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Review

Nitric oxide biochemistry, mode of action and signaling in plants

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Nitric oxide (NO) is a gaseous diatomic radical with a wide variety of physiological and pathological implications in animal, plant and microbes. NO reacts directly with metal complexes and other radicals and indirectly as a reactive nitrogen oxide species with DNA, proteins, and lipids. In animals, NO is a signal transduction element that is synthesized by nitric oxide synthase (NOS) enzyme from L-arginine that functions in many tissues and interacts with multiple target compounds in neurotransmission, vascular smooth muscle relaxation, and platelet inhibition. In plants; NO is synthesized enzymatically by NOS like enzyme, nitrate reductase, nitrite reductase etc. and also by non-enzymatically. NO play a diverse role in plant system including plant growth, stomatal movement, iron homeostasis, protection against biotic and abiotic stresses, senescence etc.

Key words: Nitric oxide (NO), nitric oxide synthase (NOS), nitrate reductase (NR), reactive oxygen species (ROS), c-GMP, L-Arginine.

INTRODUTION

Alfred Nobel was prescribed nitroglycerin to ease his chest pain due to heart disease. Hundred years after it was discovered that nitroglycerin - one of the key components of dynamite, acts through releasing nitric oxide as the therapeutic agent. Nitric oxide (NO) is a gaseous radical with a wide variety of physiological and pathological implications in animal, plant and microbes (Lamattina et al., 2003; Neill et al., 2003, 2008).

The biological significance of nitric oxide was recognized by scientific community in 1992. The free radical NO was named as the 'Molecule of the year' (Koshland, 1992). Subsequently, in the year 1998 the Nobel Prize in Physiology and Medicine was awarded to three distinguished physicians Robert F Furchgott, Louis J Ignarro and Ferid Murad for their discoveries concerning "the nitric oxide as a signalling molecule in the cardiovascular system"(Lamattina et al., 2003).

Plant researchers did not realize the enormous potential of NO during this period but focused NO as an atmospheric pollutant until the mid-1990s. Nitric oxide (NO) is a gaseous free radical that diffuses readily through biomembranes. The half-life of NO in biological tissues is estimated to be less than 6 s (Thomas et al., 2001). This short half-life reflects the highly reactive nature of NO. NO reacts directly with metal complexes and other radicals and indirectly as a reactive nitrogen oxide species with DNA, proteins, and lipids (Wink and Mitchell, 1998). In animals, NO is a signal transduction element that functions in many tissues and interacts with multiple target compounds in neurotransmission, vascular smooth muscle relaxation, and platelet inhibition. The roles of NO in plants may be equally diverse. To cover a wide array of events in the synthesis and action of NO is a herculean task. So, in this review, we report an upto date assessment of the biochemistry of evolution, localization, mode of action and signaling aspects of NO in plants (Figure 1).

BIOCHEMISTRY OF NITRIC OXIDE EVOLUTION IN PLANTS

In animal systems, NO is synthesized predominantly by the enzyme NO synthase (NOS). NOS converts L-Arg into L-citrulline in a NADPH-dependent reaction that releases one molecule of NO for each molecule of L-Arg.

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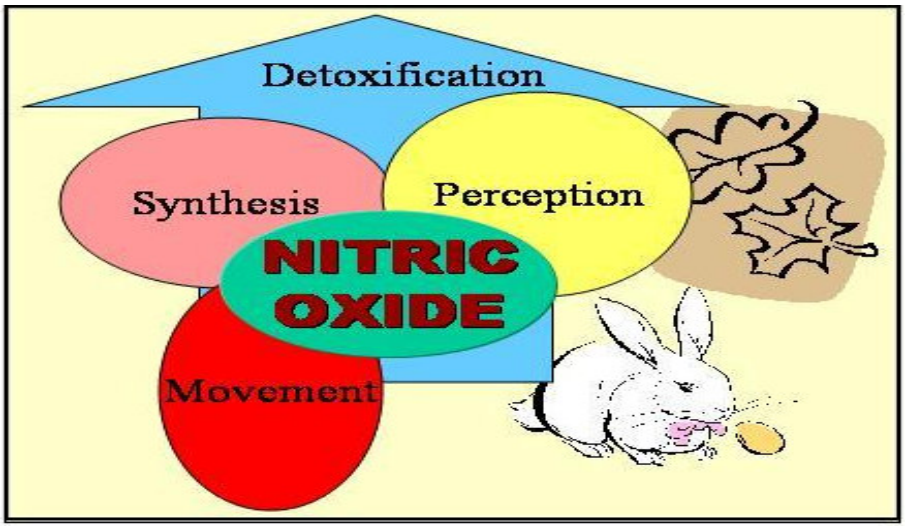

Figure 1. Schematic representation of the nitric oxide synthesis.

NOS-type enzyme also occurs in plants (Durner et al., 1998; Foissner et al., 2000). The traditional assay systems for mammalian enzymes have difficulty in the detection and estimation of NOS in plants. So EPR spectroscopy and chemiluminesence methods to detect NOS activity in plants are suggested (Corpas et al., 2004a). The plant NOS have little sequence similarity with its mammalian counterpart, but still contain domains which allow its redox functions to occur (Corpas et al., 2004a).

