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Review

Interaction between melatonin and NO: action mechanisms, main targets and putative roles of the emerging molecule NO-mela

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Abstract: Melatonin (MEL), a ubiquitous indolamine, is a molecule whose regulatory role in plant metabolism has gained interest in the last decades. Likewise, nitric oxide (NO), a gasotransmitter, can also affect plant molecular pathways due to its function as a signaling molecule. Both MEL and NO can interact at multiple levels under abiotic stress, starting by their own biosynthetic pathways and inducing a particular signaling response in plants. This review summarizes the role of these molecules during plant development and fruit ripening, as well as their interactions. Due to the impact of climate change-related abiotic stresses on agriculture, this review has also focused on their role in mediating abiotic stress tolerance and the mechanisms by which they operate, from upregulation of the whole antioxidant defence system to post-translational modifications (PTMs) of important molecules. Their interaction and crosstalk with phytohormones and H_2S is also discussed. Finally, we introduce NOmela, an emerging and very unknown molecule which seems to have a stronger potential than MEL and NO separately in mediating plant stress response.

Keywords: melatonin; NO; ROS, abiotic stress; PTMs; H_2S; RNS; NOmela

1. Introduction

N-acetyl-5-methoxytryptamine, better known as melatonin (MEL), is an indole-derivated compound similar to indole-3-acetic acid (IAA). MEL is an indolamine first discovered in bovine extracts from pineal gland in 1958, but it was isolated and identified by Lerner et al. [1]. It was named after its ability to aggregate melanin granules in skin chromatophores. Although it was believed that this compound was only present in animals, in 1995, two independent groups identified the presence of MEL in higher plants [2,3]. In the following years it was proved that MEL is also found in several Eukarya and Bacteria groups, meanwhile there is no evidence for its presence in Archea domain. Because of its ubiquitous distribution, it has been suggested that the structure of this molecule has barely changed through evolution [4,5]. Initially, this molecule had the role of acting as an antioxidant in unicellular organisms, but, throughout evolution this role changed and it started to act as an hormone in superior eukaryotes, being involved in processes as immunomodulation, circadian rhythms or seasonal reproductive regulation [6].

In 2004, the term phytomelatonin was proposed with the aim to discriminate animal MEL and plant MEL [7]. Apart from regulating plant growth, in the last few years, it has been described as a "master regulator" involved in plant cell metabolism and, moreover, regulating and increasing plant tolerance to biotic and abiotic stress. This is possible due to its ability to act as an hormone or as an antioxidant molecule, by scavenging diverse reactive oxygen species (ROS) and reactive nitrogen species (RNS) [8–10]. In addition, MEL can easily pass through cell membrane and move around the cytosol and organelle due to its amphiphilic and amphipathic character [11], thus facilitating its regulatory role of plant metabolism.

Gasotransmitters are molecules involved in the regulation of plant development and stress responses. The principal gasotransmitters are nitric oxide (NO), carbon monoxide (CO) and hydrogen sulphide (H_2S). NO is a potent signalling molecule due to its short half-life and high diffusibility across plasma membrane. It is a molecule with dual functions, as it can act both as an antioxidant or pro-oxidant, depending on its concentration, and it has important roles on flowering, plant growth, morphogenesis regulation and oxidative stress response [12]. NO interaction with target molecules results in the production of RNS, whose implication in cell metabolism still need further research [13] but we will mention some known RNS aspects in this review.

Given the importance of these two molecules (NO and MEL) in plant physiological processes and plant stress response, this review focuses on the role and putative and known interactions between NO-MEL in different contexts.

2. Interaction between MEL and NO biosynthetic pathways: positive and antagonistic interactions.

There is a crosstalk between NO and MEL in plant physiology, as MEL can affect endogenous NO levels and NO can alter endogenous MEL content (Figure 1).

MEL's precursor is tryptophan, an amino acid that plants can synthetize through shikimate pathway (which is also the synthesis route for all aromatic amino acids in plants). First, tryptophan is converted to serotonin, which can take place by two different pathways: tryptophan's decarboxylation intro tryptamine via tryptophan decarboxylase (TDC) followed by its hydroxylation intro serotonin by tryptamine 5-hydroxylase (T5H); or tryptophan hydroxylation intro 5-hydroxytryptophan by tryptophan hydroxylase (TPH) followed by its decarboxylation into serotonin by TDC, being the first route the most frequent in plants. Once serotonin is produced, it is acetylated to N-acetylserotonin by serotonin N-acetyltransferase (SNAT), which is finally methylated by O-methyltransferase (ASMT), although this last step can also be performed by caffeic acid 3-O-methyltransferase (COMT). There is also an alternative pathway that leads to MEL formation, whose first step is serotonin catalysis to 5-methoxytryptamine by ASMT or COMT, which is converted to MEL by SNAT (Figure 1A). Depending on the followed route, MEL can be synthesised in cytoplasm or chloroplast respectively [14,15]. MEL can also generate active derivatives under physiological conditions. Specifically, 2-hydroxymelatonin (2OHM) is the most abundant in plants and some authors affirm that it induces stress tolerance in plants more effectively than MEL [16,17].

Plants vary on their MEL levels, from high to undetectable concentrations [11], remarking that stress conditions enhance MEL production [4,18]. Within the plants, it has been reported that flowers are the organ with the highest MEL levels, followed by leaves and seeds, which can be explained by the necessity of maintaining a high antioxidant environment in flowers and leaves, organs which are more exposed to stress conditions [4]. MEL is mainly synthetized in chloroplasts and mitochondria, which correlates with its protective role against free radicals produced in this organelles [14,19]. At basal conditions, both ROS and MEL are kept at relatively low and constants levels, being their synthesis upregulated under stress conditions for both molecules [20].

NO production can take place via oxidative and reductive pathways. NO is synthetized through the oxidative pathway by the oxidation of L-arginine by NO synthase (NOS), polyamine by polyamine oxidase, or NADH/NADPH by cytochrome oxidase. Meanwhile, the reductive pathway is characterised by NO_3^- reduction to NO_2^- by nitrate reductase (NR), using NADH as electron donor, under anoxic conditions. This is followed by NO_2^- reduction to NO due to the action of xanthine oxidoreductase (XOR) and plasma-membrane-bound nitrite-NO reductase (Ni-NOR). Moreover, NO can be generated within the polyamine metabolism. High nitrate concentrations or high reducing environments trigger non-enzymatically reduction of nitrite to NO. Mitochondrial electron transport chain, under acidic and anoxic conditions, can also produce NO from nitrite (Figure 1B) [21–26].

