

## Review

# Nitric oxide: A ubiquitous signaling molecule with diverse role in plants

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**Nitric oxide (NO) is a gaseous di-atomic radical, readily diffusible through biological membranes and readily soluble in water is having a wide array of physiological, biochemical and molecular events in animals, plants 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 well established as a signaling molecule and is synthesized by multiple forms of nitric oxide synthase (NOS) enzyme from L-arginine. NO functions in almost all tissues and interacts with multiple target compounds in neurotransmission, vascular smooth muscle relaxation, and platelet inhibition. In plants, NO is synthesized through different enzymatic and non-enzymatic pathways, and play a diverse role in maintaining the functional homeostasis in normal (unstressed) systems as well in the protection against biotic and abiotic stresses. 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 transduction pathways. The literature available is vast. This review deals with the biosynthesis, detection and estimation methods used in plants, translocation and detoxification, and role of NO in plants. Emphasis is given to the role of NO as a signal regulating molecule in plants.**

**Key words:** Nitric oxide (NO), nitric oxide synthase (NOS), gene regulation, senescence, synthesis, signal transduction, plant growth, development, stress, programmed cell death, localization, detoxification, movement.

## INTRODUCTION

The medicinal use of nitroglycerine since the late 1940s is widespread. But the nitroglycerine-derived nitric oxide (NO) as the pharmacologically active agent is reported only in 1977 by Ferid Murad (Katsuki et al., 1977) which was recognized as an epoch making discovery of the century and Murad was awarded Nobel prize along with Furchgott and Ignarro in the year 1998 (Lamattina et al., 2003). In 1987, the endothelial-derived relaxing factor was shown to be NO by Louis Ignarro (Ignarro et al., 1987) and Salvador Moncada (Palmer et al., 1987). The biological significance of nitric oxide was recognized by

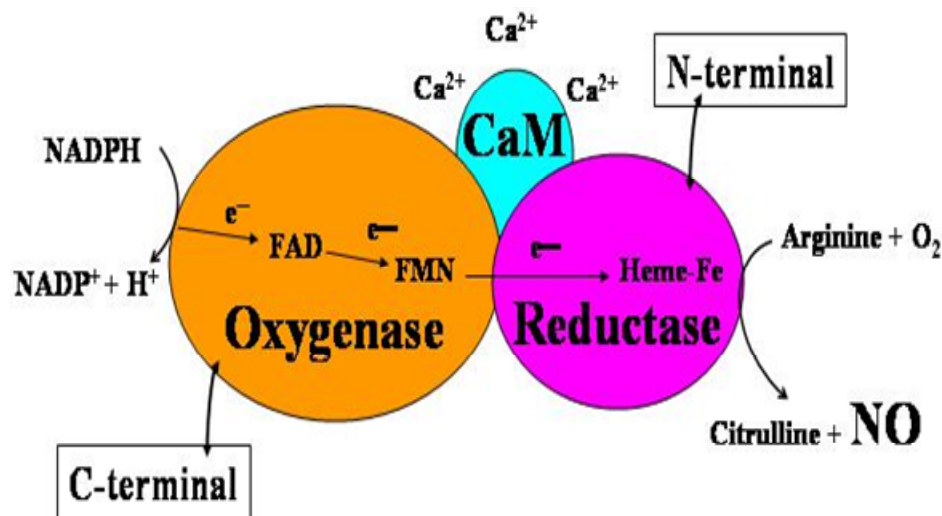
scientific community in 1992. The free radical NO was named as the 'molecule of the year' (Koshland, 1992). Much earlier to this, NO emission from plants was observed by Klepper in 1975 in soybean plants treated with herbicides (Klepper, 1979). Nitric oxide (NO) is a gaseous radical with a wide variety of physiological and pathological implications in animal, plant and microbes (Lamattina et al., 2003; Neil et al., 2003, 2008). This gaseous free radical diffuses readily through the biological membranes. The half-life of NO in biological tissues is reported to be less than 6 s (Thomas et al., 2001).

This short half-life reflects the highly reactive nature of the molecule. NO reacts directly with metal complexes and other radicals, and indirectly as a reactive nitrogen oxide species with biomolecules such as nucleic acid,

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**Table 1.** The different forms of NO synthase.

Types of NOS	Coding gene(s)	Location	Function
nNOS, NOS1	NOS1	Nervous tissue	Cell communication
iNOS, NOS2	NOS2A, NOS2B, NOS2C	Cardiovascular immune system	Immune defense against pathogens
eNOS, NOS3	NOS3	Endothelium	Vasodilation



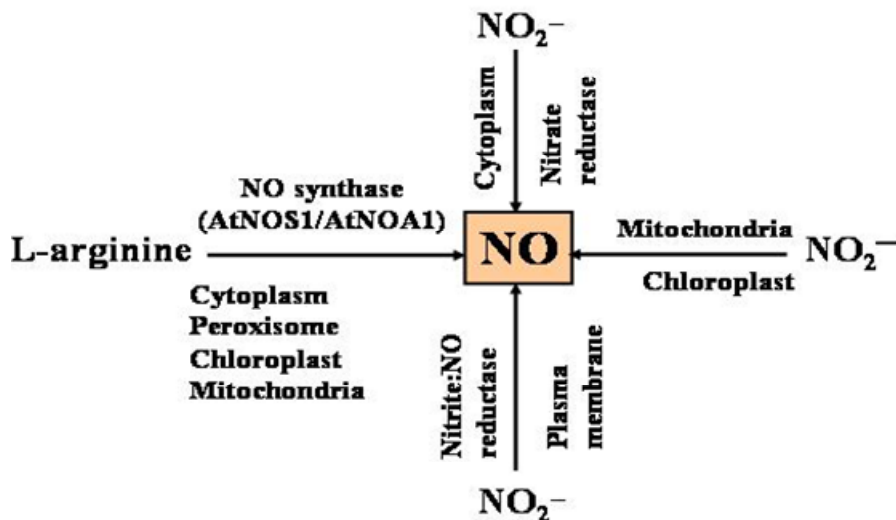
**Figure 1.** The C-terminal oxygenase and N-terminal reductase domains of nitric oxide synthase (NOS; EC1.14.13.3) with its regulatory calmodulin (CaM) domain bridging the two domains in tandem for the reduction of arginine to citrulline and the production of nitric oxide (NO).

proteins, and lipids (Wink and Mitchell, 1998). A large amount of NO may combine with superoxide ( $O_2^{\cdot-}$ ) to form peroxynitrite ( $ONOO^-$ ), which has been reported to damage lipids, proteins and nucleic acids (Yamasaki et al., 1999). In animals, NO is established as a signal transduction molecule that functions in many tissues and interacts with multiple target compounds in neurotransmission, vascular smooth muscle relaxation, and platelet inhibition (Lloyd-Jones and Bloch, 1996.). The roles of NO in plants may be equally diverse. There is an increasing evidence that NO is involved in many key physiological processes in plants also (Laxalt et al., 1997; Zhao et al., 2004). Several recent reviews covered the events in the synthesis and action of NO. In this review, we report an up to date assessment of the mode of action and signaling aspects of NO in plants.

