



Nitric Oxide as a Signal in Inducing Secondary Metabolites During Plant Stress

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Abstract

Secondary metabolites are the major defense elements of plants against biotic and abiotic stress conditions. They are diverse and valuable natural products induced by a variety of environmental and developmental cues. In recent years, NO has been successfully used as elicitor to stimulate secondary metabolite accumulation in plants. Emerging evidence has established the significant role of NO in plant growth and defense responses in plants. Several abiotic and biotic stress factors can induce NO-mediated regulation of the biosynthetic pathways of metabolites that can consequently alter their biological reaction toward the given stress. Moreover, exogenous treatments with NO donors also enhanced the

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accumulation of secondary metabolites including phenolics, flavonoids, and caffeic acid derivatives in several species suggesting the importance of NO accumulation for the secondary metabolic production. Complete elucidation of its role in the production of such secondary metabolites which are pharmaceutically significant is very essential for improving the large-scale commercial production and enhancing stress resilience in plants. Although several reports suggested the induction of secondary metabolites and NO against a range of stress factors, to establish link between NO and secondary metabolites under stress needs a deeper investigation. This compilation chiefly summarize NO biosynthesis, signaling, and functions under abiotic stress in plants highlighting what is currently known about secondary metabolite induction by NO in plants.

Keywords

Nitric oxide · Secondary metabolites · Abiotic stress · Biotic stress · Phenolics · Flavonoids

List of Abbreviations

ABA	Abscisic acid
AP2/ERF	APETALA2/ethylene response factor
APX	Ascorbate peroxidase
AsA-GSH	Ascorbic acid-glutathione
cADPR	Cyclic ADP-ribose
CAT	Catalase
Cd	Cadmium
cGMP	Cyclic guanosine monophosphate
c-PTIO	2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxid
Cu	Copper
DAF-2DA	4,5-Diaminofluorescein diacetate
eNOS	Endothelial NOS
GABA	γ -Aminobutyric acid
H ₂ O ₂	Hydrogen peroxide
iNOS	Inducible NOS
L-NAME	L-N ^G -Nitroarginine methyl ester; <i>N</i> (G)-Nitro-L-arginine methyl ester
MDA	Malondialdehyde
MeJA	Methyl jasmonate
MYB	Myb-related protein B
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate
nNOS	Neuronal NOS
NO	Nitric oxide
NO ₂	Nitrogen dioxide
NOS	Nitric oxide synthase
NR	Nitrite reductase

O ₃	Ozone
PAL	Phenylalanine ammonia-lyase
Pb	Lead
PCD	Programmed cell death
POD	Peroxidase
PSII	Photosystem II
PTMs	Posttranslational protein modifications
ROS	Reactive oxygen species
SNP	Sodium nitroprusside
SOD	Superoxide dismutase
ZN	Zinc

1 Introduction

Abiotic stress is defined as the harmful impact that nonliving factors including environment factors exert on the living systems such as plants and animals growing in the specific vicinity. Both plants and animals have evolved distinct mechanisms to survive abiotic stress imposed due to extreme climate. Plant survival is often challenged by a variety of different abiotic stress factors including drought, temperature extremes, heavy metals and salinity in soil, wounding, ozone, and UV-B stress. Water deficit and high temperatures are perhaps the two major abiotic stresses which are detrimental to crop growth and yield worldwide [1, 2]. Salts and heavy metal accumulation in soil is also prevailing in crop lands. Furthermore, recent years have seen the raising surface O₃ levels due to urbanization and industrial revolution which has also become toxic both for human health and vegetation [3]. On the other hand, the damage of the stratosphere ozone layer in turn causes an increase in UV-B exposure that leads to an increase of ion leakage, membrane protein oxidation, loss of photosynthetic efficiency, and ultimately global yield loss. Longer and severe stress episodes result in production of redox active molecules that in turn result in oxidation of proteins, lipids, and nucleic acids [4].

Given the sessile nature, plants have developed more complex mechanisms to sense and respond against the given stress condition. Plants respond to stress by activating tolerance mechanisms by perception and transmission of stress signals followed by a series of responses at multiple levels like morphological, physiological, biochemical, molecular, and anatomical adjustments [4]. These metabolic adjustments ultimately decide the stress tolerance or stress susceptibility of the plants. Although each stress induce a distinct defense response in plants, it is essential to understand the complete mechanism of plant defense to individual as well as stress in combination. Indeed in natural environments, plants may also be subjected to multiple stress responses at a time. Abiotic stresses disrupt the cellular redox homeostasis which leads to the oxidative stress or the generation of reactive oxygen species (ROS) [1]. Different plant groups may respond differently to the given stress dependent on the species, tolerance level, developmental stage, and tissue affected by the stress. This may be due to the variance in metabolic

adjustments to stress that are different in different tissues and genotypes of the same plant. The metabolic tuning of plants is usually triggered by number of defense molecules that enhance protection [5]. Although plant responses are unique to different extremes, they utilize the common components and signaling pathways to trigger defense. Recent research has revealed nitric oxide (NO) as one of the critical components in several plant acclimation responses to both biotic and abiotic stress conditions [2]. Literature demonstrated that various abiotic factors induce NO generation that lead to the activation of cellular processes for protection against oxidative stress and metabolic adjustments for survival.

1.1 Introduction to NO

NO is a lipophilic gaseous signaling molecule having versatile functions in both plants and animals. The first discovery in 1772 by Joseph Priestley described NO as an air-polluting “nitrous air” without any specific color and odor. After two centuries, Klepper observed NO emission by air purging of herbicide-treated *Glycine max* leaves followed by other observations on NO as a bacterial metabolic by-product [6]. A breakthrough study by Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad turned up the signaling functions of NO in mammalian species and secured the Nobel Prize at Physiology in 1998. From that point, there were many parallel investigations on exploration of NO generation and NO signaling in both plants and animals. However, the investigations on NO in mammals were much rapid wherein the role of NO was well demonstrated in many physiological processes including muscle relaxation, neural communication, immune responses, and programmed cell death.

A bit later, NO has been accepted as multitasking molecule with innumerable functions even in plants. The lipophilic and diffusible nature of NO makes it perfectly suitable for several signaling processes in plants [7]. NO also plays an active role in modifying the activity of enzymes and some key signaling components via posttranslational protein modifications (PTMs). NO also plays a dual role as an antioxidant and as oxidant depending on the cellular concentration and plant species and many other factors. Endogenous NO levels have been reported to get triggered by abiotic stress conditions in diverse plant species. NO play a vital role in increasing plant adaptation to stressful conditions by modifying various physiological processes. There has been sufficient data suggesting NO as an endogenous signal that mediates plant responses to various abiotic stimuli. Nevertheless NO also acts as a critical messenger during stimulation of hypersensitivity response to pathogens.

