

Phytomelatonin

Regulating Development and Stress Protection in Plants



**Edited by Chirag Maheshwari,
Muzaffar Hasan, and M. Iqbal R. Khan**



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Melatonin, once considered confined to animal systems, has now emerged as a vital bioactive molecule in plants—called **phytomelatonin**. Over the past two decades, it has gained recognition for its regulatory roles in plant growth, development, and defense. This book, *Phytomelatonin: Regulating Development and Stress Protection in Plants*, brings together the latest research on how phytomelatonin functions as a multitasking molecule, linking plant metabolism, hormonal signaling, and environmental response mechanisms.

The book offers a unique synthesis of molecular, physiological, and applied perspectives, making it the most comprehensive resource available on phytomelatonin's role in plant science. It highlights the molecule's potential in addressing pressing agricultural challenges such as climate-induced stress, reduced soil fertility, and the need for sustainable farming approaches.

Key Features:

- Covers the biosynthesis, metabolism, and transport of phytomelatonin in various plant species
- Details its regulatory role in seed germination, root architecture, flowering, fruiting, and senescence
- Explains its interaction with key phytohormones under stress conditions including drought, salinity, and extreme temperatures
- Presents recent advances in phytomelatonin-mediated gene expression and redox homeostasis

- Discusses field applications including melatonin seed priming, foliar sprays, and soil drenching to improve stress resilience
- Integrates insights from molecular biology, crop physiology, and natural farming practices

This book is an essential reference for researchers, graduate students, and professionals in plant biology, crop science, and environmental stress physiology. It serves as a foundational text and a forward-looking guide for those seeking to harness phytomelatonin in building climate-resilient and sustainable agriculture.

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CRC Press

Taylor & Francis Group

Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

Designed cover image: Getty Images (516180836)

First edition published 2026

by CRC Press

2385 NW Executive Center Drive, Suite 320, Boca Raton FL 33431

and by CRC Press

4 Park Square, Milton Park, Abingdon, Oxon, OX14 4RN

CRC Press is an imprint of Taylor & Francis Group, LLC

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ISBN: 978-1-032-86369-6 (hbk)

ISBN: 978-1-032-86463-1 (pbk)

ISBN: 978-1-003-52763-3 (ebk)

DOI: [10.1201/9781003527633](https://doi.org/10.1201/9781003527633)

Typeset in Times

by SPi Technologies India Pvt Ltd (Straive)

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Chirag Maheshwari serves as a scientist at the Division of Biochemistry, ICAR-Indian Agricultural Research Institute located in New Delhi. His present research pursuits encompass the exploration of both physiological and molecular mechanisms that underlie abiotic stress in plants. Additionally, he is engrossed in analyzing crop nutritional profiles when subjected to stress conditions. He boasts a publication record of approximately 20 research and review articles in esteemed, peer-reviewed international journals, alongside over twenty contributions to book chapters. Furthermore, his accomplishments have been recognized with several accolades, including the Young Scientist Award from SPBB in collaboration with Springer, Best Poster Award, and others. He has published numerous peer-reviewed research articles, book chapters, and popular articles, and continues actively contributing to scientific advancements. They remain deeply committed to promoting sustainable agriculture through innovation and research.

Muzaffar Hasan is a Scientist at ICAR-Central Institute of Agriculture Engineering, Bhopal, India. He received his B.Sc. degree from the G. B. Pant University of Agriculture and Technology, Uttarakhand, India, and his M.Sc. and Ph.D. from the Indian Agricultural Research Institute, New Delhi, India. He began his scientific career in the Agro Produce Processing Division at ICAR-CIAE, Bhopal 2016, and moved to the Centre of Excellence on Soybean Processing and Utilization at ICAR-CIAE, Bhopal, in 2022. During his 8 years at ICAR-CIAE, Bhopal, he taught courses ranging from basic biochemistry to food processing. His initial research

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He is on the Editorial Board of many renowned journals as the Section Editor in *Plant and Soil*, and Associate Editor in *Journal of Plant Growth Regulation*, *3-Biotech*, and *Plant Physiology Reports*. He has also served as

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1

Phytomelatonin

Discovery, Metabolic Pathways, Occurrence and Distribution in Cellular Organelles, Site of Signal Perception, Transduction, and Mode of Action in Plants

Gajra Garg and Nitin Kumar Garg

DOI: [10.1201/9781003527633-1](https://doi.org/10.1201/9781003527633-1)

1.1 INTRODUCTION

Phytomelatonin, identified as a plant hormone, plays diversified roles in the growth and development of plants, however, its exact function is not yet fully understood and is still under investigation. This well-known indolic compound has acquired an increasing interest in many physiological and biochemical aspects ([Arnao and Hernández-Ruiz 2007](#); [Arnao et al. 2023](#)). It has a pivotal role in plants as a regulatory mechanism that boosts plant tolerance and adaptation to both biotic (living) and abiotic (non-living) stressors ([Seth et al. 2023](#)). Melatonin is a biologically produced indoleamine that shares structural similarities with other important

biomolecules, including tryptophan, tryptamine, indole-3-acetic acid, and serotonin ([Figure 1.1](#)). This structural resemblance contributes to melatonin's diverse range of physiological functions that can be explained by its capacity to directly scavenge reactive oxygen species (ROS) and free radicals ([Aghdam and Arnao 2024](#)). In addition, it also regulates circadian rhythms; phytemelatonin is involved in plant development, increasing photosynthetic ability, seed germination, root growth, and amassing of carbon, etc. ([Tyagi et al. 2023](#)). The melatonin molecule functions as a potent free radical scavenger for hydroxyl radical (OH), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), nitric oxide (NO), peroxynitrite anion ($ONOO^-$), peroxy radical (ROO), and alkoxy radical (RO), etc. have been extensively investigated *in-vitro* and *in-vivo*, in plant and animal systems ([Kanwar et al. 2018](#); [Cano et al. 2023](#); [Hernandez-Ruiz et al. 2023](#)).

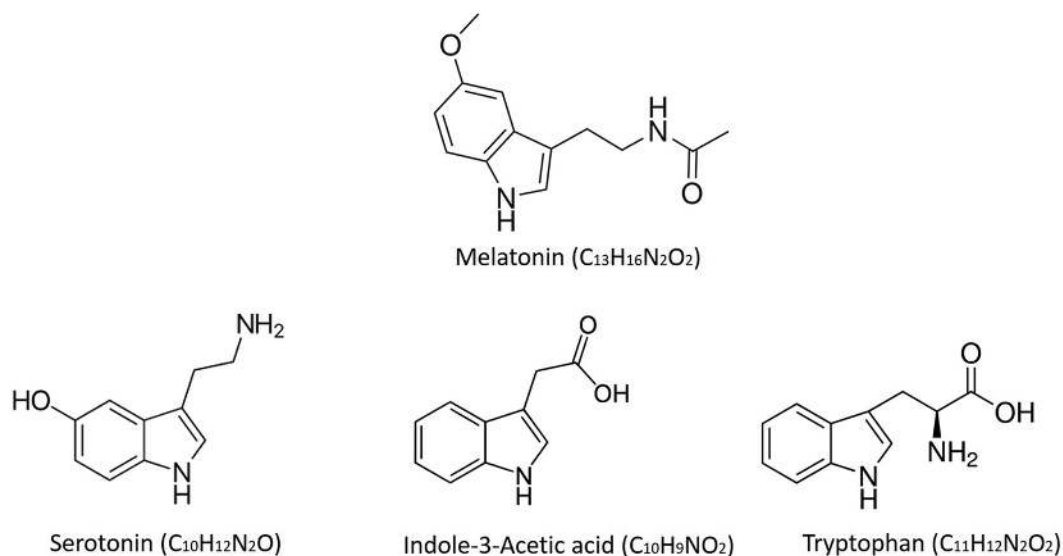


FIGURE 1.1 Impression of biomolecules, structurally similar to melatonin. [↩](#)

Phytemelatonin is reported as a functional compound during abiotic stress by regulating both kinds of antioxidant activities, i.e., enzymatic and non-enzymatic. Furthermore, it serves as a bio-stimulator, stimulating plant

tolerance to the stressful environment, thereby improvements in osmolyte production, nutrient uptake, and stability of cell membrane could also be seen ([Zhao and Hu 2023](#); [Aghdam and Arnao 2024](#)). Consequently, numerous studies have emphasized the role of phytemelatonin in mitigating stress. The endogenous phytemelatonin can be improved or enhanced by applying exogenous melatonin in a controlled way ([Hu et al. 2022](#)). This, in turn, makes plants cope with various stresses and hence increases overall tolerance (Arnao and [Hernandez-Ruiz 2021c](#)). Yet, to evaluate every layer of its study, initially, the necessity is to focus on the discovery of melatonin.

1.2 ORIGIN AND EVOLUTION

Melatonin is a fundamental compound with deep evolutionary roots tracing back to the inception of life on Earth. It is found not only in vertebrates but also in invertebrates, bacteria, unicellular organisms, and plants, demonstrating its ubiquitous distribution and evolutionary significance. The ubiquitous nature of melatonin in both animal and plant kingdoms makes it a very specialized hormone, and to discriminate against it, a new term or tag “Phytemelatonin,” was propounded in 2004 ([Murch and Erland 2021](#)). The discovery of phytemelatonin has not only expanded our understanding of its biosynthesis and roles of this compound in the plant kingdom but has also provided valuable insights regarding its successive studies. The first presence of melatonin was identified by [Lerner et al. \(1958\)](#) at Yale University, found in the bovine’s pineal gland ([Arnao et al. 2023](#)). In 1959, it was soon recognized as an N-acetyl-5-methoxytryptamine compound, furthermore, its biosynthetic pathway from the precursor amino acid tryptophan, with serotonin as an intermediate, was subsequently elucidated, provides vision into the mechanisms of melatonin production ([Arnao 2014](#)). After being discovered as a neurohormone in humans by [Lerner et al. in](#)

([1959](#)), and was later found to be present in a wide range of vertebrates, including mammals, birds, amphibians, and fish ([Lauber et al. 1968](#); [Baker et al. 1965](#); [Fenwick 1970](#)). During the 1980s, melatonin was discovered to be present in a variety of invertebrate groups, including planarians, annelids, mollusks, insects, and crustaceans ([Morita et al. 1987](#); [Callebert et al. 1991](#); [Vivien-Roels and Pevet 1993](#); [Vivien-Roels et al. 1984](#); [Abran et al. 1994](#); [Balzer et al. 1997](#)). Tassel and O'Neill identified endogenous melatonin in higher plants for the first time in [1993](#). Later on, the content of melatonin extracts was analyzed in many plant species ([Tassel et al. 1995](#); [Dubbels et al. 1995](#); [Arnao and Hernandez-Ruiz 2009](#); [Cano et al. 2024](#)).

Subsequent studies have revealed the widespread occurrence of melatonin in various plants, ranging from microalgae to angiosperms, suggesting that this indoleamine compound may have influenced evolution and adaptation. Above, we also discussed the dispersion of melatonin in primitive unicellular organisms, clearly indicating its ancient origin and persistence throughout the progression ([Hardeland and Poeggeler 2003](#); [Kolar and Machackova 2005](#)). Many believe that melatonin first appeared in bacteria before the endosymbiotic process. Following the incorporation of cyanobacteria and α -proteobacteria into early prokaryotic cells, these microorganisms subsequently differentiated into the chloroplasts and mitochondria present in all multicellular and unicellular organisms ([Figure 1.2](#)). These specialized organelles are then able to produce the most essential compound, i.e. melatonin, eventually distributed among all organisms. Plant cells have higher concentrations of melatonin (phytomelatonin) in comparison to animal cells because they carry both organelles ([Zhao et al. 2019](#)). It provides a clarification for the capacity of mitochondria to synthesize melatonin, having a function inherited from their bacterial predecessors ([Reiter et al. 2018](#)). The incredible thing is that

having billions of yearlong historical perceptions, the molecular structure of melatonin remained similar as before, despite having multiple roles. This actually shows the conservative nature of the organelles ([Tan et al. 2014](#); [Murch and Erland 2021](#)).

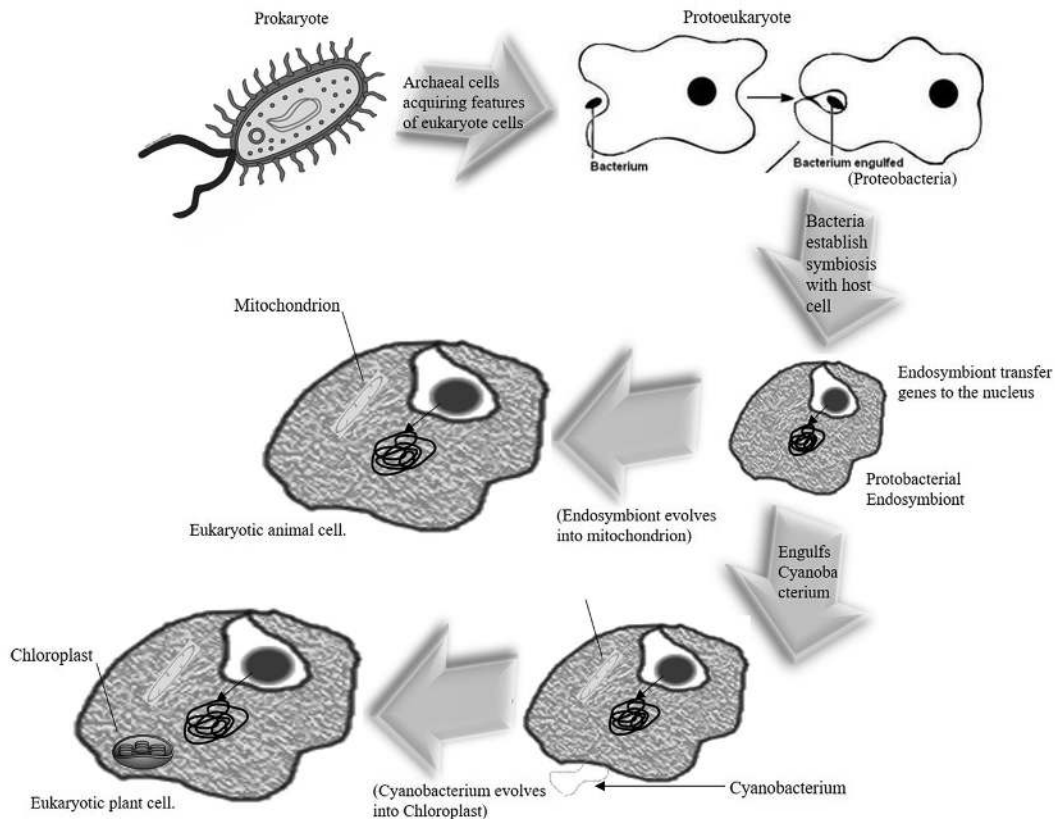


FIGURE 1.2 Illustration showing the evolutionary history behind the organelle synthesis of melatonin.

(Adapted from [Zhao et al. 2019](#).)



Since mitochondria can generate high amounts of reactive oxygen species (ROS), the capability to synthesize phytomelatonin is nearly enhanced and, hence, conserved by all the cells of eukaryotic organisms. This melatonin production machinery continuously produces the master regulator hormone. However, the quantities could vary significantly ([Tan et al. 2013](#); [Cano et al. 2024](#)). On the other hand, cyanobacteria are referred to

as a precursor of chloroplasts, so, in the case of phototropic eukaryotic organisms, they contribute to melatonin synthesis ([Zheng et al. 2017](#)). Studies also confirm the fact that chloroplast is a larger producer of higher levels of ROS and thus has a greater capacity for radical scavenging ([Schmitt et al. 2014](#); [Foyer and Hanke 2022](#)).

1.3 BIOSYNTHESIS AND METABOLISM

1.3.1 Synthesis or Anabolism of Phytomelatonin (Major Pathway)

Plants metabolize melatonin to create several significant bioactive compounds. Hence, understanding metabolism appears like a complex process including the major and alternative pathways. It initiates from tryptophan, an aromatic amino acid, synthesized by the shikimate pathway. This multi-step metabolic pathway facilitates the biosynthesis of all aromatic amino acids, including tryptophan, within plant cells. It exhibits analogous sequential enzymatic processes in both animal and plant organisms ([Liu et al. 2022b](#)). In plants, phytomelatonin is biosynthesized by at least six essential enzymes. Acetylserotonin O-methyltransferase (ASMT), caffeic acid 3-O-methyltransferase (COMT), serotonin N-acetyltransferase (SNAT) in the chloroplasts, tryptophan decarboxylase (TDC) in the cytoplasm, tryptamine 5-hydroxylase (T5H) in the endoplasmic reticulum, and an unidentified putative tryptophan hydroxylase (TPH) enzyme are among them ([Erland 2024](#)).

The biosynthetic pathway of melatonin has been intensively examined and fully characterized into primary and secondary pathways. The enzyme tryptophan decarboxylase catalyzes the decarboxylation reaction, which is

the primary method of converting tryptophan into tryptamine ([Zhou et al. 2020](#)). After that, tryptamine is hydroxylated in the presence of tryptamine-5-hydroxylase to form serotonin. According to the literature, T5H is a cytochrome P450 type of enzyme ([Kang et al. 2007](#)), and generally, it is required for serotonin synthesis in plants. However, when it was studied in rice lines, it was found that in the presence of T5H overexpressing rice lines, the content of phytomelatonin was affected and hence found lower in this case ([Park et al. 2013](#)).

Within the chloroplasts or mitochondria of plant cells, serotonin N-acetyltransferase (SNAT), a crucial enzyme in the third stage of phytomelatonin production, facilitates converting serotonin to N-acetylserotonin (NAS). The final step of the biosynthesis process involves the conversion of N-acetylserotonin (NAS) to melatonin (phytomelatonin) by the enzyme N-acetylserotonin methyltransferase (ASMT). Some species of plants lack ASMT enzyme, so the conversion of NAS could be performed in the presence of Caffeic acid O-methyltransferase (COMT) ([Aghdam and Arnao 2024](#)). Findings showed that COMT plays an advantageous role in phytomelatonin synthesis, inferring the existence of additional potential pathways capable of producing this phytochemical ([Liu et al. 2022b](#); [Erland 2024](#)).

1.3.2 Synthesis of Phytomelatonin (Alternative Pathway)

In addition, one alternative pathway also exists to study the biosynthesis that could simultaneously be regulated. It involves the methylation of serotonin to yield 5-methoxytryptamine (5-MT), which SNAT subsequently catalyzes to produce phytomelatonin. This alternative route arises from the kinship of ASMT and COMT enzymes for both serotonin and N-

acetylserotonin (NAS) ([Back et al. 2016](#)). Few studies identified a reverse enzymatic process, N-acetylserotonin deacetylase (ASDAC), that catalyzes the conversion of N-acetylserotonin back to serotonin ([Figure 1.3](#)). This deacetylase enzyme is localized within the chloroplasts, similar to the serotonin N-acetyltransferase (SNAT) enzyme ([Lee et al. 2018](#)). The presence of serotonin N-acetyltransferase (SNAT) and N-acetylserotonin deacetylase (ASDAC) enzymes confers a tightly regulated process of phytomelatonin biosynthesis, which is crucial for plants overall development. While SNAT facilitates the synthesis of phytomelatonin, ASDAC restricts it, thereby maintaining a balanced, controlled production of this key metabolite. Here, further, several studies have shown that multiple isoforms have been identified and various regulatory genes are involved in the control of phytomelatonin biosynthesis ([Aghdam and Arnao 2024](#); [Erland 2024](#)).

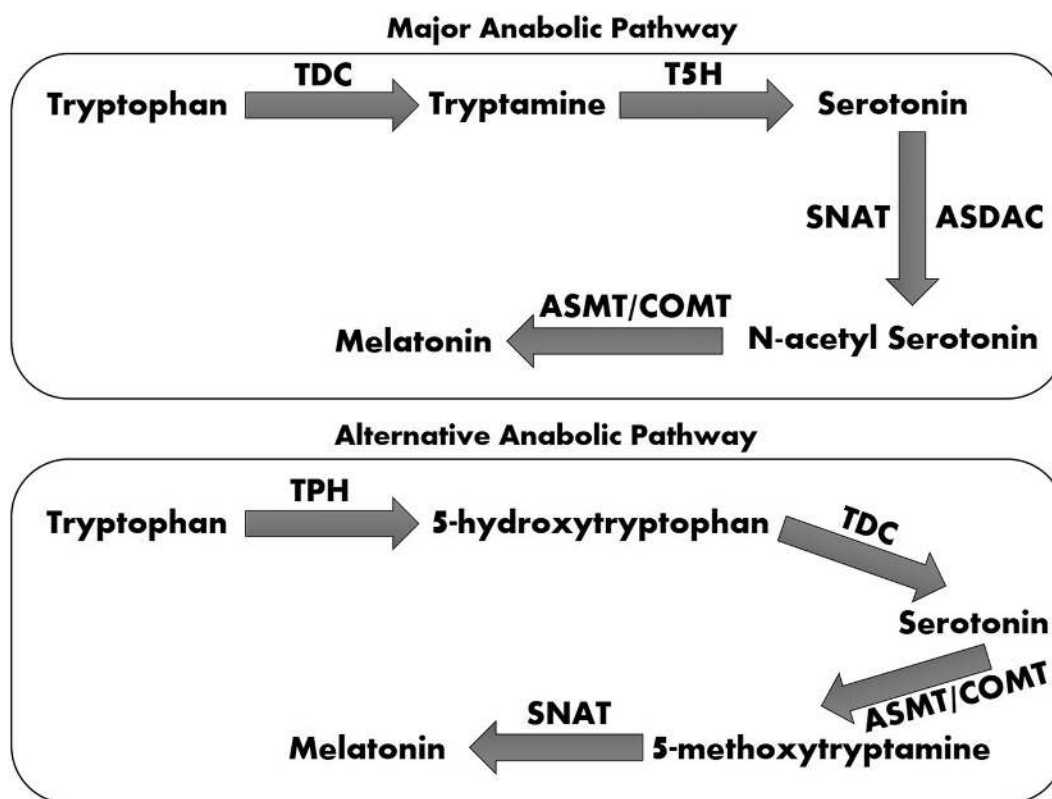


FIGURE 1.3 Biosynthesis of phytemelatonin.
(Adapted from [Liu et al. 2022b](#).)



1.3.3 Phytemelatonin Metabolism

The potent antioxidant actions of phytemelatonin have compelled researchers to investigate the distribution of metabolite across diverse plant species and even within distinct tissues of the same species ([Mishra et al. 2023](#)). Thus, after studying the process of biosynthesis, some findings related to the production of metabolites also deserve an extensive lookout. The [Table 1.1](#), demonstrates the involvement of different genes (M2H, M3H, IDO, ASDAC) related to phytemelatonin synthesis at one place, where we could easily understand the production of bioactive metabolites such as N¹-acetyl-N²-formyl-5-methoxykynuramine (AFMK), 2-hydroxymelatonin (2-OHM), cyclic 3-hydroxymelatonin (3-OHM), and 5-methoxytryptamine (5-MT), respectively ([Lee et al. 2016](#); [Byeon and Back 2015](#); [Okazaki et al. 2010](#); [Lee et al. 2018](#)). In fact, N-acetyl-5-methoxykynuramine (AMK) is also produced in this oxidation reaction in addition to N¹-acetyl-N²-formyl-5-methoxykynuramine (AFMK). In plants, both these metabolites were considered as the active metabolites to be discovered ([Tan et al. 2007](#); [Galano and Reiter 2018](#)) and possess the ability to mitigate oxidative stress by scavenging reactive oxygen (ROS) and nitrogen species (RNS). The regulation of phytemelatonin biosynthesis in plants is governed by intricate genetic mechanisms. Further investigation of phytemelatonin biosynthetic pathways will contribute to elucidating the detailed mechanisms and significance of each step, ultimately enhancing our understanding on how to improve phytemelatonin content in crop species ([Mannino et al. 2021](#); [Liu et al. 2022b](#)).

TABLE 1.1 Genes Involved in the Production of Metabolites 

Compound Formed	Genes Involved	Metabolites Produced	References
Melatonin	M2H	2-hydroxymelatonin (2-OHM)	Lee et al. 2016
	M3H	cyclic 3-hydroxymelatonin (3-OHM)	Byeon and Back, 2015
	IDO	N ¹ -acetyl-N ² -formyl-5-methoxykynuramine (AFMK)	Okazaki et al. 2010
	ASDAC	5-methoxytryptamine (5-MT)	Lee et al. 2018

The other study showed that melatonin as a precursor further catabolizes or breaks down into a few products that are present in a very lesser amount in plants, hence least studied ([Murch and Erland 2021](#)). Similarly, [Kanwar et al. \(2018\)](#) also strongly emphasized on exploration of catabolism-based research that would aid in recognizing other metabolites of phytomelatonin.

1.4 DISTRIBUTION OF PHYTOMELATONIN

The plant organelles that contain the highest levels of melatonin are the mitochondria and chloroplasts, as noted earlier. Therefore, these specific cell organelles are thought to be the primary locations for the biosynthesis of this unique indolamine. After studying the phytomelatonin contents within the same plant, it is distributed in the following order: leaves > seeds > roots > flowers > fruits ([Arnao and Hernandez-Ruiz 2009](#)).

Phytomelatonin is a compound with a molecular structure that allows it to shuttle freely within and between plant cells due to its high lipophilicity and partial hydrophilicity properties ([Yan et al. 2020](#)). It can readily traverse cell

membranes and the cytoplasm, and subsequently enter the nucleus, where it can act as a powerful antioxidant ([Reiter et al. 1997](#)). Phytomelatonin, the plant-derived form of melatonin, has been acknowledged in over 140 different plant species. Phytomelatonin levels in plants typically vary between picograms and micrograms per gram of tissue, with reproductive organs like flowers and seeds having the highest quantities ([Kanwar et al. 2018](#)). Comparative studies were also done to report that plants are capable of producing more amounts than animals. Even researchers found that in the presence of stress, such as cold or heat, soil contaminants, or any viral/bacterial infections, plants acquire the ability to enhance the production of phytomelatonin ([Arnao and Hernández-Ruiz 2013](#)). Moreover, phototrophic organisms contain very high concentrations of melatonin in their chloroplasts to scavenge more and more to protect the mechanism of photosystems. The reported average melatonin concentration is 579.38 ± 1513.28 ng per gram of fresh weight by Mannino et al. ([2021](#)). These findings highlight the significant variation across plant species. Notably, aromatic and medicinal plants typically exhibit elevated phytomelatonin levels compared to fleshy fruits and seeds. Some fruits, such as tomato, sweet and sour cherry fruits, and cranberry, have been identified as notable sources of phytomelatonin, with average reported concentrations of 9.44 ng/g, 15.050 ng/g, and 11.370 ng/g, respectively. Likewise, the highest phytomelatonin level, 55.55 ng/g, is found in rice (*Oryza sativa*) ([Hardeland 2016](#); [Mannino et al. 2021](#)).

1.5 SIGNAL TRANSDUCTION AND STRESS TOLERANCE

1.5.1 Site of Signal Perception

The very first fact about signaling is the need to understand that all living beings possess adaptation to environmental conditions; specifically, plants being a sessile organism have evolved intricate and distinctive mechanisms to cope with different threats and defend against abiotic as well as biotic stress ([Mishra et al. 2023](#); [Kumari et al. 2024](#)). Plant growth, development, and productivity may be negatively impacted by these unfavorable circumstances ([Tan 2015](#); [Jahan et al. 2021](#)). Basically, phytohormones function as crucial signaling molecules that enable cells to react rapidly to a variety of internal and external stimuli. But in the case of plants, to activate stress-response pathways and coordinate many environmental signals, phytomelatonin is essential, thereby enabling the development of defense mechanisms and enhanced plant resilience ([Khan et al. 2023b](#)). This is probably due to functionally redundant signal perception, which may be the part of the molecular basis of this particular type of resistance ([Zhao et al. 2021a](#); [Khan et al. 2023a](#)). From an evolutionary standpoint, it appears that melatonin has maintained its primary function as an antioxidant, while also acquiring diverse and crucial signaling transduction roles during plant evolution ([He and He 2020](#); [Back 2021](#)). As we studied above, the plant cells containing mitochondria and chloroplasts can produce melatonin, and similarly, the CAND2/PMTR1 receptors were first invented in *Botryococcus braunii* and *Coccomyxa subellipsoidea*, the single-celled green algae ([Liu et al. 2022a](#)). In the presence of these receptors on the outer membrane or the cell membrane, abiotic stress like heat ([Jahan et al. 2021](#)), cold ([Chang et al. 2021](#)), salt ([Zhan et al. 2019](#)), heavy metals ([Awan et al. 2023](#)) and drought ([Zhao et al. 2021a](#); [El-Bauome et al. 2022](#)), mitigated by activation of the potential scavenging system. [Ikram et al. \(2024\)](#) elucidated that most of the phytomelatonin is stored in the mitochondria and chloroplast, ultimately confirming the fact that these two

organelles produce phytemelatonin. They explained the sites of signaling, which are subjected to the phytemelatonin's receptor PMTR1/CAND2. During the signaling pathway, melatonin activates the membrane channels and hence these receptors. Further, it is associated with MAP Kinase cascades or related pathways, leading to gene expressions in the nucleus and side-by-side signaling in other subcellular compartments such as chloroplast, endoplasmic reticulum, and mitochondria, as well as in the cytoplasm ([Back 2021](#)).

1.5.2 Molecular Mechanisms of Plant Stress Regulation

Melatonin orchestrates a wide range of developmental processes and defense mechanisms through both autonomous and synergistic interactions with other phytohormones as well as with other secondary messengers ([Khan et al. 2023a](#)). Here, receptors of phytemelatonin play a crucial role in recognizing signals and initiating an intracellular downstream signal transduction pathway, named MT-crosstalk by [Gu et al. \(2022\)](#). Various functions of melatonin have been studied that are subjected to melatonin crosstalk with different signaling molecules to maintain oxidative homeostasis ([Plokhovska et al. 2022](#)). Following are the coordination mechanisms between phytemelatonin and secondary messengers under abiotic stress to study plant tolerance.

1.5.2.1 Synergistic Function of Melatonin and Ca^{+2}

Calcium (Ca^{+2}) is a multifunctional signaling molecule that can play a role inside the cell to control multicellular mechanisms in the plant's usual and stress conditions ([Khan et al. 2021](#)). The [Figure 1.4](#) clearly shows that **under cold stress** conditions, the membrane-associated phospholipid phosphatidylcholine (PC) is broken down by the enzyme phospholipase D

(PLD), resulting in the production of phosphatidic acid (PA) ([Xu et al. 2020](#)). This phosphatidic acid then goes on to regulate the activities of protein phosphatase 2A (PP2A), nitric oxide (NO), nitrate reductase (NR), and hydrogen sulfide (H₂S) ([Khan et al. 2023a](#)). The exogenous/external addition of melatonin molecules induces the release of Ca⁺² through Ca⁺² channels, and the liberated Ca⁺² subsequently binds directly to the N-terminal region of respiratory burst oxidase homologs (RBOHs). The cold-induced influx of Ca⁺² stimulates the activity of RBO, generating H₂O₂ and triggering Ca⁺² fluctuations within plant cells ([Wei et al. 2018](#)). This, in turn, elicits downstream responses involving C-repeat binding factors (CBF) -dependent signaling pathways that enhance cold tolerance mechanisms ([Chang et al. 2021](#); [Khan et al. 2023a](#)).

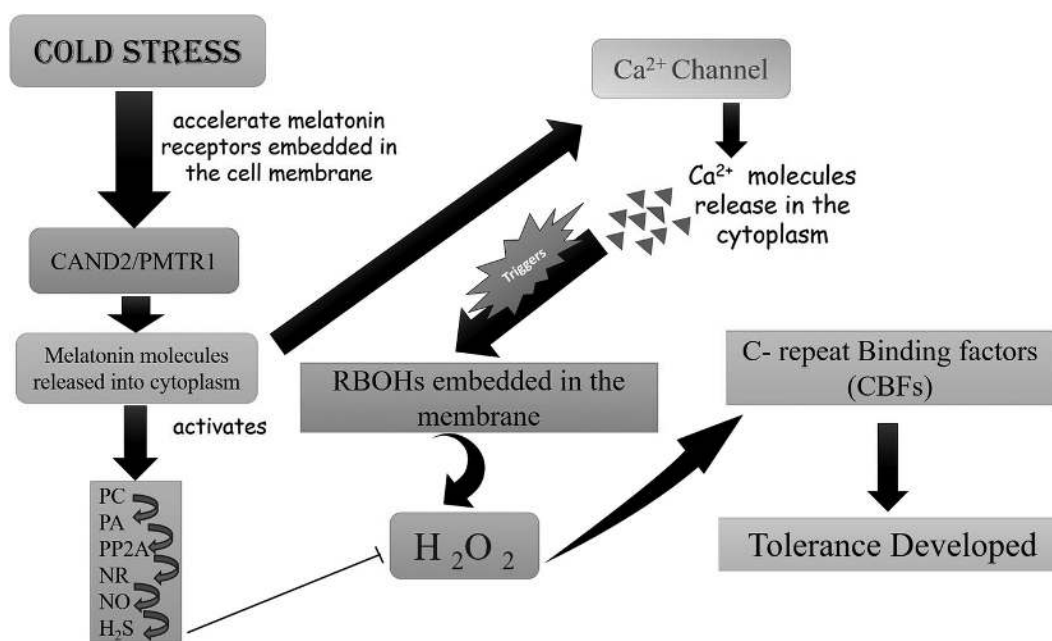


FIGURE 1.4 Putative model of interaction between phytomelatonin and secondary messengers under cold stress conditions.

(Adapted from [Khan et al. 2023a](#).)



Under salt stress conditions, phosphatidic acid (PA) undergoes phosphorylation, yielding cytidine diphosphate-diacylglycerol (CDP-DAG), phosphatidylinositol (PtdIns), and phosphatidylinositol biphosphate (PtdInsP2) as products ([Figure 1.5](#)). Phospholipase C (PLC) activity is stimulated by exogenous melatonin administration, which cleaves phosphatidylinositol biphosphate into soluble inositol 1,4,5-trisphosphate (InsP3). This inositol trisphosphate is then further phosphorylated, resulting in the production of phytic acid (InsP6), which in turn triggers the release of calcium ions within the cytoplasm ([Khan et al. 2023a](#)). The phytomelatonin receptor CAND2/PMTR1 also plays a role in the accumulation of calcium ions (Ca^{+2}) by activating the G-protein $\text{G}\alpha$, which then stimulates the production of ROS and further Ca^{+2} accumulation. Elevated levels of MT and Ca^{+2} influx subsequently up-regulate transcription factors (AP2, bHLH, WRKY) to alleviate the detrimental effects of salt stress ([Zhang et al. 2021](#)).

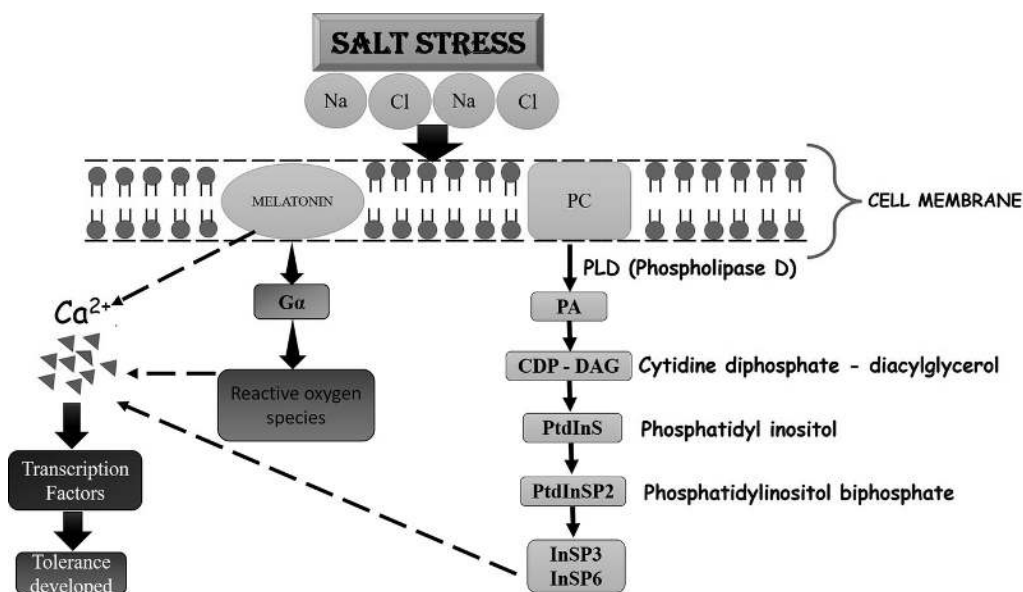


FIGURE 1.5 Putative model of interaction between phytomelatonin and secondary messengers under salt stress conditions.

(Adapted from [Khan et al. 2023a](#)).



1.5.2.2 Synergistic Function of Melatonin with ROS and RNS

An intracellular redox homeostasis is maintained through a complex interconnected network of oxidative and antioxidative reactions ([Mahajan et al. 2023](#)). These processes are crucial for preserving cellular equilibrium and safeguarding cells against oxidative stress-induced injury ([Ding et al. 2020](#)). Phytomelatonin also interacts with ROS and RNS, and aids in restoring redox balance within plant cells by producing intracellular antioxidants, including ascorbic acid (ASA), catalase (CAT), glutathione peroxidase (GPX), peroxiredoxin (PRX), superoxide dismutase (SOD), nitrate reductase (NR), nitric oxide synthase (NOS), and glutathione (GSH) ([Ikram et al. 2024](#)) ([Figure 1.6](#)).

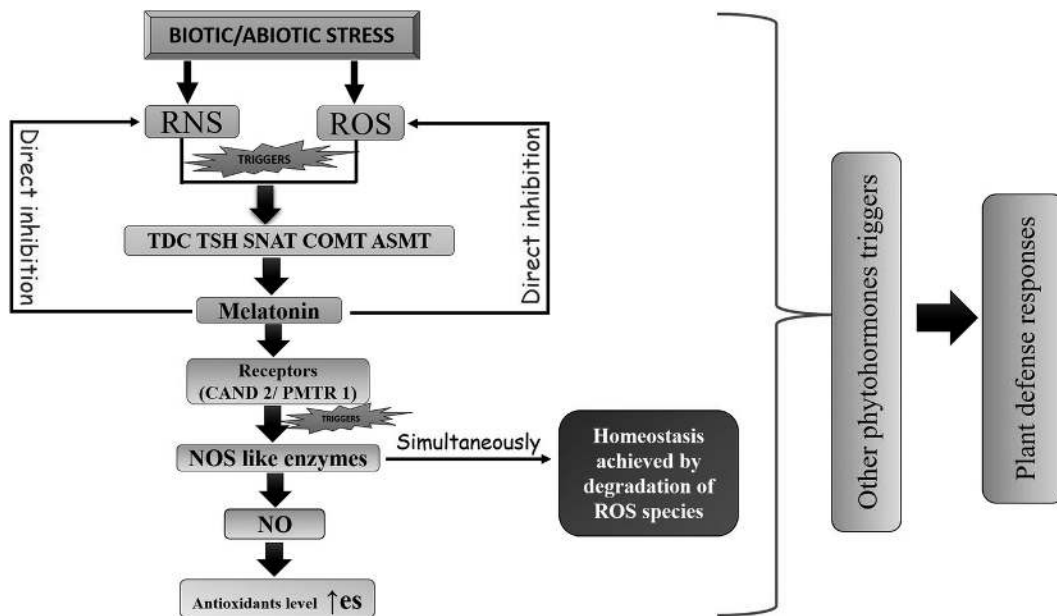


FIGURE 1.6 Putative model of interaction between phytomelatonin and ROS/RNS. (Adapted from [Khan et al. 2023a](#)).



With the help of [Figure 1.6](#), one can easily able to understand the plant defense responses triggered by the balance generated by the redox species. This balance is initially induced by the abiotic /biotic stressors that aid in increasing the ROS/RNS levels that in turn trigger the production of melatonin and its receptors such as CAND2 and PMTR1. The elevation of NO levels and antioxidant enzymes induced NOS-like enzymes and NR. Subsequently, the generation of these enzymes is targeted to destroy the reactive oxygen species to stabilize homeostasis. In turn, the increased level of ROS/RNS is inhibited by the direct action of melatonin and its metabolites. This synergistic action of melatonin with both the reactive species induces the signaling cascade responses to trigger other phytohormones (ABA- Absciscic acid; ETH- Ethylene; SA- Salicylic acid; GA- Gibberellic acid; JA- Jasmonic acid; IAA- indole acetic acid; CTK- Cytokinins; BR- Brassinosteroids; SL-Strigolactone) and plant defense response as well ([Weeda et al. 2014](#); [Khan et al. 2023a](#); [Ikram et al. 2024](#)).

1.6 MODE OF ACTION IN PLANTS

Phytomelatonin is a versatile molecule that has been discovered as a biostimulator, playing a vital role in enhancing plant tolerance. It plays multiple roles in the series of processes, from an environmental signal to the defense responses. Moreover, it has been observed to enhance important physiological roles in plants, such as root development, seed germination, photosynthetic capacity, seed and fruit production, delaying leaf senescence, and overall growth ([Ikram et al. 2024](#)). The most interesting mode of action of phytomelatonin is related to the interactions with a variety of plant hormones. In response to stress, melatonin and its metabolic derivatives actively scavenge and neutralize ROS and RNS, thereby preserving the cellular balance of these reactive molecules and initiating the

mitogen-activated protein kinase (MAPK) signaling cascade to mount an optimal defense mechanism against a varied range of stresses ([Back 2021](#)). It has also been found to mitigate pathogen infection by stimulating the SAR (Systemic Acquired Resistance) mechanisms and thus participated in crop improvement. After studying its crucial roles, it has been considered a plant master regulator by several scientists because it acts as a regulator for plant hormones, exhibiting both synergistic and antagonistic interactions, thereby influencing a range of physiological processes in plants. The most important action of phyto melatonin was the identification of its receptor in Arabidopsis, i.e., PMTR1/CAND2. This transmembrane receptor interacts with Gα protein, sometimes with Ca⁺² and NOS-like enzymes, to demonstrate regulatory functions ([Khan et al. 2023a](#)). Thus, it is sufficient to clear that phyto melatonin is involved in many plant actions. The crosstalk between phyto melatonin and varied hormones is discussed as follows.

1.6.1 Interaction with Primary Metabolites and Secondary Metabolites Metabolism

This includes the study of photosynthesis in terms of various activities performed by this master regulator. Here, pointing out the defensive role of melatonin that acts with the pigment system during the light reaction as well as with the Rubisco enzyme. Hence, phyto melatonin showed its mode of action in increasing photosynthetic proficiency ([Meng et al. 2014](#)).

Whilst similar work has been done with several other crops such as tomato ([Liu et al. 2015](#)), pea ([Szafranska et al. 2016](#)), rice ([Han et al. 2017](#)), tea ([Li et al. 2018](#)), soybean ([Zhang et al. 2019](#)), brown mustard ([Mir et al. 2020](#)), canola ([Ayyaz et al. 2021](#)), sorghum ([Zhang et al. 2022](#)), and many more. An extensive study was also performed when plants are being silenced for

melatonin production, resulting in lower tolerance against stress conditions ([Wang et al. 2020](#)). Although up-regulation and down-regulation have also been examined and found surprising results regarding the photosynthetic machinery and stress tolerance. Phytomelatonin has been found responsible for the overexpression of the genes of several enzymes being utilized during metabolic pathways ([Fan et al. 2015](#); [Arnao et al. 2021](#); [Khan et al. 2023a](#)). There are a few additional studies regarding phytomelatonin's protective effect in primary metabolism, conducted to examine the crucial role of phytomelatonin in secondary metabolism too. The first related scientific study demonstrated the Mung bean for germination studies and the growth of seedlings ([Szafranska et al. 2012](#)). Later on, several studies have described the excellent results found by inducing melatonin in plants and hence increasing levels of total phenolics, flavonoids, anthocyanin, and total antioxidant activity such as in Indian ginseng ([Adil et al. 2015](#)), Spinach ([Moussa and Algamal 2017](#)), Watercress ([Oloumi et al. 2018](#)), fruits of plum ([Bal 2019](#)), basil ([Bahcesular et al. 2020](#)), Safflower ([Heshmati et al. 2021](#)) and rice ([Samanta et al. 2022](#)). Mostly, melatonin enhances the total phenols and anthocyanin content during stressful environments. However, glucosinolate metabolism is also triggered by melatonin ([Teng et al. 2022](#)).

1.6.2 Interaction with Plant Hormones

The regulatory roles of melatonin on various hormones have been discussed by several researchers ([Arnao and Hernández-Ruiz 2021a](#); [Arnao and Hernández-Ruiz 2021b](#)). It works in such a way that it modifies the endogenous balance of every plant hormone and influences the biochemical pathway. Melatonin has been shown to collaborate with auxin-named plant hormones, also known as IAA (Indole acetic acid), in inhibiting or

promoting root growth and also in plant's overall growth as well as several genes related to auxin biosynthesis are regulated by melatonin ([Yang et al. 2021](#)). According to [Liu et al. \(2022a\)](#), it completely depends on the concentration of melatonin. Similarly, [Mao et al. \(2020\)](#) described that melatonin was found to promote the activity of GA (Gibberellic acid). Transcriptome analysis revealed that the administration of exogenous melatonin significantly upregulated the expression of various key genes notably responsible for the biosynthesis of gibberellic acid ([Liu et al. 2022a](#)). On the other hand, phytemelatonin regulates the abscisic acid biosynthetic gene expression and catabolic genes to alleviate cold stresses ([Zhao et al. 2017](#)). Few studies have shown the crosstalk effects of phytemelatonin with IAA, gibberellic acid, and abscisic acid in studying the process of germination ([Lv et al. 2021](#)). This implies that phytemelatonin plays a crucial part in extending the fruit's shelf life by delaying their softening and ripening ([Liu et al. 2020](#)). It confirms that melatonin was treated with abscisic acid, ethylene, and other important hormonal compounds such as jasmonic acid, salicylic acid, and brassinosteroids promoting tolerance to biotic and abiotic stressors.

1.7 CONCLUSION

This chapter focused on the broad spectrum of phytemelatonin's history, origin, distribution, metabolism, signaling, and mode of action in plants. Melatonin exhibits multifunctional capabilities with a wide range of roles in both plant and animal systems. It has been demonstrated to promote plant growth, resulting in improvements in leaf structure, root architecture, chlorophyll content maintenance, and fruit production, etc. Melatonin showed its primary role as a radical scavenger or an antioxidant that enhances tolerance and adaptation to both biotic & abiotic stressors. The

deep historical aspect has provided detailed evolutionary significance and also showed the responsible organelles for the production or synthesis of melatonin. Melatonin was found to be the master regulator that appeared powerful during metabolic studies as well as in the production of different metabolites. It regulates a huge number of enzymes. Phytomelatonin seems not only to regulate the signal transduction but also to modulate the redox species network. Studies in the mode of the action of phytomelatonin in plants have uncovered a wide spectrum of global actions. Further research on the mechanisms of crosstalk of phytomelatonin could enhance our knowledge to be used and benefitted in the agriculture sector, which will lead to understanding the novel part that is needed for crop productivity.

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2

Phytomelatonin

Isolation, Methods of Extraction, Detection, and Quantification through High-throughput Technological Interventions

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DOI: [10.1201/9781003527633-2](https://doi.org/10.1201/9781003527633-2)

2.1 INTRODUCTION

Melatonin (N-acetyl-5-methoxy-tryptamine) is a versatile neurohormone, antioxidant, and signaling molecule that was first identified in plants in 1995 ([Dubbels et al., 1995](#); [Hattori et al., 1995](#)). Initial studies on plant melatonin approached it from a human perspective, focusing on measuring its levels in foods ([Dubbels et al., 1995](#); [Hattori et al., 1995](#)) and medicinal plants ([Manchester et al., 2000](#)), to understand whether its presence could help explain the medicinal properties of certain plants ([Murch et al., 2001](#); [Chen et al., 2003](#)). In mammals, melatonin serves a wide range of

physiological processes, acting as a hormone that regulates mood, sleep, body temperature, retinal function, and sexual behavior. Many of these processes are tied to its role in circadian rhythm regulation ([Fuller et al., 2006](#); [Jan et al., 2009](#); [Maronde & Stehle, 2007](#); [Hardelan et al., 2007](#); [Reiter, 2003](#); [Zmijewski et al., 2009](#); [Slominski et al., 2008](#)). Its relevance extends further into the field of Food Chemistry and Nutrition, where it is essential for the regulation of the gastrointestinal tract, being extensively produced by cells in the digestive tract. Melatonin from dietary sources, whether plant-based or otherwise, is easily absorbed into the bloodstream. Consequently, understanding the bio-assimilation rate of phytomelatonin has become a key objective, particularly given the diversity of human diets across different geographic regions and communities. Additionally, its presence in juice extracts and its formation during fermentation processes, as seen in wine and beer, has introduced innovative opportunities for the development of functional foods ([Baenas et al., 2023](#); [Pranil Moongngarm and Loypimai, 2020](#)). Beyond its nutritional significance, melatonin acts in both plants and animals as a strong antioxidant, where It has the ability to scavenge free radicals, particularly targeting reactive species of nitrogen and oxygen (ROS/RNS; [Seth et al., 2023](#); [Tyagi et al., 2023](#)). In plants, phytomelatonin regulates the equilibrium between ROS/RNS and antioxidant defenses, including ascorbate-glutathione cycle enzymes, peroxidases, catalases, superoxide dismutases, and respiratory burst oxidases ([Arnao et al., 2022](#)). Moreover, Under different circumstances, it regulates the expression of hormone-responsive genes and plant hormone levels ([Arnao & Hernández-Ruiz, 2021](#)). Biochemically, melatonin (N-acetyl-5-methoxytryptamine) is synthesized from serotonin (5-hydroxytryptamine). While its biosynthesis in animals follows a well-established pathway from tryptophan, the process in plants differs in several

ways. In plants, melatonin and indole-3-acetic acid (IAA), a major auxin, share a common biosynthetic precursor, tryptamine, through the tryptophan-dependent IAA biosynthetic pathway.