Due to NO short half-life (~30 sec), NO transport and accumulation in plants is mediated by NO carriers (which are more stable in solution), mainly S-nitrosothiols [27]. S-nitrosothiols (SNOs) are non-protein sulphydryl-containing compounds which are formed by its reaction with NO. They are more stable than NO and they can be transported and act as NO storage. In addition, SNOs can carry out post-translational modifications (PTMs) mediated by cell signalling, especially during stress response [28]. SNOs can be classified by their molecular mass into high-molecular-mass S-nitrosothiols (HMM-SNOs) or low-molecular-mass S-nitrosothiols (LMM-SNOs), being GSNO (S-nitrosoglutathione) the most abundant LMM-SNO in biological systems, and it is generated by NO interaction with reduced glutathione [29]. GSNO constitutes a NO reservoir that can be degraded by S-nitrosoglutathione reductase (GSNOR). Lee et al. [30] showed that plant development and stress response can be defected after mutations in *GSNOR* gene, confirming NO role in plant metabolism.

As previously commented, MEL can either induce NO production or scavenge NO production. MEL is capable of increasing NO levels by upregulating *NOS* gene expression [31,32]. Experiments performed in tomato seedlings show that exogenous MEL inhibit S-nitrosoglutathione reductase (GSNOR) activity and upregulates NR activity, which elevates endogenous NO levels [33].

On the other hand, NO can also promote MEL biosynthesis by inducing the expression of *TDC*, *T5H*, *SNAT* and *COMT* genes, which codify for the main enzymes from MEL biosynthesis pathway. In addition, NO also increases MEL levels by modulating the activity of MEL-synthesis enzymes [8,34].

The crosstalk between NO and MEL is especially relevant during plant response to unfavourable situations. Under stress conditions, MEL triggered NO accumulation by modulating the activity of NR and NOS, via the arginine pathway, as well as the expression of related genes [31,35].

However, NO and MEL also have antagonistic actions. MEL can inhibit NOS, decreasing NO levels, through various mechanisms [36], but it can also promote NO accumulation via arginine pathway [31] or upregulating NOS-related genes, by increasing NOS activity and, thereby, NO levels [32].

3. MEL and NO action on plant growth and development: physiological responses and effect on fruit ripening

MEL plays a role modulating plant growth development, senescence, flowering and fruit ripening [37]. On the other hand, NO is able to regulate plant growth in stressed and unstressed plants [38], as well as delaying fruit ripening [39]. Moreover, MEL is capable of regulating NO/NOS system in order to perform physiological functions [40].

3.1. Plant development

The first phytomelatonin receptor, CAND2/PMRT1, was recently discovered and identified in Arabisopsis by Wei et al. [41]. Among its functions, it has been shown that CAND2/PMRT1 union to MEL can control stomatal closure via Ca^{2+} and H_2O_2 signalling cascade. MEL binding to this receptor activates $G_{\gamma b}$ dissociation from G_{α} , triggering H_2O_2 production by NADPH-oxidase, which enhances Ca^{2+} influx and K^+ efflux, causing stomatal closure [41].

In plants, MEL is involved in plant development and growth, due to its action as an auxin-like molecule [42]. MEL is not capable of stimulating IAA synthesis, but it can affect plant growth in an auxin-independent manner (as it does not activate the expression of *DR5:GUS*, an auxin-inducible gene marker, and there is no evidence that MEL can be perceived by auxin receptors), although initially MEL was described as an auxin-mimetic molecule [43,44]. It is involved in processes such as flowering, leaf senescence, root morphogenesis and fruit ripening, as well as modulation of chlorophyll and proline levels in leaves and fruits [4,43,45–48]. In recent studies by Lee & Back (2019), mutant plants in which *SNAT* was downregulated showed a semi-dwarf stature, which confirms MEL action as a growth promoter [49].

Arnao et al. [43] demonstrated the role of MEL in improving rhizogenesis. Lately, it has been shown that exogenous MEL promoted lateral and adventitious root formation in *Lupinus albus* rice, cucumber and Arabidopsis [43,44,50,51]. Moreover, MEL has been proved to modulate root gravitropic response [52].

Exogenous MEL may cause both a promotion or inhibition on plant growth depending on its concentration, with a higher inhibitory effect at higher concentrations, due to its auxin-like effects. It has been demonstrated that high MEL concentrations (100 μM) inhibit root growth, while low MEL concentrations (0.1 μM) promote it, inducing as well an increase in endogenous IAA levels (it is thought that this IAA increase triggers root growth) [53]. Thus, high MEL concentrations are associated with a decrease in IAA biosynthesis, reducing root meristem size [54]. In addition, it has been proved that the genes regulated by low MEL concentrations are different from the ones regulated at high MEL concentrations [55].

In some plant species such as cucumber, exogenous MEL did not affect the expression of auxin-related genes [51], although experiments performed in Arabidopsis showed that high MEL concentrations downregulate the expression of genes encoding auxin-influx carrier proteins (AUX1/LAX) [55] and auxin biosynthesis [54].

In addition, NO is capable of regulating auxin responses, promoting adventitious root formation, as well as lateral roots, root hair development and root gravitropism. [56,57]. An increase in NO levels inhibited root meristem activity and it reduced the number of dividing cells in primary roots by inhibiting auxin transport and response thanks the downregulation of auxin efflux protein PIN-FORMED 1 (PIN1), as shown by Fernández-Marcos et al. [58]. Thus, the effects of high NO levels are similar to the ones observed by high MEL levels.

As shown by Wen et al. [33], in tomato, exogenous MEL triggered NO production by promoting NR activity, which induced the formation of adventitious root by modulating the expression of auxin-related genes, such as the genes involved in auxin accumulation, transport and signal transduction. MEL synthesis can also be promoted by NO treatment in tomato seedlings [42], which indicates that there may be a feedback loop between NO and MEL influencing root development via auxin signalling pathways.

It has been suggested that NO is part of a signalling cascade involving protein kinases, ROS, phytohormones and cyclic guanosinc monophosphate (cGMP), although NO transduction can occur cGMP-dependently or independently. NO is capable of regulate root growth via cGMP signalling pathway, but it can also activate the MAPK signalling cascade regulating NADPH-oxidase-dependent oxidative burst. In cucumber, MPK1/a can also induce NO production by increasing NR activity, leading to GSNO accumulation, which can trigger S-nitration of NR [59–61].

Another possible mechanism for NO action is characterised by NO capacity to induce protein PTMs, mainly S-nitrosylation, which can modulate enzyme activity and protein function, although it relationship with plant development requires further research [62].

