Melatonin and Plant Cadmium Tolerance

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Cadmium (Cd) is one of the most injurious heavy metals, affecting plant growth and development. Melatonin (N-acetyl-5-methoxytryptamine) was discovered in plants in 1995, and it is since known to act as a multifunctional molecule to alleviate abiotic and biotic stresses, especially Cd stress. Endogenously triggered or exogenously applied melatonin re-establishes the redox homeostasis by the improvement of the antioxidant defense system. It can also affect the Cd transportation and sequestration by regulating the transcripts of genes related to the major metal transport system, as well as the increase in glutathione (GSH) and phytochelatins (PCs).

antioxidant defense systems   Cd stress   hydrogen sulfide   melatonin   oxidative stress

transportation and sequestration

1. Introduction

Heavy metal pollution is the most widespread contamination resulting from anthropogenic activities in the world [1]. It has raised concerns about its various harmful risks to human health via the metal transfer along the food chain [2]. Among the heavy metals, cadmium (Cd) is a toxic element and poses a hazardous impact to living organisms, such as renal tubular dysfunction and bone disease [3]. In plants, Cd disturbs a range of important biochemical, morphological, physiological, and molecular processes, thus resulting in chlorosis and shunted growth [4][5]. Cd stress deceases the chlorophyll content, net photosynthetic rate, stomatal conductance, intracellular CO_2 concentration, and transpiration rate [4][5][6]. Cd stress induces the excess accumulation of reactive oxygen species (ROS), mainly due to the imbalance between ROS generation and scavenging [7][8]. Increased concentrations of ROS further induce the lipid peroxidation and oxidative damage, destructing plant membranes, macromolecules, and organelles [7][8]. Additionally, excessive bioaccumulation of Cd in plants inhibits Fe and Zn uptake, and disrupts the uptake and transport of K, Ca, Mg, P, and Mn [9]. In response to Cd stress, plants have evolved the complex biochemical and molecular mechanisms that modulate ROS homeostasis and Cd compartmentation and chelation [7][10][11][12]. Plant hormones (ethylene, salicylic acid (SA), abscisic acid (ABA), jasmonic acid (JA), auxin, brassinosteroids (BRs), and strigolactones (SLs)) and signaling molecules (nitric oxide (NO), carbon monoxide (CO), hydrogen sulfide (H_2S), and Ca^{2+}) are involved in plant response to Cd stress [13][14]. Moreover, recent studies have reported that melatonin acts as a master regulator in plant Cd tolerance.

Melatonin (*N*-acetyl-5-methoxytryptamine) was discovered in plants in 1995, and it is since known to act as a pleiotropic molecule to participate in multiple physiological processes, such as plant growth and development, and protection against abiotic and biotic stresses [15][16]. In recent years, numerous studies have focused on the

protective role of melatonin against Cd stress in plants [17]. Application of exogenous melatonin increased photosynthetic pigments, and improved relative water content and stomatal conductance in mallow plants upon Cd stress [18]. Many results showed that melatonin could re-establish redox homeostasis by certain enzymatic and non-enzymatic antioxidant defense systems to alleviate Cd-induced oxidative stress [19][20]. In addition, melatonin decreased Cd accumulation via regulating the transcripts of several heavy metal transporter genes to restrict Cd influx, and promote Cd efflux and chelation [19][21]. Moreover, NO and hydrogen peroxide (H_2O_2) signaling, microRNAs, heat shock factor HsfA1a and flavonoids may be involved in melatonin-mediated Cd tolerance in plants [19][22][23][24][25].

2. Role of Melatonin in Plant Abiotic Stress Responses

2.1. Melatonin Biosynthesis and Catabolism

The melatonin metabolic pathway in plants contains two major parts: biosynthesis and catabolism (**Figure 1**). Melatonin was discovered and confirmed by an isotope tracer study of St. John's wort (*Hypericum perforatum* L. cv. Anthos) seedlings [15][26]. It was found that melatonin is synthesized via four continual enzymatic reactions from tryptophan, requiring at least six enzymes: tryptophan decarboxylase (TDC), tryptophan hydroxylase (TPH), tryptamine 5-hydroxylase (T5H), *N*-acetylserotonin methyltransferase (ASMT), and serotonin *N*-acetyltransferase (SNAT) [17]. T5H-catalyzed hydroxylation of tryptamine is an important step of melatonin biosynthesis in rice (*Oryza sativa*) [27]. In animals, serotonin is initially acetylated to form *N*-acetylserotonin, and then *O*-methylated to form melatonin (named NM pathway) [28]. It has also been found that serotonin is *O*-methylated to form 5-methoxytryptamine, and then acetylated to form melatonin (named MN pathway) [28]. Both NM and MN pathways exist in plants [29].
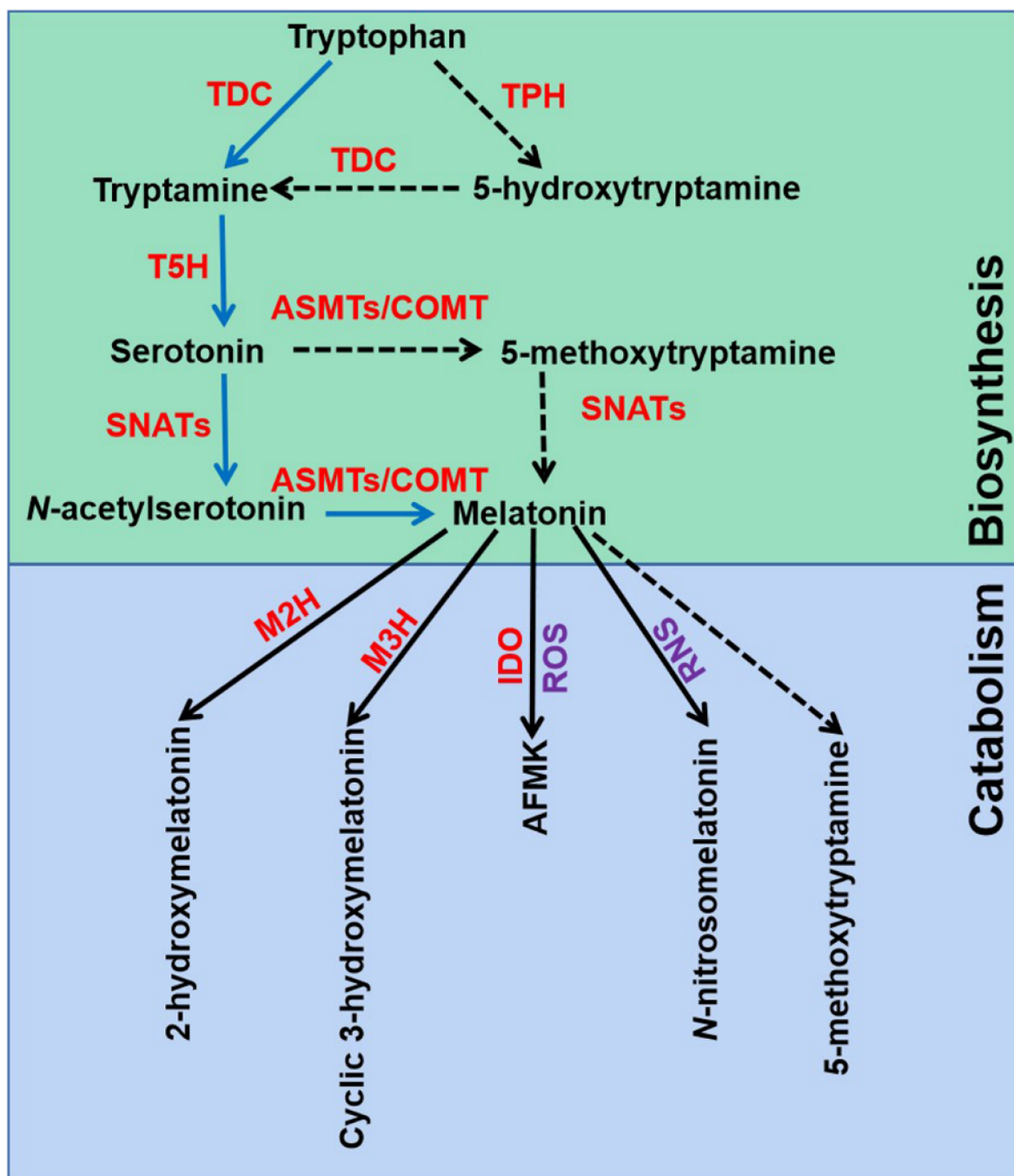
Figure 1. Melatonin biosynthesis and metabolic pathways in plants. TDC, tryptophan decarboxylase; T5H, tryptamine 5-hydroxylase; TPH, tryptophan hydroxylase; SNATs, serotonin N-acetyltransferases; ASMTs, N-acetylserotonin-O-methyltransferases; COMT, caffeic acid O-methyltransferase; M2H, melatonin 2-hydroxylase; M3H, melatonin 3-hydroxylase; IDO, indoleamine 2,3-dioxygenase; AFMK, N^1-acetyl-N^2-formyl-5-methoxykynuramine; ROS, reactive oxygen species; RNS, reactive nitrogen species. The green box indicates melatonin biosynthesis pathways, and blue box indicates melatonin metabolic pathways.

