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# Genetic and biochemical approaches used for identification and mechanistic characterization of nitric oxide-responsive plant genes

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ABSTRACT

Nitric oxide (NO) is a multiregulatory signal molecule that integrates development and stress responses. To elucidate the molecular mechanisms of NO phytoeffects and to identify NO-associated genes, both genetic screens and genome-wide transcriptome analysis have been employed in numerous studies. Forward genetic screens have linked NO signalling to key biological processes, such as photosynthesis, cytokinin metabolism, stress adaptation, and cell cycle regulation. Reverse genetics has further characterized the role of NO-related genes involved in NO biosynthesis (e.g., *NIA1/NIA2, NOA1*), signalling (e.g., *GSNOR, NPR1*), stress responses (e.g., *ABI4, RBOHD*), and development (e.g., *HO1, NOX1*). Across multiple plant species, high-throughput transcriptomic techniques have identified thousands of NO-responsive genes involved mainly in hormonal signalling, carbohydrate metabolism, cell wall formation and stress responses. Beyond transcriptional control, NO has been found to influence gene expression through epigenetic mechanisms, such as histone acetylation and methylation, as well as DNA methylation. Nitric oxide also modifies key transcription factor families, altering their stabilities, DNA-binding capacity, and protein-protein interactions. Overall, this review underscores the central role of NO in modulating gene expression through multiple regulatory layers in plants.

## 1. Introduction

Nitric oxide (NO) is an ancient molecule which has been suggested as a crucial signalling factor which can be traced back to the origin of life. The formation of NO may have been a critical defence mechanism for primitive microorganisms against the detrimental effects of reactive oxygen species (ROS) (Feelisch and Martin, 1995). In addition, NO has been suggested as a major contributor of accessible nitrogen on the early Earth (Lundberg and Weizberg, 2022). Beyond its environmental formation, NO is produced also by living cells of bacteria, fungi, animals, humans and plants. Within the plant body, NO is a key signal molecule influencing a range of physiological processes including seed germination (Zhang et al., 2023), root system development (Sanchez-Corrionero et al., 2023), photomorphogenesis (Latorre et al., 2023), flowering (Seligman et al., 2008), and senescence (Hussain et al., 2022). Further studies highlight the role of NO in plant responses to both biotic and abiotic stresses including pathogen infections, herbivore attacks, drought, salinity, extreme temperatures, nutrient deficiencies, heavy metals (Khan et al., 2023; Wani et al., 2021).

In contrast to the well-defined NO-producing enzyme system in animals, NO formation in plants occurs through a series of reactions that diverge from primary metabolic pathways. Oxidative NO formation is possible by degradation of polyamines (Wimalasekera et al., 2011; Groß et al., 2017), oxidation of hydroxylamines (Rümer et al., 2009) or oxidation of oximes involved in the synthesis of auxins (López-Gómez et al., 2024). Oximes are derived from aldehydes or ketones by reaction with hydroxylamine ($NH_2OH$) (Sørensen et al., 2018). Additionally, nitrates and nitrites can be reduced and NO produced with the direct or indirect involvement of nitrate reductase (NR) and nitrite reductase, associated with nitrogen assimilation (Rockel et al., 2002; Mohn et al., 2019, Chamizo-Ampudia et al., 2016). Recently, peroxisomal sulfite oxidase has been identified as a source of NO in the presence of nitrate

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and NADH in pepper (Corpas et al., 2025). Non-enzymatic reduction of nitrates/nitrites can also happen in the mitochondrial electrontransport chain (Kumari et al., 2023) or in acidic cell environment (Wang and Hargrove, 2013).

In living organisms, NO is a small, diatomic, membrane-permeable molecule. Its unique physical and chemical properties include high diffusibility due to its small size and gaseous state at physiological temperatures that enables NO's wide range of functions. Excellent mobility enables NO to rapidly pass through cellular membranes and diffuse over short distances within tissues (Lancaster, 1997). NO has an unpaired electron in its 2p-$\pi$ antibonding orbital that ensures its radical and redox active characteristics. In biological systems, NO has three interchangeable forms: nitric oxide (NO·) is the most radical form and this is the most basic and reactive form of NO. Loss of the unpaired electron yields nitrosonium cation ($NO^+$), while gaining an electron leads to the formation of nitroxyl anion ($NO^-$) (Stamler et al., 1992). Compared to other radicals, NO has a relatively long half-life around few seconds, and its half-life depends on its actual concentration (Neill, 2005), and on its actual radical form.

The unique nature of the NO signal molecule is further evidenced by the fact that plant cells seem to lack one distinct and specific receptor or receptor family for NO sensing (León, 2022). Instead, the perception of NO and the transfer of its biological activity leading to altered gene expressions are realized mainly by post-translational modifications (PTMs) following its redox transformations. NO can be oxidized to nitrogen dioxide ($NO_2$) or can form peroxynitrite ($ONOO^-$) in the reaction with superoxide ($O_2^{\bullet-}$). These oxidized forms of NO are involved in modifying certain proteins through nitration or nitrosation affecting their structure, subcellular localization, function, activity, protein-protein interactions (Kolbert and Lindermayr, 2021). Tyrosine nitration, involving the irreversible addition of a nitro group to tyrosine residues, modifies protein activity and serves as a marker of oxidative stress, integrating NO signalling with ROS pathways (León, 2022). NO-catalysed metal nitrosylation involves the binding of NO to metal centers in metalloproteins with few known examples in plants (Astier and Lindermayr, 2012). In the transfer of NO's bioactivity, *S*-nitrosation has been proved to have crucial role. The reversible addition of a NO group to a cysteine thiol yields *S*-nitrosothiol, a key residue in NO signalling, able to produce nitrosonium ion or NO. The microenvironment surrounding cysteine residues, including low pKa sulfhydryl groups and hydrophobic motifs, influences their NO accessibility and reactivity. Consensus motifs for *S*-nitrosation often involve acidic and basic residues flanking the target cysteine, although this is still debated (Kolbert and Lindermayr, 2021). Hundreds of endogenously *S*-nitrosated proteins have been identified in plants including numerous transcription factors (TFs), and the modification of their activity or DNA-binding is one of the major ways of NO-associated regulation of gene expression. *S*-nitrosation of wide range of target proteins can modulate various plant physiological responses, defence mechanisms and hormone signalling pathways (Borrowman et al. 2023; Saini et al., 2023).

In addition to PTMs, NO-related signalling and gene expression occurs through the bidirectional interplay with calcium ($Ca^{2+}$) homeostasis (Courtois et al., 2008). The production of NO is partly or strictly $Ca^{2+}$-dependent in plants challenged by microbe-associated molecular patterns or pathogenic microorganisms. Also, NO can trigger $Ca^{2+}$ level increase by mobilizing it from intracellular stores or facilitating its influx from the extracellular space (Jeandroz et al., 2013). This is supported by the NO-associated postharvest freshness of flowers which involves the increment of $Ca^{2+}$/calmodulin (CaM) content, and the modulation of the expressions of $Ca^{2+}$-regulated proteins (Zhang et al., 2018). Similar to this, $Ca^{2+}$/CaM proved to be downstream element of NO signalling, protecting photosynthetic system and stimulating the antioxidant defense system in osmotic-stressed plants (Niu et al., 2017). These indicate that $Ca^{2+}$ is a dowstream element in NO signalling. Furthermore, NO has been found to be involved in $Ca^{2+}$ downstream signalling towards promoting growth, photosynthesis and redox status in arsenic-stressed mustard (Singh et al., 2020). $Ca^{2+}$ and NO signalling may have synergistic effects in alleviating the detrimental effects of adverse conditions such as cadmium stress (Mir et al., 2022). Additionally, NO has been suggested to modulate $Ca^{2+}$ signalling genes at the transcriptional level, and CaM was identified as protein target of NO-related *S*-nitrosation based on bioinformatic analyses (Jeandroz et al., 2013).

NO has been shown to control the activity of protein kinases *via* the interaction with calcium signalling (Courtois et al., 2008; Rezayian and Zarinkamar, 2023), and NO signalling has been linked to lipid-associated signal transduction (Gonorazky et al., 2014; Di Fino et al., 2021). Early works suggested the interplay between NO and cyclic guanosine monophosphate signalling, which is still uncertain (León and Costa-Broseta, 2020). Moreover, NO has been shown to regulate histone acetylation altering chromatin structure and modulate expression of numerous genes implicated in plant growth, development and stress responses (Ageeva-Kieferle et al., 2019, 2021). The above listed examples indicate that NO exerts its regulatory effects on gene expression through numerous distinct mechanisms. This review aims to provide a comprehensive overview of these mechanisms, as well as to examine the approaches employed in the identification of NO-regulated and NO-associated genes.

## 2. Approaches to identify NO-related genes: genetic screens and transcriptomic analyses

Identification of NO-associated genes has been carried out by genetic screening of mutant populations or by transcriptomic analyses of exogenous NO exposed plants.

### 2.1. Genetic screens for searching NO-related genes

One effective approach for studying gene functions associated with developmental or physiological processes in an organism is to isolate the corresponding mutants with altered phenotypes. Genetic screens are key steps in the identification of mutations affecting diverse aspects of plant growth, development or responses to environmental effects. Chemical mutagens, such as ethyl methanesulfonate (EMS) can generate numerous allelic variants in any background of choice. *Arabidopsis thaliana* (T)-DNA insertion lines facilitate expeditious identification of mutant genes; however, the process of creating them in a mutant background is laborious. Nevertheless, T-DNA insertion lines are extensively used in reverse genetic strategies to identify mutations for genes of interest. Both forward and reverse genetic screens have been used to investigate NO-related plant genes. Forward genetics involves starting with an observable phenotypic trait, and then genetically dissecting it to identify the responsible genes. In contrast, reverse genetics starts with a known gene and investigates its function and associated phenotypes (Aklilu et al., 2021). The principles of forward and reverse genetics in relation to plant NO research is depicted in Fig. 1.

In the context of NO regulation, Arabidopsis forward genetic screen was first conducted to identify mutants with altered primary root growth inhibition by NO donor sodium nitroprusside (SNP), in a fast neutron-mutagenized Arabidopsis (Col-0) collection (He et al., 2004, Fig. 2). The mutant seedlings exhibiting markedly shorter primary roots were selected as putative NO overproducer (nox) mutants for subsequent characterization. Using the most specific NO probe known to date, (4, 5-diaminofluorescein diacetate), higher NO levels were detected in the selected mutant seedlings compared to wild type (WT) plants. The screen yielded six *NOX1* alleles, with *nox1–1* exhibiting the most pronounced reduction in root length in the presence of SNP. All lines had elevated NO levels relative to the WT, suggesting the overproduction of NO. Map-based cloning led to the identification of NOX1 as a homologue of chlorophyll a/b binding protein (CAB) underexpressed 1 (*CUE1*). The morphology of *nox1* exhibited a high degree of similarity to that previously documented for the *cue1* mutant, including a small plant size and pale green leaves with a reticulate pattern. Further molecular genetic

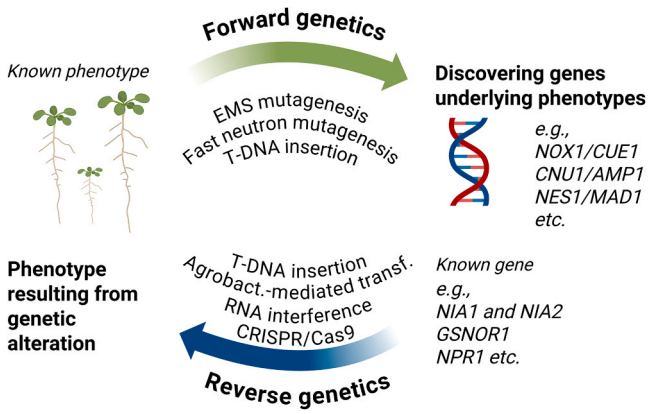


**Fig. 1.** Forward and reverse genetics in association with plant NO research. See explanation in the text. Abbreviations: *NOX1/CUE1 - CHLOROPHYLL A/B BINDING PROTEIN UNDEREXPRESSED 1; CNU1/AMP1 - ALTERED MERISTEM PROGRAM 1; NES1/MAD1 – MITOTIC ARREST DEFICIENT 1; NIA1 – NITRATE REDUCTASE 1; NIA2 - NITRATE REDUCTASE 2; GSNOR1 - S-NITROSOGLUTATHIONE REDUCTASE 1; NPR1 - NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1.*

studies have confirmed that *NOX1* is *CUE1*. The *cue1* mutants exhibited increased sensitivity to SNP and elevated NO levels, yet were unable to complement the *nox1* phenotypes. Furthermore, deletion of the *CUE1* gene was observed in all six *NOX1* alleles (He et al., 2004).

