology:a redox cue in deconvolution. *NewPhyto*, 202, 1142–1156.

Yun, B., Feechan, A., Yin, M., Saidi, N. B., Le Bihan, T., Yu, M., (2011). S-nitrosylation of NADPH oxidase regulates cell death in plant immunity. *Nature*, *478*(7368), 264-268.

Zhou, J., Jia, F., Shao, S., Zhang, H., Li, G., Xia, X., et al. (2015).Involvement of nitric oxide in the jasmonate-dependent basal defense against root-knot nematode in tomato plants. *Frontiers in Plant Science*, *6*, *193.*