Melatonin can be degraded by two distinct routes: non-enzymatic and enzymatic transformations [17]. Transgenic tomato (*Solanum lycopersicum*) plants expressing the gene encoding indoleamine 2,3-dioxygenase (IDO) in rice showed reduced melatonin levels [30]. Thus, the pathway that melatonin converts to N^1-acetyl-N^2-formyl-5-

methoxykynuramine (AFMK) exists in plants. Tan and Reiter speculated that AFMK is the product of melatonin interaction with ROS, which generated during photosynthesis [28]. This might reflect the important role of melatonin in detoxifying ROS accumulation. In addition, melatonin hydroxylation metabolites, 2-hydroxymelatonin (2-OHMel) and cyclic 3-hydroxymelatonin (c3-OHMel), have been identified in plants. Their formation is attributed to melatonin 2-hydroxylase (M2H) and melatonin 3-hydroxylase (M3H), respectively [31][32][33]. Singh et al. suggested that *N*-nitrosomelatonin (NOmela) likely served as a nitric oxide (NO) carrier that participated in the redox signal transduction [34]. Nevertheless, Mukherjee considered that NOmela served as an intracellular NO reserve in plants was questionable due to its sensitive and unstable characteristics [35]. The processes of NOmela formation and transport are not fully understood and should be thoroughly investigated. In addition, whether 5-methoxytryptamine (5-MT) formed by melatonin deacetylation is of physiological importance remains to be investigated in plants.

2.2. Melatonin Acts as a Master Regulator in Plant Abiotic Stress

As a master regulator, melatonin plays important roles in plant tolerance to abiotic stresses, such as heavy metals, drought, salinity, cold, heat, waterlogging, and pesticides [19][36][37][38][39][40][41]. This review shows schematically the melatonin-mediated responses to abiotic stresses in plants (**Figure 2**). Melatonin levels are strongly induced by the above unfavorable conditions. For instance, endogenous melatonin level in *Arabidopsis* wild-type plants was increased in response to salt stress [36]. Loss-of-function mutation *atsnat* in the *AtSNAT* gene showed lower endogenous melatonin content and sensitivity to salinity stress [36]. Cold stress induced melatonin accumulation by upregulating the relative expression of *ClASMT* in watermelon plants [38]. In tomato seedlings, Cd stress induced *COMT1* expression, and thereby improved the accumulation of melatonin [22]. Transcription factor heat shock factor A1a (HsfA1a) bound to the *COMT1* gene promoter and activated the transcription of *COMT1* gene under Cd stress [22]. However, the post-translational regulation of melatonin biosynthesis genes and modification of related proteins still remains largely unknown and should be elucidated in future.
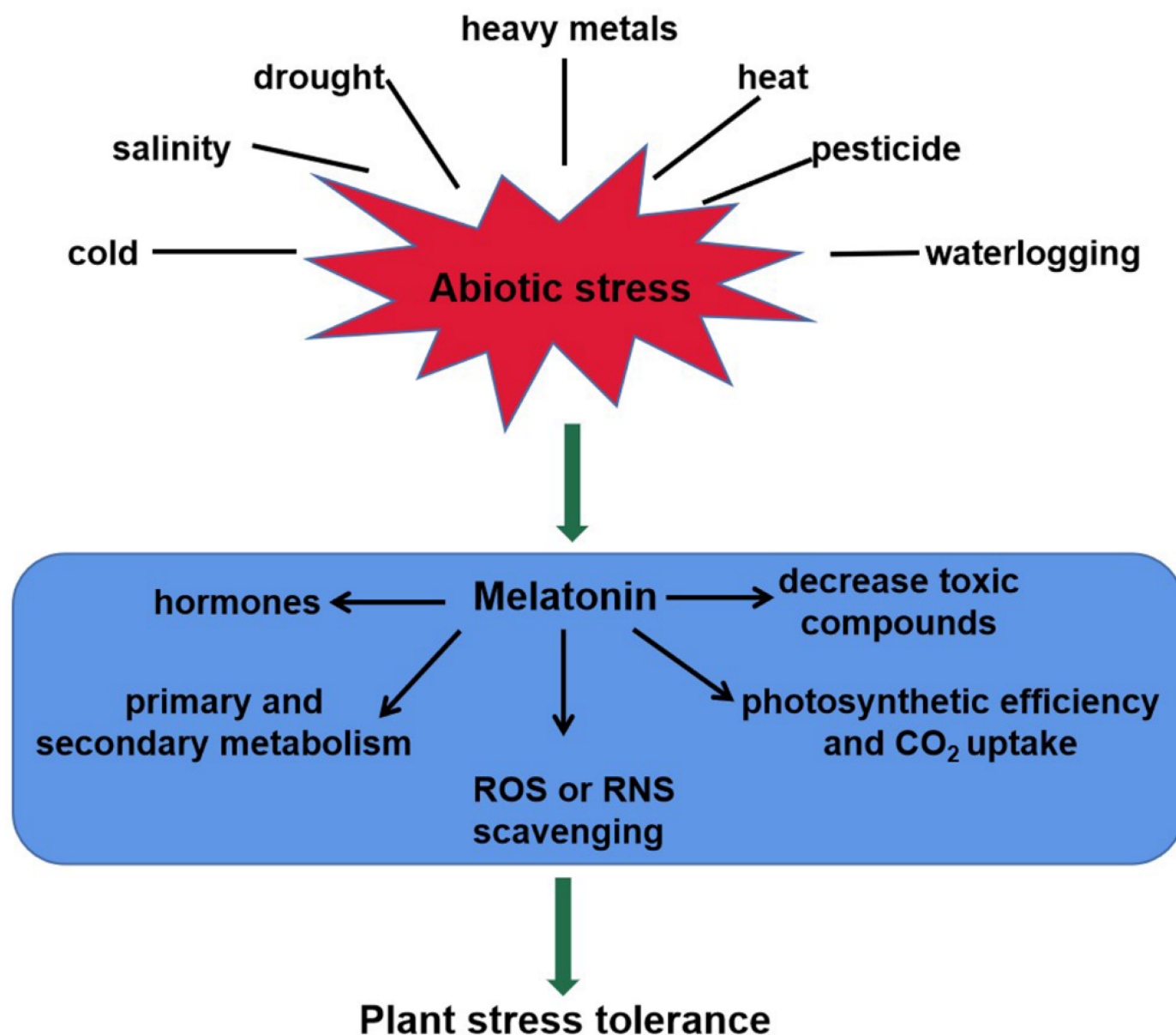
Figure 2. The roles of melatonin in plant tolerance to abiotic stress. Melatonin content of plants increases significantly in responses to abiotic stresses, such as heavy metals, salinity, drought, heat, cold, waterlogging, and pesticides. It confers plant tolerance via multiple mechanisms, including ROS or RNS scavenging, toxic compounds decrease, photosynthetic efficiency increase, interaction with hormones, and secondary metabolite biosynthesis. ROS, reactive oxygen species; RNS, reactive nitrogen species.