1.1.1 Functions of NO

Albeit NO is recognized as a toxic gas for plant foliage by early discoveries, later it was treated as a powerful signaling molecule in plant defense during pathogen infestation. NO is actively involved in a plethora of plant development responses including stomatal movement, seed germination [8], and floral transition besides

having a significant role as anti-stress compound against a plethora of abiotic and biotic stresses such as drought, salinity, temperature extremes, UV-B, and heavy metal toxicity. Several reviews delineated the functions of NO in most of environmental abiotic stresses [2]. Besides, pharmacological studies using various NO donors and scavengers also demonstrated the pivotal role of NO in increasing plant tolerance to abiotic cues [2, 9]. Till date, it has been reported that exogenous application of NO donors could enhance stress tolerance in many species of plants including reed, sunflower, wheat, rice, bitter orange, tobacco, and *Arabidopsis*. NO is critical for stress tolerance by modulating osmolytes accumulation and metabolite reprogramming [10].

Besides, number of researchers reviewed the crucial role of NO in moderating various plant hormone-mediated development and stress responses [11]. The protective effect of NO in most reports has been attributed to its antioxidant role due to its ability to activate antioxidant enzymes [2]. There are several studies that support NO inducing stimulation of major antioxidant enzymes such as ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) during stress conditions [12, 13]. Despite the emerging knowledge about NO-mediated plant stress responses including decreasing reactive oxygen species (ROS) levels and protecting membranes from oxidative damage, osmolyte accumulation, and regulation of various hormone-mediated signaling events, its functional status has been far from clarity. Nonetheless, the diffusible nature, short life, and complex chemistry in living systems of NO pose a great challenge to NO researchers [13].

1.1.2 Synthesis and Signaling of NO

In mammalian systems, NO is synthesized through well-characterized forms of nitric oxide synthase (NOS), iNOS, nNOS, and eNOS. Although the pace of investigations was bit slower in plants, initially two plant enzymes, nitric oxide synthase (NOS) and nitrate reductase (NR) [10], have been attributed for plant NO biosynthesis. Plants can also produce NO as a by-product of metabolic pathways including nitrogen fixation and respiration.

Later four major sources for NO generation have been deciphered in plants: nitrate reductase (NR) pathway, NOS pathway [14], and other enzymatic and nonenzymatic pathways [15]. Oxidative NO synthesis from *L-arginine* through NOS activity has been reported across the kingdoms including prokaryotes, unicellular eukaryotes, invertebrates, and mammals. However, the identification of NOS sequences from higher plants having high homology to already known NOS encoding genes [16] in other taxa is still awaiting. During the past decade, the first plant NOS-like gene (AtNOS1) in higher plants is identified in *Arabidopsis* having homology to a snail NOS. Additionally, the chlorotic symptoms of *Atnos1* seedlings disappeared by exogenous NO. Furthermore, the overexpression of AtNOS1 enhanced NO synthesis in *Escherichia coli* while proved to possess NOS activity by converting *L-arginine* to *L-citrulline* using commercial kits. However, its orthologs from maize and rice failed to show NOS activity indicating its function more as a regulator of NO rather than the actual gene coding for synthesis. Although few other studies build pharmacological evidence for the existence of NOS like enzyme

in various plant species, the purification of relevant protein is still underway [14]. Nonetheless, the identification of two genes in green algae *Ostreococcus tauri* and *Ostreococcus lucimarinus* share approximately 40% homology to animal *NOS* genes and also exhibit NOS-like activity [17]. NR-mediated NO synthesis is very common and known to involve in several physiological processes and plant defense against biotic and abiotic stress [16]. In several cases NO production in plant tissues occurs either through nonenzymatic light-mediated conversion of carotenoids or enzymatic catalysis through NADPH nitrate reductase. NR, in addition to its primary nitrate (NO₃) oxidoreductase activity, is capable of reducing NO₂ to NO with low efficacy [18]. Additionally, NO can also be produced through reductive pathways by assimilatory nitrate reductase, or through the mitochondrial electron transport system, or from xanthine dehydrogenase/oxidase [15]. The reductive NO synthesis from NO₂ can occur in cytoplasm, mitochondria, chloroplast, peroxisomes, and the apoplast of the plant cells [18]. Plant mitochondrial enzymes present in the matrix or the intermembrane space are also assumed to oxidize *L-arginine* to NO.

Undoubtedly, NO has the ability to modify the activity of enzymes and some key signaling components through posttranslational protein modifications including protein *S*-nitrosylation, carbonylation, and tyrosine nitration [2, 13]. While the NO-mediated protein modifications have been identified for distinct regulatory proteins such as antioxidant enzymes, there was less information on general mechanism by which NO is being sensed across multiple plant processes [2]. However, a study in *A. thaliana* suggested a unifying N-end rule pathway proteolysis mechanism involved in NO sensing in plants [2]. Sufficient data placed cGMP, cADPR, *L*-phenylalanine ammonia-lyase (*PAL*), and *PR-1* as effectors of NO levels in plants [13].

In the past decade, researchers deployed different methods to elucidate NO-dependent processes including NOS/NR activity assays, NO-binding fluorescent dyes, and various pharmacologic approaches using NO donors including sodium nitroprusside (SNP) and *S*-nitroso-*N*-acetylpenicillamine and NO scavengers 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO), and quantification of effectors by radioimmunoassay or liquid chromatography tandem-mass spectrometry [9, 15, 17, 19]. The recent use of targeted nanodonsors and nanoshutters has enhanced the specificity while reducing the pleiotropic responses and artifacts encountered with pharmacological compounds [12, 16]. Numerous genetic studies have used *Arabidopsis* mutants with altered nitric oxide levels such as *noal*, *nia1nia2*, and *nox1* to confirm the importance of NO accumulation in response to various stimuli [17]. Furthermore, the *nNOS* transgenic lines [20] also underlined the generation of NO as a vital response for increasing plant adaptation to stress [9].

1.2 Introduction to Secondary Metabolites

Plant secondary metabolites are the compounds that have vital role in plant interactions and defense. While our understanding on their role in fundamental plant

growth and development is improving, diverse arrays of secondary metabolites have been identified in higher plants. Most of them are synthesized from primary metabolites including carbohydrates, lipids, and amino acids [21, 22]. Secondary metabolites significantly contribute to develop specific aroma, tastes, and colors in plants that are in turn responsible for plant interactions with environment and defense against pathogens. In economic point of view, they are the major sources for natural products, pharmaceuticals, food additives, and flavors. The accumulation of secondary metabolites can be influenced by various genetic and environmental factors [23, 24]. Secondary metabolites including toxoids, polysaccharides, and flavones serve as key components for plant interaction with the biotic and abiotic cues in their vicinity. Indeed, these are the signals of plant communication during symbiosis, seed dispersal, and plant completion with other plants [25].

