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REVIEW

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NITRIC OXIDE IN PLANTS UNDER ABIOTIC STRESS: INVOLVEMENT IN SIGNALING NETWORKS

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Background. *Nitric oxide (NO) is recognized as one of the most vital signaling molecules in plants, involved in the control of most physiological processes under normal and, especially, stress conditions.* ***Objective.*** *This review provides data on the primary and recently discovered minor pathways of nitric oxide synthesis in plants, NO-induced protein post-translational modifications, NO involvement in a general signaling network, interaction with individual nucleotides, stress-protective action of different NO donors and metabolic precursors of nitric oxide, along with the potential applications of these compounds in phytobiotechnologies.* ***Conclusions.*** *Despite numerous unresolved questions regarding nitric oxide synthesis and signaling in plants, current evidence highlights its central role in plant adaptation and stress responses, as well as the considerable potential of NO donors and related compounds for phytobiotechnological applications.*

Keywords: nitric oxide, signaling, post-translational modification of proteins, calcium, reactive oxygen species, hydrogen sulfide, plant adaptation.

According to the Food and Agriculture Organization (FAO), the global population is projected to reach 9.0 billion by 2050, necessitating a 60% increase in food production [1]. However, this task is substantially complicated by deteriorating environmental conditions [2]. Predictive models suggest that the risk of extreme weather events, historically occurring once a century, is likely to rise to 1-in-30 by 2040 [3]. Furthermore, by the end of this century, the average global temperature could rise by 1.8–4.0°C compared to the beginning of the century [4]. Such shifts may lead to a significant decline in crop yields; for instance, maize productivity is forecasted to decrease by 24% by 2100 [2]. Concurrently, salinized areas are expected to account for approximately half of the total global agricultural land by 2050 [5].

Two primary strategies are proposed to mitigate the adverse effects of abiotic stressors. The first strategy involves developing resilient cultivars through conventional and molecular breeding, genetic engineering, and/or gene-editing approaches [6]. However, researchers increasingly note that breeding methods are inherently time-consuming and labor-intensive, often failing to keep pace with rapid climate change [7]. The second strategy relies on climate-smart agricultural technologies aimed at harnessing the full adaptive potential of existing cultivars and hybrids. A key component of this approach is the application of next-generation physiologically active substances, combined with advanced delivery methods for seeds and vegetating plants [7]. Recent studies have demonstrated that treatment with such compounds enables temporal and spatial regulation of the transcriptome and cellular metabolome, facilitating targeted responses essential for plant adaptation to environmental challenges [2, 8].

The last two decades have seen the emergence of concepts regarding the role of gasotransmitters – endogenous gaseous compounds that perform signaling functions – in plant functioning [9-11]. Among gasotransmitters, nitric oxide (NO) is the most extensively studied, with its signaling functions in plants being under investigation since the late 20th century [12, 13]. Currently, nitric oxide is considered a key participant in plant cell signaling networks, possessing vast regulatory potential. This NO potential is realized in close crosstalk with other signaling mediators, such as calcium ions, reactive oxygen species (ROS), and hydrogen sulfide, which also belong to the gasotransmitter family [14-16].

Nitric oxide (NO) is recognized as one of the most vital signaling molecules in plants, controlling a wide spectrum of processes such as seed germination, anthesis, senescence, and, notably, the development of resilience to abiotic and biotic stressors [11]. A vast body of phenomenological evidence regarding NO participation in regulating plant stress-protective reactions has been accumulated and is now becoming the subject of meta-analyses [18, 19]. In plants, NO is considered a critically important signaling molecule that activates antioxidant enzymes and/or their gene expression in response to abiotic stresses, including extreme temperatures, drought, salinity, and heavy metals [11]. Furthermore, nitric oxide is involved in regulating osmolyte accumulation, stress protein synthesis, stomatal movements, and programmed cell death [18, 20, 21].

In recent years, the focus of NO research has shifted from the phenomenology of stress-protective effects toward identifying specific targets undergoing PTMs by nitric oxide and elucidating the roles of these proteins in adaptation [22, 23]. Moreover, evidence is mounting regarding the functional interaction of NO not only with other signaling mediators but also with stress metabolites [24, 25]. Concurrently, the range of compounds used in crop production as NO donors is expanding, and their application methods are becoming more advanced; notably, nitric oxide is increasingly being administered via nanoparticle-based delivery systems [26, 27]. However, these trends in studying the stress-protective action of nitric oxide have not yet been sufficiently analyzed or synthesized, which justifies the necessity of this review.

Synthesis and metabolism of nitric oxide in plants

Two primary pathways facilitate NO synthesis in plants: an oxidative pathway, involving the oxidation of amino-containing molecules, and a reductive pathway, based on the reduction of nitrite to NO.

While L-arginine-dependent NO synthesis via nitric oxide synthase (NOS) is well-characterized at molecular and genetic levels in animals and humans, the specific enzymatic systems driving the oxidative pathway in higher plants remain elusive [28, 29]. A large-scale bioinformatic analysis of 1,300 higher plant genomes failed to identify a single NOS homolog [30]. Although a functional NOS has been characterized in the microalga *Ostreococcus tauri* [31], the lack of evidence in higher plants suggests that terrestrial species may have lost this enzyme during evolution.

Nevertheless, enzymatic oxidation of L-arginine has been observed in peroxisomes and chloroplasts of both green algae and vascular plants, yielding citrulline and NO [32]. This activity is termed “NOS-like” as it requires arginine, NADPH, FMN, FAD, CaM, and BH4, mirroring the animal [28, 33]. It is hypothesized that higher plants possess polypeptides with redox-active domains that assemble into complexes to catalyze arginine-dependent NO formation [34]. In addition to arginine conversion, the oxidative pathway in plants may involve the conversions of hydroxylamine and polyamines, which are catalyzed by Cu-amine oxidase (DAO) and polyamine oxidase (PAO) [35] (Fig. 1). The role of PAO in NO synthesis is primarily supported by inhibitor analysis. For instance, in soybean, exogenous polyamine-stimulated NO formation was abolished by a DAO inhibitor (aminoguanidine) [36]. Similarly, putrescine-induced NO generation in wheat roots was significantly suppressed by aminoguanidine [37]. Nevertheless, the biochemical possibility of NO formation from polyamines, as they are oxidized by amine oxidases, remains contentious, as the typical end-product in such reactions is rather ammonia than NO [38]. Consequently, unlike in the animal kingdom, the precise mechanisms of the oxidative pathway of NO formation (from arginine or polyamines) in plants remain a major open question in plant physiology.