MEL has also a role in regulating floral transition. It mediates the stabilization of DELLA proteins, which disturb flowering-related transcription factors, thus repressing the floral transition [63]. Excess MEL triggered the activation of *FLC* (Flowering Locus C), thus delaying flowering. However, strigolactone (a carotenoid-derived phytohormone) can act upstream MEL inducing floral transition by inhibiting MEL synthesis [64].

Lozano-Juste & León (2011) showed that NO can also disturb flowering by increasing DELLA protein levels [65]. Because of that, it is possible that flowering is regulated by a NO/MEL crosstalk, although the mechanisms involved in this process are still unknown.

MEL has been reported to have anti-senescence proprieties, which have been attributed to its role as antioxidant. Moreover, MEL can impede *Hexokinase-1*, a senescence-associated gene, and *ATGs* (autophagy-related genes) upregulation, thereby preventing leaf senescence [66–68]. In apple, during leaf senescence, MEL is capable of preventing carotenoids and chlorophylls degradation. Moreover, exogenous MEL increased fructose, sorbitol, sucrose, glucose and starch levels, as well as preventing the decline of Rubisco and soluble proteins content. [68,69].

3.2. Fruit ripening

Fruit ripening is defined as a complex process orientated to promote animal-mediated seed dispersion, which involves changes in fruit organoleptic proprieties. During ripening of climacteric fruits, both MEL and NO have been shown to inhibit ethylene biosynthesis, preventing postharvest senescence. During fruit ripening, NO can interact with ACC oxidase, a key enzyme in ethylene biosynthesis, generating an ACC oxidase-NO complex, which can also form a stable ternary ACC-ACC oxidase-NO complex via chelation by ACC. This metabolic step decreases ethylene production by inactivating ACC oxidase [70]. Moreover, NO can nitrositate ACO (also involved in ethylene production), decreasing its activity, and downregulate ACO-gene expression (*LeACO1*, *LeACOH2* and *LeACO4*) thus reducing ethylene levels [71].

Soluble sugars also play an important role in fruit ripening, as they can act as signal molecules and participate in mediating fruit ripening and senescence. Shi et al. [72] showed that exogenous NO treatment modulated sugar metabolism by enhancing sucrose phosphate synthase (SPS), sucrose synthase (SS), neutral invertase (NI) activity, thereby maintaining higher sucrose, fructose and glucose levels. Thereby, NO treatment not only acts inhibiting ethylene biosynthesis, but also regulating sugar metabolism in postharvest fruit programs [73].

Sun et al. [74] showed that, in tomato, MEL stimulated fruit ripening by up-regulating ethylene signal transduction-related genes expression, thus inducing ethylene production. Moreover, it has been shown that MEL increase ripening- and anthocyanin increase-related proteins levels [75]. This results in an amplification in ethylene signal transduction, which triggers cell wall degradation, lycopene accumulation and VOCs (volatile organic compounds) synthesis via gene regulation [76]. However, in some fruits, as banana, exogenous MEL repressed ethylene synthesis, thus delaying the ripening process [77].

In tomato, MEL acts as an antioxidant molecule scavenging RNS during fruit ripening, as well as enhancing arginine pathway-mediated NO accumulation, as well as polyamines and proline production. On the other hand, in pears, in order to delay postharvest senescence, MEL was capable of reduce ethylene production by regulating the synthesis of NO. The inhibition of NO synthesis eliminated the effect of MEL delaying fruit ripening, which may indicate that MEL acts upstream NO in this pathway [13,31,32,70,78,79]. Thereby, MEL do not directly repress ethylene biosynthesis; it inhibits ethylene production via NO-mediated mechanisms.

4. MEL and NO interaction on molecular metabolic regulation under abiotic stress

In plant physiology, stress is defined as a condition which prevents normal development, growth and metabolism [80]. Climate change is likely to increase the impact of stress factors in plants, which can limit plant production in the following years [81], thus representing a major challenge for agricultural lands.

Abiotic stresses can affect plant growth and development, as well as reproductive programs. However, plants can adapt themselves to environmental stresses through complex mechanisms. Under stress conditions, there is an immediate response characterised by an increase in ROS, RNS and malondialdehyde (MDA) levels. High concentrations of ROS and RNS may lead to membrane damage due to lipid peroxidation and electron leakage (EL), but they can also cause DNA damage, impaired enzyme activity and carbohydrate oxidation [82] (Figure 2).

ROS, such as superoxide anion, hydrogen peroxide or hydroxyl radical, are continuously produced in plants and can act as signalling molecules, although high ROS levels can lead to a situation of oxidative stress. Because of that, plants have developed both enzymatic and non-enzymatic antioxidant systems in order to protect themselves against oxidative stress. Enzymatic systems include enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR), glutathione peroxidase (GPX), etc. On the other hand, non-enzymatic systems are constituted by antioxidant molecules such as glutathione (GSH), ascorbic acid (AsA), flavonoids or carotenoids. ROS can activate stress response pathways, for example, upregulating MEL biosynthesis, which acts balancing redox homeostasis either by a direct scavenging of free radicals or indirectly by increasing the activity of key antioxidant enzymes (SOD, CAT, POD, APX and/or GPX). Moreover, MEL can also increase the concentration of GSH and AsA [34,37,83–86] (Figure 2).

On the other hand, MEL acts decreasing EL and MDA levels, thus alleviating abiotic stress-related membrane damage [87,88]. MEL also increases the transcription of stress tolerance-related genes, as well as activates downstream signalling transduction pathways [89] (Figure 2).

In terms of photosynthetic efficiency, MEL reduces chlorophyll degradation, thus improving photosynthetic efficiency during stress conditions [90]. It also regulates key proteins accumulation such as 1,5 bisphosphate carboxylase/oxygenase (Rubisco) and improves the efficiency of photosystem II reaction centres [66,90,91]. Another significant aspect is that MEL increases leaf area, which improves photosynthesis rate [85] (Figure 2). Wei et al. [92] showed that MEL can also improve the expression of ferredoxin, photosystem I subunits (PsaK and PsaG) and photosystem II-related elements (PsbO and PsbP).

Lee & Back [93] showed that MEL interacts with ROS and RNS signalling pathways, which has been described recently as a mechanism that improves plant stress tolerance via modulating the activity of key antioxidant enzymes, which resulted in an alleviation in photosynthesis inhibition and a modulation of several transcription factors [93]. It has also been proved that MEL alleviates stress effects via the increasing of NO thanks to the upregulation of NR and NO synthase-related activities, coordinating also polyamine pathway [94].