Plants also synthesize NO from nitrite. Nitrite-dependent NO production has been observed for *Glycine max* (soybean) by Delledonne et al. (1998) and *Helianthus annuus* (sunflower) by Rockel et al. (2002), green algae *Chlamydomonas reinhardtii* (Sakihama et al., 2002) and *Scenedesmus obliquus*, and the cyanobacterium *Anabaena* doliolum (Mallick et al., 1999). In some if not all of these cases, NO is likely to be produced by nitrate reductase (NR), which reduces nitrate to nitrite and can further reduce nitrite to NO.

Nitrite-dependent NO production

Several plant systems use nitrite as a substrate for NO synthesis:

$NO_2^- + e^- + 2H^+ \rightarrow NO + H_2O$

This reaction is mediated by the enzyme nitrite reuctase (NiR) localized in various compartments of the plant cell:

1. Cytosolic NiR (cNiR)
2. A plasma membrane-bound NiR (PM NiR)
3. Mitochondrial electron transport
4. Xanthine dehydrogenase/oxidase
5. Nonenzymatic NO formation at acidic pH.

NO synthesis by nitrate reductase (NR)

Nitrate reductase (NR) has long been known as a source for NO (Dean and Harper, 1988). The involvement of NR in NO production has been further established by *nia* mutants and with plants grown in NR-free media (Planchet et al., 2005). The enzyme NR, reduces nitrate to nitrite at the expense of NAD(P)H, further catalyzes a 1-electron transfer from NAD(P)H to nitrite resulting in NO formation in cell free systems also (Neill et al., 2003). NO production by NR *in vitro* was reported to be higher in anoxic condition yhat is in pure nitrogen (or argon) than in oxygenic condition – pure air (Planchet et al., 2005). The low yield of NO in oxygenic cell free system is attributed to autoxidation of NO or by its reaction with ROS produced simultaneously by NR (Yamasaki and Sakihama, 2000). Modulations of NR activity by reversible serine phosphorylation also modulate NO production (Rockel et al., 2002). Lea et al. (2004) reported a dirurnal opposite pattern to the wild type (low in day and high in night) of NO emission from plants constitutively expressing NR where serine was replaced with aspartic acid.

A plasma membrane-bound, root-specific enzyme, nitrite-NO oxidoreductase (Ni-NOR), may also function as a further source of NO. This enzyme was identified biochemically via its NO-generating activity. However, unlike NR, it does not use NAD(P)H as a cofactor, but uses cytochrome c as an electron donor in vitro and has a comparatively reduced pH optimum (Stohr and Stremlau, 2006).

Other enzymes may also be involved in NO production

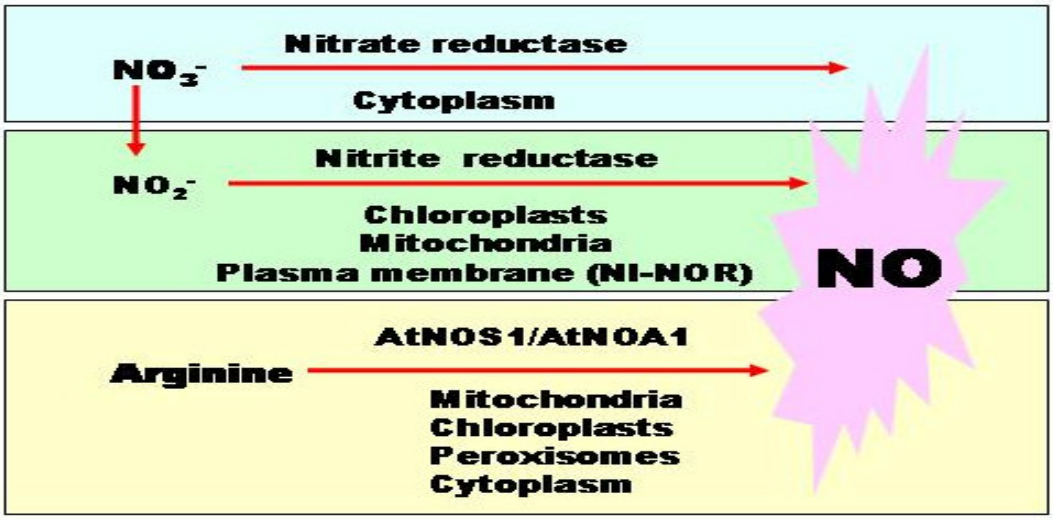

Figure 2. Enzyme sources and compartmentalization of the production of NO in plants.

(Corpas et al., 2004b). For example, in animals, xanthine oxidoreductase (XOR), under hypoxic conditions, can produce NO in preference to H_2O_2 (Millar et al., 1998). However, Planchet and Kaiser (2006b) were unable to observe any NO production from recombinant xanthine oxidase in plants. Xanthine oxidase/dehydrogenase (XDH). XDH has also been occasionally suggested as a source for NO using nitrite and xanthine as a substrate (Millar et al., 1998). However recombinant XDH, gave no evidence for NO production by the enzyme itself (Planchet et al., 2005).