In contrast, the reductive pathway is a well-characterized and established route for nitric oxide synthesis in plants. A central component of this pathway is nitrate reductase, a multifunctional cytoplas-

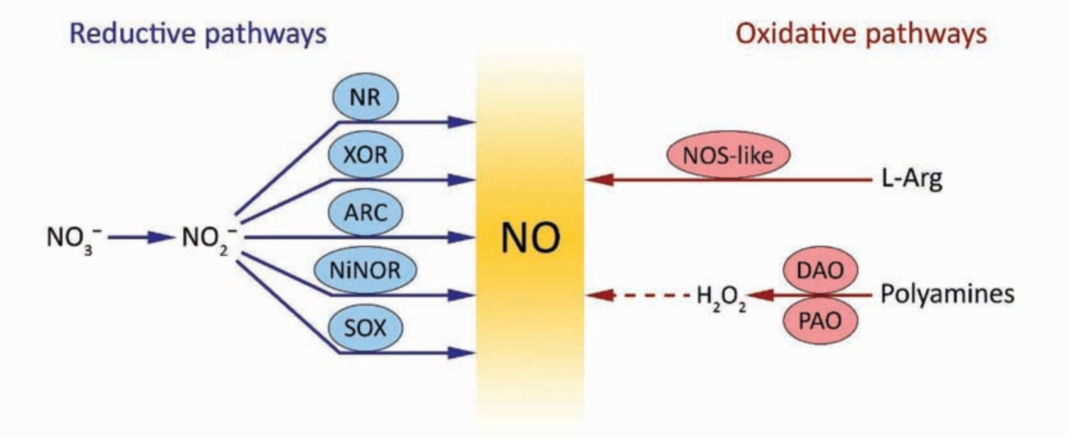

Fig. 1. Synthesis of NO in plants. NR – nitrate reductase; XOR – xanthine oxidoreductase; ARC – amidoxime reducing compound (nitric oxide-forming nitrite reductase); NiNOR – nitrite: NO reductase; SOX – sulfite oxidase; NOS-like – nitric oxide synthase-like enzyme; DAO – copper-containing diamine oxidase; PAO – flavin-containing polyamine oxidase. Other explanations are in the text

mic enzyme essential for nitrogen assimilation and metabolism. Nitrate reductase catalyzes the reduction of nitrate to nitrite using NADH or NADPH as electron donors, representing the rate-limiting step in nitrate assimilation. This active homodimeric complex requires molybdopterin, heme, and FAD as cofactors [39]. Nitrite-dependent NO generation proceeds through the subsequent reduction of nitrite, catalyzed either by nitrate reductase itself or via the mitochondrial electron transport chain. The latter involves the mitochondrial amidoxime-reducing component (mARC acting as a nitric oxide-forming nitrite reductase), molybdenum cofactor-dependent enzymes, xanthine oxidoreductase, and nitrite-dependent nitric oxide reductase [40, 41] (Fig. 1).

Recent evidence suggests that the nitrate reductase-dependent pathway is the primary source of NO across various plant tissues [42]. In higher plants, this enzyme exists in two isoforms: NIA1 and NIA2. The Arabidopsis double mutant *nia1nia2* exhibits a near-total loss of functional nitrate reductase activity, leading to severe impairment of NO synthesis [43]. Conversely, Costa-Broseta et al. [44] demonstrated that overexpression of *NIA1* or *NIA2* (to a lesser degree) significantly elevates NO levels, confirming the pivotal role of these genes in NO homeostasis in Arabidopsis.

Furthermore, the involvement of nitrate reductase in plant adaptation to specific stressors was reported. For instance, cold acclimation in tomato was accompanied by nitrate reductase induction and a concomitant increase in nitrate reductase-dependent NO formation. Suppression of the nitrate reductase gene was shown to inhibit the activation of one of mitogen-activated protein kinases (MAPs) – MPK1/2, thereby compromising the development of cold tolerance. Additionally, nitrate reductase activity was found to enhance the expression of a C-repeat binding factor (CBF) transcription factor genes, which are master regulators of the cold stress response [45].

Recent data point to the potential involvement of another enzyme in nitric oxide synthesis: sulfite oxidase (SOX). SOX is a peroxisomal enzyme that, utilizing a molybdenum cofactor (MoCo), catalyzes the oxidation of sulfite (SO_3^{2-}) to sulfate (SO_4^{2-}) with the simultaneous generation of H_2O_2 (Fig. 1). Using reconstructed recombinant SOX from pepper (*Capsicum annuum* L.) fruits, it was demonstrated that this enzyme can generate •NO by utilizing nitrite (NO_2^-) as a substrate and NADH as an electron donor [46].

Intracellular NO content is determined by the balance between its synthesis, metabolism, and utilization. Reduced glutathione (GSH) reacts with NO to form S-nitrosoglutathione (GSNO), which serves as a stable reservoir for NO, facilitating protein S-nitrosation and maintaining intracellular NO concentrations [47]. GSNO is subsequently metabolized into oxidized glutathione disulfide and ammonia by S-nitrosoglutathione reductase (GSNOR). Interestingly, GSNOR itself is a target for S-nitrosation [48]. GSNOR-mediated control of NO homeostasis is fundamental to a wide range of physiological processes, including responses to abiotic stressors. Through the fine-tuning of intracellular NO and S-nitrosothiol

(SNO) levels, this enzyme prevents nitrosative stress while preserving the cell's capacity for NO signal transduction [49]. In addition to GSNO conversion, the pool of free nitric oxide in plants can be regulated through its oxidation to nitrate, a process mediated by hemoglobins [50].

Another key mechanism regulating NO levels involves the sequestration of nitric oxide by melatonin to form N-nitrosomelatonin (NOmela). This reaction occurs in aerobic environments at physiological pH [51] and is considered a defense strategy against nitrosative stress caused by the accumulation of reactive nitrogen species (RNS) [52]. Notably, both nitrosothiols and NOmela can act as NO donors for PTMs of proteins [53]. *In vitro* studies demonstrated the efficacy of NOmela in the transnitrosylation of cysteine residues in proteins [52].

Post-translational modifications of proteins under the influence of nitric oxide and its interaction with nucleotides

The most prevalent and significant mode of protein PTMs by nitric oxide is the selective S-nitrosation of cysteine residues (Fig. 2). S-nitrosation modulates the functional activity of hundreds of proteins, thereby governing numerous cellular processes, including the activation of stress-protective systems [23]. Conceptually, S-nitrosation is often compared to phosphorylation – the most extensively studied post-translational modification [54]. However, unlike phosphorylation, S-nitrosation of proteins typically occurs non-enzymatically [55]. The unique properties of the sulfur atom within cysteine sulfhydryl groups are central to the signaling effects associated with S-nitrosation [56]. This PTM is also highly selective, determined by both the amino acid sequence and the spatial conformation of the target protein. *In vitro* studies identified several mechanisms for (SNO formation. Nitric oxide, as a free radical ($NO^•$), can undergo electron loss or gain to form the oxidized nitrosonium cation (NO^+) or the reduced nitroxyl anion ($NO^–$). Furthermore, NO can react with superoxide radicals ($O_2^{•–}$) and molecular oxygen (O_2) to generate peroxynitrite ($ONOO^–$) and nitrogen oxides (N_{2O3}/NO_x), which serve as S-nitrosating agents [56]. Additionally, NO radicals can react directly with thiyl radicals ($RS^•$), metal–NO complexes, or protein thiols to yield SNO.

The S-nitrosation of numerous proteins has been documented across various plant species under diverse stress conditions [22]. For instance, a nitrosoproteomic analysis of tomato plants under alkaline stress (sodic-alkalinity) revealed 334 S-nitrosated proteins involved in regulating calcium homeostasis, NO and ROS levels, ethylene signaling, and the MAPK cascade [57]. Similarly, studies on various crops showed that cold stress triggered the S-nitrosation of over 240 proteins, primarily associated with stress signaling, redox homeostasis (e.g., SOD, glutathione S-transferase, cysteine synthase, and dehydroascorbate reductase), photosynthesis, and cell wall modifications [58] (Table 1).