### SYNTHESIS OF NITRIC OXIDE IN PLANTS

The synthesis of NO in animals is primarily accomplished by three different isoforms of nitric oxide synthases (NOS) (Stuehr, 1999; Alderton et al., 2001). The three isoforms of NOS are iNOS (for inducible NOS), eNOS (for

endothelial NOS), and nNOS (for neuronal NOS) (Table 1). All three forms are bi-domain enzymes related to cytochrome P450 enzymes (Stuehr, 1999; Alderton et al., 2001; Li and Poulos, 2005). The C-terminal reductase domain contains the NADPH binding site as well as FAD and FMN cofactors (Knowles and Moncada, 1994; Alderton et al., 2001). The C-terminal oxygenase domain contains a proto-porphyrin IX haem iron and tetrahydrobiopterin ( $H_4B$ ) and the binding sites for arginine and oxygen. Electrons are shuttled from NADPH through the flavins to the haem and then to oxygen, which then reacts with guanidino nitrogen of arginine producing N-hydroxyarginine (NOHA). NOHA is oxidized further to produce NO and citrulline. Between the two domains is a site that binds calmodulin, which activates the enzyme (Moncada et al., 1991; Ignarro, 2002). These enzymes vary from 130 to 160 kDa in size, form dimers, and are about 50 to 60% identical in mammals (Crawford, 2006). The overall reactions for all these three isoforms are same as shown in Figure 1. The main function of the subunits of the enzyme polypeptide is (i) NADPH-dependent oxidation of L-arginine to N-hydroxy arginine and (ii) then to NO and citrulline (Figure 1). The location, regulation, properties, and discovery of these enzymes



**Figure 2.** Different biochemical pathways and their localization for the synthesis of NO in plants.

differ, and correspond to their respective functions. Since 1996 there have been an increasing number of reports showing the presence of nitric oxide synthase activity in plants similar, to a certain extent, to mammalian NOS. 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).

Although, in animal systems, NO is synthesized predominantly by the enzyme Nitric oxide synthase (NOS), but in plants it is synthesized by three different mechanisms viz: (i) NOS, (ii) Nitrate reductase (NR) and (iii) Nitrite: NO reductase (NiR) as shown in Figure 2. There are also other enzymatic and non-enzymatic processes in plants through which NO is generated in plant systems. The details of these pathways are illustrated in Figure 2.

### NOS mediated NO synthesis

To demonstrate the existence of NOS in plants, different approaches were used, based on biochemical and physico-chemical and immunological and methods. Cueto et al. (1996) and Ninnemann and Maier (1996) were the first to show the presence of NOS activity in higher plants by using the method of conversion of radiolabelled arginine, the substrate of NOS, into radiolabelled citrulline. Another method which has been widely used is the measurement in crude extracts incubated with L-arginine plus all the NOS cofactors, the NO production sensitive to NOS inhibitors by fluorometry or chemiluminescence. In crude extracts from sorghum embryonic axis the NOS activity-derived production of NO in the reaction mixture has been recently determined

by spin trapping electron paramagnetic resonance (EPR) spectroscopy, using as spin trap a complex formed by Fe(II) and dithiocarbamate  $[\text{Fe}(\text{MGD})_2]$  (Simontacchi et al., 2004). Using all these methods together, the occurrence of NOS activity was demonstrated in peroxisomes from pea plants (Barroso et al., 1999; Corpas et al., 2002, 2004). Different antibodies raised against NOS from mammalian origin were used to study the existence of NOS in plants by western blot analysis and immunogold electron microscopy (Kuo et al., 1995; Sen and Cheema, 1995). However, Butt et al. (2003) in a proteomic study in extracts from maize embryonic axis with polyclonal rabbit antibodies against human nNOS and mouse iNOS found that many apparently NOS-unrelated proteins were recognized by the antibodies. On this basis, these authors cast doubts upon the results of NOS presence in plants obtained using immunological techniques with mammalian NOS antibodies. The lack of specificity of antibodies can be sometimes a problem in immunochemical assays and should always be carefully considered.

### NR mediated NO synthesis

Nitrate reductase (NR) is one of the oldest known enzyme for NO generation (Dean and Harper, 1988). Using recent techniques in plant biology, the involvement of NR in NO production has been further established by growing *nia* mutants in NR-free media (Planchet et al., 2005). The enzyme NR, reduces nitrate to nitrite in the expense of NAD(P)H, further catalyzes a 1-electron transfer from NAD(P)H to nitrite resulting in NO formation in the cell free systems also (Neill et al., 2003; Planchet

et al., 2005). However, *in vivo* oxygenic cell free system suffers from the autooxidation of NO or by its reaction with ROS produced simultaneously by NR thereby yielding low NO (Yamasaki and Sakihama, 2000).

### NiR mediated NO synthesis

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 cyano-bacterium *Anabaena doliolum* (Mallick et al., 1999). A plasma membrane-bound, root-specific enzyme, nitrite-NO oxidoreductase (Ni-NOR), using cytochrome c as an electron donor *in vivo* and having a comparatively reduced pH optimum is reported by Stohr and Stremlau (2006). In the most recent study Kaisers group (Ruemer et al., 2009a, b, Gupta and Kaiser 2010) showed experimental evidences for NO production in plant cells by Nitrite-dependent NO production under anoxic condition, and localized in and mediated by the electron transport chain in the mitochondrial membranes.

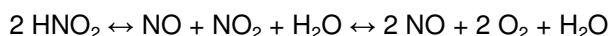
### Other sources of NO production in plants

**(a) Enzymatic:** 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). Other heme proteins that have been proposed as good candidates for the enzymatic generation of NO are cytochromes P<sub>450</sub>. These proteins are present in plants as well as in animal systems and have been shown to catalyze the oxidation of NOHA by NADPH and O<sub>2</sub> with the generation of NO (Boucher et al., 1992; Mansuy and Boucher, 2002). Hemoglobin and catalase were also reported to produce NO and other nitrogen oxides by catalyzing the oxidation of NOHA (N-hydroxyarginine) by cumyl hydroperoxide (Boucher et al., 1992).

**(b) Non-enzymatic NO production:** The generation *in vivo* of NO by the reaction of H<sub>2</sub>O<sub>2</sub> and L-arginine is reported by Nagase et al. (1997) and Gotte et al. (2002). Nitrification/denitrification cycles provide NO as a by-product of N<sub>2</sub>O oxidation into the atmosphere (Wojtaszek, 2000). It is known that the non-enzymatic reduction of nitrite can lead to the formation of NO, and this reaction is favoured at acidic pH when nitrite can dismutate to NO and nitrate (Stohr and Ullrich, 2002). Nitrite can also be chemically reduced by ascorbic acid at pH 3 to 6 to yield NO and dehydroascorbic acid (Henry et al., 1997). This reaction could occur at microlocalized pH conditions in

in the chloroplast and apoplastic space where ascorbic acid is known to be present (Horemans et al., 2000). In barley aleurone cells, NO can also be synthesized by reduction of nitrite by ascorbate at acidic pH (Beligni et al., 2002). A light-mediated reduction of NO<sub>2</sub> by carotenoids is also reported (Cooney et al., 1994).

