

Cryptic Roles of Nitric Oxide, a Key Mediator of Salicylic Acid and Jasmonic Acid Signalling under Abiotic Stress in Plants

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ABSTRACT

Nitric oxide (NO) is a multitasking, gaseous reactive molecule; it is implicated in signalling responses of plants to biotic and abiotic factors, and increasingly known for interactions with several stress alleviators. It has been studied extensively in animals due to its role as a neurotransmitter. Given its recently discovered activities in plants, NO has great possibilities in inflecting plant stress responses and prompting signalling molecules against stressful conditions. It is known for its multifaceted responses in stress alleviation which comprises plant growth regulation, germination of seed, wound suppression, and other defensive responses. The functional interactions of NO among different stress signaling cascades lead to enhancement in the expression of genes that regulate biosynthesis of phytohormones. It interacts with auxin (AUX), gibberellin (GA), abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA) and results in powerful responses under stress. This review article focuses on the SA-NO-JA interactions and how their interactions trigger the re-programming of stress-responsive gene(s) expression.

Key words: C-repeat binding factor, heat shock response, pathogenesis-related genes, programmed cell death, sodium nitroprusside, systemic acquired resistance

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Introduction

Plants are stationary, they are incapable of shifting places in response to any environmental or human-induced hazard, and therefore, have to cope with many stress conditions. The stress can be abiotic or biotic; abiotic stress includes heavy metals, salinity, heat, water (drought or flood). The adversity of these stresses results in inadequate plant growth and compromise the photosynthetic efficiency of plant (Parihar et al., 2015). For instance, the initial effect of drought mainly consists of poor seed germination and loss of turgor, this limits the growth of the plant to a very great extent. This ultimately causes wilting and reduction in the number of leaves and reduces the size of leaves as well (Ullah et al., 2022). Another severe stress is heat, it mostly affects the plant meristem and brings on early senescence of the leaves due to unremitting ambient temperature of around 45°C, it leads to abscission of leaves and decline in the rate of photosynthesis (Akter & Islam, 2017). The sluggish deposition of salts in the soil brings in salinity stress. Salt lodgement in the soil is attributed to both environmental and human-induced practices comprising weathering of parent rocks and irrigation of crop-fields using salt-rich water respectively (Yadav et al., 2022). This affects the morphological

and physiological parameters of the plants including reduction in leaf growth and a minor reduction in the root growth along with inhibiting the enzyme activities by building up of salts in the cytoplasm of plant and increasing the water potential of soil due to confined water uptake (Parihar et al., 2015).

To persist and reproduce, plants have to develop various defense strategies, as tenacious stress can bring in substantial plant's yield loss and economic losses for the farmers; these stressful conditions also pose a threat to the sustainability of the agricultural land (Wang et al., 2021). Among various signalling molecules that govern abiotic stresses in plants NO acts as an influential molecule that combats several complications emerging due to stress. It interacts with several plant growth regulators, which coordinate with different defense mechanisms of plants to enforce limits on the overproduction of reactive oxygen species (ROS) and its intermediates which are produced under stress (Wani et al., 2016). Salicylic acid (SA) is one of the many plant growth regulators which have been recognized to maintain the physiological and biochemical regulation of plants along with coordinating plant defense responses against various abiotic stresses (Khan et al., 2014). Another plant growth regulator which effectively works

during stress is jasmonic acid (JA) and its derivatives (jasmonates), which include methyl jasmonate (MeJA), jasmonyl isoleucine (JA-Ile), JA-Ile methyl ester. They are lipid-derived signalling molecules unlike SA, which are phenolic (Ghorbel et al., 2021). With a briefing about implications of NO, SA, and JA under different abiotic stresses, this review article mainly focuses on the mechanism for stress mitigation in plants by NO and its interactions with SA and JA, and briefly, with a few other signalling molecules.

Nitric oxide-mediated activity in plant growth, seed germination, and coping with salinity stress

Stresses present in the environment reduce crop productivity, and sometimes may reduce the rate of germination. Antioxidant activity a defensive plant strategy and is a prime method of stress alleviation activated in plants. NO is a plant signalling molecule that is involved in accumulating antioxidant molecules that has been testified in mediating seed germination (Fatima et al., 2021). The NO donor sodium nitroprusside (SNP) has been shown to enhance the rate of seed germination in *Brassica chinensis* L. (Ren et al., 2020) and to alleviate NaCl-generated abnormalities in *Pisum sativum* L. alongwith promoting growth in the radicle and plumule (Fatima et al., 2021). Augmenting a plant with NO donor in either stress or non-stress conditions resulted in the stimulation of seed germination in *Brassica chinensis* L. and *Suaeda salsa* L. under salt stress (Li et al., 2019; Ren et al., 2020). Other than regulating seed germination, NO induces plant growth and development by providing protection from oxidative damage and enhancing maintenance of ion homeostasis (Fancy et al., 2017).