S-nitrosation is a critical component not only in the regulation of systems mediating plant adaptation to stressors but also in hormone-induced signaling pathways. For instance, the S-nitrosation of the GTPase ROP2 reduces auxin transport rates, thereby inhibiting root growth in Arabidopsis [78]. Furthermore, the positive effect of nitric oxide on seed germination may be partially attributed to the

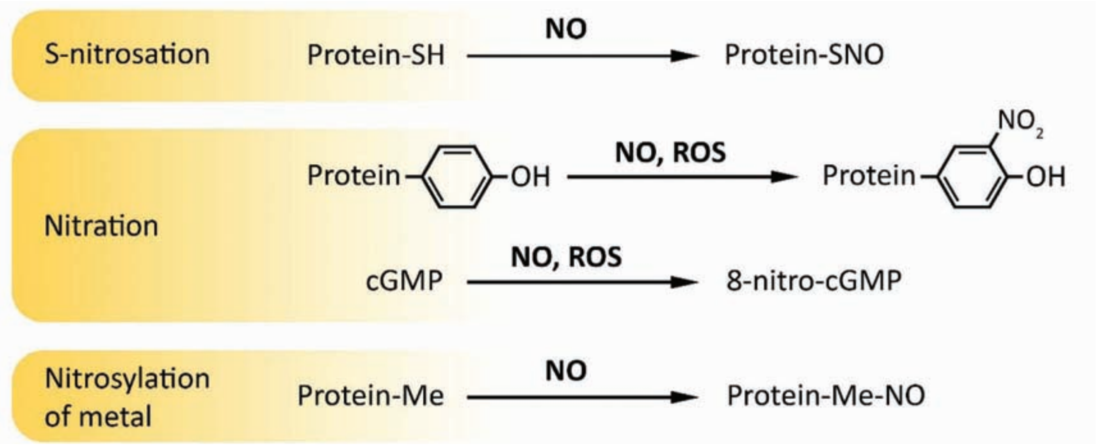

Fig. 2. Nitric oxide-mediated reactions of S-nitrosation, nitration and nitrosylation of metal

NO-induced S-nitrosation of the key ABA-signaling protein kinase, SnRK2.6, at the Cys-137 residue (Table 1). This modification diminishes SnRK2.6 kinase activity, which in turn inhibits ABA signaling and promotes germination [76]. Numerous other examples of proteins involved in phytohormone transport and signaling that are regulated via S-nitrosation are detailed in a recent review by Lin et al. [79]. They hypothesized that such protein modifications occurred not only through direct interaction between NO and thiol groups but also via transnitrosylation reactions – the transfer of nitroso groups from one protein to another [79].

Another (less studied) NO-caused PTM is the nitrosylation of metals (Fig. 2). NO is capable of interacting with transition metal ions, such as iron and copper, in metalloproteins, including phytohemoglobin, catalase, and cytochrome oxidase [22] (Fig. 2). Formation of metal-nitrosyl complexes causes reversible conformational changes in proteins, altering their structures and/or functional activities. However, the physiological roles of these processes remain poorly understood [55].

In addition to S-nitrosation and nitrosylation of metals, which is considered the most significant NO-mediated PTM, nitric oxide ensures the nitration of tyrosine residues in proteins (Fig. 2). This selective process involves the addition of a nitro group (–NO_2) to one of the two equivalent *ortho*-carbons in the aromatic ring of tyrosine residues, resulting in the formation of 3-nitrotyrosine [80]. Traditionally, tyrosine nitration has been viewed as an irreversible modification and a hallmark of nitrosative stress [81]. However, the discovery of tyrosine denitrase – an enzyme that reduces 3-nitrotyrosine in mammalian cells – suggests that tyrosine nitra-

Table 1. Examples of post-translational modification of enzymatic proteins by nitric oxide and hydrogen sulfide

Proteins	Type of PTM and its impact on activity	Effects	Reference
RBOH	S-nitrosation (Cys890)	Inhibition	[59]
	Persulfidation (Cys825 and Cys890)	Activation	[60]
Cu/Zn-SOD	Tyrosine nitration (Tyr63)	Inhibition	[61]
Mn-SOD	Tyrosine nitration (Tyr63)	Inhibition	[62]
Fe-SOD	S-nitrosation	Activation	[63]
	Tyrosine nitration (Tyr63)	Inhibition	[62]
Catalase	S-nitrosation (Cys377)	Inhibition	[64]
	Tyrosine nitration (Tyr348 and Tyr360)	Inhibition	[65]
	Heme nitrosylation	Inhibition	[66]
	Persulfidation	Inhibition	[67]
Ascorbate peroxidase	S-nitrosation (Cys32)	Activation	[68]
	Tyrosine nitration (Tyr5 and Tyr23)	Inhibition	[69]
	Heme nitrosylation	Inhibition	[66]
	Persulfidation (Cys32 or Cys223)	Activation	[70-72]
Monodehydroascorbate reductase	S-nitrosation	Inhibition	[73]
	Tyrosine nitration (Tyr345)	Inhibition	[73]
	Persulfidation (Cys 25)	Activation	[72]
Dehydroascorbate reductase	Persulfidation (Cys 20)	Activation	[72]
Glyceraldehyde-3-phosphate dehydrogenase	S-nitrosation	Inhibition	[74]
	Tyrosine nitration	Inhibition	[75]
	Persulfidation	Activation	[70]
Snf1-related protein kinase 2.6 (SnRK2.6)	S-nitrosation (Cys 137)	Inhibition	[76]
	Persulfidation (Cys131 and Cys137)	Activation	[77]

tion may also play a regulatory role in NO-mediated signaling. Although a specific denitrase has not yet been identified in plants [80], several findings point to the biological significance of tyrosine nitration. For instance, the nitration of tyrosine residues in α-tubulin, a key component of the plant cytoskeleton, was established as a mechanism that may regulate the dynamic properties of microtubules, thereby influencing plant cell growth and division [82].

It should be noted that nitric oxide undergoes nitration reactions not only with protein tyrosine residues but also with nucleotides (Fig. 2). In animal cells, the formation of 8-nitroguanosine 3′,5′-cyclic monophosphate (8-nitro-cGMP) was detected. This molecule, characterized by potent redox and electrophilic activity, is considered a signaling molecule [83]. The electrophilic properties of 8-nitro-cGMP enable it to irreversibly modify protein thiols through a novel post-translational modification termed S-guanylation [84]. Recently, protein S-guanylation in animal cells has been viewed not only as a possible manifestation of nitrosative stress but also as a signaling-related process [85]. Information regarding the role of nitrated nucleotides in plant cell signaling remains very limited [84]. However, experimental evidence for the involvement of 8-nitro-cGMP in regulating stomatal aperture in Arabidopsis thaliana highlights the signaling potential of this molecule. Joudoi et al. [86] demonstrated that 8-nitro-cGMP induced stomatal closure under light, whereas cGMP itself did not exhibit this effect. The signaling action of 8-nitro-cGMP is mediated by the modulation of calcium channels, leading to the activation of SLOW ANION CHANNEL 1 (SLAC1) and subsequent ion efflux from guard cells, thereby promoting stomatal closure [87]. Additionally, 8-SH-cGMP, a derivative of 8-nitro-cGMP, also possesses the ability to modulate stomatal movements.

It should be noted that the mechanisms underlying the synthesis of 8-nitro-cGMP in plants remain unexplored. In animal cells, it is known that formation of 8-nitro-cGMP in vivo does not occur via nitration of cGMP. Instead, 8-nitro-cGMP is synthesized by nitration of GTP, followed by guanylate cyclase acting on 8-nitro-GTP to form 8-nitro-cGMP [88]. Although no specific studies have been conducted on the mechanisms of 8-nitro-cGMP synthesis, experimental data indicate its role in physiological processes in plants. Thus, in addition to its aforementioned involvement in stomatal closure, 8-nitro-cGMP can interact with SH groups in proteins, causing their S-guanylation and thereby influencing autophagy processes [89]. Overall, the issue of NO interacting with nucleotides and nucleic acids in plant cells is a relatively new topic that goes beyond the scope of this review. Recent trends in this field are described in two recently published reviews [88, 89].