Phytomelatonin production occurs in various cellular and subcellular compartments of plants under both normal and stress conditions. Studies indicate that abiotic stress triggers an increase in phytomelatonin levels, facilitated by soil-related mechanisms. [Mukherjee et al. \(2014\)](#) proposed that phytomelatonin not only regulates adaptive responses in plant roots to soil challenges but also functions in long-distance signaling, transmitting stress signals from roots to aerial plant parts. The accurate quantification of phytomelatonin has posed significant challenges due to difficulties in its extraction from plant tissues. Various extraction procedures, depending on the plant matrix, can significantly impact the melatonin level measurement. Melatonin concentrations in higher plants vary from picograms to micrograms per gram of tissue, but inconsistencies in extraction methods often lead to unreliable recovery rates ([Kolar & Machackova, 2005](#); [Pape & Lüning, 2006](#); [Cao et al., 2006](#); [Hardeland, 2011](#)). Current methods typically involve organic solvents like ethyl acetate, chloroform, or methanol applied to plant tissues treated with liquid nitrogen. While aqueous extractions have been attempted, they often result in lower recovery rates ([Pape & Lüning, 2006](#); [Reiter et al., 2005](#); [Bukhardt et al., 2001](#)). Because melatonin is amphiphilic, direct extraction using organic solvents without homogenizing fresh tissue has been recommended for optimal results ([Hardeland et al., 2007](#)). The earliest discovery of higher plants that naturally produce melatonin begins back to 1993 when Van Tassel and O'Neill identified it in ivy morning glory (*Pharbitis nil* L.) and tomato fruits (*Solanum lycopersicum* L.) using radioimmunoassay (RIA) and gas chromatography-mass spectrometry (GC-MS) ([Van Tassel et al.,](#)

[1993](#)). However, their findings were formally published in 1995 alongside two studies that confirmed the Melatonin's occurrence in plants using RIA and HPLC-MS in *Nicotiana tabacum* L. and several edible plants ([Dubbels et al., 1995](#)). Despite advancements in the isolation, extraction, phytomelatonin detection, and quantification, there remains a critical need for high-throughput technological interventions to enhance precision and efficiency. These innovations will be essential to furthering our understanding of phytomelatonin's roles and applications across diverse fields.

2.2 CHEMISTRY OF PHYTOMELATONIN

The chemical name of melatonin is the plant-produced N-acetyl-5-methoxytryptamine is referred to as phytomelatonin ($C_{13}H_{16}N_2O_2$) and the chemical structure is shown in [Figure 2.1](#). These phytomelatonin belong to the derivatives of indoleamine particularly the amino acid group tryptophan having amide and alkoxy groups in 3 and 5 positions respectively. The major pathway for the synthesis of phytomelatonin started from tryptophan, several enzymes play a major role starting from tryptophan decarboxylase, tryptophan hydroxylase, 5-hydroxyindole-O-methyl transferase, etc. as shown in [Figure 2.2](#) and [Table 2.1](#).



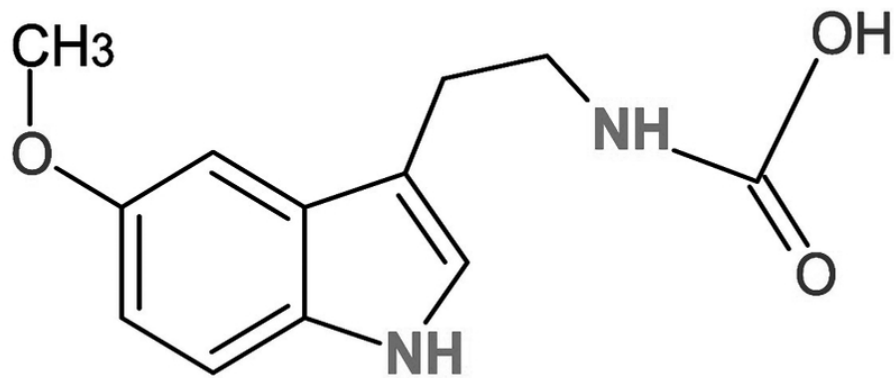
Rice



Alfa-alfa



Chlorella



Phytomelatonin
(N-acetyl-5-methoxy tryptamine)

FIGURE 2.1 Chemical structure and occurrence of Phytomelatonin. [↗](#)

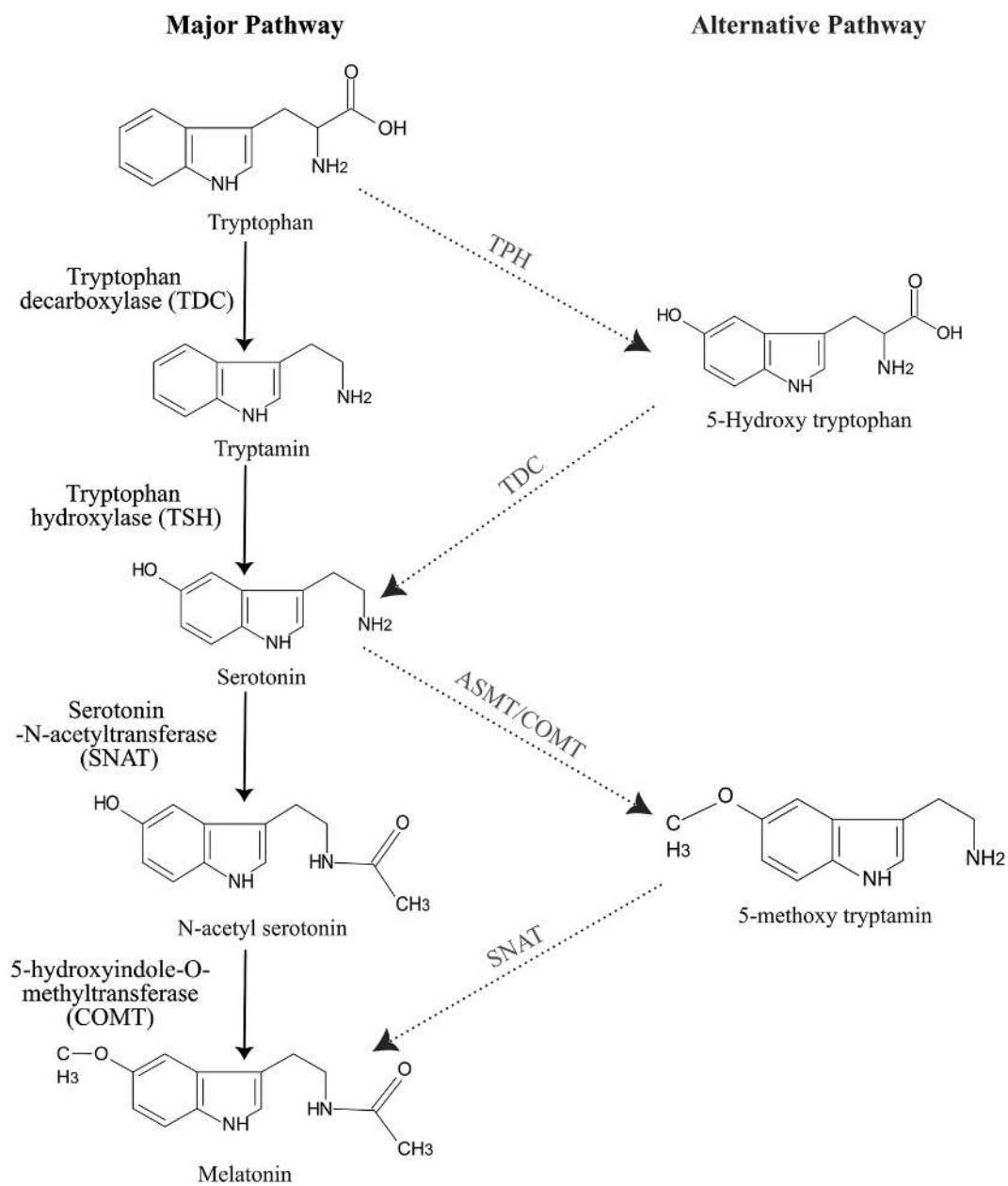


FIGURE 2.2 Biosynthesis pathway of phytomelatonin. [↗](#)

TABLE 2.1 Physical and Chemical Properties of Phytomelatonin 

Properties	Particulars
Form	Off-white powder
Molecular Weight	232.28 g/mol
Density	1.175 g/cm ³
Melting Point	116.5°C - 118°C
Boiling Point	512.8°C
IUPAC Name	N-[2-(5-methoxy-1H-indol-3-yl)ethyl]acetamide
Chemical Formula	C ₁₃ H ₁₆ N ₂ O ₂
Core Structure	Indole scaffold
Functional Groups	3-amide group, 5-alkoxy group
Chemical Classification	Indolamine compound (derived from tryptophan)
Solubility	2g/l at 20°C
Stability	High, due to resonance mesomerism

Two cell organelles in plant cells contain the highest levels of melatonin: the chloroplast and mitochondria. Serotonin the melatonin production pathway's rate-limiting enzyme is N-acetyltransferase (SNAT), and it is present in the above organelles. Therefore, these organelles were involved in the indoleamine biosynthesis.

A minimum of six or seven enzymes were involved in melatonin biosynthesis from the starting point which is tryptophan, more than one pathway can be present in the biosynthesis of melatonin. These enzymes include putative tryptophan hydroxylase (TPH), serotonin N-acetyltransferase (SNAT), acetylserotonin-O-methyltransferase (ASMT),

tryptophan decarboxylase (TDC), tryptamine-5-hydroxylase (T5H), and caffeic acid-3-O-methyltransferase (COMT). The initial step of plants' melatonin production involves converting tryptophan into serotonin. Two different pathways can facilitate this process (see [Figure 2.2](#)). In the first pathway, By decarboxylating tryptophan, TPH transforms it into tryptamine. TDC then uses hydroxylation to further convert tryptamine into serotonin. In an alternate pathway, tryptophan is first hydroxylated into 5-hydroxytryptophan by TPH, and then 5-hydroxytryptophan is decarboxylated into serotonin by TDC. Due to TDC's high affinity for tryptophan and 5-hydroxytryptophan under lab conditions, both pathways are feasible. However, it has been shown that in plants, tryptophan is more frequently decarboxylated than hydroxylated as the first step.

2.3 IDENTIFICATION METHODS OF PHYTOMELATONIN

Until 1995, melatonin was considered solely an animal hormone and was extensively studied in journals like the *Journal of Pineal Research* and *Melatonin Research*. However, in 1995, three independent research groups confirmed its presence in plants, challenging prior assumptions. Subsequent studies established that melatonin exists across all life forms, including animals, plants, and microorganisms, with plant-derived melatonin termed *phytomelatonin* ([Hattori et al., 1995](#)).

Early research focused on its potential role in plant physiology, particularly in photomorphogenesis and circadian rhythms. Czech scientists Drs. Kolář and Macháčková investigated its influence on *Chenopodium rubrum* flowering but found no conclusive results. Meanwhile, Dr. Saxena's Canadian team, working with *Hypericum perforatum* cell cultures, identified similarities between phytomelatonin and animal melatonin

biosynthesis, suggesting its auxin-like function due to its resemblance to indole-3-acetic acid (IAA). In 2004, studies on *Lupinus albus* hypocotyls confirmed melatonin's growth-promoting effects, later validated in several Poaceae species ([Murch et al., 2000](#)).

The term phytomelatonin refers to melatonin (N-acetyl-5-methoxytryptamine) of plant origin. It initially revealed in a publication by [Blask et al. \(2005\)](#) on liver cancer in rats. In higher plants, melatonin was first identified in 1993 in a communication by [Van Tassel et al. \(1993\)](#). They detected phytomelatonin in ivy morning glory (*Pharbitis nil* L., syn. *Ipomoea nil* L.) and tomato (*Solanum lycopersicum* L.) using radioimmunoassay (RIA) and gas chromatography with mass spectrometry (GC-MS). In 1995, a few other papers appeared that demonstrated the presence of melatonin in higher plants. Using RIA and high-performance liquid chromatography mass spectrometry (HPLC-MS), Dubbels and colleagues discovered melatonin in extracts of *Nicotiana tabacum* L. and a few food plants ([Dubbels et al., 1995](#)). In the same year, another report on detecting melatonin in *Chenopodium rubrum* L. came from a Czech research group that identified melatonin ([Kolar et al., 1995](#)). Since then, phytomelatonin has been detected in many vegetables, fruits, seeds, nuts, and medicinal herbs. In more than 300 plant species that have been analyzed to date it has been shown that aromatic and medicinal plants have the greatest endogenous concentrations of phytomelatonin, up to 0.3 µg/g. Generally, in edible plants, Plant tissue contains 1–10 ng/g of phytomelatonin([Arnao and Hernández-Ruiz, 2014](#); [Arnao et al., 2017](#); [Meng et al., 2017](#)). Among the various plant parts seeds generally have the highest phytomelatonin content whereas fruits tend to have the lowest. The highest phytomelatonin level to date has been reported in thyme (*Thymus*

vulgaris). Other plant species could have greater phytomelatonin levels, though, as only a small number of plant species have been studied to yet.

The analytical technique used for quantification can strongly influence the phytomelatonin content. For instance, studies made in the 1990s have reported lower melatonin levels in plants than in recent years. A possible explanation could be either the lack of control in the phytomelatonin extraction process, poor recoveries, or the use of immunological techniques, which can give rise to interferences and false negatives.

2.4 EXTRACTION METHODS OF PHYTOMELATONIN

As shown in [Figure 2.3](#), several extraction techniques have been used to extract melatonin from plants. Using the right extraction method is important for the precise quantification of any phytochemical. Improper extraction can lead to erroneous quantification of melatonin irrespective of the quantification method used ([Reiter et al., 2001](#); [Pape & Lüning, 2006](#)). The phytochemical's solubility in the solvent additionally impacts how well the extraction process works. Generally, high solubility gives better extraction and recovery of the phytochemical. Phytomelatonin is generally extracted from plant tissues using organic solvents such as methanol, chloroform, or ethyl acetate. Aqueous extraction generally gives a low recovery of phytomelatonin ([Burkhardt et al., 2001](#)). As melatonin has amphiphilic properties, its extraction is best achieved in a solvent mixture having a wide range of polarities. It was observed that the methanol: water: HCl mixture was a more efficient extraction solvent as compared to other solvents/solvent mixtures. [Murch et al. \(2010\)](#) investigated the extraction of melatonin from Chara cells and grapes using a solvent combination consisting of methanol, water, and formalic acid (80:20:1 v/v/v). [Pranil et](#)

[al. \(2020\)](#) observed the influence of different pH (1, 4, 7, 10, and 13), effects of light and temperature on melatonin stability in fruit juices. 0.1 M HCl and 0.1 M NaOH were used to alter the pH. Melatonin degradation was found to be 34% at pH 1.0, but degradation of over 95% occurred at lower pH levels. Therefore, HCl is typically added to the solvent mixes to reduce the degradation of melatonin during the extraction process. In order to extract phytomelatonin, [Uğur \(2023\)](#) examined six distinct solvents and their mixtures: ethanol: methanol, water: HCl (70:29.9:0.1 v/v/v), ethanol: water: HCl (70:29.9:0.1 v/v/v), methanol: water (50:50 v/v), and ethanol: water (50:50 v/v). Extraction time is yet another factor that influences melatonin extraction from plants. The same study by [Uğur \(2023\)](#) tested three different time intervals of 10, 20, and 30 min extraction of melatonin from cornelian cherry fruits. They observed that the extraction of melatonin increased with time It peaked at 20 minutes, following which it started to decline. [Oladi et al. \(2014\)](#) also found that the ideal duration for melatonin extraction from pistacia seeds was 20 minutes, which is in line with the results of [Uğur et al. \(2023\)](#). A greater extraction rate is the consequence of optimal exposure to the extraction solvent ([Iglesias-Carres et al., 2019](#)). However, extending the extraction period past the ideal point might cause melatonin to degrade. Ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), and solid phase extraction (SPE) are generally used for the extraction of phytomelatonin from plants. In this context, [Table 2.2](#) explains the extraction and analysis of phytomelatonin from various products through different processes.

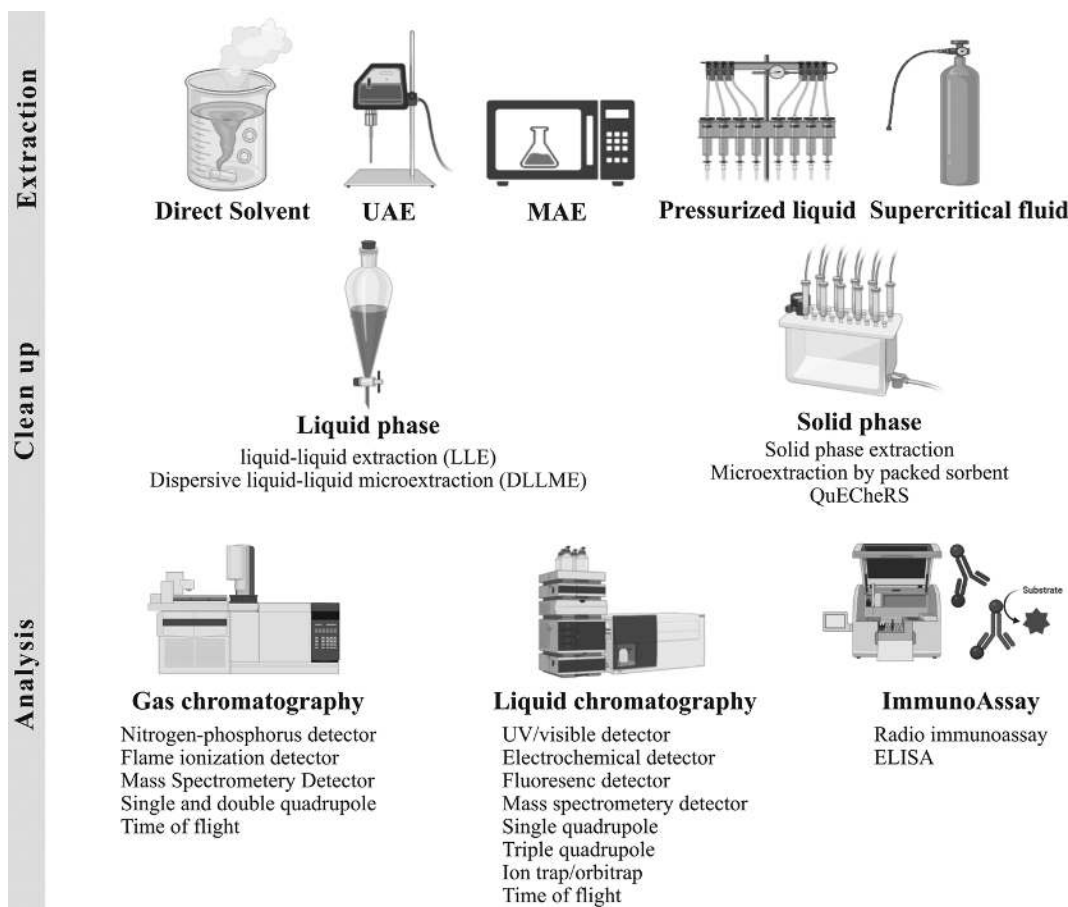


FIGURE 2.3 Extraction and identification methods for the phytomelatonin. [↗](#)

TABLE 2.2 Extraction of Phytomelatonin from Different Prod

Species/product	Extraction	Analysis	Phytomelatonin Content
Liquid-Liquid Extraction (LLE)			
<i>Glycine max</i> L.	LLE Ethyl acetate	LC-MS-MS ESI Agilent-XDB C18	0.2–95 ng/g FW
<i>Oryza sativa</i> ssp	LLE ACN or MeOH 1 M HCl Ether	LC-Orbitrap Acquity UPLC BEH phenyl column	0.58 ng/g FW
Various fruits or vegetables	LLE Sodium carbonate Diethylether	RIA GC-MS Derivatized pentafluoropropionic anhydride	0.1 –47 ng/100 g Nd in potato
Walnuts <i>Juglans regia</i> L.	LLE 40% recovery	LC-ECD 200 –900 mV	3.5 ± 1.0 ng/g
Olive oil	LLE	ELISA	50 –119 pg/mL
Direct Solvent Extraction (DSE)			
<i>Allium sativum</i> L. bulbs	DSE MeOH	Thin-layer chromatography (TLC) methods coupled with densitometric detection	4.2 –199.6 mg/kg DW

Species/product	Extraction	Analysis	Phytomelatonin Content
<i>Lupinus albu</i>	DSE 15 h Overnight dark >90% LLE	LC-FLD Spherisorb-S5 ODS2 λ_{exc} = 280 nm λ_{emi} = 350 nm Isocratic water: Acetonitrile (50:50) Flow rate of 0.2 L/min	1.8 –20 ng/g FW
<i>Hordeum vulgare</i>	DSE 15 h Overnight dark >90% LLE Na-phosphate buffer pH 7.5 UAE	LC-FLD Spherisorb-S5 ODS2 λ_{exc} = 280 nm λ_{emi} = 350 nm	0.58 –24.1 ng/g FW
Coffee tissues	DSE buffer	LC-Q-ToF ESI+ ToF (kV): 9.10 LC-FLD λ_{exc} = 280 nm λ_{emi} = 350 nm	0.407 –96.54 μ g/g FW 115.25 μ g/g DW
<i>Lupinus albus</i>	DSE Ethyl acetate BHT 15 h dark and shakin	LC-ECD RP-C18 column (ODS2) +850 mV LC-MSD QQQ ESI+ SIM 233 –174	28.1 –93 ng/g FW
Monocot species	DSE Ethyl acetate BHT 15 h dark and shaking	LC-ECD RP-C18 column (ODS2) +850 mV LC-MSD/QQQ ESI+-SIM 233 –174	26.7 –124.7 ng/g FW
Ultrasound Assisted (UAE)			

Species/product	Extraction	Analysis	Phytomelatonin Content
<i>Morus alba</i> leaves	UAE 1:2 MeOH 20 min	LC-MS QQQ ESI Inertsill ODS-3 C18	nd—9.77 µg/g extract
Brassica ssp. seeds	UAE-SPE UAE-LLE	ELISA kit LC-PDA $\lambda = 280$ nm HPLC-MS-MS ESI RP C18	176–197 ng/g DW
<i>Oryza sativa</i> grain	UAE 200 watts and 24 kHz	LC-FLD RP 18 Acquity UPLC® BEH column $\lambda_{exc} = 290$ nm $\lambda_{emi} = 334.9$ nm	7.13 –216 µg/kg
Different plant/food samples	UAE SPE DSE	LC-MS-MS APCI+ and ESI+	4.9–440 ng/g
Plant and cell culture	UAE 50% MeOH	LC-MS Q ESI+ Waters Acquity BEH column	LC-MS Q ESI+ Waters Acquity BEH column
Grape skins	UAE SPE	LC-FLD $\lambda_{exc} = 280$ nm $\lambda_{emi} = 348$ nm ELISA	0.005 –0.965 ng/g

Supercritical Fluid Extraction (SFE)

<i>Brassica campestris</i> seeds	SC-CO ₂	LC-PDA $\lambda = 280$ nm HPLC-MS-MS ESI RP C18	56.44–660 ng/g DW

Solid Phase Extraction (SPE)

<i>Prunus cerasus</i> fruits	SPE C18	LC-Q-Orbitrap ESI Kinetex C18	2.9–12.3 ng/g DW

Species/product	Extraction	Analysis	Phytomelatonin Content
Chinese medicinal herbs	SPE C18 SPE cartridge	LC-FLD RP 5 μ m Hypersil ODS (4.6 $\times$ 250 mm) λ_{exc} = 280 nm λ_{emi} = 348 nm	12-190 ng/g
Wines	SPE Strata X- polymeric reversed phase	LC-MS Q ESI and APCI LC-FLD λ_{exc} = 280 nm λ_{emi} = 350 nm	0.038–0.063 μ g/L
Must fermentation process	SPE C18	LC-Orbitrap HESI+ ZORBAX RRHD SB-C18 LOD: 0.0047 ng/mL LOQ: 0.0144 ng/mL	nd —0.14 ng/mL
Morning glory plants Tomatoes	SPE C18	RIA Radioactive melatonin (1 mCi/mL [O-methyl- ³ H] melatonin GC-MS Derivatized with pentafluoro propionic anhydride	Morning glory 20–30 pg/g RIA 5–10 pg/g GC-MS Tomatoes 3–14 pg/g RIA 0.5–3 pg/g GC-MS
QuEChERS			
Wines	QuEChERS SPE DLLME	LC-FLD	0.74–1.36 ng/mL
Sesame	QuEChERS Methanol C18 dispersant	LC-QQQ C18 column MRM: m/z 233 $\rightarrow$ 174, 159 and 130	0.41–25.18 ng/g
Microwave Assisted Extraction (MAE)			

Species/product	Extraction	Analysis	Phytomelatonin Content
<i>Oryza sativa</i> grain	Microwave-assisted extraction (MAE)	LC-FLD $\lambda_{exc} = 290$ nm $\lambda_{emi} = 330$ nm RP-18 Lichrospher column	16.0 –264.0 $\mu\text{g/kg}$ Avg.: 54.17 ± 13.48 $\mu\text{g/kg}$

2.4.1 Ultrasound-assisted Extraction, UAE (Sonication Extraction)

Electromagnetic waves at frequencies higher than sound waves that are perceptible to the human ear (20 kHz to 2000 kHz) are known as ultrasounds. The cell membrane becomes permeable due to the ultrasound's sonic cavitation, which expands the solvent-sample contact area. Thus, the UAE technique extracts bioactive compounds through the mechanical action of ultrasounds on cell walls of plants; accelerates the phytochemicals' release, and also lowers energy consumption ([Altemimi et al., 2016](#); [Bimakr et al., 2017](#); Quintero [Quiroz et al., 2019](#)). [Chakraborty and Bhattacharjee \(2020\)](#) used UAE for the extraction of melatonin from mustard seeds. They used ethanol: water mixture (1:1 v/v) as the green extraction solvent. It was optimized for sample weight to extraction solvent ratio, ultrasonication frequency, and extraction time to extract the highest amount of melatonin possible from mustard seeds. The UAE extract was further purified through liquid-liquid extraction (LLE) and solid phase extraction (SPE) to remove co-extractants before quantification of melatonin. Generally, ultrasound treatment during sample extraction enhances the extraction of phytomelatonin by 2-20% depending on the sample ([Arnao & Hernández-Ruiz, 2009](#)). [Uğur \(2023\)](#) also extracted

melatonin from cherry fruits using UAE. [Moser et al. \(2022\)](#) extracted melatonin from various samples using UAE for 20 minutes.

2.4.2 Microwave-assisted Extraction (MAE)

Microwaves (300 MHz to 300 GHz) and solvents are utilized in the extraction procedure of microwave-assisted extraction (MAE). The rapid production of pressure and heat in MAE improves compound recovery and extraction kinetics ([Hemwimon et al., 2007](#)). The efficiency of MAE depends on time, temperature, solid: liquid ratio, and the type and composition of the solvent ([Grigonis et al., 2005](#)). Since the heat generated by microwaves is directly proportional to the solvents' dielectric constant, selecting the right solvents has an impact on the extraction yield ([Bagade & Patil, 2021](#)). The extraction process is also influenced by the solvent's viscosity; lower viscosity solvents promote ion dispersion and, consequently, solvation ([Kaufmann & Christen, 2002](#)). In order to extract melatonin from rice grains, [Setyaningsih et al. \(2012\)](#) created a novel microwave-assisted extraction (MAE) method. They tested the stability of melatonin under a variety of MAE conditions, including temperature (125–175°C), microwave power (500–1000 W), time (5–25 min), solvent (10–90% ethyl acetate in methanol), and solvent to sample ratio (10:1–20:1). Response Surface Methodology (RSM) was used to optimize these extraction variables. 195 °C extraction temperature, 1000 W microwave power, 20 min extraction time, 100% MeOH as a solvent, and 10:1 ratio of solvent to sample were reported as the optimized MAE conditions for melatonin extraction from rice.

2.4.3 Solid-phase Extraction (SPE)

SPE has several benefits over other extraction techniques, including reduced organic solvent usage, quick sample loading, and excellent

recovery. A variety of polymeric adsorbents, including polyaniline, poly-N methylaniline (PNMA), polydiphenylamine, and polypyrrole (PPy). Commercial SPE adsorbents such as C8 Amprep, C18, Strata-X, and Waters Oasis HLB SPE cartridges have been used for the SPE of melatonin ([Gomez et al., 2015](#)). Molecularly imprinted polymers (MIPs) have been used for SPE applications due to their superior stability, high specificity, relative ease, and low preparation cost in order to achieve better selectivity ([Yang et al., 2005](#)). MIPs are specially designed polymeric materials that are created with a target molecule present so that, when the template is removed, complementary cavities can develop ([Glad et al., 1985](#)). There is no need for melatonin preconcentration when MIPs are used as an SPE adsorbent. For the purpose of solid phase extraction (SPE) of melatonin (MLT) from edible plants like chamomile (*Matricaria chamomilla*), fennel (*Foeniculum vulgare*), linden (*Tilia tomentosa*), and sage (*Salvia officinalis*), [Esen et al. \(2021\)](#) created melatonin (MLT)-imprinted poly (ethylene glycol dimethacrylate-N-methacryloyl-L-tryptophan methyl ester) [MLT-PEMATrp] microbeads. Using melatonin as a template, the microbeads were created using the molecular imprinting approach; the melatonin content of these samples ranged from 90 to 103.4%, and the limit of detection (LOD) was between 0.026 and 0.094 µg/ml.

2.4.4 Liquid-Liquid Extraction (LLE) and Dispersive Liquid-Liquid Microextraction (DLLME)

Liquid-liquid extraction (LLE) in conjunction with homogenization is frequently used in phytomelatonin extraction, taking advantage of the molecule's varying solubility in organic and aqueous solvents. In 1995,

Dubbels and coworkers extracted the phytomelatonin (2000 pg/mg of protein) by using the LLE with Na_2CO_3 , and diethyl ether ([Ye et al., 2017](#)).

1. Dispersive liquid-liquid microextraction (DLLME) is another kind of LLE in which a dispersant that is soluble in both organic and aqueous phases is added to the extraction medium to create a third phase that improves the mass transfer of the target component to the extraction solvent. Using acetonitrile as the disperser solvent, chloroform as the extraction solvent, and NaCl, DLLME has been used to analyze phytomelatonin in wines with good efficiency ([Pourhossein et al., 2018](#)).
2. Direct solvent extraction (DSE) is a simpler method in which samples are treated for a predetermined amount of time with a suitable organic solvent. The solvent is removed after extraction, and the sample is reconstituted before to analysis. Ethyl acetate has been used for 15 hours in the dark with continuous shaking to extract phytomelatonin using DSE in lupin tissues and monocot species ([Hernandez-Ruiz et al., 2004](#)). Pressurized liquid extraction (PLE) provides several advantages over conventional extraction methods, including reduced solvent usage, higher extraction yields, and improved reproducibility. By utilizing organic solvents at elevated temperatures and pressures, PLE enhances the efficiency of the extraction process ([Setyaningsih et al., 2015](#)).

2.4.5 Supercritical Fluid Extraction (SFE)

Chakraborty and Bhattacharjee examined the best conditions for phytomelatonin extraction from yellow mustard seeds using supercritical carbon dioxide. The optimal extraction conditions, they found, were 300 bar

and 50 °C for 120 minutes, producing 660.72 ± 41.05 ng/g of mustard powder that has been dried ([Chakraborty et al., 2017](#)).

2.4.6 QuEChERS: Rapid, Simple, Cost-effective, Productive, Robust, and Secured

For the extraction of phyto-melatonin, the more effective and ecologically friendly QuEChERS (rapid, easy, cheap, effective, robust, and safe) technique has been used. This green analytical chemistry procedure enables a quicker and less expensive solution by using less non-halogenated, low-toxicity solvents, reagents, and lab supplies ([Varela-Martinez and others, 2020](#)). QuEChERS extraction has been successfully applied to isolate phyto-melatonin from wines using acetonitrile, followed by the addition of MgSO₄ and NaCl. Following extraction, the materials were centrifuged and subjected to further purification in a dispersive solid-phase extraction (SPE) tube utilizing PSA, Bulk Carboxograph, and MgSO₄. A methanolic extraction was carried out overnight at 4 °C in the dark as part of a study looking at phyto-melatonin production in sesame plants. A C18 dispersant was then used for further ([Wang et al., 2005](#)) processing.

2.5 MELATONIN QUANTIFICATION APPROACHES

The levels of melatonin have been determined using a variety of analytical techniques. It can be detected by immunological techniques like radioimmunoassay (RIA) and enzyme-based immunoassay (ELISA) ([de la Puerta et al., 2007](#)); chromatographic techniques like GC-MS ([Gonzalez-Gomez et al., 2009](#)), HPLC-MS/MS ([Rodriguez-Naranjo et al., 2011](#)), HPLC-fluorescence detector ([Garcia Parrilla et al., 2009](#)), HPLC-ECD (Electrochemical detection) ([Reiter et al., 2007](#)); and chemiluminescent

techniques ([Garcia-Parrilla et al., 2009](#)). A topic that has not been widely addressed in the quantification of melatonin is the existence of many isomers of melatonin ([Kocadağlı et al., 2014](#)). As an outcome of improved methods, many melatonin isomers (MIs) have been discovered ([Tan et al., 2012](#)). MIs are natural molecules with structures similar to melatonin, potentially sharing some of its biological functions. However, whether these isomers are truly melatonin isomers or tryptophan ethyl esters is still controversial ([Vigentini et al., 2015](#)).

2.5.1 Radioimmunoassay (RIA)

Melatonin measurement through RIA is done by mixing a known amount of radioactive melatonin (2-I¹²⁵-iodomelatonin or ³H-melatonin) with a fixed amount of antibody against melatonin. The addition of unlabeled melatonin in increasing concentration to the mixture causes the displacement of labelled melatonin from the antibody. The free-labeled melatonin is then separated from the antibody-bound radioactive melatonin, and subsequently, radioactivity is measured. A calibration curve is prepared from a known concentration of labeled and unlabeled melatonin that allows quantification of melatonin concentration in biological samples. RIA methods have been described, wherein different antibodies (as noted above) have been used, by changing ³H-melatonin to 2-I¹²⁵-iodomelatonin which allows a lower detection limit. However, 2-I¹²⁵ is susceptible to nonspecific binding so there is a likelihood of false estimations. According to [Dubbels et al. \(1995\)](#), radioimmunoassay measurements of melatonin concentrations in nine distinct plants ranged from 0 to 862 pg melatonin/mg protein. [Fraser et al. \(1983\)](#) described a protocol for melatonin quantification using RIA in plasma that was adopted widely with slight modifications. However, when using commercial RIA kits in conjunction with a modified extraction

technique to analyze melatonin, interference from compounds such as calcium heparin is an issue.

2.5.2 Assays Utilizing Enzyme-linked Immunosorbents (ELISA)

Enzyme-linked immunosorbent assay (ELISA) uses antibodies and colorimetric enzymes ([Lequin, 2005](#)). The enzymes catalyze color reactions via an enzyme-specific chromogen. ELISA is simple to perform and relatively inexpensive. There are different types of ELISA, such as direct, indirect, competitive and sandwich ELISA ([Gan and Patel, 2013](#)). [Li & Cassone, \(2015\)](#) developed a simple, and high-throughput enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of melatonin in cell culture medium. They used a commercial monoclonal melatonin antibody and melatonin-horse radish peroxidase (HRP) conjugate, so it can be applied at a low cost compared with commercial ELISA kits. ELISA is recognized as a technically easier technique that requires a little sample amount to complete. However, the method requires an additional pre-concentration step to estimate low levels of melatonin. Unlike in animals, immunological techniques such as RIA or ELISA pose some problems in plants due to cross-reactivity with co-extractives.

2.5.3 Liquid Chromatography-based Method

Chromatographic methods have been the most widely used quantification techniques in the area of melatonin research. HPLC techniques are more economical and time-efficient when sample derivatization is not required before analysis. Most of the HPLC methods reviewed have used reverse phase columns (e.g. RP18 or RP8) for melatonin separation and fluorescence detectors (FD) were found to be sensitive and versatile for

quantifying melatonin in food samples as they showed low limits of detection and quantification ([Garcia Parrilla et al., 2009](#)). [Pérez-Llamas et al. \(2020\)](#) analyzed phytomelatonin in some herbs using liquid chromatography coupled with fluorescence detection. They described using an excitation wavelength of 280 nm and an emission wavelength of 350 nm, Waters Spherisorb-S5 ODS2 column (250×4.6 mm), an isocratic mobile phase comprising of water: acetonitrile (80:20 v/v) at a flow rate of 0.2 mL/min. In order to analyze the results, the excitation and emission spectra of standard melatonin were compared to the samples' matching phytomelatonin peak.

Phytomelatonin is typically monitored at wavelengths of 274 nm or 280 nm using a UV-Vis detector. This type of detector has been employed for quantifying phytomelatonin in mustard seeds and food supplements. However, a fluorescence detector (FLD) offers greater sensitivity than UV-Vis for the determination and quantification of phytomelatonin. For FLD, the excitation wavelength is typically adjusted at 224–298 nm, while the wavelength of emission ranges from 330 to 392 nm. Several studies on rice, Using a fluorescence detector, phytomelatonin has been successfully measured in *Lupinus albus*, *Hordeum vulgare*, wine, and dietary supplements with good sensitivity and accuracy.

Mass detectors (MSD), which combine the high sensitivity of mass spectrometry (MS) with the selectivity of liquid chromatography (LC), are one of the most used methods for detecting phytomelatonin. Low mass resolution (low-resolution mass spectrometry, or LRMS) and high mass resolution (high-resolution mass spectrometry, or HRMS) are the two primary forms of mass detectors that are used. Mass spectrometry may be used to detect and quantify certain compounds using multiple reaction monitoring (MRM) or selective reaction monitoring (SRM) modes, or it can

be used to screen unknown chemicals using full scan mode. Phytomelatonin is typically analyzed in positive ion mode, with the shifting from 233 to 216/174 being commonly determined. Considering ionization various comparisons is employed, with the most common method being electrospray ionization (ESI). Other ionization techniques that have been used in the detection of phytomelatonin by MS include droplet spray ionization (DSI), matrix-assisted laser desorption ionization (MALDI), atmospheric pressure chemical ionization (APCI), and nanospray ionization (nESI). A mass detector's primary function is to verify the analyte's mass and identify it using its molecular fragments. Although this method is quite dependable, it needs a clean extract to prevent matrix effects and interferences. Increased resolution for the analyte's mass is provided by a variety of mass detector types that have been developed over time, such as single quadrupole (Q), triple quadrupole (QQQ), time-of-flight (Q-ToF), ion-trap (IT-MS), and orbitrap-MS. All of these MS detectors have been utilized in the determination of phytomelatonin, demonstrating their versatility and effectiveness in this application.

2.5.4 Liquid Chromatography (LC)-MS-based Approaches

Recent advances in technology and reductions in the costs of MS have popularized its use for the quantification of phytochemicals. MS-based approaches present a new opportunity in phytomelatonin research, due to their sensitivity and specificity, enabling quantification in diverse tissues and species. Analysis by liquid chromatography and identification by mass spectrometry (LC-MS/MS) are among the most recommended techniques for the detection and quantification of phytomelatonin. In this regard, LC-MS/MS with positive electrospray ionization (ESI+) and multiple reaction

monitoring (MRM) is widely used. The innovative technique of liquid chromatography with time-of-flight/mass spectrometry (LC-TOF/MS) has also been applied to phytomelatonin detection. However, less specific but very sensitive techniques such as LC with electrochemical or fluorometric detection are also excellent if accompanied by identification by LC-MS/MS.

[Hernández-Ruiz et al., 2021](#) analyzed phytomelatonin content in selected herbs by LC using mass spectroscopy with time-of-flight (LC-QTOF/MS). [Pérez-Llamas et al. \(2020\)](#) also analyzed some herbs for phytomelatonin content through Time-of-Flight/Mass Spectrometry in Liquid Chromatography (LC-QTOF/MS). They used the C18 column, a flow rate of 0.4 mL/min, temperature 40°C, mobile phase A (MilliQ water with 0.1% formic acid) and B (acetonitrile with 0.1% formic acid). The mass spectrometer was run in positive ionization mode. Data were scanned in the range of 50-300 m/z range and Mass Hunter Qualitative Analysis Navigator software (Agilent Technologies, Rev. B.08.00) was used for the analysis.

[Ye et al. \(2017\)](#) used ultra performance liquid chromatography (UPLC) to create a quick and effective approach combined with orbitrap mass with high resolution spectroscopic analysis. As an internal standard, the stable isotope-labeled melatonin (MLT-d4) was utilized. Liquid-liquid extraction was used to purify the crude extracts from rice. High recovery and specificity (>96.4%) were demonstrated by the technique. A coefficient of determination (R^2) value of 0.99 revealed good linearities in the 0.01–20 ng/mL range 0.03 pg was the stated limit of detection. Compared to gas-chromatography (GC)-based techniques, which necessitate sample derivatization prior to analysis, and electrochemical detection-based techniques, which often require lengthy equilibration intervals, LC-MS procedures are further distinguished by their increased throughput and

enhanced simplicity of use. The ability to quantify numerous analytes simultaneously is another obvious benefit of LC-MS-based methods. For example, identifying many indoleamines or measuring associated signaling molecules or plant growth regulators using a via a single injection.

2.5.5 Solid Analysis Probe for Atmospheric Pressure (ASAP)

The ambient mass spectrometric method known as atmospheric pressure solid analysis probe (ASAP). It is associated with chemical ionization at atmospheric pressure (APCI). This method involves submerging a borosilicate rod in a liquid or solid sample before placing it within the ionization chamber. Analyte desorption happens when hot nitrogen is streamed, and corona discharge ionization is carried out. An ASAP technique was created by [Moser et al. \(2022\)](#) to quickly quantify melatonin in hypnotics. No chromatographic separation was carried out because they used ASAP. Thereby, a single analysis took no more than 1 minute. With a source temperature of 150°C and a scan from 100 m/z to 300 m/z, positive ionization mode was used. Standards for melatonin at a concentration of 50 mg/kg each were examined concurrently. Ultra-high-performance liquid chromatography in conjunction with a quadrupole-time-of-flight mass spectrometer was used to perform the reference measurements. Following validation, the coefficients of determination for both approaches were higher than 0.97. The boundaries of detection and measurement were less than 1 mg/kg.

2.6 CONCLUSION

Phytomelatonin has emerged as a significant compound with potential applications across multiple industries, including pharmacology,

agriculture, and nutraceuticals. The review highlights the importance of efficient isolation, extraction, detection, and quantification methods to harness the full potential of phytomelatonin. While traditional techniques such as chromatography continue to be valuable, advancements in high-throughput platforms, including LC-MS/MS and capillary electrophoresis, have significantly enhanced the sensitivity and scalability of detection. The integration of green chemistry approaches and bioinformatics tools has further optimized extraction processes and accelerated research. Despite these advancements, challenges in maximizing yield, purity, and cost-effectiveness remain, underscoring the need for ongoing improvements in technological interventions.

Future research should focus on developing more sustainable and cost-effective extraction methods, particularly those that leverage green chemistry and renewable resources. There is also a need for continued refinement of high-throughput detection methods to improve accuracy, sensitivity, and scalability. The application of advanced bioinformatics tools for the identification and quantification of phytomelatonin can help streamline research processes and enable better integration with large-scale agricultural and pharmaceutical systems. Moreover, exploring the pharmacokinetics and therapeutic efficacy of phytomelatonin in clinical settings could unlock its full therapeutic potential. As these technological and methodological advances evolve, they will likely expand the scope of phytomelatonin's use in diverse industries, offering new opportunities for therapeutic and industrial innovations.

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3

Methodological Aspects to Exogenously Source the Phytomelatonin Supplementation in Plants Exposed to Environmental Stressors

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DOI: [10.1201/9781003527633-3](https://doi.org/10.1201/9781003527633-3)

3.1 INTRODUCTION

Plants, being sessile organisms, are inherently vulnerable to a range of environmental stressors, including drought, extreme temperatures, salinity, and pathogen attacks ([Iqbal, 2023](#); [Zhang, 2015](#)). These stresses can significantly impact plant health, growth, and productivity, posing a major threat to global agriculture and food security ([Kumari et al., 2024](#)). The increasing intensity and frequency of such stress events either abiotic or biotic, exacerbated by climate change, have led to substantial yield losses in

major crops, highlighting the urgent need for effective strategies to enhance plant resilience.

The impact of abiotic stress on plants is extensive, affecting them at all levels from molecular to morphological and throughout all stages of development. For instance, drought stress can disrupt water transport within plants, leading to reduced turgor pressure, which hinders cell elongation and overall growth ([Nazir et al., 2024](#)). This stress results in smaller leaf areas, shorter plant heights, and lower crop yields. Similarly, cold stress can cause cellular dehydration, crystallization of cellular water, and oxidative damage, significantly inhibiting plant growth and productivity.

Among all abiotic stresses, heat stress stands as a primary contributor to worldwide agricultural losses, driven largely by climatic conditions. Rising global temperatures are projected to exacerbate heat-related crop damage, as elevated thermal conditions trigger excessive reactive oxygen species (ROS) generation. These unstable molecules damage cellular components such as lipids, DNA, and proteins, impairing vital physiological processes and hindering plant development ([Poór et al., 2022](#); [Yamagishi, 2022](#); [Zhou et al., 2020](#); [Khan et al., 2013](#)).

Similarly, cold stress poses a critical challenge for horticultural productivity, particularly in temperate regions. Prolonged exposure to low temperatures induces leaf wilting, chlorophyll degradation, and reduced photosynthetic capacity. It also destabilizes cell membrane integrity, disrupts enzymatic functions, and creates metabolic imbalances, ultimately stunting plant growth and diminishing yields ([Zhu et al., 2023](#)).

Plants unlike animals are dependent on their innate ability to cope stress on various physical, biochemical and molecular levels. Plants have developed various mechanisms to resist abiotic stress, including strategies of escape, avoidance, and tolerance ([Kumari et al., 2023](#)). They may adjust

osmotic balance to retain water within cells, thus preserving cell turgor and alleviating stress impacts ([Khatoon et al., 2024](#)). But surely more effective strategies need to be incorporated to curb the loss of food crops. One such promising avenue for mitigating the effects of environmental stressors is the use of melatonin, a naturally occurring indoleamine compound widely known for its role in regulating sleep in animals ([Seth et al., 2023](#)). In plants, melatonin has been found to play a critical role in various physiological processes, including growth regulation, antioxidant defense, and stress response ([Seth et al., 2023](#); [Tyagi et al., 2023](#)). Recent research has demonstrated that melatonin can enhance plant tolerance to abiotic stresses by modulating a range of biochemical and molecular pathways.

This chapter explores the multifaceted role of melatonin in plant stress physiology, with a focus on methodological approaches to enhance resilience against environmental stressors. We begin by examining the nature and impact of various abiotic stresses on plant systems, followed by a detailed discussion on the approaches involved in supplementing plants with melatonin through various methods, including foliar sprays, soil drenching, seed priming, hydroponic solutions and genetic modifications. The efficacy of these methods can vary based on factors such as the concentration of melatonin used, the timing of application, the specific plant species, and the nature of the stressor. Understanding the optimal conditions and mechanisms by which exogenous melatonin exerts its protective effects is crucial for developing effective agricultural practices. By providing a comprehensive overview of current research and practical guidelines, this chapter aims to equip researchers and agricultural practitioners with the knowledge needed to effectively harness the benefits of melatonin in enhancing plant resilience and productivity.

Through a comprehensive review of current literature, this chapter aims to provide a deeper understanding of how melatonin can be harnessed to improve plant tolerance to environmental stressors, offering new insights into the development of climate-resilient crops. By leveraging the protective properties of melatonin, it is possible to mitigate the adverse effects of climate change on agriculture, thereby contributing to global food security and sustainability.

3.2 METHODOLOGIES FOR MODULATING MELATONIN CONCENTRATION IN PLANTS

Melatonin concentration in plants can be modulated in plants by using either of the given methods:

1. Seed priming with melatonin
2. Foliar application of melatonin
3. Soil application of phytomelatonin
4. Genetic transformation

Methodologies to be included for the crop improvement against stress and food security are discussed in brief in this chapter.

3.2.1 Seed Priming with Melatonin

Seed germination is a vital developmental response of a fertilized seed embryo reactivates its metabolic machinery influenced by internal seed dormancy levels, physicochemical and biochemical regulations as well as multiple external environmental factors which ultimately determines the field production of any crop by ensuring an optimal plant stand ([Ma, 2024](#);

[Kaur et al., 2023](#)). Crop productivity depends largely on following good agricultural practices for rapid and uniform seedling emergence and root growth, as slow germination frequently leaves crop plants vulnerable to harsh environmental factors ([Joshi, 2024](#)). Thus, agricultural scientists have been developing strategies and techniques to reactivate metabolic processes that were switched off during dormancy to enhance the germination of seeds and promote crop development. One way to enhance seed germination is pre-sowing seed treatment or seed priming ([Lutts, 2016](#)). The concept of seed priming originates from delayed seed-release desert plants (serotinous plants). These seeds experience multiple cycles of wetting and drying, which improve their germination following the ultimate seed dispersal triggered by intense rainfall ([Santini, 2013](#)).

Seed priming is the exogenous uniform application of natural or synthetic growth regulator on a seed lot, causing the start of a biological mechanism through which a plant readies itself to react more swiftly or robustly to impending environmental challenges leading to better seed hydration, protection, increased seed vigor, plant growth and crop yield ([Fernandes, 2022](#)). Scientists have developed various seed priming methods in diverse environments to provide economic and agronomic advantages to cultivated plants. Melatonin application at an optimized dose has demonstrated potential as a bio-stimulator by improving several critical aspects of seed germination, overall plant growth and boosting antioxidant activity under stress conditions ([Tyagi et al., 2023](#)). Apart from the involvement of melatonin in stimulating growth, the exogenous administration of melatonin modulates the gene expression and significantly enhances plant resilience under various stress conditions. Recent research has found seed priming to be beneficial against both biotic stress (pathogenic bacteria and fungi) and abiotic stress (salinity, drought, cold, heavy metal) and also escalate the

growth, seed vigor, yield, disease resistance and physio-biochemical parameters (endosperm softening, mobilization of stored compounds, stimulation of respiratory processes, repair of DNA, RNA, proteins, and cellular membranes, and enhancement of antioxidant defenses) of the crop plants ([Tyagi, 2023](#); [Paul & Rakshit, 2022](#); [Becerra-Vázquez, 2020](#)). Non-toxic, cost-effective and eco-friendly techniques like seed priming which improve pre-germinative metabolic processes and enhance seed germination, supporting global food security and combat environmental constraints ([Paul et al., 2022](#)). Melatonin-priming has been demonstrated to boost chlorophyll levels and elongate shoot growth, sugar content, seed germination, stomatal aperture size, root length, proline content, drought tolerance, fresh seedling weight, antioxidant defense, and total soluble protein content thus improving the crop yield significantly ([Samadi, 2024](#)). Although seed priming has many advantages, the adoption of technology is hindered by conflicting reports on its variable impact on different species, cultivars, and even batches of seeds like decreased viability of desiccation-sensitive seeds, reduced long-term storage ability, reduce seed coat permeability and unequal hydration, ultimately leading to unsynchronized emergence (Wang et al., [2024a](#), [2024b](#)). Given these constraining factors, it is crucial to precisely determine the duration of priming treatment, storage conditions, temperature, medium, concentration, and water volume applied to achieve the optimal level of seed hydration and to ensure desired level of seed hydration and to ensure equal germination parameters among a defined plant species seeds ([Di Girolamo, 2012](#)). Therefore, obtaining a thorough grasp of the methods and physiological advantages of melatonin priming can enhance the optimization of this method.

3.2.1.1 Mechanism of Melatonin Seed Priming

Priming is an important seed pre-treatment method that controls seed hydration to effectively initiate seed germination through an intricate series of complex biochemical cascades inside the seed. This process activates enzymes, synthesizes growth-inducing substances, degrades substances that hinder germination, and restores cellular damage ([Amir, 2024](#)). When a dry seed is exposed to water, it progresses through three unique stages prior to sprouting: Phase I, known as the imbibition phase; Phase II, referred to as the activation or lag phase; and Phase III, the growth and cell elongation phase.