Later, Liu et al. (2013) applied forward genetics to screen for NO-insensitive mutants in Arabidopsis, employing T-DNA insertion-mutagenized and EMS-mutagenized Arabidopsis mutant collections. Similar to the previous experimental system, the externally administered NO was also SNP (120 μM), selecting NO insensitive large green leaves as mutant phenotypes. The selected lines were designated as continuous NO-unstressed (*cnu*) mutants. The *cnu1–1* and *cnu1–2* mutant alleles were isolated from T-DNA insertion and EMS pools, respectively, and were shown as allelic through genetic analysis. The *cnu1–1* and *cnu1–2* were previously identified as *altered meristem program 1* (*amp1*) with elevated levels of cytokinins and reduced levels of NO compared to the WT. Subsequent *in vivo* and *in vitro* experiments indicated the intriguing possibility that cytokinins may suppress the action of NO, potentially through a direct interaction between the two regulatory pathways (Liu et al., 2013, Fig. 2).

In another forward genetic screen, seed collection of T-DNA insertion mutants was employed, and agar-grown seedlings were treated with SNP in a manner that ensured the plants were not in contact with the donor, but only with the NO gas released from it in a closed Petri dish. To ensure the effectiveness of the treatment, ferricyanide was used as a control to exclude the possible effect of cyanide produced during SNP

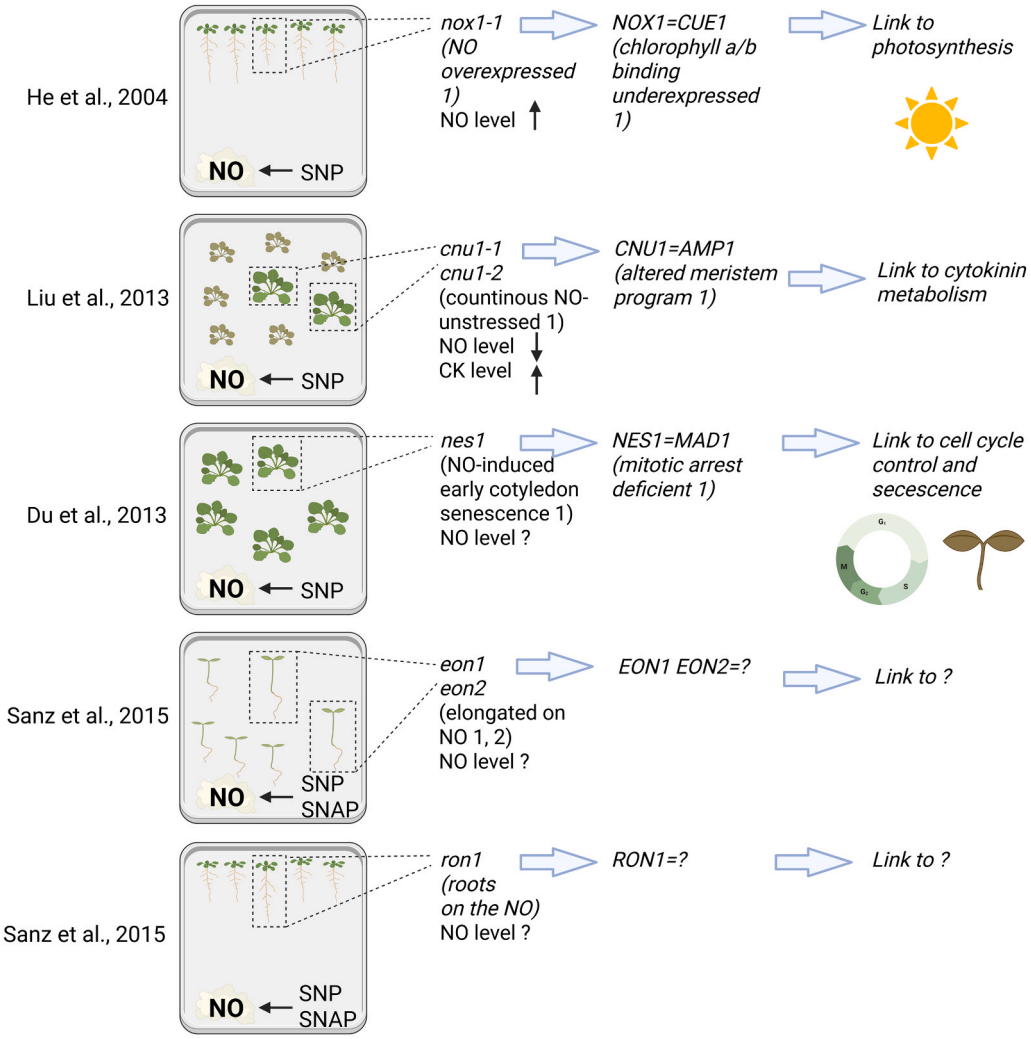


**Fig. 2.** Forward genetic screens in NO-supplemented Arabidopsis. Schematic illustration of the experimental setups and main findings of forward genetic screens applied for detecting NO-associated genes in Arabidopsis. Abbreviations: SNP - sodium nitroprusside, SNAP - S-nitroso-N-penicillamine.

decomposition. The screen identified a line designated *nitric oxide-induced early cotyledon senescence* (*nes1*) exhibiting higher degree of NO-induced cotyledon senescence than that observed in the WT. NO levels in the mutant and wild type plants were not compared in this study. Map-based cloning revealed that NES1 is allelic to the previously reported spindle assembly checkpoint protein *MAD1 (MITOTIC ARREST DEFICIENT 1)*. The NO-accelerated cotyledon senescence in *nes1–2* and delayed cotyledon senescence in the overproducer *35S::NES1* revealed the pivotal role of NO in supression of senescence. Further genetic interaction analysis between NES1 and the ethylene-associated transcription factor ORESARA1 (ORE1) indicated that ORE1 plays a dominant role and that NES1 exerts an antagonistic effect during NO-induced cotyledon senescence in Arabidopsis (Du et al., 2013, Fig. 2).

Based on germination stimulation, root growth and hypocotyl elongation, several forward genetic screens have been performed by the research group of Oscar Lorenzo using EMS-mutagenized Col-0 to search for NO-insensitive Arabidopsis mutants (Sanz et al., 2015, Fig. 2). The seeds were germinated in the presence of NO donors (SNP; S-nitroso-N-penicillamine, SNAP) or scavenger (2–4 carboxyphenyl 4,4,5,5 tetramethylimidazoline 1 oxyl 3 oxide, cPTIO). In this manner, the researchers were able to ascertain that the *roots on the NO (ron1)* line showed an insensitivity to NO-induced root shortening. Moreover, the lines *elongated on NO 1, 2 (eon1 and eon2)* were identified, and it was observed that their hypocotyl shortening in the presence of NO was negligible (Sanz et al., 2015, Fig. 2). The future comprehensive genetic characterisation of these lines may facilitate the identification of novel regulatory relationships and functional roles of NO.

During a recent screen in the EMS-mutagenized M2 population of the *gsnor1–3* mutant, *„repressor of gsnor1 (rog1)"* mutation has been identified, which specifically suppresses the semi-dwarf and bushy phenotype of *gsnor1* (Chen et al., 2020). The screen was based on scoring the restoration of developmental defects of *gsnor1–3*. ROG1 turned out to be a transnitrosylase that specifically modifies GSNOR1, and it is identical to the non-canonical catalase, CAT3. The authors proposed that as a transnitrosylase, ROG1 is a newly identified regulator of plant NO signalling (Chen et al., 2020).

Reverse genetic screening has become a powerful approach for identifying and characterizing genes involved in NO biosynthesis, signalling, and response in plants. Unlike forward genetics, reverse genetics tries to identify mutants for specific genes, and define their function after characterisation of the associated phenotype (Fig. 1). Reverse genetic screening is achieved by various molecular tools. T-DNA insertion mutagenesis uses Agrobacterium-mediated transformation to randomly insert T-DNA sequences into plant genomes, disrupting gene function (Gelvin, 2021; Koncz et al., 1992). High throughput sequencing of the T-DNA insertion sites allowed the establishment of databases of mapped mutations in the Arabidopsis genome (Sessions et al., 2002, Szabados et al., 2002, Rosso et al., 2003). Identification of mutated genes can subsequently be performed by *in silico* search or through public databases such as TAIR (https://www.arabidopsis.org). Phenotypic analysis of the mutants can contribute to the functional analysis of the investigated genes in model or crop plants (Sallaud et al., 2004, Fernie and Tohge, 2017). T-DNA insertion lines can also be screened for altered NO levels or signalling responses. NO-related mutants identified by reverse genetics have provided critical insights into the roles of NO in plant development, stress responses, and signalling pathways, significantly advancing our understanding of how plants respond to environmental stimuli and manage their growth. Reverse genetics is also used to provide accurate statistics on mutations that occur in specific genes. From these screens it is possible to determine how fortuitous the mutations are, and how often the mutations occur. RNA interference (RNAi) involves silencing specific gene transcripts by introducing small interfering RNAs (siRNAs) or microRNAs that bind complementary mRNA sequences, effectively knocking down gene expression (Agrawal et al., 2003). CRISPR/Cas9 gene editing generates site specific mutations by introducing targeted double-strand breaks and subsequent errors in DNA sequences during error-prone repair mechanism. Gene editing allows knocking out or modifying specific genes involved in any biological processes including NO signalling or response pathways (Gan and Ling, 2022). These methods allow researchers to investigate how specific genes contribute to NO-related processes by observing phenotypic effects, changes in NO levels and gene expression, or alterations in NO-mediated stress or developmental responses.

In land plants, NO synthesis involves NR, and reverse genetic screens have been essential in exploring the contribution of this enzyme to NO formation. Reverse genetic studies on *NIA1* and *NIA2*, the genes encoding NR enzyme in *A. thaliana*, revealed that NR contributes to NO production, especially under stress conditions (Rockel et al., 2002). Mutants with reduced NR activity exhibited reduced NO levels, linking NR activity to NO biosynthesis in response to various stimuli (e.g., Kolbert et al., 2008, 2010; Sun et al., 2015; Pan et al., 2019; Berger et al., 2020).

NOA1 (NITRIC OXIDE ASSOCIATED1) initially thought to be a plant NOS-like enzyme, is now understood to play a role in mitochondrial function rather than directly producing NO. Studies on *noa1* knockout mutant, which exhibit diminished NO production and altered stress responses, indicate that *NOA1* affects NO levels indirectly by modulating cellular respiration and energy production (del Río et al., 2004).

S-nitrosoglutathione reductase (GSNOR) regulates *S*-nitrosation by controlling the pool of S-nitrosoglutathione (GSNO), a major NO reservoir in cells (Sakamoto et al., 2002). Knockdown of *GSNOR* leads to elevated GSNO levels and altered NO signalling, resulting in increased susceptibility to stress and disrupted development. These results indicate that GSNOR is involved in plant immune responses and developmental processes. Additionally, studies on T-DNA insertion *gsnor* mutants highlight the role of GSNOR in maintaining NO homeostasis and balancing NO's beneficial and harmful effects during different conditions (Leterrier et al., 2011; Kubienová et al., 2016; Jahnová et al., 2019; Kolbert et al., 2019; Guan et al., 2024a).

Additionally, reverse genetics has been key for identifying genes that modulate NO-related abiotic and biotic stress adaptation pathways. NPR1 (NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1) is a central regulator of plant immunity that interacts with NO in systemic acquired resistance. Reverse genetic studies of *npr1* mutants revealed that NO accumulation is necessary for NPR1 activation and defence responses, suggesting that NPR1 functions in NO-mediated signal transduction, especially during pathogen response (Tada et al., 2008).