Nitric oxide-induced physiological and molecular responses of plants under stress

Nitric oxide has been reported to mediate several physiological responses such as it upregulated ascorbate peroxidase (APX) activity, which is located in the thylakoid region of chloroplast and stimulates stress alleviation (Mahjoubi et al., 2021). It is reported that programmed cell death (PCD) is induced by NO as the main components of PCD, viz. kinase and phosphatase are influenced by ROS and NO interplay, which regulates the response of plants cells under stress (Verma et al., 2020). Besides, NO regulates the development of pollen tube during fertilization, and thus acts as an important component in pollen-stigma interaction. It also regulates photosynthesis

by increasing leaf gas exchange ($\text{CO}_2/\text{H}_2\text{O}$) through stomata and chlorophyll fluorescence (Mahjoubi et al., 2021). It has been reported that NO supplementation significantly enhanced the rate of photosynthesis in *Hordeum vulgare* L. and *Lycopersicon esculentum* L. (Sharma et al., 2020). NO has been reported to act at the molecular level, as it modulates the working of many genes in plants under stress, activates expression of several pathogenesis-related (PR) proteins which encode genes such as *PR-1*. It regulates the expression of genes encoding several enzymes like glutathione peroxidase (GPX), and alternative oxidase (AOX), which strengthen the antioxidants activity by sustaining oxidized state of the upstream electron transport chain in mitochondria (Prakash et al., 2021).

Salicylic acid in regulating plant responses under various stresses (Fig. 1, Table 1)

The synthesis of SA in plants occurs via two pathways—either isochorismate synthase (ICS) or phenylalanine ammonia-lyase (PAL) pathway. Both pathways start with chorismate, a precursor of many important compounds. It has been reported to reinforce the antioxidant system and thus accelerate the various defensive strategies along with the expression of salt transporter genes in *Pisum sativum* L. under salinity stress (Ahmad et al., 2020). It also invigorates photosynthesis process and maintains the osmotic equilibrium by reducing the K^+ leakage and increasing the activity of H^+ -ATPase, which invigorates nutrient uptake in plants under saline conditions (Jayakannan et al., 2013). It has been found to up-regulate the synthesis of total phenolics and activities of genes like *PAL* (phenylalanine ammonia lyase) in tomato and lemon plants under heat stress (Rai et al., 2020). Cadmium (Cd), a highly toxic heavy metal, it hampers shoot height and biomass gain in rice; however exogenous supplementation of SA effectively decreased Cd-instigated complications (Majumdar et al., 2020). The inhibitory effects of Cd are reversed by SA as it boosts the chlorophyll content, photosynthesis, and stomatal conductance in plants; thus, SA strengthens the defense system of plants against toxic metals (Guan et al., 2021). A unique function of SA is as an endogenous signal for systemic-acquired resistance (SAR). Several environmental stresses (including drought and cold) result in impairment of the plant's ability to fight pathogens. As reported in rice plants, cold stress suppresses the resistance to infection against *Magnaporthe grisea*, a rice blast causing fungus. Both biotic and abiotic stresses stimulate the biosynthesis

of SA in chloroplast which is then transported to cytosol, as SA is hardly permeable to the plant cuticle so it goes via long-distance transport and undergoes methylation resulting in volatile compound formation viz. MeSA acting as a defense signal to stimulate SAR. MeSA gets converted to SA by the activity of MeSA esterase (Saleem et al., 2021). Figure 1 indicates the responses of NO under various abiotic stresses along with NO mediated stress alleviating responses of SA and JA in plants.

Nitric oxide mediated stress mitigation by salicylic acid (Fig. 1, Table 1)

Both SA and NO have proven to be excellent regulators of biotic and abiotic stresses. In studies conducted on *Brassica napus* L. under nickel stress, there was loss of chlorophyll and proline contents, however, NO-SA application largely overturned this effect (Fancy et al., 2017). Photosystem II is highly responsive to heat stress; a temperature greater than 40°C results in the disassociation of light-harvesting complex-II, chlorophyll *a* and *b* proteins from PS-II, and also causes severe structural alterations in the chloroplast like disordering of thylakoid membranes and instigating cessation of photophosphorylation (Khan et al., 2014). Salicylic acid and nitric oxide are considered to be decent performers in plant heat shock response (HSR). In *Lablab purpureus* L., the interactive role of NO and

SA is apparent in ameliorating responses to heat stress by stimulation of transcriptional signalling pathways such as regulation of heat shock transcription factors. Activation of peroxidases by SA to produce NO and ROS have been discovered in the leaf guard cells under drought stress, inactivates K_{in}^{+} channels thus, preventing loss of water through stomata (Asgher et al., 2017).