Phase I: Imbibition

In the first phase the seed quickly takes up water because of its significantly reduced water potential compared to the outside environment. During this phase, water moves into the apoplastic spaces, proteins are produced using pre-existing mRNAs, and both DNA and mitochondria undergo repair. The seed gets fully hydrated by the end of phase I and makes the cell ready for ‘pre-germinative metabolism’ ([Corbineau, 2023](#)).

Phase II: Activation

Phase II initiates metabolic activities and repair processes, including food reserve mobilization, metabolism reactivation, and protein synthesis. Mitochondria are formed for fatty acid oxidation, while DNA repair and ROS scavenging pathways protect the genome ([Amir, 2024](#)). Enzyme activity for carbohydrate and lipid breakdown increases, aiding reserve mobilization and embryo expansion. Stress-responsive proteins (LEA and heat shock proteins) accumulate, and antioxidants like catalase and peroxiredoxin maintain cellular integrity ([Sen et al., 2020](#)). ABA levels decrease to break dormancy, leading to radicle protrusion through root cell elongation and division.

Phase III: Growth and seed establishment

In Phase III, upon sowing and irrigation, seeds rapidly absorb water through imbibition, triggering cell elongation and radicle protrusion. This marks full germination, with increased metabolic activity, including DNA, RNA, and protein synthesis. Growth-promoting hormones like GA stimulate seedling establishment ([Fu, 2024](#)).

Starch is converted to sugars, fueling energy production via glycolysis, while TAGs degrade through β -oxidation for additional energy ([Graham, 2008](#)). Protein hydrolysis provides amino acids for metabolism, regulated by kinases such as MAPKs and CDPKs ([Zhao, 2021](#)).

Melatonin enhances germination under stress by upregulating energy metabolism pathways like glycolysis and the glyoxylate cycle ([Wang et al., 2023](#)). It promotes TAG breakdown and β -oxidation ([Yan et al., 2020b](#)), while preserving ionic balance by decreasing Na^+ and enhancing K^+ absorption ([Siddiqui, 2021](#)), and modulates ROS through antioxidative enzymes like SOD, CAT, and peroxidases ([Khan, 2020](#)).

Under drought stress, melatonin lowers malonaldehyde levels, preserving membrane integrity ([Campos, 2019](#)), and regulates aquaporins (GmPIPs, GmTIPs) to optimize water uptake ([Jahan, 2023](#)). Additionally, it enhances photosynthesis by increasing PSII reaction center protein gene expression, ensuring steady nutrient supply for seedling growth ([Zhang et al., 2021b](#)).

3.2.1.2 Methods of Seed Priming

Seed priming is an important strategy designed to enhance seed vigor, accelerate germination, and alleviate environmental stresses. Various methods have been established to accomplish these goals effectively: [1] Traditional seed priming methods include (i) Hydro-priming, (ii) Halo-priming, (iii) Osmo-priming, (iv) Hormone-priming, (v)

Foliar application, also known as foliar feeding, involves spraying nutrients directly onto plant leaves, allowing for plants uptake nutrients via their leaves and bypassing the root system. This method enables plants to utilize nutrients more rapidly and correct observed deficiencies more quickly than seed or soil applications while requiring smaller quantities of nutrients and avoiding phytotoxicity ([Kaya & Ashraf, 2024](#)). Foliar application of melatonin is essential for achieving sustainable agriculture by improving plant resilience, increasing crop yields, and ensuring long-term agricultural productivity. The melatonin levels in exogenously applied seed coatings and soil solutions are relatively low because they predominantly exist in insoluble forms, fixed to the seed or soil complex. Otherwise, the more soluble form of melatonin is readily washed deeper into the soil layers. The age of the leaf is important for foliar absorption ([Alshaal & El-Ramady, 2017](#)). Plant leaves have an innate ability to absorb nutrients through their stomatal pores, a biological mechanism that enables them to supplement their nutrient intake through rainwater. In agricultural practices, this principle is applied by administering foliar sprays containing diluted melatonin solutions. This approach helps mitigate the detrimental effects of abiotic stressors, such as drought or salinity, on plant growth and development ([Hasnain, 2023](#)). Melatonin is applied during the most sensitive stages of plant growth, where it reaches the leaf cells the leaf cells by crossing the cuticular barrier, from where it is translocated to neighboring tissues via the plasmodesmata channels that enable cytoplasmic exchange. Additionally, some compounds may enter the xylem, that facilitate nutrient movement to other parts of the plant ([Figure 3.2](#)).

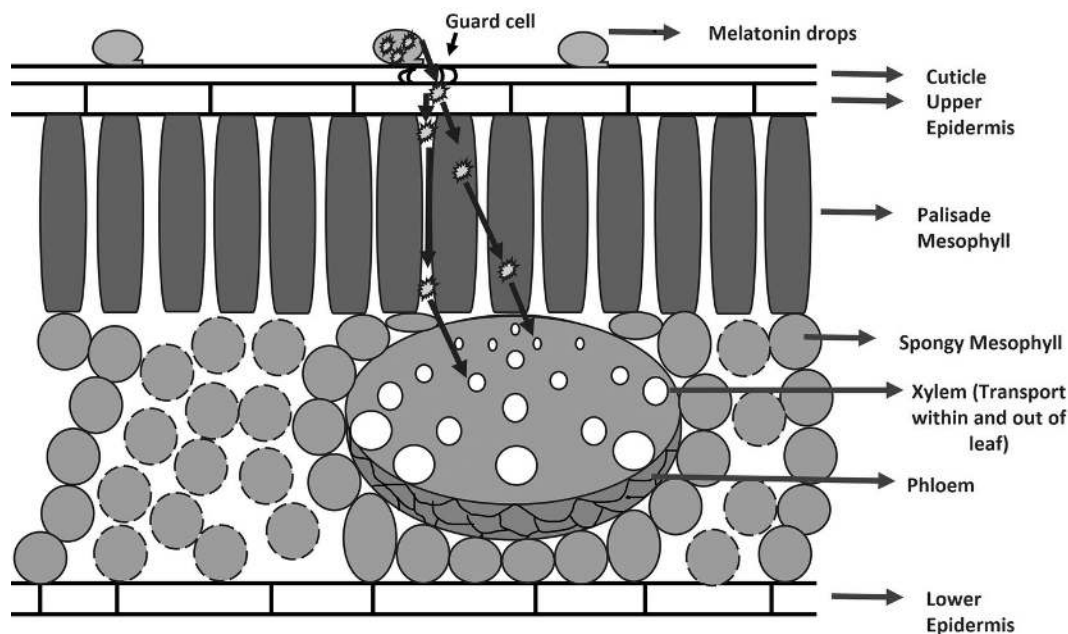


FIGURE 3.2 Stages of Foliar Nutrient Absorption

(Adapted from [Alshaal & El-Ramady, 2017](#)). (1) Application of melatonin solution to moisten the leaf surface; (2) absorption of the melatonin through traversal of the cuticle and epidermal cell wall to enter the leaf tissue; (3) diffusion into the leaf apoplastic space; (4) cellular uptake into the symplastic system (cytoplasm); (5) intra-leaf mobilization and systemic distribution via vascular tissues out of the leaf.



Abiotic stress is associated with disturbing cellular redox homeostasis, leading to the induction of oxidative stress which causes irreversible damage to the plant by forming reactive oxygen species (ROS). ROS are by-products of plant cellular metabolism, such as singlet and triplet oxygen, hydrogen peroxide, superoxide anions, hydroxyl radical, peroxy radical, alkoxy radical, and lipid hydroperoxides generated during metabolic processes. In cases where cell ROS generation is higher than detoxification, melatonin boosts the plant's inherent enzymatic and non-enzymatic antioxidant mechanisms to detoxify and scavenge excess ROS, improving plant physiology and increasing survival rates under abiotic stress ([Gu, 2022](#)). Exogenous foliar application offers several benefits such as rapid delivery of nutrients during critical stress phases when immediate action is

required and soil-based delivery is ineffective or delayed. This method also enhances nutrient accessibility by bypassing root uptake limitations, supplying essential micronutrients like zinc and iron that may have restricted bioavailability in the soil, all of which enhance plant resistance to salinity stress through various physiological processes.

A recent study showed that foliar melatonin treatment improves physiological and biochemical responses in cotton plants (*Gossypium hirsutum* L.) subjected to concurrent low-temperature and salinity stress, enhancing their resilience to these combined abiotic challenges via its effect on reduced membrane injury, enhanced antioxidant enzyme activity, and improved ion balance as well as nutrient uptake efficiency in cotton seedlings under dual stress conditions ([Fu, 2023](#)). The exogenous foliar application of melatonin alleviated nickel toxicity in pepper plants via the reducing the translocation of nickel from roots to shoots, suppressing the buildup of oxidative stress markers like malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), and enhancing the activity of key antioxidant enzymes—including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR), ascorbate peroxidase (APX), and glutathione S-transferase (GST)—in both leaves and roots ([Saqib, 2024](#)). Typically, melatonin is applied to plant foliage at a concentration of 100 μ M to deliver targeted physiological benefits. For instance, in tomato crops, spraying leaves with melatonin has been shown to reduce malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) accumulation, while simultaneously enhancing fruit yield and elevating ascorbic acid concentrations, thereby improving both productivity and nutritional quality ([Ibrahim, 2020](#)). In alfalfa (*Medicago sativa*) plants, a nightly foliar spray of melatonin significantly reduced oxidative damage under saline conditions by lowering hydrogen peroxide (H_2O_2),

malondialdehyde (MDA), and electrolyte leakage, as demonstrated by [Cen \(2020\)](#). Heat stress arises when ambient temperatures surpass a plant's physiological tolerance limits. Similarly, in wheat seedlings subjected to elevated temperatures, foliar melatonin treatment over seven days has been shown to improve thermotolerance by diminishing MDA and H₂O₂ accumulation. Concurrently, it boosted photosynthetic pigments, proline synthesis, and antioxidant activity (enzymatic and non-enzymatic), enhancing stress resilience in young plants ([Buttar, 2020](#)). Photosynthesis, an essential process carried out by plants, algae, and certain bacteria, converts light energy, water, and carbon dioxide into glucose and oxygen. It is vital for determining crop yield but is highly sensitive to environmental changes. Melatonin foliar application improves the photosynthesis by shielding the photosynthetic system from stress-induced damage, thus ensuring sustained crop yield and growth. Foliar spray in coffee plants every week reduced malondialdehyde levels and membrane permeability while elevating enzymatic antioxidant activity, thus effectively suppressing chlorophyll degradation and increasing the photosynthesis rate ([Cherono, 2021](#)). Foliar application of melatonin mitigates oxidative stress and enhances antioxidant enzyme activity, chlorophyll content, and photosynthetic efficiency in two-year-old tea plants exposed to cold stress ([Li, 2018](#)) ([Table 3.1](#)).

TABLE 3.1 Melatonin Concentrations with Foliar Application for Different

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Chromium toxicity	Tomato	10 days seedling	100 μM	Twice a week

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Vanadium toxicity	Green and purple mustard	30 days after sowing	100 $\mu\text{M L}^{-1}$	Applied to leaves twice with a 7-day gap between applications

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Drought	Broccoli	After 20 days of transplanting (height of 15 cm broccoli seedlings)	100 μ M	3 sprays w done at 20-day intervæ
Injury caused by Herbicide	Potato	First leaf of main stem unfolded (> 4 cm)	1 mM	single folia applicatiør

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Salinity	sage (<i>Salvia officinalis</i> L.)	Seedlings with at least two nodes	50 μ M and 100 μ M	Treatment was administered weekly for total of five applications
Waterlogging stress	tomato	four-leaf stage of the seedlings	50, 100, 200 μ M	Applied every other day over a 10-day period

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Heat stress	Tomato	Conducted 40 days after sowing tomato seeds	25 μ M	Single time foliar
Salt stress	Silybum marianum L.	After 7 days of planting	100 μ M	First 3 day continuous

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Saline condition	Maize	Applied prior to seed planting and during the vegetative growth phase	0.5 mM and 1mM	Single dose
Salt induced oxidative stress	<i>Gerbera jamesonii</i>	32 d of germination (i.e., two days after salinity stress)	0.1 to 0.2 mM	Single time

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Low temperature and salinity stress	Cotton	Administered at the two-leaf and one-heart-leaf growth stage	0, 50, 100, 150, and 200 μ M	Applied on the 1st, 4th and 9th day of stress treatment
Drought and waterlogging stress	Hot pepper	two- to four-leaf seedlings	500 μ M	lasted for 2 months.
Drought	African Capsicum	Vegetative stage	50 μ M	Single dose

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Drought stress	Ranunculus asiaticus	eighth true-leaf stage	50, 100, and 200 μM	Applied at 45, 60, 75, and 90 day after planting <i>Ranunculus asiaticus</i>
Lead and oxidative stress	Spinach	fifteen days of plant emergence	100 $\mu\text{M L}^{-1}$	Single dose

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Drought stress	turnip	30 days old <i>B. rapa</i> on foliar parts	100 μ M, 150 μ M, and 200 μ M)	Single dose
Semi-arid	maize	Applied when plants reached the ninth leaf stage (V9)	0, 25, 50, and 75 μ M	Administered for three consecutive days after sunset

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Salinity stress	white beans	After fifteen days of salinity application in seedlings (third leaf stage)	0, 25, 50, and 100 μ M	Single dose

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Drought stress	Lentil	30 days of drought stress	3 mM	Single dos

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Salt stress	Camelina sativa	Applied two days after initiating salinity stress treatments to the upper leaf surfaces of all plants	90 μ M	Single dose

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Wastewater polluted soil	Wheat	germinated seeds	100 $\mu\text{mol}\cdot\text{L}^{-1}$	With irrigation foliar spray
Salt stress	cucumber	fourth-fifth leaf seedlings stage	100 and 200 μM	Spraying repeated biweekly until the experiment concluded (115 days post-transplantation)

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Cadmium toxicity	rice	30 day growth	40 mg/mL	Sprayed or on rice leaf

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Waterlogging stress	Maize	7th leaf stage, foliar	100 µM	Single dose
Drought stress	<i>Citrus</i> species	One-year-old seedlings	100 µM	Single dose

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Waterlogging stress	Sorghum	five-leaf developmental stage	100 $\mu\text{mol L}^{-1}$	Single dose

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Salt stress	Wheat	Wheat seedlings	70 μM	Single dos

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Storage conditions	Barhi Date Palm Trees	12 year old tree, beginning of each season, the number of inflorescences was adjusted to 10 per tree	10, 20, and 50 ppm	Applied twice—two months and one month before harvest

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Salt stress	Maize	7th leaf stages	60 μM	Single dose
Salinity stress	strawberry	5 fully expanded leaves	100 mg L^{-1}	Sprays repeated weekly for total of five applications during the growth period

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Freezing stress	pistachio	30day-old seedlings	0.1, 0.25 and 0.5 μ M	Single dose

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Oxidative damage	Tomato	5-week-old tomato transplants	20 and 40 ppm	Applied in two doses: the first two weeks after transplanting and the second one month later
Drought stress	Moringa oleifera L.	Transplanted adult plant	50, 100, and 150 mM	Two foliar treatments at 45 and 60 days after <i>M. oleifera</i> cutting

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Sandy soil	flax	Seedling	2.5 mM, 5 mM and 7.5 mM	plants were sprayed after 30 and 45 days from sowing
Water stress	wheat	90 days old plants of wheat	150 µg/g	Single dose

Stress Type	Crop	Maturation Period	Optimized Concentration	Dosage System
Salt stress	Alfalfa	One month old seedlings (15 cm height)	50µM	Seed primi and foliar spray after month

3.2.3 Soil Application of Phytomelatonin

Soil pollution by heavy metals due to rapid industrial development and agricultural pesticides is an escalating concern globally resulting in a variety of ecological and public health challenges. The increasing level of pollutants has caused health concerns, decreased crop productivity, and plant stress ([Khan et al., 2021](#); [Briffa, 2020](#)). The pollution from heavy metals frequently stems from activities such as industrial operations, mining, inadequate waste management, and the utilization of polluted water sources for irrigation. Addressing this issue is crucial, necessitating effective pollution control measures, soil remediation strategies, and stricter environmental regulations to safeguard the environment and public health. While various methods have been explored to remove heavy metal pollutants from the environment, these approaches often face challenges such as high costs, lengthy processing times, geological complexities, and political barriers ([Rajendran, 2022](#)). Melatonin, a growth-promoting molecule, is known to function as an antioxidant during abiotic stress. A

recent study identified melatonin in *Exophiala pisciphila*, providing compelling evidence that melatonin may function as a novel growth regulator in plants and play a role in reducing heavy metal accumulation, promoting plant development and improving resistance to heavy metal-induced stress by mitigating oxidative stress ([Yu, 2021](#)). Exogenous soil application of melatonin increases plant survival under heavy metal toxicity, and additional environmental stresses like salinity, low temperatures, high heat, and nitrogen shortages by enhancing antioxidant capacity and boosting the levels of protective molecules (chlorophyll and accessory pigments) and increasing photosynthesis efficiency ([Yang, 2023](#)).

3.2.3.1 Direct Soil Application Techniques of Melatonin

3.2.3.1.1 Soil Drenching

This involves applying a melatonin solution of a known concentration directly to the soil around plant roots. Recent studies have shown that this method can significantly enhance drought resistance in crops, support plant growth (shoot mass), and improve early yield under cold stress conditions in plants like pepper by enhancing photosynthetic efficiency (increased leaf area), improving water dynamics, and photosynthetic characteristics, while reducing malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) levels, decreasing membrane permeability, and altering hormonal profiles ([Korkmaz, 2021](#)). The impact varies with the melatonin dosage applied; pretreating roots with melatonin has been shown to mitigate NaCl-induced reductions in photosynthesis and oxidative damage in a dose-specific way ([Ahmad, 2021](#)).

3.2.3.1.2 Combination with Fertilizers

Integrating melatonin with fertilizers can offer a controlled release, helping plants continuously combat stress conditions by enhancing their antioxidant capacities and regulating stress-related biochemical pathways. The

application of melatonin-coated slow-release fertilizer has shown several positive effects on tomato growth and quality like increased biomass accumulation, root characteristics, phosphorus content, phosphatase activity, yield, and better fruit quality under both reduced and partial phosphorus conditions ([Liu, 2023](#)). Similarly, Melatonin mixed fertilizers treatment in maize crop supported early development and associated physiological and biochemical traits under cadmium stress ([Ma, 2021](#)).

Plants take up melatonin from the growth medium via their root systems, and it is transported through the plant's vascular system. This movement can occur via the xylem (from roots to leaves) and the phloem (throughout the plant), facilitating its distribution to various plant tissues, including roots, stems, leaves, and fruits which is affected by elements such as the plant's developmental phase, nutrient supply, and environmental conditions ([Tan, 2012](#)). Within plant cells, maintaining stable proportions of various mineral nutrients is essential for healthy growth and development. Abiotic stress can upset this nutrient equilibrium, limiting crop growth. Melatonin enhances nutrient absorption and adjusts the elemental makeup of plants, fostering growth even in tough conditions. It achieves this by improving nutrient uptake and movement, altering root structure alongside phytohormones like auxin and ethylene, aiding cell wall adjustments, and preserving redox balance. Melatonin also appears to play a role in nitrogen nutrition by influencing key transporters and enzymes critical for nutrient uptake and processing. Regarding its effect on nitrate (NO_3^-) absorption, studies have explored genes likely encoding major nitrate and ammonium transporters (NRTs and AMTs) in plants treated with melatonin under diverse conditions. In Chinese crab apple trees, applying melatonin at low ammonia levels leads to the coordinated upregulation of genes such as AMT1.2, AMT2.1, and NRT2.7 enhancing nitrogen absorption and

transport ([Du, 2022](#)). The increased activity and expression of nitrate and nitrite reductase enzymes due to melatonin lead to the production of NH_4^+ , which is then incorporated into amino acids via the GS-GOGAT cycle or the glutamate dehydrogenase (GDH) pathway ([Erdal, 2019](#)). Furthermore, melatonin also boosts symbiotic nitrogen fixation in legumes and vegetables; for instance, [Alinia, \(2022\)](#) and [Elseedy, 2023](#) observed that melatonin increases both the quantity and volume of nodules in soybeans. Moreover, melatonin facilitates potassium (K^+) and sodium (Na^+) uptake by upregulating high-affinity K^+ transporters and specific channels, which promotes the absorption of K^+ / Na^+ into root cells. It also aids in transferring K^+ from root parenchyma cells to the xylem, ultimately reaching the shoots ([Yan et al., 2020a](#)). [Chen, 2017](#) and [Li, 2016](#) observed that melatonin-treated bermudagrass had elevated K^+ levels due to increased expression of genes for antioxidant enzymes involved in the ascorbate–glutathione cycle (*MdcAPX*, *MdDHAR1*, *MdDHAR2*, *MdMDHAR*, and *MdcGR*) and K^+ transporter genes such as *CdHAK9*, and *CdKT23*.

Melatonin also influences how plants cope with iron deficiency, affecting both physiological processes and molecular mechanisms. It stimulates root hair development and boosts iron uptake by increasing the expression of genes linked to iron acquisition, such as ferric reduction oxidase 7 (FRO7), vacuolar iron transporter 1 (VIT1), and ferric reduction oxidase 4 (FRO4). Simultaneously, it suppresses genes associated with vacuolar iron storage and transport, including vacuolar iron transporter homolog 4 (VITH4) and natural resistance-associated macrophage protein 3 (NRAMP3), thereby enhancing internal iron recycling during shortages ([Lin, 2022](#)). In a separate study on peach plants, [Lin \(2023\)](#) observed that applying exogenous melatonin enhances iron absorption and movement by elevating ferric-

chelate reductase (FCR) activity and raising both total and active iron levels across various plant tissues, regardless of whether iron was scarce or abundant. Analysis of differentially expressed genes (DEGs) in melatonin-treated peach plants revealed significant enrichment in pathways like aminoacyl-tRNA biosynthesis, cell wall metabolism, lignin synthesis, and iron translocation, reinforcing melatonin's role in optimizing iron uptake and distribution within plants.

In *Arabidopsis*, comparative studies of genomic and physiological responses have shown that soil-applied melatonin enhances tolerance to iron deficiency by improving iron absorption and storage, by increasing the expression of genes like FRO2, IRT1, and FIT1, which are involved in iron uptake, leading to elevated phyto-melatonin levels, higher iron accumulation and reduced leaf chlorosis ([Ahammed, 2020](#)). Melatonin plays a role in maintaining iron homeostasis in plants, partly through its interaction with nitric oxide (NO). NO acts as a signaling molecule that interacts with melatonin to regulate plant growth and up-regulates defense-related transcription factors, enhancing stress resistance. NO act as a positive regulator by further increasing endogenous phyto-melatonin levels, which activate the MAPK cascade which then increases sugar and glycerol production, leading to higher levels of salicylic acid promoting lateral root growth, delaying ageing, and improving iron homeostasis ([Zhu, 2019](#)). Melatonin enhances the expression of genes involved in cell wall metabolism, which modifies the composition and structure of cell walls, including components such as pectin and hemicellulose. This modification can affect the release and availability of iron bound to cell wall components. For example, iron deficiency induces the production of nitric oxide (NO) in plants, which in turn positively regulates the expression of

genes like FRO2, IRT1, and FIT, thereby improving the efficiency of iron utilization ([Zhu, 2016](#)) ([Figure 3.3](#)).

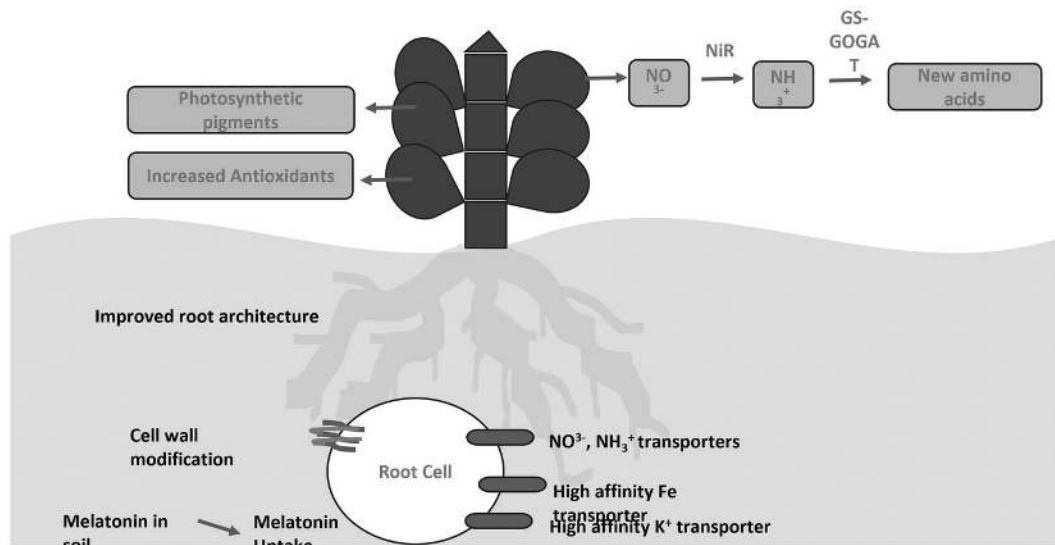


FIGURE 3.3 Schematic diagram illustrating the common mechanisms induced by melatonin under nutrient disorders. (Adapted from [Sun, 2022](#).)



3.2.4 Genetic Transformation for Phytomelatonin Enhancement

Recent research has elucidated many of its roles and mechanisms of action, establishing melatonin as a master regulator in plants and a valuable marker for responses to various stresses. Enhancing phyto-melatonin levels through genetic transformation involves introducing or modifying genes in plants to boost melatonin production. This strategy can improve plant stress tolerance, growth, and immunity.

Conventional breeding programs have traditionally focused on increasing yield, but these efforts have become less effective due to the complexity of stress tolerance traits ([Ahmar, 2020](#)). As a result, plant scientists have been striving to enhance plant growth under diverse environmental conditions,

including stresses from abiotic and biotic factors, without compromising yield and nutrient efficiency. Transgenic approaches offer a promising solution to develop plants with better yield, improved quality, and enhanced resistance to environmental stresses, pests, and diseases.

In recent advancements, dependable and consistent methods have been developed to genetically modify various crops for enhanced melatonin production through genetic engineering. Through transcriptional profiling, researchers have pinpointed the primary regulatory elements (candidate genes) that govern melatonin biosynthesis. Additionally, the ectopic expression of specific transcription factor genes has significantly boosted plant regeneration capabilities, increasing efficiency several-fold. Moreover, a CRISPR/Cas9-based genome editing system has been tailored for use in model plants like *Arabidopsis thaliana* and various cereal crops, enabling precise adjustments to melatonin levels.

3.2.4.1 Candidate Genes and Vectors for Genetic Engineering

3.2.4.1.1 Candidate Genes

Phytomelatonin levels within plant cells are highly responsive to external environmental cues, undergoing rapid changes in response to a variety of stressors. Research has shown that plant species exhibiting naturally elevated melatonin (MEL) concentrations demonstrate enhanced tolerance to stress, with melatonin playing a regulatory role in critical developmental stages such as flowering, fruit development, root growth, biomass accumulation, yield enhancement, and the delay of senescence, particularly under adverse conditions ([Wang et al., 2020](#)). Consequently, manipulating the expression of endogenous melatonin biosynthetic genes has emerged as a promising strategy to achieve these beneficial outcomes in crops.

To develop high-melatonin crop varieties through genetic engineering, six key biosynthetic genes stand out as prime candidates: tryptophan

decarboxylase (TDC), tryptamine 5-hydroxylase (T5H), serotonin N-acetyltransferase (SNAT), N-acetyl-5-hydroxytryptamine methyltransferase (ASMT), aralkylamine N-acetyltransferase (AANAT), and caffeic acid O-methyltransferase (COMT) ([Zhao et al., 2022](#); [Back, 2016](#)). Each of these genes contributes uniquely to the melatonin synthesis pathway. TDC initiates the process by converting tryptophan into tryptamine, which T5H then hydroxylates to form serotonin. SNAT and AANAT facilitate the acetylation of serotonin into N-acetylserotonin, while ASMT and COMT catalyze the final methylation steps to produce melatonin. Targeting these genes for overexpression or fine-tuning their regulation could significantly boost melatonin levels, enhancing crop resilience to stressors like drought, heat, salinity, and oxidative damage. Moreover, combining modifications across these genes could amplify synergistic effects, tailoring melatonin production to specific agricultural needs and improving overall plant vigor and productivity in challenging environments ([Table 3.2](#) and [Figures 3.3](#) and [3.4](#)).

TABLE 3.2 Phytomelatonin Effects and Response Elements in
Different Abiotic Stress 

Stress	Phytomelatonin Effects	Response Elements
Drought stress	Reactive Oxygen Species (ROS) Scavenging and Synergistic interaction with other phytohormones	MYBs, NCEDs, WRKYs, CYP707s, DREBs
Heat stress	Activate the regulatory network associated with heat stress and boost the activities of antioxidant enzymes.	HSPs, HSFs, POD, SOD, CAT, RBOH
Salt stress	Activate the H ⁺ pump and enhance the ion homeostasis	Na-K ⁺ transporters, APX, CAT, POD, TRXs, PET and D1 protein, SLAC, MYBs; ABF; AREB, MAPK
Cold stress	Increase the expression of cold-response genes and cooperate with other phytohormones	ICE, CBF, miRNAs, CDPKs, bHLHs, DREBs, WRKYs, MYBs, NCEDs
UV stress	Activate the key components of UV-B signaling pathway	COP1, HY5, HYH, RUP1/2, GPXs
Heavy metal toxicity stress	Reduce accumulation of heavy metal in plants and enhance antioxidant enzyme activities	MT1, IRT1, Nramp1, PT2, PT8, Lsi1, SGT1
Oxidative stress	Scavenges the ROS and alleviates direct oxidative stress	APX, CAT, POD, SOD

Stress	Phytomelatonin Effects	Response Elements
Waterlogging stress	Optimize metabolism in roots and regulate hypoxia-related genes	ACO, ACS, ADC, DAO, SAMDC, SPDS, SPMS

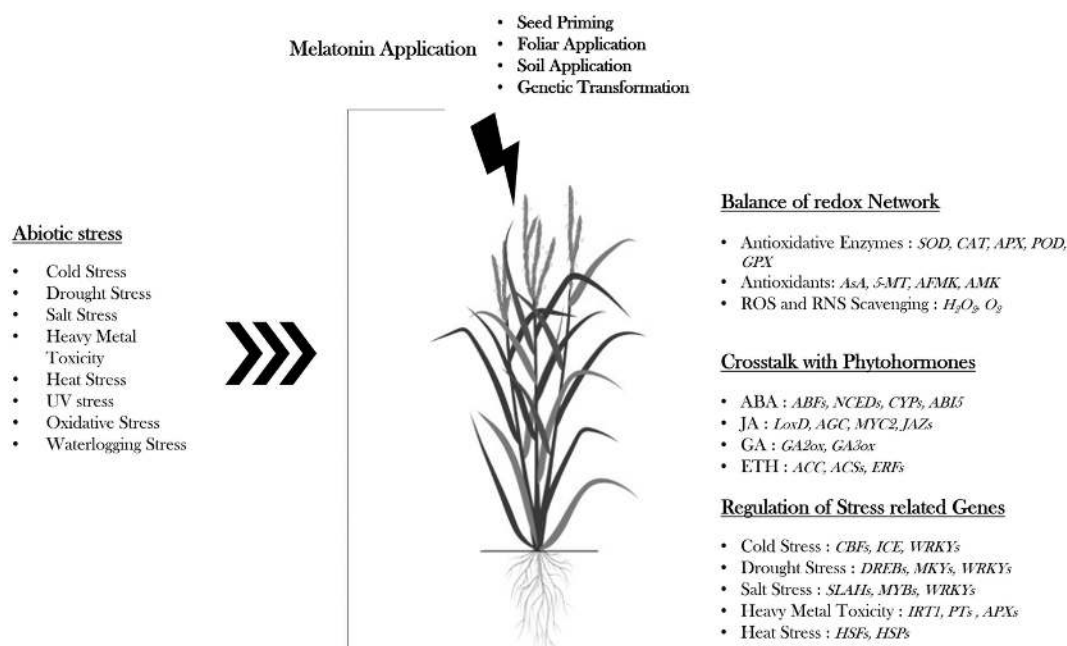


FIGURE 3.4 Candidate genes for genetic transformations for enhanced melatonin production. [\[4\]](#)

3.2.4.1.2 Tryptophan Decarboxylase (TDC)

TDC is the enzyme catalyzing the conversion of 5-hydroxy tryptophan into serotonin (4). Gibberellin (GA) as well as other phytohormones also promote the synthesis of melatonin in rice by inducing the expression of the TDC ([Hwang and Back, 2022](#)). Overexpression can increase the substrate availability for downstream melatonin synthesis. The overexpression of TDC delays senescence in transgenic rice ([Lee and Back, 2017](#)). The suppression of TDC expression by RNA interference (RNAi) led to a reduction in serotonin production and advanced the senescence process.

3.2.4.1.3 Tryptamine 5-Hydroxylase (T5H)

Tryptamine 5-hydroxylase (T5H) plays an essential role in the melatonin biosynthesis pathway by catalyzing the conversion of tryptamine into serotonin, a key precursor to melatonin. Elevating T5H activity can expand the serotonin reservoir within plant cells, thereby promoting greater melatonin production. This enhancement not only amplifies the availability of serotonin but also strengthens the plant's capacity to synthesize melatonin, potentially improving its ability to respond to environmental stresses and regulate physiological processes effectively.

3.2.4.1.4 Serotonin N-Acetyltransferase (SNAT)

SNAT genes convert serotonin to N-acetylserotonin whose overexpression can increase the rate of this conversion, pushing the pathway towards melatonin synthesis. Studies by [Hwang and Back \(2020\)](#) and [Wang et al. \(2020\)](#) indicate a positive correlation between the expression levels of SNAT genes and melatonin levels in plants. This suggests that higher expression of SNAT genes might lead to increased melatonin production. Research has revealed that disrupting the serotonin N-acetyltransferase (SNAT) genes in the model plant *Arabidopsis thaliana* significantly lowers melatonin concentrations and delays flowering, underscoring SNAT's critical role in both melatonin production and developmental timing. Conversely, introducing an allogeneic SNAT gene into *A. thaliana* enhances melatonin synthesis within mitochondria, mitigating oxidative stress from reactive oxygen species (ROS) during drought conditions ([Wang, 2017](#)). This suggests that SNAT-mediated melatonin production could serve as a protective mechanism against environmental stressors. Additionally, in tomatoes, the melatonin biosynthesis enzyme SNAT1 has been shown to physically interact with HSP40, a DnaJ-type chaperone located in chloroplasts, to fine-tune melatonin levels and bolster heat tolerance ([Wang, 2020](#)). Furthermore, SNAT's influence extends beyond stress mitigation to

broader physiological processes. For instance, its activity in chloroplasts and mitochondria highlights its compartmentalized role in melatonin synthesis, potentially optimizing cellular responses to abiotic challenges like heat and drought. The interaction between SNAT1 and HSP40 in tomatoes further indicates a sophisticated regulatory network, where SNAT not only drives melatonin production but also collaborates with molecular chaperones to stabilize protein function under high-temperature stress. These insights position SNAT as a pivotal enzyme in plant adaptation, offering opportunities for genetic manipulation to enhance crop resilience and productivity in the face of climate-related adversities.

3.2.4.1.5 N-Acetyl serotonin O-Methyltransferase (ASMT)

ASMT catalyzes the final step, converting N-acetylserotonin to melatonin. Enhancing the ASMT activity can directly increase melatonin levels. Overexpression of the ASMT gene has been shown to enhance autophagy and increase the accumulation of heat shock proteins (HSPs) in tomato plants. These HSPs help refold proteins that have been denatured by high temperatures, thereby promoting cellular protection under heat stress ([Xu et al., 2016](#)). Further research by [Qi et al. \(2018\)](#) suggests that phytomelatonin alleviates high temperature-induced pollen abortion in tomatoes through a similar mechanism.

3.2.4.1.6 Caffeic Acid-O-methyltransferase (COMT)

Caffeic acid O-methyltransferase (COMT) plays a crucial role in melatonin biosynthesis by catalyzing the methylation of N-acetylserotonin into melatonin, effectively exhibiting N-acetylserotonin O-methyltransferase (ASMT) activity. This ASMT function of COMT was initially identified in *Arabidopsis thaliana* COMT (AtCOMT) by [Byeon \(2015\)](#), marking a significant discovery in plant melatonin production pathways. Further research has demonstrated that tomato plants engineered to overexpress

COMT exhibit reduced cadmium (Cd) movement from roots to shoots, suggesting a protective role against heavy metal toxicity ([Hasan, 2019](#)). Conversely, silencing the melatonin synthesis gene COMT1 in tomatoes led to notable declines in chlorophyll levels, photosystem efficiency, and electron transport rates, while also exacerbating the negative impacts of heat stress on photosynthesis ([Ahammed, 2018](#)). Studies have also shown that cloning and overexpressing SlCOMT1, a COMT gene linked to melatonin production in tomatoes, enhances plant resilience. [Sun \(2022\)](#) reported that SlCOMT1 overexpression not only boosted melatonin levels but also upregulated several stress-responsive genes, including AREBI, AIMI, MAPKI, WRKY33, and CDPKI. This genetic modification significantly improved tomato tolerance to high salinity stress, specifically at concentrations of 800 mmol L⁻¹ NaCl. Additionally, COMT's multifaceted role extends beyond melatonin synthesis, as it contributes to lignin biosynthesis and stress adaptation, making it a key target for improving crop performance under adverse environmental conditions. These insights underscore COMT's potential as a versatile tool in agricultural biotechnology for enhancing plant vigor and resistance to diverse stressors.

3.2.4.1.7 Arylalkylamine N-acetyltransferase (AANAT)

Arylalkylamine N-acetyltransferase (AANAT) serves as the terminal enzyme in the melatonin synthesis pathway in many organisms, but its direct homologue is notably absent in the genomes of higher plants. However, significant strides have been made by introducing AANAT from other sources into plant systems. For instance, [Okazaki \(2009\)](#) successfully isolated an AANAT gene from the unicellular green alga *Chlamydomonas reinhardtii* and integrated it into the genome of the Micro-Tom tomato variety. This ectopic overexpression dramatically elevated melatonin levels

in the transgenic tomatoes, highlighting the enzyme's potential to enhance melatonin production in plants lacking a native AANAT gene. In contrast, higher plants like rice rely on serotonin N-acetyltransferase (SNAT) as a substitute for AANAT, encoding the penultimate enzyme in their melatonin biosynthesis pathway ([Kang et al., 2013](#)).

Further research has illuminated the broader implications of AANAT in plant resilience. Transgenic rice plants engineered to express the human SNAT/AANAT gene exhibited markedly higher melatonin concentrations, which correlated with increased chlorophyll production under cold stress, indicating melatonin's protective role against low-temperature damage ([Kang et al., 2009](#)). Similarly, rice seedlings modified with the ovine AANAT gene demonstrated improved seminal root elongation ([Park et al., 2012](#)), accelerated seedling growth ([Byeon and Back, 2014](#)), and greater resistance to oxidative stress induced by herbicides ([Park et al., 2013](#)). Microarray analysis of these transgenic lines revealed a significant reduction in the expression of senescence-associated proteins, suggesting that melatonin may delay aging processes in plants ([Byeon et al., 2013](#)).

Environmental factors also influence melatonin synthesis involving AANAT. High temperatures and darkness were found to boost melatonin levels in rice by enhancing the activities of the final two enzymes in the pathway, pointing to melatonin's involvement in heat stress defense ([Byeon and Park, 2013](#)). Additionally, transgenic Micro-Tom tomatoes overexpressing both ovine AANAT and hydroxyindole-O-methyltransferase (HIOMT) genes exhibited altered growth patterns, including the loss of apical dominance, alongside improved tolerance to drought conditions ([Wang et al., 2014](#)). These findings collectively underscore AANAT's versatility in bolstering plant adaptability to a range of abiotic stresses, from cold and heat to drought and oxidative challenges, while also influencing

developmental traits. This makes AANAT a promising candidate for genetic engineering aimed at improving crop performance in diverse and adverse environments ([Table 3.3](#)).

TABLE 3.3 A Summary of the Crops Modified by Transgenic Approaches to Sustain Environmental Stresses by Modulating The Phytomelatonin Biosynthesis Pathway 

Plant Species	Stress Type	Gene	Transgenic Method	Outcome
Arabidopsis thaliana	Drought	ASMT	MzASMT gene under 35S promoter	Higher melatonin levels, more lateral roots, greater fresh weight.
	Cadmium	SNAT	MsSNAT (from alfalfa) overexpressed via 35S	Slightly increased melatonin, less root length reduction, lower cadmium buildup, reduced miR398 levels.
	Salt	SNAT	VvSNAT1 (from grapes) overexpressed via 35S	Markedly higher melatonin, better growth, greener foliage.
	UVB	SNAT	AtSNAT overexpressed; SALK_032239 mutant	Overexpression: Faster UV-B signaling gene response. Mutant: Delayed response under intense UVB.

Plant Species	Stress Type	Gene	Transgenic Method	Outcome
	Drought	COMT	TaCOMT (from wheat) overexpressed via 35S	Increased melatonin, higher fresh weight, more lateral roots, longer total root length.
	Salt, Cold, Drought	COMT	CICOMT (from watermelon) overexpressed via 35S	Greatly elevated melatonin, improved survival under salt, cold, and drought-like conditions.
Solanum lycopersicum (Tomato)	Heat	ASMT	SlASMT overexpressed via 35S	Less wilting, lower electrolyte leakage, better photosynthesis, enhanced heat shock and autophagy genes.
	Salt	COMT	SICOMT1 overexpressed via 35S	Higher melatonin, less leaf drooping, reduced ROS, increased proline.

Plant Species	Stress Type	Gene	Transgenic Method	Outcome
	Heat	SNAT	SlSNAT overexpressed via 35S	Elevated melatonin, improved chlorophyll fluorescence, less wilting after recovery.
Nicotiana tabacum (Tobacco)	Salt	ASMT	MzASMT1 (from crab apple) overexpressed via 35S	Increased melatonin, reduced leaf wilting, greater fresh weight, height, root length, and water content.
Cucumis sativus (Cucumber)	Salt	SNAT	CsSNAT overexpressed; CsSNAT silenced (RNAi)	Overexpression: Higher melatonin, less ROS, better PSII efficiency. Silencing: Lower melatonin, more ROS.

Note: COMT—caffeic acid 3-O-methyltransferase, SNAT—serotonin N-acetyltransferase, ASMT—acetylserotonin O-methyltransferase.

3.2.4.2 Methods for Genetic Transformation in Plants

3.2.4.2.1 Agrobacterium-mediated Transformation

Agrobacterium-mediated transformation is a widely used technique in plant biotechnology to introduce foreign genes into plant genomes. This method

leverages the natural ability of *Agrobacterium tumefaciens*, a soil bacterium, to transfer a portion of its DNA (the T-DNA) into the genome of a host plant. For enhancing melatonin synthesis in plants, this method can be employed to introduce genes involved in the melatonin biosynthesis pathway. Melatonin, a well-known hormone in animals, is also present in plants, where it plays a crucial role in growth regulation, stress tolerance, and defense mechanisms. The engineering of plants to overproduce melatonin can confer significant agronomic benefits, such as enhanced resistance to environmental stresses, improved growth, and yield.

Serotonin N-acetyltransferase (SNAT) and hydroxyindole-O-methyltransferase (HIOMT), two enzymes essential to the melatonin synthesis pathway, are genes that are extracted and placed into a plasmid vector in the *Agrobacterium* system. Strong constitutive promoters often drive these genes to ensure high levels of expression. *Agrobacterium* introduces T-DNA and the genes for melatonin production into the plant's genome when it co-cultivates with plant tissues. After that, the altered plant cells are chosen and grown again into entire plants that express the new genes, producing more melatonin. Study on rice (*Oryza sativa*) utilized *Agrobacterium*-mediated transformation to overexpress the SNAT gene ([Lee and Back, 2017](#)). The transgenic rice plants demonstrated significantly higher levels of melatonin compared to the wild-type plants. This increase in melatonin not only improved the plants' tolerance to oxidative stress and drought conditions but also enhanced their overall growth and yield. Similar approaches have been applied in other crops, such as tomato (*Solanum lycopersicum*) and tobacco (*Nicotiana tabacum*), with promising results.

3.2.4.2.2 CRISPR/Cas9 Genome Editing

Genome editing has emerged as a powerful tool for achieving specific plant traits through precise modifications to their genetic material. Several

techniques have been developed to facilitate this process in plants, such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the widely recognized clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system ([Voytas and Gao, 2014](#)). Among these, the CRISPR/Cas system has gained prominence due to its affordability, adaptability, and exceptional precision in genetic alterations ([Yin et al., 2017](#)). This technology has proven effective across a diverse range of cereals and economically significant crops, including rice (*Oryza sativa*) ([Shan et al., 2013](#)), wheat (*Triticum aestivum*) ([Shan et al., 2013](#)), maize (*Zea mays*), potato (*Solanum tuberosum*) ([Wang et al., 2015](#)), cassava (*Manihot esculenta*) ([Odipio et al., 2017](#)), chrysanthemums (*Chrysanthemum morifolium*) ([Kishi-Kaboshi et al., 2017](#)), European chestnut (*Castanea sativa*) ([Pavese et al., 2021](#)), Kabuli chickpea (*Cicer arietinum*) ([Badhan et al., 2021](#)), poinsettias (*Euphorbia pulcherrima*) ([Nitarska et al., 2021](#)), and roses (*Rosa hybrida*) ([Wang et al., 2022](#)). The CRISPR/Cas9 system utilizes guide RNA paired with the Cas9 nuclease to target specific genomic sites, enabling precise edits that can either activate or disable genes, including those involved in melatonin biosynthesis. This capability holds immense potential for enhancing plant resilience, improving crop quality, and tailoring traits to meet agricultural needs.

The CRISPR/Cas genome editing system, as outlined by [Wiedenheft et al. \(2012\)](#), originated from the adaptive immune mechanisms of bacteria and archaea, which defend against viral and plasmid invasions. At the core of the CRISPR/Cas9 system is a single endonuclease, the Cas protein, paired with a synthetic single-guide RNA (sgRNA), a fusion of trans-activating crRNA and CRISPR RNA (crRNA) ([Barrangou et al., 2007](#)). This streamlined design enhances its precision and usability. Cas protein complexes are categorized into two main classes based on their structural

and functional properties: Class 1, encompassing types I, III, and IV, relies on multi-protein complexes to cleave foreign nucleic acids, while Class 2, which includes types II, V, and VI, utilizes a single protein effector for the same purpose ([Makarova et al., 2020](#)). Among the most widely adopted systems within Class 2 are those driven by the nucleases Cas9 and Cas12a (also known as Cpf1), both of which operate with a single protein and have become pivotal tools in genetic engineering due to their efficiency and versatility ([Ran et al., 2013](#); [Makarova et al., 2020](#)). These advancements have significantly broadened the scope of genome editing, offering powerful options for targeted genetic modifications across various organisms.

Melatonin (N-acetyl-5-methoxytryptamine) has been identified as an important bioactive molecule in a variety of plant and animal processes ([Zhang et al., 2015](#)). A crucial part of melatonin production is played by the caffeic acid O-methyltransferase gene ClCOMT1 ([Kang et al., 2013](#)). Watermelon calli's melatonin content dropped as a result of a clcomt1 mutation driven by CRISPR/Cas9 ([Chang et al., 2021](#)). Furthermore, transgene-free bg1 mutants with smaller and lighter seeds have been produced by targeting the ABA hydrolyzation β -glucosidase (BG) gene using the CRISPR/Cas system ([Wang et al., 2020](#)).

3.3 CONCLUSION

In conclusion, genetic transformation for enhancing phytomelatonin involves strategic selection of genes and vectors, employing advanced transformation techniques, and balancing the advantages with the inherent limitations and challenges. This approach holds promise for improving plant health and productivity, contributing to sustainable agricultural practices.

In recent years, researchers have explored the idea of using melatonin externally to determine if it produces effects comparable to those observed in transgenic plants rich in melatonin. Additionally, applying melatonin to plants from an external source could be viewed as an environmentally friendly agricultural technique, falling under the category of bio-stimulation. The European Bio-stimulants Industry Council (EBIC) describes bio-stimulants as “substances and/or microorganisms that, when used on plants or their root zones, enhance natural processes to improve nutrient absorption, nutrient efficiency, resistance to environmental stress, and/or crop quality, regardless of their nutrient content.” Thus, treating plants with melatonin aligns well with this definition. Furthermore, since both plants and humans naturally produce melatonin, and it shows no significant toxicity even at elevated doses, it can be regarded as a safe compound.

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4

Root Morphogenesis

The Role of Phytomelatonin Under Plant Stress

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DOI: [10.1201/9781003527633-4](https://doi.org/10.1201/9781003527633-4)

4.1 PHYTOMELATONIN: A MULTIFUNCTIONAL MOLECULE IN PLANTS

Melatonin is chemically N-acetyl-5-methoxytryptamine. It is a low molecular weight biological indolamine that readily soluble in both lipids and water, providing relatively easy access across the plasma membrane. Melatonin was initially discovered as melanophore-associated hormone that triggers the synthesis of melanophore pigment in amphibian skin melanocyte ([Sabir et al. 2024](#)). It was later found in all vertebrates, secreted rhythmically by the pineal gland, and involved in regulation of circadian

and seasonal rhythms by signaling darkness. Beyond vertebrates, melatonin is present in diverse life forms, including bacteria, protozoa, plants, fungi, and invertebrates, indicating its ancient evolutionary origin. Over time, its known functions have expanded to include roles in immune regulation, antioxidant defense, and stress responses in plants and other organisms ([Seth et al. 2023](#)). Later, in 1995, two groups of researchers identified melatonin in plants simultaneously for the first time as potent antioxidant compounds capable of quenching ROS and RNS species. Structural similarity with indole acetic acid (IAA) brought this versatile molecule into focus in plants ([Arnao and Hernández-Ruiz 2019](#)).

4.1.1 Biosynthesis in Plants

Melatonin biosynthesis four-step biochemical pathway requiring six genes. Phosphoenolpyruvate (PEP) through shikimic acid pathway to form chorismite in chloroplast which enters into tryptophan biosynthetic pathway for synthesis of tryptophan. As presented in [Figure 4.1](#) tryptophan acted upon with two set of enzymes tryptophan decarboxylase (EC 4.1.1.105) and tryptophan 5-hydroxylase (EC 1.14.16.4) forming tryptamine and 5-hydroxy tryptophan respectively ([Back, Tan, and Reiter 2016](#)). Further, two set of enzyme L-Tryptophan-5-hydroxylase (EC 1.14.16.4) on tryptamine and L-Tryptophan decarboxylase (TDC; EC 4.1.1.105) on 5-hydroxy tryptophan produces serotonin which undergoes acetylation and subsequent methylation by Serotonin N-acetyltransferases (SNATs; EC 2.3.1.87) and Acetylserotonin O-methyltransferases (ASMTs; EC 2.1.1.4) to form melatonin in plants. Alternatively, serotonin may be first methylated and then acetylated to form melatonin. Melatonin reported to self-regulate its biosynthesis wherein accumulation of ROS species leads to expression of its biosynthetic genes. N-acetylserotonin-O-methyltransferase (ASMT) is a

specific cytosolic enzyme, its overexpression in *Malus zumi* enhances the melatonin level over 24 hr dark/light cycle. Constitutive expression of ASMT in *Arabidopsis* achieves 2–4 times accumulation and exhibit greater tolerance to drought stress ([Zuo et al. 2014](#)).