The transcription factor ABI4 is a principal component of abscisic acid (ABA) signalling. Reverse genetic experiments revealed that ABI4 interacts with NO singalling during stress responses. Mutants of *ABI4* display reduced NO accumulation and impaired responses to abiotic stress, such as drought, due to altered stomatal behaviour. This finding suggests that ABI4 plays a role in NO and ABA signalling crosstalk, which is essential for stress adaptation (Neill et al., 2008). Another example is RBOHD (RESPIRATORY BURST OXIDASE HOMOLOG D) which is involved in ROS production and works alongside NO in response to pathogens. Reverse genetic studies in *rbohd* mutants indicate that ROS and NO jointly trigger defence responses, with RBOHD as a key component in NO and ROS signalling crosstalk (Torres et al., 2006).

Beyond stress responses, reverse genetic screens identified various NO-related genes being associated with developmental phenotypes. For instance, HO1 (HEME OXYGENASE 1) involved in heme breakdown, is indirectly related to NO signalling, as its by-products interact with NO pathways. *Atho1* mutants display delayed flowering and altered root development, suggesting that heme catabolites participate in NO-mediated developmental regulation (Li et al., 2013).

Reverse genetics have practical applications in crop improvement, particularly in developing crops with enhanced stress tolerance. Crop improvement by genome editing involves the targeted alteration of genes to improve plant traits, such as stress tolerance, disease resistance or nutritional content (Li et al., 2024). Furthermore, by manipulating NO-related genes, crop resilience to environmental stressors can be

engineered. For instance, *OsNR2*, a nitrate reductase gene in rice (*Oryza sativa*), is implicated in NO production. Mutants with increased NR activity have higher NO levels, which helps protection against low-oxygen stress and can enhance rice tolerance to submergence and hypoxic conditions (Kabange et al., 2021). Modifying *GSNOR* expression has potential for balancing NO levels under stress and modulate tolerance levels. Increased GSNOR activity helps prevent excessive NO accumulation, enhancing tolerance to stresses while keeping NO's level in the beneficial range (Liu et al., 2024; Rasool et al., 2021; Hussain et al., 2019). The depletion of GSNOR function using RNAi technique in

**Table 1**
Transcriptome analyses performed in NO supplemented plants.

| Plant Species | NO treatment | Main results and conclusion | Reference |
|---|---|---|---|
| Arabidopsis thaliana Col−0 cell culture (wild type) | 0.5 mM NOR−3 (2 h or 24 h) | Pathogenesis-related genes, antioxidant genes (*POD, GSTs, APX, CAT, GPX*) | Huang et al., 2002 |
| Arabidopsis thaliana Col−0 (wild type) | 0.1 mM and 1 mM SNP | 342 up-regulated disease-resistance genes (WRKY, ZnF proteins, TFs, GSTs, ABC transporters, kinases, ET, JA signalling, lignin, alkaloid biosynthesis). | Parani et al., 2004 |
| *Arabidopsis thaliana* Col−0 (wild type) | 1 mM GSNO (3 h) | 1945 GSNO-responsive genes expressed differently in leaves and roots. | Begara-Morales et al., 2014 |
| *Arabidopsis thaliana* Col−0 (wild type) | 1 mM CysNO | NO activates genes in stress response (*APX1, GSH2, CAT3*) and hormone signalling (*NCED3, ABA2, LOX3*). | Hussain et al., 2016 |
| *Arabidopsis thaliana* Col−0 (wild type) | 3 ppm NO gas | NO activates genes linked to biotic stress (PRs), hormone metabolism, secondary metabolism, photosynthesis. | Kuruthukulangarakoola et al., 2017 |
| *Arabidopsis thaliana* Col−0 (wild type) | 1 mM CysNO | 637 NO-responsive TF genes (*bHLH, AP2, EREBP)* linked to hormone signalling, protein degradation, development, biotic and abiotic stress. | Imran et al., 2018a |
| *Arabidopsis thaliana* Col−0 (wild type) | 1 mM CySNO | 33 *AtWRKY* TFs: 31 up- and 2 down-regulated by NO. | Imran et al., 2018b |
| *Arabidopsis thaliana* Col−0 (wild type) | 300 ppm pure NO gas for 15 min, 30 min, 60 min. | Time dependent activation/repression of hormone- and oxygen-related genes by NO gas. | Castillo et al., 2018 |
| Rice (*Oryza sativa*) | 25 μM NaAsO2, 30 μM SNP | DEGs implicated in metal transport (*NIP, ABC, NRAMP, PEZ*), stress response (*GSTs, GRXs, HSPs, PODs*), metabolism (*NR, GS*), NO TFs (*ERFs, MYBs, WRKYs*). | Singh et al., 2017 |
| Sunflower (*Helianthus annuus*) | 700 μM cPTIO (18 h) | 330 root genes regulated by NO depletion, upregulation of genes involved in lignin synthesis. | Corti Monzón et al., 2014 |
| Birch (*Betula platyphylla*) cell culture | 1 mM SNP (12 h) | 403 up-regulated and 971 down-regulated genes in carbohydrate metabolism (*BXL1,2; GALM, SUS2*), cell wall synthesis (*CESA9, XETs*). | Zeng et al., 2014 |
| Upland cotton (*Gossypium hirsutum*) | 100 μM or 250 μM SNP | 157 DEGs in 36 TF families (*bHLH, DBP, MYB, C3H, AP2-EREBP, NAC, WRKY*), 72 DEGs related to hormones (ET, ABA, AUX, SA, BR, JA, GA, CK). | Huang et al., 2018 |
| Tomato (*Solanum lycopersicum*) | 500 μM SNP (48 h), hypoxia (induced by $N_2$ gas, 48 h) | 792 down- and 352 upregulated genes, 395 DEGs related to hypoxia and SNP. 251 DEGs under both conditions (hormone signalling: *IAA14, PIN2, LOX1*, TFs: *NAC, AP/EREBP, bHLH, MYB, WRKY*). | Safavi-Rizi et al., 2020 |
| Maize (*Zea mays*) | 1 mM nitrate plus 1 mM cPTIO | NO-dependent and independent nitrate signalling pathways regulate root development. | Ravazzolo et al., 2021 |
| Kiwifruit (*Actinidia chinensis*) | 15 μl $L^{-1}$ NO gas | 736 DEGs, down-regulated: *PG, PL, PE, ACO, ERS1, ETR2, ERFs*; up-regulated: cellulose synthase. | Yang et al., 2021 |
| Okra (*Abelmoschus esculentus*) | 0.5, 1.0, 1.5, 2.0 mM SNP | DEGs in hormone signalling and lignin synthesis: *SAMS, ACS, ACO, ABA1, NCED, ABA2, AAO3, PAL, C4H, 4CL, CCR, CAD*. | Sun et al., 2021 |
| Watermelon (*Citrullus lanatus*) | 1200 μmol/L $Al_2(SO_4)_3$ plus 100 μmol /L SNP | 511 DEGs involved in nitrogen and phenylpropane metabolism, photosynthesis, antioxidant defences (*CAT, POD)*. | Zheng et al., 2021 |
| Mangrove (*Kandelia obovata*) root | 200 μM SNP | 1593 DEGs in starch and sugar metabolism (*SUS, HK, TPP*), hormone signalling (*Aux/IAA, ABI5, ACS, ACO, BRI1*), cell wall formation (*CESA, GAUT8, XTH*) | Wei et al., 2022 |
| Cucumber (*Cucumis sativus*) | 200 μM SNP plus low temperature (LT) (10/6 °C) | 121 DEGs of *b-ZIP, HD-ZIP TFs, implicated in LHCs*, flavonoid and lignin synthesis (*CHS, F3H, POD*), hormone signalling (*EIN2, CTR1, GA3ox*). | Wu et al., 2022 |
| Kenaf (*Hibiscus cannabinus*) | 150 μM SNP plus 200 μM $CdCl_2$ | 256 DEGs in Cd toxicity, related to hormone signalling, carbohydrate metabolism, terpenoid synthesis. 22 NO-induced TFs (*WRKY*, *ERF*, *bZIP* and *Dof*). | Cao et al., 2024 |

**Abbreviations: NOR-3 -** (*E*)-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexene-amide; **GSNO -** S-nitrosoglutathione; **SNP -** sodium nitroprusside; **cysNO -** S-nitroso-L-cysteine; **ET –** ethylene; **CK –** cytokinins; **JA –** jasmonates; **BR –** brassinosteroids; **AUX –** auxins; **GA –** gibberellins; **ABA –** abscisic acid; **SA -** salicylic acid; **CESA -** cellulose synthase A; **GAUT8 -** galacturonosyltransferase 8; **XTH -** xyloglucan endotransglucosylase/hydrolase; **Aux/IAA -** auxin/indole-3-acetic acid protein; **ABI5 -** abscisic acid insensitive 5; **ACC -** 1-aminocyclopropane-1-carboxylate acid; **ACS -** 1-aminocyclopropane-1-carboxylate acid synthase; **ACO -** 1-aminocyclopropane-1-carboxylate acid oxidase; **BRI1 -** brassinosteroid insensitive 1; **bHLH –** basic helix-loop-helix; **EREBP** - ethylene-responsive element binding protein; **LHC** – light harvesting complex; **F3H** – flavanone 3-hydroxylase; **POD** – peroxidase; **APX1** - ascorbate peroxidase 1; **GSH2** - glutathione synthetase 2; **CAT3** - catalase 3; **NCED3** - nine-cis-epoxycarotenoid dioxygenase 3; **ABA2** - abscisic acid deficient 2; **LOX3** - lipoxygenase 3; **AP2** - Integrase-type DNA-binding superfamily protein; **NIP** - Nodulin 26-like intrinsic aquaporin channels; **GST** – glutathione S-transferase; **GRX** – glutaredoxin; **HSP** – heat shock protein; **NRAMP** – metal ion transporter; **NR** – nitrate reductase; **GS** – glutamine synthetase; **ERFs** – Ethylene Response Factors; **cPTIO** - an NO scavenger (2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; **BXL1,2** - beta-xylosidase 1,2; **SUS2** - sucrose synthase 2; **CESA9** - cellulose synthase A9; **XETs** - xyloglucan endotransglycosylases; **bHLH** – Basic helix loop helix; **DBP** – DNA binding protein; **MYB** - myb domain protein; **C3H** - zinc finger CCCH domain; **AP2** - integrase-type DNA-binding superfamily protein; **EREBP** - ethylene-responsive element binding factor; **NAC** - NAC domain containing protein 6; **WRKY** - WRKY DNA-binding protein; **DEGs** – differentially expressed genes; **IAA14** - indole-3-acetic acid inducible 14; **PIN2** - auxin efflux carrier family protein; **LOX1** - lipoxygenase 1; **NAC** - No Apical Meristem domain; **PG** - Phospholipid/glycerol; **ERS1** - ethylene response sensor 1; **ETR2** - ethylene response sensor 2; **ERFs** - ethylene response factors; **SAMS** - *S*-adenosylmethionine synthetase-encoding genes; **ACS** - acetyl-CoA synthetase; **ACO** - ACC oxidase; **ABA1** - zeaxanthin epoxidase; **NCED** - nine-cis-epoxycarotenoid dioxygenase; **AAO3** - abscisic aldehyde oxidase 3; **PAL** - peptidoglycan-associated lipoprotein; **C4H** - cinnamate-4-hydroxylase; **4CL** - 4-coumarate:CoA ligase; **CCR** - cinnamoyl coa reductase; **CAD** - cinnamyl alcohol dehydrogenase; **CAT** – catalase; **HK** - histidine kinase; **TPP** - thylakoid processing peptide; **b-ZIP** - basic region/leucine zipper motif (bZIP) transcription factors; **LHCs** - light-harvesting complex**; CHS** - chalcone and stilbene synthase family protein; **F3H** - flavanone 3-hydroxylase; **EIN2** - NRAMP metal ion transporter family protein; **CTR1** - protein kinase superfamily protein; **GA3ox** - gibberellin 3-oxidase; **CHS** - chalcone synthase; **GA2** – gibberellic acid oxidase.

tomato (*Solanum lycopersicum*) results in the loss of apical dominance, changes in leaf shape, perturbations in seed development and germination, and a diminution in the fruit yield (Hussain et al., 2019). Moreover, depletion of *SlGSNOR* levels leads to promoted disease susceptibility to *Pseudomonas syringae* pv. tomato DC3000 (Pst DC3000), while overexpression of *SlGSNOR* induced disease resistance due to enhanced salicylic acid (SA) levels and expression of SA-dependent genes (Hussain et al., 2019). Transgenic tomato line overexpressing *SlGSNOR* had reduced seed size and germination rate, compromised plant growth, delayed flowering, altered leaf and fruit shape. Furthermore, *SlGSNOR* overexpressing lines showed improved resistance against *Alternaria solani*, and reduced hypersensitive response-associated cell death against Pst DC3000 (Rasool et al., 2021). In a recent study, virus-induced gene silencing (VIGS) was used to decrease *SlGSNOR* expression in tomato, which delayed transition of fruit skin color, improved total chlorophyll level and reduced total fruit carotenoid and lycopene contents. Fruit softening was postponed by *SlGSNOR* silencing, due to the decline in cell wall composition, resulted from inferior activities of cell wall-related genes and enzymes. SlGSNOR therefore positively promotes tomato postharvest fruit ripening, which may be primarily due to its negative regulatory role on endogenous NO level (Liu et al., 2024). These examples indicate that genetic manipulation of *GSNOR* expression may influence important agricultural traits in crops such as tomato.