Interaction of nitric oxide with key cellular signaling mediators and its role in plant adaptive responses

Calcium. Cytosolic calcium, recognized as one of the most vital intracellular messengers, serves as a nexus for numerous signaling pathways, ensuring the integration of the plant cell signaling network [90]. The involvement of calcium in stress signal transduction stems from its ability to interact differentially with various cellular proteins. The diversity of calcium sensors in plants and the interconnections between multiple signaling routes form intricate networks stimulated to ensure plant survival [91]. Calmodulin is one of the most significant calcium receptors [92, 93]. Proteins regulated by calmodulin include various protein kinases, some transcription factors, ion channel proteins, cytoskeletal components, chaperones, metabolic enzymes, and proteins involved in phytohormone signaling [93, 94]. Extensive experimental evidence indicates that calcium participates in both enhancing nitric oxide synthesis and transducing its downstream signals [10].

It is hypothesized that upon perception of various stress signals, the elevation of cytosolic calcium concentration may be an earlier event compared to the induction of NO synthesis, which subsequently acts in concert with calcium to foster adaptive responses (Fig. 3). For instance, cytosolic Ca^{2+} levels rise within seconds of exposure to salt stress [5]. In the ensuing events involving calcium as a signaling mediator, nitric oxide may play a crucial role (Fig. 3). For example, the alleviation of cadmium-induced growth inhibition in *Vigna radiata* by exogenous calcium was accompanied by the induction of endogenous NO synthesis [95]. Furthermore, treatment of wheat seedlings with various calcium antagonists – including an extracellular calcium chelator (EGTA), a non-specific calcium channel blocker (lanthanum chloride), and an inhibitor of phospholipase C (neomycin – abolished the increase in NO content in roots typically triggered by short-term heat acclimation and prevented the development of thermotolerance [96].

It is well-established that animal nitric oxide synthase (NOS) is activated by calcium/calmodulin [97, 98]. However, molecular and genetic evidence for the presence of typical animal-like NOS in higher plants remains elusive. Nevertheless, it is hypothesized that higher plant peroxisomes contain proteins distinct from NOS that generate NO using L-arginine as a substrate. Similar to the reaction catalyzed by NOS, this process requires calmodulin and calcium ions [99]. For instance, the joint application of exogenous arginine (a substrate for the oxidative pathway) and calcium synergistically enhanced the growth of the microalga *Haematococcus pluvialis* and stimulated astaxanthin synthesis [100].

Exogenous calcium treatment was also shown to alleviate oxidative stress, chlorophyll loss, and growth inhibition induced by arsenic toxicity in mustard seedlings [101]. This mitigation was accompanied by a calcium-triggered increase in leaf NO content, while the stress-protective effects of calcium were abolished by NO antagonists 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO) and N-nitro-L-arginine methyl ester (L-NAME).

The reductive pathway of NO synthesis in plants, primarily associated with nitrate reductase activity, also appears to be calcium-dependent. The activation of plant nitrate reductase by calcium ions and its inhibition by calcium chelators were demonstrated *in vitro* some time ago [102], and increased nitrate reductase activity in intact plants under exogenous calcium influence was also documented [103].

Thus, nitric oxide is intrinsically involved in mediating the stress-protective effects of calcium. At the same time, many effects of NO are likely to be mediated by changes in the calcium channel function and modulation of cellular calcium homeostasis. A critical aspect of signaling network integration is the S-nitrosation of calcium channel proteins, which enhances calcium influx into the cytosol [104]. However, the functional interaction between calcium and NO can be more complex. For example, a “calcium–nitric oxide loop” was recently identified as an important factor in the cold adaptation of watermelon (*Citrullus lanatus*) [105]. According to the authors, calcium promotes NO generation, which in turn triggers further cytosolic Ca^{2+} influx by upregulating the expression of calcium-permeable cyclic nucleotide-gated ion channel (ClCNGC) genes. Knockout of the nitrate reductase 1 gene (*ClNR1*), a key enzyme for

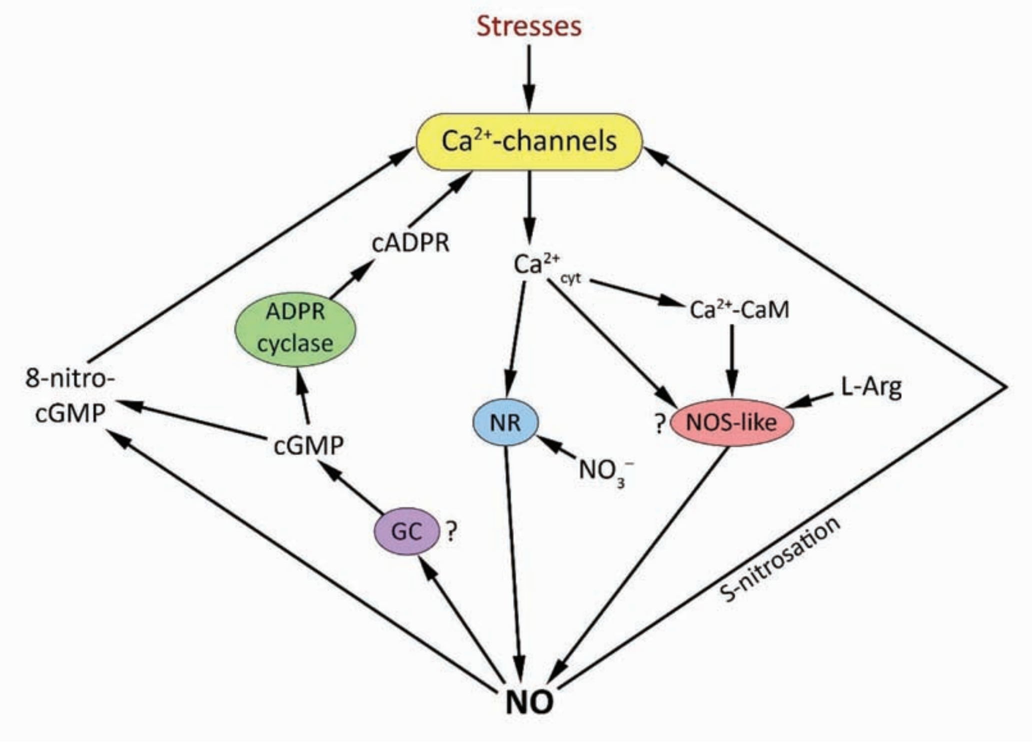

Fig. 3. Interaction between nitric oxide and calcium signaling. Ca^{2+}-CaM – calcium calmodulin; NR – nitrate reductase; NOS-like – nitric oxide synthase-like enzyme; GC – guanylate cyclase; cADPR – cyclic ADPribose; ADPR cyclase – ADPribosyl cyclase. Other explanations in the text

NO synthesis, led to reduced *ClCNGC* gene expression and compromised cold tolerance of watermelon plants [105]. This suggests that the influence of nitric oxide on calcium channel functioning may be linked to modulated expression of the corresponding channel proteins.

Furthermore, nitric oxide can be integrated into signaling cascades as a secondary messenger functionally linked to calcium through cyclic guanosine 5′-monophosphate (cGMP), cyclic ADP-ribose (cADPR), and potentially other mediators [20] (Fig. 3). Despite the lack of definitive molecular and genetic evidence for a canonical cGMP-dependent signaling pathway in higher plants [106] – such as the one characterized in green algae [107] – bioinformatic analyses suggested the existence of a nitric oxide-binding guanylyl cyclase 1 (NOGC1) in *A. thaliana*. Experimental data also indicate that the recombinant NOGC1 protein is capable of synthesizing cGMP in an NO-dependent manner, albeit in minimal quantities [108]. Finally, as previously noted, the status of calcium channels in stomatal guard cells can be modulated by a cGMP derivative – 8-nitro-cGMP, which is formed via the nitration of cGMP by nitric oxide [87] (Fig. 3). The prevalence of this compound and the extent of its contribution to calcium homeostasis regulation in plant cells remain largely unexplored.