Salicylic acid activates nitric oxide in Arabidopsis

Assessment of SA's ability to induce NO was done by analysis of *Arabidopsis* seedlings using a cell-permeable fluorescent probe and the results indicated that there was a significant increase in fluorescence, demonstrating NO production. This outcome was confirmed in *Arabidopsis* roots using cPTIO (NO scavenger); its application prominently reduced the increase in fluorescence which emerged as a result of NO production (Kaya et al., 2020). The application of SA for two hours resulted in an increment of NO, both in wild type and mutant *Arabidopsis* impaired in NO production (*Atnos1*). The degree of production was different in the two, and the role of exogenous SA application was confirmed. there are also reports of NO induction by SA in soybean leaves (Prakash et al., 2021). NO was also induced by SA under stress due to arsenic toxicity in maize plants, resulting in the up-regulation of the ascorbate-glutathione cycle and glyoxalase system (Kaya et al., 2020).

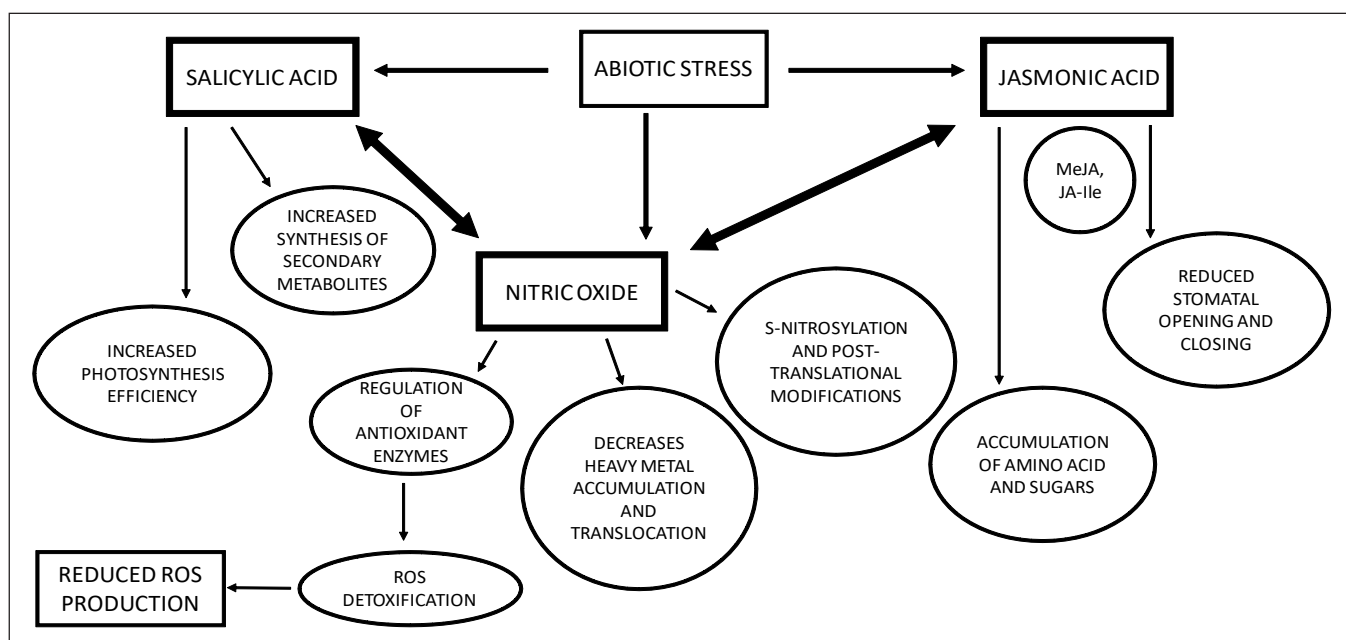


Figure 1: Stress alleviating responses of NO and mediation of various defensive and stress mitigation strategies of SA and JA by NO in plants under abiotic stresses.

Table 1. Summary of studies applying phytohormones – NO or in combination with salicylic acid (SA) or jasmonic acid (JA) -- under abiotic stresses, showing targeted mechanisms and plant responses.