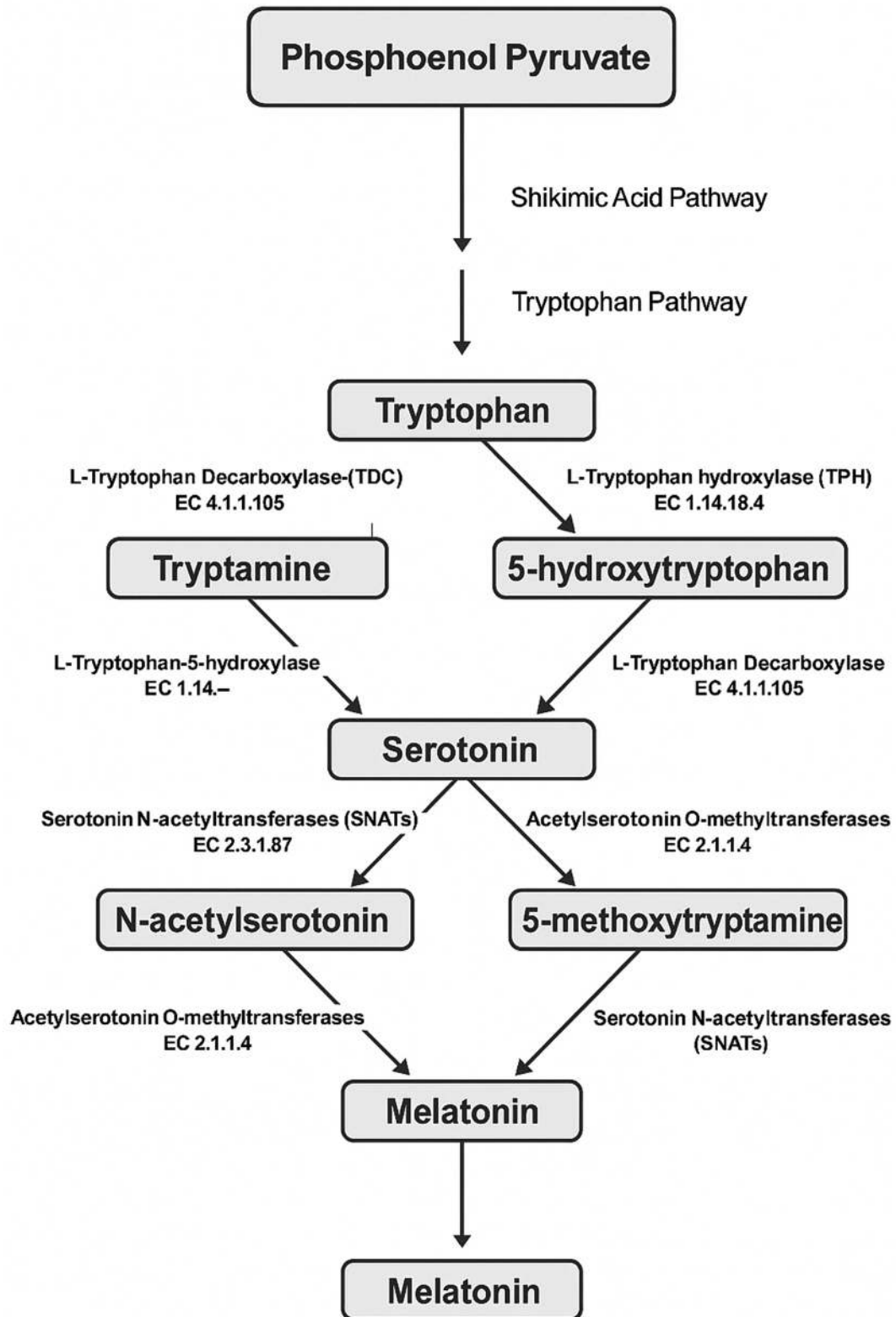


FIGURE 4.1 Biosynthetic pathway of melatonin in plants. [📄](#)

4.1.2 Cellular and Physiological Function

A range of cellular functions and physiological effects of this multifunctional melatonin molecule include intracellular spiking in Ca^{2+} ion concentrations, ion transporter led maneuvering of membrane permeability, regulation of stomatal aperture independently of abscisic acid, effect on primary and secondary metabolism. Its physiological effects on seed germination, growth stimulants, roots morphogenesis, fruit development and ripening, phototropism, photosynthesis, senescence, regulation of circadian rhythm have also been demonstrated in diverse crops. its exogenous application in many crops especially under abiotic and biotic stress conditions has been well appreciated owing to the growth promoting attributes

Melatonin delays senescence in dose dependent manner evident from the protection of chlorophyll content and enhancement in photosynthetic machinery in barley ([Arnao and Hernández-Ruiz 2009](#)). It promotes biosynthesis of lycopene, beta-carotene, and other carotenoid by upregulating transcriptional factor RIN/NOR/AP2a associated with carotenoid biosynthetic gene ([Sun et al. 2020a](#)). Exogenous application elucidates better shoot growth (like leaf area, stem diameter, plant height, chlorophyll content, net photosynthetic rate etc.), curtailed yield loss mainly due to increase in boll weight ([Sharma et al. 2024](#)). Study shows that melatonin biosynthesis in rice leaves during senescence is strongly influenced by light. Under constant light, melatonin levels and its biosynthetic precursors, such as tryptamine, serotonin, and N-acetylserotonin, are significantly higher compared to constant darkness. In darkness, these intermediate and the mRNA levels of melatonin biosynthetic genes are notably reduced. The most significant drop is seen in the enzyme tryptamine 5-hydroxylase, crucial for melatonin synthesis

indicating the light-dependent production during leaf senescence ([Byeon et al. 2012](#)). Melatonin synthesis in shoot of *chenopodium rubrum* observed to be highly regulated with photoperiod and its accumulation was higher in night/dark while remain low to undetectable in day or light period ([Kolář et al. 1997](#)). High resolution mass spectroscopy-based quantification shows that concentration of melatonin fluctuates during day and night cycle. Diurnal decay of melatonin in fruit berry is observed in *vitis vinifera* ([Boccalandro et al. 2011](#); [Liu et al. 2025](#)).

Serotonin N-acetyltransferase (SNAT) is a rate limiting enzyme in melatonin biosynthesis. Ectopic expression of in rice seedling leads to accumulation of melatonin in T3 homozygous seedling. Transgenic seeds exhibit enhanced seminal and adventitious roots on MS media suggesting the role of melatonin in regulation seminal root growth and root biomass ([Park and Back 2012](#)). Two biosynthetic enzymes of melatonin biosynthesis SNAT and ASMT exhibited light and thermal sensitivity. Biosynthesis of melatonin was more in dark and at higher temperature. The sensitivity were not due to abundance in transcripts but in the activities of both the enzymes([Byeon and Back 2014](#)). Melatonin (MT) also improves heavy metal tolerance against Vanadium, Chromium, Cadmium, Nickel, and lead by enhancing the enzymatic and non-enzymatic antioxidant defense system, Chlorophyll content, photosynthetic efficiency, osmolyte accumulation, and secondary metabolites ([Altaf et al. 2023](#)). Its application induces successful symbiosis with microbes and influencing the roots system architecture which enhances the uptake of heavy metals such as Lead (X. [Zhang et al. 2020](#)), Cadmium ([Yu et al. 2024](#)), Aluminum ([Sun et al. 2020b](#)). Exogenous application of MT upregulates the genes of ascorbate-glutathione cycle and ROS species for enhanced cadmium tolerance ([Xu et al. 2023](#)) ([Figure 4.2](#)).

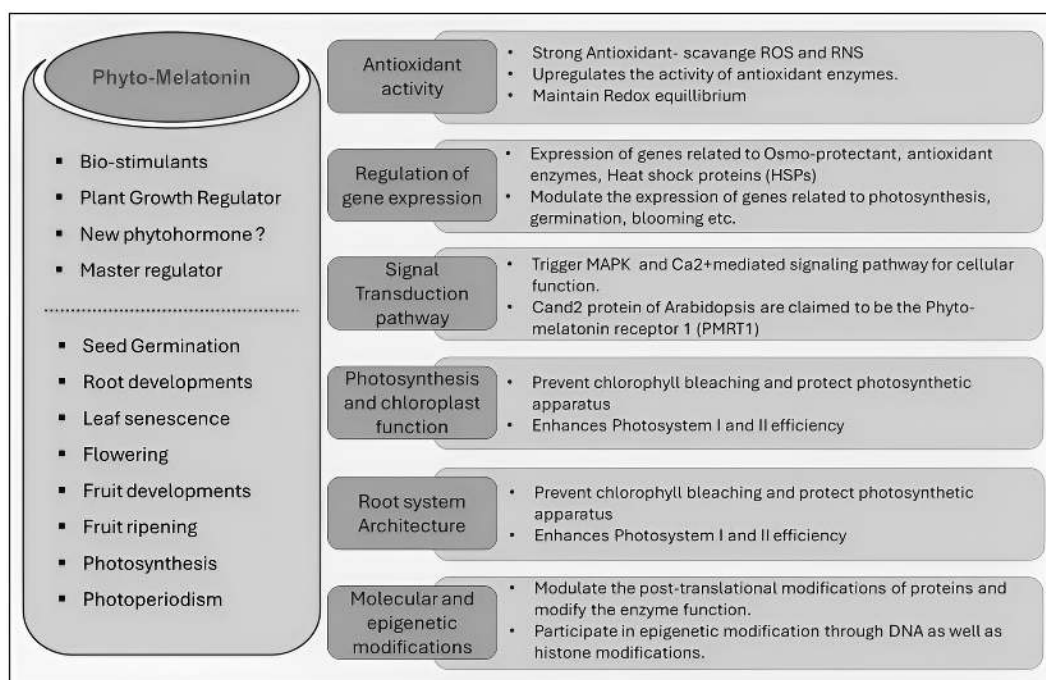


FIGURE 4.2 Cellular functions and role of melatonin in plants growth and developments. [↗](#)

4.1.3 Plant Stress

Melatonin reported to provide resilience under biotic and abiotic stress in plants. Physiologically, its application improves root-shoot biomass, photosynthetic efficiency, membrane stability index and enhances relative water content. It prevents the ROS induced damages to the chlorophyll pigments and reported to upregulate the biosynthetic genes (Chlorophyllase *CHLASE*) and downregulates the genes involved in breakdown of chlorophyll (A. [Sharma et al. 2020](#); Yunzhi [Liu et al. 2024](#)). Melatonin enhances accumulation of osmolytes such as proline, trehalose quaternary amino acids like betaines. It elevates the biosynthesis of primary antioxidant enzymes such as superoxide dismutase, peroxidase, catalase, and enzyme of ascorbate-glutathione cycle. It also reduces the accumulation of methylglyoxal by activating the glyoxalase I and glyoxalase II enzymes ([Li et al. 2019](#)). Melatonin application improves the thermotolerance in

maize seedling by maneuvering the glutathione-ascorbate cycle and methylglyoxal detoxification. It also enhances osmolytes proline, trehalose, and total soluble sugar. [Kaya et al. \(2023\)](#) reported significant improvements in shoot-root biomass, chlorophyll, relative water content under abiotic stress. It also promotes the accumulation of non-enzymatic antioxidants and osmolytes under abiotic and biotic stress in plants. They also observed that enhanced L-cysteine desulfhydrase activity promotes H₂S and NO production, potentially accelerating S-nitrosylation, while an improved glyoxalase system reduces methylglyoxal accumulation and its oxidative damage. Under drought stress, MT application in potato improves RWC, cell membrane stability, enzymatic defense and regulates tuberization process ([El-Yazied et al. 2022](#)). Similarly in tomato, it enhanced the chlorophyll content, root architecture, photosynthetic rate and the level of antioxidants metabolites such as ascorbate, glutathione as well as antioxidant enzymes peroxidase (POD), catalase, dehydroascorbate reductase (DHAR), Glutathione-S-transferase (GST), Glutathione Reductase (GR), Monodehydroascorbate Reductase (MDHAR), Superoxide dismutase (SOD) under drought stress ([Altaf et al. 2022](#)). Biological and physiological role of melatonin application on root morphogenesis under different abiotic stress in various crops has been presented in [Table 4.1](#).

TABLE 4.1 Biological and Physiological Role of Melatonin Application on Root Morphogenesis Under Different Abiotic Stress in Various Crops



Type of Stress	Crop	Impact on root System Architecture	Other Role of Melatonin	Reference
Drought	Tomato	Root architecture ↑↓ Root growth & biomass ↑ Water uptake efficiency ↑	Osmolytes (Proline) ↑ Chlorophyll content ↑ Antioxidant Enzymes ↑ MDA ↑	(Altaf et al. 2022)
	Rice	Root length, ↑ Root surface area, ↑ Root volume ↑ Root-to-Shoot ratio ↑ Lateral root formation ↑	Germination ↑ Chlorophyll content ↑ Radical activity ↑	(Tyagi et al. 2023)
	Cucumber	Root generation ↑ Root vitality ↑ Root-to-shoot ratio ↑	Chlorophyll degradation ↓ Osmolyte ↑ Antioxidants enzymes ↑	(Zhang et al. 2013)

Type of Stress	Crop	Impact on root System Architecture	Other Role of Melatonin	Reference
	Wheat	Root length ↑ Root surface area ↑ Root volume ↑ Root density ↑	Photosynthetic rate Chlorophyll content	(Zhu et al. 2023)
	Cotton	Root length ↑ Root surface area ↑ Root volume ↑	Antioxidant enzymes ↑ H2O2 and MDA accumulation ↓ Osmolytes ↑ K ⁺ /Na ⁺ ratio ↑	(Duan et al. 2022)
Salinity	Tomato	Root growth	Chlorophyll biosynthesis ↑ Antioxidants ↑ Methylglyoxal detoxification ↑	(Siddiqui et al. 2020)
	Cotton	Root development ↑ Root senescence ↓	Regulates ion balance	(Yu et al. 2024)

Type of Stress	Crop	Impact on root System Architecture	Other Role of Melatonin	Reference
	cucumber	Root growth ↑	Photosynthetic rate ↑ Chlorophyll content ↑ Antioxidant enzymes ↑ Non-enzymes antioxidants ↑ Glutathione and Ascorbate ↑	(Wang et al. 2016)
	Tomato	Root development ↑ Water and nutrient uptake ↑	Enhances root architecture ↑ Ion toxicity ↓ Maintaining water balance Antioxidant defense ↑	(Ali et al. 2021)

Type of Stress	Crop	Impact on root System Architecture	Other Role of Melatonin	Reference
Temperature stress	Tomato	Root growth ↑ Lateral root formation ↑ Oxidative damage ↓	Key regulator in mitigating heat-induced leaf senescence. Balance between GA and ABA pathways. Stress tolerance ↑ Maintaining photosynthetic efficiency.	(Jahan et al. 2020)
	Cherry radish	Root growth ↑ Antioxidant activity ↑ Protect root cells	Oxidative stress ↓ Improved photosynthetic efficiency Auxin levels ↑ Biomass, and hormonal balance ↑ Chlorophyll content ↑	(Jia et al. 2020)

Type of Stress	Crop	Impact on root System Architecture	Other Role of Melatonin	Reference
	mung bean	Root growth ↑ Root cell expansion and elongation ↑ Root functionality ↑ Lateral root ↑	Antioxidant activity ↑ Maintain cellular turgor and ion homeostasis Water uptake ↑ Early root development ↑ Protects root tissues from stress-induced damage.	(Kuppusamy et al. 2023)
Oxidative stress	Arabidopsis	Lateral root formation ↑ Root functionality ↑	Lateral root development ↓ H ₂ O ₂ and superoxide ↑ Radical signaling ↑	(Chen et al. 2019)
	Rice	Lateral root ↑ Seminal root growth ↑ Root biomass ↑	Protect root tissues from oxidative stress Positively regulates the elongation of seminal roots	(Park and Back 2012)

Type of Stress	Crop	Impact on root System Architecture	Other Role of Melatonin	Reference
	Alfalfa	Lateral root formation ↑ Root biomass ↑	Promotes lateral root development through ROS signaling. Antioxidant activity ↑ Auxin levels ↑	(Z. Chen et al. 2018)
	Arabidopsis	Root growth ↑ Elongation ↑ Root functionality ↑	Protect root tissues. Oxidative damage ↓	(Zuo et al. 2014)
	Cucumber	Root growth ↑ Antioxidant activity ↑ Root biomass ↑	Enhances root growth. Oxidative damage ↓	(Zhang et al. 2013)

Note: Upward arrow suggest accumulation or enhanced expression or activity while Downward arrow shows reduction in activity or expression.

4.2 MELATONIN AND ROOT MORPHOGENESIS

Root is the primary and vital organ of plant that primarily provides anchorage, helps in absorption of nutrients and water, establishes interactive

relationships with flora and fauna of soil and may serve as storage of organic reserves. Water and nutrient uptake are so crucial for plants growth and development that plants have evolved and adapted to the potential water availability in the soil ([Khan et al. 2023](#)). Plants make immediate physiological adjustments to limit the water losses through stomata ([Kumari et al. 2024](#)). Root length, its diameter, surface area and volume determines the efficiency of not only water but a key determinants in nutrients absorptions.

Most parts of root developed post embryonically. Embryonic roots called radicles form the single primary root or tap root in dicot plants which develop several orders of lateral roots while the radicles in case of monocots are short lived to the significance at seedling stages and adventitious roots takes up rest of the physiological function at later stage. Postembryonic roots originate from non-root tissues which include lateral roots in eudicots and adventitious roots in monocots. Postembryonic roots with origin other than roots are termed as adventitious roots which originate from root-hypocotyl junction. Root system developed from primary embryonic roots (dicots) are also termed as allorhizic system or tap root while in monocots, these are adventitious roots are termed as homorhizic or fibrous roots system. Surface layer of roots is involved in phosphorous uptake while deeper layer is involved in nitrate uptake. Root developments are focused on raising the surface area and providing anchorage as well as optimizing the nutrient uptake. Eudicots adopt an extensive lateral root system while monocots and cereals rely on adventitious and crown roots. Growth rate of primary roots, its location, spacing, orientation, and rate of lateral root development are complex developmental processes influenced by many intrinsic as well as extrinsic factors. An optimized dose of 120 ppm melatonin enhances the germination rate, improves root and shoot

biomass, chlorophyll content, total antioxidant activity and profoundly reshapes the roots system architecture in rice genotypes ([Tyagi et al. 2023](#)).

Root architecture is shaped by the combined growth of primary and lateral roots, influenced by both genetic factors and external signals ([Mahajan et al. 2025](#)). A key focus for root biologists is understanding how plants regulate the number and emergence of lateral root primordia along the main root. Root growth is determined by the proliferative activity in the root apical meristem (RAM), subsequent cell expansion and its differentiation in the zone of elongation and maturation of roots. Lateral roots (LRs) form de novo from founder cells in the pericycle along the main root axis. These cells, specified in the basal meristem just above the RAM, can resume cell division to initiate primary lateral root primordia (LRP). In response to cellular signaling, they undergo anticlinal cell divisions, triggering the formation of root primordia in mature root regions.

4.3 ROOT MORPHOGENESIS-AN ESTABLISHED ROLE OF MELATONIN

Under drought stress condition, the length, root surface area, volume root were more pronounce with exogenous application of melatonin ([Zhu et al. 2023](#)). Similarly in rice the exogenous application significantly promoted the lateral root development and inhibited the embryonic root growth. Gravitropic response analysis revealed that it interferes with the auxin-regulated processes roots of rice seedling. Melatonin enhances the level of plant hormone auxin, gibberellin, and brassinosteroids and positively regulates the root growth and development under stress conditions ([Duan et al. 2022](#)). Exogenous application of 0.1 uM melatonin exhibit the stimulatory effect on root growth and IAA concentration in etiolated seedling of wild mustard (Q. [Chen et al. 2009](#)). Melatonin treated cucumber

seed shows better germination performance under drought stress conditions. It showed profound impact on root growth by improving the root generation, vitality, and root to shoot ratio. It prevent chlorophyll degradation, enhanced photosynthetic performance and improves the antioxidant defense system to mitigate the detrimental effect drought stress in cucumber seedling (N. [Zhang et al. 2013](#)). Melatonin application improves the root morphological indicators such as root length, specific root, root surface area, root volume, root length density, and diameter of wheat plants. The influence of drought stress on shoot morphology was significantly enhanced, as evidenced by increased plant height, overall dry weight, photosynthesis rate, and the regulation of leaf stomatal openings. Additionally, it improves the duration and percentage survival of fine roots, lateral roots, and root hairs. It seems to promote vegetative growth in etiolated *Lupinus albus* hypocotyl. Response is similar with auxin but HPLC-ECD quantification of melatonin and auxin suggest differential distribution of both the molecules in hypocotyl suggesting a auxin independent action ([Hernández-Ruiz, Cano, and Arnao 2004](#)). Exogenous application up to 1000 μM impedes the growth of primary root but promotes development of lateral root. RNAseq data reveals 750 upregulated and 590 downregulated genes which includes the genes coding for transcription factor viz. Zinc Finger Proteins (*ZFPs*), Calmodulin-like (*CML*), NAC (conserved N-terminal) protein such as *NAM* (No Apical Meristem), Arabidopsis transcription activator factor 1/2 (*ATAF1/2*) and Cup-shaped cotyledon 2 (*CUC2*) and proteins related to ubiquitination (*RING/U-box and F-box*) along with genes involved in biosynthesis of ethylene, strigolactone an gibberellins were upregulated ([Sun, et al. 2020a](#)). Melatonin's role in root growth and development is well established, but its precise mode of action remains unclear. It may regulate root branching, as

well as secondary, lateral, and adventitious root formation, through an auxin-independent pathway, or it could influence primary root growth and development via an auxin-dependent mechanism.

4.3.1 Auxin Dependent Inhibition of Primary Roots

Auxin dependent action melatonin for growth and development of roots is also well reported. Melatonin treatment has been shown to regulate the development of the mitotic spindle in root tips, thereby suppressing cellular replication. This provides a foundational mechanism explaining how melatonin inhibits primary root growth. Overall, melatonin tends to suppress the elongation of primary roots while of secondary and lateral roots are promoted, leading to a shorter, more branched root architecture. This adaptation likely benefits plants by enhancing nutrient acquisition in nutrient-rich upper soil layers, where a highly branched root system offers improved resource uptake ([Murch and Erland 2021](#)).

Genome-wide expression profiling through RNA sequencing revealed that melatonin treatment significantly activated auxin-related genes. Additionally, several transcription factors and cis-regulatory elements associated with root growth and auxin signaling were highly enriched among both upregulated and downregulated differentially expressed genes. This suggests that melatonin influences root development by modulating auxin-responsive pathways. The findings highlight a complex regulatory network where melatonin interacts with auxin signaling components, reinforcing its role in root morphogenesis and adaptation to environmental conditions ([Liang et al. 2017](#)). Several studies suggest that melatonin influences auxin signaling and transport rather than mimicking auxin directly, particularly in primary root growth. It inhibits polar auxin transport

by regulating auxin transport proteins (PIN) and its biosynthesis, thereby inhibiting the primary root meristem and overall primary root growth and development while enhancing the lateral root development in Arabidopsis. In *St. John's wort*, melatonin shows a different localization pattern with auxin in primary roots and can be induced by auxin treatment, indicating a complex interplay in root organogenesis ([Ren, Rutto, and Katuuramu 2019](#)). Root growth and development were more prominent in triple *pin1pin3pin7* mutant than wild under melatonin treatment suggesting the relevance of PIN protein in melatonin mediated root growth. Combined treatment with Triiodobenzoic acid (TIBA) fail to exhibit the root inhibitory effect of meristem size reduction indicating the essentiality of polar transport of auxin (Q. [Wang et al. 2016](#)). Although, it is not an substitute to auxin but acts in auxin dependent manner ([Yang et al. 2021a](#)). Melatonin regulates the expression and spatial distribution of the auxin efflux transporters PIN2 (PIN-FORMED 2) and PIN3, as well as the auxin influx transporter AUX1 (AUXIN RESISTANT 1), while simultaneously suppressing cytokinin signaling components, including AHK4 (ARABIDOPSIS HISTIDINE KINASE 4), AHP2/3/5 (ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 2, 3, and 5), and type A ARR15 (ARABIDOPSIS RESPONSE REGULATOR 15). This modulation likely establishes a hormonal balance favoring auxin-mediated root development while restraining cytokinin-driven inhibitory effects. By fine-tuning the auxin-cytokinin crosstalk, melatonin plays a crucial role in optimizing root architecture and enhancing plant adaptability under varying environmental conditions ([Wang et al. 2023](#)) ([Figure 4.3](#)).

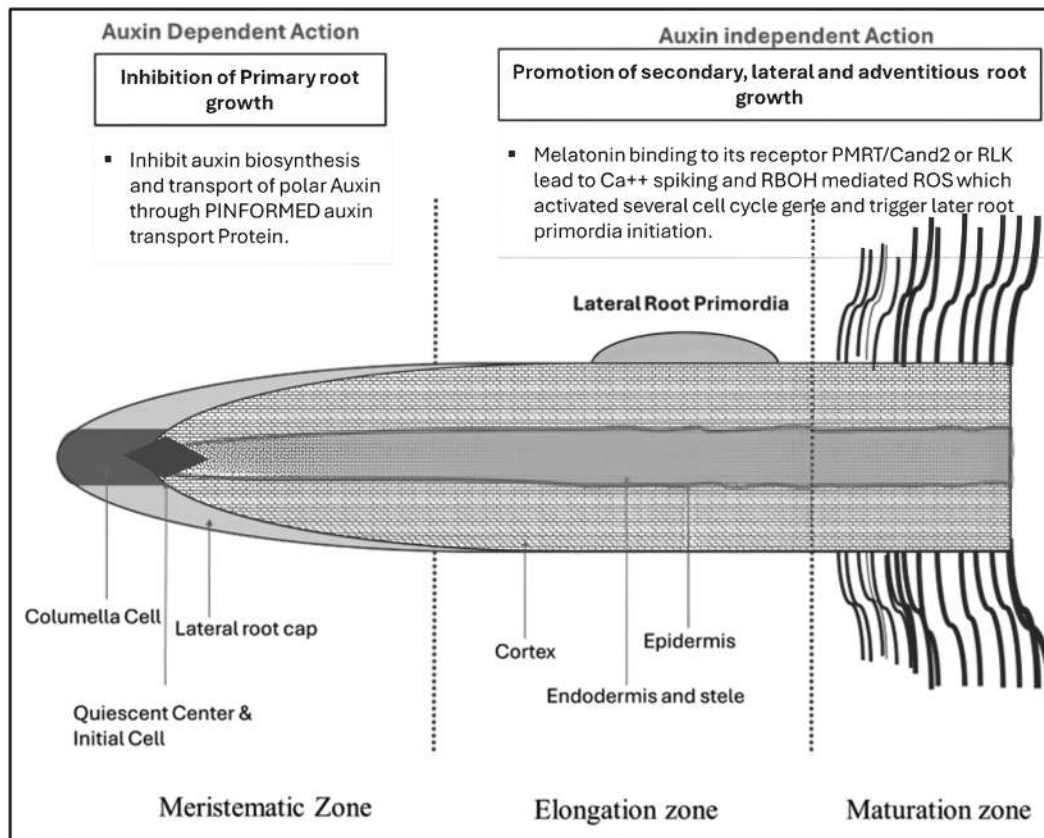


FIGURE 4.3 Auxin dependent and independent action of melatonin in root morphogenesis. [↗](#)

4.3.2 Auxin Independent Action for Later Root Development

Melatonin modulates the root system architecture by enhancing the lateral root primordia initiation and its development without impacting the primary root growth and root hair development. Overexpression of one of the biosynthetic gene ASMT enhanced the melatonin level and reduced the IAA (auxin) level induces the lateral root formation but couldn't evidence any role of auxin in LR development ([Zuo et al. 2014](#)). Exogenous application significantly enhances the H_2O_2 content, induces the LR initiation and LRP development. The response was completely blocked in presence of a NADPH oxidase inhibitor (diphenyleneiodonium DPI) and

H₂O₂ scavenger DMTU (N,N'-dimethylthiourea). An *Arabidopsis* line overexpressing a melatonin biosynthetic gene MsSNAT, increases the H₂O₂ production, accumulation and LR formation which could be blocked by DPI and DMTU. Melatonin dependent expression of cell cycle gene were also impaired in presence of H₂O₂ scavenger and fail to induce LR initiation in alfa-alfa and transgenic *Arabidopsis* (Z. [Chen et al. 2018](#)). RNAseq data showed that 77 genes are regulated in melatonin dependent manner and genes of peroxidase family is highly represented suggesting its role in lateral root initiation and development (N. [Zhang et al. 2014](#)). Auxin and melatonin both enhance the lateral root primordia (LRP) emerging from pericycle and modified the pattern/distribution of lateral roots, number and length of adventitious roots (Marino B. [Arnao and Hernández-Ruiz 2007](#)).

An Investigation of auxin activity using auxin responsive marker suggest that melatonin neither activate auxin inducible gene expression of DR5:uidA, BA3:uidA nor induces degradation of HS::AXR3NT-GUS suggesting auxin independent signaling of melatonin involved in lateral root development ([Pelagio-Flores et al. 2012](#)). Supporting this, research in maize revealed that melatonin does not exhibit auxin-like activity in any of the three classical auxin bioassays: inhibition of 1-aminocyclopropane-1-carboxylate synthase (ACC) activity, coleoptile elongation, and root growth. These findings led the researchers to reject the hypothesis that melatonin functions as an auxin ([Kim et al. 2016](#)). Development of lateral roots encompass complicated signaling networks involving several endogenous and external factors which is not well understood. Melatonin induces LRP/LR formation and involves regulation of the expression of cell cycle gene (SlCDKA1, SlCYCD3;1 and SlKRP2) by stimulating polyamine oxidase (PAO)-dependent H₂O₂ production and respiratory burst oxidase homologous (RBOH)-dependent O₂⁻ production respectively. Screening

and expression pattern of PAO and RBOH genes suggest that melatonin upregulate these gene in LR emerging zone. Melatonin mediated ROS signaling cascade regulates LR formation in rice and fungi. PAO-H₂O₂ and RBOH3/4-O₂^{·-} acts downstream to the melatonin mediated signaling cascade responsible for LRP induction and LR formation by modulating the cell cycle genes (J. [Chen et al. 2019](#)).

4.4 CROSS TALK WITH OTHER PLANT HORMONE (CK AND AUXIN)

Exogenous application of melatonin in the range of nano to micro mole exhibit IAA function. It significantly promotes saponin accumulation, primary root elongation, thickness, and overall root biomass. It also elevates phytohormone such as salicylic acid, Jasmonic acid, abscisic acid, strigolactone etc. It promotes primary root growth in *Arabidopsis* wild type and pin mutant. Transcriptome analysis reveal the coregulation of many auxin-responsive genes by melatonin. melatonin partially rescued the primary root growth defects in pin single and double mutant of auxin. Melatonin is not an substitute to auxin but acts in auxin dependent manner ([Yang et al. 2021b](#)). Melatonin reported to downregulate the ethylene biosynthetic genes ACC synthase, ACC oxidase and suppress the accumulation of its immediate precursor 1-aminocyclopropane-1-carboxylic acid (ACC) in many crops ([Zhai et al. 2018](#)). Exogenous application of melatonin reportedly blocks Ethylene signaling and accumulation in pear. Nitric Oxide (NO) treatment suppresses the ethylene biosynthesis and accumulation in pear suggesting the regulatory role of NO in melatonin mediated biosynthesis of ethylene ([Liu et al. 2025](#)). NO decreases the expression of ethylene biosynthesis gene ACS and ACO and reduces the respiration and ethylene production in pear (J. [Liu et al. 2019](#)). Genome-

wide expression profiling through RNA sequencing revealed that melatonin treatment significantly activated auxin-related genes. Additionally, several transcription factors and cis-regulatory elements associated with root growth and auxin signaling were highly enriched among both upregulated and downregulated differentially expressed genes. This suggests that melatonin influences root development by modulating auxin-responsive pathways. The findings highlight a complex regulatory network where melatonin interacts with auxin signaling components, reinforcing its role in root morphogenesis and adaptation to environmental conditions ([Liang et al. 2017](#)). Six days old de-rooted hypocotyl of lupin were emerged in different concentration of melatonin and auxin. Auxin and melatonin both enhance the lateral root primordia (LRP) emerging from pericycle and modified the pattern/distribution of lateral roots, number and length of adventitious roots.(Marino B. [Arnao and Hernández-Ruiz 2007](#)). Melatonin act as positive regulator of root growth, while exogenous application of cytokinin or overexpression of *isopentenyltransferase 8* inhibit the primary root growth ([Wang et al. 2023](#)). Melatonin masks the inhibitory effects of cytokinin on primary root growth and regulates the expression and spatial distribution of the auxin efflux transporters PIN2/3 and the influx transporter AUX1 while simultaneously suppressing cytokinin signaling components, including AHK4, AHP2/3/5, and type A ARR15. This modulation likely establishes a hormonal balance favoring auxin-mediated root development while restraining cytokinin-driven inhibitory effects. By fine-tuning the auxin-cytokinin crosstalk, melatonin plays a crucial role in optimizing root architecture and enhancing plant adaptability under varying environmental conditions. Melatonin downregulates the biosynthetic gene 9-cis-epoxycarotenoid dioxygenase (NCED) and blocks the signaling pathway thereby delaying the fruit senescence (S. [Liu et al. 2020](#)). Contrary

to this it is reported to promote ABA accumulation and fruit ripening in strawberry ([Mansouri et al. 2021](#)), sweet cherry([Mansouri, Koushesh Saba, and Sarikhani 2023](#)) and grape. MT application reduces the expression of ABA biosynthetic genes through nucleus-localized transcriptional activators ABF/ABI in Chinese flowering cabbage ([Tan et al. 2019](#)). Melatonin and GA3 application enhances the chlorophyll, proline content and glycine betaine biosynthesis and improved the ROS homeostasis and methyl glyoxylate detoxification under salt stress ([Siddiqui et al. 2020](#)) ([Figure 4.4](#)).

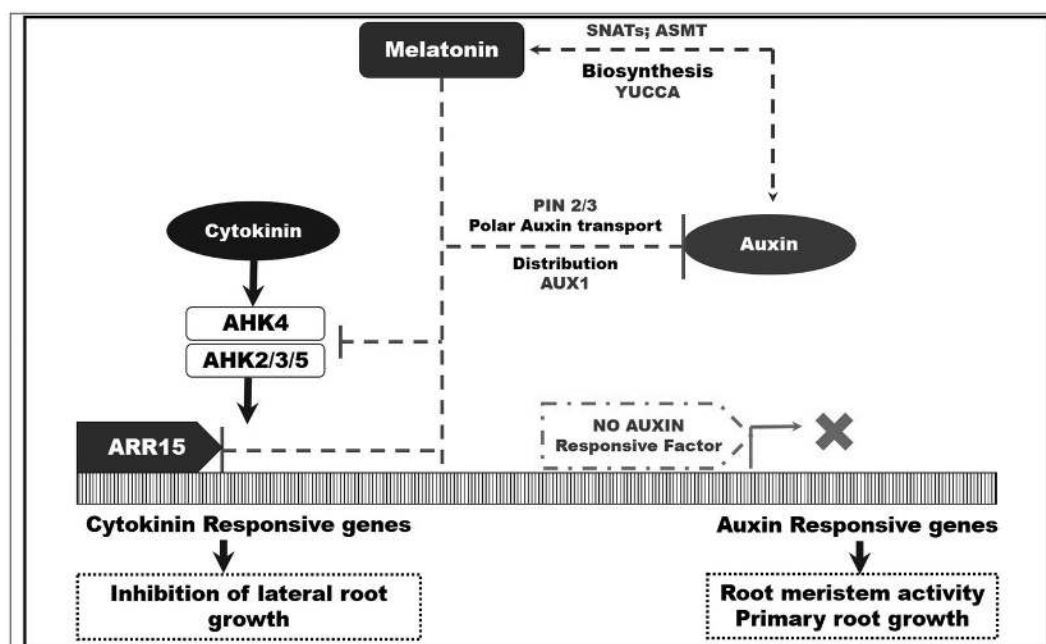


FIGURE 4.4 Hormonal cross talk of melatonin with cytokinin and auxin for root morphogenesis in plants. [↗](#)

4.5 CONCLUSION

Phytomelatonin is multifunctional biostimulator widely discussed for many cellular and physiological roles in plants under both biotic as well as abiotic conditions. Root architecture is shaped by the combined growth of primary and lateral roots, influenced by both genetic factors and external signals.

Melatonin's role in root growth and development is well established, but its precise mode of action remains unclear. It may regulate root branching, as well as secondary, lateral, and adventitious root formation, through an auxin-independent pathway, or it could influence primary root growth and development via an auxin-dependent mechanism. Lateral root (LR) branches from primary root are crucial for water and nutrient absorption. Reactive oxygen species (ROS) act downstream to signaling of melatonin for development of lateral primordia and lateral roots. Signaling cascade involve recently identified receptor Cand2, G protein, Ca^{++} and ROS. It modulates the expression and distribution of auxin efflux transporter PIN2/3 and influx transporter AUX1 and contrarily downregulated the cytokinin signaling components AHK4, AHP2/3/5, and type A ARR15. Thus, Melatonin plays a crucial role in optimizing root architecture and enhancing plant adaptability under varying environmental conditions.

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5

Phytomelatonin Regulates Uptake, Transport, and Assimilation of Mineral Nutrients and Aquaporins to Attenuate Ionic and Osmotic Stability with Redox Modifications Under Environmental Stressors

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DOI: [10.1201/9781003527633-5](https://doi.org/10.1201/9781003527633-5)

5.1 INTRODUCTION

Plants are the primary producers in most ecosystems and they rely on a complex interplay of physical, chemical, and biological processes to thrive. Among the various factors contributing to their growth and development,

phytohormones play a pivotal role. These small molecules are crucial in regulating a plethora of physiological processes, including cell division, elongation, and differentiation. Particularly, phytohormones orchestrates a complex network of regulatory mechanisms to optimize water uptake, nutrient acquisition, and mineral absorption, which are essential for plant health and productivity ([Rajendran *et al.*, 2022](#)). Plants face the constant challenge of acquiring sufficient water, nutrients, and minerals from their environment. In these circumstances, phytohormones are crucial in modulating the pivotal plant processes, ensuring plant adaptation in response to environmental stresses ([Kumari *et al.*, 2024](#)). The key phytohormones influence these critical processes, includes auxins, cytokinins (CK), gibberellins (GA), ethylene, abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), brassinosteroids (BRs), and strigolactones and many other. Among various phytohormones, phytomelatonin (PM; *N*-acetyl-5-methoxytryptamine) has emerged as a novel and universal signaling molecule that influences plant growth and stimulates defense responses against abiotic as well as biotic stresses. Understanding these hormonal interactions provides valuable insights into plant adaptive strategies and offers potential applications in agriculture to enhance crop productivity and stress resilience.

5.2 IMPORTANCE OF MINERAL NUTRIENTS AND AQUAPORINS IN PLANTS

Mineral nutrients are vital for plant growth, development and physiological functioning, and also play critical roles in various biochemical and structural functions ([Khan *et al.*, 2023](#)). Micronutrients and macronutrients

both play a role in controlling cellular processes, stress tolerance, plant growth and development, and overall production. For instance, nitrogen is vital for the synthesis of amino acids, proteins, nucleic acids, and chlorophyll. Furthermore, phosphorous is essential for energy transfer through adenosine triphosphate (ATP), nucleic acids, and phospholipids in cell membranes. Potassium is crucial for flowering and root development, also regulates osmotic balance, enzyme activity, and photosynthesis. Calcium is important for cell wall structure, membrane stability, and signal transduction ([White & Broadley, 2003](#)). Magnesium and iron are central components of the chlorophyll molecule and also act as cofactors for several enzymes involved in photosynthesis and respiration ([Marschner, 2012](#)). Zinc is necessary for the function of many enzymes and also involved in the synthesis of phytohormones. Copper takes part in photosynthesis, respiration, and lignin synthesis. Copper deficiency can cause wilting and stunted growth. Manganese also functions in photosynthesis, respiration, and nitrogen assimilation and its deficiency can result in interveinal chlorosis and reduced growth. Boron is essential for cell wall formation and reproductive growth. Molybdenum helps in nitrogen fixation and nitrate reduction and chlorine is vital for osmoregulation and photosynthesis ([Marschner, 2012](#)).

Aquaporins are a family of membrane proteins that facilitate the transport of water and small solutes across cellular membranes. They play crucial role in different physiological processes especially in plant water relations. Aquaporins increase the permeability of cell membranes to water, facilitating rapid water uptake and transport. During drought stress, these helps to maintain cellular water balance by regulating water flow into and out of cells. Some aquaporins are upregulated in response to drought, enhancing the plant's ability to cope with water scarcity. Some of the

aquaporins can also transport small solutes, such as urea and glycerol, contributing to nutrient uptake and distribution within the plant ([Maurel et al., 2008](#)). Additionally, aquaporins also play a significant role in maintaining ion homeostasis under salt stress by facilitating the movement of water and possibly small ions, helping to alleviate osmotic stress caused by high salt concentrations ([Boursiac et al., 2008](#)). These are also involved in various developmental processes, which includes seed germination, root elongation, and leaf expansion. They regulate the water flow necessary for cell elongation and expansion ([Maurel et al., 2015](#)). Aquaporins can participate in signaling pathways related to stress responses. For instance, alterations in aquaporin expression could promote signal adjustments in water transport in response to environmental cues ([Maurel et al., 2015](#)). Understanding the roles of mineral nutrients and aquaporins is important for improving plant growth, abiotic stress tolerance, and higher productivity, which is required for sustainable agriculture.

5.3 IMPORTANCE OF IONIC AND OSMOTIC STABILITY IN PLANT STRESS TOLERANCE

Ionic and osmotic stability play crucial roles in plant stress tolerance, particularly under drought, salt, and extreme temperatures. These mechanisms help plants maintain cellular homeostasis and survive adverse environmental conditions. High salt stress conditions often lead to the accumulation of toxic ions *viz.*, sodium (Na^+) and chloride (Cl^-), which could potentially disrupt cellular functions. Plants maintain ion homeostasis by regulating ion uptake, compartmentalizing toxic ions in vacuoles, and ensuring essential ions like potassium (K^+) to retain in the cytoplasm ([Zhu,](#)

[2003](#)). The maintenance of adequate levels of K^+ is essential for enzyme activation and protein synthesis under stress conditions ([Mäser *et al.*, 2002](#)). The calcium ions (Ca^{2+}) act as secondary messengers in various signaling pathways, including those triggered by stress. Maintaining appropriate Ca^{2+} levels is crucial for activating stress response mechanisms ([Sanders *et al.*, 2002](#)). Plants use specific ion transporters to control the uptake and distribution of ions. These transporters help to mitigate toxic effects of salt stress.

Osmotic stress causes water to move out of cells that leads to dehydration. Plants produce osmoprotectants like proline and glycine betaine to maintain cell turgor and water potential, enabling them to cope with water deficit ([Ashraf & Foolad, 2007](#)). Osmotic stress can lead to cell shrinkage or swelling, affecting cellular integrity and function. Thus, maintaining osmotic balance is essential for preserving cell volume and structure ([Hasegawa *et al.*, 2000](#)). Osmoprotectants and compatible solutes stabilize proteins and membranes, preventing denaturation and dysfunction under osmotic stress. This helps sustain metabolic activities during stress ([Yancey *et al.*, 1982](#)). Osmotic stress could cause stomatal closure in order to prevent water loss, reducing carbon dioxide (CO_2) uptake and photosynthetic efficiency. By maintaining osmotic stability, plants can sustain photosynthesis and growth under stress conditions ([Chaves *et al.*, 2009](#)). As aforementioned ionic and osmotic stability are vital for plant stress tolerance, prompting plants to evolve various genetic, biochemical mechanisms which includes the expression of stress-responsive genes, synthesis of stress proteins, and activation of antioxidant systems ([Mittler, 2002](#)). The physiological processes, such as modifying root architecture, altering water uptake and hormonal signaling play a crucial role to enhance these stabilizing processes to enhance stress tolerance ([Davies *et al.*, 2005](#)).

5.4 SIGNIFICANCE OF REDOX MODIFICATIONS IN PLANT STRESS RESPONSES

The redox modifications play a significant role in plant stress responses by regulating various physiological and biochemical processes. These modifications involve changes in the redox state of molecules, which can act as signals to trigger adaptive responses to environmental stresses. Under stress conditions, plants generate ROS like superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH). While high levels of ROS can be damaging, controlled production acts as a signal to activate stress response pathways ([Mittler *et al.*, 2004](#)). Antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidases (POD) help to detoxify ROS, protecting cells from oxidative damage. The activity of these enzymes is often regulated by redox modifications ([Apel & Hirt, 2004](#)). Non-enzymatic antioxidants such as ascorbate, glutathione, and tocopherols also maintain redox balance. The redox state of these molecules influences their ability to scavenge ROS and protect cellular components ([Noctor & Foyer, 1998](#)). Redox changes in the chloroplast can affect photosynthetic efficiency. The thioredoxin system modulates the activity of Calvin cycle enzymes in response to light conditions, optimizing photosynthesis under varying environmental conditions ([Schürmann & Buchanan, 2008](#)). The mitochondrial redox status influences respiration rates and ATP production. During stress, redox modifications could shift metabolic fluxes to support energy production and stress adaptation ([Raghavendra & Padmasree, 2003](#)). The redox changes can induce the expression of stress-responsive genes. For example, genes encoding heat shock proteins (HSPs) and pathogenesis-related (PR) proteins are often

upregulated in response to oxidative stress ([Foyer & Noctor, 2005](#)). Redox modifications, such as the formation of disulfide bonds, could alter protein structure and function, enhancing their stability and activity under stress conditions ([Meyer *et al.*, 2008](#)). Redox changes interact with hormonal signaling, which is crucial for stress responses such as stomatal closure during drought. ROS production in guard cells mediates ABA-induced stomatal closure, reducing water loss ([Mittler & Blumwald, 2010](#)). Redox state influences the signaling pathways of phytohormones which are involved in coordinating complex stress responses ([Foyer & Noctor, 2005](#)). The redox modifications are central to plant stress responses, acting as signals and regulators of various adaptive processes. They modulate enzyme activities, gene expression, metabolic pathways, and hormonal signaling, enabling plants to cope with and adapt to adverse environmental conditions.

As a phytohormone, PM plays a crucial role in enhancing plant stress tolerance by regulating various physiological processes. It modulates the uptake, transport, and assimilation of essential mineral nutrients and influences aquaporin activity to maintain water balance. PM helps in maintaining ionic and osmotic stability and activates antioxidant defense through redox modifications. This multifaceted hormone ultimately supports plant resilience under environmental stressors like drought, salinity, and extreme temperatures. The current book chapter aims to provide a comprehensive understanding of the multifaceted roles of PM in regulating mineral nutrient dynamics, water transport, ionic and osmotic stability, and redox balance, ultimately enhancing plant stress tolerance.

5.5 PHYTOMELATONIN AND ITS SYNTHESIS

Phytomelatonin (N-acetyl-5-methoxytryptamine) is an evolutionarily conserved chemical molecule and was first discovered in the bovine pineal glands in 1958 ([Lerner et al., 1958](#)) and was initially considered as important hormone only in mammals. PM has various physiological impacts in mammals including effects on the circadian rhythm, sleep, immune system, and body temperature. The hormone PM refers to melatonin of plant origin and was first discovered in 1995 by several independent researchers ([Dubbels et al., 1995](#); [Hattori et al., 1995](#); [Vantassel et al., 1995](#)). It is ubiquitous and present in all plant taxa from algae to angiosperms. It regulates plant growth and development, root growth, seed germination, flowering and fruiting, senescence, and photosynthesis ([Chen et al., 2022](#); [Sun et al., 2021](#); [Zhao et al., 2021c](#)). In addition, it also plays a significant role in the response of plants to various abiotic stresses ([Arnao and Hernandez-Ruiz, 2014](#); [Zhao et al., 2021a](#); [Huang et al., 2022](#); [Khan et al., 2022](#); [Mushtaq et al., 2022](#)). The first putative PM receptor, AtPMTR1 (Phytomelatonin Receptor 1), was identified in *Arabidopsis thaliana* (Arabidopsis) by [Wei et al. \(2018a\)](#). Recently, it has also been discovered that Arabidopsis employs mitogen-activated protein kinase (MAPK) cascades or GPA1/NADPH oxidase-mediated ROS using AtPMTR1-mediated PM signaling mechanisms to control the circadian stomatal rhythm and stomatal immunity ([Yang et al., 2021](#)). Lately, several PMTR1 homologs have been identified by different research groups. These homologs collectively have significant functions in PM signaling for seedling growth, leaf senescence, and plant innate immunity, as well as drought and salt stress tolerance ([Wang et al., 2021b](#); [Yang et al., 2021](#); [Bai et al., 2022](#)).

The synthesis of melatonin in mammals occurs mainly within mitochondria ([Tan and Reiter, 2019](#)) and involves four sequential steps

namely, hydroxylation, decarboxylation, acetylation, and methylation. The process starts with the hydroxylation of tryptophan, which is catalyzed by tryptophan hydroxylase (TPH) to form 5-hydroxytryptophan. Then 5-hydroxytryptophan is decarboxylated to produce 5-hydroxytryptamine (serotonin, 5-HT). Further followed by acetylation and methylation sequentially. In the final step 5-HT is converted into melatonin ([Arnao and Hernandez-Ruiz, 2014](#); [Zhao et al., 2019a](#)). The biosynthetic pathways of PM in plants are similar to those of melatonin in animals, with the exception of some unique characteristics involving tryptophan and its four synthesis steps, which are catalyzed by at least six enzymes: tryptophan decarboxylase (TDC), tryptophan hydroxylase (TPH), tryptamine 5-hydroxylase (T5H), serotonin *N*-acetyltransferase (SNAT), *N*-acetylserotonin methyltransferase (ASMT), and caffeic acid *O*-methyltransferase (COMT) ([Arnao and Hernandez-Ruiz, 2006](#); [Back et al., 2016](#); [Sun et al., 2021](#)). The melatonin produced in the mitochondria binds to the MT1 receptors localized on the outer mitochondrial membrane, which leads to the activation of mitochondrial MT1/G protein signaling and the inhibition of stress-mediated cytochrome c release that blocks caspase activation. Mitochondria are the major organelles that produce ROS and reactive nitrogen species (RNS) in the cell; melatonin plays a dual role as an antioxidant to diminish excess ROS and/or RNS and to mediate GPCR signaling to block cytochrome c release. This automitocrine signaling mechanism allows for both the synthesis and signaling of specific ligands and the precise control of organelle-based intracellular signaling ([Suofu et al., 2017](#)).