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



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

A comprehensive summary of the production of NO and compartmentalization of NO synthesis in plants is given in Figure 3.

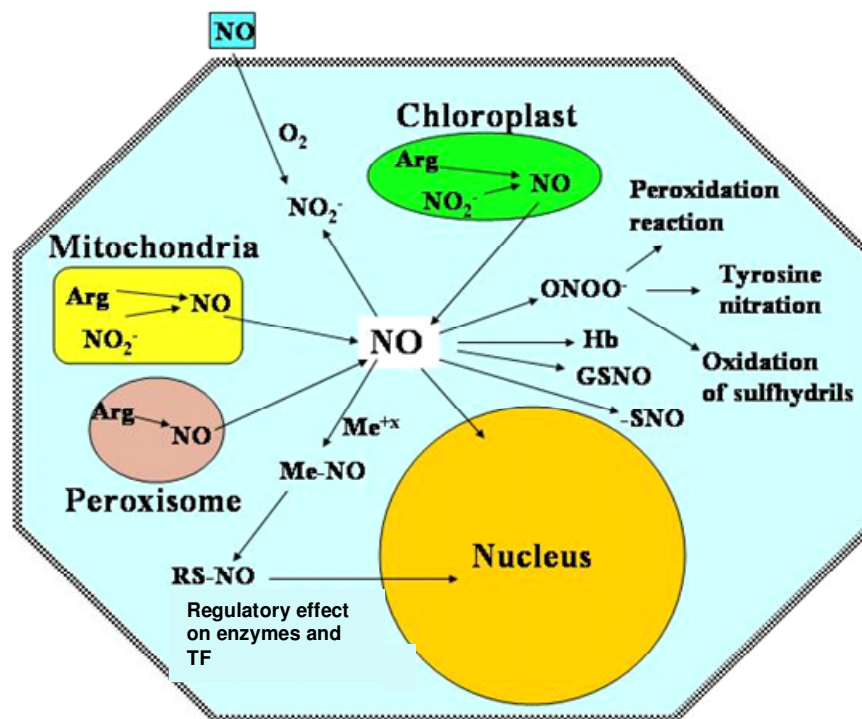
### NO MOVEMENT AND DETOXIFICATION IN PLANTS

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 molecules. It can diffuse out of the cell across the plasma membrane into adjacent cells. NO is lipophilic and may accumulate preferentially in membranes and could move through such a passage or barrier (Liu et al., 1998). NO is a reactive free radical molecule. So, NO synthesized in living systems must be rapidly removed or metabolized after inducing the initial signalling events, if any. Nitric oxide is unstable and readily reacts 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) possibly leading to protein S-nitrosylation in the NO signaling pathway. 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.

### METHODS FOR NO DETECTION AND MEASUREMENT IN PLANTS

Nitric oxide is a gaseous and highly unstable free



**Figure 3.** Compartmentation of nitric oxide synthesis, reaction, perception and signaling in plant. The nucleus, chloroplast, mitochondria and peroxisome compartment is highlighted with different colours. The cytoplasm is coloured blue. NO is readily diffusible through the matrix of plant cell wall.

radical and its detection and quantification in plants involves methodological difficulties. In comparison with mammalian tissues, there are not many reports of direct measurement of NO in plants and the methods used came from studies in animal systems with some adaptations to different plant tissues. The main methods used to assay NO in plants include: gas chromatography and mass spectrometry (Neill et al., 2003); spectrophotometric measurement of the conversion of oxyhemoglobin to methemoglobin (Orozco-Cardenas and Ryan, 2002); laser photo-acoustic spectroscopy (Leshem and Pinchasov, 2000); spin trapping electron paramagnetic resonance (EPR) spectroscopy (Caro and Puntarulo, 1999; Pagnussat et al., 2002; Corpas et al., 2002, 2004; Dordas et al., 2004; Huang et al., 2004); the nitric oxide electrode (Leshem and Haramaty, 1996; Yamasaki et al., 2001); and ozone chemiluminescence (Morot-Gaudry-Talarmain et al., 2002; del Rio et al., 2003a). Nevertheless, the use of 4, 5-diaminofluorescein diacetate (DAF-2 DA) as fluorescent probe has become a common and very sensitive technique to detect NO in plant systems (Nakatsubo et al., 1998; Nagano and Yoshimura, 2002). This probe has been used in plant cells to obtain realtime bioimaging of NO with fine temporal and spatial resolution (Foissner et al., 2000; Pedroso et al., 2000a; Neill et al., 2002b; Corpas et al.,

2002; Lamattina et al., 2003; Gould et al., 2003). A new alternative to this fluorescent probe is 3-amino-4-(N-methylamino)-20,70-difluorofluorescein diacetate (DAF-FM DA) which fluoresces in almost all pH range within the cell and *in vivo* is also suggested by Zhang et al. (2003). Using these techniques, studies have been done in plants for the possible action of NO in various biochemical and physiological processes in plants.

### SEED TO SEED - NO EFFECT ON PLANT GROWTH AND DEVELOPMENT

Leshem (1996) first reported the effect of NO on plants. NO is reported to break seed dormancy in *Arabidopsis* and barley (Bethke et al., 2004), and reduced seed dormancy in lettuce (Beligni and Lamattina, 2000), *Arabidopsis* (Bethke et al., 2006), and barley (Bethke et al., 2004a). NO also promoted seed germination in lupin (Kopyra and Gwozdz, 2003), canola (Zanardo et al., 2005) and *arabidopsis* (Libourel et al., 2006). It regulates 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, 2005), and gravitropic bending (Hu et al., 2005) is also regulated by NO. Greening of etiolated leaves is studied as a model for chloroplast development and its regulation in plants (Dash et al., 1999; Misra et al., 1998; Misra and Misra, 1987, 1992). NO stimulates chlorophyll biosynthesis and chloroplast differentiation (Graziano et al., 2002; Zhang et al., 2006). NO also activated the growth of root segments of maize comparable to that by indole acetic acid (Gouvêa et al., 1997).

High concentrations of NO inhibited the growth of tomato whereas low concentrations stimulated the growth of tomato, lettuce (Hofton et al., 1996) and pea seedling (Leshem and Haramaty, 1996). Stomatal closure which is regulated by abscisic acid (ABA) is also regulated by NO signal. Involvement of NO produced by NOS (Guo et al., 2003), NR (Desikan et al., 2002; Garcia-Mata and Lamattina, 2003), protein S-nitrosylation (Sokolovski and Blatt, 2004) and Ca<sup>2+</sup>-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.