Secondary metabolites are not essential to life but essential for survival. Indeed the specific phytochemical profile of species can be used for systematic classification of species in chemotaxonomy. Majority of the plants have four chemically distinct metabolite groups such as terpenes, phenolics, nitrogen, and sulfur-containing secondary metabolites [25]. Terpenes constitute the largest group of secondary metabolites usually derived from acetyl-coA or glycolytic intermediates. Terpenes are structurally diverse group including monoterpenes, sesquiterpenes, diterpene, triterpenes, and polyterpenes that constitute toxins and feeding deterrents in plants. Carotenoids, insecticides like pyrethroid, and phytohormone abscisic acid are the most popular examples of terpenes in plants [26]. Phenols are aromatic compounds derived from the shikimic acid pathway, having a significant role in plant defense against various bacterial, fungal pests, and disease. Few examples of phenols which include lignin, flavonoids, isoflavonoids, and coumarin derivatives play effectively against a range of plant pathogens, protect cells from UV-B radiation and oxidative stress, and promote symbiotic associations. Phytoalexins, thionins, defensins, and glutathiones are the well-known sulfur-containing secondary metabolites [26–28]. They are useful in plant growth as source of reduced sulfur, in stress responses as volatiles defensive substances. The nitrogen-containing secondary metabolites including alkaloids and cyanogenic glucosides and nonproteins amino acids such as canavanine and azetidine-2 carboxylic acid are biosynthesized from common amino acids. These metabolites are mostly toxic and offer defense against pathogenic microbes and herbivorous animals and insects.

2 Production of Secondary Metabolites in Plants Under Abiotic Stress

Plants have potential to adopt some strategies to neutralize the effects of various abiotic stresses. External stress factors such as high and low temperature, salinity, alkalinity, UV, heavy metals, and drought can significantly affect the synthesis of secondary metabolites profiles (Table 1; [27]). The released secondary metabolites

Table 1 Representative studies on the effect of abiotic stresses on synthesis of secondary metabolites in plants

Stress	Plant species	Target metabolites	References
Salt	<i>Carthamus tinctorius</i> L., <i>Lycopersicon esculentum</i> , <i>Oryza sativa</i> L., <i>Solanum lycopersicum</i>	Proline, glycine betaine, total phenolic contents, total flavonoids, sorbitol, polyamines	[21, 23, 29]
Drought	<i>Labisia pumila</i> , <i>Oryza sativa</i> , <i>Salvia officinalis</i> , <i>Cichorium intybus</i> , <i>Papaver somniferum</i> , <i>Hypericum brasiliense</i> , <i>Brassica juncea</i>	Phenols, monoterpenes, essential oils, inulin, flavonoids, anthocyanin, polyphenols, rosmarinic, ursolic, oleanolic acids	[30–35]
Heavy metal	<i>Lepidium sativum</i> , <i>Abelmoschus esculentus</i>	Lepidine, thiol, proline, total phenolics, ascorbic acid content	[36, 37]
High temperature	<i>Dukus carota</i> , <i>Quercus rubra</i> , <i>Medicago sativa</i> L., <i>Camptotheca acuminata</i> , <i>Crucifers</i>	Terpenes- α -farnesene β -caryophyllene, terpenes-isoprene, quercetin, kaempferol, agmatine and putrescine, 10-hydroxycamptothecin	[38–42]
Low temperature	<i>Salix</i> , <i>Triticum aestivum</i> , <i>Medicago</i> , <i>Papaver somniferum</i> , <i>Catharanthus roseus</i> , <i>Pringlea antiscorbutica</i> , <i>Prisms sativum</i> , <i>Vaccinium myrtillus</i>	Terpenoids, putrescine, spermidine, alkaloids-vindoline, phenols-pelargonidin, agmatine and putrescine, flavonoids	[28, 43–45]
Light	<i>Vanilla planifolia</i> , <i>Zingiber officinale</i> , <i>Lactuca sativa</i> , <i>Ipomoea batatas</i> L.	Vanillin, gingerol, zingiberene, caffeoylquinic acids, Chlorogenic acid, hydroxybenzoic acids, flavonoids	[46–49]
UV-B	<i>Passiflora quadrangularis</i> , <i>Fagopyrum esculentum</i> and <i>Fagopyrum tataricum</i> , <i>Populus trichocarpa</i>	Flavonoids, phenolics	[50–52]

are involved in protective functions in response to both biotic and abiotic stress conditions. Abiotic stress-induced accumulation of phenyl amides, anthocyanin, and polyamines has been reported in the literature [22, 25]. The change in the accumulation and composition of secondary metabolites in response to stress factors has been considered as an adaptive strategy leading to tolerance. For instance, the enhanced synthesis of saponins in *Panax ginseng* [22], serotonin in cold-exposed *Datura* flowers, and enhanced lignification of cell walls in many plant species are the examples of stress-induced accumulation of secondary metabolites [43–45]. A number of stresses are capable of redirecting the metabolism toward the accumulation of biologically active secondary metabolites. Besides, a number of researchers have applied various elicitors for enhancement of secondary metabolite production in cultures of plant cell, tissue, and organ [21].

2.1 Influence of Temperature Stress on Secondary Metabolites

Both low and high temperatures effect the metabolic process in plants. Plants often face challenges with high and low temperatures. While high temperatures induce premature leaf senescence and reduce membrane integrity, the rate of photosynthesis and biomass production in plants and low temperature leads to osmotic injury, desiccation, oxidative stresses, etc. [27, 38, 39, 41, 43]. In order to maximize their temperature tolerance, plant species adjust the metabolism to either increase or decrease the secondary metabolites. Low temperature induces the synthesis of several types of cryoprotectant compounds including nitrogenous compounds like proline, glycine and betaine; sugar alcohols like sorbitol, ribitol, and inositol; soluble sugars like saccharose, raffinose, stachyose, and trehalose; and low molecular weight to maintain the osmotic balance [53]. Leaves of wheat and alfalfa accumulate putrescine and spermidine when exposed to a low-temperature stress. Temperature stress also modulates alkaloid and phenolic compound production in several plant species [40, 41, 45]. For instance, cold acclimation in apple tree was reported to be associated with a marked increase in the accumulation of chlorogenic acid. In several plants, the enhanced phenolic production in turn results in the cell wall lignification or suberization [54]. Similarly, higher levels of phenolic acid, anthocyanin, flavones, and antioxidant capacities were observed in strawberry, sugarcane, and lettuce when grown under elevated temperatures [54]. In addition, the total phenol level and especially the genistein levels were observed highest after cold temperature treatment in soybean roots [55]. However, the low temperature reduced the accumulation of alkaloids such as morphine and benzyloisoquinoline in *Papaver somniferum* [44, 45]. The anthocyanin and flavonoid biosynthesis was also promoted by low temperature in *Zea mays* seedlings and leaves of *A. thaliana*, *Petunia hybrid*, and *Rosa hybrid* [55, 56]. It was further supported by the observed increase in the transcript accumulation of phenylpropanoid pathway genes including phenylalanine ammonia-lyase and chalcone synthase in *Arabidopsis*. Conversely, anthocyanin and carotenoid accumulation was reduced by high temperature in several species including *Vitis vinifera* and in *Brassicaceae*. This inhibition was partly attributed to the pigment degradation and reduced gene transcription under elevated temperature [56].

