



NITRIC OXIDE: A MASTER PLAYER IN PLANTS

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Review Article

Received: 19.03.2019

Revised: 26.03.2019

Accepted: 08.04.2019

ABSTRACT

Nitric oxide (NO) is a gaseous reactive oxygen species (ROS) that regulates each step of plant life. It acts as signalling molecule during abiotic and biotic stress. However, the knowledge of NO production and signal transduction remains largely unclear. Therefore in this review we will try to uncover the current knowledge of NO production and its action in different physiological responses.

Keywords: Nitric oxide, abiotic stress, metal, proteins, sulfhydryl group, free radicals.

INTRODUCTION

The world's population is expected to grow 10 billion by mid of the century i.e. 2050, boosting agricultural demand by almost 50 percent compared to 2013. Income growth in low- and middle-income countries would hasten a dietary transition in relation to cereals towards higher consumption of meat, fruits and vegetables, which will add enormous pressure on natural resources. Similarly, much remains to be done to fulfil the vision of Food and Agriculture Organization (FAO, 2017): to create 'a world free of hunger and malnutrition and one in which food and agriculture contribute to improving the living standards of all, especially the poorest, in an economically, socially and environmentally sustainable manner'.

On the other hand, to survive under adverse environmental condition, plants respond at the cellular, molecular and physiological level by interfering with ionomics, genomics, transcriptomics and proteomics level which involves a multifaceted network following perception and transmission of stress signals, which consequently initiate a plethora of responses (Nakashima *et al.*, 2017). A deep understanding of the mechanism reinforcing the plants for stress adaptation might provide novel opportunities to

develop crops with an increased ability to stand against environmental fluctuations that eventually lead to enhanced productivity. Among plant responses to abiotic stresses, the common factor is the generation of redox active molecules i.e. reactive oxygen species and reactive nitrogen species (ROS and RNS) (Mittler 2002). Though, excessive amount of ROS and RNS can be fatal to cell. Among these redox active molecules, nitric oxide (NO) is the chief one, which is present in the environment since the time ~2.7 billion years ago when molecular oxygen (O₂) was introduced into our atmosphere by O₂-evolving photosynthetic organisms. During the last few decades, NO emerged as a signalling molecule in plants, since then it became associated with a large number of phenomena. Being a ROS or free radical, NO jobs as a gasotransmitter-diffusible multitasked messenger, which easily can cross the membrane without any carrier. It was first defined in mammals, where it plays various functions ranging from blood vessel relaxation, neurotransmission, participation in the fertilization process and immune defense responses (Zhou and Zhu, 2009). In plants, both NO and other ROS found associated in mediating signalling responses in tip growing cells, where they are involved in regulating polarity and growth (Wudick and Feijo, 2014). NO may act

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as a positive as well as a negative regulator during stress. According to Mur *et al.* (2013) this two fold effect of NO either as a promoter or as an inhibitor is predominantly depend on its concentration and production. Once generated, NO can react with other redox-related molecules and potentially regulate protein function (S-nitrosylation) via distinct

mechanisms. During the past decade, NO function and S-nitrosylation have emerged as major regulatory mechanisms for abiotic stress signalling. Therefore, in this review we focus on the current state-of-the-art regarding the role of NO and S-nitrosylation in abiotic stress responses in plants.

Table 1: Depicting the nitric oxide (NO) induced effects on plants under abiotic stresses.