Plant Species	Stress applied	Phytohormones applied for mitigation	Targeted mechanism	Plant characteristics improved	References
<i>Cucumis sativus</i> L.	100, 200, 400 mM NaCl	NO	Effect on germination of seeds and ROS metabolism	Exogenous SNP improved seed germination; SOD and CAT activities enhanced	Fan et al., 2013.
<i>Brassica oleracea</i> L.	12 mM NaCl	NO	Effect on chlorophyll and proline content	Chlorophyll content increased significantly; foliar spray of NO resulted in increased proline content	Fatima et al., 2021.
<i>Jatropha curcas</i> L.	100 mM NaCl	NO	Effect on seedling growth and toxin accumulation	Improved seedling growth and reduced oxidative damage; lower toxin accumulation	Sharma et al., 2020.
<i>Oryza sativa</i> L.	As-25, 50 μ M	NO	Effect on root	Architecture of root and coleoptile modified to reduce as uptake and increase antioxidant levels.	Singh et al., 2017
<i>Zea mays</i> L.	50ml 10% PEG solution for 48hrs	NO+SA	Effect on plasmalemma H ⁺ -ATPase activity and inorganic osmolyte content	SA, along with endogenous NO, significantly increased activity of plasmalemma with increased osmoregulators	Wang et al., 2016.
<i>Pisum sativum</i> L.	100 mM and 400 Mm NaCL	NO+SA	Effect on radicle length and biomass accumulation	Significant increases in the radicle length and biomass	Yadav et al., 2016.
<i>Glycine max</i> L.	100 mM NaCl	NO+SA	Effect on antioxidant defense system	Improved enzyme activities in the antioxidant system; maintenance of ionic homeostasis	Simaei et al., 2012
<i>Solanum lycopersicum</i> L.	200 mM NaCl	NO+JA	Effect on osmolytes and antioxidant system	Upregulation of antioxidant metabolism, increase in the synthesis of osmolytes and increment in accumulation of metabolites	Ahmad et al., 2018.
<i>Triticum aestivum</i> L.	15% PEG	NO+JA	Effect on MDA and EL in leaves	Significant reduction in MDA content and EL in leaves; cPTIO application reversed the effect	Shan et al., 2015

Collaborative roles of nitric oxide and salicylic acid under biotic stress

Pathogen attack often makes a plant more susceptible to abiotic stress but it has been established that co-treatment with SA and NO donor SNP, at concentrations of 1.5 mM and 100 μ M respectively, resulted in stimulation of defense components such as defensive enzymes (chitinase, peroxidase, etc), total phenol and flavonoid contents in tomato. Tomato plants when treated with SA in addition with NO scavenger exhibited weaker expression of plant defense-related genes like *PR5*,

PR2, etc (Chakraborty, 2021). The combined action of SA and NO results in downregulation of *NPR1* gene (Non-expressor of pathogenesis-related genes) and its translocation to the nucleus via S-nitrosylation-mediated monomerization indirectly activates *PR gene* expression, hence increasing stress tolerance (Prakash et al., 2021). The beneficial impacts of SA are further enhanced by the addition of SNP but diminished under c-PTIO application. These results suggest the interaction of SA and NO in plants. Increase in the pectin and lignin synthesis in rice plants as a part of defense strategy under Cd toxicity has also been studied

under SA and NO interplay; lignin synthesis regulates cell wall thickening and protects plants against any damage (Pan et al., 2021).

Nitric oxide and salicylic acid working together under abiotic stress

Abiotic stress can be caused by salinity, heat, drought, or heavy metals. SA and NO together can ameliorate these aforementioned plant stresses as radicle length and biomass accumulation have been reported to increase greatly under their amalgamation in plants under drought stress (Fancy et al., 2017). Drop in the accumulation of protein carbonyl, and 4-hydroxy-2-nonenal (an aldehyde product of lipid peroxidation in cell, and acting as a biomarker of damage) has also manifested a decline under SNP and SA cocktail (Asgher et al., 2017). Priming of radicles by soaking in SA+SNP stimulates salt stress tolerance in the *Pisum sativum* L. with increasing POD, APX, SOD, and CAT activities (Yadu et al., 2017).

Heat stress may lead to the activation of NO by SA, as suggested by some evidence, that there is an increase in nitrate reductase (NR) activity and glutamyl kinase activity after SA treatment, this eventually leads to inhibition of ethylene synthesis in *Triticum aestivum* L. (Khan et al., 2014). Interaction between SA and NO has been reported to induce the accumulation of antioxidant compounds under heat and regulates behaviour of heat shock (HS) genes, regarded as “retention of stress memory”, referring to transcriptional memory about the ability of plants in recognising same or similar stresses (Rai et al., 2020).

Drought stress results in lower soil water potential than plant cell water potential; it disturbs the overall water balance of plant (Ilyas et al., 2017). Exogenously applied SA induces NO production, which is involved in the transduction of signals for SA and ethylene to promote closing of stomata and prevent water loss. NO, when induced by SA increases the contents of osmoregulants under drought stress. The NO signalling induced by SA results in the regulation of H⁺-ATPase activity of the plasmalemma in roots, thus maintaining root hydraulic conductivity in maize plants during drought (Wang et al., 2021).