Melatonin confers plant tolerance via multiple mechanisms, including photosynthetic efficiency increase, ROS or RNS scavenging, toxic compounds decrease, interaction with hormones, and secondary metabolite biosynthesis (**Figure 2**). Melatonin stimulated stomatal conductance and improved photosynthesis, thus enhancing tolerance to water-deficient stress in grape cuttings [42]. Another fact is that the photosynthetic efficiency was maximized by higher rates of CO_2 assimilation and stomatal conductance after application of melatonin [43]. Several stresses can induce ROS or RNS accumulation, causing oxidative damage to plants [44]. In this case, melatonin re-establishes the redox balance via activating enzymatic antioxidant defense systems, as well as the ascorbate–glutathione

(AsA-GSH) cycle [45]. In plants, the Salt-Overly Sensitive (SOS) pathway mediates ionic homeostasis and contributes to salinity tolerance [46]. This pathway comprises three crucial genes, Salt-Overly Sensitive1 (*SOS1*), Salt-Overly Sensitive2 (*SOS2*) and Salt-Overly Sensitive3 (*SOS3*), which function together to initiate transport of Na^+ out of the cell, or activating other transporters, thus leading to the sequestration of Na^+ in the vacuole [47]. Melatonin reduced ion toxicity and improved salinity tolerance via the SOS pathway [36]. ABA and H_2O_2/NO signaling transduction pathways were also modulated for plant tolerance in response to abiotic stress [36][37][45][48]. In addition, melatonin could increase primary and secondary metabolites including amino acids, organic acids and sugars, and thus improving plant cold tolerance [49].

3. Melatonin Improves Cd Tolerance in Plants

It has been found that Cd affects the ecosystem, causing stress and toxicity in plants. Melatonin acts as a key role in protecting plants from Cd stress. **Table 1** summarizes that Cd treatment up-regulates the transcripts of melatonin biosynthesis genes, such as *TDC*, *T5H*, *SNAT*, *ASMT*, and *COMT* in *Arabidopsis thaliana*, *Oryza sativa* L., *Solanum lycopersicum*, *Triticum aestivum* L., *Nicotiana tabacum* L., and *Agaricus campestris* [48][50][51][52][53][54][55][56]. Therefore, melatonin contents are significantly increased. Notably, four *M2H* genes, involved in melatonin degradation, were also induced [54]. Byeon et al. suggested that both melatonin degradation and melatonin synthesis occurred in parallel, and 2-hydroxymelatonin of melatonin metabolite also acted as a signaling molecule in plant stress tolerance [54]. As melatonin catabolism is complicated, other pathways and the role of their metabolites should be investigated in plants under Cd stress.

Table 1. Summary table explaining the effect of Cd on genes related to melatonin metabolic pathway.

Plant Species	Cd Stress and Duration	Impact on Genes Related to Melatonin Metabolic Pathway	References
Solanum lycopersicum	100 µM Cd^{2+} for 15 d	*TDC*, *T5H*, *COMT* genes (leaves)	[22]
Oryza sativa L.	500 µM Cd^{2+} for 3 d	*TDC1*, *TDC3*, *SNAT1*, *SNAT2*, *ASMT*, *COMT*, *M2H*, *M3H* genes (seedlings)	[23]
Triticum aestivum L.	200 µM Cd^{2+} for 1 d	*ASMT*, *COMT*, *TDC* genes (root and shoot)	[51]
Nicotiana tabacum	10 mg/kg Cd^{2+} for 1,	*SNAT1* gene (leaves)	[52]

Plant Species	Cd Stress and Duration	Impact on Genes Related to Melatonin Metabolic Pathway	References
L.	4, and 7 d		
Agaricus campestris	2, 5, or 8 µM Cd^{2+} for 5 d	*TDC*, *T5H*, *SNAT*, *ASMT*, *COMT* genes	[53]
Oryza sativa L.	200 µM Cd^{2+} for 6, 12, 24, 72 h	*SNAT*, *ASMT*, *COMT*, *TDC*, *T5H* genes (leaves)	[54][56]
Arabidopsis thaliana	300 µM Cd^{2+} for 2, 3, 4 d	*SNAT*, *COMT* genes (leaves)	[55]

Most studies showed that melatonin alleviated Cd-induced seedling growth inhibition, including the biomass (fresh weight and dry weight) and root length [19]. Melatonin improved the photosynthesis rate (Pn), transpiration rate (E), intracellular CO_2 concentration and stomatal conductance (Gs) upon Cd stress in *Nicotiana tabacum* L. [6]. That melatonin enhanced stomatal opening and conductance capacity ultimately favored the photosynthesis in plants. Melatonin also prevented the degradation of the chlorophyll and carotenoid molecules in Chinese cabbage seedlings [57]. Similarly, application of melatonin improved chlorophyll and the maximum quantum efficiency of photosystem II (Fv/Fm) levels of wheat plants [20]. In chloroplasts, superoxide anion ($O_2^{\bullet-}$) in photosystem I (PSI) is generated by two molecules of O_2 with two electrons from photosystem II (PSII), and disproportionated to H_2O_2 catalyzed with superoxide dismutase (SOD) [58]. The better potential in melatonin treated plants under Cd stress can aid in chlorophyll protection, improve photosynthesis, and mediate redox homeostasis from oxidative damage.

# 4. A Possible Role for H_2S in Melatonin-Mediated Tolerance against Cd Stress

Acting as a signaling molecule, NO interacts with other molecules (H_2O_2, CO, and H_2S) to mediate plant growth and development, as well as abiotic stress responses [59]. Among the molecules, H_2S is also involved in almost all physiological plant processes [60][59]. To date, there is considerable research on the role of NO in melatonin-modulated plant abiotic stress tolerance. However, the functions of H_2S have been largely unknown. It will become a research hotspot to contribute to precise analysis of the collaboration between H_2S and melatonin, and provide deeper insight into melatonin-mitigated signaling mechanisms.

4.1. H_2S Action in Plant Tolerance against Cd Stress

H_2S acts as a signaling molecule in modifying various metabolic processes in plants, especially Cd stress (**Figure 3**, [60]). Endogenous H_2S production was induced via expression of *LCD*, *DCD*, and *DES1* under Cd stress [61][62]