| Stressor | Plant | NO induced response | Reference |
|--|---|--|---|
| Drought/osmotic stress | <i>Nicotiana tabacum</i> , <i>Pisum sativum</i> | Involving in ABA signalling, stomatal closure induction of ABA synthesis, LEA expression. | Gould <i>et al.</i> (2003), Leshem and Haramaty (1996) |
| Heavy metal toxicity (Al) | <i>Hibiscus moscheutos</i> , | Increased the root elongation; reduced the NOS activity (Reduced NO level). | Tian <i>et al.</i> (2007), |
| Cd | <i>Triticum aestivum</i> | Increased antioxidants activities; reduced the ROS and indices levels; Improved PSII photochemistry. | Kaya <i>et al.</i> (2020) |
| Herbicide | <i>Scenedesmus obliquus</i> , <i>Chlamydomonas-reinhardtii</i> | Promoted the activity of antioxidant enzymes. | Mallick <i>et al.</i> (2000), Sakihama <i>et al.</i> (2002) |
| High temperature | <i>Medicago sativa</i> , <i>N. tabacum</i> | Increased tolerance of seedlings; rapid NO release. | Leshem <i>et al.</i> (1998), |
| Low temperature | <i>S. obliquus</i> | Decline the ROS level. | Gould <i>et al.</i> (2003) Mallick <i>et al.</i> (2000) |
| Mechanical injury | <i>Arabidopsis thaliana</i> , <i>Taxus brevifolia</i> | NO burst result in cell death. | Garces <i>et al.</i> (2001), Pedroso <i>et al.</i> (2000) |
| Nurient deficiency | <i>S. obliquus</i> | ----- | Mallick <i>et al.</i> (2000) |
| Salinity/salt | <i>N. tabacum</i> , Maize, <i>Ocimum basilicum</i> | Increased osmotic tolerance; induce expression of Na ⁺ /H ⁺ antiporter gene. Improved the morphological parameters. | Gould <i>et al.</i> (2003), Zhang <i>et al.</i> (2006), Gohari <i>et al.</i> (2020) |
| UV-B radiation | <i>A. thaliana</i> , <i>Paulownia tomentosa</i> | Induced the expression of CHS gene. Inhibited pollen germination and tube growth (exogenous NO). | Mackerness <i>et al.</i> (2001), He <i>et al.</i> (2007) |
| Iron deficiency | <i>A. thaliana</i> , | Upregulation of the expression of iron uptake-related genes. | Koen <i>et al.</i> (2012), |
| Iron deficiency and salt stress | <i>Capsicum annuum</i> | Decreased G6PDH and NADP-ICDH activity, decreased fruit ripening. | Muñoz-Vargas <i>et al.</i> (2020) |

NO role under abiotic stressed conditions

Since past decades, a number of articles regarding endogenous NO production/ reduction in response to different abiotic stresses have been addressed (Rodríguez-Serrano *et al.*, 2009; Besson-Bard *et al.*, 2009; De Michele *et al.*, 2009) Table 1. In many of

these studies, the assessment of exact location and quantity of NO in plants with the timing and

2009), whereas upon long-term Cd exposure (50 μM), NO induced senescence process (Rodriguez-Serrano *et al.*, 2009).

Drought is another highly studied stress that interferes with crop productivity but the role of NO under stress is unclear (Gould *et al.*, 2003; Zhang *et al.*, 2007). During drought stress, stomata closure, an essential process controlled by abscisic acid (ABA) where NO helps ABA but during dehydration involvement of NO is not necessary. This suggests that NO is involved in regulation of stomatal closure in turgid leaves which occurs in response to environmental fluctuations (Neill *et al.*, 2008; Wilson *et al.*, 2009). NO also plays significant role under herbicides, high temperatures, ozone, UV-B, salinity, mechanical damage (Neill *et al.*, 2008; Gohri *et al.*, 2020; Kaya *et al.*, 2020). However, more studies are needed to define NO signalling especially with transcriptomic analysis (Besson-Bard *et al.*, 2009).

NO have also been reported to arbitrate hormone-regulated (ABA, salicylic acid (SA), ethylene, auxins or DELLAs) processes in plants, and a cross-talk in NO and hormones may also involve secondary messengers like Ca or kinases, under varying environmental conditions (Simontacchi *et al.*, 2013). The equilibrium between ROS and NO is important to decide the fate of the cell especially under abiotic stressed conditions in context of ROS generation and antioxidant defence system of plants (Rodriguez-Serrano *et al.*, 2009). Future studies are focuses on NO-dependent protein regulation, mainly through nitration and S-nitrosylation (Vandelle and Delledonne, 2011).

Biological effects of NO

To understand the framework of different events of plant system, the study of chemical biology of NO with different biological molecules is necessary (Wink and Mitchell, 1998). Being a paramagnetic molecule NO has an unpaired π^* electron and easily can diffuse across the membranes. The nitrosonium anion (NO^+) formed after oxidation contributes in nitrosation reactions when added to thiol, amine, or hydroxyl aromatic group. When second electron is added to $2p-\pi$ orbital of NO nitrosyl anion (NO^-) is formed. According to Hughes (1999), interconversion of NO, NO^+ and NO^- prevails in cellular conditions and by reacting with free radicals, metals, oxygen molecule and thiols, NO regulates plant responses.

