Nitric oxide ameliorates stress responses in plants

A.N. Misra, M. Misra, R. Singh

Department of Biology and Biotechnology, School of Biotechnology, Fakir Mohan University, Nuapadhi Campus, Balasore, India

ABSTRACT

Nitric oxide (NO) is a gaseous diatomic molecule with a wide variety of physiological and pathological implications in plants. Presence of unpaired electron in its molecular orbital makes it highly reactive; it can react directly with metal complexes, radicals, DNA, proteins, lipids and other biomolecules. Nitric oxide (NO) and reactive oxygen species (ROS) are known to play essential role in a number of important plant physiological processes. This manuscript reviews the role of NO on these processes during various biotic and abiotic stresses.

Keywords: nitric oxide (NO); nitric oxide synthase (NOS); reactive oxygen species (ROS); stress

Nitric oxide (NO) is a well established as a signaling molecule involved in many physiological processes in animals (Wendehenne et al. 2001). In animals, NO is responsible for signal transduction and is synthesized by three different isoforms of nitric oxide synthase (NOS) enzyme from L-arginine. In animals NO has subsequently been identified as a critical signaling molecule in maintaining blood pressure in the cardiovascular system, stimulating host defenses in the immune system, regulating neural transmission in the brain, regulating gene expression, platelet aggregation, learning and memory, male sexual function, cytotoxicity and cytoprotection etc. (Lamattina et al. 2003). Through a series of studies NO has emerged as an important signalling molecule also in plants (Lamattina et al. 2003, Neil et al. 2003, Delledonne 2005). Experimental evidence in supporting such signalling roles for NO was obtained through the application of either gaseous NO or NO donors. Reports obtained so far reflect that NO is involved in almost every stress response in plants. This review summarises the role of NO that regulates the development and enables plants to tolerate the different stress responses including gene regulation in plants.

Nitric oxide synthesis, localization and transport in plant

NO is synthesized by NO synthase (NOS) in animal cells, which converts L-arginine into L-citrulline in a NADPH-dependent reaction releasing equimolar quantity of NO (Neil et al. 2003). NOS-type enzyme also occurs in plants (Del Rio et al. 2004). Plants also synthesize NO through other biochemical routes like the reduction of nitrite and nitrate. In these cases, NO is likely to be produced by nitrate reductase (NR), which reduces nitrate to NO through nitrite (Planchet and Kaiser 2006). NO production by NR in vitro under pure nitrogen (or argon) is higher than in oxygenic conditions or in pure air (Planchet et al. 2005). The low yield of NO in oxygenic cell-free system is attributed to auto-oxidation of NO or by its reaction with ROS produced simultaneously by NR (Yamasaki and Sakihama 2000). Several plant systems synthesise NO by cytosolic or plasma membrane-bound NiR, mitochondrial electron transport, Xanthine dehydrogenase/oxidase and nonenzymatic NO formation at acidic pH in the apoplasts. Other enzymes may also be involved in NO production. NO can diffuse within a cell

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from the site of synthesis to other regions of the cell where it might induce an effect by interaction with specific target proteins. NO is lipophilic and may accumulate or move through membranes (Leshem 1996) or can be stored as transportable compounds like SGNO (S-nitrosoglutathione) (Valderrama et al. 2007).

Regulation of plant growth and development by NO

Nitric oxide also regulates several plant processes like seed dormancy, leaf expansion, vegetative growth of the shoot, cell division, xylem differentiation, root development, plant-rhizobacterium interaction, gravitropic bending (Leshem 1996, Lamattina et al. 2003, Besson-Bard et al. 2008), flowering time (Simpson 2005), pollen tube growth (Prado et al. 2004) and nutritional status, especially iron homeostasis (Murgia et al. 2004). Stomatal closure which is regulated by abscisic acid (ABA) is also regulated by NO signal (Desikan et al. 2002).