For PM synthesis, plants have two different biosynthetic pathways: the major pathway (MP) and the alternate pathway (AP). PM synthesis is initiated in the shikimic acid pathway and carbon dioxide used as the initial

reactant ([Arnao and Hernandez-Ruiz, 2022](#)). The MP uses the tryptophan derived from shikimic acid pathway as a precursor to produce tryptamine using TDC. Tryptamine is then converted to 5-HT (serotonin) in a hydroxylation reaction catalyzed by T5H. In the final steps, serotonin is acetylated by SNAT to form N-acetylserotonin, which is then converted into PM by ASMT in aquatic algae or caffeic acid-3-O-methyltransferase (COMT) in land plants ([Sun *et al.*, 2021](#); [Zhao *et al.*, 2021b](#); [Figure 5.1](#)). However, in AP, the shikimic acid pathway-derived tryptophan is initially hydroxylated to 5-hydroxytryptophan by TPH and then decarboxylated to form serotonin by TDC. Similarly, serotonin can be catalyzed by ASMT or COMT to form 5-methoxytryptamine (5-MT), which is then converted into PM by SNAT ([Arnao and Hernandez-Ruiz, 2014](#); [Figure 5.1](#)). Under normal conditions MP predominates over AP whereas, the AP becomes dominant when plants are exposed to environmental stresses ([Tan and Reiter, 2020](#)). In mammals, melatonin is metabolized into 4-hydroxymelatonin (4-OHM) and 6 hydroxymelatonin (6-OHM) by the cytochrome P450 enzyme. However, PM can be metabolized into other metabolites in an enzymatic or other biochemical manner ([Back, 2021](#)). The predominant forms of these PM metabolites are 2-hydroxymelatonin (2-OHM) and cyclic 3-hydroxymelatonin (3-OHM), which are catalyzed by melatonin 2-hydroxylase (M2H) and melatonin 3-hydroxylase (M3H), respectively ([Byeon and Back, 2015](#); [Lee and Back, 2016](#)).

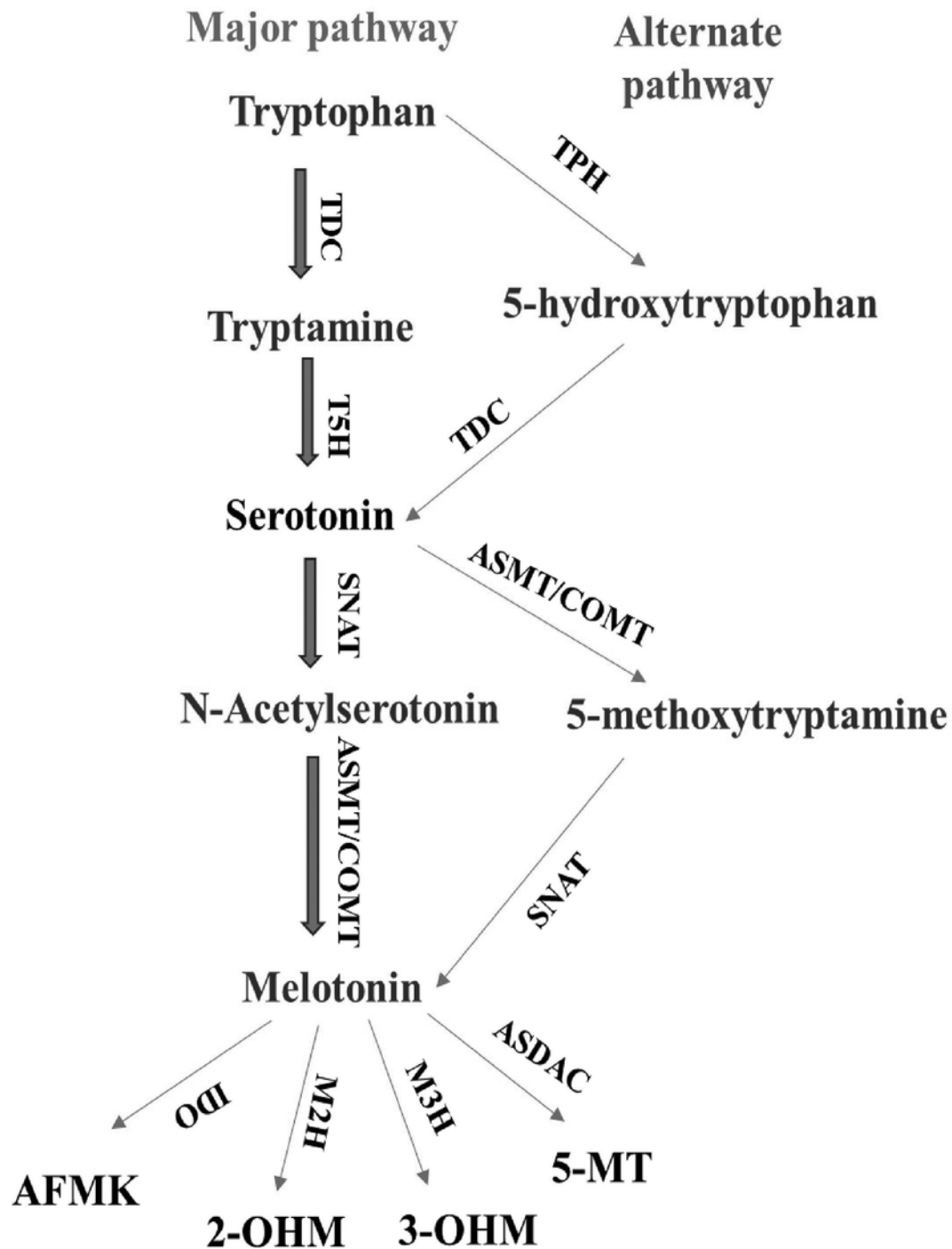


FIGURE 5.1 Biosynthesis and metabolism of phytomelatonin in plants. The major pathway of phytomelatonin synthesis is indicated by solid blue arrows, whereas the alternative pathway is indicated by solid black arrows. The phytomelatonin metabolism pathway is marked by dashed arrows. All enzymes in these pathways are indicated in red. TDC, tryptophan decarboxylase;

TPH, tryptophan-5-hydroxylase; T5H, tryptamine-5-hydroxylase; SNAT, serotonin Nacetyltransferase; ASMT, N-acetylserotonin methyltransferase; COMT, caffeic acid Omethyltransferase; ASDAC, N-acetylserotonin deacetylase; M2H, melatonin 2-hydroxylase; M3H, melatonin 3-hydroxylase;; AFMK, N1-acetyl-N2-formyl-5-methoxyaminourea; IDO, indoleamine 2,3-dioxygenase; 2-OHM, 2-hydroxymelatonin; 3-OHM, cyclic 3-hydroxymelatonin. [↵](#)

PM interacts with H_2O_2 results in the oxidative cleavage of its indole ring and produces AFMK ([Reiter et al., 2007](#)). AFMK is then convert into N-acetyl-5-methoxykynuramine (AMK) with the help of electron/hydrogen-abstracting radicals or by reacting with indoleamine 2,3-dioxygenase (IDO; [Okazaki et al., 2010](#)). This reaction will form N1-acetyl-5-methoxy-3-nitrokynuramine (AMNK) in the presence of nitric oxide (NO) or 3-acetoamidome-thyl-6-methoxycinnolinone (AMMC) in the presence of peroxynitrite ([Reiter et al., 2007](#); [Okazaki et al., 2010](#)). It has also been shown that PM can be deacetylated into 5-MT by N-acetylserotonin deacetylase (ASDAC), which is also responsible for the conversion of N-acetylserotonin into serotonin. The 2-OHM and 3-OHM that are generated from these pathways in the chloroplasts and in the cytosol are the primary products of PM hydroxylation and are implicated in senescence, seed germination, and blooming in Arabidopsis ([Lee and Back, 2021](#); Lee and Back, [2022a, b](#)) The PM and its metabolites are present in multiple organelles or sub-cellular compartments, including the mitochondria, chloroplasts ([Zheng et al., 2017](#)), and the cytosol ([Back et al., 2016](#)). This sub-cellular compartmentalization of PM in plants may have two main benefits. The first advantage is that acetyl-CoA, which is a cofactor of PM biosynthesis is synthesized in the mitochondria and chloroplasts which potentially increases the biosynthetic efficiency of PM as its synthetic

enzymes are also found in these organelles. Second, the biosynthesis of PM in the mitochondria and chloroplasts protects them from excess ROS production in these organelles ([Tan and Reiter, 2020](#)).

5.6 PHYTOMELOTONIN AND ITS RELATION WITH PLANT MINERAL NUTRITION

Plant growth and development are substantially influenced by constantly changing environmental conditions and by multiple stress factors, such as variable soil mineral nutrient bioavailability. Plant mineral nutrition is critically important for agricultural productivity. However, the bioavailability of mineral elements is strongly influenced by soil characteristics and other environmental factors ([Bouain *et al.*, 2019](#); [Gondal *et al.*, 2021](#)). Long-term intensification of agricultural practices and inadequate nutrient inputs have changed the properties of soils and this can lead to different plant disorders including nutrient deficiencies and toxicities. Under these fluctuating external conditions, plants have developed sophisticated morphological, physiological, and biochemical responses to cope with nutrient disorders. Increasing evidence indicates that some universal signaling molecules participate in the regulation of plant responses to nutrient deficiencies and toxic conditions ([Kiba *et al.*, 2011](#); [R. Zhang *et al.*, 2017a](#); [Zhu *et al.*, 2017](#); [Buet *et al.*, 2019](#)). Among these PM is a very important signal molecule participates in the uptake, transport, homeostasis of minerals and also nutrient use efficiency by affecting root system architecture and/or inducing redox-based modifications of nutrient transporter proteins ([Zhang *et al.*, 2017b](#); [Qiao *et al.*, 2019](#); [Bawa *et al.*, 2020](#); [Chen *et al.*, 2021](#)). Earlier studies over the past decade suggests that

PM can stimulate plant growth and yield ([Arnao and Hernandez-Ruiz 2014](#); [Sun et al., 2021](#)), and adequate acquisition with the appropriate balance of mineral nutrients play an important role in this. Thus, it's reasonable to conclude that strong interaction between PM and mineral nutrition exists in plants.

Exogenous application of PM in plants has influenced overall mineral nutrition as understood by ionomics ([Turk and Erdal, 2015](#); R. [Zhang et al., 2017b](#); [Bawa et al., 2020](#); [Chen et al., 2021](#); [Hosseini et al., 2021](#); [Altaf et al., 2022](#)). Furthermore, it is found that the biosynthesis and degradation of PM may be self-regulated to transduce the signals of specific mineral nutrition disorders arising from Nitrogen(N), Phosphorous(P) and Potassium(K) deficiencies and from Iron(Fe) and Zinc(Zn) toxicities ([Arnao and Hernandez-Ruiz, 2009, 2013](#); [Chen et al., 2017](#); [Meng et al., 2021](#); [Zheng et al., 2021](#)). Hence, mineral element uptake, transport, and utilization are mediated by PM to support plant growth. Scientific evidence suggests that PM levels can be considerably altered by adverse environmental conditions, such as changes in salinity, extreme temperatures, and metal stress. For example, PM content has been shown to significantly increase in the roots of lupin (*Lupinus albus*) exposed to drought stress ([Arnao and Hernandez-Ruiz, 2013](#)). The altered PM levels in relation to plant responses to a nutrient imbalance were first described by [Arnao and Hernandez-Ruiz \(2009\)](#). They reported that excessive ZnSO₄ induces the biosynthesis and accumulation of PM in roots of barley (*Hordeum vulgare*). Later, enhanced production of PM by as a result of exposure to excessive Zn was also observed in lupin roots ([Arnao and Hernandez-Ruiz, 2013](#)). Later many research groups reported PM levels are affected in plants by exposure to specific mineral nutrients. For example, PM levels have been reported to be increased in plants under limited K

supply ([Chen et al., 2017](#); [Li et al., 2021](#)), Fe deficiency ([Zhou et al., 2016](#); [Zheng et al., 2021](#)), sulfur (S) starvation ([Hasan et al., 2018](#)), and excess nitrate ([Meng et al., 2021](#)) and copper (Cu; [Cao et al., 2019](#)).

The increased abiotic stress influence mineral uptake and distribution in plants ([Barzana et al., 2021](#)). Under such adverse conditions, melatonin regulates mineral homeostasis. Generally, salinity reduces the levels of cationic nutrients, such as K, Ca, and Mg, due to competition for available transport sites. [Sezer et al. \(2021\)](#) reported that priming sweet corn (*Zea mays var. saccharata*) seeds with 100 μ M PM restores the K and Ca contents under saline soil conditions. The potential of melatonin to increase K and Ca levels has also been reported in field bean (*Vicia faba*; [Dawood and El-Awadi, 2015](#)). Drought stress significantly affects the transport of nutrients throughout the plant owing to reduced transpiration rates. The PM also modulates the mineral nutrient composition; it can mitigate drought stress by improving the uptake of nutritional elements ([Liang et al., 2018](#)). Furthermore, PM significantly ameliorates decreases in K, P, S, Mg, Fe, Cu, Mn, and Zn, and promotes increases in Ca and B in plants exposed to low-temperature stress ([Turk and Erdal, 2015](#); [Bawa et al., 2020](#)). The metal toxicities reduces the uptake of nutrients and this inhibition is not as severe when melatonin is supplied ([Kaya et al., 2019](#); [Jahan et al., 2020](#); [Altaf et al., 2021](#)). In addition to macronutrients, micronutrients also significantly affect the accumulation of other essential nutrients owing to their synergisms and antagonisms. The Cu toxicity decreases the contents of Fe, K, Mg, Ca, and P; however, PM application reverses the deleterious effects induced by Cu ([Cao et al., 2019](#)). Accumulation and analyzing of data from diverse studies might be a promising approach for identifying the pathways by which melatonin regulates mineral nutrient uptake and homeostasis, especially under single and combined nutrient disorders. Fortunately, the

development of ionomics techniques in combination with ample knowledge of transcriptomics provides unique opportunity to decipher the mechanisms of PM-mediated mineral nutrient homeostasis. Understanding the roles of melatonin in nutrient uptake under stress conditions is important for agricultural purposes, and extensive knowledge will enhance the pace of plant adaptability to abiotic stress. The underlying physiological and molecular mechanisms by which PM influences the uptake of specific mineral nutrients and maintains their stability are discussed in more detailed in the next section.

5.7 INFLUENCE OF PHYTOMELATONIN ON NITROGEN UPTAKE AND ASSIMILATION

Nitrogen is an important macronutrient for plants, and its availability is a major factor in determining growth and productivity. Plants mainly acquire N through the uptake of inorganic N as nitrate (NO_3^-) and ammonium (NH_4^+ ; [Tegeder *et al.*, 2007](#); [Bloom, 2015](#); [Tegeder and Masclaux-Daubresse, 2018](#)). The preferred N forms, NO_3^- is taken up by roots and reduced to NO_2^- by nitrate reductase (NR), which is then converted into NH_4^+ by nitrite reductase (NiR) ([Hachiya and Sakakibara, 2017](#); [Ye *et al.*, 2022](#)). Finally, NH_4^+ derived from nitrate or directly taken up by roots is assimilated into amino acids ([Lam *et al.*, 1996](#); [Xu *et al.*, 2012](#); [Krapp, 2015](#)). However, NO_3^- and NH_4^+ have heterogeneous availability and vary dynamically in many ecosystems and agricultural landscapes. Plants have evolved uptake systems for both NO_3^- and NH_4^+ that have different affinities. Despite the obvious differences that have been found to exist among different species and treatments, PM seems to consistently

participate in nitrogen nutrition by regulating key transporters and enzymes involved in N uptake and assimilation.

In a study on winter wheat (*Triticum aestivum*), [Qiao et al. \(2019\)](#) reported that PM-treated seedlings maintained high N content and NO_3^- levels in both shoots and roots under N-deficient but not N-sufficient conditions. [Talaat \(2021\)](#) also studied that melatonin increases the content of total N and NO_3^- in salt-stressed wheat plants. In contrast, at high NO_3^- concentrations, melatonin accelerates cucumber growth by decreasing N absorption ([Zhang et al., 2017a](#)), indicating that melatonin further intensifies the switch from high- to low-affinity NO_3^- transport systems. These findings suggest that melatonin could change the N uptake strategy of plants depending on the availability of the external supply. Also, researchers reported that melatonin-mediated post-transcriptional modification of proteins is involved in N uptake and transport ([Fraisier et al., 2000](#); [Arnao and Hernandez-Ruiz, 2014](#); [Wege, 2020](#)). Several studies have highlighted the importance of PM in enhancing N assimilation in plants, particularly those under abiotic stress. PM production play a role in the regulation of N assimilation based on the reports that exogenous application of PM enhances the activities of relevant enzymes. The chlorophyll-preservation and photosynthetic-stimulation properties have been reported in PM ([Arnao and Hernandez-Ruiz, 2009](#); [Tan et al., 2012](#); [Sun et al., 2020a](#)). However, understanding of the underlying mechanisms by which PM modulates N uptake and assimilation is still in its infancy, and extensive research in plants, especially crops, is needed. Melatonin can also enhance symbiotic N fixation in legumes. For example, [Ren et al. \(2019\)](#) revealed that melatonin increases the number and size of nodules formed on soybeans. In addition, the discovery of elevated melatonin levels in

endophytic organisms ([Jiao et al., 2016](#)) might be promising area of research in relation to improving agricultural productivity.

5.8 MAINTENANCE OF POTASSIUM TRANSPORT AND HOMEOSTASIS BY PHYTOMELONIN

Potassium is a vital and abundant macronutrient that constitutes ~2–10% of plant dry matter ([Leigh and Wyn Jones, 1984](#); [Kumari et al., 2021](#)).

Concentrations of K^+ in the cytoplasm are generally around 100 mM, whereas, available K^+ levels in soil solutions are usually lower of magnitude ([Maathuis, 2009](#); [Scherzer et al., 2015](#); H. [Wang et al., 2021a](#)).

Plants have acquired different pathways to absorb and maintain the K^+ homeostasis within cells. These involve a diverse array of channels and transporters in order to ensure the movement of K^+ through plasma membrane ([Ashley et al., 2006](#); [Rodríguez-Navarro and Rubio, 2006](#)). The melatonin has multifaceted beneficial effects on the modulation of plant responses to K^+ starvation ([Figure 5.3](#)). PM and K^+ exhibit an unquestionable relationship, as its levels increase in response to K^+ deficiency and ultimately direct the absorption of K^+ transporting elements ([Li et al., 2016a, 2021](#); [Chen et al., 2017](#)). Furthermore, the promoting effects of PM on root development influence K^+ uptake, particularly under starvation conditions. Regarding the important functioning of K^+ and PM during plant responses to salt and drought stress, studies have suggested that it precisely regulates K^+ nutrition and consequently improves stress tolerance ([Li et al., 2016a](#); [Sezer et al., 2021](#)). Additional PM mediated K^+ homeostasis has been identified in guard cells, where it regulates stomatal movement by optimizing K^+ concentrations. PM promotes tolerance to K^+

deficiency by improving the capacity for uptake and transport. The molecular mechanism of melatonin-mediated K^+ uptake has been described in a study on apple under different stress conditions ([Li et al., 2016b](#)). Melatonin application was found to maintain K^+ homeostasis within the plants, and this was mainly achieved by activating the up-regulation of K^+ transporter genes such as *AKT1* and *HKT* via activation of the CBL1–CIPK23 pathway. [Chen et al. \(2017\)](#) also reported that high levels of K^+ in melatonin-treated Bermuda grass resulted from increased expression levels of K^+ transporter genes, including *CdHAK9*, *CdKT1*, and *CdKT23*. Recent work with wheat plants, has shown that melatonin improves tolerance to K^+ deficiency by up-regulation of *HAK1* and *NAC7* transporters ([Li et al., 2021](#)). The available evidence indicates that PM plays a significant role in regulating the expression of genes encoding K^+ transporters and channels. This concludes the possibility of improving plant K nutrition through the help of melatonin, which is particularly useful for plants under drought and salt stress.

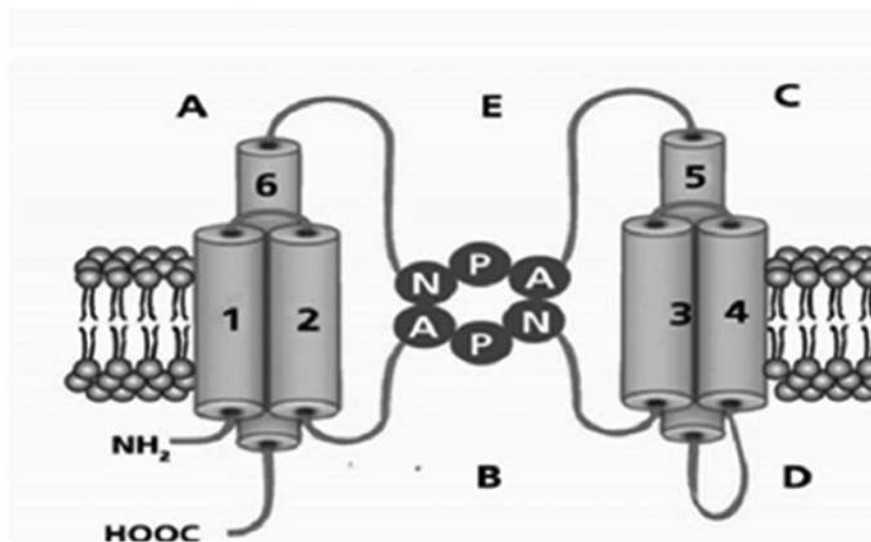
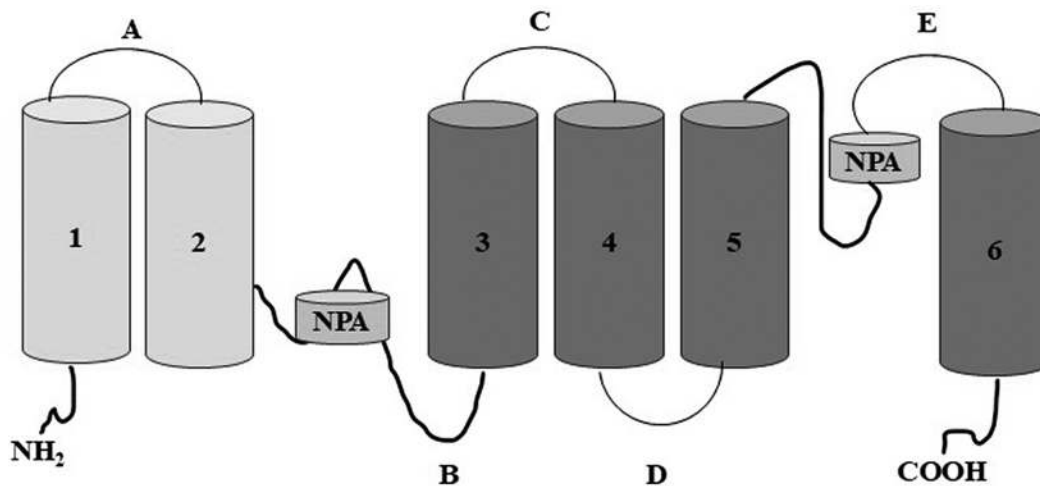


FIGURE 5.2 Protein arrangement showing the two regions of helical domains interact to form the three-dimensional structure of the protein. Pore of the AQP is composed of hemipores. MIPs consist of six transmembrane domains connected by five loops (A–E), with cytoplasmic N- and C-termini. Locations of NPA (Asn-Pro-Ala) motifs are at the loops B and E. Functional AQP formed by the interaction of the two hemipores. [↗](#)

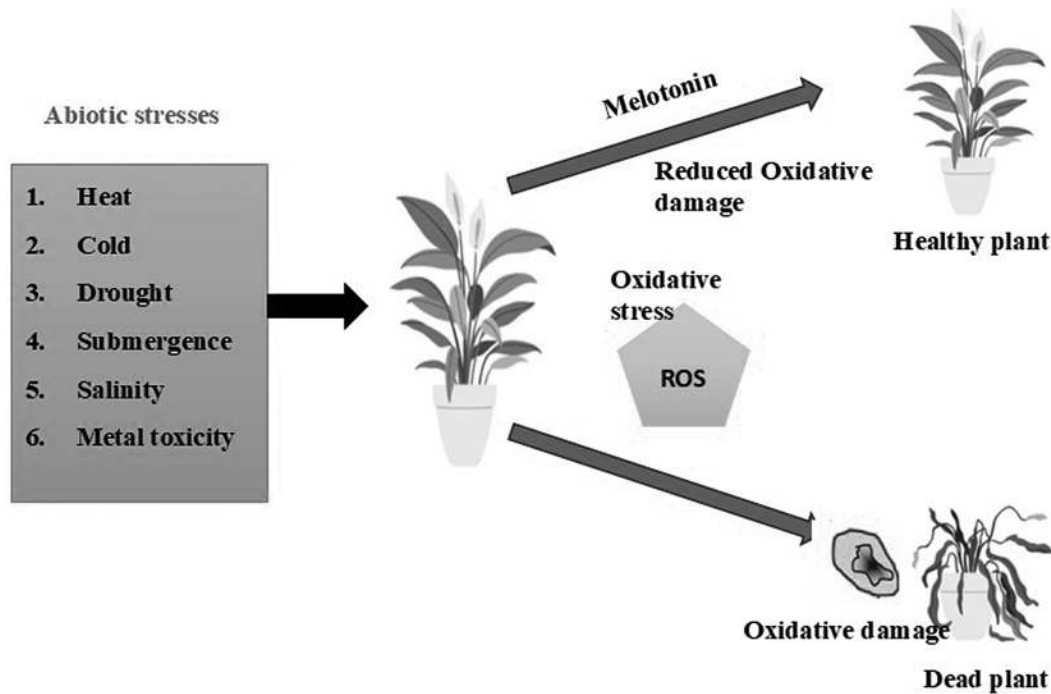


FIGURE 5.3 Role of phyto-melatonin in alleviating abiotic stress-induced oxidative damage in plants. [↗](#)

Under salt stress condition, application of exogenous melatonin enables K^+ retention in plants, which in turn is positively associated with salinity tolerance ([Dawood and El-Awadi, 2015](#); [Li et al., 2016c](#); [Sezer et al., 2021](#)). Melatonin significantly increases K^+ and decreases Na^+ contents in maize shoots under different soil salinity levels ([Sezer et al., 2021](#)). Electrophysiological experiments have shown that exogenously applied melatonin significantly reduces salt-induced K^+ efflux from the root cells and mesophyll tissues of sweet potato under salt stress ([Yu et al., 2018](#)). [Liu et al. \(2020\)](#) have explained that melatonin does not immediately inhibit K^+ efflux in rice (*Oryza sativa*), however, acts in a pronounced time-dependent manner. This suggests that melatonin may modulate the expression and activity of K^+ transporters rather than affect the receptor-like binding to these transporters. Further investigation revealed that melatonin increased

the expression levels of *OsGORK*, *OsAKT1*, and *OsHAK1/5/21* in rice roots, which maintained K⁺ homeostasis and enhanced salt tolerance.

5.9 PHYTOMELATONIN PARTICIPATES IN PLANT ACCLIMATION TO PHOSPHORUS AND SULFUR DEFICIENCY

Phosphorus is a macronutrient that is indispensable for crop production and which acts as a key component of nucleic acids, membrane lipids, and high-energy molecules. Low P availability significantly limits crop production in more than 60% of the world's arable land ([Gutiérrez-Alanís et al., 2018](#)). Plants can only take up inorganic P (Pi), and is frequently available at low concentrations (<10 µM) in the soil and it has considerably low mobility. Plants have developed multiple mechanisms to cope with P scarcity ([Vance et al., 2003](#); [Xu et al., 2020](#); [Dissanayaka et al., 2021](#); [Paz-Ares et al., 2021](#)). Recent findings have revealed that PM may modulate plant responses to P starvation at both the physiological and molecular levels. In tomato, transgenic plants overexpression of *COMT1* show increased PM levels and increased P concentration, and they exhibit tolerance to low Pi conditions ([Sun et al., 2020c](#)). The authors suggest that although increase in PM by genetic manipulation of *COMT1* will enhance Pi uptake and transport, the intrinsic molecular regulatory mechanisms of the various transporters have not been studied. Using barrel *Medicago truncatula* (clover), Zhang et al. ([2021](#)) found that melatonin application increases the expression of *MtPT4*, a low-affinity Pi transporter in arbuscule-colonized cells, to enhance Pi uptake. However, there is limited knowledge about transcriptional regulation of transporters related to Pi nutrition that are

influenced by melatonin in plants. The observed increase in P content and enhanced tolerance to P-deficient conditions following melatonin treatment highlight the need for further investigation into the molecular mechanisms regulating phosphorus uptake and transport. Root hairs are known to facilitate the uptake of Pi ([Zhang et al., 2018](#); [Kumar et al., 2019](#)), and whether melatonin stimulates their development and contribution to P nutrition should be investigated. It is crucial to conduct genomic, proteomic, and transcriptomic analyses to uncover the PM-mediated acclimation mechanisms involved in phosphorus acquisition.

Sulfur is essential for the maintenance of normal metabolic processes in plants. Plant S metabolism is particularly important for human nutrition because methionine is an essential amino acid ([Kopriva et al., 2019](#)). S-deficiency has become more prevalent in recent years, with its frequency ranking third after N and P deficiency ([Wilhelm, 2009](#); [Zhang et al., 2021](#)). Hence, the management of S homeostasis and metabolism is important not only for crop yield, also for human health. Sulphate (SO_4^{2-}) is the primary substrate for S metabolism, and a series of membrane transporters participates in its uptake into root tissues and its subsequent distribution within plant cells ([Takahashi, 2019](#)). In plants, two SULFATE TRANSPORTER (SUT) genes, *SUT1;1* and *SUT1;2*, and these help roots to absorb SO_4^{2-} from soils. Inside the cells, SO_4^{2-} enters metabolic pathways by triggering ATP sulfurylase (ATPS), adenylylsulfate reductase (APSR), and sulfite reductase (SiR) to produce sulfide. Finally, cysteine is formed by the condensation of sulfide and *O*-acetylserine as well as the catalysis of *O*-acetylserine(thiol)lyase (OAS-TL; [Kopriva et al., 2019](#)). There are various phytohormones and signaling molecules play important roles in regulating plant responses to S deficiency. Implying comparative transcriptomics, [Teng et al. \(2022\)](#) recently found that PM treatment

significantly elevated the expression of three genes related to S assimilation in *Brassica rapa* (cabbage) namely *APSR1*, *APSR3*, and glutathione synthetase2. The resulting enhanced S assimilation promotes the synthesis of glucosinolate, an S-containing compound with antioxidant, anticancer, and anti-inflammatory properties ([Grubb and Abel, 2006](#)). PM-mediated up-regulation of *SUT1;1* and *SUT1;2* expression levels has also been observed in tomato plants, with enhanced S content. Simultaneously, the activities of ATPS, APSR, and OAS-TL are up-regulated to enhance S assimilation ([Hasan et al., 2018](#)). Thus, PM could improve S-uptake efficiency from soils by roots and benefit plant nutrition, thereby contributing to adaption to S deficiency. In addition, experimental evidence suggests that enhancement of S acquisition and assimilation can decrease heavy metal toxicity due to the accumulation of S-containing chelators, such as phytochelatins and glutathione. An investigation of cadmium stress in tomato seedlings has demonstrated that application of exogenous melatonin or overexpression of *COMT* leading to increased PM levels both up-regulated the expression levels of *SUT1;1* and *SUT1;2*, and enhance the activities and transcript levels of APSR, SiR, and OAS-TL ([Hasan et al., 2019](#)). These effects ultimately promote S metabolism to inhibit cadmium translocation and enhance its detoxification. It would be interesting for future research to investigate the role of melatonin in the modulation of the mechanisms implicated in S-use efficiency in order to cope with reductions in crop yield induced by S deficiency.

5.10 PHYTOMELATONIN IS INVOLVED IN IRON HOMEOSTASIS

Iron is a vital micronutrient, being a central component of electron chains and an important cofactor for many enzymes. Although iron is abundant in

soils, its availability to plants is often limited due to its low solubility, especially in calcareous soils ([Walker and Connolly, 2008](#); [Ma and Ling, 2009](#); [Briat et al., 2015](#); [Riaz and Guerinot, 2021](#)). The limitation of Fe to plants is also a critical issue for human health because crops are the major Fe source ([Welch and Graham, 2004](#)). Higher plants have developed two distinct strategies for acquiring Fe from the rhizosphere ([Hell and Stephan, 2003](#); [Morrissey and Guerinot, 2009](#); [Kobayashi and Nishizawa, 2012](#); [Raza et al., 2019](#)). Non-graminaceous plants utilize the Fe reduction strategy (Strategy I), which includes the two main processes of ferric (Fe^{3+}) reduction by membrane-bound ferric chelate reductase FRO2 at the root surface, and absorption of the generated ferrous ions (Fe^{2+}) across the root cell plasma membrane by the high-affinity Fe transporter IRT1. In Arabidopsis, *FRO2* and *IRT1* are transcriptionally regulated by FIT, a Fer-like transcription factor (TF) induced by iron deficiency ([Riaz and Guerinot, 2021](#)). In contrast, graminaceous plants rely on a chelation strategy (Strategy II). This is characterized by the secretion of Fe^{3+} -chelating substances called phytosiderophores, which are secreted by transporter of mugineic acid 1 (TOM1) transporters into the soil. The chelated substances are then transported into cells by yellow stripe-like 1 (YSL1) transporters ([Kobayashi and Nishizawa, 2012](#); [Buet et al., 2019](#)). Alleviating effects of melatonin on Fe deficiency have been reported in both non-graminaceous and graminaceous plants including maize ([Turk and Erdal, 2015](#)), tomato ([Altaf et al., 2021](#)), and Arabidopsis ([Zhou et al., 2016](#)). However, the molecular mechanisms mediated by PM in Fe acquisition are less common in graminaceous plants. In Arabidopsis, comparative analyses of physiological responses and the transcriptome have indicated that application of exogenous melatonin improves tolerance to Fe-deficiency by enhancing acquisition ([Wan et al., 2018](#)). [Kaya et al. \(2020\)](#)

also found that melatonin application enhances the tolerance of pepper (*Capsicum annuum*) to Fe deficiency. Both transcriptome data and qRT-PCR results showed that PM markedly increases the expression of *FRO2*, *IRT1*, and various Fe-deficiency response genes, leading to accumulation and the alleviation of deficiency-induced leaf chlorosis. The rapid expression of PM synthesis genes and increased its accumulation observed under Fe deficiency further indicates its regulatory functions. It has been reported that Arabidopsis and cucumber plants cultivated under low-Fe conditions show increased PM content and higher expression levels of genes related to Fe acquisition such as *FRO2*, *IRT1*, and *FIT1* after melatonin treatment compared to controls without treatment ([Zhou et al., 2016](#); [Ahammed et al., 2020](#)). In addition, under conditions of limited supply, melatonin can enhance the reutilization of Fe stored in the roots, thereby facilitating plant growth. [Zhou et al. \(2016\)](#) reported that melatonin alleviated Fe deficiency by promoting reutilization of root cell-wall Fe in Arabidopsis. Further fractionation and characterization of the Fe content in different cell wall components revealed that the melatonin mainly targets pectin and hemicellulose, as the binding of Fe to these two components decreased after treatment. Melatonin is emerging as an important player in Fe uptake, sequestration, trafficking, and metabolism. Hence, improving our understanding of the molecular basis for the amelioration of Fe deficiency by melatonin may be important for designing and engineering crop plants that can cope with Fe deficiency.

Plants are sometimes exposed to high levels of Fe that toxic owing to its high redox activity ([Becana et al., 1998](#)). This situation usually occurs in acidic soils or under waterlogged conditions ([Wheeler et al., 1985](#)). PM also protects plants from this toxicity. When in excess, free Fe readily reacts with H_2O_2 to generate hydroxyl radicals ($OH^\cdot$), which are the most active

form of ROS. Consequently, excessive Fe disrupts cellular redox homeostasis and causes oxidative damage. Owing to its universal antioxidant capacity, melatonin can protect plants from this toxicity ([Sun et al., 2021](#)). For example, [Ahammed et al. \(2020\)](#) found that under excessive Fe conditions, melatonin decreased the Fe content in the leaves and roots of cucumber, and this was associated with a significant down-regulation of expression of *FRO2* and *IRT1*. Combined with its functions when Fe is limited, we can say that PM not only regulates Fe uptake and translocation depending on the external availability, but that it also protects plants from oxidative stress induced by Fe disorders. Interestingly, numerous studies have shown that uptake systems activated by Fe deficiency lead to enhanced uptake of cadmium ([Luo et al., 2012](#); [Sterckeman and Thomine, 2020](#)). It is known that the Fe transporter *IRT1* is primarily responsible for the entrance of cadmium into root cells ([Lux et al., 2011](#); [Ismael et al., 2019](#)). Analysis of PM-treated *Brassica rapa var. chinensis* (Chinese cabbage) under cadmium stress has shown that the expression of *IRT1* is inhibited, which reduces the accumulation and toxicity of cadmium ([Wang et al., 2021b](#)). Consistent with this, melatonin also decreases the Fe status in the Chinese cabbage seedlings under cadmium stress. Overall, this evidence adds more complexity to the function of PM in regulating plant Fe homeostasis, especially when other metals are present that compete with Fe for the same membrane transporters, such as cadmium. Melatonin probably maintains Fe homeostasis in plants through its crosstalk with nitric oxide, an important regulator of Fe homeostasis ([Graziano et al., 2002](#); [Graziano and Lamattina, 2005](#)). For example, Fe deficiency triggers nitric oxide production in various plants, and nitric oxide positively regulates the expression of *FRO2*, *IRT1*, and *FIT*, as well as improving Fe utilization efficiency ([Chen et al., 2010](#); [Zhu et al., 2016](#)). During plant growth and

stress responses, melatonin and nitric oxide act together in both synergistic and antagonistic ways ([Mukherjee, 2019](#); [He and He, 2020](#)). Recent evidence from [Kaya *et al.* \(2020\)](#) suggests that melatonin-induced pepper tolerance to Fe deficiency might be reliant on downstream nitric oxide signaling. The interaction between PM and nitric oxide in Fe nutrition at the genomic, proteomic, and transcriptomic levels requires further investigation through extensive experiments to determine the optimal plant Fe nutrition for benefiting human health.

5.11 PHYTOMELATONIN AND ADAPTATION OF PLANTS UNDER OTHER NUTRIENT DISORDERS

Several studies have reported functions of PM in plant adaptations to the availability of other mineral elements, including micronutrients and other beneficial elements. Cu is a micronutrient and its toxicity causes oxidative damage and inhibits growth. Pre-sowing seed treatment of red cabbage with melatonin confers tolerance to Cu toxicity in the seedlings ([Posmyk *et al.*, 2008](#)). Melatonin alleviates Cu toxicity and enhances plant growth through multiple mechanisms, and most studies ascribe this to its antioxidant properties. In addition, based on transcriptomic and metabolic analyses, [Hu *et al.* \(2020\)](#) have reported that JA biosynthesis is associated with the melatonin-mediated alleviation of Cu toxicity in roots of melon (*Cucumis melo*). [Cao *et al.* \(2019\)](#) have shown that in cucumber, melatonin does not influence Cu²⁺ uptake, as the Cu content and expression of the initial Cu²⁺-uptake transporter COPT do not change in the root tissues. In contrast, the contents of glutathione and phytochelatin increase after melatonin treatment, and these chelated Cu²⁺ and promote its trapping in the root cell

walls. Based on these results, melatonin might not only protect plants from Cu^{2+} -induced oxidative damage, however, also decrease toxicity through the conjugation and sequestration of Cu to insensitive locations, such as vacuoles and cell walls. The application of melatonin to non-food plants capable of detoxification might be a feasible strategy to improve phytoremediation in Cu-contaminated soils.

Zinc was the first mineral micronutrient reported to induce PM production in plants ([Arnao and Hernandez-Ruiz, 2009](#)). Most studies on the interactions between melatonin and Zn have reported increases in PM levels in response to Zn toxicity. Exogenous PM can protect plants against Zn toxicity. For example, in safflower (*Carthamus tinctorius*), PM reduces Zn uptake and diminishes its above-ground accumulation ([Goodarzi et al., 2020](#); [Eisalou et al., 2021](#)). The protective effects of melatonin in alleviating Zn toxicity are attributed to its effects of enhancing antioxidant defense and decreasing uptake and accumulation. Future research should focus on further exploring the effects of melatonin on the sub-cellular distribution of Zn. Boron, another micronutrient, is essential for plant growth and productivity ([Rerkasem et al., 2020](#)). Plants require minimal amount of B in their lifecycle, and toxicity frequently occurs naturally in arid and semi-arid soils ([Landi et al., 2019](#)). Melatonin enhances B uptake under normal conditions, and decreases its accumulation during toxicity ([Turk and Erdal, 2015](#); [Moussa and Algamal, 2017](#); [Sarafi et al., 2017](#); [Al-Huqail et al., 2020](#)).

Melatonin also influences distinct functions related to non-essential beneficial elements that are not required by all plants but could be important for particular taxa. These include aluminum (Al), cobalt (Co), sodium (Na), selenium (Se), and silicon (Si), which are the most-studied beneficial elements in particular plants under certain conditions ([Pilon-](#)

[Smits et al., 2009](#)). For example, Al is essential for the root growth and development of tea (*Camellia sinensis*) plants ([Sun et al., 2020b](#)), but it is toxic to crops in acid soils, even at low levels ([Kochian et al., 2015](#)). Although only a few studies have reported the interaction between melatonin and Al in tea, several studies have found that melatonin can protect sensitive plants from Al toxicity through multiple pathways, including the augmentation of antioxidant responses, enhancement of Al exclusion from cell walls, and increases in the exudation of organic acid anions ([Zhang et al., 2017a](#); [Sun et al., 2020a](#); [Ren et al., 2022](#)). It is well known that Si is a beneficial element for rice growth and development, and Si fertilizers are effective at reducing heavy metal concentrations in rice ([Wang et al., 2016a](#)). [Bao et al. \(2021\)](#) found that Si combined with foliar melatonin application is more effective at decreasing cadmium and arsenic uptake and transport in rice than application of Si alone. This interesting observation suggests that melatonin could potentially reduce heavy metal accumulation and risks in crops. In addition, melatonin exerts a regulatory effect on Co homeostasis in black pine seedlings and protects Arabidopsis from Se-induced toxicity ([Ulhasan et al., 2019](#); [Celik, 2021](#)). However, the functioning and underlying mechanisms of the beneficial effects of melatonin in combination with such elements are relatively under-studied compared with the toxic effects of these elements.

PM is increasingly thought to participate in the regulation of plant mineral nutrition, especially in conditions of mineral disorders. Its biosynthesis and/or degradation is rapidly regulated in response to the fluctuating availability of mineral elements in the rhizosphere. Additionally, it serves as an antioxidant and a protector against environmental stresses, including variable soil mineral nutrient bioavailability. In addition, its role in influencing root architecture and regulating nutrient uptake through

transcriptional and post-transcriptional modification of specific transporters optimizes the elemental composition to promote plant growth. Despite considerable progress related to understanding PM synthesis in plants, the effects of nutrient disorders on its biosynthesis have not been well studied and extensive research has to be done under this area of study. PM can participate in plant mineral nutrition by regulating key transporters and enzymes involved in element uptake and assimilation. It should be noted that the uptake and assimilation of mineral nutrients are regulated by complex processes including transcriptional and post-transcriptional pathways. However, most studies have emphasized the importance of transcriptional regulation and have paid little attention to changes in post-transcriptional modifications, which might also participate in melatonin-mediated nutrient uptake. Therefore, more precise and detailed studies on melatonin-mediated biological and molecular mechanisms need to be conducted, particularly with regard to protein modifications. In addition, there are strong relationships among different nutrients, with an imbalance of one element affecting the balance of others ([Rouached *et al.*, 2010](#); [Bouain *et al.*, 2019](#)). Several studies of plant ionomics have shown that melatonin can maintain nutrient homeostasis as an environmental adaptation; however, the mechanism by which melatonin integrates different nutrient signals needs to be elucidated. Crop microbiomes can strengthen the capacity and flexibility of crops to cope with abiotic stresses. The discovery of melatonin in plant growth-promoting rhizobacteria and endophytic organisms provides a promising avenue for further study. Melatonin may be an intercommunicating agent that harnesses the power of crop microbiomes, offering opportunities to boost crop productivity and improve human nutrition.

5.12 AQUAPORINS AND WATER TRANSPORT: STRUCTURE AND FUNCTION OF AQUAPORINS IN PLANTS

Aquaporins (AQPs) are transmembrane proteins that create channels in intracellular and plasma membranes, enabling the rapid bidirectional movement of water ([Maurel, 2007](#)). As part of the major intrinsic protein family, AQPs share a common structure consisting of six transmembrane helices, with their N and C termini oriented toward the cytosol. These helices are organized into compact helical domains ([Figure 5.2](#)).

Additionally, AQPs possess five connecting loops (A–E) that link the transmembrane helices. Loops B and D are positioned within the cytoplasm, while loops A, C, and E are located on the extracellular side ([Murata *et al.*, 2000](#); [Chaumont *et al.*, 2005](#)). The N terminus of each AQP protein resides on the cytoplasmic side of the membrane. Four AQP monomers assemble to form a tetrameric holoprotein, stabilized through hydrogen bonding and interactions between monomeric loops ([Murata *et al.*, 2000](#)). A distinguishing feature of AQPs is the presence of two highly hydrophobic conserved loops (B and E), which contain an internal Asparagine-Proline-Alanine (NPA) motif. This motif extends into the pore from both sides of the membrane and plays a crucial role in maintaining AQP function across all members of the family. The hydrophobic NPA motif is situated within the first intracellular loop (loop B) and the third extracellular loop (loop E), forming short helices. Additionally, loop C links loop B and loop E, a connection that is functionally essential for facilitating water permeability.

Aquaporins are integral membrane proteins that facilitate the transport of water and in some cases, small solutes across cell membranes. They play numerous critical roles in plant physiology. Here are the key functions of aquaporins in plants:

5.12.1 Functions of aquaporins in plants:

1. Water transport and homeostasis:

- **Facilitating water movement:** Aquaporins increase the permeability of cell membranes to water, enabling efficient water uptake, transport, and distribution throughout the plant.
- **Maintaining cellular hydration:** They help maintain cell turgor and hydration, which are essential for cell expansion, growth, and maintaining structural integrity.

2. Regulation of root water uptake:

- **Root water absorption:** Aquaporins are highly expressed in root cells, where they facilitate the absorption of water from the soil into the roots.
- **Adaptation to soil conditions:** They help roots adapt to varying soil moisture conditions by adjusting water uptake rates in response to changes in soil water availability.

3. Control of stomatal movement:

- **Regulating transpiration:** In guard cells, aquaporins regulate the opening and closing of stomata, controlling transpiration and gas exchange.
- **Response to environmental cues:** They enable rapid responses to environmental signals like light, humidity, and CO₂ concentration, optimizing water use efficiency.

4. Facilitating long-distance water transport:

- **Xylem and phloem transport:** Aquaporins assist in the movement of water through the xylem and phloem, facilitating long-distance transport from roots to leaves and other parts of the plant.

5. Nutrient Transport and Uptake:

- **Transporting small solutes:** Some aquaporins can transport small solutes such as urea, boron, and silicon, playing a role in nutrient uptake and distribution.
- **Enhancing nutrient efficiency:** They help in the efficient use of nutrients by regulating their movement within the plant.

6. Stress response and adaptation:

- **Drought tolerance:** Aquaporins are involved in the plant's response to drought stress by regulating water retention and minimizing water loss.
- **Salt stress adaptation:** They help maintain osmotic balance and ion homeostasis under salt stress conditions, protecting plant cells from damage.

7. Cell growth and development:

- **Cell expansion:** Aquaporins facilitate water influx necessary for cell expansion, crucial for plant growth and development.
- **Tissue development:** They are involved in the development of various tissues, including roots, leaves, and reproductive organs.

8. Response to temperature changes

- **Cold and heat stress:** Aquaporins help plants cope with temperature extremes by adjusting water transport to prevent cellular dehydration during cold stress and enhancing water uptake during heat stress.

9. Hydraulic conductivity:

- **Regulating water flow:** Aquaporins modulate the hydraulic conductivity of plant tissues, affecting the movement of water within and between cells.
- **Dynamic adjustment:** They can dynamically adjust their activity in response to changes in environmental conditions, ensuring optimal water flow.

10. Intercellular water movement:

- Symplastic pathway: Aquaporins facilitate water movement through the symplastic pathway (cell-to-cell movement via plasmodesmata), ensuring coordinated hydration of tissues.

5.13 REGULATION OF AQUAPORINS BY ENVIRONMENTAL FACTORS

Aquaporins are membrane proteins that facilitate water transport across cell membranes and are crucial for maintaining water homeostasis in plants. Their regulation by environmental factors is vital for plant adaptation and survival under various stress conditions.

5.13.1 Drought Stress

Drought stress typically reduces water availability, leading to changes in the expression and activity of aquaporins to minimize water loss and enhance water uptake. For instance, in *Arabidopsis*, drought conditions downregulate plasma membrane intrinsic proteins (PIPs) while upregulating others to optimize water transport ([Maurel *et al.*, 2015](#)). ABA, a key hormone in drought response, modulates aquaporin activity. ABA can induce the expression of specific aquaporins that enhance water uptake during water deficit ([Vandeleur *et al.*, 2009](#)).

5.13.2 Salt Stress

Ion toxicity and osmotic stress: High salt stress causes both ion toxicity and osmotic stress. Aquaporins play a role in maintaining osmotic balance by regulating water transport. Under salt stress, certain aquaporins are differentially expressed to help maintain cellular water homeostasis ([Boursiac *et al.*, 2008](#)).

ROS and redox state: Salt stress often leads to the production of ROS. Redox modifications can affect aquaporin function, with oxidative conditions generally reducing aquaporin activity to prevent excess water loss ([Horie *et al.*, 2011](#)).

5.13.3 Temperature Stress

Cold stress: Low temperatures can decrease the fluidity of membranes, affecting aquaporin activity. Some aquaporins are upregulated in response to cold stress to facilitate water movement and prevent cellular dehydration ([Aroca *et al.*, 2005](#)).

Heat stress: High temperatures can lead to increased transpiration and water loss ([Poór *et al.*, 2022](#)). Aquaporins are regulated to enhance water uptake and transport, helping plants cope with heat stress ([Martínez-Ballesta *et al.*, 2006](#)).

5.13.4 Nutrient Availability

P and N: Nutrient deficiencies can influence aquaporin expression. For example, phosphorus deficiency can alter the expression of certain aquaporins to enhance water and nutrient uptake ([Postaire *et al.*, 2010](#)). Similarly, nitrogen availability affects aquaporin activity to regulate water uptake in accordance with nutrient status ([Gao *et al.*, 2010](#)).