NO formation in response to abiotic stress is common in several plant species. NO acts as a signalling molecule which regulates key responses including osmolyte accumulation, oxidative defence, photosynthesis modulation, gene expression or PTMs in proteins [95]. NO, as well as MEL, can directly act as antioxidant by directly scavenging free radicals, alleviating oxidative damage in a receptor-independent manner [36] (Figure 2).

NO is capable of balancing redox homeostasis by inducing PTMs in essential enzymes, mainly S-nitrosylation (covalent binding of NO to cysteines thiol group) and tyrosine nitration (nitro group addition to one the aromatic ring of tyrosine residues), but also metal nitrosylation, which regulates its activity. These PTMs regulates the activity of these enzymes, specially antioxidant enzymes, but it can also affect molecules such as AsA/GSH, modulating its antioxidant capacity [96]. Among proteins regulated by NO, Ca^{2+}-sensitive channels and proteins kinases are involved in the signalling cascade that leads to the adaptive response to stresses, germination, adventitious root formation and stomatal closure [97].

It has been proposed that MEL action takes place thanks to a feedback mechanism modulated by H_2O_2 and NO, molecules which are essential for plant stress responses.

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Moreover, it is thought that interactions between NO and MEL cause PTMs of key stress-related proteins, which could be confirmed using proteomics analysis [37].

Plant exposition to over optimal temperatures (5ºC above its optimal temperature) leads to heat stress. MEL boosts the levels of antioxidant molecules such as phenolic compounds, flavonoids (via NO-dependent pathways) and carotenoids [85,98] under this conditions. In addition, in kiwifruit, Liang et al. [99] showed that exogenous MEL increased the expression of variety of glutathione S-transferase genes, which alleviated the oxidative stress caused by high temperatures. NO can also alleviate heat stress by maintaining the activity of 1,5 bisphosphate carboxylase/oxygenase (Rubisco) and enhancing photosynthetic nitrogen and sulfur-use efficiency [100].

On the other hand, suboptimal temperatures are also harmful. Exogenous MEL is capable of increasing plant, seeds, callus and explants cold resistance [10]. In tomato, MEL improved chilling tolerance by upregulating arginine pathway, which leads to higher NO levels. This lead to the maintenance of membrane integrity thanks to a decrease in EL and MDA accumulation [31]. MEL treatment, in *Arabidopsis*, was capable of modulating gene expression, causing the upregulation of *CBFs, COR15a*, *CAMTA1* and *ZAT10/12*, thus alleviating cold stress [101].

In tomato plants, under both heat and cold stress, MEL was capable of increasing the activity of at arginine pathway, which led to a higher polyamines levels [31,94].

Salt stress causes excessive Na^+ accumulation, which leads to osmotic stress and high ROS levels. Zhao et al. [102] showed that MEL and NO-releasing compounds can maintain Na^+/K^+ ratio during salt stress by modulating NHX1 (sodium hydrogen exchanger) and *SOS2* (salt overly sensitive 2) transcription levels. Thereby, NO is required for mediating MEL action in this situation [102]. GSH/GSSG ratio is an indicator of oxidative stress regulated by GR. During salt stress, both MEL and NO are capable of differentially regulating GR activity (thus, GSH levels), alleviating stress damage in sunflower seedlings [34,103].

Under stress conditions, MEL acts regulating NO levels. Stress induced by NaCl, $ZnSO_4$ and H_2O_2 resulted in an increase in MEL content in a time-dependent manner in barley roots [104]. In rapeseed, MEL induced NR and NOA1 (NO associated 1)-dependent NO formation, although NO was not responsible for MEL formation under salt stress. This condition also triggers S-nitrosylation, induced by NO [102]. On the other hand, Arora and Bhatla [105] showed that NO was capable of reducing growth inhibition induced by salt stress in sunflower by triggering MEL accumulation, which modulated the expression of Cu/Zn SOD and MnSOD genes. Moreover, the interaction between MEL and NO also reduced deleterious effects of salt stress by decreasing tyrosine nitration of proteins and decreasing peroxynitrite content.

As water is essential for plants, the lack of it causes major damage to plants, leading to a situation known as drought stress [106]. It has been shown that exogenous MEL is capable of alleviating drought stress response by increasing cell turgor, photosynthetic rate and water-retention capacity [35].

Sharma et al. [85] demonstrated that *Carya cathayensis* plants subjected to drought stress that have been pre-treated with MEL showed less negative effects than untreated plants. In addition, MEL upregulated primary and secondary metabolisms, such as carotenoids pathway [85]. In alfalfa, MEL is capable of alleviating oxidative damage due to drought stress [35].

MEL can also act alleviating drought stress by improving plant photosynthesis. Liu et al. [107] showed that, in tomato, exogenous MEL increased stomatal conductance, net photosynthetic rate, conductance, transpiration rate, quantum yield of PSII, maximum quantum yield (Fv/Fm) and electron transport. In cucumber seedlings, exogenous MEL alleviated drought effects by reducing chlorophyll degradation and increasing photosynthetic rate [108].

Rhizospheric application of MEL also improves stress tolerance. For instance, in alfalfa, Antoniou et al. [35] showed that this type of MEL application enhanced drought tolerance by regulating ROS and RNS via the modulation of SOD, GR, CAT, APX, NR and

NADH dehydrogenase activity and/or transcription. In this example, MEL application caused a downregulation of NR, decreasing NO levels, thus pointing out that drought tolerance has been associated to reduce NO accumulation [109].

Several studies also show that both NO and MEL have a role during metal toxicity stress response [110].

In *Catharanthus roseus*, MEL and SNP (sodium nitroprusside, a NO donor) improved seedling growth under Cadmium (Cd) stress via the increase of photosynthetic pigments, Cd translocation, proline concentration and antioxidant enzymes activity (SOD, POD, APX and CAT), leading to a decrease in lipid peroxidation and H_2O_2 content. Moreover, seed germination and root antioxidant response is modulated by NO, downstream of MEL [111–113]. Also, in wheat, Cd toxicity led to an increase in NO levels. Kaya et al. [114] showed that MEL treatment was capable of enhance Cd tolerance, effect which was reversed after cPTIO (NO scavenger) addition, which suggested that MEL action might take place via NO increase. Conversely, under Cd toxicity, Wang et al. [115] showed that, in Chinese cabbage, NO upregulated IRT1 (a Cd absorption regulation related transport gene) expression, increasing Cd absorption, which led to an intensified stress situation. However, MEL acted inhibiting NO synthesis, therefore reducing Cd levels.