2.2 Influence of Salt on Secondary Metabolites

Salt stress is a global problem limiting agricultural production throughout the world [56]. Salt stress lead to cellular dehydration, ionic and osmotic stress in plants that subsequently results in accumulation or decrease of specific secondary metabolites. Salt stress is known to either induce or reduce the production of secondary metabolites to maximize the tolerance in plants [21, 23, 29]. Increased production of anthocyanins in *Vitis vinifera* cultures, polyphenols in *Aegiceras corniculatum*,

tropane alkaloid in *Datura innoxia*, glycine betaine in *Triticum aestivum*, vincristine in *Catharanthus roseus*, and polyamines in *Helianthus annuus* are some examples of salinity-induced regulation of secondary metabolites [24, 57, 58]. Similarly a positive correlation between proline accumulation and salinity tolerance has been reported in tomato [29]. Salt-induced ABA is also a player in decreasing photosynthesis due to stomatal closure and plant growth inhibition under salt stress. Furthermore, higher salt concentration in growth media resulted in accumulation of high levels of terpenoids, phytoalexins, and zealexins, while lower salt concentration substantially induced the content of kauralexins in maize roots [56].

2.3 Influence of Drought on Secondary Metabolites

Drought stress is the major abiotic stress that can impact food production across the world [59]. Active accumulation of compatible solutes and osmoprotectants is the most common drought-induced metabolic adjustment in majority of the plants [32, 60]. Besides the osmotic adjustment, reprogramming of plant metabolism also occurs in drought-stressed plants leading to multiple other changes in plant secondary chemistry [30, 33]. Drought is known to induce an increase in secondary metabolites such as phenols, saponin, anthocyanin, and flavonoids in several plant species [30, 34, 35]. For instance, moderate water deficit enhanced saikosaponins in *Bupleurum chinense* and salvianolic acid in *Salvia miltiorrhiza* roots [31]. The content of glycine betaine and the total alkaloids in *C. roseus* plants increased due to drought in comparison with the unstressed control plants [61]. Drought-induced changes in secondary metabolite composition, including elevated tocopherol and carotenoid contents, have been associated with improve photoinhibition tolerance in several plants. Hence, the plants or plant tissues with anthocyanin or flavonoids are protected from drought [25]. Drought stress is also known to change the ratio of chlorophyll “a” and “b” and carotenoids [33–35].

Drought stress also increased the production of rosmarinic, ursolic, and oleanolic acid in *Prunella vulgaris* and betulinic acid content in *Hypericum brasiliense* [34, 35]. Similarly, the accumulation of alkaloids including narkotine, morphine, and codeine in *P. somniferum* was significantly increased due to drought. Drought effects can be associated with drought severity and vary for different compound classes. For instance, the total inulin percentage in *Cichorium intybus* roots increased by mild drought stress, whereas severe drought stress decreased inulin yield [32]. Another interesting study suggested more complex and differential regulation of secondary metabolites synthesized via shikimate and isoprenoid synthesis pathways in eucalypts [62]. In these plants drought had no effect on isoprenoids, monoterpenes, and sesquiterpenes, while condensed tannins were enhanced, and concentrations of macrocarpals decreased due to drought [57, 62]. Thus, all these studies underscore the need of more focused studies on secondary chemistry under various ecotypes and drought severities [59].

2.4 Influence of Light on Secondary Metabolites

Light is an important physical factor that can affect the growth and metabolite production [46]. Light is a natural elicitor for many secondary metabolites such as gingerol and zingiberene production in *Zingiber officinale* cultures, foliar tannins, and a number of phenolic glycosides [47]. High light irradiation has been seen to induce anthocyanin production in cell suspension cultures of *Perilla frutescens*, apples, and light-colored sweet cherry [46, 62]. The effect of light was also evidenced from the digitoxin accumulation in *Digitalis purpurea* L., enhanced ginsenoside contents in American ginseng plants, and increased artemisinin production in hairy root cultures of *Artemisia annua* [62]. Light not only has stimulatory effect on the formation of secondary metabolites, including flavonoid and anthocyanins, but also influences the secretion mechanism of secondary products [47, 49, 62]. It has been reported that photoperiod also affects the secondary metabolite content in some plants such as *Hypericum perforatum*, in which maximal production of metabolites occurs at flowering stage. Similarly, blue light has stimulatory effect on the vanillin content in *Vanilla planifolia* [46].

Among various physical variables, ultraviolet (UV) irradiation was considered to be the major inducer of secondary metabolites in several plant species including peanut, rice, maize, and basil [51, 52]. UV-B has been seen to increase flavonoids in barley, several *Passiflora* species [50]. UV-B significantly increased the quercetin concentration in *F. esculentum* [51] and kaempferol in *Populus trichocarpa* leaves [52]. In rice, UV-tolerant cultivar accumulated more C-glycosylflavones compared to susceptible cultivar. Increased UV-B exposure stimulates the total phenolic content as phenolics offer UV protection in plants. Similar to UV-B, UV-C irradiation also is shown to be the stimulus for phenylpropanoid pathway-derived compounds and flavonoid synthesis [52].

2.5 Influence of Heavy Metal on Secondary Metabolites

Heavy metal contamination in soil, air, and water may alter the chemical and metabolite composition of plants leading to poor production and quality [15]. Metals such as aluminum, cadmium, lanthanum, nickel, europium, and silver are known to influence secondary metabolite production due to either inactivation or stimulation of enzymes involved in their production [36, 37]. For instance, metal ions in the growth medium regulate the anthocyanin biosynthesis by inhibiting activity of PAL [15]. Cu^{2+} and Cd^{2+} have been shown to induce secondary metabolites such as shikonin, digitalin, and betalains [63]. However, combined Cd and Cu treatment reduced the production of total phenolics, flavonoids, saponin, and overall medicinal properties due to the inhibition of PAL activity in *G. procumbens* [15, 63]. Increases in heavy metal-induced secondary metabolite biosynthesis also result from increased synthesis of precursors [64]. At times heavy metal-induced stress activates the transcription of the genes encoding the secondary metabolites synthesis which subsequently contribute to the defensive reactions of the plant [15]. In silver ion-

exposed *Brugmansia candida* root culture, there is an increase in scopolamine due to the downregulation of the enzyme hyoscyamine-6- β -hydroxylase responsible for scopolamine release [65]. This can also be partly due to the metal-induced regulation of signaling molecules such as ethylene which in turn can regulate the production of tropane alkaloids such as scopolamine. Similarly heavy metals are also known to stimulate the activity of ethylene biosynthesis genes, 1-aminocyclopropane-1-carboxylic acid synthase, and oxidase either directly or through the jasmonate-mediated pathway [66]. In another study [67] also suggested a positive correlation between the increase in signal molecules with an increase of secondary metabolites under Cu^{2+} exposure. Similarly, the increase in the synthesis and metabolism of phenolic compounds under Pb stress was reported in *Phaseolus vulgaris*. The Pb-induced increase in phenolic content was thought to protect plants from oxidative damage and membrane lipid per oxidation. Plants growing in aluminum-rich soils also accumulate a lot of flavonoids in order to prevent oxidative stress [25]. It is clear from these studies that alteration in secondary metabolism may be a strategy of the plant to survive the phytotoxicity of heavy metals.

