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REVIEW

Nitric oxide, crosstalk with stress regulators and plant abiotic stress tolerance

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Abstract

Key message **Nitric oxide is a dynamic gaseous molecule involved in signalling, crosstalk with stress regulators, and plant abiotic-stress responses. It has great exploratory potentials for engineering abiotic stress tolerance in crops.**

Abstract Nitric oxide (NO), a redox-active gaseous signalling molecule, though present uniformly through the eukaryotes, maintain its specificity in plants with respect to its formation, signalling, and functions. Its cellular concentrations are decisive for its function, as a signalling molecule at lower concentrations, but triggers nitro-oxidative stress and cellular damage when produced at higher concentrations. Besides, it also acts as a potent stress alleviator. Discovered in animals as neurotransmitter, NO has come a long way to being a stress radical and growth regulator in plants. As a key redox molecule, it exhibits several key cellular and molecular interactions including with reactive chemical species, hydrogen sulphide, and calcium. Apart from being a signalling molecule, it is emerging as a key player involved in regulations of plant growth, development and plant-environment interactions. It is involved in crosstalk with stress regulators and is thus pivotal in these stress regulatory mechanisms. NO is getting an unprecedented attention from research community, being investigated and explored for its multifaceted roles in plant abiotic stress tolerance. Through this review, we intend to present the current knowledge and updates on NO biosynthesis and signalling, crosstalk with stress regulators, and how biotechnological manipulations of NO pathway are leading towards developing transgenic crop plants that can withstand environmental stresses and climate change. The targets of various stress responsive miRNA signalling have also been discussed besides giving an account of current approaches used to characterise and detect the NO.

Keywords Nitric oxide · Signalling molecule · Abiotic stress · Genetic engineering · Transcription factors · miRNA · Crosstalk

Introduction

Being sessile organisms, plants experience several challenging and stressful external conditions throughout their life cycles that include abiotic (drought, salinity, extreme temperatures, and heavy metals, beside other) and biotic stress factors (bacterial, fungal, viral, and herbivory). Plants respond to these conditions via inducing a series of different molecular, cellular and physiological changes (Yadav et al. 2020). A common consequence and response is generation of reactive chemical species that at low cellular concentrations act as signalling molecules regulating secondary messengers, enzyme activities and expression of key genes (Farnese et al. 2016). However, these reactive species may turn toxic to cells and organelles if their concentrations increase beyond threshold levels (Khare et al. 2020). These reactive species in plants include reactive

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oxygen species (ROS), reactive nitrogen species (RNS), reactive sulphur species (RSS) and reactive carbonyl species (RCS) (Zhou et al. 2021). Amongst different reactive species, ROS and RNS have gained significant attention because of their prevalent presence and dual roles in cellular metabolism, as signalling molecules or as oxidative burst causing-agents; based on their concentration in cell (Patel et al. 2019). Notable work has been done to understand and explore the roles of ROS in plant cells such as involvement in stomatal guard cell opening (Yamauchi et al. 2019), plasma membrane fluidity (Møller et al. 2007), male-sterility and tapetal programmed cell death (PCD) signalling (Neill et al. 2002; Miller et al. 2010; Suzuki et al. 2011). The RNS biology, however, is emerging as a topic of great interest with linear increase in the reports coming on various aspects of RNS in plants. Members in RNS family include nitric oxide (NO), S–nitrosothiols (RSNOs), S–nitrosoglutathione (GSNO), and peroxynitrite ($ONOO^-$). From such diverse members with contrasting characters, the only unifying character among RNS members is their derivation from nitric oxide (NO). Hence, it was necessary to investigate and decipher the NO biochemistry and physiological roles played by it to get better insights on RNS.

NO is a small diatomic gaseous molecule, with a short half-life. In early years, NO was thought to be involved mainly in air pollution and acid rains. It was not earlier than 1987, when role of NO as endothelium-derived relaxation factor (EDRF) in mammalian cells and synthesis of NO from amino acid L–arginine by enzyme NO synthase were identified (Ignarro et al. 1987; Palmer et al. 1987, 1988). Since then, extensive work has been carried out to identify the roles and functions of NO in both animals and plants. During stress, NO affect enzymes and proteins predominantly by covalently attaching to cysteine thiols by S–nitrosylation, along with other modifications such as nitrosylation of metalloproteins and tyrosine residues (Lindermayr 2018). Redox signal of NO by S–nitrosylation has recently emerged as a pioneer signal transmitter of NO bioactivity (Fernando et al. 2019). S–nitrosylation is a selective covalent post-translational modification, through which a nitrosyl group is added to reactive thiol group of a cysteine to ultimately form S–nitrosothiol, a key mechanism in transferring NO-mediated signals (Fernando et al. 2019). NO is also known to be involved in signalling for plant defence (Bellin et al. 2013), antioxidant and inhibitor of PCD (He et al. 2019a), plant-rhizobium infection (Fukudome et al. 2016), plant-fungal and oomycetes interactions (Jedelská et al. 2020), besides, symbiotic associations (Martínez-Medina et al. 2019). Along with this, NO has also been reported to be involved in plant responses to abiotic stresses such as salinity (Hasanuzzaman et al. 2018), drought (Montilla-Bascón et al. 2017), and metal stress (Sharma et al. 2019).

This review discusses the current knowledge and updates on biosynthesis, transport, and scavenging of NO in plants. A critical look on how NO interact with different stress regulators like secondary messengers, reactive chemical species, and phytohormones has been provided. Besides, this review discusses how these interactions can be used for crop improvement especially in creating abiotic stress (or climate) resilient crops using genetic engineering approaches. Recent trends in characterisation of NO along with idea of micro-RNA (miRNAs) signalling and regulations due to NO during stress conditions and adaptations are also discussed.

Nitric oxide: a dynamic entity involved in plant growth and stress responses

For the initial years after its discovery, NO was known as the ‘nitrous air’ or more commonly the ‘laughing gas’ and thus was extensively studied in mammals for its potential neurological impacts. The research shifted to plants after the report of NO generation/biosynthesis in plant cells (Klepper 1975). The NO research in animals got the Nobel Prize when Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad shared the Nobel Prize in 1998 for their discoveries concerning NO as a signalling molecule in the cardiovascular system (Nicholls 2019). Gradually, NO’s role in plants shifted from being a reactive species and a signalling molecule, to its involvement in plant defence system, changing the paradigm from cytotoxic to beneficial compound (Sahay and Gupta 2017). The role of NO varies across the strata of the organisms. For instance, it acts as a neurotransmitter in animals and regulates muscular, immunological, and respiratory responses (Esplugues 2002). In plants, it has displayed the involvement in stress response/signalling, plant growth and development and the PCD (Gupta et al. 2011). Evidence is also there that NO may protect some bacteria against oxidative stress (Gusarov and Nudler 2005). Figure 1 depicts the involvement and roles of NO in plant development and plant-environment interactions.

Being an important player in modulating plant growth under stress, NO has been reported to coordinate the activities required for plant organogenesis by participating in the pathways for production of plant hormones like jasmonic acid (JA), and brassinosteroids (BRs) (Raya-González et al. 2019). Auxins (AUX), a class of plant hormones are known to stimulate NO production in plant roots which results in lateral root development (Moni et al. 2018). Another possible mechanism is the methane-induced accumulation of NO, which leads to the increased formation and development of adventitious roots (Qi et al. 2017). Beside these roles, NO plays a vital role as a signalling molecule during the root development, nitrogen fixation, root-fungal interactions and promotion of high microbial diversity in the rhizospheric

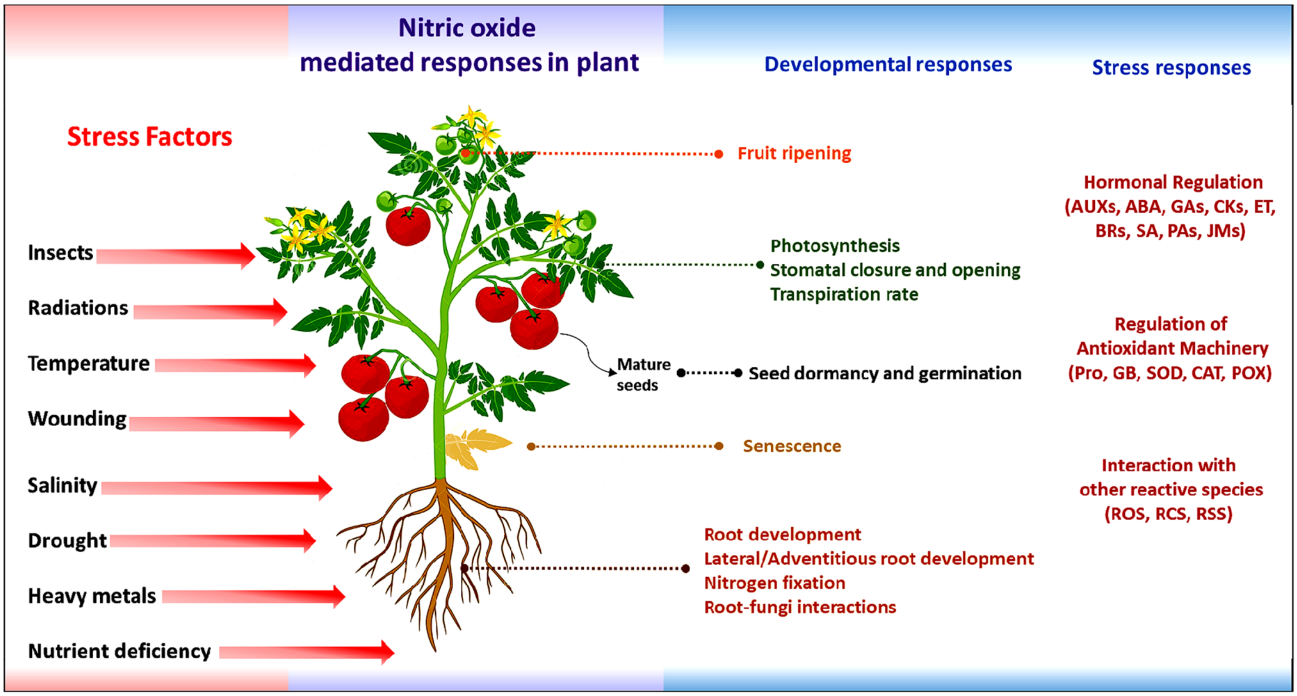

Fig. 1 Nitric oxide (NO) mediated responses during plant development and plant-environment interactions. (*AUX* auxins, *ABA* abscisic acid, *GAs* gibberellins, *CKs* cytokinins, *ET* ethylene, *BRs* brassinosteroids, *SA* salicylic acid, *PAs* polyamines, *JMs* jasmonates, *Pro* proline, *GB* glycine betaine, *SOD* superoxide dismutase, *CAT* catalase, *POX* peroxidase, ROS reactive oxygen species, *RCS* reactive carbonyl species, *RSS* reactive sulfur species)

environment (Ma et al. 2020). In *Tagetes erecta*, NO donor sodium nitroprusside (SNP) showed indirect organogenesis of shoot and root from callus, showing its role in plant growth (Jafari and Daneshvar 2020).

The role of NO in seed germination has also been described in many reports. The pre-treatment of seeds with SNP have been extensively explored. For instance, exogenous application of SNP to seeds of *Brassica chinensis* exerted positive effects on seed germination rate and seedling growth under saline conditions via enhanced scavenging activity of enzymatic antioxidants including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) (Ren et al. 2020). In *Brassica juncea,* SNP pretreated seeds showed improved seed germination rates under copper (Cu) stress due to induced antioxidant defense and increased amylase activity (Rather et al. 2020). Under chromium (Cr) stress, exogenous application of SNP relieves stress-induced impairment via promoting the activities of scavenging enzymes, amylase, and protease resulting in increased seed germination (Khan et al. 2020a). Table 1 enlists some important case studies involving exogenous applications of NO and its donor (SNP) for alleviation of negative stress impacts.

Peroxule are thin protrusions from the spherical peroxisomes, produced when the ROS levels are low. The peroxule production in a plant cell is highly dependent on the presence of NO in the cytoplasm (Terrón-Camero et al. 2020). In *Arabidopsis* under cadmium (Cd) stress, NO production and interaction were observed via electron microscopy during peroxule formation (Terrón-Camero et al. 2020). Exogenous application of NO to fresh fruits and vegetables showed delayed senescence and worked as a freshener or stabiliser during long-term storage of fruits/vegetables (Steelheart et al. 2019). In sweet pepper, the fruit ripening process was found to be NO-dependent (González-Gordo et al. 2019). Authors observed NO-dependent changes in sweet pepper fruit transcriptome, fruit ripening in the absence of NO showed changes in the abundance of 8805 transcripts, and the functional clusters associated with ROS/RNS were significantly modified. The NO treatment induced differential expression of 498 genes within these functional categories. These findings reveal the key roles of NO in the ripening of sweet pepper fruits (González-Gordo et al. 2019).