Nonenzymatic NO production can occur at pH below 4.5, since the pKa of nitrous acid is about 3.2.

$$2\ HNO_2 \leftrightarrow NO + NO_2 + H_2O \leftrightarrow 2\ NO + 2\ O_2 + H_2O$$

These conditions exist in the apoplast of plant cells (Bethke et al., 2004a).

A comprehensive summary of the enzymes for the production of NO and compartmentalization of NO synthesis in plants is given in Figure 2.

Removal of nitric oxide in plants

NO is a reactive free radical molecule. So, it is likely that NO synthesized in living systems by different pathways is rapidly removed or metabolized after inducing the initial signalling events. Also an increased in the rates of NO accumulation or emission not necessarily reflect an increased generation but it may actually reflect reduced rates of removal. Nitric oxide is unstable and readily react with oxygen to form nitrite and nitrate (Gladwin et al., 2005).

In both animals and plants, NO is often produced at the Same time and in the same place as reactive oxygen species (ROS). NO reacts readily and reversibly with either thiol groups in the cysteine residues of proteins or with the tripeptide glutathione (GSH) probably leading to protein S-nitrosylation in NO signalling. Glutathione concentrations are typically 2 to 3 mM in plant cells (Ball et al., 2004) and thus, formation of S-nitrosylated glutathione (GSNO) could have a large impact on the concentration of free NO. GSNO is metabolized by the enzyme GSNO reductase (Diaz et al., 2003) and this enzyme may be instrumental in controlling the bioavailability of NO and the formation of protein S-NO groups, thereby regulating such NO-regulated processes in plants (Feechan et al., 2005). NO can also interact with transition metals, particularly with haem as in guanylyl cyclase or in haemoglobins (Perazzolli et al., 2004). The process of NO removal in plants is shown in Figure 3.

Mode of action and signaling in plants

Nitric oxide perception in plants

Nitric oxide (NO) has emerged as an important endogenous signalling molecule in plants that mediates many developmental and physiological processes including

1. Xylogenesis,
2. programmed cell death,
3. Pathogen defence,
4. Flowering,
5. Stomatal closure, and
6. Gravitropism (Delledonne,2005; Lamattina et al., 2003; Neil et al., 2003)

Experimental evidence in support of such signalling roles for NO has typically been obtained via the application of

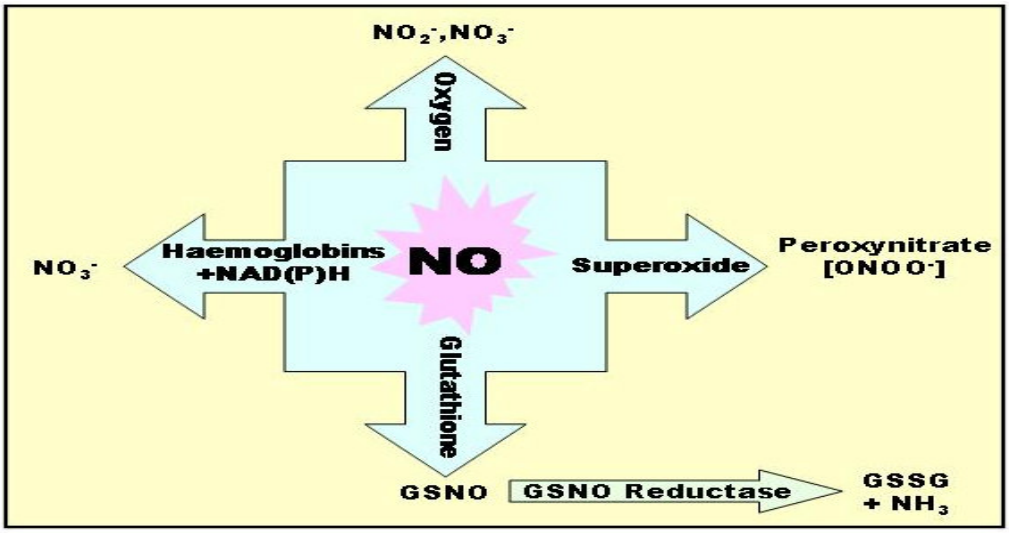

Figure 3. The overall process of NO detoxification and removal in plants.

either NO or NO donors (NO itself is a reactive gas with a short half-life in air), via the measurement of endogenous NO and through the manipulation of endogenous NO content by chemical and genetic means (Planchet and Kaiser, 2006ab). In some situations, NO can be released in far higher amounts than would probably be required to effect biological responses which raises the question of how it can actually function as a biological signal. NO also has paradoxical effects, for example, it is growth promoting at low concentrations, but quite inhibitory or toxic at high concentrations (Beligni and Lamattina, 1999) and being reactive, is perhaps unlikely to travel far between or even within cells.