[63]. SA, methane (CH_4), and WRKY DNA-binding protein 13 (*WRKY13*) transcription factor were suggested to be involved in the above process [64][65][66]. H_2S regulated the activities of key enzymes and AsA-GSH cycle involved in ROS homeostasis to alleviate Cd-induced oxidative stress [67][68][69][70][71][72][73][74]. For example, H_2S enhanced the activities of antioxidant enzymes, such as POD, CAT, APX, and SOD, and thereby decreased ROS accumulation [74]. Similarly, it also obviously increased AsA and GSH and the redox status (AsA/DHA and GSH/GSSG) levels to improve rice Cd resistance [68][70].
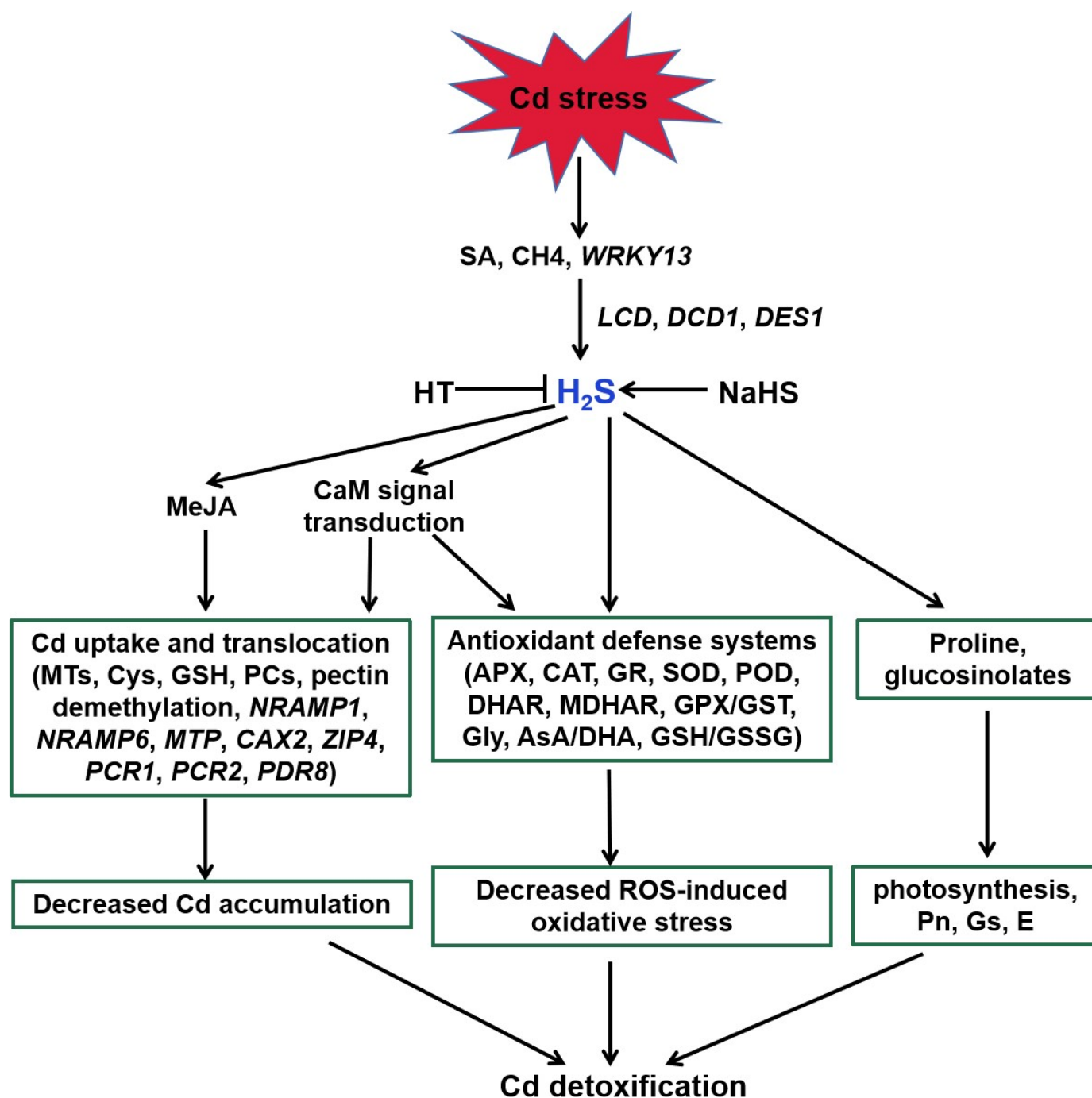

Figure 3. Function of H_2S in plant responses to Cd stress. SA, CH_4, and *WRKY13* are involved in Cd-induced H_2S generation. H_2S enhances the antioxidant defense systems to decrease the ROS accumulation, regulates the

transcripts of genes related to Cd uptake and translocation to reduce the Cd accumulation, and increases proline and glucosinolates in response to Cd stress in plants. MeJA and Ca participate in the above regulatory pathways. SA, salicylic acid; CH4, methane; HT, hypotaurine; LCD, L-cysteine desulfhydrase; DCD, D-cysteine desulfhydrase; *DES1*, L-cysteine desulfhydrase 1; MeJA, methyl jasmonate; CaM, calmodulin; *NRAMP1*, natural resistance-associated macrophage protein1; *NRAMP6*, natural resistance-associated macrophage protein6; *MTP*, metal tolerance protein; *CAX2*, vacuolar cation/proton exchanger2; *ZIP4*, zinc-iron permease4; *PCR1*, plant cadmium resistance1; *PCR2*, plant cadmium resistance2; *PDR8*, pleiotropic drug resistance8.

Increasing evidence demonstrates that H_2S also regulates Cd uptake and translocation in plants [64][71][73][75]. H_2S enhanced the expression of genes encoding metallothionein (MTs) and phytochelatin (PCS) in *Arabidopsis* roots [71]. Therefore, H_2S increased the metal chelators synthesis, contributing to Cd detoxification by binding the trace metal. In addition to enhancing the above genes expression, the protective effect of H_2S was attributed to a decrease in Cd accumulation associated with the expression of Cd transporter genes, such as *PCR1*, *PCR2*, and *PDR8* [64]. Exogenous application of NaHS weakened the expression of *NRAMP1* and *NRAMP6* genes, and intensified the expression of Cd homeostasis-related genes (*CAX2* and *ZIP4*) to enhance Cd tolerance in foxtail millet [76].

A number of studies address that H_2S can interact with other signaling molecules, such as SA, proline, MeJA, Ca, and NO during the responses of plants to Cd stress (**Figure 3** and **Figure 4**; [65][76][77]). H_2S acted as a downstream molecule of SA-transmitted signals to regulate Cd tolerance in *Arabidopsis* [65]. The endogenous production of proline and MeJA enhanced by H_2S donor NaHS responded significantly to Cd stress in foxtail millet [76][77]. H_2S also improved CaM gene expression and controlled the combination of Ca^{2+} and CaM, which act as signal transducers [78].
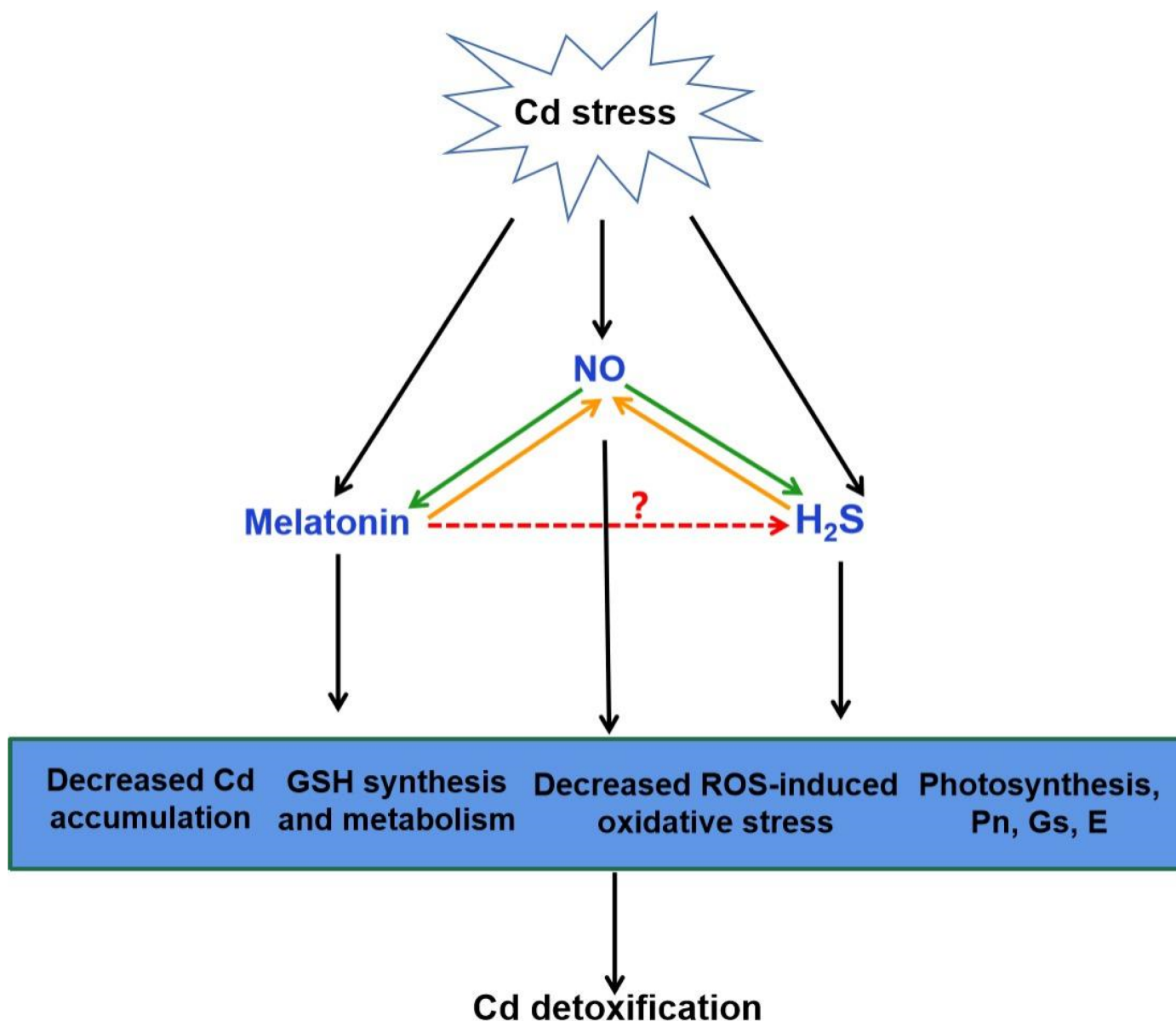
Figure 4. The possible role of H_2S in melatonin-mediated Cd detoxification. NO generation can be induced by Cd stress. Increasing evidence showed that melatonin and H_2S act as the downstream of NO in the responses to Cd stress, respectively (green arrow). It is also suggested that NO acts as a downstream of melatonin or H_2S to improve Cd tolerance (orange arrow). The combination of melatonin, NO and H_2S might be responsible for melatonin-triggered signal transduction in plant Cd tolerance via the decreased Cd accumulation, GSH synthesis and metabolism, decreased ROS-induced oxidative stress and improved photosynthesis. Red arrow, yet largely unknown. Cd, cadmium; NO, nitric oxide; H_2S, hydrogen sulfide; GSH, glutathione; ROS, reactive oxygen species; Pn, photosynthesis rate; Gs, stomatal conductance; E, transpiration.