NO also, 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). NO also regulates programmed cell death (PCD) in the aleurone layers (Beligni et al., 2002). PCD in animals can be divided into the processes such as involving (i) death receptors or (ii) 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<sub>2</sub><sup>-</sup> 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 several biotic stresses (Simons et al., 1999) or the proteinaceous bacterial elicitor Harpin (Krause and Durner, 2004). The mitochondrial respiratory electron transport chain includes abundant proteins containing transition metals such as heme, thereby being a major target of NO in animal cells (Wink and Mitchell, 1998). In fact, NO inhibits the ATP synthesis (oxidative phosphorylation) in both animal (Brookes et al., 1999) and plant (Yamasaki et al., 2001).

The progression of growth in plants can be distinctly delineated to three phases viz. development, maturation and senescence phase (Dilnawaz et al., 2001; Misra and Misra, 1989, 1991). All these phases are in common

described as aging process, although many authors used the post maturation phase of growth as aging using similar connotations in animals (Misra and Biswal, 1980, 1981, 1982, 1987; Biswal et al., 2001). However to distinguish it from other developmental processes the post maturation accelerated aging or degradative phase of plant life leading to death is called 'senescence' (Misra and Misra, 1986, 1989; Misra, 1993a). The catabolic processes are accelerated during senescence which in a sequential and orderly manner leads to death of the organelle, organ or the organism and is thus a process of programmed cell death (Thomas et al., 2003). A number of physiological, biochemical and molecular changes occur in living systems during aging or senescence. The most important is the external symptoms of leaf yellowing or chlorophyll catabolism (Misra and Biswal, 1980, 1981), loss of photosynthetic activity (Misra and Biswal, 1982, 1987; Dilnawaz et al., 2001; Biswal et al., 2001) and increase in the ROS (reactive oxygen species) (Misra, 1993b; Misra and Misra, 1989) and expression of SAG genes (Buchanan-Wollaston, 1997; Gepstein et al., 2003). Senescence implants is retarded by several growth promoters like cytokinins, auxins or gibberellins, and is promoted by growth retardant like abscisic acid (ABA), jasmonate (JA), ethylene and darkness (Misra and Misra, 1991; Misra and Biswal, 1980; Hung and Kao, 2003, 2004).

Accelerated dark-induced senescence is accompanied with increased ROS levels and protein oxidation (Guo and Crawford, 2005). NO has been shown to act as an antioxidant in other situations. NO donors protect from oxidative damage caused by methylviologen herbicides, and counteract ROS-mediated programmed cell death in barley aleurone layers (Beligni and Lamattina, 1999; Beligni et al., 2002). Thus, a probable action of NO in retardation of senescence by decreasing the ROS accumulation. As a free radical, NO reacts with superoxides in diffusion limited reaction to form peroxynitrite (Huie and Padmaja, 1993) and a subsequent fast isomerization of this toxic compound to a harmless end product like nitrate represents a possible mechanism to reduce ROS levels and cell damage through oxidative stress.

## NO AND PLANT GROWTH REGULATORS

NO induced the elongation of maize root segments in a dose-dependent manner like that of auxins (Gouvea et al., 1997). It has, therefore, been proposed that the auxin, indole acetic acid (IAA) and NO might share some common steps in the signal transduction pathway because both elicit the same responses in plants. Explants from wood species were responsive to NO treatment in inducing adventitious root formation (Lamattina et al., 2001). In cucumber explants, IAA treatment induces a transient increase in the level of endogenous NO in the basal region of the hypocotyl

(Pagnussat et al., 2002). This localized NO bulk might stimulate the GC-catalyzed synthesis of cGMP (McDonald and Murad, 1995) thereby promoting the adventitious root formation. The GC inhibitor reduced adventitious root formation in both IAA and NO-treated cucumber explants (Pagnussat et al., 2002). Earlier in tobacco, activation of defense genes by NO was also induced by cGMP (Durner et al., 1998). These genes may act via cADPR that, in turn, regulates  $Ca^{2+}$  level in plants (Leckie et al., 1998). Variations in  $[Ca^{2+}]$  might play a role in the signal transduction pathway leading to the activation of the process of mitotic differentiation to initiate rooting. NO can also act via a cGMP-independent pathway, activating phosphatases and protein kinases including MAPKs. Interestingly, a rapid and transient increase in MAPK activity in response to low level of auxins was reported in Arabidopsis seedling roots (Pfeiffer et al., 1994). IAA-induced endogenous NO bulk in roots can result in a bifurcated signal transduction pathway in which NO mediates a cGMP-dependent or independent increase in cytosolic  $Ca^{2+}$ , which in turn triggers changes in plant gene expression leading to the auxin response.

Similarly cytosolic free  $Ca^{2+}$  concentration ( $Ca^{2+}_{cyt}$ ) and cytosolic pH have been reported to participate as second messengers in ABA regulated responses (Blatt and Grabov, 1997; Blatt, 2000). An exogenous application of NO to epidermal strips to induced stomatal closure through a  $Ca^{2+}$ -dependent process (Garcia-Mata and Lamattina, 2001). Moreover, it was also reported that ABA-induced NO which mediates stomatal closure (Garcia-Mata and Lamattina, 2002; Neill et al., 2002a)

Cytokinins (CKs) stimulate photomorphogenic responses, mainly those related to the de-etiolation and pigment synthesis (Misra and Biswal, 1980, 1981; Thomas et al., 1997). In exogenous application, CKs have been reported to inhibit hypocotyl elongation in seedlings grown in the darkness (Chory et al., 1994, Beligni and Lamattina, 2000). CK has an ability to abolish the lag phase because in chlorophyll production during illumination (Dei, 1982). NO has also been reported to slightly increase the chlorophyll level in wheat seedlings grown in the dark (Beligni and Lamattina, 2000). NO plays the same role as CK action on betacyanin accumulation. Moreover, NOS inhibitor and an NO scavenger blocked the action of CKs on betacyanin accumulation (Scherer and Holk, 2000). The first evidence suggesting a direct relationship between CKs and NO production was that the exogenous application of CKs to Arabidopsis, parsley, or tobacco cell cultures leads to a rapid stimulation of NO release (Tun et al., 2001). Thus, the effect of NO is either similar or mediated through similar signaling pathway as that of CKs.

Phytochrome mediated and GA stimulated seed germination is shown also to be regulated by NO (Thomas, 1984, Beligni and Lamattina, 2000). However, the interaction of GA and NO in promoting germination

through the same or different pathways are yet to be deciphered.

Ethylene plays an active role in many plant responses (Abeles et al., 1992). NO and ethylene caused an antagonistic effect during maturation and senescence of the plant (Leshem et al., 1998), and fruit ripening (Leshem and Pinchasov, 2000). The unripe green fruits of strawberries and avocados contain high NO and low ethylene concentrations in contrast to the maturing fruits in which NO content decreased with a concomitant increase in ethylene (Leshem and Pinchasov, 2000).