NO is emerging as a vital player underlying abiotic and/or biotic stress interactions in plants. NO production during such interactions tends to alleviate in such stressful environment. NO has demonstrated to regulate the chlorophyll production and to stimulate the hypersensitive chain reactions during stress interactions (Lazalt et al. 1997; Romero-Puertas et al. 2004). Stress-mediated increase in NO production stimulated the activity of various ATPases, and ion channels for better homeostasis. In maize for instance,

Table 1 A summarised list of plant abiotic stress alleviation via exogenous application of nitric oxide and/or its donor sodium nitroprusside

Plant	Approach	Phenotypic responses	References
Citrus sinensis L.	Exogenous NO donor SNP application under cold stress	Reduced lipid peroxidation levels and hydrogen peroxide contents. NO-induced DPPH radical scavenging activity	Ghorbani et al. (2018)
Brassica napus L.	Exogenous NO and aminolaevulinic acid application under drought stress	Decrease in oxidative damage and improved growth, chlorophyll content and antioxidant enzyme activity	Akram et al. (2018)
Glycine max L.	Exogenous SNP and S–nitroso L–cysteine application under flood stress	Induced glutathione and reduced superoxide anion contents, elevated ABA contents and mitigated the negative effects of short-term flooding stress	Khan et al. (2019)
Hordeum vulgare L.	SNP treatment under Cd stress	Increased expression levels of *AOX1a* gene, and enhanced Cd stress tolerance	He et al. (2019b)
Brassica juncea L.	NO and SNP pre-treatment under oxidative stress	Advancement in seed germination, alleviation in Cu-induced photosynthetic inhibition	Rather et al. (2020)
Brassica juncea L.	SNP treatment under salt stress	Regulated the photosynthetic performance and induced salt stress alleviation	Jahan et al. (2020)
Triticum aestivum L.	Exogenously-applied NO donor SNP and H_2O_2 as seed primers under drought conditions	Reduction in H_2O_2 and MDA contents and improved drought tolerance	Habib et al. (2020)
Triticum aestivum L.	Exogenous NO donor SNP in presence of TiO_2 nanoparticles under salt stress	Enhanced seedling dry mass, relative water content, antioxidant enzymes, and proline content, coupled with reduced hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents	Faraji and Sepehri (2020)
Cucumis sativus L.	Exogenous NO treatment under low-temperature stress	Improved tolerance to low-temperature stress via enhanced efficiency of the Calvin cycle and the ascorbate–glutathione cycle	Wu et al. (2020)
Solanum lycopersicum L.	SNP treatment under chromium stress	Relieved impairment caused due to chromium by promoting activities of scavenging enzymes, amylase, and protease resulting in increased seed germination	Khan et al. (2020a)
Cucurbita pepo L.	SNP treatment under chilling stress	Enhanced chilling tolerance through S–nitrosylation of proteins and modulation of the antioxidant response	Jiménez-Muñoz et al. (2021)

NO was shown to improve salinity tolerance via regulating the plasma membrane proton pump activities (Zhao et al. 2004). The authors provided the evidence for establishing the involvement of NO in salt tolerance of *Phragmites communis* (Zhao et al. 2004). NO, produced under salt stress, served as a second messenger for the induction of plasma membrane H^+-ATPase expression, which in turn maintained high K-to-Na ratio for conferring better salt resistance (Zhao et al. 2004). SNP-treatment has been reported to exert several positive impacts like increased seed germination in *Lupinus luteus* (Kopyra and Gwóźdź 2003), restored developmental mechanisms in seedlings of sunflower (David et al. 2010), plant growth, increased ionic levels and scavenging enzymes activity in citrus during saline stress (Khoshbakht et al. 2018). Stomatal opening and closing play major roles in conferring plant abiotic stress tolerance. NO being a gasotransmitter, regulates the signalling network involved in the movement of guard cells, causing stomatal opening and closing (Nabi et al. 2019). During heat stress, the heat shock response (HSR) of the plants mediate through biosynthesis and transport of NO and salicylic acid (SA) via calcium signalling (Rai et al. 2020). This acts as a regulator of Heme Nitric oxide/Oxygen (H-NOX) motif of a heme binding protein involved in the regulation of endogenous NO synthases. High concentrations of NO and SA cause conformational changes in non-expresser of pathogenesis related (NPR) genes involved in stress-responsive genes against heat (Rai et al. 2020). These NPR genes act as master regulators of SA signalling under pathogen attack and are important in terms of providing plants innate immunity (Innes 2018). Thus after several years of extensive research on deciphering the roles and mechanisms of NO in plants, various avenues have been opened up on formulations of new research hypotheses. Owing to its dynamic nature and versatility, NO provides a great scope for its application and exploration in crops for increasing the plant efficiency and tolerance/resilience to stress conditions.

Biosynthesis, transport and scavenging of nitric oxide in plants

NO is endogenously present in the plant cells as a by-product of various catabolic reactions of nitrogen-containing compounds. Besides, it is absorbed by the plants from the surrounding environment and soil. The knowledge and understandings about the NO synthesis in plants was influenced by the findings on NO production in animal systems putting an emphasis on nitrate reductase (NR), the enzyme that usually converted nitrates to nitrites but was later observed to convert the nitrite to nitric oxide (Crawford 2006).

NO has two biosynthetic pathways as depicted in Fig. 2, an oxidative one which includes the production through oxidation of L–arginine or other polyamines (PAs), and second one a reductive pathway which heavily depends on NR and other reductive enzymes present in the mitochondrial and plasma membrane (Gupta and Igamberdiev 2011). The reductive pathway uses enzymes produced in

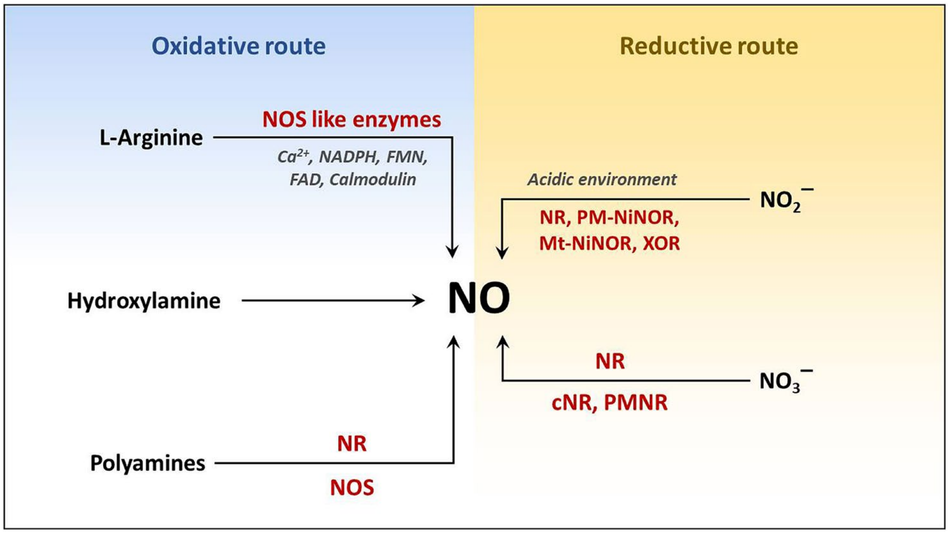

Fig. 2 Oxidative and reductive routes of nitric oxide (NO) biosynthesis. Reductive rout (observed in plasma membrane, chloroplasts, apoplast, peroxisomes, cytoplasm, and mitochondria) occur via reduction of nitrate (NO_3^-) or nitrite (NO_2^-) by nitrate reductase (NR). In oxidative route (observed in chloroplasts and leaf peroxisomes) occurs via oxidation of L-Arginine or hydroxylamine. (*cNR* cytosolic nitrate reductase, *PMNR* plasma membrane-bound nitrate reductase, *PM-NiNOR* plasma membrane-bound nitrite NO reductase, *Mt-NiNOR* mitochondrial electron transfer chain-dependent enzymatic nitrite NO reductase, *XOR* xanthine oxidoreductase, *NADPH* nicotinamide adenine dinucleotide phosphate, *Ca^{2+}* calcium ions, *FMN* flavin mononucleotide, *FAD* Flavin adenine dinucleotide)

the mitochondrial electron transport chain (ETC), mainly amidoxime reducing compound, molybdenum cofactor dependent reducers (León and Costa-Broseta 2020), and other reductases like xanthine oxidoreductase (XOR), nitrite dependent nitric oxide reductase (Ni:NOR), along with NR (Sahay and Gupta 2017). This pathway is usually dominant under normoxic conditions whereas oxidative pathway requires a hypoxic environment (León and Costa-Broseta 2020).

The production of NO involves the participation of various organelles like mitochondria, chloroplast, peroxisomes, endoplasmic reticulum, apoplast, plasma membrane, and the cytoplasm of the cell (Sahay and Gupta 2017). The mechanism of production varies between organelles depending on their preference to either of the two previously mentioned pathways. The reductive pathway occurs in the plasma membrane where lower pH converts NO_2^- to NO and O_2 and in apoplast releasing the root-specific NO using nitrite substrates present in it. Organelles like chloroplast, peroxisomes, and mitochondria switch the production pathways depending on the availability of substrates and the environmental conditions. Cytoplasm converts nitrate into nitrite in an NADPH-dependent manner for further reduction along with oxidation of PAs. It also produces intermediate NO during GSH to GSNO conversion via a glutathione-based pathway (Nabi et al. 2019).

Exogenous NO is transported inside the cell via nitrogen transporter in the form of nitrate, which then undergoes reductive pathway forming NO by nitrate NR in an NADPH-dependent manner (Zhou et al. 2021). The nitrate homeostasis is maintained by high- and low-affinity nitrate transporters (Chamizo-Ampudia et al. 2017). Along with these, transporters belonging to the chloride channel (CLC) family maintain nitrate concentrations in the cytoplasm via effluxes of various organelles.

NO, like all metabolites in a cell needs to be balanced at a particular concentration, which is done by the cell's metabolic and scavenging activities regulated by specific enzymes and substrates. In animals, proteins like flavohaemoglobin, haemoglobin and reductases degrade the excess NO while in yeast flavoglobin is the main NO degrader (Liu et al. 2000; Gardner 2005; Gupta et al. 2020b). Similarly, plants have their NO scavengers namely phytoglobins, that are highly efficient under hypoxic conditions converting NO to nitrate by oxidising itself into metphytoglobin (Gupta and Igamberdiev 2011). This is converted back to phytoglobin by a reductase mediated reaction co-factored by ascorbate (Hebelstrup et al. 2008; Gupta and Igamberdiev 2011; Gupta et al. 2020b). Reduced glutathione (GSH) reacts with NO to form nitrosoglutathione (GSNO), which can be harnessed to give back NO for nitrosylation of proteins and thus contributing to its cellular concentration maintenance (León and Costa-Broseta 2020; Kalinina and Novichkova 2021). On the other hand, a further oxidation of GSNO results in production of ammonia and glutathione disulphide by specific reductase (León and Costa-Broseta 2020). When the nitrosylated proteins are non-nitrosylated, they release NO back in the cell affecting the maintained NO-balance (León and Costa-Broseta 2020). This happens when the S–nitrosylation-related signalling is affected during pathogen attack, which triggers plant's inherent immunological response. A thioredoxin-mediated redox signalling was reported to be involved in regulation of S–nitrosylation of proteins, contributing to the homeostasis of NO and involved in plant immunity (Mata-Pérez and Spoel 2019; León and Costa-Broseta 2020). Other regulators of NO degradation are NAD(P)H dehydrogenases-type dehydrogenases, that act by converting the NO into peroxynitrite ($ONOO^-$) via forming the superoxide anions, under calcium stimulation and hindered by anoxic conditions by SOD (Gupta et al. 2020a).

Another method of NO scavenging is during reactive species reaction when NO interacts with oxygen generating nitrate and nitrite or when it forms nitro fatty acids via its reactions with lipid peroxyl radical (LOO•) (Hancock 2012; Rubbo 2013; León and Costa-Broseta 2020). Haemoglobins have been reported to contribute in NO scavenging through its reduction to FE(II)Hb which then deoxygenates the NO (Chamizo-Ampudia et al. 2017). NO is metabolised in presence of oxygen by being converted to dinitrogen trioxide (N_2O_3) and NO_2. Subsequently, in an aqueous environment, they are converted to nitrite and nitrate as part of a nitro-oxidative stress response (Corpas and Palma 2018). Overall, we can conclude that the NR is a key enzyme for nitrogen acquisition by plants. Nitrate, its main substrate, is required for signalling and is widely distributed in diverse tissues in plants. In addition, NR has been proposed as an important enzymatic source of NO (Chamizo-Ampudia et al. 2017). The homeostasis of NO depends on at least two key molybdoenzymes, NR and NO forming NR (NOFNiR) besides dioxygenase activity of haemoglobins.

Current approaches for detection and characterisation of nitric oxide

Characterisation and quantification remain two most important parameters in studying the reactive species in stress biology. Among different reactive species, measurement of NO remains challenging and tricky because of its ubiquitous, gaseous and unstable nature. Another parameter while characterising NO is inter-conversion of NO^-, NO, and NO^+ under physiological conditions; hence care should be taken while designing the experiment and choosing the NO donor to avoid misinterpretation of the results (Yamasaki et al. 2016).

NO detection method based on colorimetric bioassay, mass spectroscopy, chromatography, fluorescent probes; spin traps, electrode-based techniques have been developed (reviewed by Csonka et al. 2015). Each technique has its own advantages and disadvantages. Most frequently used bioassay technique for detection of NO via Griess method. This is a method based on nitrite-derived NO quantification based on the Griess reaction. The Griess reaction is specific for nitrite quantification. Principle behind Griess method is, in presence of O_2, NO gets converted to NO^{2-} which reacts with Griess reagent and is detected spectrometrically at 540 nm using a plate reader (Antoniou et al. 2018). Another version for detection of gaseous NO is using CrO_3 oxidizer column, CrO_3 converts NO to NO_2 which subsequently is trapped using Griess reagent solution to form NO^{2-} and detected using spectrophotometrically (Vitecek et al. 2008). Along NO, detection of S–nitrosothiols has emerged as important player. S–nitrosothiols (RSNO) detection by Saville reaction using mercury chloride and later Griess reagent has been used (Bryan and Grisham 2007).

Real-time monitoring and measuring becomes important in studies when NO is involved in signalling cascade or when formation and conversion takes place in very short time and at low quantity. In such studies bioassay or biophysical techniques are not suitable for real-time monitoring and the real time monitoring can be done using fluorescent probes, chemiluminescent detector or using electrodes. NO-specific probes that are widely used are diaminofluoresceins (DAFs), 2,3–diaminonaphthalene (DAN), 1,2–diaminoanthraquinone (DAQ or DAA), diaminorhodamine (DAR-4M AM), fluorescent nitric oxide cheletropic traps (FNOCT), acridine–TEMPO–DTCS–Fe(II), 8–(3,4–diaminophenyl)–2,6–bis (2–carboxyethyl)–4,4–difluoro–1,3,5,7–tetramethyl–4–bora–3a,4a–diaza–sindacene (DAMBO–PH) (Zhou et al. 2021). Ozone-based chemiluminescent detector (CLD) is another sensitive technique, in which NO reacts with ozone, excited NO_2 emits lights which is detected by photomultiplier (Bryan and Grisham 2007).

Biophysical techniques for measurement of NO are more sensitive. For biological detection of gaseous NO, often detection/quantification is required at very low (ppb) level, and hence high-throughput, ultra-sensitive analytic techniques are needed. NO detection based on Infra-red (IR) spectroscopy at absorbance at 5.3 µm has been reported, accordingly two systems laser photoacoustic detection (LAPD) and quantum cascade lasers (QCL) based on IR spectroscopy have been developed with high sensitivity and specificity (Mur et al. 2011). Mass spectroscopy-based detection is another technique used for detection of gaseous NO present in plant tissues. Two types of MS approaches have been developed for detection of NO, membrane inlet mass spectrometry (MIMS) and restriction capillary inlet mass spectrometry (RIMS) (Conrath et al. 2004). In MIMS/RIMS system, before MS detection, a prior step for molecule separation is done via membrane (MIMS) or through restriction capillary (RIMS) which allows only small molecules to pass through such as NO. As an advantage over other NO assays, MIMS/RIMS have advantages over other NO assays, as it discriminates nitrogen isotopes and simultaneously measures NO and O_2 (and other gases) from the same sample. This technology thus holds significance and may be used to identify cellular NO sources, besides, it can also be used for elucidating the relationships between primary gas metabolism and NO formation (Conrath et al. 2004).