Reactive oxygen species. ROS encompass partially reduced or excited forms of molecular oxygen, as well as oxygen-containing reaction products characterized by higher reactivity than atmospheric O_2 [109]. These include free radical species, such as the superoxide anion radical ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), and hydroperoxyl radical ($HO_2^{\bullet}$), as well as non-radical neutral molecules, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) [110]. Most ROS exhibit exceptionally short half-lives, ranging from nanoseconds for radical species to seconds or even several minutes for the more stable hydrogen peroxide. It is hydrogen peroxide that is considered the signaling mediator with the most extensive signaling capabilities among all ROS [111]. H_2O_2 is a molecule in close functional association with nitric oxide. Numerous findings indicate that NO is generated in plants under stress conditions with kinetics similar to those of hydrogen peroxide formation [5]. However, investigations have yet to provide a definitive answer regarding the hierarchical positioning of these two mediators in the signaling cascade. Consequently, it can be inferred that there is a tight, reciprocal functional interaction between nitric oxide and hydrogen peroxide as signaling mediators (Fig. 4).

Recently, it was discovered that the enhancement of tomato resistance to Cr(VI) via exogenous hydrogen peroxide treatment was mediated by nitric oxide synthesis. The protective effect of H_2O_2 was abolished by the addition of an NO scavenger (2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO)) and the NOS inhibitor L-NAME. These findings suggest that H_2O_2-induced plant resistance to chromium develops through NO-dependent signaling pathways [112]. Conversely, the application of ROS antagonists, such as diphenyleneiodonium and N-acetyl-L-cysteine, had only a minor impact on the stress-protective effects of exogenous NO.

It is currently understood that interactions between nitric oxide and hydrogen peroxide can be both synergistic and antagonistic [113, 114]. Current knowledge enables us to partially explain these phenomena.

There are data that ROS – particularly hydrogen peroxide – can stimulate NO synthesis in plants. This effect may be achieved through the H_2O_2-mediated modulation of phosphorylation at specific nitrate reductase sites. Arabidopsis mutants with impaired H_2O_2 synthesis, such as those with mutations of NADPH oxidase isoforms (*atrbohB, atrbohD*, *atrbohB/D*, and *RBOH1*), exhibit significantly reduced NO levels. This underscores the positive regulatory role of hydrogen peroxide in nitrate reductase-mediated NO biosynthesis [42]. Hydrogen peroxide was shown to oxidize a methionine residue (Met538) located on the protein surface adjacent to Ser534 in the hinge region 1 of nitrate reductase. This oxidation prevents the phosphorylation of Ser534, thereby disrupting the binding between nitrate reductase and its inhibitory 14-3-3 protein, ultimately leading to the enzyme activation [42].

NO and ROS may compete for identical molecular targets, primarily the thiol groups of proteins. The enzymes responsible for generating or scavenging ROS and NO can themselves be targets of PTMs [115]. For example, nitric oxide is known to induce the S-nitrosation of the NADPH oxidase subunit at Cys890, which inactivates the enzyme and inhibits ROS generation [59].

There is also evidence suggesting that nitric oxide can also enhance NADPH oxidase-dependent ROS generation [116, 117]. These effects may be attributed to the involvement of other signaling media-

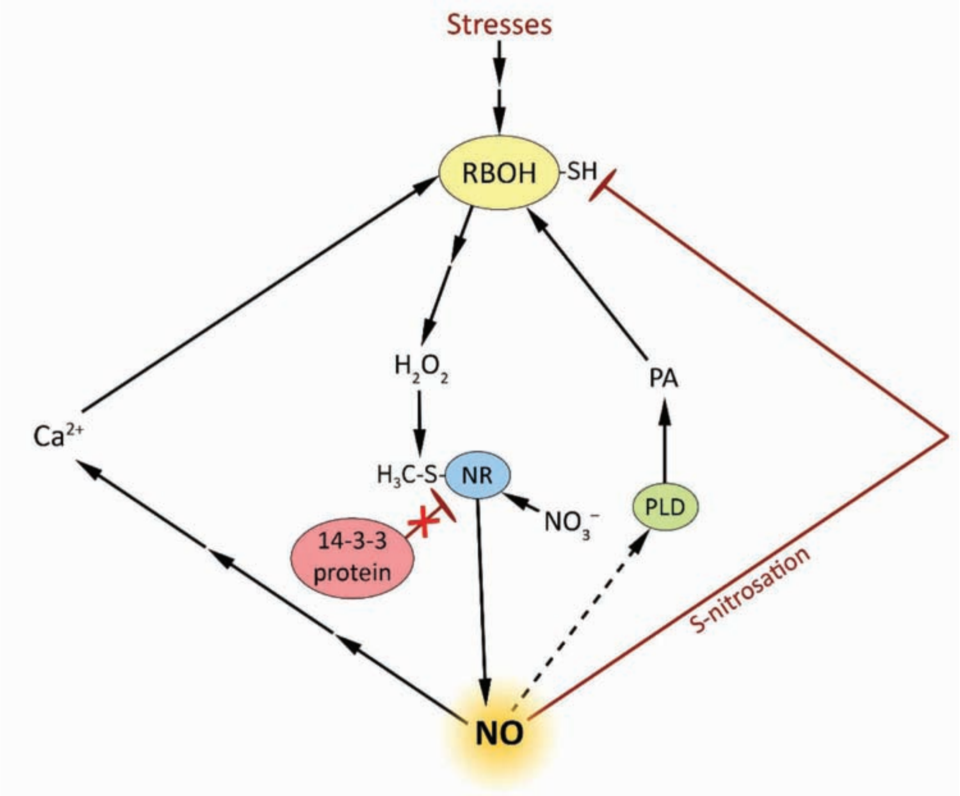

Fig. 4. Interaction between nitric oxide and ROS signaling. NR – nitrate reductase; RBOH – HADPH-oxidase; PLD – phospholipase D; PA – phosphatidic acid. Other explanations in the text

tors, particularly calcium. NADPH oxidase is a calcium-dependent enzyme [118] that can be activated either through the direct interaction of Ca^{2+} with its catalytic subunit [119] or via phosphorylation by calcium-dependent protein kinase [120]. By triggering calcium influx into the cytosol through the various mechanisms discussed above, nitric oxide can thus exert a positive regulatory influence on NADPH oxidase activity (Fig. 3). Another mechanism by which nitric oxide modulates NADPH oxidase activity may be mediated by phosphatidic acid. Nitric oxide was shown to stimulate phosphatidic acid formation by activating phospholipase D [121]. For instance, the increase in ROS generation in wheat coleoptiles induced by a nitric oxide donor was abolished by pretreatment with 1-butanol (an antagonist of phospholipase D-dependent phosphatidic acid formation), whereas its inactive counterpart, 2-butanol, had no such effect [122]. Consequently, nitric oxide can exert both direct and indirect regulatory effects on NADPH oxidase activity. This influence appears to be highly dynamic, shifting according to the presence and concentrations of other signaling molecules and ions within the signaling network.

The functional interaction between nitric oxide and hydrogen peroxide is further evidenced by studies investigating their individual and combined exogenous effects on plant resilience. For instance, the combined treatment of *Ocimum basilicum* L. plants with an NO donor (sodium nitroprusside (SNP)) and H_2O_2 exerted a more pronounced positive effect on salt tolerance than either compound applied alone. This synergy was manifested in a significantly greater enhancement of antioxidant enzyme activities and anthocyanin content in stressed [123].