SA is a key signal in the systemic acquired resistance (SAR) or the induced innate resistance (Durrant and Dong, 2004) and is independent of avr-R gene interaction and is linked to the systemic expression of genes such as PR proteins responsible for the potentiation of plant defense (Mur et al., 1996). The tobacco SIPK (SA-induced protein kinase) and WIPK (wounding-induced protein kinase) are activated upon infection, treatment with elicitors, and in response to other types of abiotic stress. SA induces SIPK by  $H_2O_2$  (Yang et al., 2001) and shows SA mediated NO inducibility (Kumar and Klessig, 2000). NO confers SAR against TMV through an SA-dependent mechanism (Song and Goodman, 2001). Probably, S-nitrosoglutathione formed in the vicinity of the HR is loaded into phloem, systemically dispersed, and unloaded to initiate systemic SA synthesis (Maldonado et al., 2002).

But this hypothesis is yet to be established and is questioned (Feechan et al., 2005). The NO-induced defence genes PR1 (pathogenesis related protein 1) and PAL1 have been linked to the synthesis of SA (Uknes et al., 1992; Mauch-Mani and Slusarenko, 1996) and NO was shown to cause SA accumulation (Durner et al., 1998), but suppressed defence signalling by JA (Orozco-Cardenas and Ryan, 2002; Huang et al., 2004). SA plays a central role in HR-mediated resistance (Delaney et al., 1994; Cao et al., 1997; Mur et al., 2000) partially through the induction of PR proteins but also through the potentiation of a wider range of defence genes and the oxidative burst (Kauss and Jeblick, 1995; Mur et al., 2000; Conrath et al., 2002). By initiating SA synthesis, NO is likely to participate in this potentiation mechanism (Van Camp et al., 1998).

However, SA-mediated defence is not effective against all plant-pathogenic organisms. For example, defence against tissue-macerating necro-trophic pathogens is mediated by JA (Thomma et al., 1998) and, predictably, resistance against the necrotrophic fungus *Botrytis cinerea*, was compromised by the application of NO donors (Malolepsza and Rozalska, 2005). The expression of 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, NO delayed wounding induced IPO expression (Jih et al., 2003).

## ROLE OF NO IN ABIOTIC STRESS RESPONSES

Studies so far show that NO is involved in stress response in plants. Among the stress factors salt (Biswal et al., 2001; Misra et al., 1990), water (Misra et al., 2002; Misra and Misra, 1991), temperature both as heat or chilling temperature (Misra et al., 1998; Misra and Terashima, 2003), oxidative stress (Mittler, 2002; Tuncz-Ozdemir et al., 2009), other abiotic and biotic stress (Misra et al., 2001, 2002) affect plant growth, development and metabolism.

Salt stress affects seed germination (Misra et al., 1990) retarded shoot growth (Misra et al., 1995, 1996, 1997a), root growth (Misra et al., 1997b), photo-synthetic efficiency (Misra et al., 1999, 2001; Sahu et al., 2008) in both monocots and dicots. Pretreatment of seed or seedlings with NO donor resulted in better growth and viability of young rice seedlings (Uchida et al., 2002), promoted seed germination and root growth of yellow lupine seedlings (Kopyra and Gwozdz, 2003), and increased the growth and dry weight of maize seedlings (Zhang et al., 2006b) under salt stress conditions. Salt stress induces oxidative damage to plant systems (Misra et al., 2003, 2006; Biswal et al., 2001a, 2001b.).

Under NaCl stress, the wild type *Arabidopsis* plants exhibited higher survival rates than *Atnoa1* plants which have a reduced level of endogenous NO (Guo et al., 2003; Zhao et al., 2007b). Exogenous NO application to *Atnoa1* mutants alleviated the oxidative damage caused by NaCl stress. NO enhanced salt tolerance in maize seedlings, through an increased level of  $K^+$  and decreased level of  $Na^+$  accumulation in all plant parts (Zhang et al., 2004). Both NO and NaCl treatment stimulated vacuolar  $H^+$ -ATPase and  $H^+$ -PPase activities, resulting in increased  $H^+$ - translocation and  $Na^+/H^+$  exchange (Zhang et al., 2006). Callus cultures are considered as a model system to study the cellular basis of stress tolerance in plants (Misra et al., 1990; Das et al., 1990, 1992) NO induced salt resistance of calluses from *Populus euphratica* under salt stress also increased by increasing the  $K^+/Na^+$  ratio, and this process was mediated by  $H_2O_2$  and was dependent on the increased plasma membrane  $H^+$ -ATPase activity (Zhang et al., 2007). In addition, NO was observed to stimulate the expression of plasma membrane  $H^+$ -ATPase in both salt-tolerant and salt-sensitive reed calluses (Zhao et al., 2004; Qiao and Fan, 2008). NO may enhance salt tolerance in plants via increasing the expression of plasma membrane  $Na^+/H^+$  antiporter gene and  $H^+$  ATPase genes that are required for  $Na^+$  homeostasis and  $K^+$  acquisition.

Water stress is one of the most critical factors affecting plant metabolism starting from seed viability, germination, seedling growth, crop plant establishment to limiting crop productivity (Misra et al., 2001). Water stress affects plants in two different ways- (i) osmotic stress and (ii) soil drought stress. The later predominantly prevails in the

field conditions for plants. But the former osmotic stress or shock can occur due to changes in atmospheric humidity, temperature, rainfall, soil moisture and ion content etc (Misra et al., 2002). Water stress affects seed germination (Misra and Misra, 1991; Misra et al., 2002), seedling vigour (Misra, 1993, 1994), root growth (Misra and Misra, 1988), photosynthetic efficiency (Misra et al., 2001, 2002) and crop productivity (Misra, 1991). Drought promoted the NO production in pea, wheat and tobacco (Leshem and Haramaty, 1996; Gould et al., 2003). NO reduced leaf dehydration, ion leakage, the transpiration rate and induced stomatal closure, thereby enhancing plant tolerance to drought stress (Garcia-Mata and Lamattina, 2001). NO scavenger could revert the above actions (Garcia-Mata and Lamattina, 2001). The NO regulation of stomatal closure may be via modulating intracellular  $Ca^{2+}$  in guard cells (Figure 5). It is reported that NO selectively activates intracellular  $Ca^{2+}$  channels in *Vicia faba* guard cells through a cGMP/cADPR-dependent signaling pathway, suggesting the involvement of NO as a signaling molecule in the ABA induced stomatal closure (Garcia-Mata et al., 2003). Desikan et al. (2004) established this hypothesis through the involvement of nitrate reductase (NR)-mediated NO synthesis in *Arabidopsis* guard cells responsive to ABA, and was shown to be required for ABA-induced stomatal closure. NO and ROS were reported to participate in the osmotic tolerance of wheat seedlings by stimulating ABA biosynthesis (Xing et al., 2004) and NO evolution in stressed reed plants impart osmotic tolerance through its protection against oxidative damage (Zhao et al., 2008).