5.13.5 Flooding and Hypoxia

Oxygen deficiency: Flooding leads to hypoxic conditions, affecting root water transport. Aquaporins are regulated to adapt to low oxygen levels, often involving downregulation of specific aquaporins to reduce water uptake and prevent cell damage ([Tournaire-Roux *et al.*, 2003](#)).

5.14 ROLE OF PHYTOMELATONIN IN AQUAPORIN EXPRESSION AND ACTIVITY

Aquaporins are essential for efficient water and nutrient management in plants, enabling them to adapt to and survive various environmental stresses. PM plays a significant role in regulating aquaporin expression and activity, thereby influencing water transport and stress responses in plants. Its regulation of aquaporin expression and activity is crucial for improving water transport and stress resilience in plants, providing a potential tool for enhancing crop performance under adverse environmental conditions.

5.14.1 Regulation of Aquaporin Gene Expression

Upregulation under stress: PM has been found to upregulate the expression of specific aquaporin genes under various stress conditions, including drought and salinity. This upregulation helps enhance water uptake and transport to mitigate stress effects ([Antonίου *et al.*, 2017](#)). In cucumber, phyto melatonin treatment has been shown to upregulate the expression of specific aquaporin genes, such as *CsPIP1;1* and *CsPIP2;1*. This upregulation helps enhance water uptake and improves drought resistance ([Zhang *et al.*, 2014](#)).

Gene promoter activation: Studies suggest that PM can activate aquaporin gene promoters, leading to increased transcription of aquaporin genes, which enhances the plant's ability to manage water efficiently under stress.

5.14.2 Modulation of Aquaporin Activity

Post-translational modifications: Phytomelatonin can influence the post-translational modifications of aquaporins, such as phosphorylation, which alters their activity and stability in the membrane, thus optimizing water transport based on environmental needs ([Arnao and Hernandez-Ruiz, 2018](#)). For instance, in *Arabidopsis*, melatonin-induced phosphorylation of aquaporins enhances their water channel activity under drought stress ([Li et al., 2016c](#)).

Protein stability: It also contributes to the stability and trafficking of aquaporin proteins within the cell, ensuring their availability and functionality during periods of environmental stress ([Zhang et al., 2014](#)).

5.14.3 Enhancement of Stress Tolerance

Drought resistance: By regulating aquaporin expression and activity, phytomelatonin enhances drought tolerance by maintaining cellular water balance and reducing water loss through transpiration ([Shi et al., 2015](#)). In apple (*Malus domestica*) plants, melatonin treatment has been observed to improve drought tolerance by regulating aquaporin expression. This regulation facilitates better water uptake and transport, helping the plant cope with water deficit ([Li et al., 2016a](#)).

Salt stress adaptation: PM-mediated regulation of aquaporins also aids in maintaining osmotic balance under salt stress, helping plants cope with high salinity conditions ([Li et al., 2016b](#)). In soybean (*Glycine max*), melatonin enhances salt tolerance by modulating the expression and activity of aquaporins, thereby maintaining osmotic balance and reducing ion toxicity ([Wei et al., 2015](#)).

5.15 MELATONIN LEVELS IN PLANTS UNDER STRESS CONDITIONS

It is extensively reported that the PM concentration inside the cell responds dynamically to external conditions. A rapid fluctuation in light, temperature and various environmental stress factors may increase PM levels (Shoaib [khan et al., 2023](#)). The level of PM in plants is also variable under different stress conditions. Arnao and Hernandez-Ruiz ([2009](#)) investigated the melatonin level in *Hordeum vulgare* (barley) roots and *Lupinus Sps.* (lupin) ([Arnao and Hernandez-Ruiz, 2013](#)) under natural or artificially induced adverse conditions. In the former experiment, treated plants with the chemical stress agent's sodium chloride (NaCl), zinc sulphate (ZnSO₄), or hydrogen peroxide (H₂O₂). Drought, anaerobic, pH, and cold stress were included in the latter experiment. However, all chemical stresses produced similar changes in melatonin levels. Low temperature and drought cause pronounced changes in the endogenous level of melatonin following the chemical stress ZnSO₄ or NaCl. Restricting the oxygen supply to roots also caused a slight increase in root melatonin levels. Under pH stress, alkalization of the medium lowered the root melatonin content, whereas acidification resulted in a slight increase. Additionally, the responses observed in both lupin and barley were clearly dose- and time-dependent.

The authors concluded that nearly all stress factors can stimulate an upregulation of melatonin biosynthesis in the studied plants.

5.16 ROLE OF PHYTOMELATONIN IN MAINTAINING IONIC AND OSMOTIC BALANCE

Ionic and osmotic homeostasis in plants involves the intricate regulation of ion concentrations and water potential to maintain cellular function, growth, and stress resilience. Plants absorb essential ions such as potassium, sodium, calcium, and magnesium through roots and regulate their distribution using transport proteins like H^+ -ATPases and ion channels. Compartmentalization within vacuoles, facilitated by transporters like NHX, prevents toxic ion build up in the cytoplasm. Osmotic homeostasis is achieved by balancing water uptake through aquaporins and transpiration, and by synthesizing osmoprotectants such as proline and glycine betaine. These processes are crucial for maintaining cell turgor pressure, particularly under stress conditions like drought and salinity, with signaling pathways involving hormones such as ABA orchestrating the responses ([Taiz *et al.*, 2015](#); [Marschner, 2011](#); [Hasegawa *et al.*, 2000](#) and [Zhu, 2001](#)). Among various phytohormones, PM is a plant-specific form of melatonin, which plays a crucial role in maintaining ionic and osmotic balance by enhancing stress tolerance and modulating various physiological processes. It acts as a powerful antioxidant, reducing oxidative stress that often accompanies ionic and osmotic imbalances. PM regulates ion transporters and channels, thereby stabilizing ion homeostasis under salt and drought conditions. It promotes the synthesis of osmoprotectants such as proline, glycine betaine, and soluble sugars, aiding in osmotic adjustment and maintaining cell

turgor. Additionally, PM influences the expression of stress-responsive genes and interacts with phytohormones like ABA, enhancing the plant's adaptive responses to adverse conditions ([Arnao and Hernandez-Ruiz, 2018](#)) and [Zhang *et al.*, 2015](#)), highlight its multifaceted role in stress physiology, underlining its potential as a bio-stimulant to improve plant resilience to environmental stresses.

5.17 REDOX BIOLOGY IN PLANTS

Abiotic stress triggers oxidative stress, disrupting cellular redox balance under extreme and recurrent conditions. This results in elevated oxidative stress levels, potentially causing irreversible damage to plants ([Raza *et al.*, 2019](#); [Sachdev *et al.*, 2021a](#)). Reactive oxygen species (ROS), natural by-products of cellular metabolism, can become toxic when excessively accumulated. There are in-built enzymatic and non-enzymatic scavenging mechanisms for ROS detoxification in plants. The antioxidant enzyme system includes enzymes such as superoxide dismutase(SOD), guaiacol peroxidase(GPX), catalase(CAT), ascorbate peroxidase(APX), thioredoxins(TRX), peroxiredoxins(PRX), glutathione peroxidase(GPX), and glutathione reductase(GR). Non-enzymatic defensive mechanisms utilize ascorbic acid, glutathione, vitamin E, and flavonoids ([Gu *et al.*, 2022](#)). When the rate of ROS generation is higher than detoxification, its accumulation in the cell leads to oxidative stress. Exposure to abiotic stress conditions leads to the elevation of ROS production. ROS participate in signal transduction in minute quantities, help the plant adjust its metabolism for proper stress response, and support seed germination, photosynthesis, flowering, and delay senescence ([Hasanuzzaman *et al.*, 2020](#); [Nazir *et al.*, 2020](#)). However, abiotic stress conditions disturb the balance between ROS production and detoxification. Cellular ROS accumulation beyond a

threshold level triggers oxidative stress resulting in membrane lipid peroxidation and generating malondialdehyde, electrolyte leakage, and ultimately, programmed cell death ([Demidchik, 2015](#)). Oxidative DNA damage is another consequence of cellular ROS accumulation. This damage is caused by DNA base modification and strand breaks which change the coding ability and cellular processes ([Gonzalez-Hunt *et al.*, 2018](#)). Oxidative stress-induced damage and the destruction of cellular components and genetic material lead to retarded plant growth, development, reproduction, and yield loss. oxidative stress is one of the primary causes of abiotic stress-induced damage in plant growth, development, and reproduction ([Hasanuzzaman *et al.*, 2020](#); [Nadarajah, 2020](#); [Sachdev *et al.*, 2021b](#)). Sessile plants cannot avoid environmental stress; thus, their defensive mechanisms depend on adjusting their metabolism to survive adverse conditions. These protective mechanisms include the production of the chemical PM, which supports the cell in neutralizing ROS by acting as a free oxygen radical scavenger and enhancing the other existing enzymatic and non-enzymatic antioxidant activities, increasing the abiotic stress tolerance ([Pardo-Hernández *et al.*, 2020](#)).

5.18 INTERACTION BETWEEN PHYTOMELATONIN AND REDOX SIGNALING PATHWAYS

Redox effect may be closely related to enhanced antioxidant activity, photosynthetic efficiency, and greater adaptability to environmental stress. A recent study showed melatonin application can increase the crop yield by 20% under environmental stress conditions and by 18% under drought

stress alone. Furthermore, the plants treated with PM exhibit increased photosynthetic rate of 44% under drought, 42% under salt, and 48% under cold stress conditions, which ultimately contributing into yield ([Yang *et al.*, 2022](#)). PM is a scavenger for ROS produced during metabolism in bacteria. In plants, the principal function of melatonin as a ROS scavenger remains conserved with added roles in plant growth, circadian rhythm modulation, and stress responses (Zhao *et al.*, [2019b](#), [2021a](#)). Due to its structural similarity to indole acetic acid and shared precursor pathways, melatonin was hypothesized to act as a phytohormone ([Hernández-Rui, 2006](#)). This hypothesis was confirmed with the discovery of the melatonin receptor CAND2/PTMR1, which regulates stomatal closure in *Arabidopsis* ([Wei *et al.*, 2018c](#)). Recent research highlights melatonin's role beyond redox homeostasis, showing its influence on plant growth, development, phytohormone regulation, and the expression of stress-responsive genes under unfavorable conditions ([Arnao and Hernandez-Ruiz, 2021](#); [Lee *et al.*, 2022](#)).

5.19 ENDOGENOUS PHYTOMELATONIN IN ABIOTIC STRESS

Plants exhibit varying endogenous PM levels depending on environmental conditions. Studies indicate that plants increase melatonin production in response to abiotic stress, enabling metabolic adjustments that enhance survival ([Zhang *et al.*, 2015](#)). PM directly improves plant tolerance to oxidative stress by scavenging ROS and stimulating enzymatic and non-enzymatic antioxidant activities ([Khan *et al.*, 2020](#); [Pardo-Hernández *et al.*, 2020](#)). Additionally, it enhances photosynthesis ([Altaf *et al.*, 2021](#)) and

reduces membrane lipid peroxidation, leading to lower malondialdehyde accumulation and electrolyte leakage, protecting the cell from programmed cell death ([Zhao *et al.*, 2021](#)). The combined effect of all the protective metabolic alterations mediated by elevated melatonin levels reduces the abiotic stress-induced damage to the plant ([Figure 5.3](#)).

5.20 EXOGENOUS PHYTOMELATONIN APPLICATION ENHANCES ABIOTIC STRESS TOLERANCE

With the accelerating climate changes, endogenous stress tolerance mechanisms in plants alone are insufficient to protect crop productivity. As a result, researchers have focused on exogenous PM application as a strategy to enhance abiotic stress resilience. Pre-treatment with external PM has been shown to mitigate damage caused by cold, heat, heavy metals, drought, waterlogging, and salt stress. The external application methods include seed priming, irrigation supplementation, growth medium incorporation, or foliar spraying at critical developmental stages. A global meta-analysis by [Muhammad *et al.* \(2022\)](#), encompassing 32 studies, revealed that exogenous melatonin application significantly increases the activity of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX). The resulting enhancement of antioxidant defense is a key factor in improving plant physiology and increasing survival under abiotic stress ([Muhammad *et al.*, 2022](#)). The interplay between PM and redox signaling is crucial in modulating plant responses to various environmental stressors.

5.20.1 Antioxidant Properties and Redox Homeostasis

PM exhibits strong antioxidant properties, directly scavenging ROS. By reducing ROS levels, PM mitigates oxidative damage to cellular components including lipids, proteins, and nucleic acids. This action is crucial under stress conditions, where excessive ROS production can lead to cellular damage and impaired physiological functions ([Arnao and Hernandez-Ruiz, 2014](#); [Zhang *et al.*, 2015](#)).

5.20.2 Regulation of Antioxidant Enzymes

PM enhances the activity and expression of antioxidant enzymes, which play a crucial role in the detoxification of ROS, thereby maintaining cellular redox homeostasis. PM's ability to upregulate these enzymes provide a robust defense mechanism against oxidative stress. A study by [Liang *et al.* \(2018\)](#) demonstrated that exogenous application of PM in rice (*Oryza sativa*) under cadmium stress significantly increased the activity of antioxidants enzymes, leading to reduced oxidative damage and improved plant growth.

5.20.3 Modulation of Redox-Sensitive Signaling Pathway

PM modulates redox-sensitive signaling pathways, including those mediated by mitogen-activated protein kinases (MAPKs) and TFs like NAC and WRKY. These pathways are essential for the regulation of gene expression in response to oxidative stress. Its influence on these pathways helps to orchestrate a coordinated response to stress, enhancing the plant's ability to adapt and survive.

5.20.4 Interaction with Other Phytohormones

PM interacts with other phytohormones, such as abscisic acid, ethylene and SA, which are also involved in redox signaling. These interactions can modulate the hormonal balance and stress response pathways, leading to improved stress tolerance. For instance, PM has been shown to alleviate ABA-induced stomatal closure, thereby enhancing photosynthetic efficiency and water use under drought conditions. [Sun *et al.* \(2018\)](#) found that PM treatment in tomato (*Solanum lycopersicum*) plants under salt stress improved photosynthetic performance and reduced ROS accumulation by modulating ABA and SA signaling pathways.

5.20.5 Induction of Stress-Responsive Genes

PM induces the expression of stress-responsive genes involved in antioxidant defense, osmotic adjustment, and cellular repair. This induction is mediated through redox signaling pathways, highlighting PM role in enhancing the plant's intrinsic defense mechanisms against stress. PM interaction with redox signaling pathways is integral to plant stress tolerance. By modulating ROS levels, enhancing antioxidant enzyme activities, regulating redox-sensitive signaling pathways, interacting with other hormones, and inducing stress-responsive genes, PM significantly contributes to the maintenance of ionic and osmotic balance under stress conditions. These multifaceted interactions underscore the potential of PM as a vital component of plant stress physiology, offering avenues for improving crop resilience through biotechnological and agronomic approaches.

5.21 ROLE OF REDOX MODIFICATIONS IN STRESS

RESPONSE AND TOLERANCE

Redox modifications refer to the changes in the oxidation-reduction state of molecules within cells, which play a crucial role in the regulation of stress responses and tolerance in plants. These modifications are primarily mediated by ROS and RNS, which function as signaling molecules to trigger adaptive responses to various stresses.

5.21.1 Generation and Scavenging of ROS and RNS

Under stress conditions such as drought, salt, and pathogen attack, the production of ROS and RNS increases significantly. While these reactive species can cause oxidative damage to proteins, lipids, and nucleic acids, they also serve as crucial signaling molecules ([Mittler, 2017](#)).

5.21.2 Redox-Sensitive Signaling Pathways

ROS and RNS modulate various signaling pathways that regulate stress responses. These pathways involve the activation of redox-sensitive TFs such as NAC, WRKY, and MYB, which control the expression of stress-responsive genes. In Arabidopsis, the NAC TF ANAC042 is activated by ROS and regulates genes involved in oxidative stress responses ([Wu *et al.*, 2012](#)).

5.21.3 Antioxidant Defense Mechanisms

Plants possess a complex antioxidant defense system to manage ROS and RNS levels, including both enzymatic and non-enzymatic antioxidants ([Noctor and Foyer, 1998](#)). Enzymatic antioxidants, includes superoxide dismutase (SOD; converts superoxide radicals into hydrogen peroxide. Further, catalase (CAT) and peroxidases (POD) antioxidant enzymes play

critical role in breaking down H_2O_2 into water and oxygen. Non-Enzymatic Antioxidants: Ascorbate (Vitamin C), detoxifies ROS, while Glutathione plays a critical redox buffer that reduces oxidative stress.

5.21.4 Redox Modifications of Proteins

Redox modifications of proteins, such as the oxidation of cysteine residues, could alter their activity, stability, and interaction with other proteins. These modifications could activate and/or deactivate signaling pathways in response to stress. Thioredoxin and glutaredoxin systems regulate the redox state of cysteine residues in target proteins, influencing stress response pathways ([Meyer *et al.*, 2012](#)).

5.21.5 Interaction with Phytohormones

Redox modifications interact with phytohormones such as ABA, SA, and jasmonic acid, which play significant roles in stress responses. ROS could modulate the biosynthesis, signaling, and action of these hormones, integrating redox signaling with hormonal control. ABA-induced stomatal closure involves ROS production, which acts as a second messenger in the signaling pathway ([Mittler & Blumwald, 2015](#)). Redox modifications are central to the plant stress response and tolerance, acting as key regulators of signaling pathways, antioxidant defenses, protein function, and hormone interactions. By modulating these processes, plants could adapt and survive in response to various environmental stresses, enhancing their resilience and overall fitness. Understanding these redox-mediated mechanisms offers potential strategies for improving crop performance and stress tolerance through genetic and biotechnological approaches.

5.22 EMERGING TRENDS IN PHYTOMELATONIN RESEARCH

Research is increasingly focusing on the role of phytomelatonin in mitigating various abiotic stresses. Phytomelatonin enhances antioxidant defense mechanisms, reduces oxidative damage, and modulates stress-responsive gene expression, thereby improving crop resilience. [Zhang et al. \(2015\)](#) demonstrated that exogenous application of PM enhances drought tolerance in *Solanum lycopersicum* (tomato) plants by regulating stomatal closure and improving water-use efficiency. [Li et al. \(2016a\)](#) showed that PM alleviates salt stress in *Cucumis sativus* (cucumber) by enhancing the antioxidant capacity and reducing lipid peroxidation. Its role in promoting plant growth and development, including seed germination, root development, and flowering is being studied. It acts as a regulator of growth hormones and influences various developmental processes. Arnao and Hernandez-Ruiz ([2014](#)) reported that PM application enhances root growth and seedling development in several plant species. [Wang et al. \(2016b\)](#) found that PM promotes flowering in *Arabidopsis* by modulating the expression of flowering-related genes.

Researchers are exploring the interaction between PM and other phytohormones such as auxins, gibberellins, ABA, and ethylene. These interactions are critical for a coordinated response to environmental stimuli and developmental cues. [Wei et al. \(2018b\)](#) investigated the crosstalk between PM and auxin in root development, showing that the former one enhances root formation by modulating auxin signaling pathways. [Liang et al. \(2017\)](#) studied the interaction between PM and ABA in drought stress responses, revealing that PM alleviates ABA-induced stomatal closure and promotes photosynthesis. There is growing interest in the role of PM in

post-harvest physiology, particularly in extending the shelf life and improving the quality of fruits and vegetables. PM treatments are being evaluated for their potential to delay senescence, reduce decay, and maintain post-harvest quality. [Sun et al. \(2018\)](#) reported that PM application delays senescence and maintains the quality of post-harvest tomato fruits by regulating antioxidant enzyme activities and reducing ethylene production. [Liu et al. \(2019\)](#) found that PM treatment extends the shelf life of post-harvest cherries by enhancing the antioxidant system and delaying ripening. Implications of genetic engineering and biotechnology to manipulate PM biosynthesis and signaling pathways have also been promoted to improve stress tolerance and crop productivity. Transgenic approaches are being explored to enhance endogenous PM levels in plants. [Park et al. \(2013\)](#) engineered rice plants to overproduce PM by introducing the *serotonin N-acetyltransferase* gene, resulting in improved tolerance to abiotic stresses. [Zuo et al. \(2018\)](#) developed transgenic *Arabidopsis* with elevated PM levels, showing enhanced resistance to oxidative stress and pathogens. The emerging trends in PM research highlight its significant potential in enhancing plant stress tolerance, growth, development, and post-harvest quality. The integration of PM into agricultural practices and biotechnological applications offers promising avenues for improving crop resilience and productivity in the face of environmental challenges. Ongoing research continues to uncover the complex regulatory networks and multifaceted roles of PM, paving the way for innovative strategies in plant science and agriculture.

5.23 KNOWLEDGE GAPS AND UNANSWERED QUESTIONS IN

PHYTOMELATONIN PLANT RESEARCH

Despite the significant advancements in understanding the role of PM in plant physiology, there are several knowledge gaps and unanswered questions remain. One major area of uncertainty is the precise molecular mechanisms through which PM exerts its effects on stress tolerance and growth regulation. While its antioxidant properties and interaction with stress-responsive genes are well-documented, the specific signaling pathways and receptor proteins involved are not fully elucidated.

Additionally, the cross-talk between PM and other phytohormones, such as auxins, ABA, and ethylene, requires further exploration to understand the integrated hormonal responses under stress conditions. Another gap lies in the diversity of PM functions across different plant species and environmental contexts. The variability in PM biosynthesis and its physiological impacts among various crops suggests a complex, species-specific regulation that is not yet comprehensively mapped. This variability extends to the effectiveness of exogenous PM application, where the optimal concentrations, timing, and methods of delivery need to be standardized for different plants and stress scenarios. The long-term effects of PM on plant development and productivity, particularly in field conditions, remain largely unexplored. Understanding how PM impacts the overall life cycle of plants, including reproductive development and yield, is crucial for practical agricultural applications. Correspondingly, the potential environmental and ecological impacts of widespread PM use in agriculture are not well explored. As research progresses, it will be essential to assess whether the benefits of PM application outweigh any unintended consequences on plant ecosystems and soil health.

5.24 POTENTIAL STRATEGIES FOR ENHANCING PLANT RESILIENCE THROUGH PHYTOMELATONIN

Improving plant resilience with PM includes several promising approaches. One approach is the exogenous application of PM to plants, which has been shown to improve tolerance to abiotic stresses like drought, salt, and extreme temperatures by modulating antioxidant defense and stress-responsive gene(s) expression. Genetic engineering techniques offer another strategy, where genes involved in PM biosynthesis, such as serotonin N-acetyltransferase, are over-expressed in transgenic plants to elevate endogenous PM levels, thereby increasing stress tolerance and growth. Additionally, optimizing agricultural practices to include PM treatments during critical growth stages could enhance crop yield. Combining PM with other phytohormones and signaling molecules to create synergistic effects further supports plant adaptation to environmental challenges ([Park *et al.*, 2013](#); [Zhang *et al.*, 2015](#)). Altogether, these approaches highlight the potential of PM as a versatile tool for sustainable agriculture and crop enhancement.

5.25 CONCLUSION

The current book chapter delves into the multifaceted role of PM in enhancing plant resilience to environmental stress. The chapter outlines how PM modulates the uptake and transport of essential mineral nutrients, ensuring their optimal assimilation even under stress conditions. By regulating aquaporins, PM improves water transport and retention, thereby maintaining osmotic stability. The chapter also highlights the significance of redox modifications, where PM acts as an antioxidant, reducing

oxidative stress by maintaining cellular structures. These insights underscore the potential of PM as a crucial signaling molecule in plant stress responses, offering innovative strategies for improving plant performance under challenging environmental conditions. The integration of these mechanisms into agricultural practices could lead to development of climate resilient crops and enhanced sustainability in agriculture.

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6

Exploring Phytomelatonin-Induced Physiological and Biochemical Responses to Heavy Metal Stress in Plants

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DOI: [10.1201/9781003527633-6](https://doi.org/10.1201/9781003527633-6)

6.1 INTRODUCTION

Phytomelatonin, a pleiotropic and stress activated signal molecule, is essential for plant growth and environmental stress tolerance ([Arnao & Hernández-Ruiz, 2019](#); [Seth et al. 2023](#)). Our knowledge of phytomelatonin's function has advanced significantly since its discovery in plants in 1995 ([Dubbels et al. 1995](#); [Hattori et al. 1995](#)). The direct role of phytomelatonin in promoting plant growth was demonstrated by [Hernández-Ruiz et al. \(2005\)](#), who reported that phytomelatonin treatment encouraged the coleoptiles extensions of wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), oat (*Avena sativa*), and canary grass (*Phalaris canariensis*). Additionally, it has been known to act as a phytohormone-like substance (Arnao & Hernández-Ruiz, [2019](#), [2021](#); [Tyagi et al. 2023](#)), and

crucially functions as an antioxidant under stressful conditions ([Arnao & Hernández-Ruiz, 2021](#); [Sun et al. 2021](#); [Seth et al. 2023](#)). Additionally, it controls fruit ripening and flowering, acting as a plant stimulant that promotes root formation, seed germination, and biological development ([Tan & Reiter, 2020](#); [Sun et al. 2021](#)).

In recent times, heavy metal stress has been considered as one of the leading environmental stressors ([Thao et al. 2015](#); [Khan et al. 2021](#)). The primary driver behind the surge in environmental contaminants, which negatively impact both plant and human health, is the rapid expansion of global industry and urbanization. This has greatly contributed to the contamination of ecosystems, along with other anthropogenic activities including contemporary farming methods and poor farm management ([Kumari et al. 2024](#)). Pollutants from these activities, such as heavy metals and chemical residues, are released into the air, water, and soil. These pollutants worsen environmental deterioration and pose major risks to human health and food security ([Thakur et al. 2022](#); [Khan et al. 2021](#)). Even though the Earth's crust naturally contains heavy metals, human activity has greatly increased the amount of these pollutants that are released into ecosystems. This influx of heavy metals often pushes their concentrations beyond permissible levels, and in many cases, far beyond what ecosystems can safely handle ([Thakur et al. 2022](#); [Niekerk et al. 2024](#)). Among the most hazardous heavy metals are arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu) and lead (Pb) ([Thao et al. 2015](#); [Khan et al. 2021](#); [Mahajan et al. 2023](#)). Heavy metals may result in oxidative stress-induced cellular damage by generating reactive oxygen species (ROS) because of their pervasiveness in the soil environment and potential for plant absorption ([Thao et al. 2015](#); [Khan et al. 2021](#); [Tyagi et al. 2024](#)). Consequently, this impacts key plant functions such as mineral nutrition

homeostasis, osmotic balance, and primary and secondary metabolisms ([Khan et al. 2021](#); [Khan et al. 2023](#); [Faizan et al. 2024](#)). Metal exclusion, limited foliar translocation, metal sequestration and compartmentalization, chelation, and antioxidant enzyme-mediated free radical scavenging potential are some of the natural defense mechanisms that plants use to combat heavy metal stress ([Thao et al. 2015](#)). Plants use a large amount of plant resources to respond to heavy metal stress by carrying out a sequence of precisely timed reactions ([Faizan et al. 2024](#); [Tyagi et al. 2024](#)). This resource-intensive defense tactic, however, may take focus away from development and growth, which could have an impact on the general health and productivity of plants. To cope with this situation, external modulators can provide benefit by accelerating the defense-process. The supplementation of phyto melatonin has emerged as an effective strategy to help plants deal with heavy metal stress. Phyto melatonin-mediated responses interact with multifaceted phytohormones to mitigate and overcome the unfavorable consequences of the underlying stressors ([Sun et al. 2021](#)) ([Figure 6.1](#)).

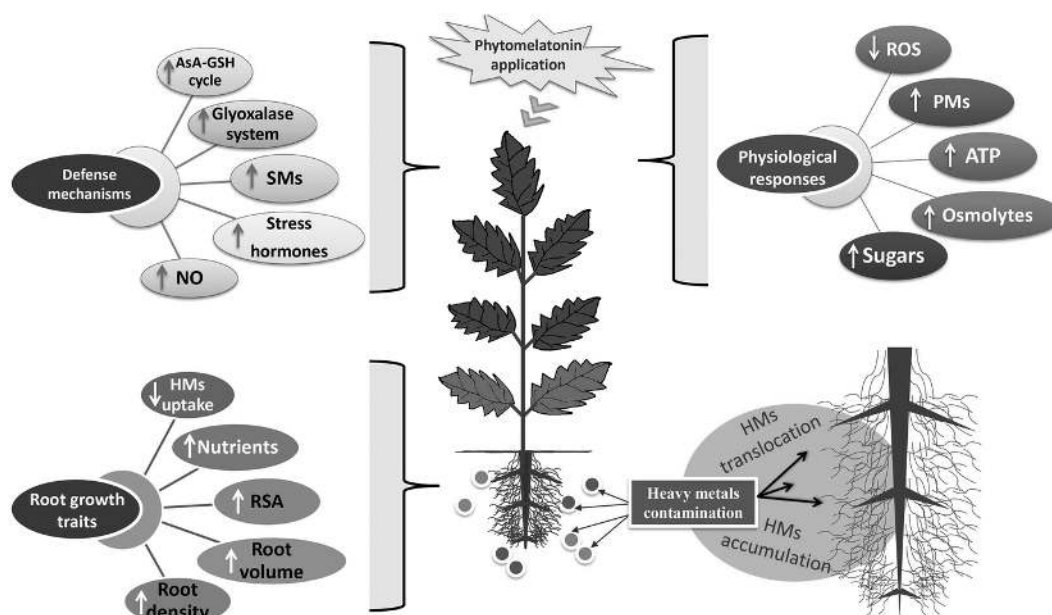


FIGURE 6.1 Potential beneficial impacts of phyto melatonin on growth and physiological defense in heavy metal-stressed plants. Phyto melatonin aid ameliorating heavy metal stress accompanying oxidative stress-induced damages through up-regulating defense mechanism to limit cellular membrane damage. Phyto melatonin ameliorates root damages imposed by heavy metal stress by way of enhanced root surface area, volume and density to facilitate the acquisition of mineral nutrients that culminate in restricting the uptake of heavy metals or potent contaminants through soil in stressed plants. Exposure to heavy metal stress deviate regular physiological responses causing enhanced generation of reactive oxygen species, which could be terminated with the activation of primary and secondary metabolites to mediate energy transduction processes through ATP generation. Improved plant defense activity amplifies ascorbate-glutathione pathway, glyoxalase system, stress hormones and nitric oxide biosynthesis, thus augmenting cytosolic tolerance. The activated vital growth and physiological processes, including osmolyte adjustment and sugar homeostasis in response of heavy metal stress. White/blue color arrow heads lines facing upwards and downwards are showing positive and negative plant responses, respectively.

Abbreviation: AsA, arscorbate; ATP, adenosine triphosphate; GSH, reduced glutathione; HMs, heavy metals; NO, nitric oxide; PMs, primary metabolites; ROS, reactive oxygen species; RSA, root surface area; SM, secondary metabolite.



The relation between phyto melatonin and heavy metal stress tolerance in plants has been taken into consideration in the latest studies (Altaf et al. [2023a](#), [2023b](#); [Li et al. 2023](#); [Xing et al. 2023](#); [Yang et al. 2023a](#)). This highlights the importance of studies showing that plants under heavy metal stress could gain significant benefits from phyto melatonin's ability to restrain heavy metals in root cell walls and impound them within root cell vacuoles (Altaf et al. [2023a](#), [2023b](#)). This decreases the heavy metal's transfer from roots to shoots tissues, and encourages plant mineral nutrients

acquisition in reaction to contaminated environments ([Li et al. 2023](#); [Xing et al. 2023](#)). Evidently, phytomelatonin has been known to stimulate photosynthesis, osmolytes biosynthesis, and activation of antioxidant enzymes, which improve cellular redox homeostasis in response to the heavy metal toxicities and enable plant to process and invest their energy on developmental responses (Altaf et al. [2023a](#), [2023b](#); [Li et al. 2023](#); [Xing et al. 2023](#); [Yang et al. 2023a](#)). Thus, it could be deduced that in soils challenged by heavy metals, crop productivity could be maintained by comprehending the phytomelatonin-mediated signaling transduction ([Ayyaz et al. 2022](#); [Yang et al. 2023a](#)). However, additional investigation is required to fully understand how phytomelatonin controls plant physiological activities in the presence of heavy metal toxicity. To guide future study, it is necessary to evaluate the sparseness of this information and evaluate it promptly in order to determine the level of achievements and shortcomings.

To enhance agricultural productivity in soils affected by heavy metal stress, this chapter aims to deepen the understanding of how phytomelatonin mediates heavy metal tolerance in plants. It also explores the interactions between phytomelatonin and various plant growth and physiological mechanisms that regulate defense adaptations, stabilize proteins, and influence downstream gene expression related to stress tolerance against heavy metal stressors. In conclusion, we emphasize the need to identify phytomelatonin receptors and the associated signaling pathways to better manage heavy metal stress in susceptible plant genotypes, cultivars, and varieties.

6.2 PHYTOMELATONIN UNDER DIFFERENT HEAVY METAL

STRESSES

6.2.1 Arsenic Stress

Arsenic (As) being the toxic metalloid enters the food chain majorly through anthropogenic activities including As-based fertilizers, insecticides and irrigation practices that causes threat to all organisms ([Khatun et al. 2022](#)). Almost more than 2.5 billion human population is dependent on ground water which gets intoxicated by As posing serious health concerns ([Shaji et al. 2021](#)). It affects plants and animals either precisely through intake of As contaminated water or subtly through food webs ([Singh et al. 2023](#)). Plants exhibit morphological and physio-biochemical disadvantages due to As toxicity by building up ROS leading to oxidative outburst ([Nazir et al. 2023](#)). Phytomelatonin being a potential plant hormone has been discovered to alleviate heavy metal stresses by chelation and boosting antioxidative defense system ([Altaf et al. 2023a](#)). Phytomelatonin (250 mM) alleviated As stress (25 μ M) in rice (*Oryza sativa*) plants by increasing the reduced glutathione (GSH), phytochelatin content and dry mass content thereby reducing hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content ([Nazarian et al. 2020](#)). Experiment analysis reported that As (75 mg kg^{-1} soil) caused increase in oxidative stress markers such as H_2O_2 and MDA levels in rosemary (*Rosmarinus officinalis*) plants. However, the exogenous treatment of phytomelatonin at a concentration of 50 μ M extensively boosted the antioxidative defense system by enriching the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). This treatment also augmented the production of secondary metabolites, including phenols, flavonoids, and ascorbic acid (AsA), while improving chloroplast structure and elevating levels of chlorophylls and essential ions. As a result, these enhancements

help maintain cell growth and membrane integrity under As stress in rosemary ([Farouk and Al-Amri, 2019](#)). Further, in tea (*Camellia sinensis*) plants when exposed to As stress (25 μ M) lead to severe oxidative stress by reducing maximum photosynthetic effectiveness of photosystem-II and boosted ROS accumulation and lipid peroxidation. But, exogenous application of phytomelatonin (100 μ M) alleviated As toxicity by enhancing anthocyanin content through selective upregulation of *chalcone synthase* (*CsCHS*) and *anthocyanidin synthase* (*CsANS*) genes ([Li et al. 2021](#)). Experiments reported by [Samanta et al. \(2022\)](#) uncovered that supplementation of phytomelatonin (20 μ M) alleviated As stress (150 μ M) by reducing endogenous As content, lowering electrolyte leakage, decreasing chlorophyll loss, reducing protein carbonylation, MDA accumulation, lipoxygenase (LOX), NADPH oxidase (NOX) and protease activity, thereby improving membrane stability index in rice plants. Furthermore, by restoring the levels of pyruvic acid, citric acid, and malic acid, improving the activities of pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase, and stimulating the accumulation of total soluble sugars, reducing and non-reducing sugars linked to higher enzymatic activities of sucrose synthase, acid invertase, and sucrose phosphate synthase, the application of phytomelatonin (20 μ M) also decreased As toxicity (150 μ M) ([Samanta et al. 2020](#)) ([Figure 6.2](#); [Table 6.1](#)).

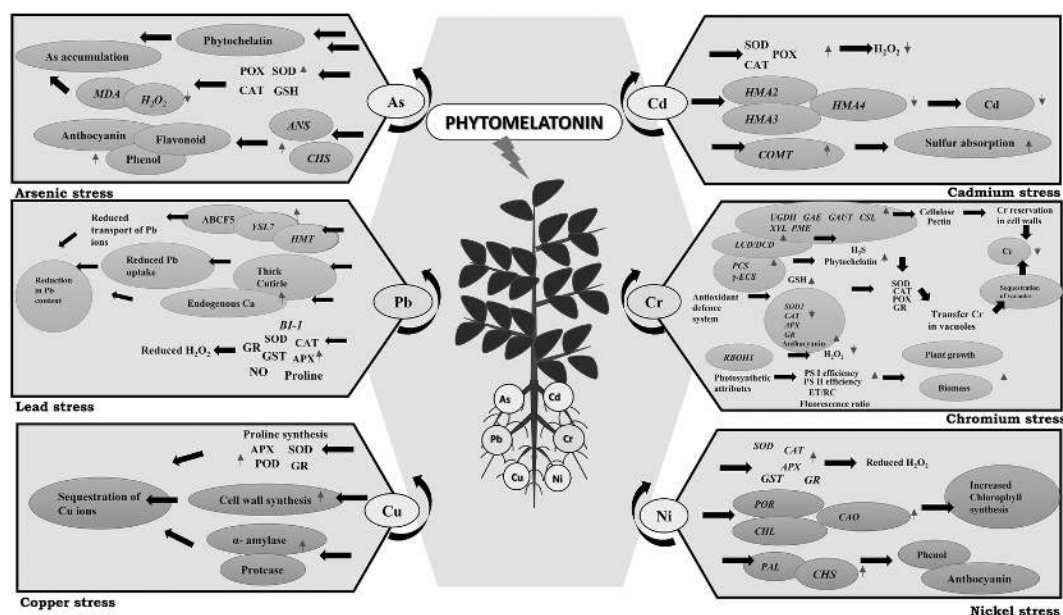


FIGURE 6.2 Schematic representation of exogenous phytomelatonin induced impacts on genetic and enzymatic expression under arsenic, cadmium, chromium, copper, nickel and lead stress.

Abbreviation: Cr, Chromium; Cd, Cadmium; As, Arsenic; SOD, superoxide dismutase; POX, peroxidase; CAT, catalase; APX, ascorbate peroxidase; GR, Glutathione reductase; GSH, reduced glutathione; H₂O₂, hydrogen peroxide; MDA, malondialdehyde; Chl, Chlorophyll; ET/RC, electron transport per reaction center; PME, pectin methylesterase; *HMA2*, *HMA3*, *HMA4*, heavy metal ATPase; *COMT*, caffeic acid O-methyltransferase; *UGDH*, UDP-glucose 6-dehydrogenase; *GAE*, 4-epimerase; *GAUT*, galacturonsyl-transferase; *CSL*, cellulose synthase-like; *XYL*, beta-xylosidase; *LCD/DCD*, L-/D-cysteine desulfhydrase; *PCS*, phytochelatins synthase; *RBOH1*, Respiratory Burst Oxidase Homolog 1; *ANS*, anthocyanidin synthase; *CHS*, chalcone synthetase; *BI-1*, Bax inhibitor-1; *HMT*, Heavy metal transport protein; *ABCF5*, *YSL7*, ABC transporter gene.



TABLE 6.1 Phytomelatonin-Mediated Responses of Different Plant Species Exposed to Different Concentrations of Arsenic, Cadmium, Chromium, Copper, Lead and Nickel Stress [↩](#)

Plant species	Phytomelatonin (conc.)	Heavy metal stress (conc.)	Response	Reference
Arsenic (As) stress				
Rice (<i>Oryza sativa</i>)	250 mM	25 μ M	Increased GSH, phytochelatin, dry mass and reduced H ₂ O ₂ , MDA contents	Nazarian and Ghanati, 2020
Rosemary (<i>Rosmarinus officinalis</i>)	50 μ M	75 mg/kg soil	Increased SOD, POD, CAT, phenols, flavonoids, AsA levels	Farouk and Al-Amri, 2019
Tea (<i>Camellia sinensis</i>)	100 μ M	25 μ M	Enhanced anthocyanin content, upregulation of <i>CsCHS</i> and <i>CsANS</i> genes	Li et al. 2021
Rice	20 μ M	150 μ M	Reduced endogenous As content, lowered electrolyte leakage, reduced MDA content, decreased NOX and protease activities	Samanta et al. 2022

Plant species	Phytomelatonin (conc.)	Heavy metal stress (conc.)	Response	Referenc
Rice	20 μ M	150 μ M	Restored levels of pyruvic acid, citric acid, malic acid, improved pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase, succinate dehydrogenase and malate dehydrogenase activities	Samanta al. 2020
Cadmium (Cd) stress				
Rice	10 μ M & 50 μ M	10 μ M & 50 μ M	Reduced ROS production, enhanced SOD, POD, CAT and MDA expression	Lv et al. 2019

Plant species	Phytomelatonin (conc.)	Heavy metal stress (conc.)	Response	Reference
Alfalfa (<i>Medicago sativa</i>)	50 μ M	100 μ M	Increased endogenous melatonin content, reduced Cd content, enhanced upregulation of <i>ABC</i> transporter and <i>PCR2</i> transcripts, increased microRNA-mediated redox homeostasis	Gu et al. 2017
Tobacco (<i>Nicotiana tabacum</i>)	100 μ M	100 μ M	Reduced Cd accumulation, enhanced APX, SOD and CAT activities and lowered <i>IRT1</i> , <i>Nramp1</i> , <i>HMA2</i> , <i>HMA4</i> , and <i>HMA3</i> gene expression	Wang et al. 2019

Plant species	Phytomelatonin (conc.)	Heavy metal stress (conc.)	Response	Referenc
Kaido crab apple (<i>Malus micromalus</i>)	100 μ M	30 μ M	Lowered root Cd uptake, reduced leaf Cd accumulation and enhanced root, stem and leaf endogenous phytomelatonin levels	He et al. 2020
Cucumber (<i>Cucumis sativus</i>)	150 μ mol/l	100 μ M	Increase Pn, enhanced Gs, Tr, improved photochemical fluorescence quenching coefficient	Wu et al. 2019
Mallow (<i>Malva parviflora</i>)	50 μ M	50 μ M	Improved RWC, Gs, Pro, enhanced PAL, SOD, CAT and guaiacol peroxidase activities	Tousi et al. 2020
Wheat (<i>Triticum aestivum</i>)	100 μ M	100 μ M	Improved growth attributes, increased leaf K ⁺ and Ca ²⁺ levels	Kaya et al. 2019

Plant species	Phytomelatonin (conc.)	Heavy metal stress (conc.)	Response	Referenc
Quickweed (<i>Galinsoga parviflora</i>)	100 µmol/l	10 mg/kg	Improved Cd remediation efficiency, increased shoot biomass, enhanced SOD, POD, CAT, APX activities	Xu et al. 2020
Chromium (Cr) stress				
Maize (<i>Zea mays</i>)	50 µM	100 µM	Upregulation of <i>LCD/DCD</i> gene for H ₂ S synthesis and reduced oxidative stress	Yang et a 2024
Wheat	100 µM	50 µM	Upregulation of genes involved in phytochelatin synthesis such as <i>TaPCS</i> and <i>Tay- ECS</i> Activation of antioxidant enzymes leading to reduction in ROS	Sun et al. 2023a

Plant species	Phytomelatonin (conc.)	Heavy metal stress (conc.)	Response	Referenc
Mustard (<i>Brassica juncea</i>)	0.15 μ M	0.2 mM	Decreased <i>RBOH1</i> gene expression, and enhanced antioxidant defence system	Kour et al 2024
Tomato (<i>Solanum lycopersicum</i>)	1 μ M	200 μ M	Enhanced anthocyanin content	Sun et al. 2023b
Maize	100 μ M	100 μ M	Enhanced chlorophyll and carotenoid content leading to improved growth	Malik et ; 2021
Canola (<i>Brassica napus</i>)	5, 10 μ M	50 μ M	Enhanced PSI and PSII efficiency and electron transfer per reaction center, improved growth and biomass, and fluorescence ratios	Ayyaz et al. 2020; Ayyaz et al. 2021

Plant species	Phytomelatonin (conc.)	Heavy metal stress (conc.)	Response	Referenc
Maize	50 μ M	100 μ M	Upregulation of genes involved in synthesis of cellulose and pectin such as <i>UGDH</i> , <i>GAE</i> , <i>GAUT</i> and <i>XYL</i> for immobilization of Cr ions in cell walls Chelation of Cr ions via enhanced phytochelatin and GSH synthesis	Yang et a 2023b
Copper (Cu) stress				
Melon (<i>Cucumis melo</i>)	10 nmol/l	80 μ M	Enhanced SOD, APX, POD, GR activities, increased chelation	Hu et al. 2020
Rapeseed (<i>Brassica napus</i>)	100 μ M	10–100 μ M	Increase in plant biomass, enhanced photosynthetic efficiency and increased proline content	Kholodov et al. 201

Plant species	Phytomelatonin (conc.)	Heavy metal stress (conc.)	Response	Referenc
Tomato	100 μ M	100 μ M	Activated CAT and APX activities, increased GSH/GSSG and AsA/DHA ratios	Zhang et al. 2022
Rice	100 μ M	20 μ mol/l	Enhanced Cu sequestration and improved reserve mobilization by promoting the α -amylase and protease activity	Li et al. 2022a
Cucumber	80 μ mol/l	80 μ mol/l	Improved Cu ²⁺ sequestration, carbon metabolism and ROS scavenging by increasing GSH and phytochelatin levels and promoting cell wall trapping	Cao et al. 2019
Tomato	100 μ mol	192 ppb Cu	Increased Chl content, CAT and SOD activities	Brengi, 2024
Lead (Pb) stress				

Plant species	Phytomelatonin (conc.)	Heavy metal stress (conc.)	Response	Reference
Red amaranth (<i>Amaranthus cruentus</i>)	400 μ M	20 mM	Thickened cuticle and epidermis	David et al. 2021
<i>Medicago truncatula</i>	10 μ M	800 mg kg ⁻¹	Increased mycorrhizal growth	Zhang et al. 2020
Safflower (<i>Carthamus tinctorius</i>)	300 μ M	50 μ M	Inhibiting Pb uptake, increasing root cuticular deposition	Namdjoy et al. 202
Bermudagrass (<i>Cynodon dactylon</i>)	100 μ M	2000 mg/kg	Enhanced SOD, APX, CAT activities and increased Proline content	Xie et al. 2018
Radish (<i>Raphanus sativus</i>)	50 μ M	200 mg L ⁻¹	Reduced Pb accumulation, Upregulated antioxidant genes, Reduced ROS levels	Tang et al. 2021
Tobacco	200 nM	15 μ M	Upregulation of antioxidant genes namely <i>RsABCF5</i> , <i>RsYSL7</i> and <i>RsHMT</i> leading to reduce Pb ion accumulation	Kobyliński and Posmyk 2016

Plant species	Phytomelatonin (conc.)	Heavy metal stress (conc.)	Response	Reference
Nickel (Ni) stress				
Tomato	100 μ M	50 Mm	Upregulation of genes for stress defense such as <i>SOD</i> , <i>CAT</i> , <i>APX</i> , <i>GR</i> , <i>GST</i> , <i>MDHAR</i> and <i>DHAR</i> to reduce ROS	Jahan et al. 2020
Tomato	100 μ M	50 μ M	Enhanced antioxidant machinery, improved root architecture	Altaf et al. 2021
Fenugreek (<i>Trigonella foenum-graecum</i>)	75 μ M	80 mg kg^{-1} of soil	Up regulation of enzymes such as SOD, POX, CAT and APX and enhanced stress defense metabolites such as phenol, flavonoid, alkaloids and tannins with ROS reduction	Parwez et al. 2023

Note: **Abbreviations:** APX, ascorbate peroxidase; As, arsenic; AsA, ascorbic acid; Ca^{2+} , calcium ions; Cd, cadmium; CAT, catalase; Gs, stomatal conductance; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2 , hydrogen peroxide; K^+ , potassium ions; MDA, malondialdehyde; NOX,

NADPH oxidase; PAL, phenylalanine ammonia lyase; Pn, net photosynthetic rate; POD, peroxidase; ROS, reactive oxygen species; RWC, relative water content; S, sulfur; SOD, superoxide dismutase; Tr, transpiration rate; Hydrogen sulphide (H₂S); *L*-/*D*-cysteine desulhydrase (*LCD/DCD*); *UDP-glucose 6-dehydrogenase (UGDH)*, *UDP-glucuronate 4-epimerase (GAE)*, *galacturonosyl-transferase (GAUT)*, *cellulose synthase-like (CSL)*, and *beta-xylosidase (XYL)*; *Respiratory Burst Oxidase Homolog 1 (RBOH1)*.