Stress due to heavy metals such as Cd and Zn result in impairment of plants physiological attributes. Net photosynthetic rate along with transpiration rate have been reported to rise in ryegrass plants under Cd stress,

after SA and SNP application. Iron (Fe) concentration also increased in both root and shoot on the application of SA+SNP and the rate of enhancement was significant as it facilitated in augmenting the overall growth of the plant. Iron is one of the macronutrients in plants and enhances plant growth so its upsurge in plant under stress is significant (Mostofa et al., 2019). Unlike other heavy metals, zinc does not have deleterious effects on plant, but at higher concentrations could prove to be detrimental. Zinc affects cell division by disturbing mitotic activity, disrupts membrane integrity and permeability, can sometimes even kill cells (Kalaivanan & Ganeshamurthy, 2016). Exogenously applied SA+SNP increased the performance of glyoxalase systems and ascorbate–glutathione cycle, and ultimately protected the safflower plant from excess ROS damage under Zn-stress (Namdjoyan et al., 2017). The combination of SA and SNP has been reported to be more effective in alleviating Cd toxicity than either SA or SNP alone which indicates the potential role of cooperative effects of SA and NO in decreasing Cd toxicity in rice (Mostofa et al., 2019). These evidences show that phytoremediation of heavy metals using co-application of SA and SNP is an effective method.

Hence, the interaction between signalling of NO and SA has significant effects in lowering stress due to salinity, heat, drought, and heavy metals.

Jasmonic acid-mediated abiotic stress responses in plants

Jasmonic acid is well known for its role in activating defense responses under biotic and abiotic stresses. It plays a vital role in the stress management of plants by regulating many biological processes: stomatal opening, regulation of the cell cycle, and transportation of glucose (Ahmad et al., 2018). A brief discussion about role of JA under cold, salinity and heat stresses along with its role in wound induced signalling is given hereunder.

Cold stress is the condition when the temperature goes below 16°C. This is a severe environmental issue because, e.g., such low temperature drastically challenges the plant's ability to perform photosynthesis as various enzymes activity get affected because of the high vulnerability of enzymes to extreme temperatures (Saleem et al., 2021). Cold stress also induces chlorosis. In acclimation, JA plays an important role in regulating downstream signalling of cold-responsive genes, such as in upregulating the C-repeat binding factor (CBF)

pathway. CBF are transcriptional factors that regulate responses to low temperature stress in plants, thereby inducing cold tolerance (Du et al., 2013).

Salinity tolerance is enhanced by the application of JA. Endogenous JAs maintain homeostasis of ROS in tomato plants under stress; this effect is also seen when applied exogenously. Jasmonic acid can turn over the deleterious effects of high salt concentration and brings about an increase in the rate of photosynthesis, as well as increases ABA content in the plants (Ahmad et al., 2018). The increase in the transcript levels of crucial antioxidative enzymes such as SOD, POD, CAT, and APX have been reported (Wang et al., 2021). Different concentrations of JA have proven to be useful in moderating salt stress. In *Ocimum basilicum* L. 0.5 mM of MeJA raised the levels of volatile compounds such as linalool and 1.8-cineole along with enhancing the activities of antioxidant enzymes under salinity stress (Talebi et al., 2018). In *Brassica napus* L. salt concentrations of 40, 70, and 100 μ M were mitigated using 0.5 mM MeJA. JA hampered the Na⁺ intake into cells, ultimately diminishing the negative consequences of salinity in plants (Farhangi-Abriz et al., 2019).

Jasmonic acid plays an important defense-inducing role in plants during drought; different concentrations of endogenous JAs have proven to be useful in preventing leaf senescence and water loss. Supplementation of 100 μ M JA has increased the shoot length, osmolyte content and enhanced the growth of wheat by 27% under drought (Ilyas et al., 2017). JA is also involved in a wound-suppressing response as reported in vascular wilt in tomato caused by *Fusarium oxysporum* f. sp. *Lycopersici* (FOL). Magnesium oxide treated roots upregulated MYC2, a master regulator of JA signalling, resulting in the decline of FOL inoculation and restoring the plant growth (Fujikawa et al., 2021).