The most reliable, specific and sensitive technique, however, for detection of NO can be considered as electron spin resonance (ESP) also called as electron paramagnetic resonance (EPR). EPR detects only free radicals, making it specific for NO detection over other radicals (Kleschyov et al. 2007; Mur et al. 2011). It is the most specific method, due to NO-specific spin traps used in it. Major advantage associated with the use of EPR for NO detection is that it only detects the paramagnetic molecules, and the EPR spectrum presents a unique fingerprint of the chemical/electronic structure around the unpaired electron.

Crosstalk of nitric oxide with stress regulators

Crosstalk with signalling molecules

The interaction of NO with signalling molecules such as receptors, secondary messengers, transcription factors (TFs) and enzymes during physiological and environmental responses of the plant cells has led to the study of crosstalk of NO with ions like Ca^{2+}, K^+, and Na^+. During salinity-induced stress responses, the activities of signalling molecules including ROS, NO and Ca^{2+} are altered in the guard cells leading to the regulatory changes in opening and closing of stomata (Hasanuzzaman et al. 2018). Salinity-driven responses in the mangrove plants revealed the interaction of H_2O_2, ATP, and Ca^{2+} increase the Na^+ extrusion via NO-induced Na^+/H^+ antiporters (Lang et al. 2014). Garcia-Mata et al. (2003) studied the regulation of Ca^{2+} ion channels in *Vicia faba* guard cells mediated through NO by releasing Ca^{2+} from inner cell stores to the cytosol, leading to the controlled guard cell movements and indicating the NO-induced Ca^{2+} activation of cellular signalling. These results confirmed the NO action decisively in one branch of Ca^{2+} signalling pathways engaged by ABA and defined the boundaries of parallel events in control of guard cell movements (Garcia-Mata et al. 2003). Mitogen-activated protein kinase (MAPK) signalling pathway can be activated via phytohormones; however, reports have shown the H_2O_2–NO or NO-mediated cGMP-independent pathway stimulation of

MAPK with the help of secondary messengers during physiological processes and abiotic stress responses (Hasanuzzaman et al. 2018; Shen et al. 2019). Reports have revealed the crosstalk of NO and MAPK–1/2 via NO generation by NR, the activity of S–nitrosylated glutathione reductase, and activation of MAPK1/2 enzymes during cold stress tolerance in tomato plants (Lv et al. 2017).

An important polyamine spermidine is considered crucial for plant adaptations to the environmental stresses (Alcázar et al. 2006). Besides, spermidine is reportedly involved in the induction of H_2S signalling via increasing the L/D–cysteine desulfhydrate (L/DCD) activities (Li et al. 2019). The TFs such as *bZIP37*, *bZIP107*, *DREB2*, *DREB4*, and *WRKY108715* are regulated by the spermidine during H_2S downstream signalling and crosstalk between NO, H_2S, and Ca^{2+} signalling resulted in water stress tolerance in white clover plants (Li et al. 2019). The WRKY TFs are actively involved in the environmental stress responses. In tomato, SlWKRY81 reported to repress the transcription of *NR* and to lower the NO accumulation for modulating the stomatal closure and subsequently the drought tolerance (Ahammed et al. 2020). Hajihashemi et al. (2020) studied the crosstalk between NO, H_2O_2 and Ca^{2+} at the germination stages in *Chenopodium quinoa* plants subjected to salinity stress. The findings revealed stimulated amylase activities in the seeds pre-treated either with SNP, H_2O_2 or $CaCl_2$. This resulted in high starch breakdown rates and thus increased availability of water-soluble sugars; which can be attributed to the improved germination under the saline conditions (Hajihashemi et al. 2020).

Crosstalk with reactive species

The inevitable production of the reactive species during the stress interactions in plants is well-established. The evidences have suggested the interplay between NO and the ROS (such as H_2O_2) as well as RSS (such as H_2S). Aerobic metabolic processes in subcellular-compartments of various organelles results in ROS generation in mitochondria, chloroplasts, apoplasts, and plasma membrane, which is formed by partial reduction of oxygen. Amongst these, the hydrogen peroxide (H_2O_2), superoxide anion $\left(O_2^{*-}\right)$ and hydroxyl radical ($^{\cdot}OH$) are the major reactive species (Kaur et al. 2019; Kalia et al. 2017). ROS are major signalling components involved in plant growth, development, and abiotic/biotic stress responses, which interplays with the RNS generation and NO signalling (Hancock et al. 2002; Wrzaczek et al. 2013). NO and ROS play vital roles in biological- and cross-talk processes involved in plant responses to environmental stresses (Kohli et al. 2019). In plants, NO acts as iron ligand in heme-containing enzymes which leads to their activation/inhibition. During stress-interactions, the ROS generation induces NO signalling, where NO binds with the heme group of the enzymatic ROS scavengers (like CAT). The highly-reactive, lipophilic NO binds to the thiolic- and metallic-groups of enzymes, which may modulate the enzyme activities (Arora et al. 2016). Further, at low NO concentration, the scavenging of $^{\cdot}OH$ confers the antioxidant properties to NO. Besides, NO is also known as the strong inhibitor of protein oxidation and lipid peroxidation (Fancy et al. 2017).

Several reports have established melatonin (MET) as one of the amphiphile multi-functional signalling molecules involved in physiological responses, abiotic responses, ROS and RNS scavenging, cell antioxidant activities, and oxidative-stress improvement (Debnath et al. 2019). The MET levels can regulate the ROS concentrations in two ways; firstly, by chemical interaction with ROS leading to the ROS inactivation, or secondly, by MET induced activities of enzymatic antioxidants including SOD, CAT and POX (Arnao et al. 2019; Khan et al. 2020a, b). Exogenous MET associate with CAND2/PMTR1 (CANDIDATE G-PROTEIN COUPLED RECEPTOR 2/Phytomelatonin Receptor 1) receptors, resulting in triggering of responses against stressors and regulation of ROS and RNS (Pardo-Hernández et al. 2020). Liu et al. (2020) reported reduced trehalose-mediated lipid–peroxidation levels in tomato leaves treated with H_2O_2 and NO scavengers, dimethyl thiourea (DMTU) and 2–(4–carboxyphenyl)–4, 4, 5, 5–tetramethylimidazoline–1–oxyl–3–oxide (cPTIO). The results indicated that the trehalose might act as H_2O_2 and NO inducer that modulates the expression of genes from antioxidant defense system for conferring cold tolerance. On similar lines, the interactions between H_2O_2 and NO were studied during the BR-induced stress responses in *Medicago truncatula* by Arfan et al. (2019). The results shown the participation of H_2O_2 in NO generation, and NO was found key in BR-induced alternative oxidase capacity, which protected the photosystem under cold stress (Arfan et al. 2019).

Chemical priming is emerging as an effective approach for conferring stress tolerance in plants. For instance, *Citrus aurantium* L. roots pre-treated with SNP (NO donor), enhanced the NO- and H_2O_2-induced salinity and drought acclimation in plants (Molassiotis et al. 2016). Priming of wheat roots (using polyethylene glycol 6000) stimulated the production of H_2O_2 and NO which was attributed to the priming-induced drought tolerance (Wang et al. 2020c). The report also suggested the direct relation of H_2O_2 biosynthesis with respiratory burst oxidase homologs (RBOH) and the downstream working of NO during the H_2O_2 mediated accumulation of proline and glycine betaine (Wang et al. 2020c).

The nutrient recycling event of the leaf senescence is also linked with the redox balance in plants (Jing et al. 2008). During the senescence, there is marked increase in the H_2O_2 level in leaf tissues. Apart from the damaging role of the macromolecular oxidisation that promotes the cell death,

H_2O_2 also functions as a signal to induce the expression of senescence related genes (Cui et al. 2013). On the other hand, NO can provoke or impede the senescence process depending on its subcellular localization and concentration. The toxicity of ROS may get alleviated by the NO via enhancing the activity of antioxidant enzymes (Liu and Guo 2013). Similarly, the intracellular NO and nitrosothiol levels act as vital mediators during the H_2O_2-induced leaf cell death (Lin et al. 2012; Wang et al. 2013a). Collectively, both NO and H_2O_2 can induce the cell death in leaves during which there is observable interplay between NO and H_2O_2 (reviewed by Wang et al. 2013b).

Besides H_2O_2, the H_2S (RSS) is also a vital signalling molecule during seed germination, photosynthesis and environmental stress responses (Chen et al. 2015a, b). The NO and H_2S jointly regulate the key events during the plant growth and development (seed germination, root development, stomatal movement and fruit ripening) and stress tolerance (reviewed by Mukherjee and Corpas 2020). Studies have revealed the interactive effects of NO and H_2S during Cd-induced stress interactions in wheat, which resulted in the reduced oxidative stress and Cd uptake, attributable to the activated antioxidant defence and uptake of essential minerals like Zn and Fe (Kaya et al. 2020). Further, the interaction between NO and H_2S resulted into the generation of persulfide (intermediate product) which regulate the ROS and RNS contents in plant cells (Lisjak et al. 2013). The seedlings of sweet potato exposed of sodium hydrosulfide (NaHS, H_2S donor) displayed the increased levels of H_2S and NO (Zhang et al. 2009). The plants also displayed H_2S-stimulated adventitious root formation mediated through NO, which authors attributed to H_2S activity upstream of the NO signal-transduction pathways (Zhang et al. 2009). However, further studies are still required to establish a deeper understanding of the crosstalk between NO and H_2S (Chen et al. 2016).

Crosstalk with phytohormones

Like the reactive species and secondary messengers, NO also interacts with the phytohormones such as ABA, AUX, cytokinins (CKs), ethylene (ET), gibberellins (GAs), BRs, salicylic acid (SA), γ-aminobutyric acid and jasmonates during different plant metabolic and physiological conditions and abiotic stress responses (Asgher et al. 2017). Studies have shown the signalling pathways of NO and AUX being interrelated with each other during abiotic stress responses and during the root growth and development (reviewed by Sanz et al. 2015). NO improved Cd-tolerance in *M. truncatula* roots via reducing the oxidative damage, enhancing the ion uptake (K^+ and Ca^+) and maintaining the AUX equilibrium (Xu et al. 2010). Santos et al. (2020) studied ABA-NO-AUX interactions in roots of *Solanum lycopersicum*, and observed ABA and molybdenum dependent enzymes as decisive factors for the salt stress driven NO production. The study also highlighted the integration of NO in the ABA–indole acetic acid (IAA) signalling network during salt stress response along with the ABA mediated and NO-dependent activity of the antioxidant system (Santos et al. 2020). Cd and As treatments decreased the endogenous NO concentration in roots of *Oryza sativa* along with high rate of H_2O_2 formation and altered AUX biosynthesis (Piacentini et al. 2020). The SNP-formed NO proved to mitigate the Cd/As toxicity by reducing the H_2O_2 production and induced expression of *AUX1* (IAA–influx carrier) (Piacentini et al. 2020). Application of SNP in the root systems of *Oryza sativa* under Cd/As stress resulted in the alleviation of IAA and reduced H_2O_2 generation, leading to the increased lateral root production and NO generation, ultimately mitigating the toxic impacts of these toxic heavy metals (Piacentini et al. 2020). Figure 3 highlights the interplay between the NO and phytohormones, TFs, miRNAs, reactive species and ions.

GAs are known to be involved in various physiological and/or developmental processes in plants including seed germination, leaf expansion, stem elongation, initiation of flowering and development of fruits. Several reports have supported the possible interplay between GAs and NO (Asgher et al. 2017). In *Arabidopsis thaliana*, antagonistic interaction between the NO and the GA resulted in the regulation of root growth during fluctuating levels of phosphorous (Wu et al. 2014). Lozano-Juste and León (2011) studied the crosstalk between NO and GA involving the phytochrome-interacting factors (PIFs) and GA-regulated DELLA proteins; where NO synchronise with PIF genes, increases the DELLA proteins content, and promotes photo-morphogenesis. Beside GAs, CKs also play important roles in the plant developmental processes (such as chloroplast biogenesis, leaf senescence and cell division) (Fahad et al. 2015). Both synergistic and antagonistic interactions have been observed during NO and CKs crosstalk (Asgher et al. 2017). The effectiveness of CKs in photosynthesis processes was studied under drought stress in *Zea mays*, which revealed the CK-stimulated conversion of excitation energy to electron transfer due to the probable interaction between NO and plastoquinone. Results also indicated CK-induced NO formation apparently via NR (Shao et al. 2010). Further, Liu et al. (2013) investigated the interactions of CK and NO in *Arabidopsis* under metal stress, where CK suppressed the action of NO probably via direct interaction between them, resulting in the overall reduced levels of endogenous NO levels.