Reactions of NO with free radicals

NO reaction with superoxide anion ($\text{O}_2^{\cdot-}$), is diffusion limited because NO is not too fast to react (Henry and Guissani, 1999). This reaction between reactive nitrogen and oxygen species leads to the formation of a toxic powerful oxidant peroxynitrite (ONOO^-), which reacts majorly with macromolecules (Wink and Mitchell, 1998). NO is a strong inhibitor of lipid peroxidation and scavenges peroxy radical (LOO^\cdot) (Hogg and Kalyanaraman, 1999). Lipid peroxidation is a toxic component formed due to oxidative injuries, when antioxidant defence system of plants surrenders under abiotic stresses, while NO accumulation under these conditions shows its protective behaviour (Patel *et al.*, 1999). Formation of NO^- derived species and nitrolipids (nitro fatty acids) have been reported by Fazzari *et al.* (2014), which suggests their role as signal transduction mediator.

Reactions of NO with metals

NO after reacting with transition metals forms coordination complexes like with Fe it forms nitrosyl iron complex (a NO^+ carrier). Through Fenton's reaction toxic iron catalyzes the formation of hydroxyl radical (HO^\cdot). According to Jasid *et al.* (2008), NO have the ability to protect plants from oxidative injuries by inhibiting Fenton chemistry binding ferrous iron (by directly reacting with Fe, NO forms nitrosyl Fe in presence of sGC and catalase). NO after reacting with iron-heme component of sGC enzyme, increases its activity and leads to the production of cGMP, which triggers different responses in animals (Neill *et al.*, 2008).

Reactions of NO with tyrosine in protein

Nitration is the process of addition of nitro group (NO_2^+) tyrosine residues in proteins, which interferes tyrosine phosphorylation, a general process to control the enzymatic activities. The nitration reaction is mainly influenced by RNS and CO_2 concentration, in different plant cells (Santos *et al.*, 2000). Camejo *et al.* (2013) have reported that in salt stressed pea plant protein nitration increases with NO increase, where PSI, PSII, cytochrome b_6/f and ATP synthase complex are the major tyrosine nitration sites in chloroplast (Galetskiy *et al.*, 2011). Nitration targets were identified in sunflower hypocotyls (Chaki *et al.*, 2009) and in *Arabidopsis* under hypersensitive (Cecconi *et al.*, 2009) as well as non-stressed (Lozano-Juste *et al.*, 2011) condition. When nitroproteomic analysis was

done in salt stressed root and leaves of citrus plants, defence/ disease related proteins were the majorly affected group in roots while photosynthesis related proteins were chiefly affected group in leaves (Tanou *et al.*, 2012), where 86 and 88% proteins underwent tyrosine nitration, respectively.

Reactions of NO with sulfhydryl groups

Due to its strong electrophilic nature, NO⁺ reacts with most of biological –SH (Gaston 1999), resulting the formation of S-nitrosothiols (SNO). Generally, nitrosogluthathione (GSNO) and SNO are NO⁺ carrier and reservoirs in biological system. GSNO with the help of GSNOR activity transform into oxidized glutathione (GSSG) and ammonium, but the higher concentration of NO inhibits GSNOR activity via S-nitrosylation reactions and therefore, excessive NO inhibits GSNO degradation with probable reduction in nitrate uptake rate (Frungillo *et al.*, 2014). The reaction between NO⁺ and cysteinyl sulfhydryl moieties (S-nitrosylation), is an important signalling event which involves in various plant physiological processes. In addition to NO levels, protein nitrosylation have also been linked with GSNO accumulation, as in case of low GSNOR/reduced thioredoxin5 (TRXh5) activities (Kneeshaw *et al.*, 2014). TRXh5 activity in plants reveals a strong protein-SNO reductase activity, which is the basis for salicylic acid-dependent plant immune signalling (Kneeshaw *et al.*, 2014).

Concluding remarks

Nitric oxide defends plants from oxidative injuries, by maintaining photosynthetic capacity, antioxidant defence system and other major metabolic processes; either by interacting with plant hormones or other molecules/ messengers. Although, NO-mediated responses to abiotic stresses have been well documented; however, the multiple signalling pathways implied in morphological and physiological stress responses needs further studies which could help to develop strategies to improve the plant growth and yield under adverse conditions that also will help to combat human nutrition problems.

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