In terms of toxicity by other metals, as shown by Zhang et al. [116], exogenous MEL abolished NO production, alleviating aluminium-induced root growth inhibition. Meanwhile, in maize, Okant & Kaya [117] demonstrated that NO increased antioxidant enzyme activity, alleviating Pb toxicity.

Both NO and MEL can alleviate sodic alkaline stress via reduction of Na^+ levels and increase of K^+ uptake, as well as the enhancement of antioxidant enzymes activity [84].

Although light is essential for plants, under high light stress conditions, NO can also interact with other molecules such as H_2O_2 (which induces stomatal closure) or inositol, mediating UV-B-initiated oxidative stress situation [118,119]. In maize seedlings, NO application improved high light tolerance by increasing flavonoids, anthocyanins, MDA and UV-B absorbing-compounds levels, as well as increasing CAT and APX enzyme activity [120].

The climate predictions for the coming years tend to be more adverse, with devastating increases in temperature, salinity and water scarcity. In nature, stresses do not act isolated, but in combination [121], which means that the plant is simultaneously subjected to two or more abiotic stresses [122]. It has been shown that the plant response to combined stress cannot be elucidated from the study of single stresses [123,124], so NO and MEL role under abiotic stress combination may different from what has been described under single stress experiments.

Recent studies by Martinez et al. [125] made in tomato plants showed that, under heat and salt stress combination, MEL enhances stress tolerance by protecting photosynthetic apparatus and promoting ROS detoxification. Plants subjected to MEL treatment showed less lipid peroxidation and protein oxidation than non-treated plants. Exogenous MEL modulated the expression of key oxidative-metabolism related genes, such as the genes coding APX, GR, GPX and Ph-GPX enzymes, which lead to a reduction is ROS levels [125].

MEL also modulates osmoregulators concentration in high temperature stress conditions, mainly carbohydrates (as trehalose) and amino acids (as proline) [126]. This response was also found under stress combination (cold and drought stress) in rice plants [17]. During cold and drought stress combination, in cucumber, tomato and tobacco, 2-hydroxymelatonin alleviated cell damage by lowering MDA production [127].

Given the few studies carried out in the field of abiotic stress combination, it is necessary to continue investigating into the role of MEL and NO in the response to stress combination, and moreover, into the signaling mechanisms involving the interaction of both molecules in the light of increasing plant stress tolerance to climate change.

4.1. NO and MEL interaction with hormones during stress response

Both MEL and NO interact with the main plant hormones, such as auxins (AUXs), cytokinins (CKs), ethylene and abscisic acid (ABA), modulating stress response.

ABA is an essential phytohormone during stress response, so its interaction with NO and MEL could provide a better understanding of the mechanisms involved in stress tolerance [128]. MEL can act via ABA signalling transduction pathways, modulating the response to salinity and drought stress respectively [84,85]. In cucumber or apple plants, under stress situations, exogenous MEL can downregulate ABA biosynthesis and upregulate its catabolism by promoting the expression of ABA catabolism-related genes such as *CYP707* and repressing the expression of ABA biosynthesis-related genes such as *NCED2* or *MdNCED3* [129,130]. Contrary, in water-stressed maize, exogenous MEL had no effect on ABA levels [131] and, in chilling-stressed cucumber, MEL treatment even triggered ABA production during the firsts 4 days [132].

As described, MEL effect in ABA levels is still controversial, although it is clear that MEL modulates ABA signalling transduction pathways, as MEL is capable of regulating ABA receptors either inducing or repressing them [87,133].

ABA can also act mediating NO-MEL signalling and Li et al. [129] also proved that MEL is capable of activating ABA-mediated signalling pathways. NO metabolism is also responsible of regulating ABA homeostasis via PTMs, which also modulate the activity of proteins from ABA-mediated signalling pathways. Moreover, NO and ABA are both capable of regulating antioxidant systems’ activity, although other molecules such as H_2S and MEL can also be responsible of regulating these pathways [134]. Moreover, ABA can also induce PMTs during stress response. Specifically, ABA induces S-nitrosylation of SnRK6.2/OST1 at Cys-137, inhibiting its kinase activity, which also be induced by NO. As this kinase is part of ABA signalling pathways, this results suggest that both ABA and NO regulate ABA signalling via a negative regulatory loop [168].

MEL, due to its auxin-like activity, is able to stimulate and modulate root generation and growth, as well as enhancing adventitious root formation [42]. Under stress conditions, MEL effect stimulating plant growth is higher than under favourable conditions, as shown in salt-stressed maize or cold-stressed Arabidopsis [101,135]. There is also evidence of NO action during auxin signalling pathway. During Fe deficiency, in roots, Chen et al. [136] showed a correlation between auxin availability and NO levels, enhancing root ferric-chelate reductase activity, thus improving Fe uptake.

Ethylene not only intervenes during fruit ripening, but also during stress response. In alfalfa, MEL inhibited ethylene biosynthesis by downregulating ethylene biosynthesis-related genes. Under these conditions, MEL also promoted the accumulation of polyamines by upregulating polyamine metabolism-related enzymes, thus alleviating waterlogging stress [137].

MEL can also interact with gibberellins (GAs). Zhang et al. [130] demonstrated that cucumber seedlings subjected to salt stress treated with MEL showed higher GA content due to the upregulation of *GA20ox* and *GA3ox*, GA biosynthesis genes, and downregulation of ABA biosynthesis genes.

During heat stress, exogenous MEL increased CK levels by upregulating *LpIPT2* and *LpOG1*, key CK biosynthesis-related genes, while, under non-stress conditions, MEL treatment did not modify CK levels. Moreover, CK signalling pathway was also altered by MEL via the modulation of A-ARRs and B-ARRs, transcription factors involved in CK signalling pathways [138]. NO also mediate tolerance to drought stress by modulating CK-induced photosynthetic resistance, enhancing parameters such as PSII electron donation capacity or plant photosynthetic performance index (PI). Moreover, during this response, CK increased NO synthesis via NR, which suggests that there is a crosstalk between CK and NO during stress response [139].

4.2. Interaction with H_2S in mediating stress tolerance

As it has been previously mentioned, hydrogen sulfide (H_2S) is a reactive gaseous molecule which modulates stress tolerance, fruit ripening and antioxidant-enzymes action

[140]. Therefore, due to the similarity between its role and the one played by NO and MEL, it is interesting to mention the interaction between these three molecules (Figure 3).