3 Abiotic Stress and Nitric Oxide

NO has gained significant attention in recent years due to its potentiality in enhancing tolerance of plants to various environmental stresses [2, 12, 13]. As a redox molecules, NO can function both as a positive and negative regulator of stress responses depending on the local concentration. Being a free radicle, NO plays a powerful role in activating ROS-scavenging enzyme activities and protecting from oxidative damage under abiotic stress. Studies in the recent past have established the role of NO in resistance to salt, drought, extreme temperature, UV-B, and heavy metal stress (Table 2; [12, 13, 74]). Although the complete mechanism by which NO reduces abiotic stress is yet to be deciphered, a definite role of NO is suggested in several physiological processes. In fact, an enhancement of endogenous NO accumulation has been observed in several plant species exposed to wide variety of stress responses providing evidence that endogenous NO could be actually involved in plant stress responses [2]. Although accumulation of NO during various stress conditions appears to be a general response in diverse plant species and tissues, its specificity has been established by using various inhibitors/scavengers such as c-PTIO or L-NAME which reversed these NO-mediated effects in many such studies [2, 69]. Furthermore, exogenous supplementation of NO donors including SNP, SNAP, and diethylenediamine have reported to offer protective actions against abiotic stress, while NO scavengers/inhibitors reversed these actions [12, 84]. Most of the abiotic stresses lead to oxidative burst that disrupt the cellular redox homeostasis. NO may act as a chain breaker and provide protection against oxidative damage under given stress. Being lipolytic small molecules, NO can easily cross cell barriers and enhance cell communications under stress. NO can directly or indirectly interact with a wide range of targets due to the number of posttranslation modifications such as *S*-nitrosylation and nitration [79]. Several transcription factors involved in the regulation of abiotic stress responses in plants including MYB family

Table 2 Various studies describing the involvement of nitric oxide (NO) in plant abiotic stress tolerance

Stress	Plant species	NO response	References
Drought	<i>Arabidopsis thaliana</i> , <i>Medicago truncatula</i> , <i>Tagetes erecta</i> , <i>Oryza sativa</i>	Synthesis of ROS and NO, involved in ABA signaling, stomatal movement, late embryogenesis abundant protein expression, enhanced antioxidant defense and osmolytes, increased adventitious root length, reduced lipid peroxidation	[2, 20, 68–72]
Salt	<i>Cucumis sativas</i> , <i>Oryza sativa</i> , <i>Brassica nigra</i> , <i>Glycine max</i> , <i>Gossypium hirsutum</i>	Survival of more green leaf tissue, and increased quantum yield for photosystem II, increased germination rate and root growth, reduced lipid peroxidation, enhanced antioxidants, altered gene transcription, enhanced photosynthesis	[73–77]
Low temperature	<i>Helianthus annuus</i> , <i>Capsicum annuum</i> , <i>Oryza sativa</i> , <i>Citrus sp.</i> , <i>Cucumis sativus</i> L.	Increase in endogenous NO production in wild types, decline the ROS level, synthesis of osmolytes, reprogramming of lipid signaling, negatively regulates sphingolipid phosphorylation, increases spermidine and spermine levels	[78–82]
High temperature	<i>Oryza sativa</i> , <i>Citrus sp.</i> , <i>Cucumis sativus</i> L., <i>Festuca arundinacea</i>	NO acts as signal molecule for the stress response, protects the plant from heat stress-induced oxidative stress, plays an important role in H ₂ O ₂ metabolism ROS-scavenging enzymes, alleviated the expression of HSPs, and acts as signal molecule for the stress response	[12, 74, 78, 82, 83]
Metal	<i>Triticum aestivum</i> , <i>Glycine max</i>	Noticed that SNP pretreatment significantly reduced O ₂ -induced-specific fluorescence, increased the root elongation, reduced the NOS activity	[84–87]
Ozone stress	<i>Arabidopsis thaliana</i> , <i>Popules sp.</i>	Exogenous application or endogenous synthesis of NO reduces the damaging effects of ozone by activating active oxygen scavenging enzymes	[88, 89]
UV	<i>Betula pendula</i> , <i>Arabidopsis thaliana</i> , <i>Zea mays</i>	Increased accumulation of putrescine, spermine, and spermidine, reduced lipid peroxidation, activation of antioxidant enzymes, increased osmotic tolerance	[90–92]

transcription factors and protein kinases are regulated through S-nitrosylation. It can also trigger several redox-based signaling while altering expression of several genes involved in plant defense. NO upregulated the activity and transcription of APX and GR, the two key enzymes in the ascorbic acid-glutathione (AsA-GSH) cycle in *Nicotiana tabacum* and *Cucumis sativus* leaves, and conferred resistance to abiotic stress [93].

It has been reported that even mild water deficit also leads to the accumulation of NO in cucumber roots [2]. Moreover, accumulation of NO as a result of application of exogenous donors in many reported studies also correlated well with the amelioration of drought stress, while the use of NO scavengers/inhibitors reversed this effect [68]. Exogenous NO improved drought tolerance by reducing stomatal opening, membrane damage, and lipid peroxidation in water-stressed plants [2]. Application of SNP enhanced plant tolerance to drought by inducing stomatal closure, reducing transpiration rate, thereby lowering water loss in leaves and protein synthesis, enhancing photosynthesis rate, and increasing the activities of ROS-scavenging enzymes [69]. A good number of studies confirmed the generation of NO in guard cells in response to drought and ABA by using a NO-sensitive fluorescent dye DAF-2DA [70]. The increase in NO production under drought stress has been correlated significantly to the decrease in stomatal conductance in *Vitis vinifera*. Additionally, NO decreased drought-induced reduction in photochemical quenching during adventitious rooting in explants of *Tagetes erecta* [71]. Similarly, NO-treated *Dendrobium huoshanense* plants maintained high levels of antioxidant enzyme activities and less lipid peroxidation under drought stress [2]. Not only that, NO also help in maintaining high vacuolar concentrations of osmotically active solutes and amino acids under drought. NO promoted drought-induced free proline accumulation in *Oryza sativa*, *Ginkgo biloba*, and *Triticum aestivum* [72]. Similarly, accumulation of glycine betaine was also promoted by NO-mediated stimulation of *betaine aldehyde dehydrogenase* activity in the leaves of drought-stressed *Zea mays* [72]. Moreover, transgenic plants overexpressing the rat *neural nitric oxide synthase* gene in *A. thaliana* and *O. sativa* exhibit enhanced drought tolerance than their respective untransformed controls [2, 20].