There exists a complicated and synergistic relationship between H_2S and NO in response to Cd stress in plants (**Figure 4**; [69][72][79][80]). Exogenous NO and H_2S application increased the Cd tolerance in plants [69][79][81]. Subsequent pharmacological experiments proved that H_2S donor NaHS triggered NO production, which might act as a signal for alleviation of Cd-induced oxidative damage in alfalfa seedling roots [79]. Nevertheless, H_2S

production activated by NO is essential in Cd stress response of bermudagrass [69]. As a second messenger, Ca acted both upstream and downstream of NO signal, and crosstalk of Ca and NO regulated the cysteine and H_2S to mitigate Cd toxicity in Vigna radiata [81]. Moreover, application of sodium nitroprusside (SNP), the donor of NO, increased H_2S generation, and thus enhanced Cd stress tolerance in wheat [72]. However, this protective effect was reversed by hypotaurine (HT), the scavenger of H_2S [72]. These results suggested that H_2S and NO can function in a coordinated way under certain signaling cascades in plants under Cd stress.

4.2. Crosstalk of Melatonin and H_2S in Plants

The interaction between melatonin and H_2S plays a beneficial role in abiotic stress response [82]. Exogenous melatonin regulated the endogenous H_2S homeostasis by modulating the L-DES activity in salt-stressed tomato cotyledons [83]. Moreover, an endogenous H_2S-dependent pathway was involved in melatonin-mediated salt stress tolerance in tomato seedling roots [84]. Synergistic effects of melatonin and H_2S regulated K^+/Na^+ homeostasis, and reduced excessive accumulation of ROS by enhancing the activity of antioxidant enzymes. Inhibition of H_2S by HT reversed the melatonin-modulated heat tolerance by inhibiting photosynthesis, carbohydrate metabolism, and the activity of antioxidant enzymes in wheat [85]. Recent investigation has revealed that melatonin-induced pepper tolerance to iron deficiency and salt stress was dependent on H_2S and NO [72]. It was further confirmed that H_2S and NO jointly participated in melatonin-mitigated salt tolerance in cucumber [86]. Thus, these results postulate that H_2S might act as a downstream signaling molecule of melatonin. Combined with the roles of H_2S and melatonin in alleviating Cd stress, it is easy to speculate that H_2S might be involved in melatonin-mediated Cd tolerance in plants (**Figure 4**).

GSH plays a critical role in plant Cd tolerance. It is synthesized from glutamate, cysteine and glycine by γ-glutamyl cysteine synthetase (γ-ECS, encoded by *GSH1/ECS* gene) and glutathione synthetase (GS, encoded by *GSH2/GS* gene) [87]. The catalysis of GSH1 is the rate-limiting step of GSH biosynthesis [88]. Cd stress induced the transcripts of *GSH1* and *GSH2* in *Arabidopsis*, as well as *ECS* and *GS* in *Medicago sativa* [68][89][90][91]. It was suggested that H_2S could be quickly incorporated into cysteine and subsequently into GSH [92]. Application of NaHS re-established (h)GSH homeostasis by further strengthening the up-regulation of *ECS* and *GS* genes [68]. Similar results were also found in strawberry and cucumber plants [93][94]. Interestingly, exogenous melatonin also increased the GSH content by inducing the transcript levels of *SlGSH1* in tomato [95]. Hence, there might be a certain connection between H_2S and melatonin in regulating the GSH homeostasis at the transcriptional regulatory pathway. This will provide an interesting direction for further research on the complex interactions between melatonin and H_2S in improving Cd tolerance in plants.

References

1. Weissmannová, H.D.; Pavlovský, J. Indices of soil contamination by heavy metals-methodology of calculation for pollution assessment. Environ. Monit. Assess. 2017, 189, 616.

2. Clemens, S. Safer food through plant science: Reducing toxic element accumulation in crops. J. Exp. Bot. 2019, 70, 5537–5557.

3. Clemens, S.; Aarts, M.G.; Thomine, S.; Verbruggen, N. Plant science: The key to preventing slow cadmium poisoning. Trends Plant Sci. 2013, 18, 92–99.

4. DalCorso, G.; Manara, A.; Furini, A. An overview of heavy metal challenge in plants: From roots to shoots. Metallomics 2013, 5, 1117–1132.

5. Ismael, M.A.; Elyamine, A.M.; Moussa, M.G.; Cai, M.; Zhao, X.H.; Hu, C.X. Cadmium in plants: Uptake, toxicity, and its interactions with selenium fertilizers. Metallomics 2019, 11, 255–277.

6. Wang, M.; Duan, S.; Zhou, Z.; Chen, S.; Wang, D. Foliar spraying of melatonin confers cadmium tolerance in Nicotiana tabacum L. Ecotoxicol. Environ. Saf. 2019, 170, 68–76.

7. Sharma, S.S.; Dietz, K.J. The relationship between metal toxicity and cellular redox imbalance. Trends Plant Sci. 2009, 14, 43–50.

8. Pérez-Chaca, M.V.; Rodríguez-Serrano, M.; Molina, A.S.; Pedranzani, H.E.; Zirulnik, F.; Sandalio, L.M.; Romero-Puertas, M.C. Cadmium induces two waves of reactive oxygen species in Glycine max (L.) roots. Plant Cell Environ. 2014, 37, 1672–1687.

9. Khaliq, M.A.; James, B.; Chen, Y.H.; Saqib, H.; Li, H.H.; Jayasuriya, P.; Guo, W. Uptake, translocation, and accumulation of Cd and its interaction with mineral nutrients (Fe, Zn, Ni, Ca, Mg) in upland rice. Chemosphere 2019, 215, 916–924.

10. Noctor, G.; Mhamdi, A.; Chaouch, S.; Han, Y.; Neukermans, J.; Marquez-Garcia, B.; Foyer, C.H. Glutathione in plants: An integrated overview. Plant Cell Environ. 2021, 35, 454–484.

11. Cobbett, C.; Goldsbrough, P. Phytochelatins and metallothioneins: Roles in heavy metal detoxification and homeostasis. Rev. Plant Biol. 2002, 53, 159–182.

12. Rizwan, M.; Ali, S.; Adrees, M.; Rizvi, H.; Zia-ur-Rehman, M.; Hannan, F.; Qayyum, M.F.; Hafeez, F.; Ok, Y.S. Cadmium stress in rice: Toxic effects, tolerance mechanisms, and management: A critical review. Environ. Sci. Pollut. Res. Int. 2016, 23, 17859–17879.