Although there is no doubt that plants perceive and respond to NO, the mechanisms by which such perception occurs still require clarification. There is now considerable research interest concerning this question, but as no specific plant NO receptor has been identified, work in this area has taken its lead from mammalian research. Comprehensive step in NO perception mechanism in plants is shown in Figure 4. NO may be perceived in plants by a number of mechanisms that differ depending on the cell type, intracellular location, biochemical microenvironment, and environmental stimuli. NO can bind to the haem domain in proteins such as guanylate cyclase and with metals to form metal-nitrosyl complexes. It can also react with the SH group of low molecular weight thiols such as glutathione to form S-nitrososglutathione (GSNO) and, either directly or via GSNO, nitrosylate proteins to form S-nitrosylated proteins. S-nitrosylation induces conformational changes and is reversible. NO reacts with superoxide to form peroxynitrite which can then nitrate proteins on tyrosine residues. It is not yet known whether this reaction has signalling consequences.

NO movement in plants

NO can diffuse within a cell from the site of synthesis to other regions of the cell where it might induce an effect by interaction with specific target proteins. It can diffuse out of the cell across the plasma membrane into adjacent cells and thereby create a small region of cells responding to NO. However, this remains unknown. But comprehending the reactivity of NO, such diffusion can be limited. NO is lipophilic and may accumulate preferentially in membranes and could move through such a passage or barrier (Liu et al., 1998).

Alternate hypothesis is that NO precursors or 'NO storage compounds' may be transported with either NO generation or release occurring at distant sites in a manner analogous to the transport of the ethylene precursor ACC. SGNO is proposed to be such a molecule in plants (Valderrama et al., 2007) as glutathione is present at high (e.g. millimolar levels) concentrations in phloem cells. Arginine and nitrite could also serve as transported NO precursors (Rockel et al., 2002; Modolo et al., 2005).

Nitric oxide and gene regulation in plants

The participation of NO in plant signalling pathways is established. However, in order to decipher NO signalling pathways, its targets or inductive or repressive effects on gene expression level is inevitable (Figure 5).

Whole genome approaches

Polverari et al. (2003) studied the NO induced changes of Expression profiles of 2500 transcripts of

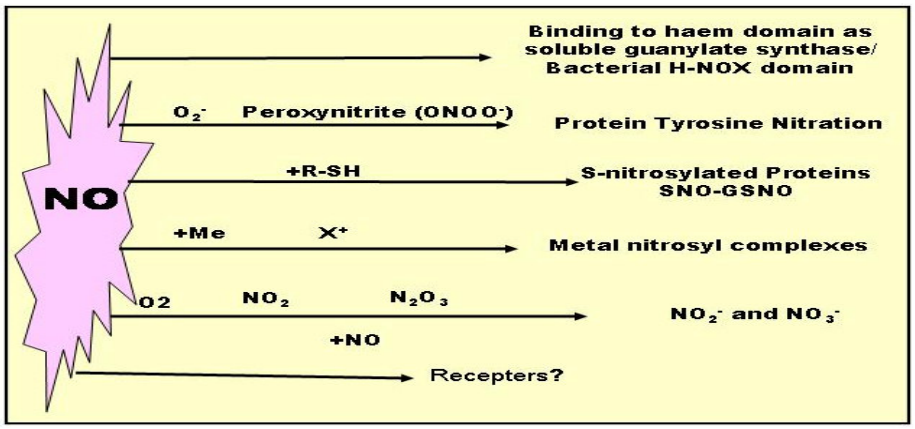

Figure 4. Mechanisms of NO perception in plants.

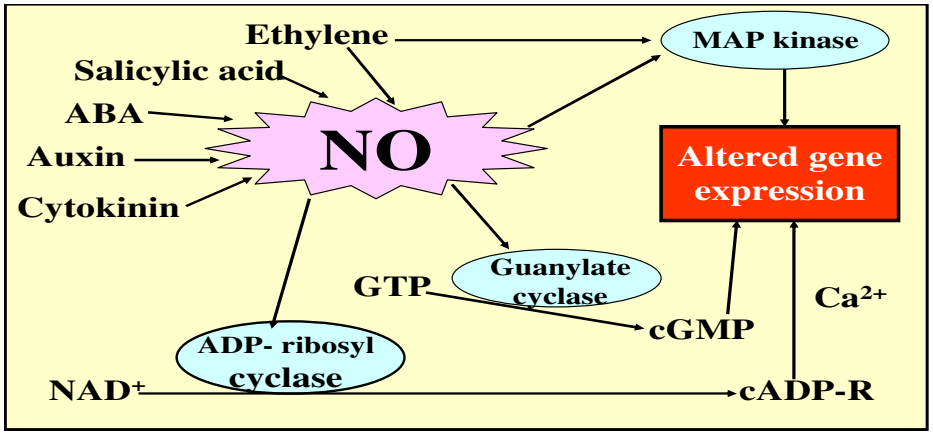

Figure 5. Plant growth regulator induced NO burst and NO induced signal transduction in plants.