Nitric oxide-mediated responses of jasmonic acid under stressful regimes (Fig. 1, Table 1)

Together JA and NO appropriately mediate the growth of plants and mitigates NaCl induced decline in the growth to a considerable extent. Interactions between JA and NO under abiotic stress are less explored but on integrating with NO, JA has proven to alleviate various stress responses more efficiently. Both NO and JA are involved in regulating numerous metabolic pathways, under normal as well as stressed conditions. JA is involved in cellular functioning, e.g., JA represses leaf expansion by inhibiting the activity of mitotic

cyclin and affects cell division without affecting cell size and in maintaining developmental events, for e.g., JA regulates leaf and root morphogenesis in soybean (Farhangi-Abriz et al., 2019). When a plant under salinity stress is treated with JA and NO it has been testified to increase plant biomass, root and shoot lengths significantly; chlorophyll *a*, *b*, and carotenoid contents also increase (Shan et al., 2015). It has been reported that, under salinity stress, combined exposure to JA and NO up-regulates the activities of SOD, CAT, ADX, GR, and results in a 70% enhancement in the flavonoid content in *Solanum lycopersicum* L. (Ahmad et al., 2018). Drought stress has been mitigated using the stress suppression ability of the JA+NO cocktail. Drought stress and JA both have been reported to enhance the level of endogenous NO, which induces stomatal closure (Ghorbel et al., 2021). Enzyme activity of the AsA-GSH cycle were increased by JA-induced NO production when JA was exogenously applied in wheat plants under drought stress, lowering ROS damage (Zhou et al., 2021). JA, in addition to stimulating NO synthesis, also results in the increase of ROS scavenging enzymes under chilling stress in *Cucumis sativus* L. (Shan et al., 2015).

Interactions between NO and JA are both synergistic and antagonistic. The various steps involved in JA biosynthesis are modulated by NO on activating enzymes, such as allene oxide synthase and allene oxide cyclase, associated with JA biosynthesis. Nitric oxide results in down-streaming of H₂O₂ and regulates ascorbate and glutathione metabolisms in the leaves of *Agropyron cristatum* L. through JA signalling. This report suggests the part played by NO in showing the role of JA in the regulation of antioxidants under cold stress, showing synergistic relationship. NO downregulates genes encoding JA biosynthetic enzymes, such as LOX2 which in turn downregulates JA by masking the production of H₂O₂ along with inhibiting protease gene, which reduces expression of pathogenesis genes under biotic stress, showing antagonistic relationship. A rapid increase in NO concentration is induced by JA in the cytosol of guard cells, while the NO-scavenger, cPTIO, inhibited responses of NO in guard cells of *Vicia faba* L. confirming NO production. Guard cell considered as a model system for investigating the interplay among NO, SA, and JA (Xin et al., 2019). Table 1 enumerates the importance of NO solitarily and in association with SA, JA signalling for various

physiological responses and their mechanisms involved under abiotic stresses.

Molecular perception of nitric oxide interactions with signalling molecules in plants under stress (Fig. 2)

Interactions of NO with other phytohormones during stress involves protein modifications through the mechanism of nitration and S-nitrosylation. Whenever a plant is under abiotic stress, then NO either present endogenously or when applied exogenously, reacts with the superoxide radical (O_2^-) and is converted into peroxynitrite ($ONOO^-$). This conversion plays a noteworthy role in stress management because of its influential reactive nitrogen species (RNS) molecule which is yet again used up in the nitration process and results in post-translation modifications in plants (Sami et al., 2018). Nitric oxide regulates the expression of genes that encode enzymes such as GST (Glutathione S-transferase), CHS (Chalcone synthase), GPX (glutathione peroxidase), and AOX1a (alternative peroxidase), all these enzymes are intricately involved in the detoxification of ROS thus, protecting plant (Khator & Shekhawat, 2019).

Studies confirm the rise in endogenous SA levels on NO treatment and vice-versa, dose-dependent stimulation of NO production on SA treatment has been observed in investigations of *nia1* and *nia2*

mutants of *Arabidopsis* (Jayakannan et al., 2013). Regulation of NR and nitric oxide synthase (NOS) activity implicates NO production under SA treatment. Evolution of NO induced by SA in soybean leaves in the presence of photosynthesis inhibiting herbicides suggested production of NO due to stimulating action of NR, later an attempt was made to define role for NR activity in the induction of NO by a genetic approach using an NR-deficient *Arabidopsis* mutant (*nia1*, *nia2*). When wild-type and mutant seedlings were incubated in the presence of SA, NO synthesis was clearly induced in both lines, showing that NR activity was not involved in this process but the participation of NOS-type enzymatic activities in SA-induced NO production in wild-type and *nia1*, *nia2* seedlings was confirmed with the NOS inhibitor L-NMMA (Kaya et al., 2020). In plants lacking SA, the role of JA comes into play, as these plants carry *NahG* transgene. In the plants carrying *NahG* transgene, salicylate hydroxylase converts salicylic acid (SA) to catechol. *Arabidopsis NahG* plants are defective in non-host resistance, under such conditions NO induces JA production for relieving stress in plants by regulating JA-responsive genes. Nitric oxide has recently emerged as a ubiquitous signal in plant, displaying its role in stress mitigation responses owing to its redox signalling by S-nitrosylation and known to be involved in inhibiting PCD