Hydrogen sulfide. Similar to nitric oxide, hydrogen sulfide is recognized as a key gasotransmitter in both plant and animal cells [11, 124]. It has become evident that H_2S plays a role in nearly all physiological processes in plants, particularly in the development of responses to abiotic and biotic stresses [125]. Hydrogen sulfide is recognized as being as vital as other key signaling molecules, such as nitric oxide and hydrogen peroxide [126]. The primary mechanisms of action for the two leading gasotransmitters, NO and H_2S, are remarkably similar: both molecules modulate protein function through PTMs – specifically S-nitrosation and persulfidation of cysteine residues. They interact either synergistically or competitively within shared signaling networks [125, 127]. Furthermore, hydrogen sulfide, like nitric oxide, interacts with ROS, calcium, and phytohormones. The interaction between hydrogen sulfide and the same target proteins that undergo NO-mediated PTM appears to be a critical factor in physiological regulation [127] (Table 1). Specifically, the persulfidation of antioxidant enzymes may be a mechanism by which H_2S modulates the antioxidant system. In this context, H_2S and NO can act in conjunction, either synergistically or antagonistically [127]. For instance, ascorbate peroxidase and monodehydroascorbate reductase are activated by persulfidation but inhibited by S-nitrosation and tyrosine nitration [71, 73] (Table 1). Catalase is inhibited by modifications induced by both hydrogen sulfide and nitric oxide [64, 65, 70]. Redox homeostasis may also be influenced by PTMs of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a key enzyme for maintaining the NADPH pool. GAPDH is inhibited by tyrosine nitration [75] and S-nitrosation [57] but is activated by persulfidation [70] (Table 1). Persulfidation also promotes the nuclear localization of this enzyme [127]. The effects of NO- and H_2S-caused PTMs on NADPH oxidase (RBOHD) activity are also opposing: S-nitrosation leads to the inhibition of an RBOHD isoform, whereas persulfidation results in its activation [127]. Hence, the direct modifications of enzymes involved in ROS generation, scavenging, and the maintenance of the reductant pool through the combined action of H2S and NO are essential for the regulation of oxidative stress and redox homeostasis (Table 1).

Furthermore, modulations by hydrogen sulfide and nitric oxide target the key enzymes responsible for levels of these gasotransmitters. For instance, nitrate reductase, a major source of NO generation, can be inhibited by two PTMs induced by NO itself – tyrosine nitration and S-nitrosation – as well as by H_2S through persulfidation [127] (Table 1).

The functional interaction between nitric oxide and hydrogen sulfide extends beyond their involvement in PTMs of shared protein targets. For example, the induction of heat tolerance in wheat seedlings by the hydrogen sulfide donor NaHS was accompanied by a transient increase in hydrogen peroxide and nitric oxide contents in the roots [128]. Notably, the H_2S-induced rise in NO content was abolished in the presence of an antioxidant (dimethylthiourea) or an NADPH oxidase inhibitor (imidazole). In contrast, the elevation of H_2O_2 levels triggered by exogenous H_2S was not eliminated by an NO scavenger (PTIO) or a nitrate reductase inhibitor (tungstate). These findings suggest a specific hierarchical arrangement in the signaling cascade: hydrogen peroxide likely acts upstream of nitric oxide within the hydrogen sulfide-mediated pathway that induces the development of thermotolerance. The involvement of nitric oxide was also demonstrated in the induction of salt tolerance in alfalfa by exogenous hydrogen sulfide. The effects of the H_2S donor NaHS on plant resilience and the expression of antioxidant enzyme genes were abolished by the NO scavenger PTIO [129]. One cannot exclude that this phenomenon is not due to the direct influence of H_2S on NO-synthesizing enzymes but is instead mediated by other components of the signaling network.

Conversely, there are data indicating that hydrogen sulfide can act as a mediator for nitric oxide's effects. For instance, treatment of maize seedlings with an NO donor, which induced the development of thermotolerance, was associated with upregulation of the gene encoding L-cysteine desulfhydrase (LCD1) and an increase in its enzymatic activity [130]. Furthermore, a hydrogen sulfide scavenger (hypotaurine) eliminated the influence of the NO donor on the primary H_2S-synthesizing enzyme (L-cysteine desulfhydrase) and the subsequent development of thermotolerance in maize seedlings. In addition, hypotaurine abolished the stress-protective effect of the NO donor SNP on rice plants under prolonged heat stress, highlighting the role of H_2S as a mediator of the physiological action of exogenous NO [131]. Another study investigated the positive (synergistic) impact of NO and H_2S on the tolerance of wheat plants to sustained heat stress and revealed that their co-application significantly enhanced the effects of these substances on the antioxidant system, specifically the activities of SOD, catalase, and enzymes of the ascorbate-glutathione cycle [132].

In summary, the functional interaction between hydrogen sulfide and nitric oxide can encompass at least several regulatory levels: (a) Competition for protein thiol groups, which serve as the primary targets for PTMs induced by NO, H_2S, and H_2O_2; (b) Differential modulation of protein activities resulting from nitric oxide’s action on tyrosine residues and thiol groups as well as from hydrogen sulfide’s action on thiol groups; (c) Reciprocal regulation of NO and H_2S levels via direct modification of enzymes catalyzing the syntheses and metabolisms of these gasotransmitters; (d) Modulation of cellular concentrations of other mediators, primarily calcium and ROS, which in turn regulate the activities of NO and H_2S synthases and the expression of the genes encoding these enzymes; (e) Direct chemical interaction between nitric oxide and hydrogen sulfide (or more precisely, between reactive nitrogen and sulfur species), potentially further influenced by their interaction with ROS.

It is hypothesized that the specific type of thiol group modification – and ultimately the resulting protein activities – depends on local concentrations of H_2S, NO, and ROS [133]. Such "stochastic" modifications likely govern a wide array of enzymatic and signaling proteins.

Nitric oxide donors and their application for inducing plant stress tolerance

The high biological activity of nitric oxide across various groups of living organisms drives its extensive biotechnological application [134]. Fumigation with gaseous nitric oxide was proposed as a technique to mitigate the effects of stressors during the storage of fruits and vegetables [135]. However, on an industrial scale, gaseous NO is not widely utilized due to safety concerns [134]. Decades of research and practice have demonstrated that different nitric oxide donors are significantly more practical and technologically viable [136]. Currently, about 15 classes comprising more than 300 chemical compounds are known to act as nitric oxide donors, with applications in various fields, including medicine, crop production, and related scientific research [137]. Among these, S-nitrosothiols (RSNOs), N-diazeniumdiolates (NONOates), nitrates, furoxans, metal nitrosyl complexes, and several others are most frequently used [26] (Table 2). Although L-arginine – the substrate for nitric oxide synthesis in living organisms – is not formally classified as a nitric oxide donor, it is considered a natural NO precursor. It is used in pharmaceuticals, dietary supplements [138], as well as in experimental plant biology and crop science [139-141].

Despite the vast number of available nitric oxide donors, research in plant physiology and biotechnology primarily utilizes a very limited range of compounds. Furthermore, there is a distinct lack of studies comparing the effects of different NO donors or evaluating various application methods on plant objects. Additionally, insufficient attention is often given to the potential side effects associated with the most commonly used NO donors. Below, we provide a concise analysis of these aspects within the context of how nitric oxide donors influence plant tolerance to abiotic stresses.