Arabidopsis thaliana and reported NO-induced alterations in 120 transcripts. Sequence analysis of 71 differentially expressed cDNAs and their comparison to microarray results showed that most NO-modulated genes are also affected by other abiotic or biotic stress-related conditions. These transcripts belong to the functional categories of signal transduction, defence or cell death, ROS generation and removal, photosynthetic processes, cellular trafficking, and basic metabolism. Almost one-third of them consist of unclassified proteins.

Subsequent studies by Parani et al. (2004) using a whole-genome microarray (MicroArray Suite 5.0, Affymetrix, Inc.) representing approximately 24,000 genes and showed NO-induced 342 up-regulated 342 and down-regulated 80 genes in *A. thaliana*. In addition to the findings of Polverari et al. (2003), the transcript level of several plant defence response modulating transcription factors, like WRKYs, EREBPs (ethylene responsive element-binding proteins) several zinc finger proteins, and dehydration responsive element binding proteins (DREB1 and DREB2), were also induced by NO. Other interesting induced transcripts were coding for oxidative stress-related proteins (GSTs, ABC transporters), iron homeostasis proteins (e.g. ferritin genes), signal transduction factors (e.g. members of the defence-related MAP kinase modules), and plant development. However, these studies did not reflect any spatio-temporal aspects of NO signalling in plants. Nevertheless, these genes belong to a wide range of different physiological functions regulated by diverse signal transduction pathways.

NO's role in stress responses

Gene induction or suppression does not induce metabolic change as such, but the end result is a physiological reaction of the cell. So of different signaling pathways interacting directly or indirectly with NO through cause-and-effect chain may reflect the possible mode of action of NO in plant cells. Reports obtained so far reflect that NO is involved in almost every stress response analysed for NO so far in plants (Figure 6).

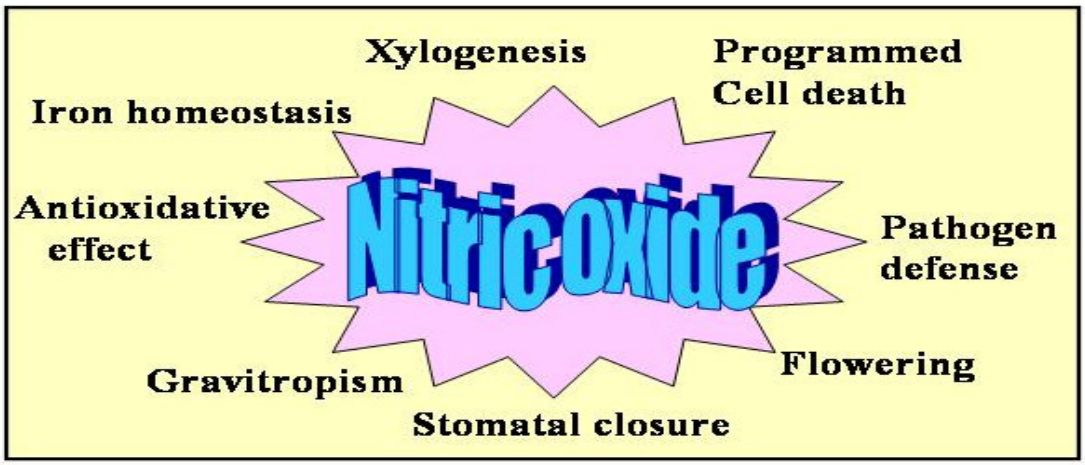

Figure 6. The physiological role of NO in plants.

Wound induced stress

Wounding of the leaf epidermis in *Arabidopsis* induced a burst in NO within minutes. Direct treatment of NO to plants enhanced the expression of key enzymes of the octadecanoid pathway, like AOS, LOX2, or OPR3 (Huang et al., 2004) but had no effect on either the jasmonic acid (JA) levels or JA responsible genes, like PDF1.2 (Glazebrook, 2001). NO increases the SA level (Durner et al., 1998; Durner and Klessig, 1999; Huang et al., 2004) and in SA-deficient *NahG* plants, NO treatments led to elevated JA levels along with the induction of PDF1.2 and JIP, which were non-responsive in wild-type plants. Durner et al. (1998) presented evidence for the increase of total SA levels and the induction of Pr-1- and Pal-expression in NO-treated tobacco leaves. Astonishingly, the induction of Pr-1 was shown to be SA-dependent, whereas Pal-expression was not. Nevertheless, SA does not always play a role in NO-induced gene expression. The Ipomoelin gene (IPO) in sweet potato was shown to be enhanced by methyl jasmonate (MeJA) and mechanical wounding (Imanishi et al., 1997). Although NO and H_2O_2 accumulation were both enhanced, but NO delayed wounding-induced IPO expression (Jih et al., 2003). The authors suggest two important wound-response-related effects of NO: Initiation of the cell death cycle together with H_2O_2, and delay of IPO-expression. H_2O_2 accumulation and expression of the proteinase inhibitors Inh1, Inh2, cathepsin D inhibitor (CDI), and metallocarboxypeptidase inhibitor (CPI) were inhibited by NO, but not the expression of AOS or LOX. Thus the authors suggest that NO is inhibiting signalling downstream from JA, but still upstream from ROS generation. These contradictory results correlate with several reports on the basic differences in wound-induced signalling pathways in Arabidopsis and those in the Solanaceae (Leon et al., 2001). Nevertheless, they demonstrate clearly that the accumulation of one signaling substance alone is not sufficient to induce any physiological changes.