It was shown that both in tobacco leaf peels and suspension cells, high temperature generated a rapid and significant surge in NO levels (Gould et al., 2003). Leshem (2001) reported that short term heat stress increased the NO production in alfalfa, which negatively correlated with ethylene production. Heat stress accelerates chlorophyll bleaching (Misra, 1980, 1981) and affected photosynthetic efficiency in rice (Misra and Misra, 1986), *V. faba* (Misra et al., 2001, 2007; Misra and Terashima, 2003) and pothos leaves (Misra et al., 1997). NO reduced heat-induced damage in rice seedlings and prevented the impairment of photo-system II (PSII) (Uchida et al., 2002). Lamattina et al. (2001) observed that treatment with SNP increased the survival rate of leaves of wheat and maize seedlings under extreme temperatures. The role of NO during extreme temperature stress might be through decreasing the ROS level caused by heat or lower temperature (Neill et al., 2002b).

Herbicides are one of the major sources of soil, air and water pollution in the environment and one of the primary target of action of herbicides is photosystem II (Vladkova et al., 2009). Exogenous NO has been shown to reduce herbicide toxicity by its protective effects on chloroplast membrane and by retarding herbicide induced loss of chlorophyll (Beligni and Lamattina, 1999; Hung et al.,



2002). The paraquat induced reduction in leaf protein content was prevented by NO (Huang et al., 2002). Diquat-triggered lipid peroxidation, ribulose-1, 5-biphosphate carboxylase/ oxygenase (rubisco) and D1 protein loss and mRNA breakdown were markedly reduced by NO (Beligni and Lamattina, 2000b). Moreover, diquat caused an increase in the rate of photosynthetic electron transport in isolated chloroplasts, and NO restored it back to the control levels (Beligni and Lamattina, 2000b).

Mechanical stress or damage was reported to elicit NO production through NOS activity in Arabidopsis leaves (Garces et al., 2001), *Kalanchoeda igremontianna* and *Taxes brevifolia* callus cells, followed by DNA fragment and cell death (Pedroso et al., 2000a,b,c). However, such events were not observed in *Lycopersicon* or *Nicotiana* (Orozco-Cardenas and Ryan, 2002; Gould et al., 2003).

Wounding of the leaf epidermis in Arabidopsis induced a burst in NO within minutes (Huang et al., 2004) but had no effect on either the jasmonic acid (JA) levels or JA responsible genes (Glazebrook, 2001). NO increases the SA level (Durner et al., 1998; Durner and Klessig, 1999; Huang et al., 2004; Durner et al., 1998). But, SA does not always play a role in NO-induced gene expression. The results from the wound induced signaling pathways and gene regulation by NO focuses a network regulation on oxidative stress during wounding and wound induced metabolic changes in plants (Imanishi et al., 1997; Jih et al., 2003; Leon et al., 2001).

## NO ROLE IN BIOTIC STRESS RESPONSES

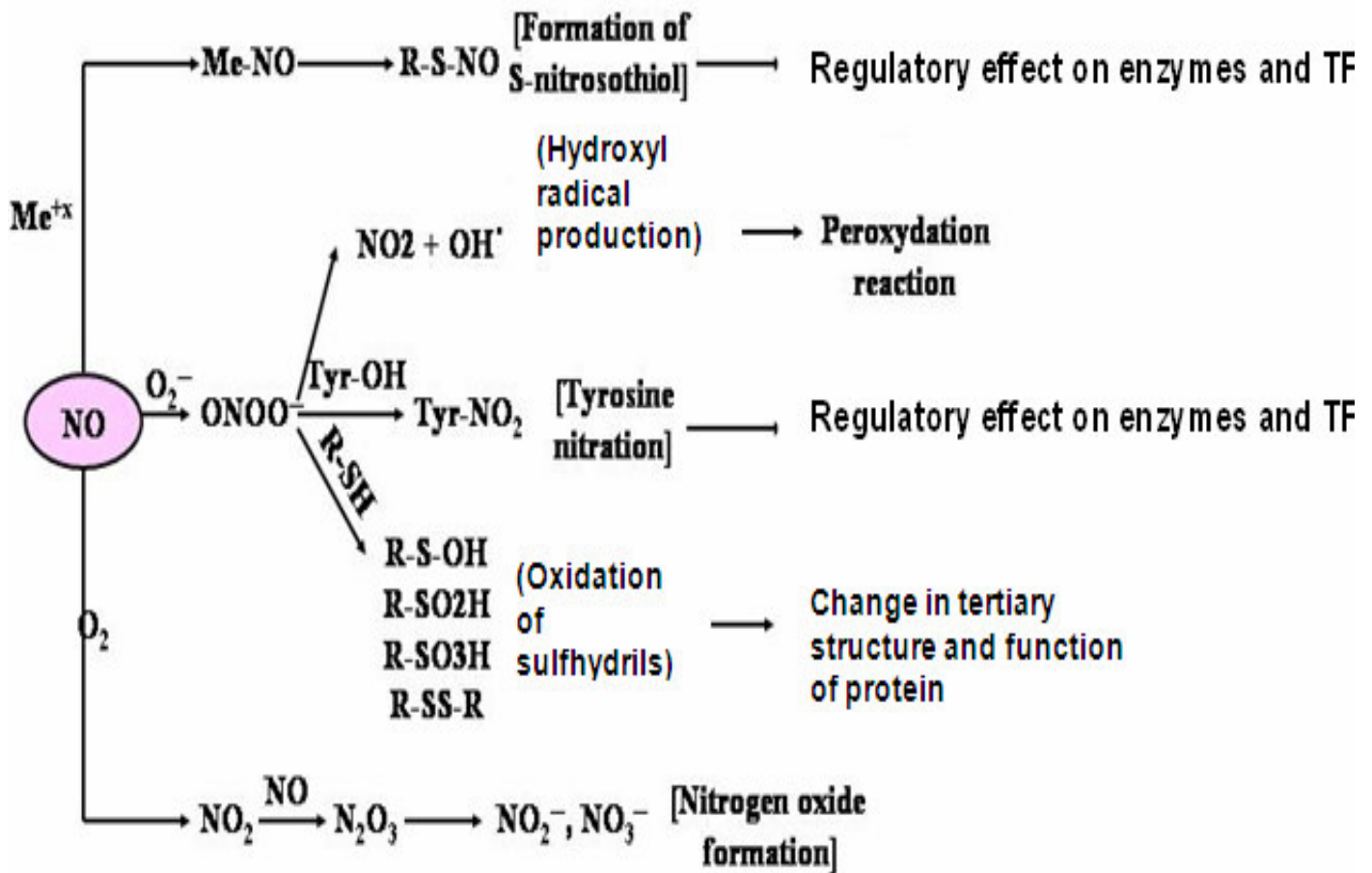
Plants respond to pathogen infection by inducing local and systemic defence reactions. The local hypersensitive response is characterized by the development of lesions through PCD or cellular apoptosis restricts the growth and spread of pathogens (Delledonne et al., 1998; Krause and Durner, 2004; Tada et al., 2004). The hypersensitive reactions induce defense-related gene expression for the synthesis of antimicrobial enzymes and toxic secondary metabolites 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 pathogen combating molecules *in situ*. In animals, ROS (generated by NADPH oxidase) simultaneously with NO is generated mainly by inducible NO synthase (NOS) to regulate apoptosis and kill 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 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, example, (i) in tobacco with a tobacco-mosaic virus (Durner et al., 1998; Modolo et