In terms of the total number of studies (including medicine and pharmacology), RSNOs are the most popular nitric oxide donors [26]. However, in plant physiology, SNP, which belongs to the class of metal nitrosyl complexes, has dominated for many decades [142]. Nevertheless, the physiological effects of this donor can be ambiguous because, in addition to NO, SNP can release cyanide and iron ions as toxic byproducts [136]. The inconsistency of results on using SNP may also be linked to the varying rates of NO release depending on light intensity [134]. To reduce the likelihood of incorrect conclusions, additional experimental controls are used to prove the specificity of SNP as an NO donor. These include the parallel use of ferricyanides – which have a structure similar to SNP but do not release nitric oxide – or the preparation of a "exhausted" SNP solution, from which NO is removed by exposing the solution to light in an open container for several days [98]. Furthermore, the reliability of results can be increased by including treatments with nitric oxide scavengers in additional experiments.

Generally, the use of RSNOs, particularly GSNO, is considered more methodologically sound in research compared to SNP [134]. However, the effects of S-nitrosothiols can be influenced by plant metabolites, such as reducing agents like ascorbic

Table 2. Most common groups of NO donors

Type	Mechanism of nitric oxide release	Example
S-nitrosothiols (RSNO)	$RSNO \rightarrow NO^+ + RS^-$	S-nitroso cysteine, S-nitroso glutathione
Metal nitrosyl	$[M]\text{-}NO \rightarrow NO + [M]$	SNP
Nitrates	$NO_3^- \rightarrow NO_2– \rightarrow NO$	Nitroglycerin
NONOates	$R_1\text{-}O\text{-}N\text{-}(N^+O^-)\text{-}R_2 \rightarrow –O\text{-}N\text{-}(N^+O^-)\text{-}R_2 \rightarrow 2NO$	N-diazeniumdiolate

acid and reduced glutathione, which can be present in plant tissues [134].

Nitrates represent the third most frequently used type of NO donors after RSNOs and metal nitrosyls [26]. Their distinctive feature is the presence of a nitrate functional group. While nitroglycerin has been used in biomedical research and practice for decades, inorganic nitrates are more common in experimental plant biology. However, these also serve as a primary form of nitrogen nutrition for plants and possess their own signaling effects [143, 144]. To distinguish the effects of nitrates as NO donors from their role as mineral nutrients, studies utilize NO scavengers. For instance, it was demonstrated that the positive effect of sodium nitrate on the heat tolerance of wheat seedlings was abolished by the nitrate reductase inhibitor sodium tungstate [144, 145].

L-arginine, the substrate for nitric oxide formation via the oxidative pathway, can also be utilized as an inducer of plant stress tolerance. For instance, soaking wheat seeds in arginine solutions was shown to mitigate the harmful effects of drought. Arginine enhanced the syntheses of sugars, proline, other amino acids, phenols, and flavonoids in wheat plants under both normal and stressful conditions [139]. Similarly, L-arginine treatment significantly improved the growth of maize seedlings under heat stress [146].

However, there are still relatively few studies that specifically demonstrate the role of L-arginine as a source of nitric oxide rather than merely an amino acid. Nevertheless, there is compelling evidence linking the physiological effects of L-arginine to enhanced NO production in tissues. Exogenous L-arginine applied via foliar treatment mitigated drought-induced growth inhibition in maize by increasing the photosynthetic rate and antioxidant activity [147]. The amino acid's effect was abolished by the NOS inhibitor L-NAME. Treatment of *Carthamus tinctorius* seedlings with L-arginine increased their tolerance to lead toxicity by mitigating oxidative stress [148]. These effects of L-arginine were eliminated by the nitric oxide scavenger methylene blue and the NOS inhibitor L-NAME. Furthermore, it was shown that the enhancement of heat tolerance in wheat seedlings by L-arginine was accompanied by a transient increase in NO content in the roots. However, in the presence of L-NAME, NO levels did not rise, and the positive effect of L-arginine on seedling survival after heating was significantly reduced [145]. These results underscore the role of NO formation via the oxidative pathway in the induction of heat tolerance by L-arginine.

In recent years, compounds known as NONOates have become commercially available. These consist of a diolate group [N(O-)N=O] linked to a primary or secondary amine, or to a polyamine (such as spermidine), via a nitrogen atom [149]. Through spontaneous decomposition at physiological pH and temperatures, these compounds produce two NO molecules (Table 2). The presence of amines in these compounds, which are themselves stress metabolites, may cause additional physiological effects, potentially distinct from their role as NO donors [149]. However, the potential stress-protective impacts of these compounds on plants remain little studied.

Currently, the action of N-nitrosomelatonin (NOMela) – a natural compound for living organisms – is being actively investigated as an exogenous nitric oxide donor. Research has shown that the time required for the total release of NO from a 100 μM NOMela solution is approximately twice as long as that needed for the release of equivalent NO volumes from equimolar CySNO and GSNO solutions [150]. According to the authors, this slow and steady release is a crucial property of NOMela as an NO donor, as it may ensure prolonged effects under physiological conditions. Furthermore, preferential uptake of NOMela by Arabidopsis roots was reported in comparison to another natural NO donor – GSNO [151]. A long-distance transport of NOMela through vascular bundles, accompanied by the release of larger amounts of NO into tissues compared to GSNO, was also documented. Consequently, NOMela is considered a more effective NO donor than GSNO at equimolar concentrations when applied exogenously [150]. However, due to the high cost of synthesis, NOMela is not yet utilized in large-scale crop production, and studies on its exogenous effects on plants remain limited.

The efficacy of long-established inorganic NO donors, such as SNP, and newly synthesized organic compounds that release NO *in vivo* in practical plant cultivation applications is a complex issue that goes beyond the scope of this review. Several meta-analyses and other special reviews are devoted to the quantitative aspects of the effects of NO donors as stress-protective agents. These studies generally confirm the efficacy of SNP and other compounds on cultivated plants under stress conditions [17, 19]. However, it should be noted that publications more often report the results of studies on the most effective concentrations of NO donors. Only a few studies demonstrate the concentration-dependent action of the compounds under investigation. Meanwhile, the most commonly available NO donor, SNP, exhibits a stress-protective effect within a relatively narrow concentration range [152]. For instance, a study investigating the impact of SNP on soybean drought tolerance revealed that its concentrations two to five times higher than the optimal level considerably diminished the activity of key enzymes involved in nitrogen metabolism and exhibited phytotoxic properties [153]. One reason for the somewhat "unpredictable" effects of NO donors may be that they lead to varying levels of endogenous NO. Changes in NO levels in plant tissues are associated both with the direct release of NO from the incoming donor compound, whose decomposition depends on many factors, and with the ability of exogenous NO to induce endogenous NO synthesis [154-156]. Different concentrations of endogenous NO can induce protein PTMs with varying mechanisms of action and outcomes, including protective ones. As previously noted, different PTMs can activate or inhibit antioxidant enzymes. The interaction of NO with other signaling molecules (e.g., ROS) also depends on its endogenous concentration and can produce various effects accordingly. It is sufficient to mention that, depending on its concentration, nitric oxide, as a signaling molecule, can both inhibit and induce programmed cell death [152, 157, 158]. Moreover, it has been demonstrated that SNP can enhance the phytotoxic effects of the herbicides 2,4-D and carfentrazone [159]. In this case, treating *Raphanus sativus* model plants with SNP increased hydrogen peroxide accumulation caused by these herbicides. At the same time, the NO donor reduced H_2O_2 level in leaves in the absence of herbicides [159].