It is well-established that ABA is involved in seed germination, dormancy and abiotic stress responses. The ABA mediated enhancement in NO biosynthesis in guard cells was observed, indicating the strong interplay between ABA and NO for provoking the stomatal closure whenever required (Joudoi et al. 2013). NO regulated the stomatal closure via

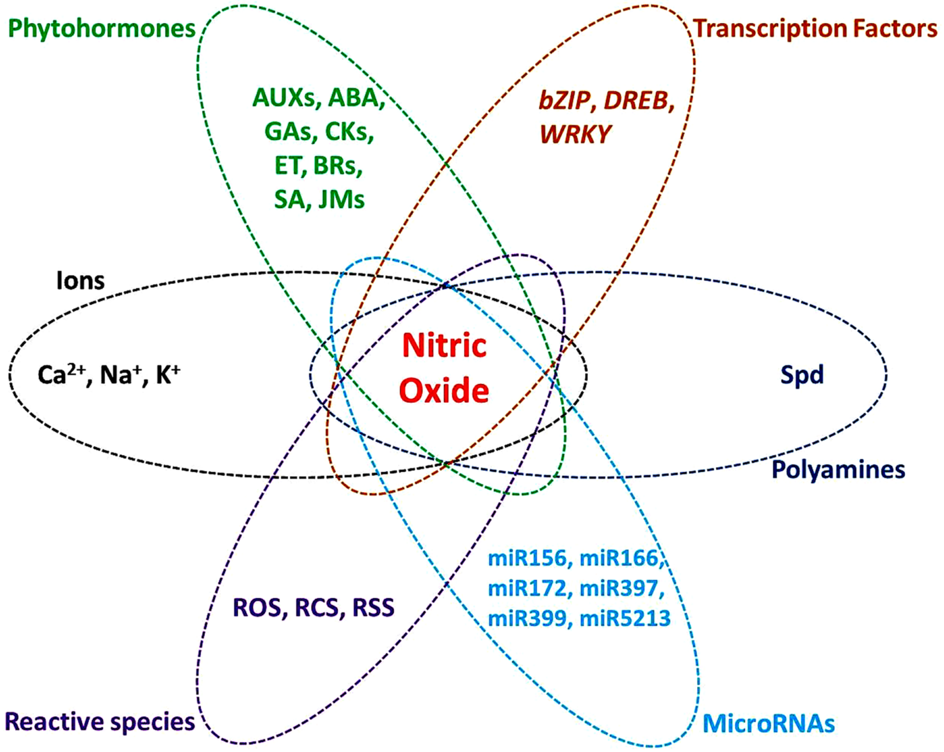

Fig. 3 Interplay between the nitric oxide (NO) and phytohormones (*AUXs* auxins, *ABA* abscisic acid, *GAs* gibberellins, *CKs* cytokinins, *ET* ethylene, *BRs* brassinosteroids, *SA* salicylic acid, *JMs* jasmonates), transcription factors, Polyamines (*Spd* spermidine), microRNAs, reactive species (*ROS* reactive oxygen species, *RCS* reactive carbonyl species, *RSS* reactive sulfur species) and ions

modulating the K^+ and Ca^{2+} in guard cells (reviewed by Asgher et al. 2017). ABA-induced NO synthesis pathway was reported in *Z. mays* leaf with increased concentrations of H_2O_2 inhibited by the NADPH oxidase inhibitor, called diphenylene iodonium (DPI), and activation of NO mechanisms for the maintenance of cell homeostasis (Tossi et al. 2009). Various studies have been done in *Arabidopsis* to study the crosstalk between NO and ABA or ET. In *Arabidopsis*, MoCo-sulfurase ABA3 enzyme converts the de-sulfo MoCo form (cofactor of NR enzyme) to sulfo MoCo form (cofactor of aldehyde oxidase) for the production of NO and ABA in the plants (Arc et al. 2013). H_2O_2 is produced endogenously via ABA signalling, leading to the generation of NO for the regulation of stomata movements (Bright et al. 2006). Increased UV-B treatment can lead to the production of ROS and RNS and induction of various signalling pathways during the stress responses in plants; increased production of ABA causes the generation of NO and H_2O_2, leading to the maintenance of cellular homeostasis and cellular damage alleviation (Prakash et al. 2019).

ET is known to play major roles in fruit ripening, flower senescence, and adventitious root hair formation where crosstalk of NO and ET is involved antagonistically during stress conditions (Wei et al. 2020). During abiotic stresses, NO signalling is recognised by S–nitrosation regulated by the production of ET, leading to the antagonism in various plant developmental and physiological processes such as Cd-induced cell death, stomatal opening, and UV–B induced stomatal closure (Kolbert et al. 2019). The S–adenosyl–methionine (S–AdoMet), precursor of ET and polyamine; is involved during the negative feedback regulation between ET and polyamine dependent synthesis of NO (Arc et al. 2013); ABA signalling and NO synthesis is regulated by the Cu-amine oxidase enzyme. In *Arabidopsis thaliana* and *Cucumis sativus*, up-regulation of genes such as *AtFIT* (TF), *AtFRO2* (ferric reductase), and *AtIRT1* (iron transporter) during cross-talk of NO and ET was observed in acquisition of iron (García et al. 2010). SA is widely known for its involvement in the immune responses under various abiotic stress responses showing both synergistic and antagonistic effects (Asgher et al. 2017). Key roles played by SA and NO signalling in plant growth and physiological processes beside environmental stress responses, have always attracted the scientific community. The non-expresser of pathogenesis related (NPR) gene signalling is mediated by SA and NO during the stress interactions via switching the SA and NO biosynthesis through Ca^{2+} signalling (Rai et al. 2020). SA/NO-mediated heat stress resistance and stress memory has been studied using epigenetically modified plants for the regulations of cellular immunity, leading to the activation of various heat stress-responsive TFs and genes for the better understanding of signalling cascades (Rai et al. 2020). In *Vigna angularis,* the exogenous application of SA triggered the interactions between SA and NO, which resulted into the enhanced photosynthesis, coupled with upregulation of amino acid and carbohydrates as well as antioxidant enzymes (SOD, CAT), and ultimately improved the salt stress tolerance (Ahanger et al. 2020). Wang et al. (2020a) observed SA-induced stomatal closure, controlled by ROS and NO generation, which was mediated by ET.

Similarly, NO also interact with BRs and play key role in plant stress responses. Zhou et al. (2018) observed that the BR treatment increased NO accumulation which resulted the reduction of virus accumulation in Arabidopsis plants. Furio et al. (2019) in an attempt to evaluate the protective effects of 24–epibrasinolide (EP24) and a formulation based on a brassinosteroid spirostanic analogue DI–31 (BB16) against the fungal pathogen *Colletotrichum acutatum*, the causal agent of anthracnose disease, and the treatment with both the BR compounds induced a defence response in strawberry plants which was mediated through enhanced NO levels. In another interesting study, BR treatment of water-stressed *Z. mays* leaves induced the NO levels and upregulated the expression levels of ABA pathway gene, which enhanced the water stress tolerance (Zhang et al. 2011). Further, Arfan et al. (2019) reported that H_2O_2 and NO crosstalk mediates BR-induced cold stress tolerance in *Medicago truncatula.*

Nitric oxide and miRNA signalling

Non-coding small RNAs, particularly miRNAs play important regulatory roles at transcriptional and/or post-transcriptional levels in plant responses and adaptation to biotic and abiotic stresses (Shriram et al. 2016; Xu et al. 2019; Zhou et al. 2020). The miRNAs are being extensively investigated owing to their direct effect on sequence complementary based degradation of target mRNAs that often encode TF families. The roles of both miRNAs and NO under environmental stresses have been observed in hormone-dependent pathways (Singh et al. 2017). During stress, NO usually targets mitochondrial and chloroplast complexes and the proteins present in them by participating in signal transduction, regulation of Ca^{2+} and TFs (Singh et al. 2017). Recent studies have shown the interaction between biosynthesis of miRNAs and actions of post-transcriptional modifications (Iki et al. 2018; Szweykowska-Kulinska and Jarmolowski 2018; Wang et al. 2018). Chromatin remodelling factor CHR2 acts on a post-transcriptional level in *Arabidopsis* to inhibit formation of miRNA (Wang et al. 2018). The primary-miRNAs (pri-miRNAs) have been observed to undergo post-transcriptional splicing. Factors like stabilized1 (STA1) and GLYCINE-RICH RNA-BINDING PROTEIN (GRP7), etc. in *Arabidopsis* and barley are involved with the splicing of pri–miR168a, pri–miR162a, pri–miR842 (Stepien et al. 2017).

During drought stress, ABA signalling is heightened which results in activation of SnRK2 leading to inactivation of channels regulating efflux in the cell, and triggering H_2O_2-mediated activation of NO. ABA also mediates inositol 1,4,5–trisphosphate (IP3) to increase Ca^{2+} influx causing NO production. As seen earlier NO plays an important role in regulating protein kinases like CDPKs, MAPKs leading to an indirect control on genes responsive to ABA signalling. These genes have an interactive network with the miRNAs as seen with miR393, miR160, miR168 from *Arabidopsis*, poplar, rice and *Brachypodium* plants and that play important roles in stress responses via upregulating the expression of their targets, ABA-related genes via auxin response factors (ARFs) and auxin receptor transport inhibitor response I (TIR1), involved in cellular homeostasis (Bright et al. 2006; Courtois et al. 2008; Neill et al. 2008; Singh et al. 2017; Prakash et al. 2019). These are involved in AUX signalling, down-regulation of stress-related genes, and regulating plant growth and development (Bright et al. 2006; Courtois et al. 2008; Neill et al. 2008; Singh et al. 2017; Prakash et al. 2019). In Alfalfa, it was observed that exogenous application of NO down-regulated miRNAs like mtr–miR156a, mtr–miR399a, mtr–miR399c, mtr–miR399q, and mtr–miR5213–5p, targeting SPL genes (SQUAMOSA promoter binding like proteins genes), phosphate transporter genes (miR399a, c, q) and disease resistant protein gene, respectively, playing key role in drought tolerance showing a positive regulation and increasing plant growth and development under drought conditions (Zhao et al. 2020).

NO production and synthesis depend on the temperature of the environment in which the plant coexists. During cold, NO production is stimulated in some plants by activation of ion channels leading to sequential activation of inducer of CBF expression (ICE) protein and C-repeat binding factor/ Dehydration-responsive element binding-1 (CBF /DREB1) TFs. The TFs are known to be regulated by different miRNA candidates like miR397, miR166, miR172, and some ABA signalling regulators like miR168 (Kumar 2014). The CBF/ DREB factors are shown to work via binding to C-repeat elements /low-temperature response elements (CRT/LTRE) regulating the cold-responsive genes involved in giving cold stress tolerance to plants (Kumar 2014). In plants including *Arabidopsis*, *Brachypodium*, *O. sativa*, *Lotus japonica*, *Pisum sativa*, and poplar, these miRNAs (miR166, miR168, miR172, miR397) were found to be NO-responsive and were attributed for conferring cold tolerance to these plants (Kumar 2014; Singh et al. 2017; Prakash et al. 2019).

The interplay between oxidative stress and miRNAs are well known. The most common miRNA with their targets involved during stress are miR171—GRAS TF (Huang et al. 2017), miR396—Growth Regulating Factor (GRF) (Chen et al. 2015a, b; Yuan et al. 2019), miR159—SlMYB TF genes (López-Galiano et al. 2019), miR474—proline dehydrogenase (PDH) (Wei et al. 2009), miR528—polyphenol oxidase (PPO) (Zhu et al. 2020), observed in various plants like *Arabidopsis*, *Z. mays*, *O. sativa* and are up-/

down-regulated. It has been observed that cellular ROS production and accumulation leads to enhanced NO-production, contributing to the activation of stress-responsive genes and ROS scavenging enzymes. The specific miRNA like miR398 regulating SOD is involved in the down-regulation of copper chaperone during Cu^{2+} stress (Singh et al. 2017; Prakash et al. 2019). The miRNA family belonging to miR399 is also involved in the regulation of stress-responsive genes during mineral deficient conditions like phosphate starved environment inhibiting UBC24 in roots of *Arabidopsis* (Fujii et al. 2005; Aung et al. 2006; Bari et al. 2006; Park et al. 2018). The absence of above-mentioned miRNAs contributes to NO production, as they regulate the oxidative stress. During nutrient stress like lower concentrations of phosphate, sulphate, ferrous, etc., the plant cell undergoes anaerobic conditions due to imbalances in the catabolic and anabolic pathways causing increased production of NO, which sequentially activate genes responsive to stress-causing feedback activation of such miRNAs that regulate these genes. (Singh et al. 2017; Prakash et al. 2019).

Biotechnological manipulations of NO and its interactions for engineering abiotic stress tolerance in plants

Biotechnological manipulations of metabolicte pathways and their applications have opened new vistas of research in plant abiotic stress tolerance and crop improvement. The studies aimed on interactions of NO with other signalling molecules, phytohormones, and reactive species have revealed the crosstalk mechanisms in plant growth and physiological developments. Studies on the exploration of NO interactions during stress responses and tolerance in crop and model plants have suggested the use of various mutants for the production of stress-tolerant plants. Lechón et al. (2020) developed *cue1* NO overproducer mutants with enhanced arginine content in the roots of *A. thaliana.* Similarly, *cue1–1, cue1–5, cue1–6*, and *nox1* alleles resulted in the accumulation of NO during seed germination, primary root elongation, and salt stress resistance revealing potential roles NO play in plant physiological developments. When plants are exposed to external environmental stimuli, they synthesize/upregulate peptides for maintaining plant growth and development. The CLAVATA3/Embryo Surrounding Region-Related (CLE) peptides are essentially involved in the process of closing stomata. Zhang et al. (2019) showed that CLE9 acts as an essential regulator in the induction of stomatal closure. Overexpression and loss of function of CLE9 resulted in enhanced resistivity and sensitivity in *Arabidopsis thaliana* during drought stress via increased NO/ H_2O_2 generation and ABA signalling (Zhang et al. 2019). Further, CLE9 peptides upregulated the H2O2 and NO synthesis associated with stomatal closure, which was stopped in the NADPH oxidase-deficient mutants or NR mutants, respectively. Collectively, these results indicated the role of CLE9 in the regulation of stomatal apertures, and in turn stress acclimatisation of plants (Zhang et al. 2019).

The haemoglobin 1 (Hb1) proteins play an essential role in the plant environmental stress responses. The overexpression of *Nicotiana tabacum* non-symbiotic class 1 haemoglobin gene (NtHb1) in *A. thaliana* resulted in NO-scavenging, reduction in ROS/NO accumulation, and increase in antioxidant enzyme activity, Cd export transporters (PDR8), and Ca^{2+}/H^+ exchangers (CAXs) during Cd stress (Bahmani et al. 2019). Nitric reductase enzyme is primarily involved in the NO signalling mechanisms, following NO crosstalk with anthocyanin, Li et al. (2020) studied NO generation in lyceum fruits by cloning a *Lyceum barbarum* NR gene leading to inhibition of anthocyanin synthesis and enhancement of proanthocyanin accumulation. During salt stress, modulations in 1–aminocyclopropane–1–carboxylic acid (ACC)–oxidase enzyme activity in sunflower seedling resulted in the regulation of lateral-root formation via ACC–oxidase–NO complex formation and reduced ethylene biosynthesis, leading to the development of enhanced salt stress tolerant plants (Singh and Bhatla 2018). *S*–nitrosoglutathione reductase (GSNOR) enzyme plays a putative role in NO homeostasis and is primarily involved in plant biotic and abiotic stress responses. The overexpression of *GSNOR* in tomato plants resulted in increased sodic alkaline stress tolerance via *GSNOR* regulated NO signalling activation and ROS scavenging efficiency (Gong et al. 2015). Suppression of GSNOR1 enzyme in plants by S–nitrosylation via NO generation revealed the NO scavenging modulation of GSNOR1 activity (Frungillo et al. 2014). Induction of Delta–1–pyrroline–5–carboxylate synthase 1 (P5CS1) gene resulted in NO-dependent signalling between proline and PAs during abiotic stress responses (Shi and Chan 2014). Two mutants, *nia1nia2* (nitrate reductase [NR]-defective double mutant) and *Atnoa1/rif1* (nitric oxide associated1/resistant to inhibition by fosmidomycin1) resulted in overproduction of NO, upregulation of P5CS1, and down-regulation of proline dehydrogenase (ProDH) genes during cold stress and acclimation (Zhao et al. 2009). These results thus clearly demonstrated that NR-dependent NO production play critical role in cold stress tolerance mediated through proline accumulation in *Arabidopsis*.