13. Thao, N.P.; Khan, M.I.R.; Thu, N.B.A.; Hoang, X.L.T.; Asgher, M.; Khan, N.A.; Tran, L.S.P. Role of ethylene and its cross talk with other signaling molecules in plant responses to heavy metal stress. Plant Physiol. 2015, 169, 73–84.

14. Rabia, A.; Faiza, M.; Ghulam, K.; Tooba, I.; Maryam, K. Plant signaling molecules and cadmium stress tolerance. Cadmium Toler. Plants 2019, 367–399.

15. Hattori, A.; Migitaka, H.; Iigo, M.; Itoh, M.; Yamamoto, K.; Ohtani-Kaneko, R.; Hara, M.; Suzuki, T.; Reiter, R.J. Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. Biochem. Mol. Biol. Int. 1995, 35, 627–634.

16. Arnao, M.B.; Hernandez-Ruiz, J. Melatonin: A New Plant Hormone and/or a Plant Master Regulator? Trends Plant Sci. 2019, 24, 38–48.

17. Sun, C.; Liu, L.; Wang, L.; Li, B.; Jin, C.; Lin, X. Melatonin: A master regulator of plant development and stress responses. J. Integr. Plant Biol. 2021, 63, 126–145.

18. Tousi, S.; Zoufan, P.; Ghahfarrokhie, A.R. Alleviation of cadmium-induced phytotoxicity and growth improvement by exogenous melatonin pretreatment in mallow (Malva parviflora) plants. Ecotoxicol. Environ. Saf. 2020, 206, 111403.

19. Gu, Q.; Chen, Z.; Yu, X.; Cui, W.; Pan, J.; Zhao, G.; Xu, S.; Wang, R.; Shen, W. Melatonin confers plant tolerance against cadmium stress via the decrease of cadmium accumulation and reestablishment of microRNA-mediated redox homeostasis. Plant Sci. 2017, 261, 28–37.

20. Kaya, C.; Okant, M.; Ugurlar, F.; Alyemeni, M.N.; Ashraf, M.; Ahmad, P. Melatonin-mediated nitric oxide improves tolerance to cadmium toxicity by reducing oxidative stress in wheat plants. Chemosphere 2019, 225, 627–638.

21. He, J.; Zhuang, X.; Zhou, J.; Sun, L.; Wan, H.; Li, H.; Lyu, D. Exogenous melatonin alleviates cadmium uptake and toxicity in apple rootstocks. Tree Physiol. 2020, 40, 746–761.

22. Cai, S.Y.; Zhang, Y.; Xu, Y.P.; Qi, Z.Y.; Li, M.Q.; Ahammed, G.J.; Xia, X.; Shi, K.; Zhou, Y.; Reiter, R.; et al. HsfA1a upregulates melatonin biosynthesis to confer cadmium tolerance in tomato plants. J. Pineal Res. 2017, 62, e12387.

23. Lee, K.; Choi, G.H.; Back, K. Cadmium-induced melatonin synthesis in rice requires light, hydrogen peroxide, and nitric oxide: Key regulatory roles for tryptophan decarboxylase and caffeic acid O-methyltransferase. J. Pineal Res. 2017, 63, e12441.

24. Kyungjin, L.; Jin, H.O.; Reiter, R.J.; Kyoungwhan, B. Flavonoids inhibit both rice and sheep serotonin N-acetyltransferases and reduce melatonin levels in plants. J. Pineal Res. 2018, 65, e12512.

25. Lu, R.; Liu, Z.; Shao, Y.; Sun, F.; Zhang, Y.; Cui, J.; Zhou, T. Melatonin is responsible for rice resistance to rice stripe virus infection through a nitric oxide-dependent pathway. Virol. J. 2019, 16, 141.

26. Murch, S.J.; KrishnaRaj, S.; Saxena, P.K. Tryptophan is a precursor for melatonin and serotonin biosynthesis in in vitro regenerated St. John's wort (Hypericum perforatum L. cv. Anthos) plants. Plant Cell Rep. 2000, 19, 698–704.

27. Park, S.; Lee, K.; Kim, Y.S.; Back, K. Tryptamine 5-hydroxylase-deficient Sekiguchi rice induces synthesis of 5-hydroxytryptophan and N-acetyltryptamine but decreases melatonin biosynthesis during senescence process of detached leaves. J. Pineal Res. 2012, 52, 211–216.

28. Tan, D.X.; Reiter, R.J. An evolutionary view of melatonin synthesis and metabolism related to its biological functions in plants. J. Exp. Bot. 2020, 71, 4677–4689.

29. Ye, T.; Yin, X.; Yu, L.; Zheng, S.J.; Cai, W.J.; Wu, Y.; Feng, Y.Q. Metabolic analysis of the melatonin biosynthesis pathway using chemical labeling coupled with liquid chromatography-mass spectrometry. J. Pineal Res. 2019, 66, e12531.

30. Okazaki, M.; Higuchi, K.; Aouini, A.; Ezura, H. Lowering intercellular melatonin levels by transgenic analysis of indoleamine 2,3-dioxygenase from rice in tomato plants. J. Pineal Res. 2010, 49, 239–247.

31. Byeon, Y.; Back, K. Molecular cloning of melatonin 2-hydroxylase responsible for 2-hydroxymelatonin production in rice (Oryza sativa). J. Pineal Res. 2015, 58, 343–351.

32. Lee, H.J.; Back, K. 2-Hydroxymelatonin promotes the resistance of rice plant to multiple simultaneous abiotic stresses (combined cold and drought). J. Pineal Res. 2016, 61, 303–316.

33. Lee, K.; Zawadzka, A.; Czarnocki, Z.; Reiter, R.J.; Back, K. Molecular cloning of melatonin 3-hydroxylase and its production of cyclic 3-hydroxymelatonin in rice (Oryza sativa). J. Pineal Res. 2016, 61, 470–478.

34. Singh, N.; Kaur, H.; Yadav, S.; Bhatla, S.C. Does N-nitrosomelatonin compete with S-nitrosothiols as a long distance nitric oxide carrier in plants? Biochem. Anal. Biochem. 2016, 5, 262.

35. Mukherjee, S. Insights into nitric oxide-melatonin crosstalk and N-nitrosomelatonin functioning in plants. J. Exp. Bot. 2019, 70, 6035–6047.

36. Chen, Z.; Xie, Y.; Gu, Q.; Zhao, G.; Zhang, Y.; Cui, W.; Xu, S.; Wang, R.; Shen, W. The AtrbohF-dependent regulation of ROS signaling is required for melatonin-induced salinity tolerance in Arabidopsis. Free Radical. Biol. Med. 2017, 108, 465–477.

37. Imran, M.; Shazad, R.; Bilal, S.; Imran, Q.M.; Lee, I.J. Exogenous melatonin mediates the regulation of endogenous nitric oxide in Glycine max L. to reduce effects of drought stress. Environ. Exp. Bot. 2021, 188, 104511.

38. Li, H.; Guo, Y.; Lan, Z.; Xu, K.; Chang, J.; Ahammed, G.J.; Ma, J.; Wei, C.; Zhang, X. Methyl jasmonate mediates melatonin-induced cold tolerance of grafted watermelon plants. Hortic. Res. 2021, 8, 57.

39. Xia, H.; Zhou, Y.; Deng, H.; Lin, L.; Deng, Q.; Wang, J.; Lv, X.; Zhang, X.; Liang, D. Melatonin improves heat tolerance in Actinidia deliciosa via carotenoid biosynthesis and heat shock proteins expression. Physiol Plant. 2021, 172, 1582–1593.

40. Zheng, X.; Zhou, J.; Tan, D.X.; Wang, N.; Wang, L.; Shan, D.; Kong, J. Melatonin improves waterlogging tolerance of Malus baccata (Linn.) Borkh. seedlings by maintaining aerobic respiration, photosynthesis and ROS migration. Front. Plant Sci. 2017, 8, 483.

41. Pang, Y.W.; Jiang, X.L.; Wang, Y.C.; Wang, Y.Y.; Hao, H.S.; Zhao, S.J.; Du, W.H.; Zhao, X.M.; Wang, L.; Zhu, H.B. Melatonin protects against paraquat-induced damage during in vitro maturation of bovine oocytes. J. Pineal Res. 2019, 66, e12532.