### 2.2. Transcriptome analyses for searching NO-regulated genes

Whole genome transcriptome analysis provides a powerful tool for quantitatively assessing changes in plant gene expression on genomic scale under specific physiological conditions and at defined time points. This approach enables the elucidation of complex regulatory networks at the whole-genome level, and facilitates the identification of novel genes involved in key biological processes, such as NO signalling (Wang et al., 2020).

High-throughput transcriptomic techniques, such as microarray analysis and RNA sequencing (RNA-seq), allow for a comprehensive examination of genome-wide transcriptional changes in response to NO supplementation or depletion as well as to identify gene sets whose activity is modulated by mutations or transgenes altering NO response. These methods provide insights into differential gene expression patterns, aiding in the identification of NO-responsive genes and regulatory pathways (regulons). The majority of transcriptomic studies have been performed in *A. thaliana* exposed to exogenous NO supplementation (mostly SNP, GSNO, *S*-nitrosocysteine [CysNO] or pure NO gas). Most studies were conducted with plants cultured in standard conditions, leading to the identification of hundreds of differentially expressed NO-response genes (DEGs). The picture emerges that in healthy plants, NO primarily regulates phytohormone-mediated signalling (Parani et al., 2004; Hussain et al., 2016; Imran et al., 2018a, Castillo et al., 2018; Table 1). Additionally, genes encoding pathogenesis-related proteins are targeted by NO in unstressed plants (Huang et al., 2002; Kuruthukulangarakoola et al., 2017). Also, carbohydrate metabolic genes are among the main targets of NO-related regulation (Zeng et al., 2014; Cao et al., 2024; Table 1). Transcriptome analyses also revealed that cell wall-associated genes are regulated by NO leading to changes in cell wall composition (Parani et al., 2004; Sun et al., 2021; Wei et al., 2022; Table 1). RNA-seq demonstrated that GSNO-responsive genes differ between roots and leaves of WT Arabidopsis (Begara-Morales et al., 2014). Transcriptomic studies in stress-exposed plants suggest that NO regulates specific set of genes in the presence of a stress factor. For example, in arsenic-treated rice, NO modulates the activity of metal- and iron transporters (*NIP, ABCs, NRAMP, PEZ*) and stress related genes (e.g., *GSTs, GRXs, HSPs, PODs*) (Singh et al., 2017). When plants were exposed to low temperature, aluminium or cadmium, NO was found to modulate secondary metabolism by regulating genes involved in terpenoid, flavonoid, and lignin biosynthesis (Zheng et al., 2021; Wu et al., 2022; Cao et al., 2024, Table 1).

These studies collectively highlight NO's versatile role in plant biology, encompassing growth regulation mainly *via* phytohormone-related pathways, primary and secondary metabolic control and tolerance mechanisms against stress factors.

## 3. Mechanisms of NO-related gene expression regulation

The above mentioned studies identified NO-related plant genes but did not give information about the mechanisms by which NO modifies the expression of target genes. More recent research; however, suggests that NO regulates the expression of target genes primarily by two mechanisms, through PTM of transcription factors and/or by modifying the chromatin structure.

### 3.1. Nitric oxide as an epigenetic modulator: A focus on histone and DNA methylation modifications of gene expression

Epigenetic regulation in plants occurs through three main mechanisms: PTMs of histone proteins, DNA methylation on cytosine residues, and RNA-based processes. These mechanisms work together to influence the structure and accessibility of DNA, thereby providing an additional layer of regulation over gene expression. Histone proteins undergo various modifications, such as acetylation, methylation, ubiquitination and phosphorylation, which impact interactions with DNA, other histones, and non-histone proteins (Wurm and Lindermayr, 2021).

Histone acetylation is a key epigenetic process that influences gene transcription in both plants and animals. This modification is catalysed by histone acetyltransferases (HATs), which transfer acetyl groups from acetyl-CoA to lysine residues on histones. This neutralizes the positive charge of lysine, weakening the interaction between histones and DNA, leading to a more relaxed chromatin structure that facilitates transcription. On the other hand, histone deacetylases (HDAs) remove acetyl groups, causing chromatin to condense and repressing transcription (Hollender and Liu, 2008; Luo et al., 2012). In *A. thaliana*, 18 HDAs are grouped into three families: RPD3-like (HDA2, HDA5–10, HDA14–15, HDA17–19), HD-tuins (HDT1–4), and sirtuins. The members of the RPD3 superfamily contain multiple conserved cysteine residues, which serve as targets for *S*-nitrosation across different species. Specifically, in Arabidopsis, HDA6 contains conserved cysteines similar to those found in human HDA2, which interact with NO. Structural modelling of the HDA domain in HDA6 and HDA19 in soybean (*Glycine max*) shows strong similarity to human HDA2, suggesting a conserved regulatory mechanism (Ageeva-Kieferle et al., 2019). Ageeva-Kieferle et al. (2021) demonstrated that NO affects histone acetylation in Arabidopsis, particularly H3K9 and H3K9/K14, under different light conditions. In *gsnor1–3* and *hda6* mutants, these changes were absent, highlighting the importance of GSNOR and HDA6 in light-induced histone acetylation. *In vitro* assays confirmed HDA6's sensitivity to NO, and ChIP-seq analysis revealed that GSNOR and HDA6 regulate growth and stress genes, promoting acetylation in stress-related genes and deacetylation in growth genes under low light. Moreover, NO-mediated acetylation has been shown to enhance the expression of pathogen-related genes in *Phytophthora infestans*, highlighting NO's role in histone acetylation as an epigenetic regulator (Guan et al., 2024b).

In addition to histone acetylation, histone methylation plays a significant role in modulating chromatin structure and transcriptional activity. Histone methylation occurs on lysine and arginine residues, and its effects on chromatin accessibility depend on the location and degree of methylation (mono-, di-, or tri-methylation) (Feng and Jacobsen, 2011). Unlike acetylation, methylation does not alter the overall negative charge of histone proteins and can be associated with both euchromatin and heterochromatin, affecting both silenced and active transcription regions (Wurm and Lindermayr, 2021). Histone methylation is regulated by histone methyltransferases (HMTs), which add methyl groups, and histone lysine demethylases (KDMs), which remove

them (Pikaard and Scheid, 2014). NO has been implicated in the redox regulation of both HMTs and KDMs, suggesting that histone methylation is also influenced by NO signalling (Nott et al., 2008). GSNOR1 plays a critical role in maintaining *S*-adenosylmethionine (SAM) homeostasis, a key methyl donor for DNA and histone methylation. *gsnor1–3* mutants accumulate SAM, leading to increased histone H3K9me2 levels and elevated global DNA methylation at CG, CHG, and CHH sites, particularly affecting transposable element repression (Rudolf et al., 2021). Mass spectrometry and bisulfite sequencing further confirmed that GSNOR1 regulates chromatin accessibility and epigenetic silencing by modulating the histone methylation index. Protein arginine methylation, catalysed by protein arginine methyltransferases (PRMTs), represents another essential histone modification. PRMT5, a well-studied plant methyltransferase, is positively regulated by NO through *S*-nitrosation at Cys 125, enhancing its enzymatic activity of arginine methylation and contributing to salt stress tolerance (Hu et al., 2017). Histone lysine methylation is carried out by SET-domain-containing methyltransferases, while demethylation is performed by lysine-specific histone demethylase-like proteins or those containing a Jumonji-C (JmjC) domain. The conservation of epigenetic mechanisms across species, including the regulation of histone methylation, suggests common regulatory principles in plants and animals (Lindermayr et al., 2020).

During DNA methylation, a methyl group is attached to the fifth position of cytosine, forming 5-methylcytosine. When this modification occurs in promoter regions, it is associated with gene silencing by altering chromatin structure, DNA conformation, stability, and DNA-protein interactions, ultimately affecting gene expression. This widely distributed epigenetic mechanism plays a crucial role in transcriptional regulation and is considered a key example of epigenetic gene silencing and heterochromatin formation (Wurm and Lindermayr, 2021). Plant DNA methyltransferases can be classified into three main categories: methyltransferases, chromotransferases, and structural domain rearrangement methyltransferases (Hao et al., 2020). In plants, DNA demethylation is primarily regulated by Demeter, Repressor of Silencing 1, Demeter-like 2, and Demeter-like 3, which influence the plant's stress response (Li et al., 2018). Hou et al. (2024) found that both NO and DNA methylation play key roles in regulating flowering in plants. Their results confirmed that the DNA methylation inhibitor, 5-AzaC accelerated flowering in tomatoes, and the application of GSNO enhanced the positive effects of 5-AzaC on flowering, suggesting that exogenous NO may participate in the 5-AzaC-mediated DNA demethylation response during tomato flowering. Furthermore, exogenous NO significantly increased the expression of DNA demethylation genes (*DML1*, *DML2*, and *DML3*) in tomato shoot tips, while the expression of methyltransferase-related genes, such as *DRM6* and *CMT4*, changed only slightly, indicating that NO potentially regulates DNA methylation levels by influencing the expression of demethyltransferase enzymes. Exogenous NO application was reported to reduce damage caused by cold storage of peach (Guo et al., 2023). NO treatment alleviated the cold-dependent decrease in DNA methyltransferase (DNMT) activity being responsible for regulating DNA methylation, while cPTIO enhanced DNMT activity at transcript levels. Their research suggests that NO can enhance the cold tolerance of ripening peaches through mediating DNA methylation. NO signalling was found to regulate shoot stem cell homeostasis in Arabidopsis by controlling genome-wide DNA methylation through modulating the expression and activity of Argonaute 4 (AGO4) (Zeng et al., 2023). Interaction between the TF WUSCHEL (WUS) and AGO4, is disrupted by NO, suggesting that part of WUS's repressive activity may be mediated by DNA methylation at its target sites.