Beside abiotic stress responses and tolerance attributed to NO manipulations, plant–insect interactions are being studied to explore the NO interactions for conferring biotic stress tolerance in plants. Xu et al. (2020) reported that NO boosted the *Bemisia tabaci* performance via suppressing the JA signalling pathway in tobacco plants. The results suggested that the NO signalling got activated by *B. tabaci* infestation, NO was found to be involved in

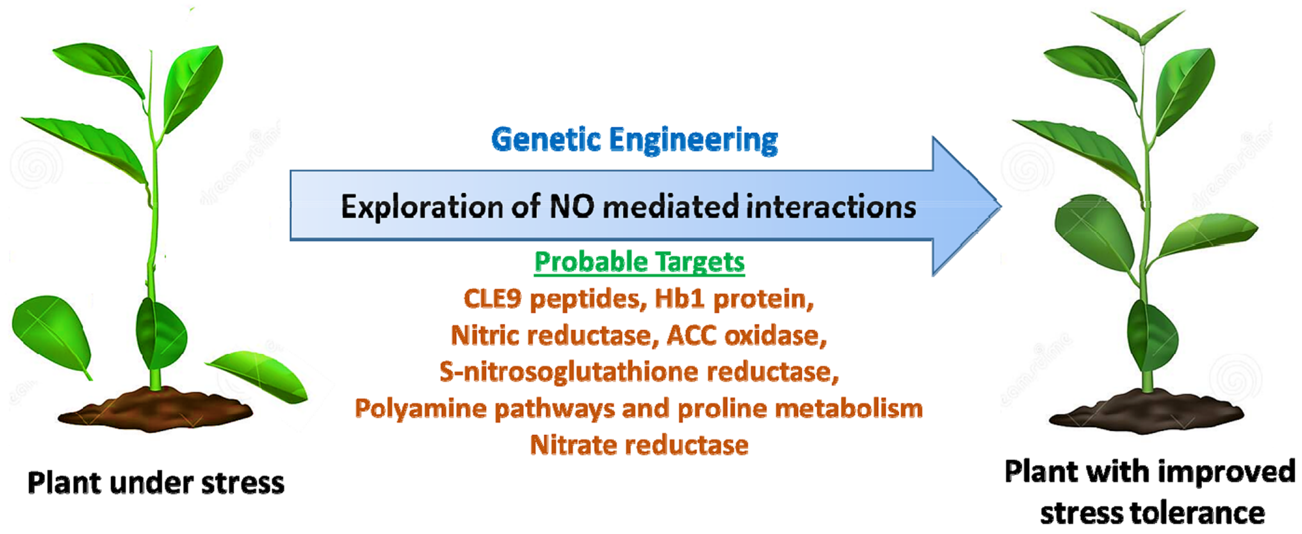

Fig. 4 Exploration of nitric oxide (NO) mediated interactions in for engineering stress tolerance in plants. (*CLE9* CLAVATA3/ESR (CLE)-related protein 9, *Hb1* hemoglobin 1, *ACC* 1–aminocyclopropane–1–carboxylic acid) [The probable targets were presented based on the literature: Zhang et al. (2019), Bahmani et al. (2019), Li et al. (2020), Singh and Bhatla (2018), Gong et al. (2015), Rodríguez-Ruiz et al. (2019), Frungillo et al. (2014), Shi and Chan (2014), Zhao et al. (2009)]

the suppression of JA-dependent plant defence, and, consequently, NO improves *B. tabaci* performance on host plants (Xu et al. 2020). *S*–nitrosoglutathione reductase (GSNOR) was silenced in *Arabidopsis thaliana* for investigating the plant–pathogen interactions, and it resulted into the increased expression levels of the NO and S–nitrosothiol (Rustérucci et al. 2007; Rodríguez-Ruiz et al. 2019). Figure 4 represents the approaches for exploration of NO-mediated interactions in plants for developing stress-tolerant plants, while Table 2 enlists events of developing transgenic plants via manipulating the NO pathways for enhanced abiotic stress tolerance.

Conclusion

After 40 years from identification of NO in plant cells, extensive research on this dynamic reactive and signalling molecule has opened up various functional capabilities in plant growth and development. Being so dynamic and versatile, NO provides a great scope for its application in crops for various purposes using different approaches to increase the plant's efficiency and tolerance under stress conditions. NO crosstalk between different signalling molecules has given us glimpse on involvement of NO in process such as stomata closure, salinity tolerance, cGMP pathway, seed germination, beside others. Interactions between NO and plant hormones like GA, AUX, CKs and BRs have shown us influence of NO in photo-morphogenesis, leaf senescence, root growth and photosynthesis, although deeper and more comprehensive research is needed for interpreting whether it is because of direct involvement of NO or RNS and to know the exact mechanism of action. Interaction of NO with different signalling molecules, and stress regulators have shown potential role of NO pathway in crop improvement and creating stress resilient crops. Accordingly, overexpression studies have shown promising results for future crop improvement programme. Interplay between NO and miRNA has recently gained attraction among researchers. Feedback activation and regulation of genes controlled by miRNA under presence of NO has potential for keeping cellular homeostasis in cell and ultimately using it crop improvement.

Table 2 A list of transgenic plants developed via manipulating NO pathways for enhanced abiotic stress tolerance

Gene of interest	Source organism	Transgenic plant	Description	References
No synthase (*NOS*)	Rat	*Oryza sativa*	Overexpression resulted in increased NO levels, higher salt and drought tolerance	Cai et al. (2015)
NOS-like gene	*Ostreococcus tauri*	*Arabidopsis*	Transgenic plants showed higher NO content and increased tolerance to stresses including salinity, drought, and oxidative stress	Foresi et al. (2015)
PRMT5C125S transgene, carrying a non-nitrosylatable mutant *Cys-125*	*Arabidopsis*	*Arabidopsis prmt5* mutant	Rescued the developmental defects, but not the stress hypersensitive phenotype and the responsiveness to NO during stress responses	Hu et al. (2017)
MdHY5	*Malus hupehensis*	Orin' apple	Increased nitrate reductase activities, anthocyanin accumulation and nitrate contents	An et al. (2017)
S–adenosylmethionine decarboxylase	*Cynodon dactylon*	*Eremochloa ophiuroides*	Improved cold tolerance through involvement of H_2O_2 and NO signalling	Luo et al. (2017)
Glutathione synthetase (*OsGS*)	*Oryza sativa*	*Oryza sativa*	Improved stress tolerance and productivity in transgenic rice	Park et al. (2017)
OtNOS	*Ostreococcus tauri*	*Nicotiana tabacum*	Accumulation of higher NO levels compared to siblings transformed with the empty vector, and displayed accelerated growth in different media containing sufficient nitrogen availability	Nejamkin et al. (2020)
S–nitrosoglutathione reductase	*Spinacia oleracea*	*Nicotiana tabacum*	lower malondialdehyde content, reactive oxygen species and higher activities and transcript level of superoxide dismutase, catalase, peroxidase under nitrate stress	Wang et al. (2020b)
SlWRKY81	*Solanum lycopersicum*	*Solanum lycopersicum*	Transcription factor inhibited the stomatal closure by attenuating nitric oxide accumulation in the guard cells	Ahammed et al. (2020)
Glucose–6–phosphate dehydrogenase (*G6PD* or *G6PDH*)	*Glycine max L*	*Arabidopsis thaliana*	Increased salinity tolerance by decreasing ROS accumulation	Jin et al. (2021)
NR1 and *NR2*	*Arabidopsis*	*Arabidopsis*	Increased NO content	Costa-Broseta et al. (2021)

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Declarations

Conflicts of interest The authors declare no conflict of interest.

References

Ahammed GJ, Li X, Mao Q, Wan H, Zhou G, Cheng Y (2020) The SlWRKY81 transcription factor inhibits stomatal closure by attenuating nitric oxide accumulation in the guard cells of tomato under drought. Physiol Plant. https://doi.org/10.1111/ppl.13243

Ahanger MA, Aziz U, Alsahli AA, Alyemeni MN, Ahmad P (2020) Influence of exogenous salicylic acid and nitric oxide on growth, photosynthesis, and ascorbate- glutathione cycle in salt stressed *Vigna angularis*. Biomolecules 10:42. https://doi.org/10.3390/biom10010042

Akram NA, Iqbal M, Muhammad A, Ashraf M, Al-Qurainy F, Shafiq S (2018) Aminolevulinic acid and nitric oxide regulate oxidative defense and secondary metabolisms in canola (*Brassica napus* L.) under drought stress. Protoplasma 225:123–128. https://doi.org/10.1007/s00709-017-1140-x

Alcázar R, Marco F, Cuevas JC, Patron M, Ferrando A, Carrasco P, Tiburcio AF, Altabella T (2006) Involvement of polyamines in plant response to abiotic stress. Biotechnol Lett 28:1867–1876

An JP, Qu FJ, Yao JF et al (2017) The bZIP transcription factor MdHY5 regulates anthocyanin accumulation and nitrate assimilation in apple. Hort Res 4:17023. https://doi.org/10.1038/hortres.2017.23

Antoniou C, Savvides A, Georgiadou EC, Fotopoulos V (2018) Spectrophotometric quantification of reactive oxygen, nitrogen and sulfur species in plant samples. In: Methods in Molecular Biology. Humana Press Inc., pp 155–161. https://doi.org/10.1007/978-1-4939-7398-9_16

Arc E, Sechet J, Corbineau F, Rajjou L, Marion-Poll A (2013) ABA crosstalk with ethylene and nitric oxide in seed dormancy and germination. Front Plant Sci 4:63. https://doi.org/10.3389/fpls.2013.00063

Arfan M, Zhang DW, Zou LJ, Luo SS, Tan WR, Zhu T, Lin HH (2019) Hydrogen peroxide and nitric oxide crosstalk mediates brassinosteroids induced cold stress tolerance in *Medicago truncatula*. Int J Mol Sci 20:144. https://doi.org/10.3390/ijms20010144

Arora D, Jain P, Singh N, Kaur H, Bhatla SC (2016) Mechanisms of nitric oxide crosstalk with reactive oxygen species scavenging enzymes during abiotic stress tolerance in plants. Free Rad Res 50:291–303. https://doi.org/10.3109/10715762.2015.1118473

Asgher M, Per TS, Masood A, Fatma M, Freschi L, Corpas FJ, Khan NA (2017) Nitric oxide signaling and its crosstalk with other plant growth regulators in plant responses to abiotic stress. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-016-7947-8

Aung K, Lin S-I, Wu C-C, Huang Y-T, Su C, Chiou T-J (2006) pho2, a phosphate overaccumulator, is caused by a nonsense mutation in a MicroRNA399 target gene. Plant Physiol 141:1000–1011. https://doi.org/10.1104/pp.106.078063

Bahmani R, Kim DG, Na JD, Hwang S (2019) Expression of the tobacco non-symbiotic class 1 hemoglobin gene hb1 reduces cadmium levels by modulating Cd transporter expression through decreasing nitric oxide and ROS level in *Arabidopsis*. Front Plant Sci 10:201. https://doi.org/10.3389/fpls.2019.00201

Bari R, Datt Pant B, Stitt M, Scheible W-R (2006) PHO2, MicroRNA399, and PHR1 define a phosphate-signaling pathway in plants. Plant Physiol 141:988–999. https://doi.org/10.1104/pp.106.079707

Bellin D, Asai S, Delledonne M, Yoshioka H (2013) Nitric oxide as a mediator for defense responses. Mol Plant Microbe Interact 26:271–277. https://doi.org/10.1094/MPMI-09-12-0214-CR

Bright J, Desikan R, Hancock JT, Weir IS, Neill SJ (2006) ABA-induced NO generation and stomatal closure in *Arabidopsis* are dependent on H_2O_2 synthesis. Plant J 45:113–122. https://doi.org/10.1111/j.1365-313X.2005.02615.x

Bryan NS, Grisham MB (2007) Methods to detect nitric oxide and its metabolites in biological samples. Free Rad Biol Med 43:645–657. https://doi.org/10.1016/j.freeradbiomed.2007.04.026

Cai W, Liu W, Wang W-S, Fu Z-W, Han T-T et al (2015) Overexpression of rat neurons nitric oxide synthase in rice enhances drought and salt tolerance. PLoS ONE 10(6):e0131599. https://doi.org/10.1371/journal.pone.0131599

Chamizo-Ampudia A, Sanz-Luque E, Llamas A, Galvan A, Fernandez E (2017) Nitrate reductase regulates plant nitric oxide homeostasis. Trends Plant Sci 22:163–174. https://doi.org/10.1016/j.tplants.2016.12.001

Chen J, Wu FH, Shang YT, Wang WH, Hu WJ, Simon M, Liu X, Shangguan ZP, Zheng HL (2015a) Hydrogen sulphide improves adaptation of *Zea mays* seedlings to iron deficiency. J Exp Bot. https://doi.org/10.1093/jxb/erv368

Chen L, Luan Y, Zhai J (2015b) Sp-miR396a-5p acts as a stress-responsive genes regulator by conferring tolerance to abiotic stresses and susceptibility to *Phytophthora nicotianae* infection in transgenic tobacco. Plant Cell Rep 34:2013–2025. https://doi.org/10.1007/s00299-015-1847-0

Chen J, Shangguan ZP, Zheng HL (2016) The function of hydrogen sulphide in iron availability: sulfur nutrient or signaling molecule? Plant Signal Behav 11(6):e1132967. https://doi.org/10.1080/15592324.2015.1132967

Conrath U, Amoroso G, Köhle H, Sültemeyer DF (2004) Non-invasive online detection of nitric oxide from plants and some other organisms by mass spectrometry. Plant J 38:1015–1022. https://doi.org/10.1111/j.1365-313X.2004.02096.x

Corpas F, Palma J (2018) Assessing nitric oxide (NO) in higher plants: an outline. Nitrogen 1:3. https://doi.org/10.3390/nitrogen1010003

Costa-Broseta Á, Castillo M, León J (2021) Post-translational modifications of nitrate reductases autoregulates nitric oxide biosynthesis in *Arabidopsis*. Int J Mol Sci 22:549. https://doi.org/10.3390/ijms22020549

Courtois C, Besson A, Dahan J, Bourque S, Dobrowolska G, Pugin A, Wendehenne D (2008) Nitric oxide signalling in plants: interplays with Ca^{2+} and protein kinases. J Exp Bot 59:155–163. https://doi.org/10.1093/jxb/erm197

Crawford NM (2006) Mechanisms for nitric oxide synthesis in plants. J Exp Bot 57:471–478. https://doi.org/10.1093/jxb/erj050

Csonka C, Páli T, Bencsik P, Görbe A, Ferdinandy P, Csont T (2015) Measurement of NO in biological samples. Br J Pharmacol 172:1620–1632. https://doi.org/10.1111/bph.12832

Cui MH, Ok SH, Yoo KS, Jung KW, Yoo SD, Shin JS (2013) An *Arabidopsis* cell growth defect factor-related protein, CRS, promotes plant senescence by increasing the production of hydrogen peroxide. Plant Cell Physiol 54:155–167. https://doi.org/10.1093/pcp/pcs161

David A, Yadav S, Bhatla SC (2010) Sodium chloride stress induces nitric oxide accumulation in root tips and oil body surface accompanying slower oleosin degradation in sunflower seedlings.