NO and H_2S have been reported to act both synergistically and antagonistically to each other. H_2S is capable of reducing NO accumulation by triggering stomatal opening [141]. However, during salt stress, H_2S reduced oxidative damage by inducing NO production [142]. Moreover, NO was capable of alleviating hypoxia stress by triggering the activation of enzymes involved in H_2S biosynthesis [143].

In banana, H_2S enhanced chilling stress tolerance by inhibiting ethylene production, function that can be also made by NO and MEL. It can also promote cold-stress tolerance by decreasing EL and MDA levels, as well as upregulating Ca^{2+}-ATPase activity, a key secondary signalling enzyme involved in energy metabolism [144].

As shown by Li et al. [145], Cd stress induced H_2S expression by upregulating *LCD*, *DCD* and *DES1*. This molecule could act as a signalling molecule modulating antioxidant enzymes, such as SOD, CAT, POD and APX, alleviating Cd-induced oxidative stress [145]. As mentioned, both exogenous H_2S and NO can alleviate Cd stress response. H_2S was capable of increasing NO synthesis in alfalfa seedlings [146]. On the other hand, NO also enhanced H_2S production in bermudagrass and wheat [147,148]. These responses have been seen during Cd stress, which indicates that there is an NO/H_2S crosstalk that enhances Cd stress tolerance.

In addition, MEL can also interact with H_2S during abiotic stress response. In tomato cotyledons subjected to salt stress, MEL can modulate L-DES activity, thus regulating H_2S homeostasis [149]. In addition, Siddiqui et al. [150] showed that, in tomato seedlings, MEL-mediated salt stress tolerance involved a H_2S-dependent pathway.

Very recently, it has been suggested that stress tolerance induced by phytomelatonin might take place through H_2S and NO cascade. During salt stress, in pepper, both NO and H_2S were essential for stabilising MEL-induced stress tolerance. Similar results have been seen in cucumber under salt stress, confirming that NO and H_2S act downstream MEL during stress response [151,152].

5. NOmela: an emerging molecule with important stress signaling roles

As suggested during this review, the convergence of MEL and NO in many physiological, biochemical and molecular events in plant cells is not fortuitous, and recently there has been increased interest in a new emerging molecule with important role in plant cells. In situations involving the presence of oxygen, MEL can be NO-nitrosated in the nitrogen atom of the indole ring, giving N-Nitrosomelatonin (NOmela). This nitrosated form of MEL is an effective NO donor and is involved in redox signalling in plants [27,153–155]. The transference of nitroso groups from N-nitrosotryptophan derivates to MEL can also derivate in the formation of NOmela. This transnitrositation reaction is very strong and it can’t be inhibited by RNS scavengers, as shown in Kirsch & De Groot [156].

An advantage provided by NOmela as NO-releasing compound is that NO release from NOmela is independent from buffer composition, meanwhile it does affect NO release from GSNO. Due to that, it is believed that NOmela is a better NO precursor in cell-culture. Moreover, the MEL generated by NOmela is a strong antioxidant that can protect cells in culture. This molecule is capable of releasing both NO and MEL, thus combining the beneficial proprieties of both molecules. Due to the simultaneous releasing of NO and MEL during NOmela breakdown, cells are protected from harmful effects of the RNS formed by NO autoxidation thanks to MEL. *In vivo*, NOmela is capable of releasing NO, MEL and SNOs without promoting the generation of hydroxyl radicals, avoiding the cytotoxic effects given by them [27,157].

In mammals, NOmela has been proved to modulate circadian rhythms. The NO released from this compound was capable of enhancing its photic synchronization via increasing inmunoreactivity of key genes, as shown by Baidanoff et al. [158]. Therefore, it is possible that this compound is also capable of regulating various responses and physiological processes in other eukaryotes, as plants.

Roots are the first organs in being affected by soil-mediated abiotic stress. For example, sunflower seedlings show higher MEL concentration in roots than cotyledons after being exposed to salt stress. It has been also proved that abiotic stress situations can trigger long distance signalling mediated by MEL from roots to aerial parts [159]. Due to NO short half-life, NO transport from roots to aerial parts still needs further research, although molecules such as NOmela or S-nitrosothiols as GSNO could participate in NO long distance transport. Moreover, *in vivo* studies performed by Singh et al. [160] in *Arabidopsis* seedlings show that NOmela facilitates NO transport from roots to leaves, being a more efficient NO donor and transporter than GSNO (Figure 4A).

As mentioned previously, NO can modify protein function or activity via PTMs, so our thoughts are that NOmela could also act through this mechanism, which it will be detailed explained as follows. The main PTM generated by NO is a reversible redox modification called S-nitrosylation, which is characterised by the addition of a nitroso group to a thiol group present in cysteines (Cys) [161]. It can modulate protein stability, activity, subcellular localization, conformation and protein-protein interactions. Most of the times, S-nitrosylation takes place as a non-enzymatic process, thanks to the mediation of NO, SNOs, $ONNOO^-$ or higher nitrogen oxides (NO_x), without the interplay of any co-factor or enzyme-like protein. However, transnitrosylation do involve enzymes called transnitrosilases, which can transfer a nitroso group to a Cys residue [62]. This PTM is involved in important physiological processes, such as xylem vessel cell differentiation, as shown by Kawabe et al. [162]. Moreover, it can regulate plant growth and development by positively regulating auxin signalling and negatively regulating cytokinin signalling [163,164]. Plant response to abiotic stress is also regulated by S-nitrosylation; for instance, in response to hypoxia conditions during seed germination, GSNOR1 is subjected to S-nitrosylation, which leads its degradation [62,165]. Tyrosine nitration is another common PTM mediated by NO, which can alter the activity of SOD, modifying ROS signalling balance [166]. During stress response, protein arginine methylation was also modulated by NO-mediated S-nitrosylation, which indicates that cellular signalling could be regulated via interactions between different PTMs [167].

Given the versatility of NO inducing PTMs, NOmela could have a similar role, since NO carriers, such as LMM-SNOs, can also transnitrosate cysteine residues of proteins, altering their activity and functionality [168]. In fact, it has been demonstrated that NOmela is capable of transnitrosating proteins, as well as vitamins and aromatic amines, modulating their activity. Moreover, NOmela can also react with thiols, which makes NOmela able to transnitrositate several proteins in Cys residues, as shown by Kirsch & de Groot [169] (Figure 4B). Due to the importance of PTMs in regulating plant development and stress response, it would be key to deepen in NOmela ability to induce protein PTMs.