al., 2005) and (ii) 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; Planchet et al., 2004). These reports suggest that NO plays a crucial role in the early events of pathogen resistance responses in plants. So far the evidences suggest that NO alone may not be sufficient for the induction or propagation of PCD but it influences gene expression leading to PCD.

## NO ACTION THROUGH ANTIOXIDATIVE EFFECTS

Most of the stress responses described above induces the formation of ROS and thereby causes damage to plant systems (Mittler, 2002; Tuncz-Ozdemir et al., 2009). UV-light increase ROS accumulation and 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 (Shi et al., 2005). Exogenous NO provide some protection against the oxidative damage associated with water stress (Garcia-Mata and Lamattina, 2001). Several other studies have also shown that exogenous NO ameliorates the oxidative stress induced by a range of abiotic conditions including those resulting from the presence of heavy metal ions, salinity, high temperatures, H<sub>2</sub>O<sub>2</sub>, dehydration, UV irradiation, and paraquat (Cheng et al., 2002; Hung et al., 2002; Uchida et al., 2002; Kopyra and Gwozdz, 2003; Zhang et al., 2003, 2006; Hsu and Kao, 2004; Hung and Kao, 2005; Laspina et al., 2005). Some of the antioxidant effects of NO may be due to its direct interaction with ROS such as superoxide to form peroxynitrite that might then be scavenged by other cellular processes (Figure 4). In other cases NO may enhance the antioxidant capacity of cells by increasing the activities of antioxidant enzymes such as superoxide dismutase, which converts superoxide to H<sub>2</sub>O<sub>2</sub>, and catalase and ascorbate peroxidase, which both remove H<sub>2</sub>O<sub>2</sub> (Cheng et al., 2002; Hung et al., 2002; Uchida et al., 2002; Kopyra and Gwozdz, 2003; Zhang et al., 2007). Whether or not endogenous NO has an antioxidant function is debatable. Guo and Crawford (2005) detected greater levels of oxidized proteins and lipids in the *Atnos1* mutants with enhanced sensitivity to the oxidative stress induced by either methyl viologen or salinity. This can be ameliorated by exogenous NO (Zhao et al., 2007; Zhao et al., 2007). Moreover, in the case of stress-tolerant and stress-sensitive plants, stress induces endogenous NO generation in the tolerant, but not in the sensitive ecotype (Zhao et al., 2004; Song et al., 2006). A similar study in *Populus callus* and *Stylosanthes guianensis* showed that stress tolerance is associated with the stress-induced endogenous NO synthesis (Zhou et al., 2005, F Zhang et al., 2007). Given that various stresses induce ABA and, as a consequence NO, and given that NO appears to



**Figure 4.** NO perception, complexation and interaction with biological molecules before inducing signaling events in plant.

enhance antioxidant enzyme activity, one might expect a signaling link between ABA, NO, and oxidative stress tolerance.

**MODE OF ACTION AND NO MEDIATED SIGNALING IN PLANTS**

Nitric oxide (NO) has emerged as an important endogenous signalling molecule in plants that mediates many developmental and physiological processes including xylogenesis, programmed cell death, pathogen defence, flowering, stomatal closure etc. (Delledonne, 2005; Lamattina et al.,2003; Neil et al.,2003). NO is growth promoting at low concentrations, but is inhibitory or toxic at high concentrations (Beligni and Lamattina, 1999) and being reactive, is perhaps unlikely to travel far between or even within cells. No doubt plants perceive and respond to NO but as no specific plant NO receptor has been identified, postulates from mammalian receptors are used as models for plants. 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 nitrosylate proteins on tyrosine residues. It is not yet known whether this reaction has signalling consequences. Comprehensive steps in NO perception mechanism in plants are shown in Figure 3.

**NO 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.

Polverari et al. (2003) studied the NO induced changes of expression profiles of 2500 transcripts of *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 up-regulated 342 but 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 transcripts induced by NO were those coding for oxidative stress-related proteins (GSTs, ABC transporters), iron homeostasis proteins (example, ferritin genes), signal transduction factors (example, 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-derived induction of defence-related genes was shown for Pr-1 and Pal in tobacco (Durner et al., 1998) and PAL and CHS in soybean (Delledonne et al., 1998). 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. NO was shown to induce the expression of the *LjHb1* gene coding for non-symbiotic haemoglobin in *Lotus japonicus* (Shimoda et al., 2005).

## SECOND MESSENGER MEDIATED NO SIGNALLING

In mammalian systems cGMP is produced when NO bind to heme in the guanylate 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. Taking this corollary, Modolo et al. (2005) suggested that plants and animals probably use