### 3.2. Modulation of transcription factor activity by NO-associated PTMs

NO often modulates gene expression through PTMs of transcription factors. Among NO-associated PTMs, the reversible *S*-nitrosation seems to have a pivotal role in TF regulation. TFs are key regulators of gene expression by binding to specific DNA sequences, thereby controlling transcription initiation and various biological processes in plants. The Plant Transcription Factor Database (PlantTFDB) provides a comprehensive resource for plant TFs, identifying 320,370 TFs across 165 species and classifying them into 58 distinct families (http://planttfdb.gao-lab.org) (Guo et al., 2008). Several TF families have been identified as NO targets or as responsive to NO signalling such as WRKY, NAC, ERF/AP2, MYB, ZnF and TGA-type factors, including NPR1 and ABI5. WRKY TFs are central regulators of biotic stress responses (Saha et al., 2024), and several WRKY proteins are activated in response to NO during pathogen attack. For instance, NO-mediated *S*-nitrosation enhances the DNA-binding ability of certain WRKY TFs, promoting the transcription of defence-related genes and functions downstream of NO signalling in systemic acquired resistance (Imran et al., 2018b). NAC (NAM, ATAF1/2, and CUC2) is a large family of plant-specific transcription factors which are involved in abiotic stress tolerance and developmental processes (Chen et al., 2025; Xiong et al., 2025). NO regulates hundreds of TFs during stress responses, modulating their ability to activate genes. NAC-type TFs can be involved in antioxidant defence, regulating genes encoding superoxide dismutase and catalase (Xiong et al., 2025). Ethylene-responsive factors (ERF) are key players in NO and ethylene crosstalk during stress responses. Group VII ERFs control responses to anoxia and were shown to be destabilized in the presence of NO *via* the N-end rule pathway, while they are stabilized in the absence of NO. Such N-end rule regulation provides a flexible and precise mechanism for perception and transduction of NO signal (Gibbs et al., 2014). More recently, the *in vitro S*-nitrosation of *Medicago truncatula* ERF75 N-terminal part has been evidenced, and it was suggested that *S*-nitrosation is the first step in the oxidation of the Cys residue peptide, and it occurs in peptides where the Cys residue is exposed (Rovere et al., 2023). MYB TFs regulate a broad range of processes, including secondary metabolism and stress responses. Regarding their NO-associated modifications, the DNA binding activity of AtMYB2 is altered by NO due to *S*-nitrosation of a conserved Cys in its DNA-binding domain (Serpa et al., 2007). Similarly, AtMYB30 proved to be directly affected by the *S*-nitrosation leading to a prohibition of DNA binding (Tavares et al., 2014). Recently, NO has been found to *S*-nitrosate MYB30 at Cys 49 and enhance its transcriptional activity by interfering the interaction of MYB30 with the PYL4 repressor (Zhao et al., 2024). Further experiments revealed that S-nitrosation of MYB30 regulates the balance between seed dormancy and germination induction (Zhao et al., 2024). The basic leucine zipper (bZIP) transcription factor ABA-Insensitive 5 (ABI5) is a key regulator of ABA-mediated seed germination and early seedling development. ABI5 has been identified as a target for NO-dependent *S*-nitrosation at Cys 153 which facilitates its degradation by CULLIN4-based and KEEP ON GOING E3 ligases promoting seed germination (Albertos et al., 2015). Recently, the Arabidopsis ABI5 homolog bZIP67 has been observed to be a target of *S*-nitrosation also, leading to its stabilization enabling its accumulation. The bZIP67 induces the expression of fatty acid desaturase3 which catalyses the conversion of linoleic acid (18:2) into linolenic acid (18:3). Moreover, the PTM of bZIP67 proved to be reversible by the *trans*-denitrosylation activity of peroxiredoxin IIE providing evidence for a precise feedback regulation (Sánchez-Vicente et al., 2024). TGA TFs belong to bZIP family and bind to their target DNA sequence as dimers through the conserved bZIP domain. TGA activity is often connected to hormonal pathways (Tomaž et al., 2022). Several TGA factors are involved in the regulation of defence-related genes through interaction with NON-EXPRESSOR OF PR-1 (NPR1) cofactor (Zhang et al., 1999). NPR1 and TGA1 were shown to be *S*-nitrosated by NO. In TGA1, Cys residues 260 and 266 are *S*-nitrosated and *S*-glutathionylated by low GSNO dose. The presence of elevated NO levels prevent oxygen-mediated modifications of TGA1 and enhance its DNA binding activity in the presence of NPR1 (Lindermayr et al., 2010). Sustained NO synthesis triggers the *S*-nitrosation at Cys 87 of zinc finger TF, SRG1, relieving both SRG1 DNA binding and transcriptional repression activity. Thus, the *S*-nitrosated form of SRG1 may be involved in a negative

feedback loop that reduces the plant immune response (Cui et al., 2018).

## 4. Conclusion and future perspectives

Nitric oxide serves as a key signalling molecule in cellular communication, stress responses, and metabolic regulation across biological systems. In plants, NO integrates multiple hormonal and environmental signalling pathways, influencing every stages of development and diverse stress responses. Genetic screens and genome-wide transcriptome analyses were employed in a number of studies to decipher the molecular mechanism of NO effects and to identify NO-associated genes. Forward genetic screens linked NO signalling to photosynthesis, cytokinin metabolism, stress responses and cell cycle regulation. Moreover, reverse genetic approaches contributed to the understanding of NO-related gene functions in plants, characterizing genes involved in NO biosynthesis (e.g., *NIA1/NIA2*, *NOA1*), signalling (e.g., *GSNOR*, *NPR1*), stress responses (e.g., *ABI4*, *RBOHD*), and development (e.g., *HO1*, *NOX1*). High-throughput transcriptomic techniques such as microarray and RNA-seq analyses have identified thousands of NO-responsive genes involved in various processes from growth regulation to stress adaptation, across multiple plant species. GSNO or nanoforms of NO donors can be considered to achieve NO specific effects in future genetic screens and transcriptome analyses. Similar to animals, NO has been found to function as an epigenetic regulator in plants, influencing gene expression through histone acetylation, methylation, and DNA methylation. Through *S*-nitrosation, NO influences histone deacetylase and methyltransferase activities, thereby modulating chromatin structure and transcriptional responses to environmental signals. Nitric oxide has also been shown to influence key TF families, including WRKY, NAC, ERF, MYB, NPR1, ABI5, and TGA, by altering their stabilities, DNA-binding abilities, and protein-protein interactions. Overall, NO has been proven to intervene eukaryotic gene regulation at the level of epigenetics, transcription and post-translational modifications as summarized by Fig. 3. Future research should focus on elucidating the precise molecular mechanisms of NO biosynthesis, long-distance (inter-organ, inter-plant) signalling, and its interaction with ROS, phytohormones and calcium signalling. The majority of the studies identifying NO-responsive genes have focused on a single stress condition so far. However, considering climate change, there is increasing interest in understanding the synergistic effects and interconnections between biotic and abiotic stresses. Examining the NO-associated gene networks under combined stress conditions is a promising research direction which will deepen our understanding of NO signalling and regulation at the core of plant responses. Disentangling these interactions requires precise genetic tools and combinatorial approaches to target multiple genes simultaneously. Advanced "omics" technologies and computational modelling will enhance our understanding of NO-mediated PTMs and chromatin remodelling. By integrating multi-omics, genome editing, and functional genomics, future research is expected to unravel the complexity of NO signalling. As genomic tools and gene editing methods are increasingly available for crop plants, improving important traits such as stress tolerance through engineering NO-related regulatory pathways is becoming available for a number of cultivated species. Efforts to adapt our knowledge on NO signalling obtained on model plants to crops is becoming a reality and pave the way for improved agricultural productivity and generation of stress-resilient crops.

## CRediT authorship contribution statement

**Caetano Da Silva Rafael:** Writing – review & editing. **Szabados László:** Writing – review & editing. **Lindermayr Christian:** Writing – review & editing. **Kolbert Zsuzsanna:** Writing – review & editing,

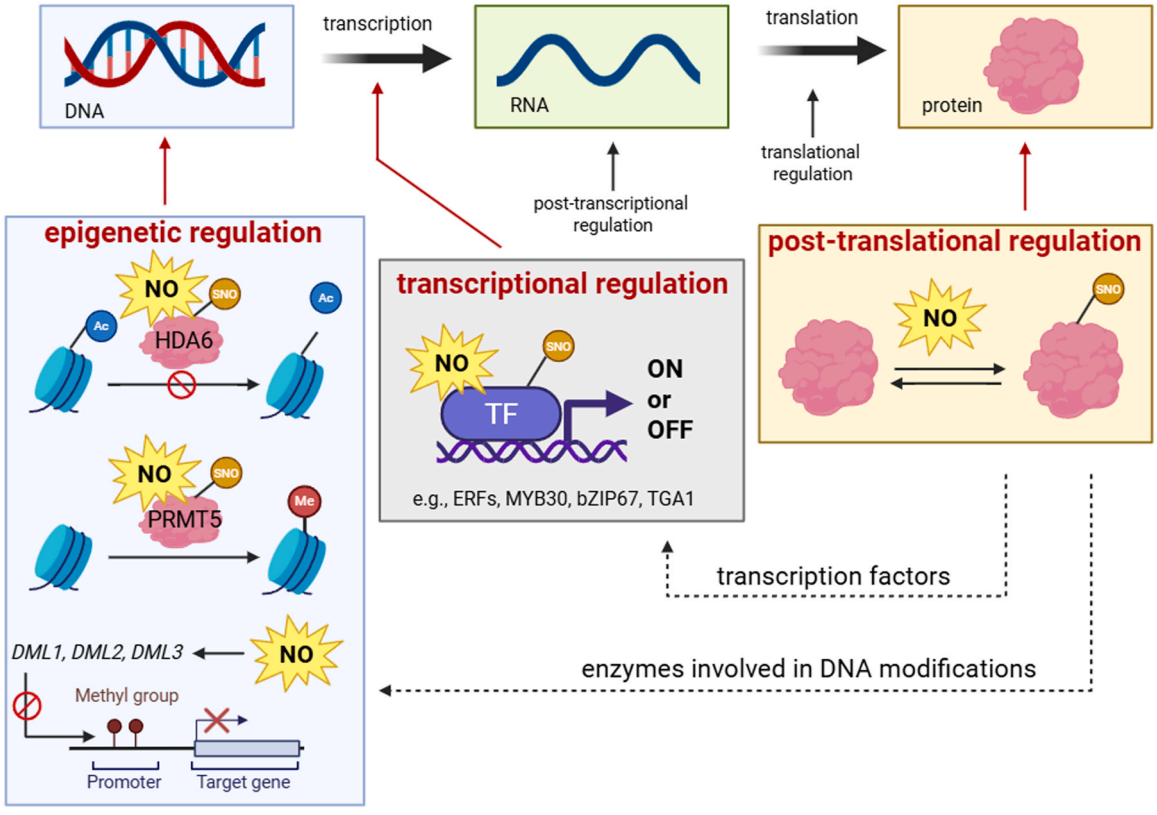


**Fig. 3.** Different levels of eukaryotic gene expression regulation involve NO signalling. NO is involved in histone acetylation, methylation and DNA methylation (the level of epigenetic regulation). At the transcriptional level, NO regulates transcription factor stability, DNA-binding ability, and protein-protein interaction. At the post-translational level, NO *S*-nitrosates specific target proteins including transcription factors and enzymes involved in DNA modifications. Abbreviations: HDA6-histone deacetylase 6, PRMT5- protein arginine methyltransferase 5, DML1,2,3- DNA demethylases, TF- transcription factor.

Writing – original draft, Visualization, Funding acquisition, Conceptualization. **Széles Eszter:** Writing – review & editing, Writing – original draft, Data curation. **Kondak Dóra:** Writing – original draft.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Eszter Széles reports financial support was provided by Hungarian Academy of Sciences. Given Zsuzsanna Kolbert's role as Executive Guest Editor, she had no involvement in the peer review of this article and had no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to another journal editor. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability

No data was used for the research described in the article.

## References

A. Ageeva-Kieferle, E.E. Rudolf, C. Lindermayr, Redox-dependent chromatin remodeling: a new function of nitric oxide as architect of chromatin structure in plants, Front. Plant Sci. 10 (2019) 625, https://doi.org/10.3389/fpls.2019.00625.

A. Ageeva-Kieferle, E. Georgii, B. Winkler, A. Ghirardo, A. Albert, P. Hüther, A. Mengel, C. Becker, J.P. Schnitzler, J. Durner, C. Lindermayr, Nitric oxide coordinates growth, development, and stress response via histone modification and gene expression, Plant Physiol. 187 (2021) 336–360, https://doi.org/10.1093/plphys/kiab222.

N. Agrawal, P.V. Dasaradhi, A. Mohmmed, P. Malhotra, R.K. Bhatnagar, S.K. Mukherjee, RNA interference: biology, mechanism, and applications, Microbiol. Mol. Biol. Rev. 67 (2003) 657–685, https://doi.org/10.1128/MMBR.67.4.657-685.2003.

E. Aklilu, Review on forward and reverse genetics in plant breeding, All Life 14 (2021) 127–135, https://doi.org/10.1080/26895293.2021.1888810.

P. Albertos, M.C. Romero-Puertas, K. Tatematsu, I. Mateos, I. Sánchez-Vicente, E. Nambara, O. Lorenzo, S-nitrosylation triggers ABI5 degradation to promote seed germination and seedling growth, Nat. Comm. 6 (2015) 8669, https://doi.org/10.1038/ncomms9669.

J. Astier, C. Lindermayr, Nitric oxide-dependent posttranslational modification in plants: an update, J. Mol. Sci. 13 (2012) 15193–15208, https://doi.org/10.3390/ijms131115193.

J.C. Begara-Morales, B. Sánchez-Calvo, F. Luque, F. Luque, M.O. Leyva-Pérez, M. Leterrier, F.J. Corpas, J.B. Barroso, Differential transcriptomic analysis by RNA-Seq of GSNO-responsive genes between arabidopsis roots and leaves, Plant Cell Physiol. 55 (2014) 1080–1095, https://doi.org/10.1093/pcp/pcu044.