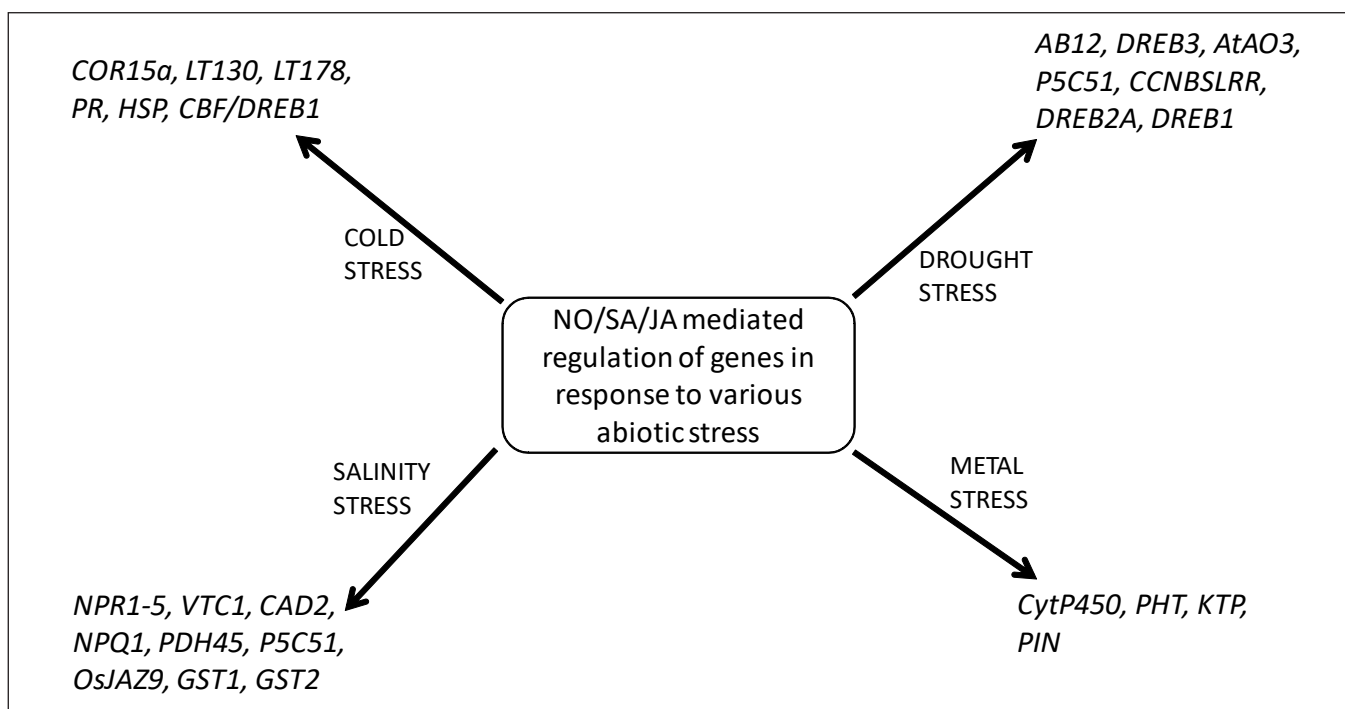


Figure 2: Upregulation of a range of genes (details in the text) induced by nitric oxide, salicylic acid, and jasmonic acid in response to different abiotic stresses.

(He et al., 2019). It is also referred to as an “artificial plant hormone” because of its recently discovered abilities against plant biotic and abiotic stress. The interaction of NO with various phytohormones such as ABA, AUX, cytokinin (CK), ethylene (ET), gibberellin (GA), BRs, SA, JA and their responses have been reported in plants during stress (Verma et al., 2020). Interaction between ABA-NO-IAA has been recently reported in roots of *Solanum lycopersicum* L. and it was found that ABA and molybdenum-dependent enzymes are important factors for NO production in plants under salt stress. On loading plant roots with NO fluorescent dye 4,5-diaminofluorescein diacetate (DAF-2A) for investigating NO production and results indicated accumulation of NO in wilt type tomato roots but opposite effect on *sitiens* (ABA-deficient mutants), similarly effect of NO on IAA signalling was also confirmed using auxin response reporter *DR5::GUS*, it was shown that NaCl and NO reduced auxin response but cPTIO treatment increased *DR5::GUS* signal. These results together suggest an ordered hormonal interaction between the ABA-IAA signalling network under high salt concentration in tomato plants (Santos et al., 2020). The possible interaction between GA and NO is well reported, GA and NO together control phytochrome interacting factors (PIFs), and augmentation of DELLA proteins occurs due to high NO concentration whereas its degradation by GA helps in hypocotyl growth, together it leads to the possibility of NO-GAs-light interplay in the regulation of seed germination events, which regulate the expression of gibberellin target genes, and promote photomorphogenesis. Cytokinins (CKs) also interact with NO both synergistically and antagonistically (Asgher et al., 2017).