The progression of development in plants is through growth, maturation and senescence phases. Leaf senescence is accompanied with an increase in the reactive oxygen species-ROS and expressinon of SAG genes (Gepstein et al. 2003). The probable action of NO in retardation of senescence is by reacting with ROS to form peroxynitrite, and subsequent fast isomerization to harmless product like nitrate (Mishina et al. 2007). NO is also reported to be an antagonist of the senescence-promoting plant growth regulator – ethylene and acts through the inhibition of ethylene synthesis (Leshem 1996).

Major sources of NO in plants synthesized through different biochemical pathways, and regulation of various plant growth and development processes under biotic and abiotic stress are shown in Figure 1.

Role of NO in abiotic 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 different signaling pathways interacting directly or indirectly with NO reflect the possible mode of action of NO in plants under stress conditions as described in the following sections:

(i) Wound-induced stress. Wounding is a common damage that occurs to plants as a result of stress factors such as wind, rain, hail, and insect feeding. Wounding of the leaf epidermis in *Arabidopsis* induced a burst in NO within minutes. Direct treatment of NO to plants enhanced

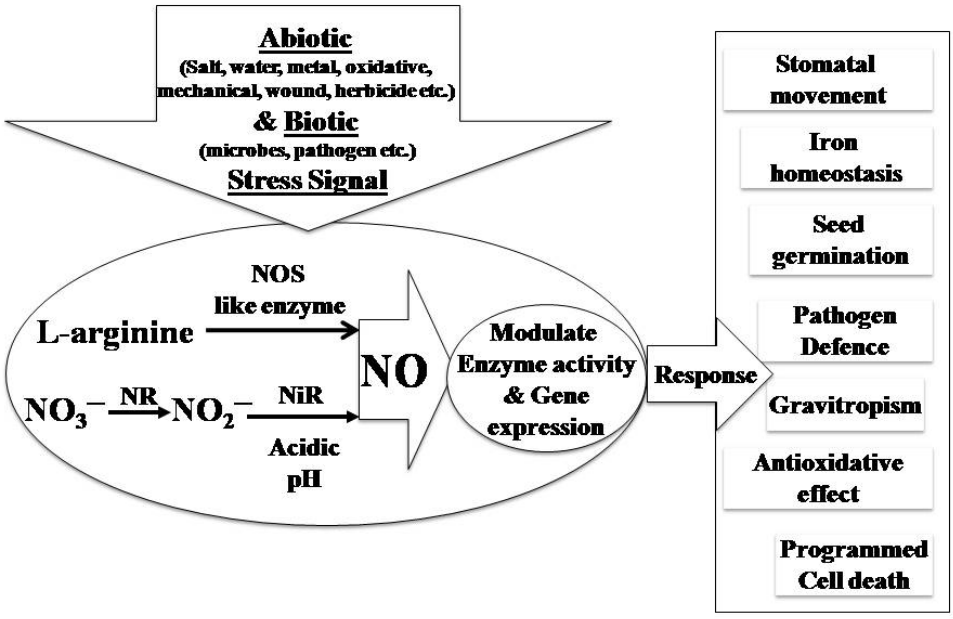

Figure 1. The effect of abiotic and biotic stresses on synthesis of NO in plant cells and its effect on the modulation of enzyme activities and gene expression, resulting (response) in the regulation of plant processes

the expression of key enzymes of the octadecanoid pathway, like allene oxide synthase (AOS), lipoxygenase (LOX2), or 12-oxophytodienoate reductase (OPR3) (Huang et al. 2004) but had no effect on either the jasmonic acid (JA) levels or JA-responsible genes, like defensin gene (PDF1.2) (Glazebrook 2001). NO increases the salicylic acid (SA) level (Durner and Klessig 1999).

(ii) Metal stress. Heavy metal pollution is a major environmental concern; some reports showed that NO exhibited an antioxidative role in the context of copper, Cd^{2+}, Al^{3+} toxicity (Singh et al. 2009). Graziano and Lamattina (2007) reported that NO produced in iron-deficient tomato roots promotes the transcriptional activation of the iron assimilation-related genes helix-loop-helix transcription factor (FER), ferric-chelate reductase (LeFRO1), and Fe(II) transporter (LeIRT1). Depending on iron availability, plant cells use NO as an intracellular signal to promote iron sequestration or uptake, highlighting a central function for NO in the control of iron homeostasis.