A. Berger, A. Boscari, Horta, N. Araújo, M. Maucourt, M. Hanchi, S. Bernillon, D. Rolin, A. Puppo, R. Brouquisse, Plant nitrate reductases regulate nitric oxide production and nitrogen-fixing metabolism during the *Medicago truncatula-sinorhizobium meliloti* symbiosis, Front. Plant Sci. 11 (2020) 1313, https://doi.org/10.3389/fpls.2020.01313.

S. Borrowman, J.G. Kapuganti, G.J. Loake, Expanding roles for S-nitrosylation in the regulation of plant immunity, Free Rad. Biol. Med. 194 (2023) 357–368, https://doi.org/10.1016/j.freeradbiomed.2022.12.009.

S. Cao, J. Pan, M. Rehman, D. Luo, Q. Wang, G. Jin, R. Li, T. Chen, P. Chen, Exogenous nitric oxide alleviates cadmium toxicity in kenaf (*Hibiscus cannabinus* L.) through modulating cd deposition and regulating key genes and involved pathways, Indust. Crop. Prod. 221 (2024) 119359, https://doi.org/10.1016/j.indcrop.2024.119359.

M.C. Castillo, A. Coego, Á. Costa-Broseta, J. León, Nitric oxide responses in arabidopsis hypocotyls are mediated by diverse phytohormone pathways, J. Exp. Bot. 69 (2018) 5265–5278, https://doi.org/10.1093/jxb/ery286.

A. Chamizo-Ampudia, E. Sanz-Luque, Á. Llamas, F. Ocaña-Calahorro, V. Mariscal, A. Carreras, J.B. Barroso, A. Galván, E. Fernández, A dual system formed by the ARC and NR molybdoenzymes mediates nitrite-dependent NO production in chlamydomonas, Plant Cell Environ. 39 (2016) 2097–2107, https://doi.org/10.1111/pce.12739.

L. Chen, R. Wu, J. Feng, et al., Transnitrosylation mediated by the non-canonical catalase ROG1 regulates nitric oxide signaling in plants, Dev. Cell 53 (2020) 444–457, https://doi.org/10.1016/j.devcel.2020.03.020.

S. Chen, W. Zhang, Q. Zhang, B. Li, M. Zhang, J. Qin, W. Shi, C. Jia, SlNAC12, a novel NAC-type transcription factor, confers salt stress tolerance in tomato, Plant Cell Rep. 44 (2025) 5, https://doi.org/10.1007/s00299-024-03400-x.

F.J. Corpas, J. Taboada, B. Sánchez-Romera, J. López-Jaramillo, J.M. Palma, Peroxisomal sulfite oxidase (SOX), an alternative source of NO in higher plants which is upregulated by $H_2S$, Plant Physiol. Biochem 225 (2025) 110000, https://doi.org/10.1016/j.plaphy.2025.110000.

G. Corti Monzón, M. Pinedo, J. Di Rienzo, E. Novo-Uzal, F. Pomar, L. Lamattina, L. de la Canal, Nitric oxide is required for determining root architecture and lignin composition in sunflower. Supporting evidence from microarray analyses, Nitric Oxide 39 (2014) 20–28, https://doi.org/10.1016/j.niox.2014.04.004.

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

B. Cui, Q. Pan, D. Clarke, M.O. Villarreal, S. Umbreen, B. Yuan, W. Shan, J. Jiang, G. J. Loake, S-nitrosylation of the zinc finger protein SRG1 regulates plant immunity, Nat. Comm. 9 (2018) 4226, https://doi.org/10.1038/s41467-018-06578-3.

L.A. del Río, F.J. Corpas, J.B. Barroso, Nitric oxide and nitric oxide synthase activity in plants, Phytochem 65 (2004) 783–792, https://doi.org/10.1016/j.phytochem.2004.02.001.

L. Di Fino, A. Di Palma, E.A. Perk, C. García-Mata, F.J. Schopfer, A.M. Laxalt, Nitro-fatty acids: electrophilic signaling molecules in plant physiology, Planta 254 (2021) 120, https://doi.org/10.1007/s00425-021-03777-z.

J. Du, M. Li, D. Kong, et al., Nitric oxide induces cotyledon senescence involving co-operation of the NES1/MAD1 and EIN2-associated ORE1 signalling pathways in arabidopsis, J. Exp. Bot. 65 (2013) 4051–4063, https://doi.org/10.1093/jxb/ert429.

M. Feelisch, J.F. Martin, The early role of nitric oxide in evolution, Trend Ecol. Evol. 10 (1995) 496–499, https://doi.org/10.1016/s0169-5347(00)89206-x.

S. Feng, S.E. Jacobsen, Epigenetic modifications in plants: an evolutionary perspective, Curr. Opin. Plant Biol. 14 (2011) 179–186, https://doi.org/10.1016/j.pbi.2010.12.002.

A.R. Fernie, T. Tohge, The genetics of plant metabolism, Annu. Rev. Gen. 51 (2017) 287–310, https://doi.org/10.1146/annurev-genet-120116-024640.

W.C. Gan, A.P.K. Ling, CRISPR/Cas9 in plant biotechnology: applications and challenges, Biotech 103 (2022) 81–93, https://doi.org/10.5114/bta.2022.113919.

S.B. Gelvin, Plant DNA repair and *agrobacterium* T-DNA integration, Int. J. Mol. Sci. 22 (2021) 8458, https://doi.org/10.3390/ijms22168458.

D.J. Gibbs, N. Md Isa, M. Movahedi, J. Lozano-Juste, G.M. Mendiondo, S. Berckhan, N. Marín-de la Rosa, J. Vicente Conde, C. Sousa Correia, S.P. Pearce, G.W. Bassel, B. Hamali, P. Talloji, D.F. Tomé, A. Coego, J. Beynon, D. Alabadí, A. Bachmair, J. León, J.E. Gray, F.L. Theodoulou, M.J. Holdsworth, Nitric oxide sensing in plants is mediated by proteolytic control of group VII ERF transcription factors, Mol. Cell 53 (2014) 369–379, https://doi.org/10.1016/j.molcel.2013.12.020.

G. Gonorazky, A.M. Distéfano, C. García-Mata, L. Lamattina, A.M. Laxalt, Phospholipases in nitric Oxide-Mediated plant signaling, in: X. Wang (Ed.), E-Publishing Inc, Phospholipases in Plant Signaling, Springer, Berlin, Heidelberg, 2014, pp. 135–158, https://doi.org/10.1007/978-3-642-42011-5_8.

F. Groß, E.E. Rudolf, B. Thiele, J. Durner, J. Astier, Copper amine oxidase 8 regulates arginine-dependent nitric oxide production in *Arabidopsis thaliana*, J. Exp. Bot. 68 (2017) 2149–2162, https://doi.org/10.1093/jxb/erx105.

M. Guan, X. Zheng, Y. Zhu, S-nitrosoglutathione reductase disfavors cadmium tolerance in shoots of arabidopsis, Sci. Rep. 14 (2024a) 26401, https://doi.org/10.1038/s41598-024-77759-y.

Y. Guan, J. Gajewska, E. Sobieszczuk-Nowicka, J. Floryszak-Wieczorek, S. Hartman, M. Arasimowicz-Jelonek, The effect of nitrosative stress on histone H3 and H4 acetylation in *Phytophthora infestans* life cycle, Plant Physiol. Biochem 216 (2024b) 109129, https://doi.org/10.1016/j.plaphy.2024.109129.

A.Y. Guo, X. Chen, G. Gao, H. Zhang, Q.H. Zhu, X.C. Liu, Y.F. Zhong, X. Gu, K. He, J. Luo, PlantTFDB: a comprehensive plant transcription factor database, Nucl. Acid. Res. 36 (2008) D966–D969, https://doi.org/10.1093/nar/gkm841.

X. Guo, D. Huang, G. Jing, J. Feng, S. Zhu, Nitric oxide-mediated DNA methylation enhances cold resistance in postharvest peach fruit, Food Chem. 404 (2023) 134660, https://doi.org/10.1016/j.foodchem.2022.134660.

X. Hao, Z. Jin, Z. Wang, W. Qin, Y. Pei, Hydrogen sulfide mediates DNA methylation to enhance osmotic stress tolerance in *Setaria italica* l, Plant Soil 453 (2020) 355–370, https://doi.org/10.1007/s11104-020-04590-5.

Y. He, R.H. Tang, Y. Hao, R.D. Stevens, C.W. Cook, S.M. Ahn, L. Jing, Z. Yang, L. Chen, F. Guo, F. Fiorani, R.B. Jackson, N.M. Crawford, Z.M. Pei, Nitric oxide represses the arabidopsis floral transition, Science 305 (2004) 1968–1971, https://doi.org/10.1126/science.1098837.

C. Hollender, Z. Liu, Histone deacetylase genes in arabidopsis development, J. Integr. Plant Biol. 50 (2008) 875–885, https://doi.org/10.1111/j.1744-7909.2008.00704.x.

X. Hou, M. Shi, Z. Zhang, Y. Yao, Y. Li, C. Li, W. Yu, C. Wang, W. Liao, DNA demethylation is involved in nitric oxide-induced flowering in tomato, J. Integr. Agric. 24 (2024) 1769–1785, https://doi.org/10.1016/j.jia.2024.09.037.

J. Hu, H. Yang, J. Mu, T. Lu, J. Peng, X. Deng, Z. Kong, S. Bao, X. Cao, J. Zuo, Nitric oxide regulates protein methylation during stress responses in plants, Mol. Cell 67 (2017) 702–710, https://doi.org/10.1016/j.molcel.2017.06.031.

X. Huang, U. von Rad, J. Durner, Nitric oxide induces transcriptional activation of the nitric oxide-tolerant alternative oxidase in arabidopsis suspension cells, Planta 215 (2002) 914–923, https://doi.org/10.1007/s00425-002-0828-z.

J. Huang, H. Wei, L. Li, S. Yu, Transcriptome analysis of nitric oxide-responsive genes in upland cotton (*gossypium hirsutum*), PLoS One 13 (2018) e0192367, https://doi.org/10.1371/journal.pone.0192367.

A. Hussain, B.G. Mun, Q.M. Imran, S.U. Lee, T.A. Adamu, M. Shahid, K.M. Kim, B. W. Yun, Nitric oxide mediated transcriptome profiling reveals activation of multiple