6. Conclusions and future perspectives

Both MEL and NO have a fundamental role in different molecular pathways present in plants. The synthesis, accumulation, transport and action mechanisms of both molecules is fundamental from the first stages of development to more advanced stages such as fruit ripening. In addition, they also play a fundamental role in modulating the molecular response to abiotic stresses, which requires further knowledge of the mechanisms by which this modulation is performed, deepening into the interaction of these molecules with phytohormones or with other signaling molecules such as H_2S.

There is evidence that both molecules are capable of interacting, both at the level of biosynthesis and in more complex molecular pathways that regulate different processes, with agonistic and antagonistic interactions between them. Despite the studies presented in this review, there is still a great lack of knowledge about the exact relationship between these two molecules in numerous physiological processes, so further research is still necessary.

Given its ability to transport NO, release MEL and induce protein PTMs, the study of NOmela promises to be a powerful source of knowledge to better understand the molecular pathways that regulate physiological processes and stress responses in plants. Despite this, NOmela detection tools are scarce and imprecise [157], and it is necessary to develop them correctly in order to properly investigate this molecule.

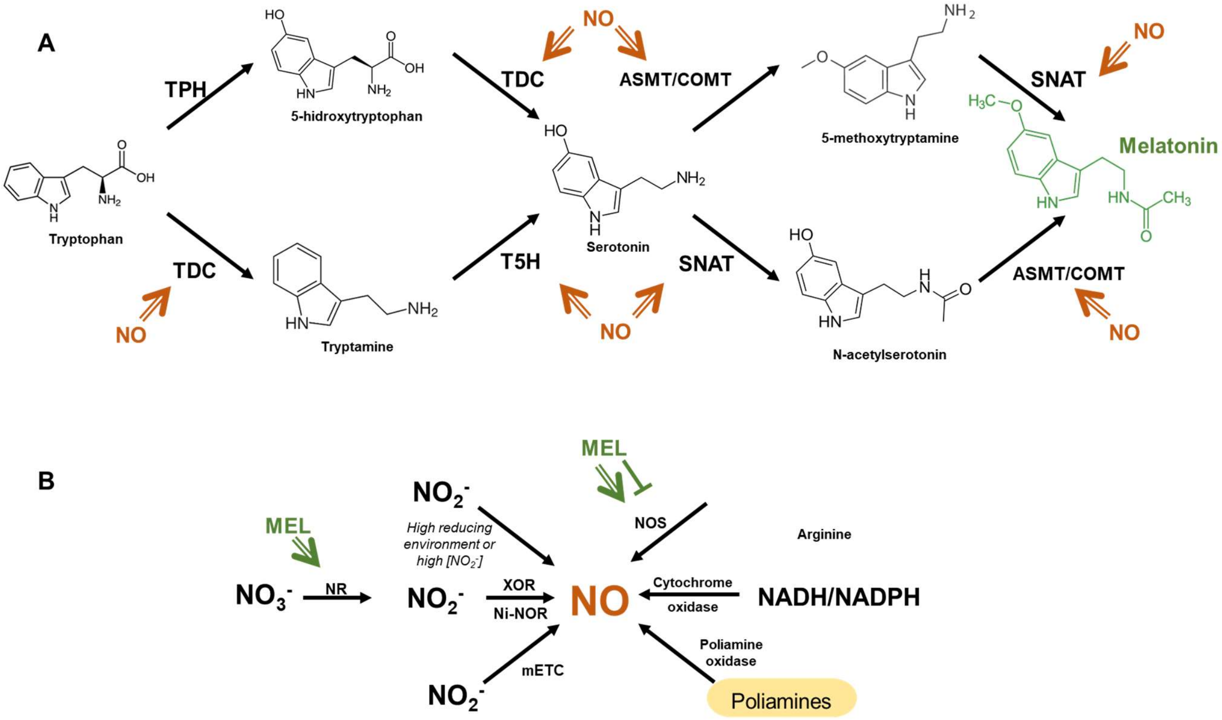

Figure 1. Melatonin (A) and NO (B) biosynthetic pathways. The figure shows the specific points in the melatonin biosynthetic pathway that NO is able to regulate, and vice versa through the modification of key enzymes within each pathway.

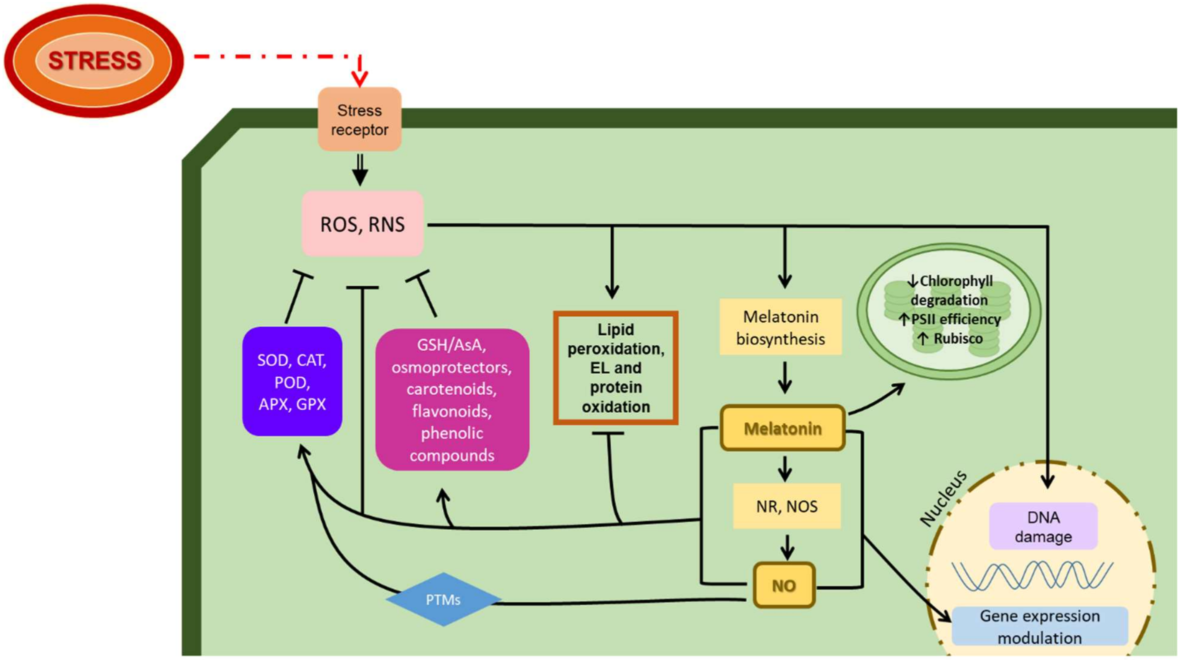

Figure 2. General mechanisms for MEL and NO-mediated stress response. Stress causes an increase in ROS and RNS levels, which are damaging for plant cells. Increased ROS levels trigger MEL production, which can also promote NO synthesis. Both MEL and NO directly scavenge ROS and RNS, as well as promote the activity of antioxidant enzymes, the accumulation of antioxidant molecules and osmoprotectors, and influence gene expression, thus alleviating stress cell effects.