6.2.2 Cadmium Stress

Due to the high mobility and toxicity, even at low concentrations, cadmium (Cd) is one of the main heavy metal stressors. According to [Manzoor et al. \(2019\)](#), anthropogenic activities such as chemical fertilizers, pesticides, and industrial and mining sources are the main causes of Cd accumulation in the environment. Numerous morphological, physiological, biochemical, and molecular facets of plant growth are impacted by Cd toxicity ([Rizwan et al. 2017](#)). Plants in Cd contaminated soils respond to plant hormones including phyto melatonin relieving stress responses, thereby, enhancing plant growth and development. Experimental evidences reported that exogenous application of phyto melatonin (10 µM & 50 µM) reduced Cd stress (10 µM & 50 µM) induced increase in ROS production, lipid peroxidation and weakened growth and development by scavenging the ROS through enhanced activity of SOD, POD, CAT and MDA expression, thereby, increasing plant biomass in rice varieties ([Lv et al. 2019](#)). Further, pretreatment with exogenous phyto melatonin (50 µM) to Cd (100 µM) stressed alfalfa (*Medicago sativa*) plants increased endogenous melatonin content, reduced Cd content, enhanced upregulation of *ABC* transporter and *PCR2* transcripts, increased microRNA-mediated redox homeostasis, thereby, reducing Cd accumulation and decreasing ROS production ultimately leading to reduced oxidative damage ([Gu et al. 2017](#)). Reports by [Wang et al. \(2019\)](#) disclosed that foliar treatment of phyto melatonin (100

μM) enhanced Cd (100 μM) tolerance in tobacco (*Nicotiana tabacum*) leaves through reduced Cd accumulation by Cd sequestration in cell wall and vacuoles, direct scavenging of ROS by enhanced ascorbate peroxidase (APX), SOD and CAT activities and lowered *IRT1*, *Nramp1*, *heavy metal ATPase* (*HMA2*, *HMA4*, and *HMA3*) gene expression. Research evidence indicates that exogenous supplementation of phytemelatonin at 100 μM effectively mitigates the effects of Cd stress (30 μM) in Kaido crab apple (*Malus micromalus*) plants. This supplementation alleviates growth and photosynthetic activity reductions induced by Cd, decreases ROS and MDA accumulation, and lowers root Cd uptake. Additionally, it reduces Cd accumulation in leaves and decreases Cd translocation, while enhancing endogenous phytemelatonin levels in the roots, stems, and leaves. These effects are mediated through the altered expression of numerous genes, including *HA7*, *NRAMP1*, *NRAMP3*, *HMA4*, *PCR2*, *NAS1*, *MT2*, *ABCC1*, and *MHX* ([He et al. 2020](#)). Also, in cucumber (*Cucumis sativus*) plants exposed to Cd stress (100 μM) there was reduced leaf area, decreased chlorophyll content, reduced quantum yield of photochemical energy conversion in PSII and decreased photochemical fluorescence quenching coefficient. This inhibitory effect of Cd was reversed by exogenous phytemelatonin (150 $\mu\text{mol l}^{-1}$) treatment which increased net photosynthetic rate (P_n) with enhanced stomatal conductance (G_s) and transpiration rate (T_r) ([Wu et al. 2019](#)). Cadmium-stressed (50 μM) mallow (*Malva parviflora*) plants demonstrated reduced harmful effects through the supplementation of phytemelatonin at 50 μM . This treatment improved relative water content (RWC) and G_s , while increasing levels of proline and phenolic compounds. Furthermore, it enriched the enzymatic activities of phenylalanine ammonia-lyase (PAL), CAT, SOD, and guaiacol peroxidase. These improvements resulted in decreased electrolyte leakage, reduced lipid

peroxidation, and lower H₂O₂ content, ultimately promoting growth, enhancing photosynthetic pigment levels, and increasing soluble protein content ([Tousi et al. 2020](#)). Also, Cd toxicity (100 µM) in wheat (*Triticum aestivum*) plants decreased plant growth, total chlorophyll, leaf water potential, potassium (K⁺) and calcium (Ca²⁺) contents and increase in H₂O₂, MDA, Cd, electrolyte leakage and nitric oxide (NO) concentrations, alleviating effect was observed by exogenous treatment with phyto melatonin (100 µM). Phyto melatonin intensified growth attributes and leaf K⁺ and Ca²⁺ levels ([Kaya et al. 2019](#)). Experimental evidences revealed that exogenous supplementation of phyto melatonin (100 µmol l⁻¹) to Cd stressed (10 mg kg⁻¹) quickweed (*Galinsoga parviflora*) plants improve the remediation efficiency of lightly Cd-contaminated soil by increasing the shoot biomass, photosynthetic pigment content and antioxidant enzyme activities of SOD, POD and CAT ([Tang et al. 2018](#)). Radish (*Raphanus sativus*) plants when subjected to Cd toxicity of 50 µM led to amplification of ROS production and disturbed cellular redox which was alleviated by exogenous treatment with phyto melatonin (50 µM) through activation of SOD, POD, CAT, APX, GR and signaling molecules including CaMs, CDPKs and MAPKs, which subsequently activated numerous metal-binding peptides and metal transporter genes to establish redox homeostasis and eliminate Cd toxicity ([Xu et al. 2020](#)). Also, exogenous treatment with phyto melatonin (100 µmol l⁻¹) alleviated Cd stress (1 mmol l⁻¹) in strawberry (*Fragaria x ananassa*) seedlings by enhancing antioxidant enzyme activities (SOD, POD, CAT, APX), increasing biomass, chlorophyll, soluble protein, and anthocyanin content, while reducing MDA levels and oxidative damage ([Wu et al. 2021](#)). Studies discovered that adding 100 µM of phyto melatonin to tomato (*Solanum lycopersicum*) plants improved their sulfur (S) metabolism by overexpressing the gene for caffeic

acid O-methyltransferase (COMT), hence relieving 100 μ M of Cd stress. As a result, there was an increase in S absorption and assimilation, which improved plant growth and Cd tolerance. Effective S metabolism and appropriate control of Cd distribution in the plant are both necessary for phyto melatonin-mediated Cd tolerance, as evidenced by the fact that COMT overexpression improved Cd tolerance but was unable to make up for reduced S uptake when the sulfate transporter (SUT1 and SUT2) genes were co-silenced ([Hasan et al. 2019](#)). Moreover, phyto melatonin (100 μ M) decreased Cd stress (100 μ M) in tomato plants by boosting the activity of H^+ -ATPase, encouraging the synthesis of phytochelatin and GSH, improving the deposition of Cd in the cell wall and vacuoles, decreasing its toxicity in essential cellular components, and ultimately increasing plant biomass and photosynthesis by boosting the activity of antioxidant enzymes and reducing oxidative damage caused by Cd-induced ROS. Treatment with phyto melatonin (50 mM) relieved Cd stress (200 mM) in wheat seedlings by regulating H_2O_2 levels by boosting the antioxidant defense system specifically APX, SOD, GSH and GSH/GSSG (oxidized glutathione) ratio, essential for redox homeostasis. Moreover, it boosted primary root growth and increased the ability of seedling roots to worsen exogenous H_2O_2 , thereby, promoting growth and Cd resistance in wheat ([Ni et al. 2018](#)) ([Figure 6.2](#); [Table 6.1](#)).

6.2.3 Chromium Stress

The seventh most prevalent element in the Earth's crust, chromium (Cr), is useful in small quantities but extremely hazardous in large ones ([Singh et al. 2013](#)). Cr can be found in two different states: Cr (III) and Cr (VI). According to [Srivastava et al. \(2021\)](#), Cr (VI) is more harmful to plants. Plants take up Cr through phosphate and sulfate receptors ([Srivastava et al.](#)

[2021](#)). Cr is extensively used in metallurgical industries and pollutes water and soil through industrial sewage ([Hayat et al. 2012](#)). Cr deteriorates plant growth by inhibiting photosynthetic machinery, water and minerals uptake, and increased lipid peroxidation ([Singh et al. 2013](#)). Phytomelatonin guards plants against several stresses, mainly heavy metal stress ([Altaf et al. 2023a](#)). Due to its shielding effect, phytomelatonin has recently come up as an effective molecule in lessening the impacts of Cr stress in plants ([Altaf et al. 2023a](#)).

For instance, 14-days old maize (*Zea mays*) seedlings pretreated with 50 μM phytomelatonin 12 h before Cr stress (100 μM $\text{K}_2\text{Cr}_2\text{O}_7$) treatment ameliorated the detrimental effects of Cr toxicity ([Yang et al. 2024](#)). Phytomelatonin upregulated the expression of *L-/D*-cysteine desulphydrase (*LCD/DCD*) accountable for synthesis of hydrogen sulphide (H_2S). According to [Yang et al. \(2024\)](#), phytomelatonin induced synthesis of H_2S was involved in Cr stress amelioration as treatment of hydroxylamine (H_2S synthesis inhibitor) reduced the phytomelatonin induced stress amelioration. Furthermore, treatment with exogenous phytomelatonin (50 μM L^{-1}) on 3 weeks old maize plants resulted in amelioration of Cr stress (100 μM $\text{K}_2\text{Cr}_2\text{O}_7$) via chelation of Cr ions and enhanced antioxidant defense system ([Yang et al. 2023b](#)). Melatonin application reduced Cr content in leaves via reservation of Cr ions in cell wall. This is done via upregulation of genes such as *UDP-glucose 6-dehydrogenase (UGDH)*, *UDP-glucuronate 4-epimerase (GAE)*, *galacturonsyl-transferase (GAUT)*, *cellulose synthase-like (CSL)*, and *beta-xylosidase (XYL)* resulting in enhanced pectin and hemicellulose content as well as activation of pectin methylesterase (PME) managing the binding of pectin ([Yang et al. 2023b](#)). Also, phytomelatonin application enhanced GSH synthesis and phytochelatin which chelates Cr content and transfers them to vacuoles for

sequestration ([Yang et al. 2023b](#)). [Yang et al. \(2023b\)](#) further proved that exogenous treatment of phytomelatonin induced endogenous melatonin synthesis which was involved in Cr resistance as melatonin treated *serotonin N-acetyltransferase (snat)* mutants depicted reduced growth and pectin than wild Arabidopsis plants under Cr stress. Similarly, in wheat, exogenous application of phytomelatonin (100 μ M) under Cr stress (50 μ M) offered resistance to plants via up regulation of genes concerned in phytochelatin synthesis such as *TaPCS* and *Ta γ -ECS* ([Sun et al. 2023a](#)). In addition to that, phytomelatonin reduced oxidative stress via upregulation of *TaSOD2*, *TaCAT*, *TaAPX*, and *TaGR* and increased activities of SOD, CAT, APX and GR ([Sun et al. 2023b](#)). In mustard (*Brassica juncea*) seedlings, application of phytomelatonin (0.15 μ M) reduced oxidative stress and decreased expression of *Respiratory Burst Oxidase Homolog 1 (RBOH1)* gene via enhanced activities of SOD, CAT, POD, Dehydroascorbate reductase (DHAR), Glutathione S-transferase (GST) and GR under Cr (0.2 mM) stress ([Kour et al. 2024](#)). Similarly, foliar application of phytomelatonin (1 mM, 2 mM) in 1 week old wheat plants enhanced enzymatic and non-enzymatic antioxidants owing to better growth of plants and lower Cr accumulation in Cr (100 mg Cr Kg⁻¹ DM soil) stressed plants ([Seleiman et al. 2020](#)). Furthermore, Sun et al. ([2023b](#)) suggested that application of phytomelatonin (100 μ M) enhanced anthocyanin content in tomato plants increasing resistance to Cr (100 μ M) stress. In maize plants grown hydroponically and pre-treated with melatonin (1 μ M) for 2 days before administration of Cr (200 μ M K₂Cr₂O₇) stress through Hoagland solution, reinstated the growth reduction of root and shoot ([Malik et al. 2021](#)). Moreover, phytomelatonin enhanced chlorophyll and carotenoid content under Cr stress ([Malik et al. 2021](#)). [Ayyaz et al. \(2020\)](#) showed that treatment of 5, 10 μ M phytomelatonin in 3-week-old Cr

stressed (50 μM) canola (*Brassica napus*) plants improved growth via enhancing PSI and PSII efficiency. OJIP tests parameters further depicted that phytomelatonin reduced the Cr induced lowered electron transfer to reaction center and inhibited detachment of oxygen evolving complex (OEC) from reaction center. Furthermore, phytomelatonin (10 μM) protects PSII via mediating heat dissipation through NPQ (Non photochemical quenching) and enhanced fluorescence ratio (F_v/F_m , F_v/F_m) under Cr (50 μM $\text{K}_2\text{Cr}_2\text{O}_7$) stress in canola plants ([Ayyaz et al. 2021](#)). This concludes that phytomelatonin alleviates Cr stress via enhancing photosynthetic system, reducing oxidative burst and promotes growth via enhanced antioxidant system and chelation of Cr ions ([Figure 6.2](#); [Table 6.1](#)).

6.2.4 Copper Stress

Copper (Cu) being one of the essential trace element occurs in oxides, carbonates, sulfates and sulfide forms. Due to industrial and agricultural production, its concentration have increased dramatically leading to its accumulation in the soil at toxic levels. They are a major hazard to the environment and food security because of their toxic levels, which cannot be broken down by any chemical or biological process ([Rehman et al. 2019](#)). Cu poisoning can restrict and hinder root development, which lowers the intake of nutrients and water. Additionally, because of its toxicity, plants have lower levels of calcium (Ca), manganese (Mn), and nitrogen (N) ([Mir et al. 2021](#)). Furthermore, its elevated levels resulted in decreased amounts of carotenoids, chlorophyll a, and chlorophyll b ([Singh et al. 2022](#)).

In several studies, exogenous application of phytomelatonin mitigated the negative effects occurred in the plants due to Cu toxicity. For instance, melon (*Cucumis melo*) plants exposed to 80 μM of Cu led to increased ROS production, decreased plant fresh weight and degraded cell wall stability,

however, exogenous application of 10 nmol/l phyto melatonin improved the activities of SOD, APX, POD and GR activities, increased chelation of excess Cu ions through GSH modulation and enhanced redox and cell wall related gene expression ([Hu et al. 2020](#)). Also, rapeseed (*Brassica napus*) plants exposed to 10–100 μ M Cu led to increased oxidative stress but after the exogenous application of phyto melatonin at 100 μ M, there was increase in plant biomass, enhanced photosynthetic efficiency and increased proline content ([Kholodova et al. 2018](#)). Tomato plants exposed to 100 μ M Cu led to decreased plant growth, increased ROS and disturbed cellular integrity, however, foliar application of phyto melatonin at 100 μ M impeded the decrease in plant growth by activating CAT and APX activities, amplified GSH/GSSG and AsA/DHA ratios ([Zhang et al. 2022](#)). Further, seed priming with 100 μ M of phyto melatonin decreased the Cu accumulation in rice seedlings exposed to 20 μ mol Cu by enhancing Cu and upgraded reserve mobilization by promoting the α -amylase and protease activity ([Li et al. 2022a](#)). Also, application of phyto melatonin at 80 μ mol/l to cucumber (*Cucumis sativus*) seeds exposed to 80 μ mol/l Cu stress showed improved Cu^{2+} sequestration, carbon metabolism and ROS scavenging by increasing GSH and phytochelatin levels and promoting cell wall trapping ([Cao et al. 2019](#)). Also, tomato plants exposed to 192 ppb Cu led to decreased fresh weight of roots and aerial parts, reduced fresh and dry weight of tomato plant, decreased number of fruits per plant, average fruit weight and average plant productivity, however, foliar spray of 100 μ mol phyto melatonin reversed these effects, thereby increasing Chl content, CAT and SOD activities ([Brengi, 2024](#)). However, due to few studies on this particular area, more research is warranted to tackle Cu toxicity in different plants, thereby, opening new frontlines for enhanced understanding ([Figure 6.2](#); [Table 6.1](#)).

6.2.5 Lead Stress

With the expanding rise in automobile industries, the accretion of heavy metals such as lead (Pb) is rapidly increasing ([Aslam et al. 2021](#)). Since Pb is not necessary for plants, even a small buildup of lead in the soil can be harmful to them. Its buildup impairs key metabolic functions in plants, including transpiration, enzyme activity, membrane stability, and photosynthesis ([Aslam et al. 2021](#)). Pb toxicity induces severe changes in photosynthetic apparatus leading to chlorophyll degradation and disturbance of electron transport chain ([Aslam et al. 2021](#)). Lead often gets accumulated in the plants grown on Pb-contaminated soils ([Aslam et al. 2021](#)). As a result, often humans intake Pb via their diet. This predisposes humans at major risk to illness. Humans exposed to lead often suffer from memory loss and kidney damage ([Aslam et al. 2021](#)).

In some studies, phyto melatonin has confirmed to be playing a major role in eradicating the adverse impacts of Pb stress ([Xie et al. 2018](#); [Namjodiyan et al. 2020](#); [David et al. 2021](#); [Chakraborty and Sen, 2024](#)). As, in safflower (*Carthamus tinctorius*) application of phyto melatonin (300 μ M) reduced the detrimental impacts of Pb stress (50 μ M) by obstructing the transfer of Pb ions from roots to shoots and lessening the uptake of Pb ([Namdjoyan et al. 2020](#)). In addition to inhibiting the transport of Pb from root to shoot, phyto melatonin might also reduce Pb uptake by increasing the cuticular deposition on roots to avoid damage. In *Amaranthus cruentus*, thickened cuticle and epidermis were observed in phyto melatonin (400 μ M) supplemented *Amaranthus cruentus* plants under lead (20 mM) stress conditions ([David et al. 2021](#)). Further, studies suggest that in barrel clover plants (*Medicago truncatula*) phyto melatonin (10 μ M) supplementation could increase mycorrhizal growth thereby providing additional resistance

from Pb (800 mg kg⁻¹) stress by enhancing arbuscular mycorrhizal (AM) growth ([Zhang et al. 2020](#)).

In bermudagrass (*Cynodon dactylon*), phytomelatonin (100 µM) application enhanced tolerance to Pb stress (2000 mg/kg) via an upregulated antioxidant defense system ([Xie et al. 2018](#)). Enhanced enzyme activity of SOD, APX, CAT and proline content induced by phytomelatonin lead to a reduction of H₂O₂ under lead stress ([Xie et al. 2018](#)). This suggests that phytomelatonin could act as a promising molecule in phytoremediation approaches to reduce soil Pb content ([Xie et al. 2018](#)). Similarly, in isabgol (*Plantago ovata*), pretreatment with phytomelatonin resulted in alleviation of Pb stress via up regulated antioxidant enzymes such as APX, POD, CAT and SOD ([Chakraborty and Sen, 2024](#)). Lead is a competitive inhibitor of calcium (Ca²⁺) uptake and uses Ca²⁺ channels for its uptake in plants, however, phytomelatonin supplementation reduces accumulation of Pb ions by enhancing Ca²⁺ content in plants ([Chakraborty and Sen, 2024](#)).

Furthermore, in radish (*Raphanus sativus*), phytomelatonin (50 µM) supplementation induced several transporter genes involved in transporting heavy metals such as *RsABCF5*, *RsYSL7* and heavy metal transport protein (RsHMT) leading to reduced accumulation of Pb ions under Pb-stressed (200 mg L⁻¹) conditions ([Tang et al. 2021](#)). Also, phytomelatonin reduced DNA methylation under Pb stress via upregulated antioxidant genes *RsAPX2*, *RsPOD52* and *RsGST* leading to a reduction ion ROS levels ([Tang et al. 2021](#)). Moreover, in tobacco plants, [Kobylińska and Posmyk \(2016\)](#) observed that phytomelatonin supplementation (200 nM) induced the expression of Bax inhibitor-1 (BI-1) protein under lead stress (15 µM.) conditions. BI-1 is a protein which inhibits the stress induced cell death. This opens up a new dimension of phytomelatonin induced stress tolerance

via an indirect approach. Phytomelatonin and NO both play a significant function in alleviating heavy metal stresses. In plants, application of NO inhibitor (cPTIO) in phytomelatonin supplemented Ni stressed plants resulted in reversal of tolerance ([Okant and Kaya, 2019](#)). This suggests that NO might play a major part in phytomelatonin stimulated stress tolerance mechanism ([Okant and Kaya, 2019](#)) ([Figure 6.2](#); [Table 6.1](#)).

6.2.6 Nickel Stress

Nickel (Ni) is beneficial for plant growth at lower concentration, however, due to increasing anthropogenic activities, the cases of nickel toxicity has become a serious concern ([Hassan et al. 2019](#)). At higher concentrations, Ni poses detrimental impacts on plant growth via inhibiting physiological processes leading to cell death ([Hassan et al. 2019](#)). However, phytomelatonin has recently posed as a promising signaling molecule to alleviate various heavy metal stresses ([Ali et al. 2022](#)).

According to several research, phytomelatonin may help lessen the negative effects of Ni toxicity ([Jhana et al. 2020](#); [Altaf et al. 2021](#); [Saqib et al. 2024](#)). For instance, in Ni-exposed (50 μM $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$) hydroponically grown tomato seedling, foliar supplementation of 100 μM phytomelatonin depicted alleviation in Ni stress via reduction in oxidative stress markers (H_2O_2 , O_2^-) accumulation ([Jahan et al. 2020](#)). Phytomelatonin lowered oxidative stress via up regulation of genes responsible for stress defense such as *SOD*, *CAT*, *APX*, *GR*, *GST*, *MDHAR* and *DHAR* ([Jahan et al. 2020](#)). In addition, phytomelatonin supplementation enhanced photosynthesis system via up regulating the chlorophyll synthesis genes such as *POR*, *CAO* and *CHL* ([Jahan et al. 2020](#)). Also, significant increase in flavonoid, phenol and anthocyanin content was reported in phytomelatonin supplemented plants via up regulated *PAL* and *CHS* expression ([Jahan et al. 2020](#)).

Similarly [Altaf et al. \(2021\)](#) reported that phytomelatonin (100 μM) supplementation inhibited Ni-induced (50 μM) damage in tomato plants via enhancing antioxidant enzymes, nutrient uptake, protecting photosynthesis machinery and better root architecture.

Furthermore, in Ni-exposed (80 mg kg^{-1} of soil) fenugreek (*Trigonella foenum-graecum*) plants, phytomelatonin supplementation (75 μM) through foliage enhanced stress tolerance through enhanced antioxidant machinery and stress-defense metabolites ([Parwez et al. 2023](#)). Up regulated enzymes namely SOD, POX, CAT and APX was seen concomitant with a decrease in H_2O_2 content. Other stress defense metabolites such as phenol, flavonoid, alkaloids and tannins were amplified in reaction to phytomelatonin supplementation ([Parwez et al. 2023](#)). In addition to improvement in Ni stress tolerance, phytomelatonin supplementation enhanced trigonelline content in Ni stressed plants ([Parwez et al. 2023](#)).

Accumulation of heavy metals more than permissible for human diet has become a major concern. However, phytomelatonin supplementation has proven as a major signaling molecule in reducing accumulation of Ni in pepper (*Piper nigrum*) crops ([Saqib et al. 2024](#)). In pepper, phytomelatonin supplementation restricted the accumulation of Ni ions via preventing the translocation of Ni from roots to shoots ([Saqib et al. 2024](#)). In addition, significant increase in antioxidant enzymes such as SOD, CAT, APX, POD, GR and GST was observed in phytomelatonin supplemented pepper plants which reduced the lipid peroxidation ([Saqib et al. 2024](#)). Similarly, exogenous phytomelatonin (30 μM) induced inhibition of Ni (200 mg kg^{-1}) accumulation was observed in bell pepper (*Capsicum annum*) plants and resulted in enhanced growth ([Alomran et al. 2024](#)). In another study, where delivery of phytomelatonin in rice was done via porous silicon (pSi), the Ni stressed plants alleviated the negative impacts and restored the plants

growth ([Li et al. 2022b](#)). This must be due to better delivery of phytomelatonin via pSi-MT in plants cells, and stimulation of the antioxidant machinery ([Figure 6.2](#); [Table 6.1](#)).

6.3 CONCLUSION AND FUTURE PROSPECTS

The relative amounts of phytomelatonin in plants have sparked a strong interest among plant scientists in examining the regulatory role of this hormone in plant growth and development. Present evidences strongly elucidated the role of phytomelatonin being the crucial molecule in imparting resilience to heavy metal stresses such as As, Cd and Cr. Phytomelatonin decreases the heavy metal damage by re-establishing the redox homeostasis and reducing heavy metal accretion, but its underlying mechanisms continue to be established. By scavenging ROS, chelating heavy metal, transporting heavy metal, reducing photosynthetic inhibition, and controlling transcription factor processes, phytonelatonin reduces the effects of heavy metal stresses. Even with the discoveries about the molecular and metabolic mechanisms by which phytonelatonin controls plant growth and development, there are yet a plethora of unsolved problems in this field. These problems include the identification of new receptors, transcription factors, and metabolites formed during its metabolism, which have specific roles in controlling the different upstream and downstream signaling cascades in plants under heavy metal stressful conditions. Supporting this knowledge with molecular and mutant studies would undoubtedly open new perspectives on the ability of phytonelatonin to design plant growth in unpredictable conditions, particularly for sustainable agriculture.

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Role of Phytomelatonin in Uptake, Transport and Assimilation of Mineral Nutrients in Plants

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DOI: [10.1201/9781003527633-7](https://doi.org/10.1201/9781003527633-7)

7.1 INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is a pleiotropic signaling molecule plays an important role in plant growth and development such as stress tolerance, seed germination, root development, photosynthesis and antioxidant defense role, stomatal regulation, flowering, leaf senescence retardation, and fruit quality and its quality ([Mumithrakamatchi et al., 2024](#); [Zhang et al., 2024](#)). Role of melatonin in mitigating heat stress has been reported in rice by modulating net photosynthetic rate, chlorophyll content and antioxidant defense response ([Barman et al., 2019](#)). Recently, homolog of Arabidopsis melatonin receptor (AtCAND2/PMTR1) has been reported in rice (OsPMTR) through comparative in-silico analysis ([Barman et al.,](#)

[2023](#)), indicated its significant role in plant growth and development. Current research has also highlighted that melatonin plays significant role in nutrient uptake, transport, and assimilation in plants ([Qiao *et al.*, 2019](#)). Thus, the understanding the role of melatonin in nutrient uptake, transport and its assimilation will give us an excellent opportunity to take advantage of such a useful molecule for strengthening plant tolerance and adaptability to nutrient deficiency and toxicity stress. In addition, numerous genes involved in the regulation of pathways for nutrient uptake and its assimilation can be altered for further improvement by gene editing tools like CRISPR-Cas9 technology.

Melatonin controls numerous basic metabolism pathways in plants, primarily those involving lipids, carbohydrates (starch and sucrose), and nitrogen compounds. It also affects secondary metabolism pathways, which include phenolics, flavonoids, and terpenoids (Arnao *et al.*, [2021](#), [2022a](#), [b](#)). Melatonin acts as master regulator of plant development and stress response ([Sun *et al.*, 2021](#)). It has been documented that in some crops, it regulates tolerance mechanisms against biotic and abiotic challenges (Shi *et al.*, [2015a](#), [2015b](#); [Zhao *et al.*, 2017](#); [Naghizadeh *et al.*, 2019](#)). Role of melatonin in delaying senescence in grape seedling has been reported ([Yang *et al.*, 2024](#)), indicated its multifaceted functions. As a pleiotropic molecule, phytemelatonin is involved in uptake and transport of mineral nutrient by affecting root system architecture (RSA) and bringing redox-modification of nutrient transport proteins ([Zhang *et al.*, 2017b](#); [Qiao *et al.*, 2019](#); [Bawa *et al.*, 2020](#); [Chen *et al.*, 2021](#)). In this book chapter, role of phytemelatonin in maintaining nutrient homeostasis has been discussed.

7.2 MINERAL NUTRIENTS AND THEIR FUNCTIONS-PHYSIOLOGICAL

ASPECT

The essential mineral nutrients have been grouped into macronutrients and micronutrients based on their concentration in plant dry matter. The macronutrients are consisting of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S). A condition of deficiency will result when a nutrient is not available in adequate levels to maintain its functional responsibilities ([van Maarschalkerweerd and Husted, 2015](#)). Physiological function and deficiency of important mineral elements has been discussed below briefly.

Nitrogen is the most vital mineral element that plants require in more amounts. Under nitrogen deficiency, chlorosis (yellowing of leaves) of older leaves occur due to its mobile nature in plants. When nitrogen deficiency develops slowly, plants may develop slender and woody stem. Phosphorus (P) is an important component of nucleotides used in plant energy metabolisms (ATP) and in DNA and RNA as well. Under P deficiency, dark green coloration with necrotic spots appears in leaves. Potassium (K) plays an important role in regulation of osmotic potentials in plant cell. The first observable symptom of K deficiency is mottled or marginal chlorosis, which then develop at the margins and between the veins. Calcium (Ca) is an important constituent of middle lamella of plant cell wall. It is also playing a role of secondary messenger, where it binds to calmodulin proteins to initiate different signaling process in plants. Calcium deficiency include necrosis (death) of young meristematic regions such as tips of the roots and young leaves. In the plant cell, magnesium (Mg^{2+}) plays an important role in activation of enzymes involved in respiration, photosynthesis, and in the synthesis of DNA and RNA. A characteristic symptom of Mg^{2+} deficiency includes chlorosis between the leaf veins in

older leaves due to its higher mobility. However, unlike N, sulfur (S) deficiency causes chlorosis in younger leaves in preference to older leaves.

Iron (Fe) is important component of enzymes involved in the transfer of electrons such as cryptochromes with oxidation of ferrous (II) form to ferric (III) form. Like Fe, copper (Cu) is implicated in the transfer of electrons through plastocyanin during light reaction of photosynthesis ([Yruela, 2009](#)). Unlike magnesium, Fe deficiency causes interveinal chlorosis in younger leaves. Manganese (Mn^{2+}) activates several enzymes of citric acid cycle such as decarboxylase and dehydrogenase. It is also an important component of oxygen evolving complex (OEC) of PSII complex. In addition, Mn^{2+} causes interveinal chlorosis in younger or older leaves depending upon plant species and growth rate. Zinc (Zn^{2+}) is required for chlorophyll biosynthesis in plants ([Broadley et al., 2007](#)). Zinc is also needed for tryptophan biosynthesis in plants. Zinc deficiency is characterized by reduction in internodal growth, causing rosette growth habit. Boron plays a vital role in cell elongation, hormone responses, nucleic acid synthesis, cell cycle regulation and membrane function ([Brown et al., 2002](#); [Reguera et al., 2009](#)). Deficiency of B causes black necrosis of young leaves and terminal buds. Molybdenum (Mo) ions are important components of several enzymes such as nitrate reductase and nitrogenase involved in nitrogen assimilation ([Schwarz and Mendel, 2006](#)). As Mo is involved in nitrate assimilation and nitrogen fixation, its deficiency may cause nitrogen deficiency if plant depends on nitrate and symbiotic nitrogen fixation only.

7.3 PHYTOMELATONIN AND MINERAL NUTRIENTS

7.3.1 Role of Phytomelatonin in Nitrogen Metabolism

The predominant absorbed form of nitrogen in plant is nitrate (NO_3^-) and ammonium (NH_4^+), though root also absorb amino acids from symbiotic nitrogen fixation ([Tegeder *et al.*, 2007](#), [Tegeder and Masclaux-Daubresse, 2018](#); [Bloom, 2015](#); [Taria *et al.*, 2022](#)). Inside the cell, NO_3^- is converted into NH_4^+ through sequential action of nitrate reductase (NR) and nitrite reductase (NiR) ([Hachiya and Sakakibara, 2017](#); [Ye *et al.*, 2022](#)). Finally, NH_4^+ is assimilate into glutamine and glutamate through GS/GOGAT cycle ([Lam *et al.*, 1996](#); [Xu *et al.*, 2012](#); [Krapp, 2015](#)). Plant has developed different mechanism for absorption of nitrogen such as low affinity transporter (NRT1) which operates at high concentration of NO_3^- ($> 1 \text{ mM}$) and high affinity transporter (NRT2) which operates at low concentration of NO_3^- at micromolar range ([Sun *et al.*, 2022](#)). In this context, Phytomelatonin appears to be involved in N metabolism by regulating key transporter and enzymes involved in N uptake and assimilation ([Figure 7.1](#)).

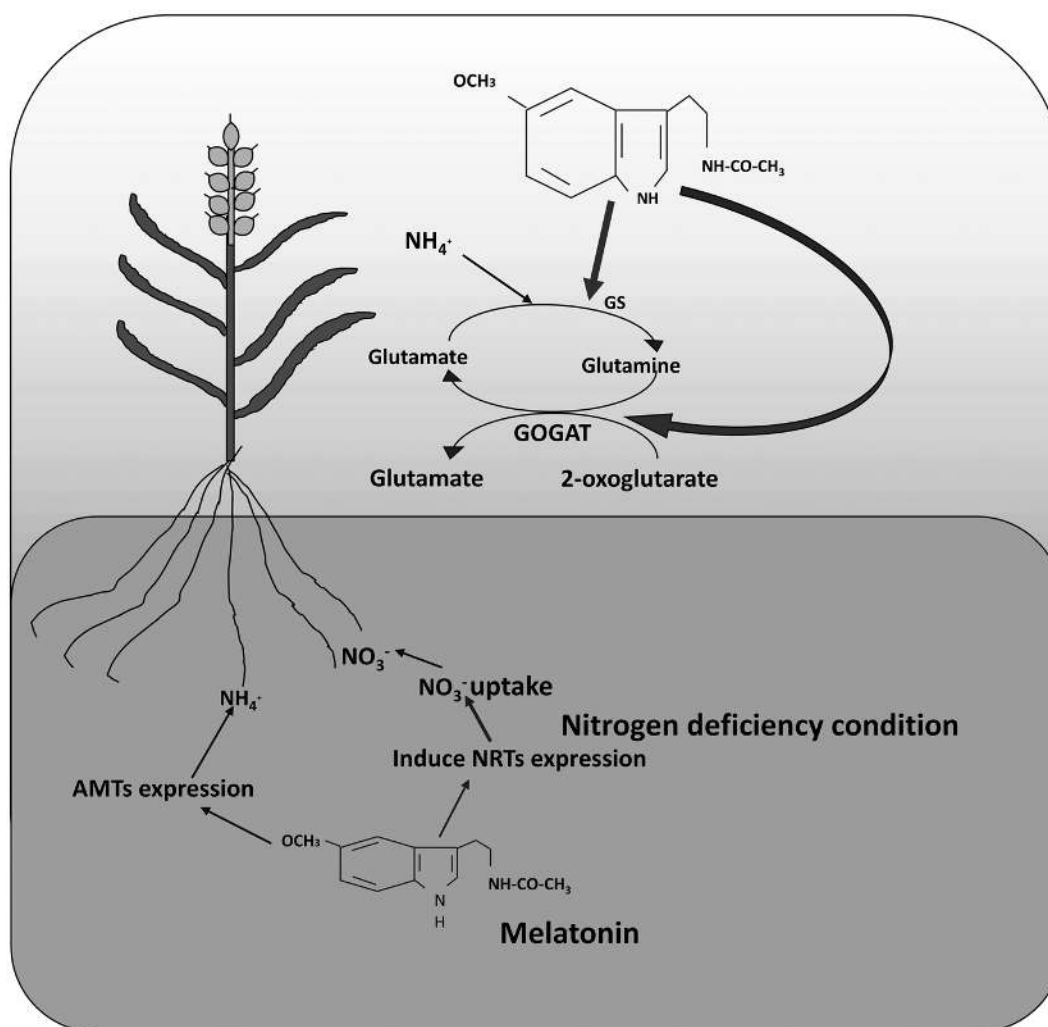


FIGURE 7.1 Phytemelatonin and nitrogen (N) metabolism under N starvation condition. [📄](#)

It was observed that application of melatonin maintained higher level of NO_3^- and N content under N starvation conditions in winter wheat seedlings ([Qiao et al., 2019](#)). In addition, melatonin application activates OsbZIP79-OsABI5 module to alleviate nitrogen limiting stress in rice by regulating reactive oxygen species (ROS) homeostasis ([Jiang et al., 2023](#)). In addition, total N and NO_3^- level were found to be increased by application of melatonin in salt stressed wheat plants ([Talaat, 2021](#)). In contrast, melatonin hastened the cucumber growth by decreasing the N absorption at high NO_3^- concentration ([Zhang et al., 2017a](#)), indicating the

shifting from high affinity to low affinity transport system. This indicates that melatonin application change/modulate the N uptake strategy in plants depending upon the availability of N. Moreover, under excess nitrogen condition, melatonin in association with nitric oxide (NO) overturned the surplus nitrogen inhibition of root growth, and upregulate several genes involved in IAA and ABA metabolism, as well as melatonin biosynthesis genes ([Zhang et al., 2022](#)). Exogenous melatonin dramatically boosted the expression of NITRATE TRANSPORTER (NRT) genes in the roots and leaves of nitrate-deficient plants in *Malus hupehensis*, which lowered the inhibitory effect of low nitrate levels ([Du et al., 2022](#)).

Foliar application of melatonin induced the drought tolerance in pepper (*Capsicum annum*) by activating various enzymes such as nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), glutamate synthase (GOGAT) and glutamine dehydrogenase (GDH) ([Kaya and Shabala, 2023](#)). In addition, role of melatonin in alleviating salt stress-induced decline in growth and photosynthesis by improving mineral uptake in soybean was reported ([Alharbi et al., 2021](#)). Exogeneous application of melatonin decreased the ammonium N content by activating glutamine synthetase (GS) and glutamate synthase (GOGAT) in sub-low temperature melon seedling ([Gao et al., 2016](#)). Exogenous application of melatonin also enhanced the nitrogen fixation in soybean and thereby reducing the negative effect of nitrogen deficiency in soybean yield ([Wang et al., 2022](#)). This enhanced nitrogen fixation was also due to the improved activity of GS/GOGAT enzymes along with glucose dehydrogenase activity ([Wang et al., 2022](#)). Furthermore, [Antoniou et al. \(2016\)](#) observed that application of melatonin in drought stress could maintain the nitro-oxidative homeostasis at enzymatic as well as at transcript levels in *Medicago sativa*.

7.3.2 Role of Phytomelatonin in Phosphorus Deficiency Acclimation

Phosphorous (P) is a well-recognized indispensable mineral nutrient for crop production, however lower availability of P limits crop production ([Gutiérrez-Alanís et al., 2018](#)). Only inorganic P (Pi) can be absorbed by plants, and it is often found in the soil at very low concentrations (<10 µM) with very less mobility. Plant has developed numerous mechanisms to cope with P deficiency ([Vance et al., 2003](#); [Péret et al., 2011](#); [Xu et al., 2020a](#); [Dissanayaka et al., 2021](#); [Paz-Ares et al., 2022](#)). Phosphate uptake is mediated by various transporter like AtPT1 (AtPHT1;1) and AtPT2 (AtPHT1;4) ([Karthikeyan et al., 2002](#)). Studies showed that AtPHT1;1 is involved in the uptake of P under high phosphorous condition, while AtPHT1;4 is involved in the uptake of Pi in P starved roots ([Wang et al., 2021](#)).

According to recent findings, phytomelatonin may alter the physiological and molecular responses of plants to P deprivation. It has been found that overexpression of caffeic acid *O*-methyltransferase (COMT), an enzyme of melatonin biosynthesis in tomato showed augmented phytomelatonin concentration along with increased P concentration and, which also exhibited tolerance to low P_i level ([Sun et al., 2020a](#)). [Zhang et al. \(2020\)](#) also found that application of melatonin enhanced the expression of (*MtPT4*), a low affinity P_i transporter in arbuscule-colonized cell to increase the P_i uptake in *Medicago truncatula*. It has been also found that exogenous melatonin induces the low phosphorous tolerance in barley roots by increasing the root length ([Ma et al., 2023](#)). Generally, little is known about the transcriptional regulation of transporters related to Pi nutrition that are influenced by melatonin in plants. However enhanced tolerance to Pi deficiency along with increased Pi content after melatonin application

indicated further exploration in the direction of molecular mechanism involved in Pi uptake with application of phytomelatonin ([Sun et al., 2022](#)).

7.3.3 Role of Phytomelatonin in Potassium Transport

Among various essential nutrients, potassium (K^+), is one of the imperative nutrients required by the plants for its proper growth and development ([Chen et al., 2017](#); [Liu et al., 2019](#)). Potassium (K) is a vital and abundant macronutrient that constitutes ~2–10% of plant dry matter ([Leigh and Wyn Jones, 1984](#)). Concentrations of K^+ in the cytosol are generally maintained at approximately 100 mmol/L. This relatively high, stable level induces several physiological processes in plants such as enzyme activation, protein biosynthesis, and the maintenance of membrane potential.

Potassium content in soil ranges between 0.1 and 1 mmol/L and its uptake in plants mainly dependent on two types of K transporters; high-affinity transporters (KT/HAK/KUPs) and low-affinity transporters (LATs). High-affinity transporters (HATs) work at low external K concentrations (<0.2 mmol/L) whereas low-affinity transporters (LATs) work at high K concentrations (>0.5 mmol/L). Most of the circumstances, HAKs play major roles in soil with low K supply conditions. HAKs are universally present in the plant kingdom, whereas their numbers are plant species-specific. Such as *Arabidopsis* and rice have 13 and 27 HAKs, respectively. Melatonin influences the K^+ uptake in plant, while regulating the process of root development ([Li et al., 2016a](#); [Sezer et al., 2021](#)). Study found that melatonin application induced potassium deficiency tolerance by regulating HAK1 transporter including upstream transcription factor NAC71 in wheat ([Li et al., 2021](#)).

Generally, the cytoplasmic concentration of K^+ is around 100 mM, whereas the levels of K^+ in soils are 1 to 4 times lower as compared to cytoplasmic concentration ([Scherzer *et al.*, 2015](#); [Wang *et al.*, 2021](#)). Plants have acquired different pathways to absorb and maintain the homeostasis of K^+ within cells; these involve a diverse array of channels and transporters in order to assured the passage of K^+ through the plasma membrane ([Ashley *et al.*, 2006](#); [Rodríguez-Navarro and Rubio, 2006](#)). Melatonin application was found to maintain K^+ homeostasis within the plants by activating the up-regulation of K^+ transporter genes such as AKT1 and HKT *via* activation of the CBL1–CIPK23 pathway. [Chen *et al.* \(2017\)](#) also reported that high levels of K^+ in melatonin-treated bermudagrass resulted from augmented expression levels of K^+ transporter genes, including CdHAK9, CdKT1, and CdKT23.

Earlier studies evidenced that melatonin treated plants show better tolerance and adaptability to salt and nutrition deficiency stresses. The exogenous application of melatonin regulates ROS signaling, followed by activation of the CBL1-CIPK23 pathway to regulate the expression of a potassium channel protein gene, which then promotes K^+ absorption. The increased K^+ and Ca^{2+} level in salt-stressed MT treated plants improve the salt tolerance by reducing Na^+ uptake and accumulation in leaves ([Altaf *et al.*, 2021](#)). Melatonin enhances salinity tolerance by inducing the expression of ion-channel genes in leaves, contributing to the maintenance of ion homeostasis ([Ren *et al.*, 2019](#)).

Another type of melatonin-mediated K^+ homeostasis has been identified in stomata, where it regulates stomatal movement by optimizing K^+ concentrations. In Arabidopsis, [Wei *et al.* \(2018\)](#) found that melatonin induced K^+ efflux across the guard-cell plasma membrane but this effect was eradicated in seedlings of the *cand2-1* receptor mutant. However, the

signaling network intricate during this process remains to be clarified, and it needs to be determined whether the effect of or not melatonin on stomatal movement is accompanied by changes in ROS and nitric oxide, which are two important components of stomatal movement ([Liu et al., 2021](#)). Moreover, using K⁺-channel blocker and H⁺-ATPase inhibitor, it was observed that application of melatonin improves the K homeostasis by regulating H⁺-ATPase activity under chromium toxicity ([Khan et al., 2023c](#)), indicating melatonin regulate the K⁺ homeostasis by interacting with other nutrients.

7.3.4 Phytomelatonin Function Synergistically with Calcium (Ca)

Melatonin along with Ca is involved in increasing tolerance to metalloid toxicity, and it was demonstrated that melatonin in combination with Ca induced the tolerance to arsenic toxicity (As) in *Vicia faba* ([Siddiqui et al., 2020](#)). Moreover, melatonin in combination with Ca is involved with abiotic stress tolerance in plants ([Khan et al., 2023b](#)). Similarly, when exposed to cold stress, MT treatment significantly increases the uptake of calcium (Ca), indicating that MT protected membranes from the cold by promoting Ca uptake. Additionally, enhanced Ca absorption after MT treatment protect the membranes from cold stress and lowers MDA formation and electrolyte leakage ([Turk and Erdal, 2015](#)). Melatonin improves resistance to low-calcium stress by modulating auxin and brassinosteroids signals in wax gourd ([Chang et al., 2024](#)). Recently, it was observed that exogenous application of melatonin along with calcium chloride (CaCl₂) mitigate negative effect of salinity stress in alfalfa by improving cytosolic Ca²⁺ level, antioxidant mechanisms, K⁺/Na⁺ ratio and endogenous MT level ([Guo et al., 2025](#)).

7.3.5 Phytomelatonin and Sulfur (S) Deficiency Acclimation

It is well established that sulfur (S) is required for normal metabolic processes in plants. Since methionine is an essential amino acid, sulfur gains in importance in human health as well ([Kopriva et al., 2019](#)). In plants, sulfur uptake through root is mediated by two sulfur transporter genes, SUT1;1 and SUT1;2 ([Barberon et al., 2008](#)). Inside the cell, SO_4^{2-} is metabolized to cysteine through sequential action of ATP sulfurylase (ATPS), adenosine-5-phosphosulfate reductase (APSR), sulfite reductase (SiR) and O-acetyl serine(thio) lyase (OAS-TL) ([Saito, 2004](#); [Kopriva et al., 2019](#)).

Melatonin alleviates the sulfur deficiency by upregulating the genes involved in sulfur transport and metabolism ([Arnao et al., 2022a](#)). Melatonin treatment significantly elevated sulfur assimilation genes in *Brassica rapa* ([Teng et al., 2022](#)). Similarly, melatonin upregulated SUT1;1 and SUT1;2 with upregulated ATPS, ATPR and OAS-TL in tomato ([Hasan et al., 2018](#)), suggesting the possible involvement of phytomelatonin in sulfur assimilation. Furthermore, under cadmium (Cd) stress in tomato, it was observed that overexpression of COMT enhanced the expression level of SUT1;1 and SUT1;2, and transcript levels of APSR, NiR and OAS-TL, thereby promoting sulfur metabolism to inhibit Cd translocation ([Hasan et al., 2019](#)). This again raise the possibility of mechanistic regulation of phytomelatonin in mitigating heavy metal toxicity. Furthermore, effect of melatonin in inducing S assimilation in the presence of excess sulfur by enhancing ATP sulfurylase and serine acetyl transferase activity in mustard was also reported ([Iqbal et al., 2023](#)). Foliar application of melatonin enhanced the sulfur metabolism in plant by enhancing its translocation

along with higher chlorophyll content and photosynthetic rate in soybean ([Marques et al., 2024](#)).

7.3.6 Phytomelatonin and Iron (Fe) Homeostasis

Iron (Fe) is an important mineral nutrient, being a vital component of chloroplast and mitochondrial electron transport chain (ETC) and co-factors of numerous enzymes ([Sun et al., 2022](#)). Fe is a rich element in soils, but because of its limited solubility, especially in calcareous soils, its availability is often insufficient for plants ([Walker and Connolly, 2008](#); [Ma and Ling, 2009](#); [Briat et al., 2015](#); [Riaz and Guerinot, 2021](#)). Two distinct mechanisms have been established by higher plants to obtain iron from the rhizosphere zone ([Hell and Stephan, 2003](#); [Morrissey and Guerinot, 2009](#); [Kobayashi and Nishizawa, 2012](#); [Riaz and Guerinot, 2021](#)). Plants use two different strategies for uptake of Fe such as (i) strategy-I and (ii) strategy-II ([Figure 7.2](#)). Non-graminaceous plants uses Fe reduction strategy (strategy-I), where ferric iron (Fe^{3+}) is reduced to ferrous (Fe^{2+}) form by membrane bound ferric-chelate reductase FRO2 at root surface and resulting Fe^{2+} ion is absorbed by high affinity Fe transporter, IRT1. Graminaceous plants uses strategy-II, where secretion of Fe^{3+} -chelating substance, called phytosiderophore are secreted in to the soil and chelated iron is subsequently uptake by yellow stripe-like 1 (YSL1) transporters ([Kobayashi and Nishizawa, 2012](#); [Murata et al., 2015](#); [Buet et al., 2019](#)).

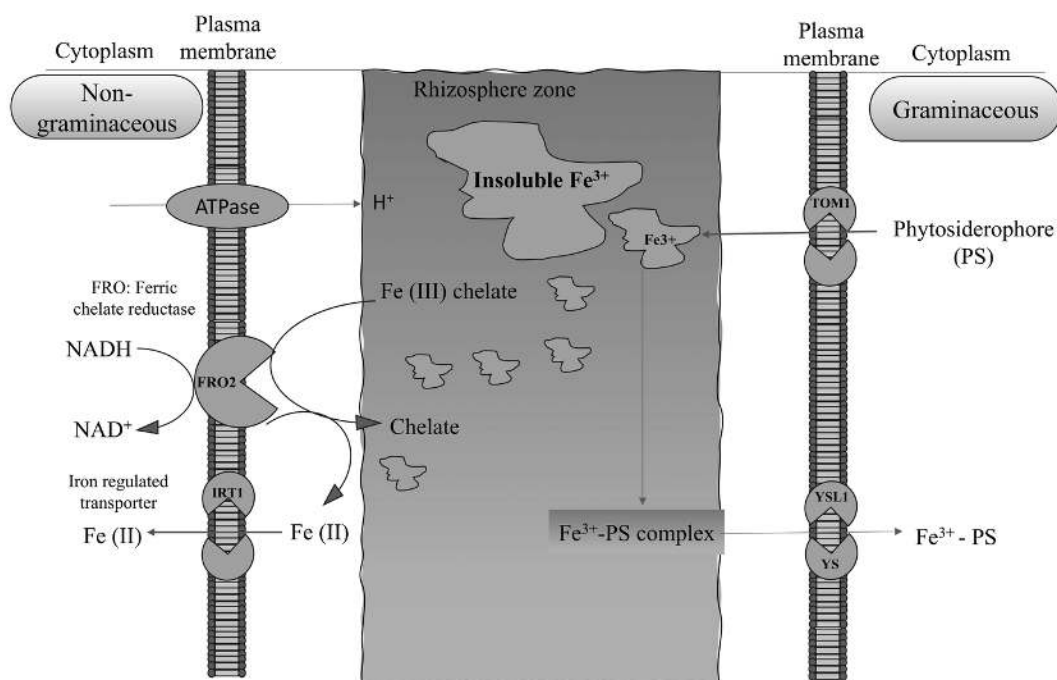


FIGURE 7.2 Two different strategies use by the plants for uptake of Fe. [↗](#)

Comparative studies of the transcriptome and physiological responses in *Arabidopsis* have shown that applying exogenous melatonin increases acquisition of the plant's tolerance to Fe-deficiency ([Wan et al., 2018](#)). The role of melatonin in inducing tolerance to iron deficiency in pepper (*Capsicum annuum* L.) by involving NO and H₂S signaling network was also reported ([Kaya et al., 2020](#)). According to reports, after being treated with melatonin, *Arabidopsis* and cucumber plants grown in low-Fe environments exhibited higher levels of phytomelatonin and the expression of genes linked to the acquisition of Fe, including FRO2, IRT1, and FIT1 ([Zhou et al., 2016](#); [Ahammed et al., 2020](#)). Fe deficiency activates the production of nitric acid (NO) production in various plant species, thereby NO positively regulate the expression of FRO2, IRT1 and FIT and improves the Fe utilization efficiency ([Chen et al., 2010](#); [Zhu et al., 2016](#)). Furthermore, it was found that application of melatonin (100 μ M and 150 μ M) in peach upregulated the expression of genes involved in Fe

absorption, including the ferric reduction oxidase 7 (*FRO7*), vacuolar iron transporter 1 (*VITI*), and ferric reduction oxidase 4 (*FRO4*), while downregulating the vacuole genes, vacuolar iron transporter homolog 4 (*VITH4*) and natural resistance-associated macrophage protein 3 (*NRAMP3*) that are related to vacuolar iron transport ([Lin et al., 2022](#)). According to transcriptome analysis, in MT-treated peach plants, the differentially expressed genes (DEGs) associated with iron metabolism were predominantly linked to aminoacyl-tRNA production pathway during iron-deficient conditions ([Lin et al., 2023](#)). Recently, it is reported that, collective action of nanoscale zero-valent iron and melatonin improved the grain Fe content and reduced the grain cadmium and arsenic level in rice ([Bao et al., 2024](#)).