More than 45 million hectares of cultivated land globally has been contaminated with high salinity limiting the plant water and mineral uptake. Previous research suggested that exogenous application of NO donors could enhance salinity tolerance in a number of plant species including *Phragmites communis*, *Lupinus luteus*, tobacco, sunflower, cucumber, wheat, and rice [73, 74, 76, 77]. In most cases, an enhancement of endogenous NO levels is followed by Na^+ exclusion and improved K^+/Na^+ ratios. On the other hand, *Arabidopsis noa1* mutant with lower NO level was more sensitive to NaCl further supporting the need of NO in salinity tolerance. Further SNP-induced antioxidant enzymes provided resistance to salt stress by alleviating the oxidative damage in many plant species including rice seedlings, cucumber, maize, etc. [73, 74, 76]. Besides, NO participates in enhancement of photosynthesis by inducing the photosynthetic pigments and adenosine triphosphate synthesis, by quenching excess energy, and by increasing in quantum yield of PSII by using exogenous NO in *Solanum melongena* seedlings under salt stress [75, 77].

Participation of NO in plant response to temperature extremes is also well documented in literature [12]. An increase in NO synthesis associated with cold acclimation was observed in *Helianthus annuus* and *Capsicum annuum* [12, 74, 79, 80]. Transgenic cucumber plants overexpressing *CsNOA1* constitutively had greater accumulation of soluble sugars and starch and a lower chilling damage index, while suppression of *CsNOA1* expression resulted in opposite effects [78]. Furthermore, exogenous application of an NO donor can induce cold acclimation through synthesis of osmolytes such as glycine betaine and proline and reprogramming of lipid signaling and composition [74, 81]. Similarly, high-temperature treatment increased NO levels in leaves of *Nicotiana tabacum* and *Medicago sativa* [9]. While exogenous NO has been able to reduce heat-induced cellular damage, depleting endogenous NO levels by cPTIO reversed these beneficial effects [12, 84]. SNP treatment recovered relative water content, chlorophyll content, and electrolyte leakage in heat-stressed *Zingiber officinale*, *Festuca arundinacea*, *Triticum aestivum*, and *Zea mays* [12, 82, 83]. NO plays a significant role in mitigating heat-induced oxidative stress in plants by maintenance of cellular redox hemostasis and through moderation of carotenoid content [68].

NO mitigate heavy metal stresses in plants mainly by upregulation of antioxidant defense, by regulating cellular free metal concentration, or by excluding the heavy metal in the root zone, thereby preventing the accumulation at toxic concentrations [15, 84–86]. SNP supplementation decreased Cd accumulation in roots and stems while increasing the photosynthetic and antioxidant activity in *Arachis hypogaea* [15]. In rice, exogenous NO treatment has increased Cd tolerance by increasing pectin and hemicellulose content in the root cell walls and decreasing Cd sequestration in leaf soluble fractions [85]. Moreover, the involvement of NO has also seen in protection of chlorophyll against Cd stress in *H. annuus* and Cu stress in *Lolium perenne* [86]. Similarly, NO treatment raised photosynthetic rate, antioxidant activity, and reduced MDA content in *Vigna unguiculata* and antioxidant gene transcription in *Triticum aestivum* under Al stress [87]. NO also plays a critical role in promoting antioxidant enzymes activities and inducing the activity of H^+ -ATPase under metals stress in tomato plants. The role of NO in alleviating other heavy metals has been reviewed by few authors in recent years [15, 86].

Ozone exposure induced NO generation and flavonol accumulation in *Ginkgo biloba* cells [3]. Further, a study in poplar has indicated increased activity of phenylalanine ammonia-lyase (PAL) due to de-nitrosylation and also *S*-nitrosylation of nearly 172 proteins due to ozone fumigation [89]. Similarly studies indicate that upregulation of flavonoids and chalcone synthase gene responsible for flavonoid production by UV-B requires NO in *A. thaliana* and *Betula pendula* plants [90]. Pretreated with SNP prevented the oxidative stress progression in UV-B-exposed *Phaseolus vulgaris* seedlings by decreasing H_2O_2 content, increasing the thiol group content, and upregulation of active oxygen scavenging genes [91]. These studies suggest that UV-B-enhanced NO levels protect the microtubule organization as well as microtubule-related processes by in-plant cells against disrupting effects of UV-B [92]. All these evidence presents NO as a key regulator in maintaining cellular osmotic and redox status in plants under stress.

4 Role of NO in Inducing Secondary Metabolites

Biosynthesis of plant secondary metabolites is regulated by multiple endogenous signaling pathways. NO has been widely utilized as elicitor to stimulate secondary metabolite accumulation in several plants ([94, 95]; Table 3). Priming with SNP has enhanced the phenolic and flavonoid content in fenugreek seeds [116]. Similarly, SNP priming of ripe litchi fruits enhanced the shelf life due to the enhancement of the total phenolic content during postharvest storage [117]. NO is known to regulate the production of many pharmaceutically important secondary metabolites in plants [95]. *Rhodiola sachalinensis* A. Bor. is a perennial herb popularly known for its traditional medicinal properties in China. Nitric oxide induced the bioactive metabolites including salidroside in this endangered plant while increased the total content of phenolic and flavonoid compounds in lemon balm seedlings under in vitro conditions [101]. In another study, the effect of NO donor was studied on the content of secondary metabolites in *Calendula officinalis* L. SNP treatment had significant role in production of total phenolic and flavonoid content, antioxidant activity, and essential oil of capitule, while it had no effect on other pigment content. The application of NO donors for induction of the secondary metabolite production in plant cultures is also becoming increasingly popular [95]. To give few examples, SNP treatment induced catharanthine production in *Catharanthus roseus* cells [94, 96]. Similarly, the hypericin production by *Hypericum perforatum* was significantly enhanced by at least fourfold after eliciting with NO [96, 105]. The accumulation of secondary metabolite such as tannins, saponins, phenols, and total flavonoids is significantly enhanced by high doses of SNP in *Ginkgo biloba* callus cultures [106]. The accumulation of phenolic compounds and glycosides is subsequently followed by an oxidative burst and subsequent activation of specific enzymes activities such as PAL, SOD, and APX in *Ginkgo biloba* [118]. SNP treatment elicited the accumulation of secondary metabolites in *Echinacea purpurea* adventitious roots. Exogenous treatments with SNP also enhanced the accumulation of phenolics, flavonoids, and caffeic acid derivatives in this species suggesting the importance of NO accumulation for the secondary metabolic production [100]. Moreover, the involvement of NO was also suggested in the accumulation of artemisinin in hairy root cultures of *Artemisia annua* L. and taxol production from *Taxus chinensis* cell cultures [119, 120]. Many previous studies have shown that NO is being involved in elicited production of secondary metabolites such as ginseng saponin, hypericin, puerarin, catharanthine artemisinin, and taxanes in plant cell and tissue cultures [94, 96, 101–103, 105, 120, 121]. These metabolites are highly valuable components of pharmaceuticals and nutraceuticals. The production and isolation of secondary metabolites from *Ficus religiosa* L. in tissue culture are often challenged by callus browning [122]. Adding SNP to the MS medium along with 2,4-dichlorophenoxyacetic acid and 6-benzyl amino purine significantly reduced the accumulation of hydrogen peroxide and phenolic compounds in the callus tissues. Similarly, exogenous NO promoted callus induction and reduced browning of Chinese yam. Hence, complete elucidation of its role in the production of such pharmaceutically significant secondary metabolites is crucial for improving the large-scale commercial production.