Plant defense responses and programmed cell death (PCD)

Plants respond to pathogen infection by inducing local and systemic defence reactions. The local hypersensitive response is characterized by the development of lesions through programmed cell death or cellular apoptosis which restrict pathogen growth and/or spread (Delledonne et al., 1998; Krause and Durner, 2004; Tada et al., 2004). The hypersensitive reactions induce defense-related gene expression for the synthesis of antimicrobial enzymes and toxic secondary metabolites, such as phytoalexins, which kill pathogens. During the hypersensitive response, a sudden burst in the synthesis of ROS was reported (Delledonne et al., 1998; Krause and Durner, 2004; Tada et al., 2004). ROS act as both cellular signals and direct weapons against pathogens. In animals, ROS (generated by NADPH oxidase), collaborate with NO and related species, generated mainly by inducible NO synthase (NOS) to regulate apoptosis and kill invading pathogens (Hippeli and Elstner, 1998). The discovery of plant homologs of the NADPH oxidase (Murgia et al., 2004b) prompted several groups to examine whether NOS also plays a role during plant–pathogen interactions and programmed cell death (PCD) in plants (Zhang et al., 2003a; Tada et al., 2004).

More recently, NO and possibly NOS is reported to play a vital role in defense against microbial pathogens. Examples of such roles are:

1. In tobacco with a tobacco-mosaic virus (Durner et al., 1998; Modolo et al., 2005).
2. In soybean cells and *Arabidopsis* in response to either a bacterial pathogen or an elicitor (a signaling molecule that indicates the presence of a pathogen) (Delledonne et al., 1998; Plancet et al., 2004).

These experiments clearly suggest that NO plays an essential role in the early events of plant resistance responses. Although NO alone may not be sufficient for the induction or propagation of PCD, it influences gene expression.

NO signaling in plants

Second messenger mediated signalling

NO not only acts against stress and defence responses, but also acts as an important signaling molecule. In mammalian systems, guanylate cyclase. cGMP, is produced when NO bind to heme in the cyclase, and thus regulate many cellular functions (Planchet et al., 2005). In plants, cGMP can accelerate the induction of stress-associated gene expression and biosynthesis of secondary metabolites involved in defense responses (Perazzolli et al., 2006). cGMP and cADP-ribose induced similar defence related genes in tobacco as that by NO (Zaccolo, 2006). These two molecules, cGMP and cADP-ribose, are reported to serve as second messengers for NO signaling in mammals. Thus, Modolo et al. (2005) suggested that plants and animals probably use common mechanisms to transduce NO signals. SA which is characterised as a secondary messenger in plant-pathogen interactions and might serve as a general redox signal. NO activity is shown to be partially SA-dependent (Modolo et al., 2005). The relations among NO, SA, and ROS in the activation of defense genes and/or induction of host cell death are probably through the redox signaling network (Plancet et al., 2004; Tunc-Ozdemir et al., 2009).

cGMP-dependent signaling

The evidence that cGMP is an NO signalling intermediate has been obtained in several systems (Neill et al., 2003; Delledonne, 2005). Both salt and osmotic stress, two conditions which would both be expected to induce ABA synthesis, induced a rapid increase in the cGMP content of Arabidopsis seedlings (Donaldson et al., 2004). Using a sensitive radioimmunoassay technique, the cGMP content of pea epidermis and Arabidopsis guard cell fragments has been similarly measured as being in the pmol $g^{-}1$ range, and transient increases in cGMP levels following either ABA or SNP treatment have been observed could be prevented by co-incubation of the treated tissues with PTIO (Wilson et al., 2009). Further evidence that cGMP can mediate the effects of ABA in stomatal guard cells has resulted from pharmacological work using the cell-permeable cGMP analogue 8-bromo cGMP (8BrcGMP) and inhibitors of NO-sensitive sGC such as 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one (ODQ). The ABA- or NO-induced closure of pea (Neill et al., 2002) stomata was inhibited by ODQ. This inhibition could be prevented by coincubation of the ABA-/NO-stimulated, ODQ-treated guard cells with 8BrcGMP. However, 8BrcGMP alone did not induce stomatal closure. Thus, it would appear that although an elevated level of cGMP is required for effective ABA-induced stomatal closure, additional signaling pathways stimulated by ABA must operate in concert for such an increase to mediate its effects. In non-plant systems, cADP ribose (cADPR), an agent that mobilizes Ca^{2+} from internal stores, is a downstream messenger of NO. Nicotinamide, a potential inhibitor of cADPR synthesis, blocks ABA- and NO-induced stomatal closure (Neill et al., 2002). Garcia-Mata et al. (2003) have also shown that NO-induced intracellular Ca^{2+} release and the regulation of guard cell plasma membrane K^{+} and Cl^{-} channels are mediated by a cGMP- and cADPR-dependent pathway (Figure 7).