Physiol Plant 140:342–354. https://doi.org/10.1111/j.1399-3054.2010.01408.x

Debnath B, Islam W, Li M, Sun Y, Lu X, Mitra S, Hussain M, Liu S, Qiu D (2019) Melatonin mediates enhancement of stress tolerance in plants. Int J Mol Sci. https://doi.org/10.3390/ijms20051040

Esplugues JV (2002) NO as a signalling molecule in the nervous system. Br J Pharmacol 135(5):1079–1095. https://doi.org/10.1038/sj.bjp.0704569

Fahad S, Hussain S, Matloob A, Khan FA, Khaliq A, Saud S et al (2015) Phytohormones and plant responses to salinity stress: a review. Plant Growth Regul 75:391–404

Fancy NN, Bahlmann AK, Loake GJ (2017) Nitric oxide function in plant abiotic stress. Plant Cell Environ. https://doi.org/10.1111/pce.12707

Faraji J, Sepehri A (2020) Exogenous nitric oxide improves the protective effects of TiO_2 nanoparticles on growth, antioxidant system, and photosynthetic performance of wheat seedlings under drought stress. J Soil Sci Plant Nutr. https://doi.org/10.1007/s42729-019-00158-0

Farnese FS, Menezes-Silva PE, Gusman GS, Oliveira JA (2016) When bad guys become good ones: the key role of reactive oxygen species and nitric oxide in the plant responses to abiotic stress. Front Plant Sci 7:471. https://doi.org/10.3389/fpls.2016.00471

Fernando V, Zheng X, Walia Y, Sharma V, Letson J, Furuta S (2019) S-Nitrosylation: an emerging paradigm of redox signaling. Antioxidants 8:404. https://doi.org/10.3390/antiox8090404

Foresi N, Mayta ML, Lodeyro AF, Scuffi D, Correa-Aragunde A et al (2015) Expression of the tetrahydrofolate-dependent nitric oxide synthase from the green alga *Ostreococcus tauri* increases tolerance to abiotic stresses and influences stomatal development in Arabidopsis. Plant J 82:806–821. https://doi.org/10.1111/tpj.12852

Frungillo L, Skelly MJ, Loake GJ, Spoel SH, Salgado I (2014) S-nitrosothiols regulate nitric oxide production and storage in plants through the nitrogen assimilation pathway. Nat Commun 5:401. https://doi.org/10.1038/ncomms6401

Fujii H, Chiou T-J, Lin S-I, Aung K, Zhu J-K (2005) A miRNA involved in phosphate-starvation response in *Arabidopsis*. Curr Biol 15:2038–2043. https://doi.org/10.1016/j.cub.2005.10.016

Fukudome M, Calvo-Begueria L, Kado T, Osuki KI, Rubio MC, Murakami EI, Nagata M, Kucho KI, Sandal N, Stougaard J, Becana M, Uchiumi T (2016) Hemoglobin LjGlb1-1 is involved in nodulation and regulates the level of nitric oxide in the *Lotus japonicus-Mesorhizobium loti* symbiosis. J Exp Bot 67:5275–5283. https://doi.org/10.1093/jxb/erw290

Furio RN, Albornoz PL, Coll Y et al (2019) Effect of natural and synthetic Brassinosteroids on strawberry immune response against *Colletotrichum acutatum*. Eur J Plant Pathol 153:167–181. https://doi.org/10.1007/s10658-018-1551-3

García MJ, Lucena C, Romera FJ, Alcántara E, Pérez-Vicente R (2010) Ethylene and nitric oxide involvement in the up-regulation of key genes related to iron acquisition and homeostasis in *Arabidopsis*. J Exp Bot. https://doi.org/10.1093/jxb/erq203

Garcia-Mata C, Gay R, Sokolovski S, Hills A, Lamattina L, Blatt MR (2003) Nitric oxide regulates K+ and Cl- channels in guard cells through a subset of abscisic acid-evoked signaling pathways. Proc Natl Acad Sci USA 100:11116–11121. https://doi.org/10.1073/pnas.1434381100

Gardner PR (2005) Nitric oxide dioxygenase function and mechanism of flavohemoglobin, hemoglobin, myoglobin and their associated reductases. J Inorg Biochem 99:247–266. https://doi.org/10.1016/j.jinorgbio.2004.10.003

Ghorbani B, Pakkish Z, Khezri M (2018) Nitric oxide increases antioxidant enzyme activity and reduces chilling injury in orange fruit during storage. New Zeal J Crop Hortic Sci 46:101–116. https://doi.org/10.1080/01140671.2017.1345764

Gong B, Wen D, Wang X, Wei M, Yang F, Li Y, Shi Q (2015) S-nitrosoglutathione reductase-modulated redox signaling controls sodic alkaline stress responses in *Solanum lycopersicum* L. Plant Cell Physiol 56:790–802. https://doi.org/10.1093/pcp/pcv007

González-Gordo S, Bautista R, Claros MG, Cañas A, Palma JM, Corpas FJ (2019) Nitric oxide-dependent regulation of sweet pepper fruit ripening. J Exp Bot 70:4557–4570. https://doi.org/10.1093/jxb/erz136

Gupta KJ, Igamberdiev AU (2011) The anoxic plant mitochondrion as a nitrite: NO reductase. Mitochondrion 11:537–543. https://doi.org/10.1016/j.mito.2011.03.005

Gupta KJ, Fernie AR, Kaiser WM, van Dongen JT (2011) On the origins of nitric oxide. Trends Plant Sci 16:160–168. https://doi.org/10.1016/j.tplants.2010.11.007

Gupta KJ, Kolbert Z, Durner J, Lindermayr C, Corpas FJ, Brouquisse R, Barroso JB, Umbreen S, Palma JM, Hancock JT, Petrivalsky M, Wendehenne D, Loake GJ (2020a) Regulating the regulator: nitric oxide control of post-translational modifications. New Phytol 227:1319–1325. https://doi.org/10.1111/nph.16622

Gupta KJ, Mur LAJ, Wany A, Kumari A, Fernie AR, Ratcliffe RG (2020b) The role of nitrite and nitric oxide under low oxygen conditions in plants. New Phytol 225:1143–1151. https://doi.org/10.1111/nph.15969

Gusarov I, Nudler E (2005) NO-mediated cytoprotection: instant adaptation to oxidative stress in bacteria. Proc Natl Acad Sci USA 102(39):13855–13860

Habib N, Ali Q, Ali S, Javed MT, Zulqurnain Haider M, Perveen R, Shahid MR, Rizwan M, Abdel-Daim MM, Elkelish A, Bin-Jumah M (2020) Use of nitric oxide and hydrogen peroxide for better yield of wheat (*Triticum aestivum* L.) under water deficit conditions: growth, osmoregulation, and antioxidative defense mechanism. Plants. https://doi.org/10.3390/plants9020285

Hajihashemi S, Skalicky M, Brestic M, Pavla V (2020) Cross-talk between nitric oxide, hydrogen peroxide and calcium in salt-stressed *Chenopodium quinoa* Willd. At seed germination stage. Plant Physiol Biochem. https://doi.org/10.1016/j.plaphy.2020.07.022

Hancock JT (2012) NO synthase? Generation of nitric oxide in plants. Period Biol 114:19–24

Hancock JT, Desikan R, Clarke A, Hurst RD, Neill SJ (2002) Cell signalling following plant/pathogen interactions involves the generation of reactive oxygen and reactive nitrogen species. Plant Physiol Biochem 40:611–617. https://doi.org/10.1016/S0981-9428(02)01403-1

Hasanuzzaman M, Oku H, Nahar K, Bhuyan MHMB, Al MJ, Baluska F, Fujita M (2018) Nitric oxide-induced salt stress tolerance in plants: ROS metabolism, signaling, and molecular interactions. Plant Biotechnol Rep 12:77–92. https://doi.org/10.1007/s11816-018-0480-0

He H, Oo TL, Huang W, He LF, Gu M (2019a) Nitric oxide acts as an antioxidant and inhibits programmed cell death induced by aluminum in the root tips of peanut (*Arachis hypogaea* L.). Sci Rep 9:1–12. https://doi.org/10.1038/s41598-019-46036-8

He L, Wang X, Feng R, He Q, Wang S, Liang C, Yan L, Bi Y (2019b) Alternative pathway is involved in nitric oxide-enhanced tolerance to cadmium stress in barley roots. Plants 8:557. https://doi.org/10.3390/plants8120557

Hebelstrup KH, Østergaard Jensen E, Hill RD (2008) Bioimaging techniques for subcellular localization of plant hemoglobins and measurement of hemoglobin-dependent nitric oxide scavenging in planta. Methods Enzymol 437:595–604. https://doi.org/10.1016/S0076-6879(07)37030-4

Hu J, Yang H, Mu J, Lu T, Peng J et al (2017) Nitric oxide regulates protein methylation during stress responses in plants. Mol Cell 67:702-710.e4. https://doi.org/10.1016/j.molcel.2017.06.031

Huang W, Peng S, Xian Z, Lin D, Hu G, Yang L, Ren M, Li Z (2017) Overexpression of a tomato miR171 target gene SlGRAS24 impacts multiple agronomical traits via regulating gibberellin and auxin homeostasis. Plant Biotechnol J 15:472–488. https://doi.org/10.1111/pbi.12646

Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G (1987) Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci USA 84:9265–9269. https://doi.org/10.1073/pnas.84.24.9265

Iki T, Cléry A, Bologna NG, Sarazin A, Brosnan CA, Pumplin N, Allain FHT, Voinnet O (2018) Structural flexibility enables alternative maturation, ARGONAUTE Sorting And Activities of miR168, a global gene silencing regulator in plants. Mol Plant 11:1008–1023. https://doi.org/10.1016/j.molp.2018.05.006

Jafari M, Daneshvar MH (2020) Effects of sodium nitroprusside on indirect shoot organogenesis and in vitro root formation of *Tagetes erecta*: an important medicinal plant. Polish J Appl Sci 5:14–19. https://doi.org/10.34668/PJAS.2019.5.3.03

Jahan B, AlAjmi MF, Rehman MT, Khan NA (2020) Treatment of nitric oxide supplemented with nitrogen and sulfur regulates photosynthetic performance and stomatal behavior in mustard under salt stress. Physiol Plant 168:490–510. https://doi.org/10.1111/ppl.13056

Jedelská T, Luhová L, Petřivalský M (2020) Nitric oxide signalling in plant interactions with pathogenic fungi and oomycetes. J Exp Bot 72:848–863. https://doi.org/10.1093/jxb/eraa596

Jiménez-Muñoz R, Palma F, Carvajal F, Castro-Cegrí A, Pulido A, Jamilena M, Romero-Puertas MC, Garrido D (2021) Pre-storage nitric oxide treatment enhances chilling tolerance of zucchini fruit (*Cucurbita pepo* L.) by S-nitrosylation of proteins and modulation of the antioxidant response. Postharvest Biol Technol 171:111345. https://doi.org/10.1016/j.postharvbio.2020.111345

Jin J, Li K, Qin J, Yan L, Wang S (2021) The response mechanism to salt stress in *Arabidopsis* transgenic lines over-expressing of GmG6PD. Plant Physiol Biochem 162:74–85. https://doi.org/10.1016/jplaphy.2021.02.021

Jing HC, Hebeler R, Oeljeklaus S, Sitek B, Stühler K, Meyer HE, Sturre MJG, Hille J, Warscheid B, Dijkwel PP (2008) Early leaf senescence is associated with an altered cellular redox balance in *Arabidopsis* cpr5/old1 mutants. Plant Biol 10(S1):85–98. https://doi.org/10.1111/j.1438-8677.2008.00087.x

Joudoi T, Shichiri Y, Kamizono N, Akaike T, Sawa T, Yoshitake J, Yamada N, Iwai S (2013) Nitrated cyclic GMP modulates guard cell signaling in *Arabidopsis*. Plant Cell 25:558–571

Kalia R, Sareen S, Nagpal A, Katnoria J, Bhardwaj R (2017) ROS-induced transcription factors during oxidative stress in plants: A tabulated review. In: Khan M, Khan N (eds) Reactive oxygen species and antioxidant systems in plants: role and regulation under abiotic stress. Springer, Singapore, pp 129–158. https://doi.org/10.1007/978-981-10-5254-5_6

Kalinina E, Novichkova M (2021) Glutathione in protein redox modulation through S-glutathionylation and S-nitrosylation. Molecules 26:435. https://doi.org/10.3390/molecules26020435

Kaur P, Handa N, Verma V, Bakshi P, Kalia R, Sareen S, Nagpal A, Vig AP, Mir BA, Bhardwaj R (2019) Cross talk among reactive oxygen, nitrogen and sulfur during abiotic stress in plants. In: Hasanuzzaman M et al (eds) Reactive oxygen, nitrogen and sulfur species in plants: production, metabolism, signaling and defense mechanisms. Wiley, Hoboken, pp 857–871

Kaya C, Ashraf M, Alyemeni MN, Ahmad P (2020) Responses of nitric oxide and hydrogen sulfide in regulating oxidative defence system in wheat plants grown under cadmium stress. Physiol Plant 168:345–360. https://doi.org/10.1111/ppl.13012

Khan MA, Khan AL, Imran QM, Asaf S, Lee SU, Yun BW, Hamayun M, Kim TH, Lee IJ (2019) Exogenous application of nitric oxide donors regulates short-term flooding stress in soybean. Peer J 7:e7741. https://doi.org/10.7717/peerj.7741