42. Meng, J.F.; Xu, T.F.; Wang, Z.Z.; Fang, Y.L.; Xi, Z.M.; Zhang, Z.W. The ameliorative effects of exogenous melatonin on grape cuttings under water-deficient stress: Antioxidant metabolites, leaf anatomy, and chloroplast morphology. J. Pineal Res. 2014, 57, 200–212.

43. Wang, P.; Yin, L.; Liang, D.; Li, C.; Ma, F.; Yue, Z. Delayed senescence of apple leaves by exogenous melatonin treatment: Toward regulating the ascorbate-glutathione cycle. J. Pineal Res. 2012, 53, 11–20.

44. DalCorso, G.; Farinati, S.; Maistri, S.; Furini, A. How plants cope with cadmium: Staking all on metabolism and gene expression. J. Integr. Plant Biol. 2008, 50, 1268–1280.

45. Arnao, M.B.; Hernández-Ruiz, J. Melatonin against environmental plant stressors: A review. Curr. Protein Pept. Sci. 2021, 21, 413–429.

46. Zhu, J.K. Plant salt tolerance. Trends Plant Sci. 2001, 6, 66–71.

47. Zelm, E.V.; Zhang, Y.; Testerink, C. Salt tolerance mechanisms of plants. Annu. Rev. Plant Biol. 2020, 71, 403–433.

48. Lee, H.Y.; Back, K. Melatonin is required for H2O2- and NO-mediated defense signaling through MAPKKK3 and OXI1 in Arabidopsis thaliana. J. Pineal Res. 2017, 62, e12379.

49. Shi, H.; Jiang, C.; Ye, T.; Tan, D.X.; Reiter, R.J.; Zhang, H.; Liu, R.; Chan, Z. Comparative physiological, metabolomic, and transcriptomic analyses reveal mechanisms of improved abiotic stress resistance in bermudagrass by exogenous melatonin. J. Exp. Bot. 2015, 66, 681–694.

50. Li, M.Q.; Hasan, M.K.; Li, C.X.; Ahammed, G.J.; Xia, X.J.; Shi, K.; Zhou, Y.; Reiter, R.; Yu, J.; Xu, M.; et al. Melatonin mediates selenium-induced tolerance to cadmium stress in tomato plants. J. Pineal Res. 2016, 61, 291–302.

51. Ni, J.; Wang, Q.; Shah, F.A.; Liu, W.; Wang, D.; Huang, S.; Fu, S.; Wu, L. Exogenous melatonin confers cadmium tolerance by counterbalancing the hydrogen peroxide homeostasis in wheat seedlings. Molecules 2018, 23, 799.

52. Zhang, J.; Yao, Z.; Zhang, R.; Mou, Z.; Yin, H.; Xu, T.; Zhao, D.; Chen, S. Genome-wide identification and expression profile of the SNAT gene family in tobacco (Nicotiana tabacum). Front. Genet. 2020, 11, 591984.

53. Gao, Y.; Wang, Y.; Qian, J.; Si, W.; Tan, Q.; Xu, J.; Zhao, Y. Melatonin enhances the cadmium tolerance of mushrooms through antioxidant-related metabolites and enzymes. Food Chem. 2020, 330, 127263.

54. Byeon, Y.; Lee, H.Y.; Hwang, O.J.; Lee, H.J.; Back, K. Coordinated regulation of melatonin synthesis and degradation genes in rice leaves in response to cadmium treatment. J. Pineal Res. 2015, 58, 470–478.

55. Byeon, Y.; Lee, H.J.; Lee, H.Y.; Back, K. Cloning and functional characterization of the Arabidopsis N-acetylserotonin O-methyltransferase responsible for melatonin synthesis. J. Pineal Res. 2016, 60, 65–73.

56. Byeon, Y.; Lee, H.Y.; Back, K. Cloning and characterization of the serotoninn-acetyltransferase-2 gene (SNAT2) in rice (Oryza sativa). J. Pineal Res. 2016, 61, 198–207.

57. Wang, T.; Song, J.; Liu, Z.; Liu, Z.; Cui, J. Melatonin alleviates cadmium toxicity by reducing nitric oxide accumulation and IRT1 expression in Chinese cabbage seedlings. Environ. Sci. Pollut. Res. Int. 2021, 28, 15394–15405.

58. Asada, K. THE WATER-WATER CYCLE IN CHLOROPLASTS: Scavenging of active oxygens and dissipation of excess photons. Annu. Rev. Plant Physiol. Plant Mol. Biol. 1999, 50, 601–639.

59. Mishra, V.; Singh, P.; Tripathi, D.K.; Corpas, F.J.; Singh, V.P. Nitric oxide and hydrogen sulfide: An indispensable combination for plant functioning. Trends Plant Sci. 2021, 17, S1360–S1385.

60. Zhang, J.; Zhou, M.; Zhou, H.; Zhao, D.; Gotor, C.; Romero, L.C.; Shen, J.; Ge, Z.; Zhang, Z.; Shen, W.; et al. Hydrogen sulfide, a signaling molecule in plant stress responses. J. Integr. Plant Biol. 2021, 63, 146–160.

61. Zhang, L.; Pei, Y.; Wang, H.; Jin, Z.; Liu, Z.; Qiao, Z.; Fang, H.; Zhang, Y. Hydrogen sulfide alleviates cadmium-induced cell death through restraining ROS accumulation in roots of Brassica rapa L. ssp. pekinensis. Oxidative Med. Cell. Longev. 2015, 2015, 804603.

62. Hu, L.; Li, H.; Huang, S.; Wang, C.; Sun, W.J.; Mo, H.Z.; Shi, Z.Q.; Chen, J. Eugenol confers cadmium tolerance via intensifying endogenous hydrogen sulfide signaling in Brassica rapa. J. Agric. Food Chem. 2018, 66, 9914–9922.

63. Zhang, J.; Zhou, M.J.; Ge, Z.L.; Shen, J.; Zhou, C.; Gotor, C.; Romero, L.C.; Duan, X.L.; Liu, X.; Wu, D.L.; et al. Abscisic acid-triggered guard cell L-cysteine desulfhydrase function and in situ hydrogen sulfide production contributes to heme oxygenase-modulated stomatal closure. Plant Cell Environ. 2020, 43, 624–636.

64. Zhang, Q.; Cai, W.; Ji, T.T.; Ye, L.; Lu, Y.T.; Yuan, T.T. WRKY13 enhances cadmium tolerance by promoting D-cysteine desulfhydrase and hydrogen sulfide production. Plant Physiol. 2020, 183, 345–357.

65. Qiao, Z.; Tao, J.; Liu, Z.; Zhang, L.; Jin, Z.; Liu, D.; Pei, Y. H2S acting as a downstream signaling molecule of SA regulates Cd tolerance in Arabidopsis. Plant Soil 2015, 393, 137–146.

66. Yang, X.; Kong, L.; Wang, Y.; Su, J.; Shen, W. Methane control of cadmium tolerance in alfalfa roots requires hydrogen sulfide. Environ. Pollut. 2021, 284, 117123.

67. Sun, J.; Wang, R.; Zhang, X.; Yu, Y.; Zhao, R.; Li, Z.; Chen, S. Hydrogen sulfide alleviates cadmium toxicity through regulations of cadmium transport across the plasma and vacuolar membranes in Populus euphratica cells. Plant Physiol. Biochem. 2013, 65, 67–74.

68. Cui, W.; Chen, H.; Zhu, K.; Jin, Q.; Xie, Y.; Cui, J.; Xia, Y.; Zhang, J.; Shen, W. Cadmium-induced hydrogen sulphide synthesis is involved in cadmium tolerance in Medicago sativa by reestablishment of reduced (homo) glutathione and reactive oxygen species homeostases. PLoS ONE 2014, 9, e109669.

69. Shi, H.; Ye, T.; Chan, Z. Nitric oxide-activated hydrogen sulfide is essential for cadmium stress response in bermudagrass (Cynodon dactylon (L). pers.). Plant Physiol. Biochem. 2014, 74, 99–107.