regulatory pathways in *arabidopsis thaliana*, Front. Plant Sci. 7 (2016) 975, https://doi.org/10.3389/fpls.2016.00975.
A. Hussain, B.W. Yun, J.H. Kim, K.J. Gupta, N.I. Hyung, G.J. Loake, Novel and conserved functions of S-nitrosoglutathione reductase in tomato, J. Exp. Bot. 70 (2019) 4877–4886, https://doi.org/10.1093/jxb/erz234.
A. Hussain, F. Shah, F. Ali, B.W. Yun, Role of nitric oxide in plant senescence, Front. Plant Sci. 13 (2022) 851631, https://doi.org/10.3389/fpls.2022.851631.
Q.M. Imran, A. Hussain, S.U. Lee, B.G. Mun, N. Falak, G.J. Loake, B.W. Yun, Transcriptome profile of NO-induced arabidopsis transcription factor genes suggests their putative regulatory role in multiple biological processes, Sci. Rep. 8 (2018a) 771, https://doi.org/10.1038/s41598-017-18850-5.
Q.M. Imran, A. Hussain, B.G. Mun, et al., Transcriptome wide identification and characterization of NO-responsive WRKY transcription factors in *arabidopsis thaliana* l, Environ. Exp. Bot. 148 (2018b) 128–143, https://doi.org/10.1016/j.envexpbot.2018.01.010.
J. Jahnová, L. Luhová, M. Petřivalský, S-Nitrosoglutathione Reductase-The master regulator of protein S-Nitrosation in plant NO signaling, Plants 8 (2019) 48, https://doi.org/10.3390/plants8020048.
S. Jeandroz, O. Lamotte, J. Astier, S. Rasul, P. Trapet, A. Besson-Bard, S. Bourque, V. Nicolas-Francès, W. Ma, G.A. Berkowitz, D. Wendehenne, There's more to the picture than meets the eye: nitric oxide cross talk with $Ca^{2+}$ signaling, Plant Physiol. 163 (2013) 459–470, https://doi.org/10.1104/pp.113.220624.
N.R. Kabange, S.Y. Park, J.Y. Lee, D. Shin, S.M. Lee, Y. Kwon, J.K. Cha, J.H. Cho, D. V. Duyen, J.M. Ko, J.H. Lee, New insights into the transcriptional regulation of genes involved in the nitrogen use efficiency under potassium chlorate in rice (*oryza sativa l.*), Int. J. Mol. Sci. 22 (2021) 2192, https://doi.org/10.3390/ijms22042192.
M. Khan, S. Ali, T.N.I. Al Azzawi, B.W. Yun, Nitric oxide acts as a key signaling molecule in plant development under stressful conditions, Int. J. Mol. Sci. 24 (2023) 4782, https://doi.org/10.3390/ijms24054782.
Z. Kolbert, B. Bartha, L. Erdei, Exogenous auxin-induced NO synthesis is nitrate reductase-associated in *arabidopsis thaliana* root primordia, J. Plant. Physiol. 165 (2008) 967–975, https://doi.org/10.1016/j.jplph.2007.07.019.
Z. Kolbert, L. Ortega, L. Erdei, Involvement of nitrate reductase (NR) in osmotic stress-induced NO generation of *arabidopsis thaliana* L. Roots, J. Plant Physiol. 167 (2010) 77–80, https://doi.org/10.1016/j.jplph.2009.08.013.
Z. Kolbert, Á. Molnár, D. Oláh, G. Feigl, E. Horváth, L. Erdei, A. Ördög, E. Rudolf, T. Barth, C. Lindermayr, S-Nitrosothiol signaling is involved in regulating hydrogen peroxide metabolism of Zinc-Stressed arabidopsis, Plant Cell Physiol. 60 (2019) 2449–2463, https://doi.org/10.1093/pcp/pcz138.
Z. Kolbert, C. Lindermayr, Computational prediction of NO-dependent posttranslational modifications in plants: current status and perspectives, Plant. Physiol. Biochem 167 (2021) 851–861, https://doi.org/10.1016/j.plaphy.2021.09.011.
C. Koncz, K. Németh, G.P. Rédei, J. Schell, T-DNA insertional mutagenesis in arabidopsis, Plant Mol. Biol. 20 (1992) 963–976, https://doi.org/10.1007/BF00027166.
L. Kubienová, T. Tichá, L. Luhová, M. Petřivalský, Detection of S-nitrosoglutathione reductase activity in plants, Methods Mol. Biol. 1424 (2016) 175–189, https://doi.org/10.1007/978-1-4939-3600-7_15.
A. Kumari, V.C. Kaladhar, N. Yadav, P. Singh, K. Reddy, K.J. Gupta, Nitric oxide regulates mitochondrial biogenesis in plants, Plant Cell Environ. 46 (2023) 2492–2506, https://doi.org/10.1111/pce.14637.
G.T. Kuruthukulangarakoola, J. Zhang, A. Albert, et al., Nitric oxide-fixation by non-symbiotic haemoglobin proteins in *Arabidopsis thaliana* under N-limited conditions, Plant Cell Environ. 40 (2017) 36–50, https://doi.org/10.1111/pce.12773.
J.R. Lancaster Jr., A tutorial on the diffusibility and reactivity of free nitric oxide, Nitric Oxide 1 (1997) 18–30, https://doi.org/10.1006/niox.1996.0112.
L. Latorre, M.B. Fernández, R. Cassia, Nitric oxide is a key part of the UV-B-induced photomorphogenesis in arabidopsis, Environ. Exp. Bot. 216 (2023) 105538, https://doi.org/10.1016/j.envexpbot.2023.105538.
J. León, Á. Costa-Broseta, Present knowledge and controversies, deficiencies, and misconceptions on nitric oxide synthesis, sensing, and signaling in plants, Plant Cell Environ. 43 (2020) 1–5, https://doi.org/10.1111/pce.13617.
J. León, Protein tyrosine nitration in plant nitric oxide signaling, Fron. Plant Sci. 13 (2022) 859374, https://doi.org/10.3389/fpls.2022.859374.
M. Leterrier, M. Chaki, M. Airaki, R. Valderrama, J.M. Palma, J.B. Barroso, F.J. Corpas, Function of S-nitrosoglutathione reductase (GSNOR) in plant development and under biotic/abiotic stress, Plant Signal. Behav. 6 (2011) 789–793, https://doi.org/10.4161/psb.6.6.15161.
H. Li, J.B. Song, W.T. Zhao, Z.M. Yang, AtHO1 is involved in iron homeostasis in an NO-dependent manner, Plant Cell Physiol. 54 (2013) 1105–1117, https://doi.org/10.1093/pcp/pct063.
Y. Li, S. Kumar, W. Qian, Active DNA demethylation: mechanism and role in plant development, Plant Cell Rep. 37 (2018) 77–85, https://doi.org/10.1007/s00299-017-2215-z.
B. Li, C. Sun, J. Li, C. Gao, Targeted genome-modification tools and their advanced applications in crop breeding, Nat. Rev. 25 (2024) 603–622, https://doi.org/10.1038/s41576-024-00720-2.
C. Lindermayr, S. Sell, B. Müller, D. Leister, J. Durner, Redox regulation of the NPR1-TGA1 system of *arabidopsis thaliana* by nitric oxide, Plant Cell 22 (2010) 2894–2907, https://doi.org/10.1105/tpc.109.066464.
C. Lindermayr, E.E. Rudolf, J. Durner, M. Groth, Interactions between metabolism and chromatin in plant models, Mol. Metab. 38 (2020) 100951, https://doi.org/10.1016/j.molmet.2020.01.015.
W.-Z. Liu, D.-D. Kong, X.-X. Gu, et al., Cytokinins can act as suppressors of nitric oxide in arabidopsis, Proc. Natl. Acad. Sci. USA 110 (2013) 1548–1553, https://doi.org/10.1073/pnas.1213235110.
Z. Liu, D. Huang, Y. Yao, X. Pan, Y. Zhang, Y. Huang, Z. Ding, C. Wang, W. Liao, The crucial role of SlGSNOR in regulating postharvest tomato fruit ripening, Int. J. Mol. Sci. 25 (2024) 2729, https://doi.org/10.3390/ijms25052729.
P. López-Gómez, J. Buezo, M. Urra, A. Cornejo, R. Esteban, J. Fernández de Los Reyes, E. Urarte, E. Rodríguez-Dobreva, A. Chamizo-Ampudia, A. Eguaras, S. Wolf, D. Marino, V. Martínez-Merino, J.F. Moran, A new oxidative pathway of nitric oxide production from oximes in plants, Mol. Plant 17 (2024) 178–198, https://doi.org/10.1016/j.molp.2023.12.009.
J.O. Lundberg, E. Weitzberg, Nitric oxide signaling in health and disease, Cell 185 (2022) 2853–2878, https://doi.org/10.1016/j.cell.2022.06.010.
M. Luo, C.W. Yu, F.F. Chen, L. Zhao, G. Tian, X. Liu, Y. Cui, J.Y. Yang, K. Wu, Histone deacetylase HDA6 is functionally associated with AS1 in repression of KNOX genes in arabidopsis, PLoS Gen. 8 (2012) e1003114, https://doi.org/10.1371/journal.pgen.1003114.
I.R. Mir, H. Gautam, N.A. Anjum, A. Masood, N.A. Khan, Calcium and nitric oxide signaling in plant cadmium stress tolerance: a cross talk, S. Afr. J. Bot. 150 (2022) 387–403, https://doi.org/10.1016/j.sajb.2022.07.039.
M.A. Mohn, B. Thaqi, K. Fischer-Schrader, Isoform-specific NO synthesis by *arabidopsis thaliana* nitrate reductase, Plants 8 (2019) 67, https://doi.org/10.3390/plants8030067.
S. Neill, R. Barros, J. Bright, R. Desikan, J. Hancock, J. Harrison, P. Morris, D. Ribeiro, I. Wilson, Nitric oxide, stomatal closure, and abiotic stress, J. Exp. Bot. 59 (2008) 165–176, https://doi.org/10.1093/jxb/erm293.
S. Neill, NO way to die-nitric oxide, programmed cell death and xylogenesis, N. Phytol. 165 (2005) 5–7, https://doi.org/10.1111/j.1469-8137.2004.01267.x.
L. Niu, J. Yu, W. Liao, J. Yu, M. Zhang, M.M. Dawuda, Calcium and calmodulin are involved in nitric oxide-induced adventitious rooting of cucumber under simulated osmotic stress, Front. Plant Sci. 8 (2017) 1684, https://doi.org/10.3389/fpls.2017.01684.
A. Nott, P.M. Watson, J.D. Robinson, L. Crepaldi, A. Riccio, S-Nitrosylation of histone deacetylase 2 induces chromatin remodelling in neurons, Nat 455 (2008) 411–415, https://doi.org/10.1038/nature07238.
Q.-N. Pan, C.-C. Geng, D.-D. Li, S.-W. Xu, D.-D. Mao, S. Umbreen, G.J. Loake, B.-M. Cui, Nitrate reductase-mediated nitric oxide regulates the leaf shape in *arabidopsis* by mediating the homeostasis of reactive oxygen species, Int. J. Mol. Sci. 20 (2019) 2235, https://doi.org/10.3390/ijms20092235.
M. Parani, S. Rudrabhatla, R. Myers, H. Weirich, B. Smith, D.W. Leaman, S.L. Goldman, Microarray analysis of nitric oxide responsive transcripts in arabidopsis, Plant Biotech. J. 2 (2004) 359–366, https://doi.org/10.1111/j.1467-7652.2004.00085.x.
C.S. Pikaard, O.M. Scheid, Epigenetic regulation in plants, Cold Spring Harb. Perspect. Biol. 6 (2014) a019315, https://doi.org/10.1101/cshperspect.a019315.
G. Rasool, G. Buchholz, T. Yasmin, G. Shabbir, N.A. Abbasi, S.I. Malik, Overexpression of SlGSNOR impairs in vitro shoot proliferation and developmental architecture in tomato but confers enhanced disease resistance, J. Plant Physiol. 261 (2021) 153433, https://doi.org/10.1016/j.jplph.2021.153433.
L. Ravazzolo, S. Trevisan, S. Iori, C. Forestan, M. Malagoli, S. Quaggiotti, Nitrate regulates maize root transcriptome through nitric oxide dependent and independent mechanisms, Int. J. Mol. Sci. 22 (2021) 9527, https://doi.org/10.3390/ijms22179527.
M. Rezayian, F. Zarinkamar, Nitric oxide, calmodulin and calcium protein kinase interactions in the response of *brassica napus* to salinity stress, Plant Biol. (Stuttg. ) 25 (2023) 411–419, https://doi.org/10.1111/plb.13511.
P. Rockel, F. Strube, A. Rockel, J. Wildt, W.M. Kaiser, Regulation of nitric oxide (NO) production by plant nitrate reductase in vivo and in vitro, J. Exp. Bot. 53 (2002) 103–110, https://doi.org/10.1093/jxb/53.366.103.
M.G. Rosso, Y. Li, N. Strizhov, B. Reiss, K. Dekker, B. Weisshaar, An *arabidopsis thaliana* T-DNA mutagenized population (GABI-Kat) for flanking sequence tag-based reverse genetics, Plant Mol. Biol. 53 (2003) 247–259, https://doi.org/10.1023/B:PLAN.0000009297.37235.4a.
M. Rovere, C. Pucciariello, C. Castella, et al., Group VII ethylene response factors, MtERF74 and MtERF75, sustain nitrogen fixation in *medicago truncatula* microoxic nodules, Plant Cell Environ. 46 (2023) 607–620, https://doi.org/10.1111/pce.14505.
E.E. Rudolf, P. Hüther, I. Forné, E. Georgii, Y. Han, R. Hell, M. Wirtz, A. Imhof, C. Becker, J. Durner, C. Lindermayr, GSNOR contributes to demethylation and expression of transposable elements and stress-responsive genes, Antiox 10 (2021) 1128, https://doi.org/10.3390/antiox10071128.
S. Rümer, K.J. Gupta, W.M. Kaiser, Plant cells oxidize hydroxylamines to NO, J. Exp. Bot. 60 (2009) 2065–2072, https://doi.org/10.1093/jxb/erp077.
V. Safavi-Rizi, M. Herde, C. Stöhr, Identification of nitric oxide (NO)-responsive genes under hypoxia in tomato (*solanum lycopersicum* L.) root, Sci. Rep. 10 (2020) 16509, https://doi.org/10.1038/s41598-020-73613-z.
B. Saha, J. Nayak, R. Srivastava, L. Samal, D. Kumar, J. Chanwala, N. Dey, M. Giri, Unraveling the involvement of WRKY TFs in regulating plant disease defense signaling, Planta 259 (2024) 7, https://doi.org/10.1007/s00425-023-04269-y.
L. Sanz, P. Albertos, I. Mateos, I. Sánchez-Vicente, T. Lechón, M. Fernández-Marcos, O. Lorenzo, Nitric oxide (NO) and phytohormones crosstalk during early plant development, J. Exp. Bot. 66 (2015) 2857–2868, https://doi.org/10.1093/jxb/erv213.
S. Saini, P. Sharma, P. Singh, V. Kumar, P. Yadav, A. Sharma, Nitric oxide: an emerging warrior of plant physiology under abiotic stress, Nitric Oxide 140-141 (2023) 58–76, https://doi.org/10.1016/j.niox.2023.10.001.
A. Sakamoto, M. Ueda, H. Morikawa, Arabidopsis glutathione-dependent formaldehyde dehydrogenase is an S-nitrosoglutathione reductase, FEBS Lett. 515 (2002) 20–24, https://doi.org/10.1016/s0014-5793(02)02414-6.