Khan MN, Alamri S, Al-Amri AA, Alsubaie QD, Al-Munqedi B, Ali HM, Singh VP, Siddiqui MH (2020a) Effect of nitric oxide on seed germination and seedling development of tomato under chromium toxicity. J Plant Growth Regul. https://doi.org/10.1007/s00344-020-10212-2

Khan MN, AlSolami MA, Basahi RA, Siddiqui MH, Al-Huqail AA, Abbas ZK, Siddiqui ZH, Ali HM, Khan F (2020b) Nitric oxide is involved in nano-titanium dioxide-induced activation of antioxidant defense system and accumulation of osmolytes under water-deficit stress in *Vicia faba* L. Ecotoxicol Environ Saf. https://doi.org/10.1016/j.ecoenv.2019.110152

Khare T, Srivastava AK, Suprasanna P, Kumar V (2020) Individual (Na^+ and Cl^-) and additive stress impacts of NaCl on proline metabolism and nitrosative responses of rice. Plant Physiol Biochem 152:44–52. https://doi.org/10.1016/j.plaphy.2020.04.028

Khoshbakht D, Asghari MR, Haghighi M (2018) Effects of foliar applications of nitric oxide and spermidine on chlorophyll fluorescence, photosynthesis and antioxidant enzyme activities of citrus seedlings under salinity stress. Photosynthetica 56:1313–1325. https://doi.org/10.1007/s11099-018-0839-z

Klepper LA (1975) Evolution of nitrogen oxide gases from herbicide treated plant tissues. WSSA Abstr 184:70

Kleschyov AL, Wenzel P, Munzel T (2007) Electron paramagnetic resonance (EPR) spin trapping of biological nitric oxide. J Chromatogr B Anal Technol Biomed Life Sci 851:12–20. https://doi.org/10.1016/j.jchromb.2006.10.006

Kohli SK, Khanna K, Bhardwaj R, Abd Allah EF, Ahmad P, Corpas FJ (2019) Assessment of subcellular ROS and NO metabolism in higher plants: multifunctional signaling molecules. Antioxidants 8:641. https://doi.org/10.3390/antiox8120641

Kolbert Z, Feigl G, Freschi L, Poór P (2019) Gasotransmitters in action: nitric oxide-ethylene crosstalk during plant growth and abiotic stress responses. Antioxidants. https://doi.org/10.3390/antiox8060167

Kopyra M, Gwóźdź EA (2003) Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of *Lupinus luteus*. Plant Physiol Biochem 41:1011–1017

Kumar R (2014) Role of MicroRNAs in biotic and abiotic stress responses in crop plants. Appl Biochem Biotechnol 174:93–115. https://doi.org/10.1007/s12010-014-0914-2

Lang T, Sun H, Li N, Lu Y, Shen Z, Jing X, Xiang M, Shen X, Chen S (2014) Multiple signaling networks of extracellular ATP, hydrogen peroxide, calcium, and nitric oxide in the mediation of root ion fluxes in secretor and non-secretor mangroves under salt stress. Aquat Bot 119:33–43. https://doi.org/10.1016/j.aquabot.2014.06.009

Lazalt A, Beligni MV, Lamattina L (1997) Nitric oxide preserves the level of chlorophyll in potato leaves infected by *Phytophthora infestans*. Eur J Plant Pathol 103:643–351. https://doi.org/10.1023/A:1008604410875

Lechón T, Sanz L, Sánchez-Vicente I, Lorenzo O (2020) Nitric oxide overproduction by cue1 mutants differs on developmental stages and growth conditions. Plants 9:1484. https://doi.org/10.3390/plants9111484

León J, Costa-Broseta Á (2020) Present knowledge and controversies, deficiencies, and misconceptions on nitric oxide synthesis, sensing, and signaling in plants. Plant Cell Environ 43:1–15. https://doi.org/10.1111/pce.13617

Li Z, Zhu Y, He X, Yong B, Peng Y, Zhang X, Ma X, Yan Y, Huang L, Nie G (2019) The hydrogen sulfide, a downstream signaling molecule of hydrogen peroxide and nitric oxide, involves spermidine-regulated transcription factors and antioxidant defense in white clover in response to dehydration. Environ Exp Bot 161:255–264. https://doi.org/10.1016/j.envexpbot.2018.06.036

Li G, Qin B, Li S, Yin Y, Zhao J, An W, Cao Y, Mu Z (2020) LbNR-derived nitric oxide delays lycium fruit coloration by transcriptionally modifying flavonoid biosynthetic pathway. Front Plant Sci 11:1215. https://doi.org/10.3389/fpls.2020.01215

Lin A, Wang Y, Tang J, Xue P, Li C, Liu L et al (2012) Nitric oxide and protein S-nitrosylation are integral to hydrogen peroxide-induced leaf cell death in rice. Plant Physiol 158:451–464. https://doi.org/10.1104/pp.111.184531

Lindermayr C (2018) Crosstalk between reactive oxygen species and nitric oxide in plants: Key role of S-nitrosoglutathione reductase. Free Rad Biol Med 122:110–115. https://doi.org/10.1016/j.freeradbiomed.2017.11.027

Lisjak M, Teklic T, Wilson ID, Whiteman M, Hancock JT (2013) Hydrogen sulfide: environmental factor or signalling molecule? Plant Cell Environ 36:1607–1616. https://doi.org/10.1111/pce.12073

Liu L, Zeng M, Hausladen A, Heitman J, Stamler JS (2000) Protection from nitrosative stress by yeast flavohemoglobin. Proc Natl Acad Sci USA 97:4672–4676. https://doi.org/10.1073/pnas.090083597

Liu WZ, Kong DD, Gu XX, Gao HB, Wang JZ, Xia M, Gao Q, Tian LL, Xu ZH, Bao F, Hu Y, Ye NS, Pei ZM, He YK (2013) Cytokinins can act as suppressors of nitric oxide in *Arabidopsis*. Proc Natl Acad Sci USA 110(4):1548–1553. https://doi.org/10.1073/pnas.1213235110

Liu T, Ye X, Li M, Li J, Qi H, Hu X (2020) H_2O_2 and NO are involved in trehalose-regulated oxidative stress tolerance in cold-stressed tomato plants. Environ Exp Bot 171:103961. https://doi.org/10.1016/j.envexpbot.2019.103961

López-Galiano MJ, García-Robles I, González-Hernández AI, Camañes G, Vicedo B, Real MD, Rausell C (2019) Expression of miR159 is altered in tomato plants undergoing drought stress. Plants 8:201. https://doi.org/10.3390/plants8070201

Lozano-Juste J, León J (2011) Nitric oxide regulates della content and pif expression to promote photomorphogenesis in *Arabidopsis*. Plant Physiol 156(3):1410–1423. https://doi.org/10.1104/pp.111.177741

Luo J, Liu M, Zhang C, Zhang P, Chen J et al (2017) Transgenic centipedegrass (*Eremochloa ophiuroides* [Munro] Hack.) overexpressing S-adenosylmethionine decarboxylase (SAMDC) gene for improved cold tolerance through involvement of H2O2 and NO signaling. Front Plant Sci 8:1655. https://doi.org/10.3389/fpls.2017.01655

Lv X, Ge S, Jalal Ahammed G, Xiang X, Guo Z, Yu J, Zhou Y (2017) Crosstalk between nitric oxide and MPK1/2 mediates cold acclimation-induced chilling tolerance in tomato. Plant Cell Physiol 58:1963–1975. https://doi.org/10.1093/pcp/pcx134

Ma M, Wendehenne D, Philippot L, Hänsch R, Flemetakis E, Hu B, Rennenberg H (2020) Physiological significance of pedospheric nitric oxide for root growth, development and organismic interactions. Plant Cell Environ 43:2336–2354. https://doi.org/10.1111/pce.13850

Martínez-Medina A, Pescador L, Fernández I, Rodríguez-Serrano M, García JM, Romero-Puertas MC, Pozo MJ (2019) Nitric oxide and phytoglobin PHYTOGB1 are regulatory elements in the *Solanum lycopersicum*–Rhizophagus irregularis mycorrhizal symbiosis. New Phytol 223:1560–1574. https://doi.org/10.1111/nph.15898

Mata-Pérez C, Spoel SH (2019) Thioredoxin-mediated redox signalling in plant immunity. Plant Sci 279:27–33. https://doi.org/10.1016/j.plantsci.2018.05.001

Miller GAD, Suzuki N, Ciftci-yilmaz S, Mittler RON (2010) Reactive oxygen species homeostasis and signalling. Plant Cell Environ 2010:453–467. https://doi.org/10.1111/j.1365-3040.2009.02041.x

Molassiotis A, Job D, Ziogas V, Tanou G (2016) Citrus plants: a model system for unlocking the secrets of NO and ROS-inspired priming against salinity and drought. Front Plant Sci 7:229. https://doi.org/10.3389/fpls.2016.00229

Møller IM, Jensen PE, Hansson A (2007) Oxidative modifications to cellular components in plants. Annu Rev Plant Biol 58:459–481. https://doi.org/10.1146/annurev.arplant.58.032806.103946

Moni A, Islam M, Uddin M (2018) Role of auxin and nitric oxide on growth and development of lateral root of plants: possible involvement of exogenously induced Phot1. J Adv Biotechnol Exp Ther 1:61. https://doi.org/10.5455/jabet.2018.d11

Montilla-Bascón G, Rubiales D, Hebelstrup KH, Mandon J, Harren FJM, Cristescu SM, Mur LAJ, Prats E (2017) Reduced nitric oxide levels during drought stress promote drought tolerance in barley and is associated with elevated polyamine biosynthesis. Sci Rep 7:1–15. https://doi.org/10.1038/s41598-017-13458-1

Mukherjee S, Corpas FJ (2020) Crosstalk among hydrogen sulfide (H_2S), nitric oxide (NO) and carbon monoxide (CO) in root-system development and its rhizosphere interactions: a gaseous interactome. Plant Physiol Biochem. https://doi.org/10.1016/j.plaphy.2020.08.020

Mur LAJ, Mandon J, Cristescu SM, Harren FJM, Prats E (2011) Methods of nitric oxide detection in plants: a commentary. Plant Sci 181:509–519. https://doi.org/10.1016/j.plantsci.2011.04.003

Nabi RBS, Tayade R, Hussain A, Kulkarni KP, Imran QM, Mun BG, Yun BW (2019) Nitric oxide regulates plant responses to drought, salinity, and heavy metal stress. Environ Exp Bot 161:120–133. https://doi.org/10.1016/j.envexpbot.2019.02.003

Neill S, Desikan R, Hancock J (2002) Hydrogen peroxide signalling. Curr Opin Plant Biol 5:388–395. https://doi.org/10.1016/s1369-5266(02)00282-0

Neill S, Barros R, Bright J, Desikan R, Hancock J, Harrison J, Morris P, Ribeiro D, Wilson I (2008) Nitric oxide, stomatal closure, and abiotic stress. J Exp Bot 59:165–176. https://doi.org/10.1093/jxb/erm293

Nejamkin A, Foresi N, Mayta ML, Lodeyro AF, Del Castello F, Correa-Aragunde N, Carrillo N, Lamattina L (2020) Nitrogen depletion blocks growth stimulation driven by the expression of nitric oxide synthase in tobacco. Front Plant Sci 11:312. https://doi.org/10.3389/fpls.2020.00312

Nicholls M (2019) Nitric oxide discovery Nobel Prize winners: Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad shared the Noble Prize in 1998 for their discoveries concerning nitric oxide as a signalling molecule in the cardiovascular system. Eur Heart J 40:1747–1749. https://doi.org/10.1093/eurheartj/ehz361

Palmer RMJ, Ferrige AG, Moncada S (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327:524–526. https://doi.org/10.1038/327524a0

Palmer RMJ, Ashton DS, Moncada S (1988) Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 333:664–666. https://doi.org/10.1038/333664a0

Pardo-Hernández M, López-Delacalle M, Rivero RM (2020) ROS and NO regulation by melatonin under abiotic stress in plants. Antioxidants 9:1078. https://doi.org/10.3390/antiox9111078

Park S, Kim Y, Kim J, Mok J, Kim J et al (2017) Improved stress tolerance and productivity in transgenic rice plants constitutively expressing the *Oryza sativa* glutathione synthetase OsGS under paddy field conditions. J Plant Physiol 215:39–47. https://doi.org/10.1016/j.jplph.2017.05.006

Park BS, Yao T, Seo JS, Wong ECC, Mitsuda N, Huang C-H, Chua N-H (2018) Arabidopsis nitrogen limitation adaptation regulates ORE1 homeostasis during senescence induced by nitrogen deficiency. Nat Plants 4:898–903. https://doi.org/10.1038/s41477-018-0269-8

Patel MK, Pandey S, Burritt DJ, Tran LSP (2019) Plant responses to low-oxygen stress: interplay between ROS and NO signaling pathways. Environ Exp Bot 161:134–142. https://doi.org/10.1016/j.envexpbot.2019.02.013

Piacentini D, Della Rovere F, Sofo A, Fattorini L, Falasca G, Altamura MM (2020) Nitric oxide cooperates with auxin to mitigate the alterations in the root system caused by cadmium and arsenic. Front Plant Sci 11:1182. https://doi.org/10.3389/fpls.2020.01182

Prakash V, Singh VP, Tripathi DK, Sharma S, Corpas FJ (2019) Crosstalk between nitric oxide (NO) and abscisic acid (ABA) signalling molecules in higher plants. Environ Exp Bot 161:41–49. https://doi.org/10.1016/j.envexpbot.2018.10.033

Priestley J (1772) XIX. Observations on different kinds of air. Philos Trans R Soc Lond 62:147–264. https://doi.org/10.1098/rstl.1772.0021

Qi F, Xiang Z, Kou N, Cui W, Xu D, Wang R, Zhu D, Shen W (2017) Nitric oxide is involved in methane-induced adventitious root formation in cucumber. Physiol Plant 159:366–377. https://doi.org/10.1111/ppl.12531

Rai KK, Pandey N, Rai SP (2020) Salicylic acid and nitric oxide signaling in plant heat stress. Physiol Plant 168:241–255. https://doi.org/10.1111/ppl.12958

Rather BA, Mir IR, Masood A, Anjum NA, Khan NA (2020) Nitric oxide pre-treatment advances seed germination and alleviates copper-induced photosynthetic inhibition in Indian mustard. Plants 9:776. https://doi.org/10.3390/plants9060776

Raya-González J, López-Bucio JS, López-Bucio J (2019) Nitric oxide and hydrogen peroxide in root organogenesis. In: Nitric oxide and hydrogen peroxide signaling in higher plants. Springer International Publishing, Cham, pp 157–173. https://doi.org/10.1007/978-3-030-11129-8_8