70. Mostofa, M.G.; Rahman, A.; Ansary, M.; Watanabe, A.; Fujita, M.; Tran, L.P. Hydrogen sulfide modulates cadmium-induced physiological and biochemical responses to alleviate cadmium toxicity in rice. Sci. Rep. 2015, 5, 14078.

71. Jia, H.; Wang, X.; Dou, Y.; Liu, D.; Si, W.; Fang, H.; Zhao, C.; Chen, S.; Xi, J.; Li, J. Hydrogen sulfide-cysteine cycle system enhances cadmium tolerance through alleviating cadmium-induced oxidative stress and ion toxicity in Arabidopsis roots. Sci. Rep. 2016, 6, 39702.

72. Kaya, C.; Ashraf, M.; Alyemeni, M.N.; Ahmad, P. Responses of nitric oxide and hydrogen sulfide in regulating oxidative defence system in wheat plants grown under cadmium stress. Physiol. Plantarum 2020, 168, 345–360.

73. Lv, H.; Xu, J.; Bo, T.; Wang, W. Comparative transcriptome analysis uncovers roles of hydrogen sulfide for alleviating cadmium toxicity in Tetrahymena thermophila. BMC Genom. 2021, 22, 21.

74. Li, G.; Shah, A.A.; Khan, W.U.; Yasin, N.A.; Ahmad, A.; Abbas, M.; Ali, A.; Safdar, N. Hydrogen sulfide mitigates cadmium induced toxicity in Brassica rapa by modulating physiochemical attributes, osmolyte metabolism and antioxidative machinery. Chemosphere 2021, 263, 127999.

75. Jia, H.; Wang, X.; Shi, C.; Guo, J.; Ma, P.; Ren, X.; Wei, T.; Liu, H.; Li, J. Hydrogen sulfide decreases Cd translocation from root to shoot through increasing Cd accumulation in cell wall and decreasing Cd2+ influx in Isatis indigotica. Plant Physiol. Biochem. 2020, 155, 605–612.

76. Tian, B.; Zhang, Y.; Jin, Z.; Liu, Z.; Pei, Y. Role of hydrogen sulfide in the methyl jasmonate response to cadmium stress in foxtail millet. Front. Biosci. 2017, 22, 530–538.

77. Tian, B.; Qiao, Z.; Zhang, L.; Li, H.; Pei, Y. Hydrogen sulfide and proline cooperate to alleviate cadmium stress in foxtail millet seedlings. Plant Physiol. Biochem. 2016, 109, 293–299.

78. Wang, H.R.; Che, Y.H.; Wang, Z.H.; Zhang, B.N.; Ao, H. The multiple effects of hydrogen sulfide on cadmium toxicity in tobacco may be interacted with CaM signal transduction. J. Hazard. Mater. 2021, 403, 123651.

79. Li, L.; Wang, Y.; Shen, W. Roles of hydrogen sulfide and nitric oxide in the alleviation of cadmium-induced oxidative damage in alfalfa seedling roots. Biometals 2012, 25, 617–631.

80. Khan, M.N.; Siddiqui, M.H.; AlSolami, M.A.; Alamri, S.; Hu, Y.; Ali, H.M.; Al-Amri, A.A.; Alsubaie, Q.D.; Al-Munqedhi, B.M.A.; Al-Ghamdi, A. Crosstalk of hydrogen sulfide and nitric oxide requires calcium to mitigate impaired photosynthesis under cadmium stress by activating defense mechanisms in Vigna radiata. Plant Physiol. Biochem. 2020, 156, 278–290.

81. Fang, L.; Ju, W.; Yang, C.; Jin, X.; Liu, D.; Li, M.; Yu, J.; Zhao, W.; Zhang, C. Exogenous application of signaling molecules to enhance the resistance of legume-rhizobium symbiosis in Pb/Cd-contaminated soils. Environ. Pollut. 2020, 265, 114744.

82. Huang, D.; Huo, J.; Liao, W. Hydrogen sulfide: Roles in plant abiotic stress response and crosstalk with other signals. Plant Sci. 2021, 302, 110733.

83. Mukherjee, S.; Bhatla, S.C. Exogenous melatonin modulates endogenous H2S homeostasis and L-cysteine desulfhydrase activity in salt-stressed tomato (Solanum lycopersicum L. var. cherry) seedling cotyledons. J. Plant Growth Regul. 2020, 4, 1–13.

84. Siddiqui, M.; Khan, M.; Mukherjee, S.; Basahi, R.; Alamri, S.; Al-Amri, A.; Alsubaie, Q.; Ali, H.; Al-Munqedhi, B.; Almohisen, I. Exogenous melatonin-mediated regulation of K+/Na+ transport, H+-ATPase activity and enzymatic antioxidative defence operate through endogenous hydrogen sulphide signalling in NaCl-stressed tomato seedling roots. Plant Biol. 2021, 23, 797–805.

85. Iqbal, N.; Fatma, M.; Gautam, H.; Umar, S.; Khan, N.A. The crosstalk of melatonin and hydrogen sulfide determines photosynthetic performance by regulation of carbohydrate metabolism in wheat under heat stress. Plants 2021, 10, 1778.

86. Sun, Y.; Ma, C.; Kang, X.; Zhang, L.; Wang, J.; Zheng, S.; Zhang, T. Hydrogen sulfide and nitric oxide are involved in melatonin-induced salt tolerance in cucumber. Plant Physiol. Biochem. 2021, 167, 101–112.

87. Graham, N.; Arisi, A.M.; Lise, J.; Kunert, K.J.; Heinz, R.; Foyer, C.H. Glutathione: Biosynthesis, metabolism and relationship to stress tolerance explored in transformed plants. J. Exp. Bot. 1998, 49, 623–647.

88. Jozefczak, M.; Remans, T.; Vangronsveld, J.; Cuypers, A. Glutathione is a key player in metal-induced oxidative stress defenses. Int. J. Mol. Sci. 2012, 13, 3145–3175.

89. Chen, J.; Yang, L.; Yan, X.; Liu, Y.; Wang, R.; Fan, T.; Ren, Y.; Tang, X.; Xiao, F.; Liu, Y.; et al. Zinc-finger Transcription Factor ZAT6 positively regulates cadmium tolerance through the glutathione-dependent pathway in Arabidopsis. Plant Physiol. 2016, 171, 707–719.

90. Shen, J.; Su, Y.; Zhou, C.; Zhang, F.; Yuan, X. A putative rice L-cysteine desulfhydrase encodes a true L-cysteine synthase that regulates plant cadmium tolerance. Plant Growth Regul. 2019, 89, 217–226.

91. Gu, Q.; Chen, Z.; Cui, W.; Zhang, Y.; Hu, H.; Yu, X.; Wang, Q.; Shen, W. Methane alleviates alfalfa cadmium toxicity via decreasing cadmium accumulation and reestablishing glutathione homeostasis. Ecotoxicol. Environ. Saf. 2018, 147, 861–871.

92. Kok, L.; Bosma, W.; Maas, F.M.; Kuiper, P. The effect of short-term H2S fumigation on water-soluble sulphydryl and glutathione levels in spinach. Plant Cell Environ. 1985, 8, 189–194.

93. Anastasis, C.; Manganaris, G.A.; Ioannis, P.; Vasileios, F. Hydrogen sulfide induces systemic tolerance to salinity and non-ionic osmotic stress in strawberry plants through modification of reactive species biosynthesis and transcriptional regulation of multiple defence pathways. J. Exp. Bot. 2013, 7, 1953–1966.

94. Liu, F.; Zhang, X.; Cai, B.; Pan, D.; Ai, X. Physiological response and transcription profiling analysis reveal the role of glutathione in H2S-induced chilling stress tolerance of cucumber seedlings. Plant Sci. 2020, 291, 110363.

95. Hasan, M.K.; Ahammed, G.J.; Yin, L.; Shi, K.; Xia, X.; Zhou, Y.; Yu, J.; Zhou, J. Melatonin mitigates cadmium phytotoxicity through modulation of phytochelatins biosynthesis, vacuolar sequestration, and antioxidant potential in Solanum lycopersicum L. Front. Plant Sci. 2015, 6, 601.