C. Sallaud, C. Gay, P. Larmande, M. Bes, P. Piffanelli, B. Piegu, G. Droc, F. Regad, E. Bourgeois, D. Meynard, C. Périn, X. Sabau, A. Ghesquière, J.C. Glaszmann, M. Delseny, E. Guiderdoni, High throughput T-DNA insertion mutagenesis in rice: a first step towards in silico reverse genetics, Plant J. 39 (2004) 450–464, https://doi.org/10.1111/j.1365-313X.2004.02145.x.

A. Sanchez-Corrionero, I. Sánchez-Vicente, N. Arteaga, I. Manrique-Gil, S. Gómez-Jiménez, I. Torres-Quezada, P. Albertos, O. Lorenzo, Fine-tuned nitric oxide and hormone interface in plant root development and regeneration, J. Exp. Bot. 74 (2023) 6104–6118, https://doi.org/10.1093/jxb/erac508.

I. Sánchez-Vicente, P. Albertos, C. Sanz, B. Wybouw, B. De Rybel, J.C. Begara-Morales, M. Chaki, C. Mata-Pérez, J.B. Barroso, O. Lorenzo, Reversible S-nitrosylation of bZIP67 by peroxiredoxin IIE activity and nitro-fatty acids regulates the plant lipid profile, Cell Rep. 43 (2024) 114091, https://doi.org/10.1016/j.celrep.2024.114091.

K. Seligman, E.E. Saviani, H.C. Oliveira, C.A. Pinto-Maglio, I. Salgado, Floral transition and nitric oxide emission during flower development in *arabidopsis thaliana* is affected in nitrate reductase-deficient plants, Plant Cell Physiol. 49 (2008) 1112–1121, https://doi.org/10.1093/pcp/pcn089.

V. Serpa, J. Vernal, L. Lamattina, E. Grotewold, R. Cassia, H. Terenzi, Inhibition of AtMYB2 DNA-binding by nitric oxide involves cysteine S-nitrosylation, Biochem. Biophys. Res. Commun. 361 (2007) 1048–1053, https://doi.org/10.1016/j.bbrc.2007.07.133.

A. Sessions, E. Burke, G. Presting, G. Aux, J. McElver, D. Patton, B. Dietrich, P. Ho, J. Bacwaden, C. Ko, J.D. Clarke, D. Cotton, D. Bullis, J. Snell, T. Miguel, D. Hutchison, B. Kimmerly, T. Mitzel, F. Katagiri, J. Glazebrook, M. Law, S.A. Goff, A high-throughput arabidopsis reverse genetics system, Plant Cell 14 (2002) 2985–2994, https://doi.org/10.1105/tpc.004630.

P.K. Singh, Y. Indoliya, A.S. Chauhan, S.P. Singh, A.P. Singh, S. Dwivedi, R.D. Tripathi, D. Chakrabarty, Nitric oxide mediated transcriptional modulation enhances plant adaptive responses to arsenic stress, Sci. Rep. 7 (2017) 3592, https://doi.org/10.1038/s41598-017-03923-2.

R. Singh, P. Parihar, S.M. Prasad, Interplay of calcium and nitric oxide in improvement of growth and arsenic-induced toxicity in mustard seedlings, Sci. Rep. 10 (2020) 12065, https://doi.org/10.1038/s41598-020-69172-y.

M. Sørensen, E.H.J. Neilson, B.L. Møller, Oximes: unrecognized chameleons in general and specialized plant metabolism, Mol. Plant 11 (2018) 95–117, https://doi.org/10.1016/j.molp.2017.12.014.

J.S. Stamler, D.J. Singel, J. Loscalzo, Biochemistry of nitric oxide and its redox-activated forms, Sci 258 (1992) 1898–1902, https://doi.org/10.1126/science.1281928.

H. Sun, J. Li, W. Song, J. Tao, S. Huang, S. Chen, M. Hou, G. Xu, Y. Zhang, Nitric oxide generated by nitrate reductase increases nitrogen uptake capacity by inducing lateral root formation and inorganic nitrogen uptake under partial nitrate nutrition in rice, J. Exp. Bot. 66 (2015) 2449–2459, https://doi.org/10.1093/jxb/erv030.

M. Sun, X.L. Yang, Z.P. Zhu, Q.Y. Xu, K.X. Wu, Y.J. Kang, H. Wang, A.S. Xiong, Comparative transcriptome analysis provides insight into nitric oxide suppressing lignin accumulation of postharvest okra (*abelmoschus esculentus* L.) during cold storage, Plant Physiol. Biochem 167 (2021) 49–67, https://doi.org/10.1016/j.plaphy.2021.07.029.

L. Szabados, I. Kovács, A. Oberschall, E. Abrahám, I. Kerekes, L. Zsigmond, R. Nagy, M. Alvarado, I. Krasovskaja, M. Gál, A. Berente, G.P. Rédei, A.B. Haim, C. Koncz, Distribution of 1000 sequenced T-DNA tags in the arabidopsis genome, Plant J. 32 (2002) 233–242, https://doi.org/10.1046/j.1365-313x.2002.01417.x.

Y. Tada, S.H. Spoel, K. Pajerowska-Mukhtar, Z. Mou, J. Song, C. Wang, J. Zuo, X. Dong, Plant immunity requires conformational changes of NPR1 via S-nitrosylation and thioredoxins, Sci 321 (2008) 952–956, https://doi.org/10.1126/science.1156970.

C.P. Tavares, J. Vernal, R.A. Delena, L. Lamattina, C. Raul, H. Terenzi, S-nitrosylation influences the structure and DNA binding activity of AtMYB30 transcription factor from *arabidopsis thaliana*, Biochim. Biophys. Acta 1844 (2014) 810–817, https://doi.org/10.1016/j.bbapap.2014.02.015.

Š. Tomaž, K. Gruden, A. Coll, TGA transcription factors-Structural characteristics as basis for functional variability, Front. Plant Sci. 13 (2022) 935819, https://doi.org/10.3389/fpls.2022.935819.

M.A. Torres, J.D. Jones, J.L. Dangl, Reactive oxygen species signaling in response to pathogens, Plant Physiol. 141 (2006) 373–378, https://doi.org/10.1104/pp.106.079467.

X. Wang, M.S. Hargrove, Nitric oxide in plants: the roles of ascorbate and hemoglobin, PLoS One 8 (2013) e82611, https://doi.org/10.1371/journal.pone.0082611.

X. Wang, N. Li, W. Li, X. Gao, M. Cha, L. Qin, L. Liu, Advances in transcriptomics in the response to stress in plants, Glob. Med. Genet 7 (2020) 30–34, https://doi.org/10.1055/s-0040-1714414.

K.I. Wani, M. Naeem, C.D.M. Castroverde, H.M. Kalaji, M. Albaqami, T. Aftab, Molecular mechanisms of nitric oxide (NO) signaling and reactive oxygen species (ROS) homeostasis during abiotic stresses in plants, Int. J. Mol. Sci. 22 (2021) 9656, https://doi.org/10.3390/ijms22179656.

M.Y. Wei, H. Li, Y.H. Zhong, Z.J. Shen, D.N. Ma, C. Gao, Y.L. Liu, W. Wang, J. Zhang, Y. P. You, H.L. Zheng, Transcriptomic analyses reveal the effect of nitric oxide on the lateral root development and growth of mangrove plant *Kandelia obovata*, Plant Soil 472 (2022) 543–564, https://doi.org/10.1007/s11104-021-05271-7.

R. Wimalasekera, C. Villar, T. Begum, G.F. Scherer, COPPER AMINE OXIDASE1 (CuAO1) of *Arabidopsis thaliana* contributes to abscisic acid- and polyamine-induced nitric oxide biosynthesis and abscisic acid signal transduction, Mol. Plant 4 (2011) 663–678, https://doi.org/10.1093/mp/ssr023.

P. Wu, Q. Kong, J. Bian, G.J. Ahammed, H. Cui, W. Xu, Z. Yang, J. Cui, H. Liu, Unveiling molecular mechanisms of nitric oxide-induced low-temperature tolerance in cucumber by transcriptome profiling, Int. J. Mol. Sci. 23 (2022) 5615, https://doi.org/10.3390/ijms23105615.

C.J. Wurm, C. Lindermayr, Nitric oxide signaling in the plant nucleus: the function of nitric oxide in chromatin modulation and transcription, J. Exp. Bot. 72 (2021) 808–818, https://doi.org/10.1093/jxb/eraa404.

H. Xiong, H. He, Y. Chang, B. Miao, Z. Liu, Q. Wang, F. Dong, L. Xiong, Multiple roles of NAC transcription factors in plant development and stress responses, J. Int. Plant Biol. 67 (2025) 510–538, https://doi.org/10.1111/jipb.13854.

R. Yang, X. Lin, Y. Dou, W. Zhang, H. Du, C. Wan, J. Chen, L. Zhang, L. Zhu, Transcriptome profiling of postharvest kiwifruit in response to exogenous nitric oxide, Sci. Hortic. 277 (2021) 109788, https://doi.org/10.1016/j.scienta.2020.109788.

F. Zeng, F. Sun, L. Li, K. Liu, Y. Zhan, Genome-scale transcriptome analysis in response to nitric oxide in birch cells: implications of the triterpene biosynthetic pathway, PLoS One 9 (2014) e116157, https://doi.org/10.1371/journal.pone.0116157.

J. Zeng, X. Zhao, Z. Liang, I. Hidalgo, M. Gebert, P. Fan, C. Wenzl, S.G. Gornik, J. U. Lohmann, Nitric oxide controls shoot meristem activity via regulation of DNA methylation, Nat. Comm. 14 (2023) 8001, https://doi.org/10.1038/s41467-023-43705-1.

Y. Zhang, W. Fan, M. Kinkema, X. Li, X. Dong, Interaction of NPR1 with basic leucine zipper protein transcription factors that bind sequences required for salicylic acid induction of the PR-1 gene, Proc. Natl. Acad. Sci. USA 96 (1999) 6523–6528, https://doi.org/10.1073/pnas.96.11.6523.

J. Zhang, H. Fang, J. Huo, D. Huang, B. Wang, W. Liao, Involvement of calcium and calmodulin in nitric oxide-regulated senescence of cut lily flowers, Fron. Plant Sci. 9 (2018) 1284, https://doi.org/10.3389/fpls.2018.01284.

Y. Zhang, R. Wang, X. Wang, C. Zhao, H. Shen, L. Yang, Nitric oxide regulates seed germination by integrating multiple signalling pathways, Int. J. Mol. Sci. 24 (2023) 9052, https://doi.org/10.3390/ijms24109052.

H. Zhao, L. Ma, J. Shen, H. Zhou, Y. Zheng, *s*-nitrosylation of the transcription factor MYB30 facilitates nitric oxide–promoted seed germination in arabidopsis, Plant Cell 36 (2024) 367–382, https://doi.org/10.1093/plcell/koad276.

Y. Zheng, J. Xiao, K. Zheng, J. Ma, M. He, J. Li, M. Li, Transcriptome profiling reveals the effects of nitric oxide on the growth and physiological characteristics of watermelon under aluminum stress, Genes 12 (2021) 1735, https://doi.org/10.3390/genes12111735.