Ren Y, Wang W, He J, Zhang L, Wei Y, Yang M (2020) Nitric oxide alleviates salt stress in seed germination and early seedling growth of pakchoi (*Brassica chinensis* L.) by enhancing physiological and biochemical parameters. Ecotoxicol Environ Saf 187:109785. https://doi.org/10.1016/j.ecoenv.2019.109785

Rodríguez-Ruiz M, Zuccarelli R, Palma JM, Corpas FJ, Freschi L (2019) Biotechnological Application of Nitric Oxide and Hydrogen Peroxide in Plants. In: *Nitric Oxide and Hydrogen Peroxide Signaling in Higher Plants*. In: Gupta DK, Palma JM, Corpas FJ (eds) Springer International Publishing: Cham, Switzerland, pp 245–270. https://doi.org/10.1007/978-3-030-11129-8_12

Romero-Puertas MC, Perazzolli M, Zago ED, Delledonne M (2004) Nitric oxide signalling functions in plant-pathogen interactions. Cell Microbiol 6:795–803. https://doi.org/10.1111/j.1462-5822.2004.00428.x

Rubbo H (2013) Nitro-fatty acids: novel anti-inflammatory lipid mediators. Braz J Med Biol Res 46:728–734. https://doi.org/10.1590/1414-431X20133202

Rustérucci C, Espunya MC, Díaz M, Chabannes M, Martínez MC (2007) S-nitrosoglutathione reductase affords protection against pathogens in arabidopsis, both locally and systemically. Plant Physiol. https://doi.org/10.1104/pp.106.091686

Sahay S, Gupta M (2017) An update on nitric oxide and its benign role in plant responses under metal stress. Nitric Oxide 67:39–52. https://doi.org/10.1016/j.niox.2017.04.011

Santos MP, Zandonadi DB, de Sá AFL, Costa EP, de Oliveira CJL, Perez LEP, Façanha AR, Bressan-Smith R (2020) Abscisic acid-nitric oxide and auxin interaction modulates salt stress response in tomato roots. Theor Exp Plant Physiol. https://doi.org/10.1007/s40626-020-00187-6

Sanz L, Albertos P, Mateos I, Sánchez-Vicente I, Lechón T, Fernández-Marcos M, Lorenzo O (2015) Nitric oxide (NO) and phytohormones crosstalk during early plant development. J Exp Bot 66:2857–2868. https://doi.org/10.1093/jxb/erv213

Shao R, Wang K, Shangguan Z (2010) Cytokinin-induced photosynthetic adaptability of *Zea mays* L. to drought stress associated with nitric oxide signal: probed by ESR spectroscopy and fast OJIP fluorescence rise. J Plant Physiol 167:472–479. https://doi.org/10.1016/j.jplph.2009.10.020

Sharma A, Soares C, Sousa B, Martins M, Kumar V, Shahzad B, Sidhu GPS, Bali AS, Asgher M, Bhardwaj R, Thukral AK, Fidalgo F, Zheng B (2019) Nitric oxide-mediated regulation of oxidative stress in plants under metal stress: a review on molecular and biochemical aspects. Physiol Plant 168:ppl13004. https://doi.org/10.1111/ppl.13004

Shen Q, Zhan X, Yang P, Li J, Chen J, Tang B, Wang X, Hong Y (2019) Dual activities of plant cGMP-dependent protein kinase and its roles in gibberellin signaling and salt stress. Plant Cell 31:3073–3091. https://doi.org/10.1105/tpc.19.00510

Shi H, Chan Z (2014) Improvement of plant abiotic stress tolerance through modulation of the polyamine pathway. J Integr Plant Biol 56:114–121. https://doi.org/10.1111/jipb.12128

Shriram V, Kumar V, Devarumath RM, Khare TS, Wani SH (2016) MicroRNAs as potential targets for abiotic stress tolerance in plants. Front Plant Sci 7:817. https://doi.org/10.3389/fpls.2016.00817

Singh N, Bhatla SC (2018) Nitric oxide regulates lateral root formation through modulation of ACC oxidase activity in sunflower seedlings under salt stress. Plant Signal Behav 13:e1473683. https://doi.org/10.1080/15592324.2018.1473683

Singh R, Parihar P, Singh S, Singh MPVVB, Singh VP, Prasad SM (2017) Micro RNAs and nitric oxide cross talk in stress tolerance in plants. Plant Growth Regul 83:199–205. https://doi.org/10.1007/s10725-016-0190-y

Steelheart C, Galatro A, Bartoli CG, Gergoff Grozeff GE (2019) Nitric oxide and hydrogen peroxide: signals in fruit ripening. In: Gupta D, Palma J, Corpas F (eds) Nitric oxide and hydrogen peroxide signaling in higher plants. Springer, Cham. https://doi.org/10.1007/978-3-030-11129-8_9

Stepien A, Knop K, Dolata J, Taube M, Bajczyk M, Barciszewska-Pacak M, Pacak A, Jarmolowski A, Szweykowska-Kulinska Z (2017) Posttranscriptional coordination of splicing and miRNA biogenesis in plants. Wiley Interdiscip Rev RNA 8:e1403. https://doi.org/10.1002/wrna.1403

Suzuki N, Miller G, Morales J, Shulaev V, Torres MA, Mittler R (2011) Respiratory burst oxidases: the engines of ROS signaling. Curr Opin Plant Biol 14:691–699. https://doi.org/10.1016/j.pbi.2011.07.014

Szweykowska-Kulinska Z, Jarmolowski A (2018) Post-transcriptional regulation of MicroRNA accumulation and function: new insights from plants. Mol Plant 11:1006–1007. https://doi.org/10.1016/j.molp.2018.06.010

Terrón-Camero LC, Rodríguez-Serrano M, Sandalio LM, Romero-Puertas MC (2020) Nitric oxide is essential for cadmium-induced peroxule formation and peroxisome proliferation. Plant Cell Environ 43:2492–2507. https://doi.org/10.1111/pce.13855

Tossi V, Lamattina L, Cassia R (2009) An increase in the concentration of abscisic acid is critical for nitric oxide-mediated plant adaptive responses to UV-B irradiation. New Phytol. https://doi.org/10.1111/j.1469-8137.2008.02722.x

Vitecek J, Reinohl V, Jones RL (2008) Measuring no production by plant tissues and suspension cultured cells. Mol Plant 1:270–284. https://doi.org/10.1093/mp/ssm020

Wang Y, Lin A, Loake GJ, Chu C (2013a) H_2O_2-induced leaf cell death and the crosstalk of reactive nitric/oxygen species. J Integr Plant Biol 55:202–208. https://doi.org/10.1111/jipb.12032

Wang Y, Loake GJ, Chu C (2013b) Cross-talk of nitric oxide and reactive oxygen species in plant programed cell death. Front Plant Sci 4:314. https://doi.org/10.3389/fpls.2013.00314

Wang Z, Ma Z, Castillo-González C, Sun D, Li Y, Yu B, Zhao B, Li P, Zhang X (2018) SWI2/SNF2 ATPase CHR2 remodels pri-miRNAs via Serrate to impede miRNA production. Nature 557:516–521. https://doi.org/10.1038/s41586-018-0135-x

Wang HQ, Sun LP, Wang LX, Fang XW, Li ZQ, Zhang FF, Hu X, Qi C, He JM (2020a) Ethylene mediates salicylic-acid-induced stomatal closure by controlling reactive oxygen species and nitric oxide production in *Arabidopsis*. Plant Sci. https://doi.org/10.1016/j.plantsci.2020.110464

Wang M, Dong Y, Yan J et al (2020b) Overexpression of the spinach S-nitrosoglutathione reductase (SoGSNOR) in tobacco resulted in enhanced nitrate stress tolerance. Plant Cell Tiss Organ Cult 143:173–187. https://doi.org/10.1007/s11240-020-01906-2

Wang X, Li Q, Yang M, Zhang J, Huang M, Cai J, Zhou Q, Dai T, Wollenweber B, Jiang D (2020c) Crosstalk between hydrogen peroxide and nitric oxide mediates priming-induced drought tolerance in wheat. J Agron Crop Sci. https://doi.org/10.1111/jac.12458

Wei L, Zhang D, Xiang F, Zhang Z (2009) Differentially expressed miRNAs potentially involved in the regulation of defense mechanism to drought stress in maize seedlings. Int J Plant Sci 170:979–989. https://doi.org/10.1086/605122

Wei L, Zhang M, Wei S, Zhang J, Wang C, Liao W (2020) Roles of nitric oxide in heavy metal stress in plants: cross-talk with phytohormones and protein S-nitrosylation. Environ Pollut. https://doi.org/10.1016/j.envpol.2020.113943

Wrzaczek M, Brosché M, Kangasjärvi J (2013) ROS signaling loops—production, perception, regulation. Curr Opin Plant Biol 16(5):575–582. https://doi.org/10.1016/j.pbi.2013.07.002

Wu AP, Gong L, Chen X, Wang JX (2014) Interactions between nitric oxide, gibberellic acid, and phosphorus regulate primary root growth in *Arabidopsis*. Biol Plant 58:335–340. https://doi.org/10.1007/s10535-014-0408-7

Wu P, Xiao C, Cui J, Hao B, Zhang W, Yang Z, Ahammed GJ, Liu H, Cui H (2020) Nitric oxide and its interaction with hydrogen peroxide enhance plant tolerance to low temperatures by improving the efficiency of the calvin cycle and the ascorbate-glutathione cycle in cucumber seedlings. J Plant Growth Regul. https://doi.org/10.1007/s00344-020-10242-w

Xu J, Wang W, Yin H, Liu X, Sun H, Mi Q (2010) Exogenous nitric oxide improves antioxidative capacity and reduces auxin degradation in roots of *Medicago truncatula* seedlings under cadmium stress. Plant Soil 326:321. https://doi.org/10.1007/s11104-009-0011-4

Xu J, Hou Q-M, Khare T, Verma SK, Kumar V (2019) Exploring miRNAs for developing climate-resilient crops: a perspective review. Sci Total Environ 653:91–104. https://doi.org/10.1016/j.scitotenv.2018.10.340

Xu Y, Qu C, Sun X, Jia Z, Xue M, Zhao H, Zhou X (2020) Nitric oxide boosts *Bemisia tabaci* performance through the suppression of jasmonic acid signaling pathway in tobacco plants. Front Physiol 11:847. https://doi.org/10.3389/fphys.2020.00847

Yadav S, Modi P, Dave A, Vijapura A, Patel D, Patel M (2020) Effect of Abiotic Stress on Crops. In: Sustainable crop production. https://doi.org/10.5772/intechopen.88434

Yamasaki H, Watanabe NS, Sakihama Y, Cohen MF (2016) An overview of methods in plant nitric oxide (NO) research: Why do we always need to use multiple methods? In: Methods in molecular biology. Humana Press Inc, pp 1–14. https://doi.org/10.1007/978-1-4939-3600-7_1

Yamauchi S, Mano S, Oikawa K, Hikino K, Teshima KM et al (2019) Autophagy controls reactive oxygen species homeostasis in guard cells that is essential for stomatal opening. Proc Natl Acad Sci 116:19187–19192. https://doi.org/10.1073/pnas.1910886116

Yuan S, Zhao J, Li Z, Hu Q, Yuan N, Zhou M, Xia X, Noorai R, Saski C, Li S, Luo H (2019) MicroRNA396-mediated alteration in plant development and salinity stress response in creeping bentgrass. Hortic Res 6:48. https://doi.org/10.1038/s41438-019-0130-x

Zhang H, Tang J, Liu XP, Wang Y, Yu W, Peng WY, Fang F, Ma DF, Wei ZJ, Hu LY (2009) Hydrogen sulfide promotes root organogenesis in *Ipomoea batatas*, *Salix matsudana* and *Glycine max*. J Integr Plant Biol 51:1086–1094. https://doi.org/10.1111/j.1744-7909.2009.00885.x

Zhang A, Zhang J, Zhang J, Ye N, Zhang H, Tan M, Jiang M (2011) Nitric oxide mediates brassinosteroid-induced ABA biosynthesis involved in oxidative stress tolerance in maize leaves. Plant Cell Physiol 52:181–192

Zhang L, Shi X, Zhang Y, Wang J, Yang J, Ishida T, Jiang W, Han X, Kang J, Wang X, Pan L, Lv S, Cao B, Zhang Y, Wu J, Han H, Hu Z, Cui L, Sawa S, He J, Wang G (2019) CLE9 peptide-induced stomatal closure is mediated by abscisic acid, hydrogen peroxide, and nitric oxide in *Arabidopsis thaliana*. Plant Cell Environ 42:1033–1044. https://doi.org/10.1111/pce.13475

Zhao L, Zhang F, Guo J, Yang Y, Li B, Zhang L (2004) Nitric oxide functions as a signal in salt resistance in the calluses from two ecotypes of reed. Plant Physiol 134:849–857. https://doi.org/10.1104/pp.103.030023

Zhao MG, Chen L, Zhang LL, Zhang WH (2009) Nitric reductase-dependent nitric oxide production is involved in cold acclimation and freezing tolerance in *Arabidopsis*. Plant Physiol 151(2):755–767. https://doi.org/10.1104/pp.109.140996

Zhao Y, Ma W, Wei X, Long Y, Zhao Y, Su M, Luo Q (2020) Identification of exogenous nitric oxide-responsive miRNAs from alfalfa (*Medicago sativa* L.) under drought stress by high-throughput sequencing. Genes 11:30. https://doi.org/10.3390/genes11010030

Zhu H, Chen C, Zeng J, Yun Z, Liu Y, Qu H, Jiang Y, Duan X, Xia R (2020) MicroRNA528, a hub regulator modulating ROS homeostasis via targeting of a diverse set of genes encoding copper-containing proteins in monocots. New Phytol 225:385–399. https://doi.org/10.1111/nph.16130

Zhou X, Khare T, Kumar V (2020) Recent trends and advances in identification and functional characterization of plant miRNAs. Acta Physiol Plant 42:25. https://doi.org/10.1007/s11738-020-3013-8

Zhou X, Joshi S, Patil S, Khare T, Kumar V (2021) Reactive oxygen, nitrogen, carbonyl and sulfur species and their roles in plant abiotic stress responses and tolerance. J Plant Growth Regul. https://doi.org/10.1007/s00344-020-10294-y

Zou LJ, Deng XG, Zhang LE, Zhu T, Tan WR, Muhammad A, Zhu LJ, Zhang C, Zhang DW, Lin HH (2018) Nitric oxide as a signaling molecule in brassinosteroid-mediated virus resistance to cucumber mosaic virus in *Arabidopsis thaliana*. Physiol Plant 163:196–210. https://doi.org/10.1111/ppl.12677