cGMP may also signal by binding to and directly activating cyclic nucleotide-gated ion channels (CNGCs) or by similarly activating cGMP-dependent protein kinases. To date, no cGMP-activated plant protein kinases have been identified, and the potential role of CNGCs in guard cell NO signalling awaits clarification. Although cGMP has been unequivocally identified in various plant tissues (Neill et al., 2003) and pharmacological data indicate a role for it during stomatal closure, very little is known of the mechanisms by which cGMP might be turned over in plant cells. The one plant guanylyl cyclase gene (AtGC1) cloned from Arabidopsis encodes a protein which shows many domain differences from mammalian sGC, lacks a haem-binding motif, and is insensitive to NO (Ludidi and Gehring, 2003). The biological roles of AtGC1 remain to be described and it may be that plants contain other enzymes capable of cGMP production. Interestingly and indicating that there may well be novel plant guanylyl cyclases awaiting discovery, a recent report has indicated that the Arabidopsis brassinosteroid receptor, AtBRI1, contains a domain with guanylyl cyclase activity (Kwezi et al., 2007). If cGMP is an intracellular plant signal, then mechanisms for its rapid degradation will exist. Plant cells do indeed possess cGMP hydrolysis activity and the Arabidopsis genome contains several genes for potential phosphodiesterases including that encoding a putative cGMP phosphodiesterase (Gen-Bank accession no. NM_118011) (Maathuis, 2006).

cGMP-independent signaling

NO and its related species can oxidize, nitrate, or nitrosylate proteins (Wang et al., 2006). Peroxynitrite, formed by the reaction of NO with superoxide, can oxidize proteins on cysteine, methionine, or tryptophan residues or nitrate tyrosine residues to form nitrotyrosine. These post-translational modifications may well turn out to have roles in intracellular signalling and the subse-quent physiological effects. For example, recent work

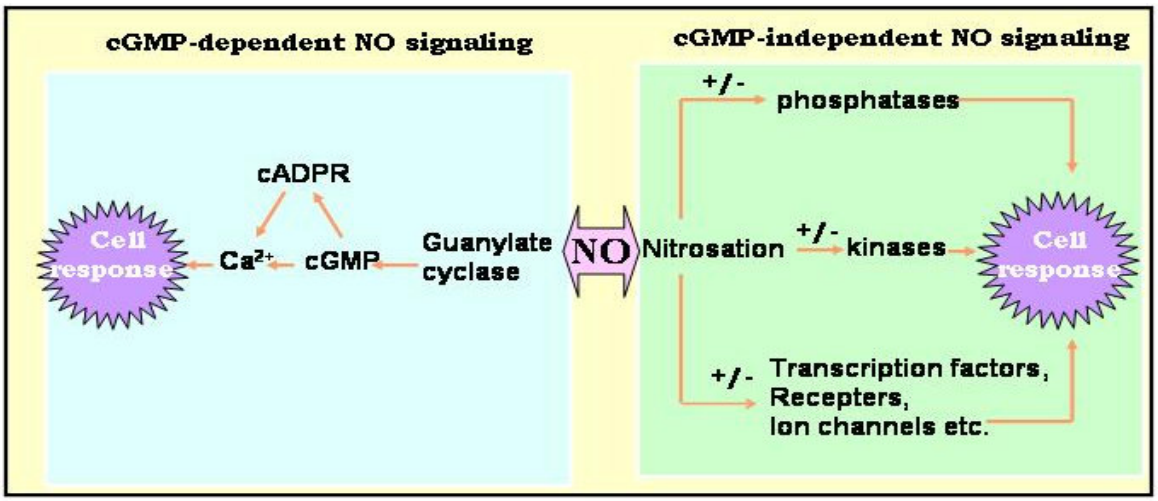

Figure 7. Cyclic GMP dependent and independent NO signaling in plants.

indicates a role for protein tyrosine nitration in plant defence responses (Saito et al., 2006).

S-nitrosylation is the reversible covalent attachment of NO to the thiol group of cysteine residues forming an S-nitrosothiol (SNO) and may well be an ancient highly conserved cell signalling mechanism (Wang et al., 2006). Nitrosylation can occur either directly through the interaction of NO and other NO-related species with the cysteine group or indirectly by trans-nitrosylation where the NO is derived from S-nitrosglutathione (GSNO) or other S-nitrosylated proteins. Some recent studies indicate that this redox-based mechanism plays a pivotal role in plant biology and will, therefore, also be important with regard to NO signalling in guard cells.