NO controls Ca^{2+} homeostasis both in animals and plants by modulating the activity of plasma membrane and all other types of intracellular Ca^{2+} permeable channels (Besson-Bard et al. 2008).

(iii) Oxidative stress. As a signaling molecule, NO is believed to be one of the first antioxidants during early evolution which can up-regulate antioxidants (Beligni and Lamattina 2002) and indirectly mediate H_2O_2 level in the cascade of events leading to alterations of antioxidant gene expression (Leshem 1996). A great variety of abiotic stresses including drought, salinity, ultraviolet light, air pollutants and heavy metals cause damage to plants, either directly or indirectly through reactive oxygen species (ROS) formation (Tunc-Ozdemir et al. 2009), such as superoxide anion (O_2^-) and hydroxyl (OH) radicals, hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$). Two mechanisms are proposed by which NO might abate oxidative stress. First, NO might function as an antioxidant by directly scavenging ROS, such as O_2^-, to form peroxynitrite anion ($ONOO^-$) (Laspina et al. 2005). Second, NO could function as a signalling molecule in the cascade of events leading to changes of gene expression (Lamattina et al. 2003, Laspina et al. 2005). Peroxynitrite was suggested as a deleterious mechanism (Leshem 1996), because it oxidizes DNA, lipids, protein thiols and iron clusters, resulting in impaired enzyme activities and cellular damage. However, in systems where the toxicity is predominantly from peroxides, these compounds are much more toxic than NO and $ONOO^-$, making NO protective against them. Thus, interaction of NO with lipid alcoxyl or lipid peroxyl radicals breaks the self perpetuating chain reaction during lipid peroxidation (Beligni and Lamattina 2002).

(iv) Drought stress. Water deficit or drought stress induces several physiological, biochemical and molecular responses in plants, and is characterized by an increased ROS level which activates expression of genes for antioxidant enzymes. The activated antioxidant systems are beneficial for plant performance under water stress, because this helps in the removal of excess ROS and inhibits lipid peroxidation. Recent studies showed that ABA induces increases in the production of NO and H_2O_2 which are involved in antioxidant defense (Lamattina et al. 2003, Arasimowicz and Floryszak-Wieczorek 2007).

(v) Salt stress. Nearly half of the irrigated land and 20% of the world's cultivated land are currently affected by salinity (Misra et al. 2001). Salt stress induces oxidative damage and regulates protein kinase activities in plants (Misra et al. 2006). Tolerant plants typically maintain high potassium (K^+) and low sodium (Na^+) in the cytosol of cells under salinity. This is mediated by H^+-ATPase, carriers (symporters and antiporters), and channels associated with plasma membranes (PMs) and tonoplasts. NO produced under salt stress could serve as a second messenger for the induction of PM H^+-ATPase expression (Zhao et al. 2004). Guo et al. (2009) suggested that NO might confer salt tolerance to plants by preventing both oxidative membrane damage and translocation of Na^+ from root to shoots

Role of NO 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 programmed cell death or cellular apoptosis which restrict pathogen growth and/or spread (Delledonne et al. 1998). To resist these pathogens, plants activate defense response in recognition to pathogen-derived molecules or elicitors. Downstream signal transduction cascades become activated upon recognition of the elicitors. The initial defense response is often manifested as the so-called hypersensitive response (HR) and the earliest event is the oxidative burst and generation of NO (Van Camp et al. 1998). These activated species are involved in the

Table 1. The stimulatory (+) and inhibitory (–) effect of nitric oxide on different enzymes in plants