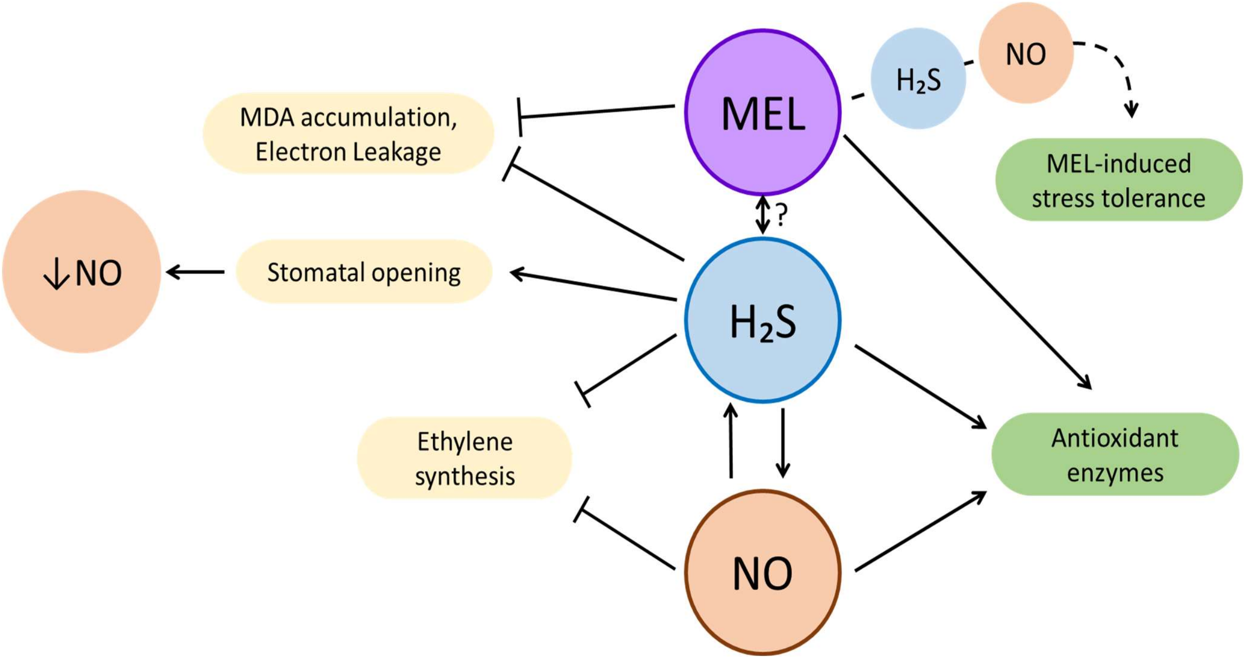

Figure 3. MEL, NO and H_2S interactions during abiotic stress response.

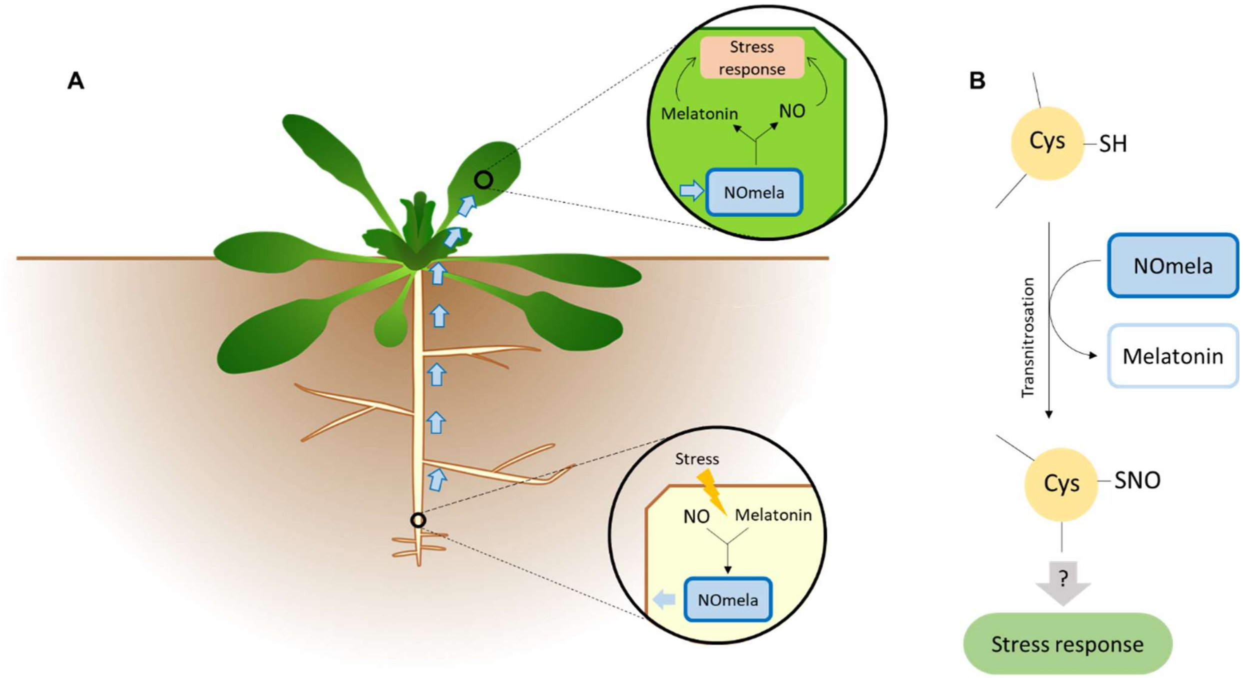

Figure 4. A) Proposed mechanism for NO and MEL transport from roots to aerial parts via NOmela synthesis during stress conditions. Stress induces NOmela formation from NO and MEL, which can be transported to aerial parts, where it breakdowns into NO and MEL for triggering stress response. B) Proposed mechanism for NOmela-mediated molecules transnitrosation mechanism under stress.

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