Table 3 Studies describing the role of NO and abiotic cues in eliciting the synthesis of secondary metabolites in plants

Plant species	Stress	NO elicitation	Target metabolites	References
<i>Catharanthus roseus</i>	–	SNP	Catharanthine	[96]
<i>Scutellaria baicalensis</i>	–	SNP	Baicalin	[97]
<i>Tagetes erecta</i>	–	SNP	Phenol and antioxidants	[98]
<i>Sophora flavescens</i>	–	SNP	Matrine	[99]
<i>Onosma paniculatum</i> Bur. et Franch	–	SNP	Shikonin products	[100]
<i>Rhodiola sachalinensis</i> A. Bor. L.	–	SNP	Salidroside	[101]
<i>Artemisia annua</i> L.	–	SNP	Artemisinin	[102]
<i>Atractylodes lancea</i>	–	SNP	Volatile oil (β -eudesmol, atractylone, and atractylodin)	[103, 104]
<i>Hypericum perforatum</i>	High temperature	SNP	Improve hypericin production	[105]
<i>Ginkgo biloba</i>	UV-B	SNP	Phenols, acids, flavonoids	[106]
<i>Zea mays</i>	UV-B	cPTIO	Flavonoids	[92]
<i>Pisum sativum</i> L.	UV-B	NO	Cell wall polysaccharides	[107]
<i>Taxus chinensis</i>	UV-B	SNP, cPTIO	Flavonoids, condensed tannins, total phenolics, and taxol	[108]
<i>Achillea species, Ginkgo biloba, Vitis vinifera</i>	Drought	–	Total phenolic, flavonoid, soluble proteins, lignin	[109–111]
<i>Spinacia oleracea, Solanum lycopersicum</i>	Salt	NO	Total phenolics, flavonoids, osmolytes, carotenoids	[112, 113]
<i>Vicia faba</i>	Arsenic	NO	Photosynthetic pigments, phenols, phytohormones, minerals	[114]
<i>Glycine max</i>	Low temperature	–	Phenols, genistein, daidzein	[55]
<i>Camptotheca acuminata</i>	High temperature	–	Alkaloids- 10-hydroxycamptothecin	[41]
<i>Helianthus annuus</i>	High temperature	NO	S-nitrosothiols	[79]
<i>Chlamydomonas reinhardtii</i>	Cu ⁺²	SNP	Proline	[115]
<i>Trigonella foenum-graecum</i>	Oxidative stress	SNP	Phenolics, flavonoids	[116]

4.1 NO-Mediated Elicitation of Secondary Metabolites Under Abiotic Stress

Several studies (mentioned in above sections) have demonstrated the alteration in the secondary metabolite profile under abiotic stress in plants. NO can also increase production of secondary metabolites and activate plant protection systems even under stress conditions [94, 114]. Several abiotic and biotic elicitors can induce NO-mediated regulation of the biosynthetic pathways of metabolites that can consequently alter growth and development in plants [95]. However, very little is known on NO signaling in the biosynthesis of plant secondary metabolites under stress. Given the production of NO in plants in response to abiotic and biotic stresses [106, 107, 111, 123], it can be presumed that NO may have the most possible and prominent role in inducing secondary metabolites in response to stress. Hence, the elicitor or stress-induced NO production is essential for triggering the biosynthesis of critical secondary metabolites in plants [95]. Cu²⁺ stress could induce NO production and subsequent proline accumulation in *Chlamydomonas reinhardtii* and in roots of *P. ginseng* [115]. In another study, ultrasound treatment for 2 min resulted in a rapid and dose-dependent NO production in *T. yunnanensis* cell cultures which in turn stimulated the production of taxol and baccatin III [120].

An interesting study suggested that NO treatment created a strong demand for cysteine synthesis as a way to reduce oxidative stress. Cysteine synthesis is one of the rate-limiting steps for the formation of glutathione which is very crucial component in cellular redox responses. In agreement with that, active synthesis of amino acids specifically α -ketoglutarate-derived amino acids of the glutamate family was evident in response to NO treatment [94]. It is known from earlier reports that the metabolism of γ -aminobutyrate (GABA) is crucial plants exposed to low oxygen or high light condition. Exposure of plants to NO showed a moderate increase in the levels of GABA and 2-aminobutyrate and the significant increase for γ -hydroxybutyrate.

A recent metabolomic data suggested a significant increase in metabolites involved in purine and pyrimidine metabolism by 6 h after NO treatment. There was a significant increase in the levels of allantoin, guanine, urate, cytidine, cytosine-2',3'-cyclic monophosphate, pseudouridine, uridine, and uracil by NO. On the other hand, NO treatment induced chlorophyll degradation as evident by an increment in the levels of pheophorbide, a breakdown intermediate product of chlorophyll in plants [19, 124].

NO is one of those key signaling molecules in elicitor-induced secondary metabolite biosynthesis in plant cells. Although pharmacological experiments with NO donor and scavenger showed that the occurrence of NO contributes to strengthening the transcription of genes encoding key enzymes involved in the biosynthesis of those target secondary metabolites such as shikonin [100], little effort has been put onto revealing the signal transduction steps underlying NO activation of plant secondary metabolism. PAL is the critical enzyme that mediates the conversion of phenylalanine into trans-cinnamate, from which many plant phenolic compounds originate [125]. In several studies, more plant phenolic compounds are produced

with increased PAL activity [120]. It has been clearly demonstrated that NO stimulates transcription of the PAL gene in plants [125]. Increased PAL production means greater efficiency in converting phenylalanine into phenolic compounds; and, therefore, in most cases, concentrations of plant phenolic compounds increase following the use of NO. In *T. chinensis* cell cultures, NO enhanced PAL activity while inhibiting the transcription of strictosidine synthase and tryptophan decarboxylase by inducing zinc finger-binding proteins [120]. Besides, exogenous NO donor SNP is known to induce the expression levels of 4-hydroxybenzoate meta-geranyltransferase and 3-hydroxy-3-methylglutaryl CoA reductase involved in shikonin biosynthesis in *O. paniculatum* cells [100].