Enzymes	Sources	Activated (+)/ inhibited (–)
Nitrate reductase	*Brassica chinensis*	+
PM H^+/-ATPase	Tomato (*Lycopersicon esculentum*)	+
Vacuolar H^+/-ATPase and H^+-PPase	Maize (*Zea mays*)	+
SOD, ascorbate peroxidases (APX) and CAT	Salt stressed barley (*Hordeum vulgare*)	+
Catalase and ascorbate peroxidase	Tobacco (*Nicotiana tabacum*)	–
Cytochrome C oxidase	Plant mitochondria	–
Methionine adenosyltransferase (d MAT1)	*Arabidopsis thaliana*	–
Coniferyl alcohol peroxidase	*Zinnia elegans*	–
Type-II metacaspase AtMC9	*Arabidopsis thaliana*	–

regulation of gene expression during the HR and they appear to play a key role in the coordination of the plant responses to pathogen challenge. NO induces defense gene expression and/or production of antimicrobial compounds e.g. phytoalexins (Delledonne 2005). NO also potentiates pathogen- and ROS-induced cell death and induces HR responses (Delledonne 2005). Expression of primary defense responses like HR, lignin deposition and defense-related enzyme phenylalanine ammonia-lyase, accumulation of salicylic acid and activation of pathogenesis related protein (PR-1) and onset of systemic acquired resistance (SAR) were enhanced by NO (Delledonne 2005, Planchet and Kaiser 2006, Arasimowicz and Floryszak-Wieczorek 2007).

Table 1 summerises different types of enzymes in plant systems, which are up- (activated) or down- (inhibited) regulated by stress and stress induced NO.

NO-regulated gene expression

NO modulates the expression of genes (Grun et al. 2006). Besides pathogen-related defence genes, NO induces gene expression of peroxidases, ferritin, and key enzymes of jasmonic acid biosynthesis (Del Río et al. 2004). NO activates the expression of glutathione-*S*-transferase (GST), chalcone synthase (CHS), glutathione peroxidase (GPX), and alternative oxidase (AOX1a) genes, and inhibits gene expression of thylakoid ascorbate peroxidase (tAPX) (Murgia et al. 2004). The *Arabidopsis* ABA-dependent SnRK2 kinase, SRK2C/SnRK2.8, improves plant drought tolerance, probably by

Table 2. Modulation of gene expression by nitric oxide to cope with different stress conditions. The up-regulation or induction and suppression of the gene is shown as + and – symbols in the 'effect' column

Genes	Effect	Function
Ferritin (FER)	+	iron storage
Pathogenesis-related protein (PR-1)	+	protection against pathogens
Phenylalanine-ammonia-lyase (PAL)	+	protection against pathogens
Chalcone synthase (CHS)	+	protection against UV-B radiation
Thylakoidal ascorbate peroxidase (t-APX)	–	elevate oxidative stress
DREB1A/CBF3 (transcriptional factor)	+	stress responsible genes
Plasma membrane intrinsic protein (PIP)	+	seed germination, seedling growth in rice (*Oryza sativa*)
ABC-transporters, cytochrome p450 genes	+	protection against pathogens
Lotus japonicus non-symbiotic haemoglobin (LJHB1)	–	symbiosis
Glutathione-*S*-transferase (GST)	+	protection against pathogens
Ipomoelin gene (IPO)	–	delayed wounding stress
Heme oxygenase (HO)	+	protection against Cd stress
Flowering locus C (FLC)	–	delayed floral induction

promoting the up-regulation of stress-responsive genes expression, including DREB1A/CBF3 encoding a transcription factor that broadly regulates stress-responsible genes (Umezawa et al. 2004). Courtois et al. (2008) suggested that NO as an early signalling compound acting upstream of SnRK2-induced pathways combating osmotic stress. A summary of genes induced and repressed by nitric oxide in plant system is summerised in Table 2.

The present knowledge on the nitric oxide evolution and mode of action against different stress conditions in plants shows that NO is one of the versatile molecules which can be transported easily to any compartment in the plant cell and elucidates its impact through various signal transduction pathways. The literature available is huge, but this review is restricted to few important aspects of NO action under stress conditions in plants.

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Corresponding author:

Prof. Amarendra Narayan Misra, Nuapadhi Campus, Fakir Mohan University, School of Biotechnology, Department of Biology and Biotechnology, Balasore-756020, India
E-mail: misraan@yahoo.co.uk