S-nitrosylated proteins can be detected using the 'biotin switch assay' which is based on specifically biotin labelling any S-nitrosylated cysteines within proteins. This assay is useful for demonstrating the existence of potentially S-nitrosylated proteins but, like many assays, may be prone to identifying false positives. However, S-nitrosylation can be demonstrated unequivocally using mass spectrometry (Lindermayr et al., 2006). The biotin switch assay has been used to show the presence of S-nitrosylated proteins in plants and to demonstrate that specific proteins can be S-nitrosylated (Lindermayr et al., 2006; Wang et al., 2006). A large number of potentially S-nitrosylated proteins have been identified which include stress-related proteins, redox-related proteins, signalling proteins, cytoskeletal proteins, and proteins involved in photosynthesis and metabolism (Lindemayr et al., 2005). Moreover, conserved protein S-nitrosylation and GSNO-binding motifs are present in plant proteins (Wang et al., 2006), and the effects of S-nitrosylation on protein activity and plant physiology are now being addressed (Lindermayr et al., 2006). It would seem likely that all cells contain nitrosylated proteins and that the spectrum and levels of these, the 'nitrosylome', will alter during NO accumulation in, for example, guard cells after their challenge with ABA. In fact some evidence that this case already exists, and data published by Sokolovski and Blatt (2004) suggest that NO regulates outward rectifying K+ channels in Vicia faba guard cells by S-nitrosylation.

NO as a signal affecting plant cell organelles

Mitochondria

Animal cell death pathways (PCD) can be divided into the processes such as involving (1) death receptors or (2) mitochondria (Brune, 2003). NO is a signalling factor in the mitochondria, where it is supposed to be synthesised. NO inhibits the activity of Cyt *c* oxidase (COX) leading to the generation of superoxide O_2^- due to the reduced ubiquinone (UQ) pool. Plant mitochondria are also a target of NO, but possess alternative oxidase, AOX (Thirkettle-Watts et al., 2003). The alternative oxidase AOX1a localized in the mitochondria is triggered by NO. AOX1a is also induced by by several biotic stresses (Simons et al., 1999) or the proteinaceous bacterial elicitor Harpin (Krause and Durner, 2004).

Chloroplasts

UV-light has an effect on plants, and affects cellular macromolecules as well increase ROS accumulation. NO protects against UV-stress induced damages in plants.
NO protects the photosynthetic apparatus in bean leaves from UV-B induced photo-oxidative stress by enhancing the activities of antioxidant enzymes like superoxide dismutases, ascorbate peroxidases, and catalases (Shi et al., 2005).

Regulation of plant growth and development by NO

Floral development and seed dormancy

NO regulates sexual reproduction processes in plants. AtNOS1-deficient Arabidopsis plants were induced to flower earlier than wild-type plants when treated with NO (Guo et al., 2003). However, there was a delayed flowering in NO-overproducing plants (nox1) than the wild type (He et al., 2004). These results showed that NO affects flowering time by reducing the amplitude, but not the rhythm of the circadian clock (Simpson, 2005). NO also regulates the growth of pollen tubes (Prado et al., 2004), programmed cell death in the aleurone (Beligni et al., 2002), and breaking of seed dormancy (Bethke et al., 2004).

Plant growth and development

Leshem (1996) first reported that NO induces leaf expansion, root growth and phytoalexin production (Leshem, 1996; Noritake et al., 1996). The vegetative growth processes of the shoot (Zhang et al., 2003b; An et al., 2005), cell division (Ötvös et al., 2005), xylem differentiation (Gabaldon et al., 2005), root development (Pagnussat et al., 2002, 2003; Guo et al., 2003), plant–rhizobacterium interaction (Creus et al., 2005), and gravitropic bending (Hu et al., 2005) is also regulated by NO.

Stomatal closure

Stomatal closure which is regulated by abscisic acid (ABA) is also regulated by NO signal. Desikan et al. (2002) using *Arabidopsis* double mutant *nia1/nia2* showed that nitrate reductase - produced NO is solely responsible for the ABA-regulated stomatal closure. However, involvement of NO produced by NOS (Guo et al., 2003), NR (Garcia-Mata and Lamattina, 2003), protein S-nitrosylation (Sokolovski and Blatt, 2004) and Ca^{2+}-sensitive ion channels (Garcia-Mata et al., 2003) in the ABA induced stomatal closure through NO signaling is reported, although the direct targets of NO are still obscure.

Iron-homeostasis

Ferritins are a class of multi-subunit proteins of plants and animals with 24 subunit proteins forming a coat for the storage of iron ions (Murgia et al., 2002). NO is shown by Murgia et al. (2002) as a factor essential for the iron-induced ferritin induction. Iron-dependent regulatory sequence (IDRS) of the Arabidopsis ferritin gene promoter (Atfer) was identified as the target sequence for NO-modulated ferritin gene expression (Murgia et al., 2004a).

The present knowledge on the biochemistry of evolution, localization, mode of action and signaling of NO in plants shows that NO is one of the versatile molecule which can be transported easily to any compartment in the plant cell and elucidate its impact through various signal transductionpathways. The literature available is huge, but this review is restricted to few aspects to focus on the over all apects of NO biosysnthesis, action and signaling in plants.

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