Some abiotic stress-eliciting responses, including ROS production, lipid peroxidation, the activation of PAL, and osmolyte production, were also mediated by NO. For example, exogenous NO treatment enhanced the production of anti-oxidation-associated compounds, total phenolic content, proline, and flavonoids in salt-stressed spinach and tomato [111–113]. Furthermore, exogenous NO application has also increased the fresh and dry biomasses of edible parts compared to salt alone treated plants. Given the results in spinach, the authors have proposed the application of nitric oxide gas as an effective strategy for boosting biomass production and nutrition quality in spinach under salt stress. NO donor has also been proven to exert a protective effect against polyethylene glycol-induced drought stress in wheat seedlings by enhancing growth, relative water content, and reducing oxidative damage [2]. Similarly, heat shock in *H. perforatum* suspension cells induced NO production subsequently resulting in hypericin production [105].

The most common protective mechanism against UV irradiation is the biosynthesis of UV-absorbing secondary metabolites [106]. Several reports indicated the protective effect of NO against oxidative stress under UV-B irradiation [90, 91]. The role of NO in the regulation of flavonoid biosynthesis in *G. biloba* leaves under the UV-B was elucidated by [106]. Additionally, the sequential occurrence of NO production via increased NOS activity and increased chalcone synthase has been suggested [94]. A similar observation was noted in pea seedlings, where UV-B induced NR activity and NO production inhibited stem elongation due to the inhibition of xyloglucan-degrading activity [107]. In *Taxus chinensis*, spraying SNP and cPTIO had significant effect on the contents of photosynthetic pigments and taxol production [108]. Interestingly high levels of flavonoids, condensed tannins, total phenolics, and taxol were noted under UV-B+cPTIO treatment suggesting the requirement of balanced levels of NO in the secondary metabolism.

Use of fungal elicitors is one of the most effective strategies for inducing economically important secondary metabolites in plants. A study by [103] has shown that NO mediates violate oil accumulation induced by the endophytic fungus *Gilmaniella* sp. through salicylic acid and H₂O₂-dependent pathways in plantlets of *Atractylodes lancea*. Furthermore pretreatment of plantlets with exogenous NO donor promoted volatile oil accumulation, while treatment with NO scavenger inhibited the burst of salicylic acid and volatile oil accumulation induced by the fungus. Likewise elicitation with another endophytic fungal *Cunninghamella* sp. also induced the NO-mediated accumulation of atractylone, hinesol, β-eudesmol, and atractylodin in suspension cells of *A. lancea* [104]. NO induced by cerebroside

elicitor from *Fusarium* was involved in the regulation of artemisinin production by increasing the gene expression of 1-deoxy-D-xylulose 5-phosphate synthase and hydroxybenzoate meta-geranyltransferase in *A. annua* hairy roots as well [102]. It was reported that oligogalacturonic acid-induced NO accumulation could improve the transcription of squalene synthase and squalene epoxidase, two early enzymes for the synthesis of triterpenoid saponins in cell cultures of *Panax ginseng* [121]. Similarly, NO burst followed by the biosynthesis of torpinoid β -thujaplicin in elicited *Cupressus lusitanica* cells which has strong antifungal, antiviral, and anti-cancer activities [126]. NO was found to reduce the transcription of genes in the monoterpenoid indole alkaloids pathway and the octadecanoid-responsive *Catharanthus* AP2/ERF domain transcription through the inhibition of type-I protein prenyltransferase gene, leading to a downregulation of the catharanthine biosynthesis [96].

NO may also interact with other signaling molecules integral of plant defense system including jasmonic acid, ethylene, salicylic acid, and ROS while taking part in elicitor-induced production of secondary metabolites [94, 126]. Although these molecules operate through distinct defense signaling pathways, they are all known to interact with NO in mediating plant secondary metabolite production [126]. The combination of elicitation with various biotic, abiotic stresses, and other signal molecules implies NO as the keypoint in the signaling network leading to the biosynthesis of some secondary metabolites [95]. Jasmonic acid induce NOS activity and subsequent NO production leading to enhanced matrine accumulation in *Sophora flavescens* suspension cells [99]. Similarly, NO-mediated accumulation of fungal elicitor-induced puerarin production in *P. thomsonii* suspension cells occurs through both SA-dependent or SA-independent signaling pathways [94, 95]. Although a direct link between methyl jasmonate and NO is yet to establish, exogenous MeJA triggered a burst of NO during the accumulation of taxol from *T. chinensis* cell cultures. Furthermore the suppression of NO by its inhibitors also suppressed the MeJA-induced taxol production suggesting a central role of NO in taxol accumulation [120]. Similarly, NO acts downstream to MeJA during the accumulation of four tanshinone compounds in hairy root cultures of *Salvia miltiorrhiza* [127]. However, SNP supplementation along with methyl jasmonate leads to the marked decrease of the catharanthine production by repressing the transcription of its biosynthetic genes, while methyl jasmonate supplementation alone stimulated the transcription of catharanthine pathway genes suggesting an antagonistic relation between NO and MeJA [127].

NO acts synergistically with reactive oxygen species to stimulate ethylene biosynthesis and stomatal closure in defense response to UV-B irradiation in maize leave [92]. In another study, brassinolide pretreatment induced the production of NO prior to the upregulation of cold-related gene expression and antioxidant enzymes activities in *Medicago truncatula* plants during cold stress tolerance. Further, brassinolide inhibitor reduced NO production and the expression of brassinolide-induced mitochondrial alternative oxidase, photosystem II efficiency, and homeostasis secondary metabolites accumulation [128]. This suggests that production of secondary metabolites or phytosignalling molecules may be the mechanism through which NO exerts its protecting effect from abiotic stress in plants.

5 Conclusions

Alteration in secondary metabolism is an effective strategy of the plants to survive and grow in adverse conditions [56]. Many studies indicated the influence of abiotic stress on the amounts of phenolic compounds, flavonoids, glucosinolates, antioxidants, osmolytes, carotenoids terpene derivatives, and phytohormones in plants. NO has been reported to be induced rapidly by abiotic and biotic elicitors in a variety of plant species. Although several studies evidence the role of secondary metabolites and NO in plant's response to various abiotic stress factors, the knowledge about NO-mediated secondary metabolome alterations in abiotic stressed plants is still in its infancy. Few recent studies have shown that exogenous addition of NO can enhance the effect of abiotic elicitors on plant secondary metabolite production. Moreover, different NO donors could be chemically synthesized to be used as a priming agents or elicitor for industrial production of important secondary metabolites in plant culture systems. The NO elicitation can be an effective strategy to significantly improve specificity and efficiency of the production of desired metabolites. Hence, complete understanding of the signal transduction pathways underlying NO-induced production of secondary metabolites not only advance our understanding but also is important for optimizing the commercial production of metabolites which are difficult to be obtained by chemical synthesis.

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