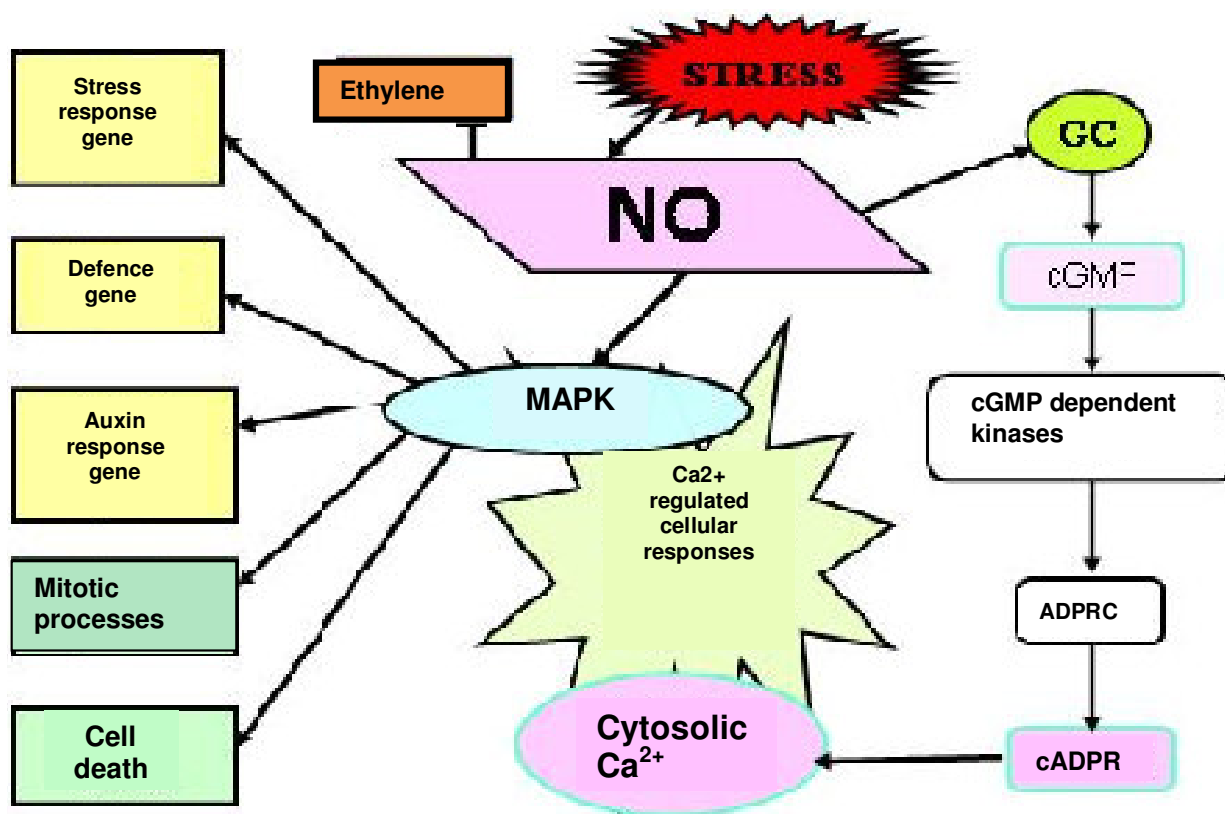
common mechanisms to transduce NO signals. SA is assigned as a secondary messenger in plant-pathogen interactions and serves as a general redox signal. NO activity is shown to be partially SA-dependent (Modolo et al., 2005). The relations among NO, SA, and ROS in the activation of defense genes and/or induction of host cell death are probably through the redox signaling network (Planchet et al., 2004; Biswal et al., 2001; Tunc-Ozdemir et al., 2009).

The indirect means involve cGMP, produced following the NO-induced activation of soluble guanylate cyclase, and/or cyclic ADP-ribose (cADPR) a  $\text{Ca}^{2+}$ -mobilizing metabolite that is synthesized from  $\text{NAD}^+$  by ADP-ribosylcyclase (Willmott et al., 1996; Hanafy et al., 2001; Allen et al., 1995; Fliegert et al., 2007). NO modulates  $\text{Ca}^{2+}$ -permeable channels in plant cells (Garcia-Mata et al., 2003; Gould et al., 2003; Lamotte et al., 2004, 2006). NO scavengers and mammalian NOS inhibitors also reduced the burst in cytosolic  $\text{Ca}^{2+}$  concentration triggered by hyperosmotic stress or elicitors of defense responses (Gould et al., 2003; Lamotte et al., 2004, 2006; Vandelle et al., 2006).

Other targets of NO are mitogen activated protein kinases (MAPKs). Similar to that in mammals, where NO modulates MAPKs activities in tumor cells and neurons (Mott et al., 1997), NO is also known to activate MAP kinase signalling pathways in plant cells (Kumar and Klessig, 2000; Pagnussat et al., 2004) which presumably results in altered gene expression. NO donors and recombinant NOS are reported to modulate tobacco pathogen-activated MAPKs (Klessig et al., 2000). A rapid transient increase of MAPK activity in response to auxins was reported in *Arabidopsis* seedling roots (Mockaitis and Howell, 2000). In plants MAPKs can be activated in response to extracellular signals such as drought, cold, phytohormones, pathogen attack and osmotic stress that cause to the activation of signal transduction pathways resulting in nuclear gene expression (Hirt, 1997).

## cGMP- DEPENDENT SIGNALING

The evidence that cGMP is an NO signalling intermediate has been reported in several plant systems (Neill et al., 2003; Delledonne, 2005). Both salt and osmotic stresses which induce ABA synthesis induced a rapid increase in the cGMP content of *Arabidopsis* seedlings and ABA induced closure of stomata (Donaldson et al., 2004; Wilson et al., 2009). Additional signaling pathways stimulated by ABA might also operate in a coordinated fashion for such actions. In non-plant systems, cADP-ribose (cADPR), an agent that mobilizes  $\text{Ca}^{2+}$  from internal stores, is said to be a downstream messenger of NO. Nicotinamide, a potential inhibitor of cADPR synthesis, blocks ABA- and NO-induced stomatal closure (Neill et al., 2002a). Garcia-Mata et al. (2003) have also shown that NO-induced intracellular  $\text{Ca}^{2+}$  release and the



**Figure 5.** NO mediated cellular signaling mechanism in plants. The colour codes are : Red - stressor (either biotic or abiotic), Pink – upregulated, Green – regulators, white – intermediate processes, Blue – critically regulated processes, yellow – gene regulation.

regulation of guard cell plasma membrane  $K^+$  and  $Cl^-$  channels are mediated by a cGMP- and cADPR-dependent pathway. 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 is yet to be confirmed, although cGMP has been unequivocally identified in various plant tissues (Neill et al., 2003). The mechanism for rapid degradation cGMP also exists in plants conforming it as an intracellular signal molecule. Arabidopsis genome contains several genes for potential phosphodiesterases including that encoding a putative cGMP phosphodiesterase that catalyse the hydrolysis of cGMP (Maathuis, 2006).

### cGMP-INDEPENDENT SIGNALING

NO can be oxidized to nitrate or form complexes with proteins to generate nitrosylated 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 (Figures 4 and 5). These post-translational modifications may well turn out to have roles in intracellular signalling and the subsequent physiological effects (Saito et al., 2006). Besides this, S-nitrosylation is the reversible covalent attachment of NO to the thiol group of cysteine residues forming an S-nitrosothiol (SNO) and is one of the ancient highly conserved cell signaling mechanism in plants (Wang et al., 2006). Nitrosylation can occur either through the direct interaction of NO with the cysteine group or indirectly by trans-nitrosylation where the NO is derived from S-nitrosoglutathione (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 plants. 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 seems likely that all cells contain nitrosylated proteins and that the spectrum and levels of these, the 'nitrosylome', will be an interesting aspect of study in addressing NO signaling in plants as well to elucidate the role of such signals in plant processes.

## CONCLUSION

The literature available for NO synthesis and action in plants so far gives an overall aspect of its action which is inconclusive, as the mode of its detection, localization and quantification in plants suffers from a major technical limitation in plants. A concrete fool proof evidence for its detection, mechanism of synthesis, cellular compartmentation, mode of action, and its role in signaling in plants appears to be still a nascent topic in plant to be studied and explored.

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