Nitric oxide interacts also with other molecules involved in stress signalling, depending on the kind and severity of stress. NO supplementation upregulates the expression of genes such as *ACC synthase*, and enzyme coding 1-aminocyclopropas-1 carboxylic acid (ACC) under certain stress conditions. NO interacts with molecules such as receptors, secondary messengers, transcription factors (TFs), and ions such as K^+ , and Na^+ , Ca^{2+} and plays an important role during salinity stress (Hasanuzzaman et al., 2018). The interaction of NO and ROS increases the Na^+ extrusion via NO-induced Na^+/H^+ antiporters under salinity (Singh et al., 2021). The TFs such as DREB2, DREB4, and WRKV108715 are regulated by spermidine (Spd), and Spd is involved in inducing H_2S signalling on

interacting with NO. Crosstalk between Spd, NO, H_2S , and Ca^{2+} is well reported in the regulation of water stress tolerance in white clover plants, Spd is an important plant growth regulator and is involved in many stress responses. Exogenously applied Spd significantly increases L/D-cysteine disulphhydrate activities resulting in quick accumulation of H_2S and up-regulation of Ca^{2+} , improving antioxidant enzymes (SOD, CAT, APX) activity in plants under stress. Further analysis revealed that H_2S signalling was inhibited by the application of NO and H_2S inhibitors i.e., c-PTIO and NH_2OH respectively (Li et al., 2019). NO and H_2S together regulate vital signalling during seed germination and environmental stress responses. NO plays an important role in regulating protein kinases such as CDPKs and MAPKs, which leads to indirect control over the genes that are responsive to ABA signalling (Zhou et al., 2021). Figure 2 describes the up-regulated expression of genes involved in providing resistance against various abiotic stress conditions such as *COR1A*, cold regulated 15a gene *LT30*, low temperature induced gene 30; *LT178*, low temperature induced gene 178; *PR*, pathogenesis related genes; *CBF1*, C-repeat binding factor; and *DREB1*, dehydration-responsive element binding protein 1 are expressed under cold stress which helps in overcoming various cold generated phenotypic symptoms such as poor germination, stunted seedlings and also prevents chlorosis and necrosis. Under drought stress genes such as *ABI2*, ABA-insensitive 2; *DREB3*, dehydration responsive element binding 3; *AtAO3*, *A. thaliana* aldehyde oxidase 3; *P5CSI*, delta (1)-pyrroline-5-carboxylate synthetase 1; *CCNBSLRR*, (CC)-nucleotide-binding site leucine rich repeat; *DREB2A*, dehydration-responsive element binding protein 2A; and *DREB1*, dehydration responsive element binding 1 are expressed which improves growth rate and development in plants which is compromised under drought stress. Under heavy metal stress genes such as *CytP450*, cytochrome P450; *PHT*, phosphorus transporter; *KTP*, potassium transporter; and *PIN*, are expressed, these genes help in controlling detrimental effects of heavy metals and improves altered water balance, nutrient accumulation and enhances biomass accumulation, The genes such as *NPRI-5*, non-expressor of pathogenesis related; *VTCL1*, vitamin C defective 1; *CAD2*, cadmium sensitive 2; *NPQ1*, non-photochemical quenching 1; *PDH45*, pea DNA helicase 45; *P5CSI*; *OsJAZ9*, *GST1*, glutathione-S-transferase 1, and *GST2*, glutathione-S-transferase 2 are expressed under salt

stress and improves the water uptake, seed germination and maintain photosynthesis.

Conclusions and Future Prospects

This review article is a critical analysis of NO as a mediator of various stress signalling molecules giving special attention to SA and JA, two important plant hormones. It is noted that NO either alone or when applied together with other stress regulator regulates many physiological processes of plant growth, stomata open/closure, photosynthesis, hypocotyl elongation. Over upcoming years further research into the action of NO under non-optimal conditions has the potential to lead to a more integrated view of plant growth and abiotic stress resistance. The ability of NO to mediate a diversity of signalling molecules makes it a proficient abiotic stress regulator. Therefore, its involvement in future crop improvement programs could prove to be very important, even though at present its involvement in stress mitigation at reproductive plant phases is not clearly understood. The investigation of role of NO as a mediator of signalling molecules can be assisted with genomic studies for better insights into gene-gene and gene-protein interactions. Biotechnological applications in manipulating or enhancing genes for better stress responses could be a prospect in studies of NO production under stress.

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