The above examples demonstrate both the potential for expanding the practical applications of exogenous NO in crop production and the complexity of controlling its dosage. To enhance the efficiency of NO donors in plants, the use of nanoparticle-based (NP) delivery systems has been intensively researched in recent years [160]. For example, a study investigated the impact of soil application of chitosan nanoparticles containing S-nitrosothiols on soybean plants under copper toxicity [161]. It was demonstrated that only the nanoencapsulated form of S-nitrosothiol served as a bioavailable source of NO for the roots. Compared to the conventional form of the same compound, it induced more significant antioxidant activity and reduced oxidative damage and showed a greater capacity to mitigate the copper stress-inflicted nutrient imbalance in the roots [161]. In another recent study, treatments of sugarcane plants with encapsulated forms of S-nitroso-N-acetylcysteine and GSNO attenuated the negative impact of water deficit on photosynthetic rates, maintained water-use efficiency at control levels and promoted growth under stressful conditions [162]. In contrast, spraying with SNP was not effective in mitigating the consequences of water deficit, as the sugarcane plants exhibited oxidative damage even after rehydration. De Souza [163] showed that utilizing chitosan/tripolyphosphate nanoparticles containing S-nitrosomercaptosuccinic acid (NP-MSNO) or S-nitrosoglutathione (NP-GSNO) was significantly more effective in inducing drought tolerance in wheat seedlings compared to hydropriming, treatment with free MSNO and GSNO, or chitosan nanoparticles without an NO donor. Specifically, the NP-MSNO and NP-GSNO treatments resulted in higher seed germination rates, improved growth parameters, and increased chlorophyll content compared to other treatments.

The exogenous physiological effects of NP-based NO in plants are highly dependent on its concentration, flux, duration of exposure, and site of action [136, 164, 165]. Protective responses in plants exposed to abiotic stress are generally induced by low concentrations of NO, typically within the nano- to micromolar range. The concentration of NPs in the solution essentially determines the density of active sites ("hot spots") and the kinetics of absorption processes, which are directly related to the availability of functional groups on the surface or within the NP matrix. In this context, not only the total number of particles but also their size (specific surface area) becomes critical [163]. The smaller the particle, the greater the accessibility of functional groups and the more intense the signal will be, even at ultra-low doses of the substance.

Unlike salt donors (for example, sodium nitroprusside), NP provide a slow and stable release of NO, which prevents a sharp toxic jump. The small size of NPs allows them to overcome the seed coat and deliver the signal molecule directly to the tissues of the embryo [166]. In this context, integrating nanotechnology with NO donor systems represents an effective strategy for controlled NO transport and release, enabling targeted delivery with minimal side effects.

Recent studies also demonstrated that encapsulating NO donors in nanoparticles made them more stable against thermolysis and photolysis, creating opportunities for more reliable preservation of active compounds [167]. Furthermore, nanotechnologies allow for a reduction of administered NO donor doses, thereby decreasing the likelihood of their toxic side effects [160].

A limitation of this review is the potential publication bias in the literature, where positive effects of nitric oxide donors on plant stress tolerance may be reported more frequently than negative ones. Consequently, the protective role of nitric oxide could appear more unequivocal than it actually is.

Conclusion. Nitric oxide is one of the most critical participants in the processes underlying plant tolerance to stress factors. This is attributed to its close crosstalk with other signaling mediators, primarily calcium, ROS, and hydrogen sulfide.

In recent years, the acquisition of extensive data regarding nitric oxide-induced PTMs of proteins has become vital for understanding its mechanisms of action. The lists of known proteins whose functional activities are altered by S-nitrosylation, tyrosine nitration, and metal nitrosylation are constantly expanding. At the same time, PTMs of protein thiol groups are currently considered one of the most important mechanisms for the functional interaction between nitric oxide, hydrogen sulfide, and ROS (hydrogen peroxide), as these groups are the primary targets for all three compounds. Notably, the PTMs of specific proteins lead to the formation of new signals. Direct inhibitory modifications can serve as a mechanism for the subsequent activation of corresponding processes. For instance, the inhibition of certain antioxidant enzymes by nitric oxide can trigger ROS signals, which in turn activate the expression of genes encoding antioxidant enzymes, leading to a subsequent increase in their activities. Nitric oxide can exert varying effects on the activity of the key enzyme in ROS signaling – NADPH oxidase. While direct S-nitrosylation of its molecules leads to activity inhibition, nitric oxide can also stimulate its activity, specifically by influencing calcium homeostasis and phosphatidic acid formation.

Despite the intensive accumulation of new knowledge regarding the effects of NO, many questions posed as far back as the late 20th century remain unresolved. Among these are the identity and nature of the enzymes responsible for nitric oxide formation via the oxidative (L-arginine-dependent) pathway in plant cells, the lack of molecular-genetic evidence for the existence of guanylyl cyclase in higher plants, and the absence of clear concepts regarding how the character of NO-induced PTMs of the same proteins might change depending on NO concentration and concomitant factors. Questions concerning the mutual influence of different nitric oxide synthesis pathways on each other and the role of such interactions in realizing NO signaling effects also remain unclear. Despite the long-known phenomenology of how L-arginine, putrescine, and several other compounds influence NO formation via the nitrate reductase-dependent pathway [10], the underlying mechanisms of these phenomena have yet to be investigated. Consequently, our understanding of the various NO synthesis pathways in plants and their functional interplay remains far from complete.

Nonetheless, despite the numerous open questions in nitric oxide synthesis and signaling in plants, the functional potential of this molecule is already being widely utilized in biotechnologies. In particular, there is a continuously expanding list of NO donors that prove effective in crop production, including as stress-protective agents [10, 26, 134]. In this context, three trends appear to hold significant potential: 1) To develop NO donors with prolonged action, which would provide a gradual increase in the content of endogenous NO over a long period of time [152]; 2) Use of NO conjugates with other biologically vital molecules (e.g., melatonin), which increases the bioavailability of nitric oxide in cells, mitigates its potential side effects, and combines its action with the vital stress-protective properties of the partner compound [150]; 3) Encapsulation of NO donors into nanoparticles, which helps avoid potential NO toxicity, maintains the bioavailability of the donors within cells over an extended period, and may have specific effects related to particle size [163]; 4) Development of simple and cost-effective application methods for classical and especially "novel" NO donors, such as seed priming techniques [139]. However, the successful development of biotechnological applications for nitric oxide in crop production requires dedicated, in-depth research into the mechanisms of action for each new donor form or application method, with mandatory comparative analysis against well-studied forms of NO.

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ОКСИД АЗОТУ У РОСЛИН ЗА АБІОТИЧНИХ СТРЕСІВ: ЗАЛУЧЕННЯ У СИГНАЛЬНУ МЕРЕЖУ

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Вступ. Оксид азоту (NO) визнано однією з найважливіших сигнальних молекул у рослин, що бере участь у контролі більшості фізіологічних процесів за нормальних та, особливо, стресових умов. **Мета.** В огляді аналізуються дані про основні та нещодавно виявлені мінорні шляхи синтезу NO у рослин, посттрансляційні модифікації протеїнів, індуковані NO, участь NO в загальній сигнальній мережі, його взаємодію з окремими нуклеотидами, стрес-протекторну дію різних донорів NO та його метаболічних попередників, а також потенційне застосування цих сполук у фітобіотехнологіях. **Висновки.** Незважаючи на численні невирішені питання щодо синтезу та сигналінгу NO у рослин, сучасні дані переконливо свідчать про його ключову роль в адаптації рослин та стресових реакціях, а також про значний потенціал донорів NO та сполук-попередників для застосування у фітобіотехнологіях.

Ключові слова: оксид азоту, сигналінг, пост-трансляційна модифікація протеїнів, кальцій, активні форми оксигену, гідроген сульфід, адаптація рослин.

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