7.3.7 Impact of Phytomelatonin in Micronutrients (Cu, Zn, B)

In addition to Fe, copper (Cu) is an important mineral element, which is chief components of photosynthetic ETC such as blue colored copper containing protein, plastocyanin (PC) and components of cytochrome complex as well. However, excessive Cu reduces the plant fresh weight, which leads to the decrease in yield. Transcriptomic and metabolomic analyses found that melatonin induces melon root development under copper stress by inhibiting jasmonic acid biosynthesis ([Hu et al., 2020](#)). Melatonin protective effect in *Vigna radiata* seedling in the presence of copper oxychloride was also reported by [Giraldo-Acosta et al. \(2023\)](#). Result found that melatonin alleviates copper toxicity by improving Cu^{2+} sequestration in vacuoles and ROS scavenging without affecting Cu^{2+} uptake under excess Cu^{2+} ([Cao et al., 2019](#)). Applying melatonin to non-

food plants with detoxifying abilities could be a workable way to enhance phytoremediation in soils contaminated with copper ion.

The first mineral micronutrient known to cause plants to produce phyto melatonin was zinc (Zn) ([Arnao and Hernández-Ruiz, 2009](#)). Exogenous application of melatonin with glutathione reduced the Zn toxicity by reducing the level of malondialdehyde (MDA), H_2O_2 as well as lipoxygenase activity in safflower seedling ([Goodarzi et al., 2020](#)). Furthermore, it was found that overexpression of *EpTDC1* and *N*-acetylserotonin *O*-methyltransferase (*EpASMT1*) enhanced the heavy metal-induced (Zn, Cd, Pb) stress tolerance in *Exophiala pisciphila* ([Yu et al., 2021](#)). Application of melatonin improved the level of phytochelatin under Zn toxicity in safflower, thereby enhanced tolerance to Zn toxicity ([Eisalou et al., 2021](#)). Subsequent investigations ought to concentrate on examining the impact of melatonin on the subcellular distribution of Zn, which can provide clear picture of interaction of phyto melatonin and Zn.

Though, plants need boron (B) in very less amount, it is vital for plant growth and productivity ([Rerkasem et al., 2020](#)). Under normal condition, melatonin enhances the uptake of boron, however it also mitigates B toxicity under high boron concentration ([Turk and Erdal, 2015](#); [Moussa and Algamal, 2017](#); [Sarafi et al., 2017](#); [Al-Huqail et al., 2020](#); [Li et al., 2023](#)). The molecular mechanism involved in alleviating the B toxicity by application of phyto melatonin needs to be explored in future research programs.

7.3.8 Impact of Phyto melatonin on Beneficial Elements (Al, Co, Si and Se)

Beneficial elements are the elements which are required for the plant growth in certain plant species, but not required by all the plants. These

include aluminum (Al), cobalt (Co), sodium (Na), selenium (Se), and silicon (Si), which are the most-studied beneficial elements in particular plants under certain scenarios ([Pilon-Smits et al., 2009](#)). Aluminum (Al) is indispensable for root growth and development of tea plants ([Sun et al., 2020b](#)); however, it is toxic to plants under acid soils, even at low concentration ([Kochian et al., 2015](#)). Studies reported that melatonin can protect the sensitive plants from Al toxicity through antioxidant responses, exclusion of Al from cell wall and enhanced organic anions exudation ([Zhang et al., 2017b](#); [Sun et al., 2020a](#); [Ren et al., 2022](#)). In addition, role of melatonin in ameliorating Al toxicity by increasing Al exclusion and rebuilding redox homeostasis in roots of the wheat has been reported ([Sun et al., 2020c](#)). It has been found that application of melatonin improved resistance to cobalt (Co) toxicity by influencing its antioxidant defense, osmotic adjustment, and stress response gene expression in *Brassica napus* ([Ali et al., 2023](#)). Application of melatonin induced the expression of Na⁺ transport genes (*SOS1*, *SOS2*, *SOS3* and *NHX1*), resulting in moderation of salinity stress in wheat ([Khan et al., 2024](#)).

It is commonly known that silicon (Si) is good for rice growth and development, and that using Si fertilizers can lower the levels of heavy metals in rice ([Wang et al., 2016](#)). Application of Si in conjugation with foliar melatonin reduces uptake and transport of cadmium and arsenic more effectively than does Si application alone in rice ([Bao et al., 2021](#)), indicating essential role of melatonin in reducing heavy metal toxicity. In addition, melatonin protects Arabidopsis from Se-induced toxicity and regulates cobalt (Co) homeostasis in black pine seedlings ([Ulhasan et al., 2019](#); [Çelik, 2021](#)).

7.3.9 Phytomelatonin and Cadmium (Cd) Tolerance

Cadmium (Cd) is an environmental contaminant that harms living organisms. Melatonin promotes Cd tolerance by improving phytochelatin synthesis and by maintaining nutrient homeostasis in *Solanum lycopersicum* ([Xing et al., 2023](#)) ([Figure 7.3](#)). Melatonin also reported to be induce the Cd tolerance through non-coding RNA mediated cell wall modification (pectin and cellulose metabolism) and by enhancing net photosynthetic rate in rice ([Qiu et al., 2024](#)). It was demonstrated that exogenous melatonin induced the Cd tolerance by activating antioxidants enzymes (ascorbate peroxidase and superoxide dismutase) in wheat seeding ([Ni et al., 2018](#)). By modulating stomatal movement through mitogen-activated protein kinase (MAPK) signaling pathway, melatonin application alleviated Cd toxicity in cotton ([Khan et al., 2023a](#)). It has been stated that overexpression of MT1 (*metallothionine-1*) gene conferred Cd tolerance in radish by modulating heavy metals chelators and transporter ([Xu et al., 2020b](#)). Furthermore, combined action of melatonin and selenium improved Cd tolerance in tomato plant as evident through reduced growth inhibition, photoinhibition and electrolyte leakage ([Li et al., 2016b](#)).

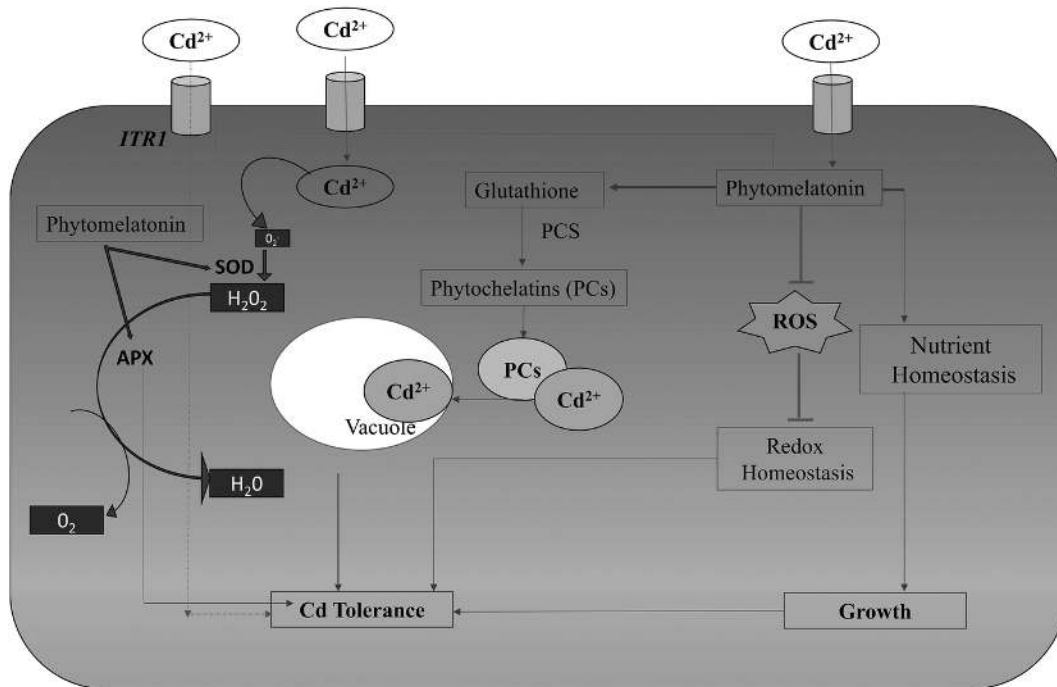


FIGURE 7.3 Phytochelatins and Cadmium (Cd) tolerance in plants-Interplay of phytochelatin and antioxidants system. [📄](#)

7.3.10 Role of Phytochelatins in Soil Microbiomes

Microbes and plants interact in natural environment; thereby promote growth of microorganisms and improve the plants fitness ([Barea *et al.*, 2002](#); [Gouda *et al.*, 2018](#)). In addition to the role of phytochelatins in regulating plant growth and development, phytochelatins also improves the relative abundance of beneficial microorganisms in soil for plant growth ([Zheng *et al.*, 2025](#)). It was reported that combined action of melatonin and plant-growth-promoting rhizobacteria alleviated the cadmium and arsenic stress, and enhanced the growth of the *Spinacia oleracea* ([Asif *et al.*, 2020](#)), indicated the interactive role of phytochelatins and soil microorganism in modulating the plant growth and development.

7.4 CONCLUSIONS AND FUTURE PERSPECTIVES

There is growing evidence that phytemelatonin plays a role in controlling the nutrition of plant minerals, particularly under mineral deficiency conditions. Still the potential role of phytemelatonin in uptake of mineral nutrient and its assimilation in plant is not fully explored. Though numerous research has been conducted to decipher the role of phytemelatonin in growth and development of crops species, no research has been explored to unveil possible role of phytemelatonin in growth and development of various trees species of agroforestry importance. More research is direly needed to uncover its potential benefits under deficiency and toxicity levels of mineral nutrients. More detailed and comprehensive studies on melatonin-mediated biological and molecular mechanisms in nutrient uptake and assimilation are also need to be conducted, particularly with regard to protein modifications. In addition, how do melatonin participates with different nutrient signals also thoroughly need to be explored.

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8

Phytomelatonin Biology Serving as a Driving Force in Regulating Plant Growth and Development under Optimal/Environmental Stressors/Biotic Interferences

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DOI: [10.1201/9781003527633-8](https://doi.org/10.1201/9781003527633-8)

8.1 INTRODUCTION

Intricate signaling pathways regulates the physiological growth in higher plants. Plant growth regulators (viz auxin, cytokinin, and ethylene) including signaling molecules (including Ca^{2+} , H_2S , and NO) assist mutually to regulate vital physiological processes in plants, including starch synthesis, physiological response including growth, responses to environmental stress, and cell death ([Khan et al., 2023](#); [Khatoon et al., 2023](#); [Kumari et al., 2024](#)). Recent studies, both basic and applied, have increasingly recognized that melatonin (N-acetyl-5-methoxytryptamine), a

highly conserved endogenous molecule across eukaryotes, plays a crucial role in various plant biological roles ([Seth et al., 2023](#)). Melatonin is a key molecule in living organisms and acts as natural bioactive product engaged in different regular, and physiological phenomena at the cellular level ([Tyagi et al., 2023](#)). It is last product of tryptophan metabolic synthesis route. In agriculture, its work is particularly significant due to its impact on several crucial processes, including enhancing biomass production, promoting seed germination, improving photosynthetic activity, regulating fruit ripening, delaying flowering, and boosting resistance to various biotic and abiotic stresses.

Recent reports demonstrated that phyto-melatonin enhances plant resistance against various stresses, like reduced toxicity against heavy metals ([Li et al., 2021](#)), scarcity of water ([Li et al., 2015](#)), salt stress ([Zhang et al., 2014](#)), variation in temperatures ([Cao et al., 2018](#)), against pest including biotic and also abiotic stress ([Arnao and Hernández-Ruiz, 2018](#)). Phyto-melatonin protects plants by through antioxidant activities having free radical scavenging potential, boosting activity of antioxidant enzymes, and enhancing the expression of antioxidant genes, thereby mitigating radical scavenging efficacy at the cellular levels ([Zhang et al., 2015](#)). Phyto-melatonin tempers a resistance by mutually in association with other compounds, including NO, ethylene, and abscisic acid (ABA), thus coordinating and controlling plant defense responses ([Wang et al., 2018](#)). Current research highlights the remarkable efficacy of melatonin in enhancing plant acclimatization to unfavorable conditions, including particular resistance mechanisms and regulatory networks involved in plant defense responses ([Zhang et al., 2015](#)). Moreover, melatonin has been shown to significantly regulate plant physiological response ([Debnath et al., 2018](#); [Sun et al., 2021](#); [Xia et al., 2021](#); [Yang et al., 2021](#)). As a growth

regulator, melatonin promotes cell expansion, stimulates root expansion, delays flowering, and enhances quantity in fruiting and qualitative traits of crops (Arnao and Hernández-Ruiz, [2020a, b](#), [Arnao and Hernández-Ruiz, 2021](#)). Furthermore, phyto-melatonin has also vital role in controlling mechanism of circadian rhythms and delayed cell death as signaling compound ([Tan et al., 2012](#); [Arnao and Hernández-Ruiz, 2015](#); [Sun et al., 2015](#)).

This chapter revealed recent research progress on the role of phyto-melatonin in plant growth, development, yield, and quality formation, as well as its molecular regulatory network. It also explores various strategies to optimize the use of melatonin to improve plant growth and productivity, offering valuable insights for future studies and applications of phyto-melatonin in agriculture.

8.2 SYNTHESIS OF MELATONIN IN PLANTS

At recent scenario, tremendous reports have been made in identifying key mechanisms of phyto-melatonin pathways ([Hardeland, 2015](#)). The traditional artificial route of phyto-melatonin in plants resembles as like of animals ([Zhao et al., 2019](#)). The idea behind concept that plant has capacity to synthesize melatonin was first confirmed through radioactive tracing experiments of *St. John's wort* (*Hypericum perforatum* L. cv. *Anthos*) seedlings ([Murch et al., 2000](#)). While it is broadly accepted that biosynthetic pathways of phyto-melatonin in higher plants is similar to fauna ([Arnao and Hernández-Ruiz, 2006](#); [Tan et al., 2013](#); [Zhao et al., 2019](#)), some controversy remains around this concept.

According to multiple reports, melatonin biosynthesis in plants initiates with the amino acid tryptophan and engage at least 4 enzymatic routes

initiated by 6 enzymes: tryptophan decarboxylase (TDC), tryptophan hydroxylase (TPH), tryptamine 5-hydroxylase (T5H), serotonin N-acetyltransferase (SNAT), N-acetylserotonin methyltransferase (ASMT), and caffeic acid O-methyltransferase (COMT) ([Back et al., 2016](#)) ([Figure 8.1](#)).

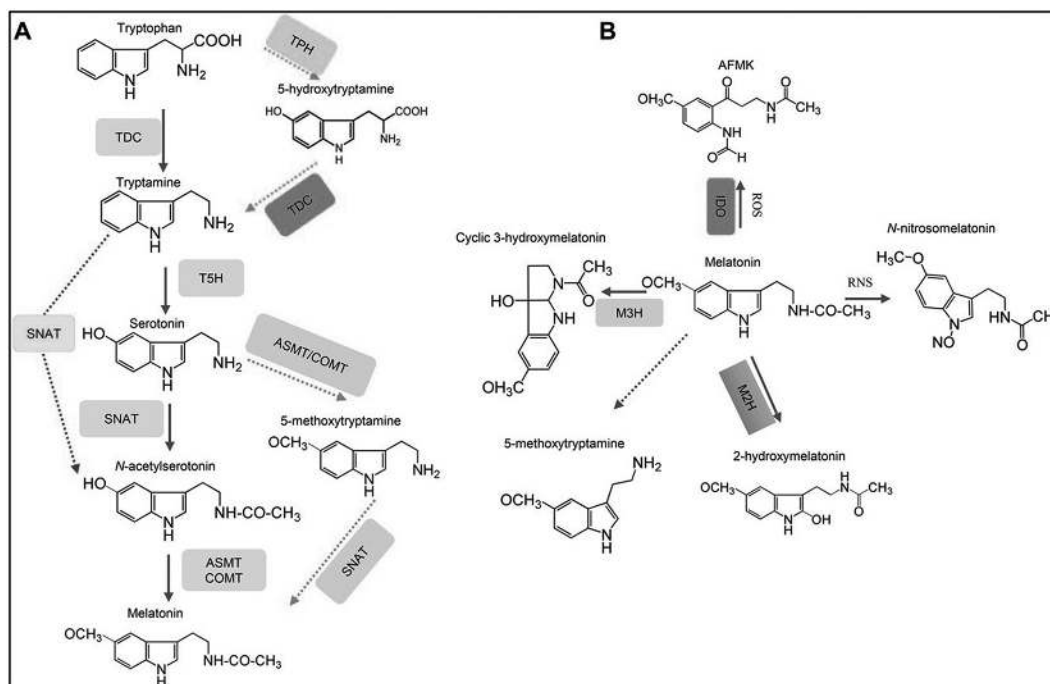


FIGURE 8.1 Melatonin biosynthesis (A) and metabolic (B) pathways in plants. [📄](#)

Initially 2 enzymes, responsible for its hydroxylation and decarboxylation, thus essential for melatonin production, also reported in plants ([Facchini et al., 2000](#); [Kang et al., 2007](#); [Park et al., 2012](#)). However, they are totally different in vertebrates. In rice (*Oryza sativa* L), the initial path in melatonin synthesis is the decarboxylation of tryptophan by TDC, followed by hydroxylation by T5H ([Park et al., 2012](#)).

Interestingly, while T5H is crucial for serotonin biosynthesis, its suppression leads to an increase in melatonin amount in genetically modified rice plants, thus proved that melatonin production in plants is not

directly comparative to serotonin amount. This finding adds complexity to our understanding of melatonin biosynthesis.

The enzymes which are involved in specific events are: AAA, aryl acylamidase; ASMT, *N*-acetylserotonin methyltransferase; COMT, caffeic acid *O*-methyltransferase; AFMK, *N*¹-acetyl-*N*²-formyl-5-methoxykynuramine; IDO, indoleamine 2,3-dioxygenase; M2H, melatonin 2-hydroxylase; M3H, melatonin 3-hydroxylase; 5-MT, 5-methoxytryptamine; ROS, reactive oxygen species; RNS, reactive nitrogen species; TDC, tryptophan decarboxylase; TPH, tryptophan hydroxylase; T5H, tryptamine 5-hydroxylase; SNAT, serotonin *N*-acetyltransferase.

8.2.1 Biosynthesis of Melatonin

The last but one path is the acetylation of serotonin, a process regulated by serotonin *N*-acetyltransferase (SNAT) in plants, which is homologous to arylalkylamine *N*-acetyltransferase (AANAT) in animals ([Back et al., 2016](#)). Studies have shown that when AANAT homolog from *Chlamydomonas reinhardtii* (crAANAT) was introduced into *Micro-Tom* tomato plants ([Okazaki et al., 2009](#)) or when human AANAT (hAANAT) was expressed in *rice* ([Kang et al., 2010](#)), melatonin levels were consistently elevated. This suggests that the *N*-acetylation of serotonin in plants is likely catalyzed by AANAT paralogs, instead of chlorophyceans or acquired through straight gene transfer ([Tan et al., 2012](#)). However, exact isoenzymes and gene modifications responsible for this process is yet to be understood and require further investigation.

O-Methylation of *N*-acetylserotonin is considered final path in melatonin biosynthesis. The ASMT gene, cloned and expressed in rice, shares homology with other plant *O*-methyltransferase genes ([Kang et al., 2011](#)). However, a contrast between rice and animal ASMT suggested notable

variations in enzyme kinetics, as rice ASMT do not have substrate reticence features observed in animal counterparts ([Nakane et al., 1983](#); [Kang et al., 2011](#)). Interestingly, the role of ASMT in rice contrasts with recent research that caffeic acid O-methyltransferase (COMT) plays a role in *Arabidopsis thaliana* (Byeon et al., [2014](#), [2015](#)). Synthesis of these gene by recombinant DNA technology presents opportunities to further explore melatonin-mediated roles. Both ASMT and COMT exhibit substrate affinities for serotonin and N-acetylserotonin. They initially add methyl at serotonin to produce 5-methoxytryptamine, which then reacts with SNAT forming melatonin ([Arnao and Hernández-Ruizm, 2015](#); [Back et al., 2016](#)).

8.3 THE ROLE OF MELATONIN IN PLANT GROWTH AND DEVELOPMENT

Phyto-melatonin is widely synthesized across various plant organs and has been shown to increase in reaction to both abiotic and biotic stresses. This stress-induced rise in phyto-melatonin helps mitigate the adverse effects of stress, particularly oxidative stress, by combating excessive reactive oxygen species (ROS), including free radicals, which are often generated under such conditions. Stress typically hampers plant growth, and phyto-melatonin serves as a critical modulator for alleviating these effects. Their diverse roles in plants range from promoting seed germination to delaying senescence, showcasing their pleiotropic effects. It has vital part in different directions of physiological development of plants, insisting seed germination, enhancing root and shoot growth, regulating photosynthetic efficiency, modulating flowering and fruiting processes, delaying leaf senescence, and strengthening stress tolerance mechanisms. These functions

highlight the importance of phyto-melatonin as vital regulator of plant development and the stress stimuli.

8.3.1 Promoting Growth

The function melatonin in physiological growth of plant was observed by [Hernández-Ruiz et al. \(2005\)](#), who demonstrated that melatonin treatment extended the coleoptiles of canary grass (*Phalaris canariensis*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and oat (*Avena sativa*). Subsequent studies have shown that melatonin promotes physiological growth in different plants, including soybean (*Glycine max*), etiolated white lupin (*Lupinus albus L.*), maize (*Zea mays*), cucumber (*Cucumis sativus*), and *Arabidopsis* ([Arnao and Hernández-Ruiz, 2007](#); [Posmyk et al., 2009](#); [Wei et al., 2015](#)). Thus, most of these studies engaged melatonin concentrations beyond physiological quantity in plants, raising questions about the natural growth-insisting effects of the molecule.

Knocking down *SNAT2* in rice or *SNAT1* in *Arabidopsis* gave v in semi-dwarf plants ([Hwang and Back, 2018](#); [Lee and Back, 2019](#)), while genetically modified tomato seedlings expressing the rice *IDO* gene showed reduced melatonin concentrations and decreased yield ([Okazaki et al., 2010](#)). These findings suggest that boosting melatonin synthesis could significantly enhance crop production. Interestingly, peripheral expression of the sheep *SNAT* gene in rice increased melatonin content and premature seedling growth but delayed flowering and dramatically reduced grain yield ([Byeon et al., 2014](#)). The potential role of melatonin in agriculture represents a promising area for future research and innovation.

8.3.2 Root Organogenesis

The chemical resemble between melatonin and indole-3-acetic acid (IAA) has gained significant consideration owing to its importance in root growth

and development. Over the past few decades, melatonin has been identified as a key regulator of root growth and development ([Arnao and Hernández-Ruiz, 2007, 2015](#); [Pelagio-Flores et al., 2012](#); [Tan et al., 2012](#); [Zhang et al., 2014](#); [Arnao and Hernández-Ruiz, 2015](#); [Wen et al., 2016](#); [Liang et al., 2017](#); [Chen et al., 2019](#)). Additionally, growing evidence highlights the role of melatonin as a central mediator in the interface between roots and rhizosphere microorganisms ([Jiao et al., 2016](#); [Ma et al., 2017](#); [Asif et al., 2019](#)). The exogenous importance of melatonin key role in root organogenesis, lateral and adventitious root development, in *Lupinus albus*, rice, cucumber, and *Arabidopsis* ([Arnao and Hernández-Ruiz, 2007](#); [Pelagio-Flores et al., 2012](#); [Zhang et al., 2014](#); [Chen et al., 2019](#)). In *Arabidopsis*, melatonin treatment increases the amount of adventitious and lateral roots twice and thrice in number, respectively ([Pelagio-Flores et al., 2012](#)). Similarly, melatonin expressively stimulates lateral root expression in cucumbers ([Zhang et al., 2014](#)). Moreover, rice seedlings overexpressing sheep *SNAT*, which resulted in melatonin levels 10 times higher than those of wild-type plants, exhibited enhanced root growth. The importance of melatonin in promoting rhizogenesis observed [Arnao and Hernández-Ruiz \(2007\)](#), demonstrated that melatonin stimulated root primordia through pericycle cells in lupine (*Lupinus albus*), resulting in the development of adventitious and lateral roots. Later, it was also reported that melatonin might also have importance in regulating gravitropic response of the roots ([Arnao and Hernández-Ruiz, 2017](#)). However, most of the evidence linking melatonin to the regulation of root morphology has been derived from observational studies. Although an apparent gradient of melatonin has been detected across various root sections in some plants ([Tan et al., 2012](#)), the precise pathways by which melatonin influences root morphology is yet to be elucidated.

It has also been reported that melatonin interacts with auxin to regulate external structure of root ([Chen et al., 2009](#); Arnao and Hernández-Ruiz, [2014](#), [2018](#); [Wen et al., 2016](#)). A crucial study found that limited concentrations of melatonin promoted root growth, while high amount inhibited it, exhibiting a bimodal concentration dependence similar to that of IAA ([Chen et al., 2009](#)). This proved that melatonin and auxin interact to control physiological growth of roots. For example, in *Brassica juncea* and tomato plants, reduced melatonin levels are associated with increased endogenous IAA production and enhanced root development ([Chen et al., 2009](#); [Wen et al., 2016](#)). It has been reported that the root-promoting effect of melatonin is induced by IAA biosynthesis; however, more recent studies have revealed complex interactions. In transgenic tomato plants expressing sheep *SNAT/HIOMT* and *Arabidopsis* plants heterologous expressing apple (*Malus domestica*) *HIOMT*, melatonin treatment increased root branching and excess lateral roots, resembling auxin responses. Interestingly, endogenous IAA content in these tomato plants decreased by 7-fold, and 1.4-fold in *Arabidopsis* ([Zuo et al., 2014](#)). Additionally, synthetic treatments have indicated that enhanced melatonin concentrations reduce root meristem size, thus correlates with reduced IAA biosynthesis ([Wang et al., 2016](#)). The molecular levels of key genes involved in IAA biosynthesis coding for YUCCA and TAA family proteins, were found to decrease significantly following melatonin treatment ([Arnao and Hernández-Ruiz, 2018](#)). Interestingly, melatonin appears to induce rhizogenesis irrespective of auxin, as *Arabidopsis* supplemented with melatonin do not have over expression of auxin-inducible DR5-GUS reporter ([Pelagio-Flores et al., 2012](#); [Koyama et al., 2013](#)), including many other auxin-related genes in cucumber roots was found to be same in presence and absence of melatonin ([Zhang et al., 2014](#)). Notably, melatonin supplemented resulted in

significant underexpression of genes encoding auxin influx carrier proteins (AUX1/LAX), engaged in physiological response and development of root ([Swarup and Péret, 2012](#)) in *Arabidopsis* ([Arnao and Hernández-Ruiz, 2015](#)). Additionally, melatonin inhibited the expression profiles of various auxin-related transcription factors, including WRKY, NAC, MYB, bHLH, and HD-ZIP families ([Liang et al., 2017](#)). Thus it has been concluded that melatonin has crucial role in regulation of root morphology, potentially thus modulating the auxin signaling pathway, as well as influencing polar auxin transference and perception.

Further it has been reported that melatonin and auxin signaling pathways operate via different cascades, which may ultimately converge at certain checkpoints, although not necessarily in a regulatory manner. One key molecule in this process is nitric oxide (NO), which has diverse functions in stimulating plant growth, development, and environmental responses ([Stöhr and Stremmlau, 2006](#); [Wilson et al., 2008](#); [Sun et al., 2014](#); [Yu et al., 2014](#); [Sanz et al., 2015](#)). NO is a critical regulator of auxin responses, influencing adventitious root formation, lateral root and root hair development, as well as root gravitropism ([Pagnussat et al., 2002](#); [Yu et al., 2014](#)). Elevated NO levels in *Arabidopsis* reduced both auxin transport and stimuli through a PIN1-dependent pathway, which concurrently inhibited root meristem ([Fernández-Marcos et al., 2011](#)). Similar results were observed in plants with increased melatonin levels ([Wang et al., 2016](#)), including tomato (*Solanum lycopersicum*). Melatonin treatment induces adventitious root formation by promoting NO production, which in turn influences the expression of various auxin-related genes, including those involved in auxin transport, signal transduction, and accumulation ([Wen et al., 2016](#)). Furthermore, NO supplementation enhances melatonin synthesis in tomato

seedlings, indicating a potential response mechanism ([Arnao and Hernández-Ruiz, 2018](#)).

8.3.3 The Floral Transition

A recently identified function of melatonin as a growth regulator is its involvement in regulation of flowering time (Arnao and Hernández-Ruiz, [2020a, b](#)). In earlier reports, disrupting signaling in *Chenopodium rubrum* leaves through current was shown to interfere with flowering, potentially owing to changes in melatonin levels ([Macháčková and Krekule, 2002](#); [Kolář et al., 2003](#)). Notably, *C. rubrum* was 1st plant to establish a circadian regulatory molecule for melatonin ([Wolf et al., 2001](#)). Later studies observed reduced flowering in genetically modified rice plants overexpressing sheep *SNAT* ([Byeon et al., 2014](#)).

It is important to note that while melatonin does not affect the flowering phase, it has crucial role in controlling several crucial phases of floral transition. The significance of melatonin in the vegetative-to-reproductive transition of *Arabidopsis* was explored by [Shi et al. \(2016\)](#), demonstrated its pathway. According to their model, melatonin reduces floral transition via interceding the steadiness of DELLA proteins, which, in turn, disrupts the function of transcription factors involved in flowering. This mechanism, along with the proposed model, builds on previous research ([Shi et al., 2016](#); [Arnao and Hernández-Ruiz, 2021](#)).

Current reports highlighted the role of correlation between melatonin and other signaling compounds in physiological response of flowering. [Zhang et al. \(2019\)](#) discovered a straight link between strigolactones, a class of carotenoid-derived phytohormones, and melatonin in the floral transition of *Arabidopsis*. It has been reported that strigolactones have over expression as compared to melatonin to suppress flowering by activating FLC when

melatonin levels in tissues surpass beyond desired amount. Interestingly, nitric oxide (NO), which is produced in response to melatonin, has been shown to increase DELLA protein levels ([Lozano-Juste and León, 2011](#)) and repress the floral transition in *Arabidopsis* ([He et al., 2004](#)). [Shi et al. \(2016\)](#), proposed that NO has crucial role in melatonin-assisted stabilization of DELLA proteins, contributing to the delay in flowering. However, further detailed studies are required to better understand how strigolactones regulate melatonin biosynthesis and the distinct role of the interaction between NO and melatonin in plant development.

Melatonin quantity in plants varies in circadian rhythm. For example, [Kolář et al. \(1997\)](#) reported oscillations in endogenous melatonin routes in *Chenopodium rubrum* under a 12-hour light:12-hour dark cycle, with reduced melatonin amount during the light period and a significant rise during the dark period. Similarly, in the macroalga *Ulva sp.*, melatonin levels oscillate with a peak at night under long-day conditions ([Tal et al., 2011](#)). Variation in melatonin amount have been observed in different floras like *Eichhornia crassipes* (water hyacinth), *Vitis vinifera* (grape berry), *Prunus avium* (sweet cherry), *Arabidopsis*, *Lupinus albus*, and *Hordeum vulgare* (barley seedlings) ([Tan et al., 2007](#); [Boccalandro et al., 2011](#); [Zhao et al., 2013](#); [Arnao and Hernández-Ruizm, 2015](#); [Li et al., 2020](#)). [Li et al. \(2020\)](#) reported particular importance of daily rhythms in phyto-melatonin amount in *Arabidopsis*, a facultative long-day plant. Reports proved that endogenous phyto-melatonin levels and the expression of biosynthetic genes exhibited tremendous oscillations, peaking in the morning (zeitgeber time 8). In contrast, AtPMRT1 expression level was maximum in afternoon. It has been suggested that AtPMRT1 may mediate ROS signaling, acting as a dark signal to initiate stomatal closure around evening and at night, thus

proved a connection between melatonin rhythms to plant responses in response to environmental proof.

The phyto-melatonin functions as a night signal, thus logical to adopt that it also helps synchronize different biological processes with diurnal and photoperiodic environmental prompts, like flowering, which is short-day response. It has been reported that melatonin decrease flowering in both *Chenopodium rubrum* and the long-day plant *Arabidopsis thaliana* ([Kolář et al., 2003](#); [Shi et al., 2015b](#); [Zhang et al., 2019](#)). Thus it has been assumed that, melatonin acts as chrono biological hormone-like regulator, thus coordinating the plant's centric circadian clock with the redox network ([Arnao and Hernández-Ruiz, 2020b](#)).

8.3.4 Autophagy

Autophagy, is biological phenomena which has crucial role in cellular homeostasis by debasing and salvaging cytoplasmic materials, is a highly conserved and strongly controlled eukaryotic pathway ([Qi et al., 2020](#)). It is crucial for physiological growth of plant and development against stress ([Qi et al., 2020](#); [Wei et al., 2020](#)). This process is often related with oxidative stress in both plants and animals ([Wei et al., 2020](#); [Qi et al., 2020](#)).

Increasing evidence suggests that melatonin plays a beneficial role in the regulation and restoration of damaged autophagic processes in plants.

[Wang et al. \(2013\)](#) reported that various autophagy-associated genes obtained from *Malus hupehensis* were tremendously upregulated in response to melatonin, which delayed physiological death of leaf. They also reported that melatonin supplementation enhances oxidative stress-stimulated autophagy in *Arabidopsis* without disrupting reactive oxygen species ([Wang et al., 2015](#)). This suggests that melatonin-assisted stimulation of autophagy is component of an endurance mechanism to cope

with cellular damage. In cassava (*Manihot esculenta*), melatonin biosynthesis enzymes, including TDC and ASMT, interrelate with autophagy-related genes to coordinate the dynamics of melatonin biosynthesis and autophagic activity ([Wei et al., 2020](#)). However, the straight connection between autophagy and melatonin in plants, is yet to be studied. Future research will be essential to study how phyto-melatonin controls the beginning and development of autophagy including about how it might terminate the process.

8.3.5 Anti-senescence and Chlorophyll Conservation Features

It has been reported that melatonin possess anti-aging property, temperately expand the lifespan of living organism ([Hardeland, 2013](#)). The positive effects of melatonin as an free radical antioxidant has been reported by several workers ([Reiter, 1995](#); [Tan et al., 2000](#); [Hardeland, 2015](#); [Manchester et al., 2015](#); [Tan et al., 2015](#)). Melatonin assist in signaling molecule which triggers antioxidant enzymes and help in synthesis of ascorbic acid (AsA), glutathione (GSH), and polyphenolics ([Zhang et al., 2013](#); [Li et al., 2015](#); [Wei et al., 2015](#)). For example, CAND2/PMTR1 assist to sustain reactive oxygen species (ROS) homeostasis by adaptable the activity and gene expression of ROS-scavenging enzymes, including SOD, CAT and POD in Arabidopsis ([Li et al., 2020](#)). Till date effects of melatonin on plant viability is yet to be eluciated, its part in leaf senescence has attracted lot of scientific communities ([Arnao and Hernández-Ruiz, 2014](#), [Arnao and Hernández-Ruiz, 2009a](#); [Tan et al., 2012](#); [Wang et al., 2018](#)). The first report of melatonin slowing dark-stimulated cell death in barley leaves was by [Arnao and Hernández-Ruiz \(2009b\)](#). This surprising outcome was later proved in leaves of the apple *Brassica rapa* var. *parachinensis*

(Chinese cabbage), *Lolium perenne* (perennial ryegrass), *Actinidia deliciosa* (kiwifruit), and *Arabidopsis thaliana* ([Wang et al., 2013](#); [Shi et al., 2015a](#); [Liang et al., 2018](#); [Tan et al., 2019](#)). The anti-senescence effects of melatonin were initially attributed to its powerful antioxidant properties.

Previous reports proved that that melatonin stimulates redox reactions, like reducing ROS enhancing the amount of ascorbate (AsA) and glutathione (GSH), thus acting as an anti-senescence agent. Further, melatonin treatment protects the overexpression of senescence-related gene *Hexokinase-1* and various autophagy-associated genes (*ATGs*), which naturally exist at the time of terminal stage of leaf senescence ([Wang et al., 2012](#); [Liang et al., 2015](#); [Shi et al., 2015a](#)). But it is not completely proved. Genomics studies of melatonin-supplemented *Arabidopsis* proved that melatonin overexpress genes engaged in the signaling of different plant which insist or stimulate cell death, like ethylene, jasmonic acid, and salicylic acid ([Jibran et al., 2013](#); [Khan et al., 2014](#)). Melatonin amount elevates leaf senescence in rice ([Park et al., 2012](#)). Independently on this factor, the interactions between melatonin plant growth regulators are crucial and preserving chlorophyll efficacy which is critical for preventing leaf senescence, which finally affects crop yields. Therefore, the effect of melatonin in the control of photosynthetic pathways needs lot of attention. [Arnao and Hernández-Ruiz \(2009b\)](#) reported that exogenous melatonin supplementation resists chlorophyll from degradation in barley. The role of melatonin in conserving chlorophyll was further proved in *Arabidopsis*, apple, rice, cucumber, cherry, and wheat, in general physiological and stress conditions ([Kang et al., 2010](#); [Sarropoulou et al., 2012](#); Wang et al., [2013](#), [2016](#); [Zuo et al., 2014](#)).

Further, melatonin also enhances the photosynthetic efficacy of plants. For example, in apple trees, melatonin treatment improves the efficacy of

PS II, permitting the leaves to sustain increased amount of CO₂ assimilation and stomatal conductance ([Wang et al., 2012](#)). In a metabolomics, melatonin enhanced the expression of genes encoding two subunits of PS I (PsaK and PsaG), and 2 subunits of PS II (PsbO and PsbP), and ferredoxin PetF during under expression the expression of genes thus results in chlorophyll dilapidation, like chlorophyllase and PaO ([Wei et al., 2015](#)). The importance of melatonin in conservation photosynthetic pigments in different stress conditions has been documented in wheat, Bermuda grass (*Cynodon dactylon*), and tomato ([Turk et al., 2014](#); [Shi et al., 2015b](#)). Collectively, these studies suggest that melatonin helps to preserve chlorophyll, aligning with its known effect in delaying leaf senescence.

8.3.6 Importance in Postharvest Maturation of Fruits

Various reports in recent years have revealed interesting insights into importance melatonin in sustaining postharvest fruit ripening, particularly in climacteric fruits like banana (*Musa acuminata*), apple, and pear (*Pyrus communis*) ([Sun et al., 2015](#); [Arnao and Hernández-Ruiz, 2020b](#)).

Melatonin amount tremendously enhance in the pericarps of tomato during the conversion from the established green stage to the red stage ([Okazaki et al., 2009](#)). The exogenous supplementation of melatonin enhanced ripening in tomato and progress its nutritional amount ([Sun et al., 2015](#)). Further studies revealed that melatonin enhances ethylene biosynthesis and exponentially induce ethylene signal transduction, thus causing accumulation of lycopene, dilapidation of cell walls, and biosynthesis of volatile compounds by sustaining the expression of several underlying genes ([Arnao and Hernández-Ruiz, 2020b](#)). These research recommend that

melatonin has importance in regulating various processes that contribute to fruit ripening, with potential applications in improving the quality and shelf life of postharvest fruit.

Although melatonin and ethylene typically work together to regulate postharvest fruit ripening, their interactions can sometimes be antagonistic. It has been assumed that, melatonin supplementation during the ripening process in bananas delays fruit ripening by decreasing ethylene biosynthesis ([Hu et al., 2017](#)). This delay may be due to factors such as the ripening temperature maintained experiments or complex reactions between melatonin and ethylene signaling. Unlike NO and H₂S, having responsible for anti-ripening agents ([Mukherjee, 2019](#)), melatonin modulates fruit ripening and influences the ethylene biosynthesis and signaling pathways. Recent research has made significant strides in clearing the role of melatonin in the regulation of postharvest fruit ripening. However, the role of NO in this process is still a relatively new area of study. In maturation of pears, when NO biosynthesis is reserved, melatonin-resulted in increase of time interval of fruit senescence is nearly eliminated. This suggests that melatonin regulates overexpression of NO biosynthesis to regulate fruit senescence ([Liu et al., 2019](#)). To fully understand the complex relationships between melatonin, NO, H₂S, and ethylene, more comprehensive genomic and proteomic approaches are needed. These studies will help clarify how these signaling molecules interact in the regulation of fruit ripening and senescence, offering insights into how melatonin can be effectively used in postharvest fruit management. Melatonin not only promotes fruit ripening but also improves fruit quality, primarily owing to its powerful antioxidant properties. Several studies have demonstrated that melatonin increase antioxidant defense routes in ripening fruits, thereby reducing oxidative stress ([Cao et al., 2018](#)). Further, the postharvest role of melatonin is related

to cause senescence in strawberry (*Fragaria ananassa*) by reducing the chance of decay and increasing resistance to cold stress ([Aghdam and Fard, 2017](#); [Liu et al., 2018](#)). The antioxidant action of melatonin protects fruit from oxidative damage during ripening.

[Aghdam and Fard \(2017\)](#) reported temporary eruption of hydrogen peroxide (H₂O₂) production in melatonin-treated strawberries, accompanied by an accretion of phenolics and anthocyanins. It proves that while melatonin is responsible for ripening, it also resist cell death in climacteric fruit. This dual role of melatonin, which promotes ripening while slowing senescence, makes it a valuable tool in postharvest fruit management. Melatonin supplementation is responsible for improvement in the yield and nutritional value of other horticultural crops. In tomato, for example, melatonin-supplemented plants produced enhanced fruit outcomes and exhibited elevated levels of sucrose, glucose, and essential nutrients in contrast to untreated plants ([Liu et al., 2016](#)). This indicates that melatonin not only enhances the sensory qualities of fruits but may also be effective in maintaining their nutritional attributes and extending their shelf life, making it a promising natural compound for improving fruit quality and postharvest storage.

8.4 IMPORTANCE OF MELATONIN IN PLANT STRESS MANAGEMENT

Melatonin has importance in various physiological roles and stress responses, making its study in controlling physiological and biochemical pathways in normal and stress environment a rapidly advancing field. It primarily mitigates the harmful effects of environmental or abiotic stresses, including water deficiency, salinity, variation in temperatures, and heavy metal toxicity. Moreover, numerous studies have demonstrated that

melatonin enhances stress resistance in plants by influencing gene expression and driving protein variations.

8.4.1 Importance of Melatonin under Abiotic Stresses

Tremendous research has been achieved made in understanding the protective effects of exogenous melatonin against abiotic stress in plants, with numerous comprehensive reviews addressing this topic ([Arnao and Hernández-Ruiz, 2015](#); [Hardeland, 2015](#); [Zhang et al., 2015](#); [Nawaz et al., 2016](#); [Fan et al., 2018](#)). This discussion focuses on variations in endogenous melatonin levels and the mechanisms driving melatonin biosynthesis in plants in unfavorable climatic conditions. Additionally, research in the mechanistic basis of melatonin's role in mitigating abiotic stress effects and its regulation of epigenetic and transcriptional changes during stress responses has been done. Generally, melatonin levels in plants are relatively stable. However, these levels are significantly influenced by stressful climate like increased light intensity, extreme temperatures (cold or heat), drought, and heavy metal toxicity ([Arnao and Hernández-Ruiz, 2014](#); [Zhang et al., 2015](#)) ([Figure 8.2](#)).

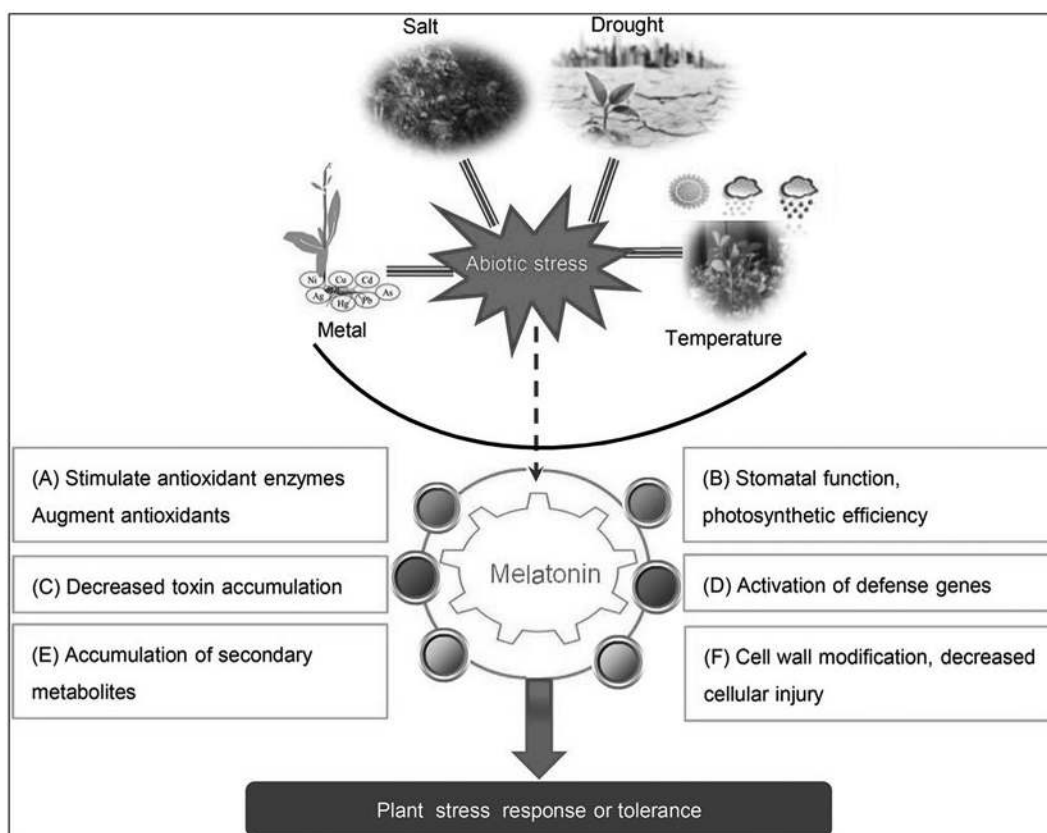


FIGURE 8.2 Generalized model of the roles of melatonin in plant resistance to abiotic stresses such as drought, salt, ambient temperature, and heavy metal stress. [↗](#)

Barley and lupine plants exposed to synthetic compounds like NaCl, ZnSO₄, and H₂O₂ exhibited elevated melatonin quantity ([Arnao and Hernández-Ruiz, 2009a, 2014](#)). Similarly, UV-B light significantly increased melatonin content in the roots of *Glycyrrhiza uralensis* ([Afreen et al., 2006](#)). Enzymes involved in melatonin biosynthesis are effected by stress response. In rice seedlings, melatonin production rose in enhanced temperature and low light intensity due to increased activity of SNAT and ASMT enzymes ([Byeon et al., 2014](#)). In salt-supplemented sunflower (*Helianthus annuus*) cotyledons, elevated HIOMT activity led to enhanced melatonin accumulation, highlighting its importance as potent free radical scavengers ([Mukherjee et al., 2014](#)). Research on the sustainability of

melatonin biosynthesis in adverse conditions has focused on upstream transcription factors that influence the activity of many rate-effected enzymes or their isoenzymes. For instance, overexpression of *AANAT* or *HIOMT* genes in tomato plants boosted melatonin levels and assisted in drought resistance ([Wang et al., 2014](#)). The upregulation of melatonin biosynthesis genes like *MdTDC1*, *MdAANAT2*, *MdT5H4*, and *MdASMT1* has been shown to modulate water balance and enhance drought tolerance in *Malus hupehensis* ([Li et al., 2015](#)). In tomato plants, Cd toxicity triggered the expression of *COMT1*, leading to increased melatonin and improved Cd resistance ([Cai et al., 2017](#)). Similarly, selenium treatment in tomato plants upregulated the overexpression of *TDC*, *T5H*, *SNAT*, and *ASMT*, resulting in elevated melatonin levels and cadmium toxicity ([Li et al., 2016](#)). Despite these insights, the control of overexpressed genes of melatonin biosynthesis remains less understood compared to animals. Moreover, the mechanisms underlying the translational and post-translational control of phyto-melatonin biosynthesis enzymes have yet to be elucidated.

8.4.1.1 Melatonin under Drought Stress

Drought stress causes significant losses in crop yield, causing a major challenge to precision agriculture. Numerous reports explored the relationship among melatonin and abiotic stress ([Wang et al., 2013](#); [Li et al., 2015](#)). Limited water availability induces melatonin biosynthesis, and exogenous melatonin application effectively mitigates drought-stimulated inhibition of physiological growth of plants ([Zhang et al., 2013](#); [Li et al., 2015](#); [Wei et al., 2015](#)). Further, genetically modified *Arabidopsis* plants heterologously induced expression of gene *MzASMT1* from *Malus zumi* Mats exhibited greater drought tolerance in contrast to wild-type, suggesting a functional importance of melatonin in drought resistance ([Zuo](#)

[et al., 2014](#)). Under drought conditions, melatonin is known to enhance the biological role of antioxidant enzymes and increase levels of antioxidants like AsA, GSH and phenolic derivatives. These changes help reduce reactive oxygen species (ROS) synthesis and mitigate oxidative disruption caused by drought stress ([Zhang et al., 2013](#); [Li et al., 2015](#); [Wei et al., 2015](#)).

In *Medicago sativa*, melatonin may act prominent source, enhancing drought resistance by maintaining nitro-oxidative and osmoprotective stability ([Antoniou et al., 2017](#)). The water scarcity generally restricts plant development and development by reducing photosynthetic activity. Melatonin has crucial role in extenuating these effects by preserving chlorophyll content and enhancing stomatal function. It interacts ABA to control stomatal closing, thereby improving drought tolerance ([Chen et al., 2020](#)). [Li et al. \(2015\)](#) demonstrated that it modulates ABA levels by downregulating the *MdNCED3* gene, which regulates enzyme in ABA production ([Iuchi et al., 2001](#)), and upregulating the ABA degrading genes, *MdCYP707A1* and *MdCYP707A2*. This regulation reduces ABA amount and helps to maintain stomatal functionality under drought stress. Enhanced stomatal performance works in concert with the ROS defense pathway to effectively combat drought-induced stress.

Drought stress adversely effects the morphophysiological and biochemical activities, ultimately leading to reduced crop outcomes ([Chen et al., 2019](#)). It induces oxidative stress by promoting the accumulation of ROS, which damages plant cells, impairs stomatal closure, reduces photosynthetic activity, and disrupts antioxidant defense systems. Excessive ROS pose a significant threat to plant cell survival, causing electron leakage, lipid peroxidation, membrane damage, and degradation of proteins and nucleic acids. To mitigate these effects, plants have evolved different

approaches to control physiological growth in adverse climatic conditions. Melatonin, a novel hormone, plays a role in enhancing drought stress tolerance ([Zhang et al., 2015](#); [Li et al., 2021](#)). For instance, drought stress reduces structural traits such as leaf extent and water conductivity in corn seedlings. However, melatonin application significantly improves these parameters ([Li et al., 2021](#)). Similarly, Ye et al. ([2016](#)) proved that melatonin enhanced shoot dry mass and leaf extent in corn seedlings, further demonstrating its potential in mitigating drought-induced damage.

In different crops key physiological phenomena in leaves, including photosynthesis, respiration, and water evaporation, are regulated by stomata. The nurture movement of stomata are governed by intricate signal transduction routes and water counter current mechanisms. During drought stress, plants conserve water level by promoting stomatal shutting and decreasing transpiration rates. However, drought stress can lead to an increase in stomatal density due to the shrinkage of guard cells and a general deterioration of stomatal structure. Melatonin application is reported to respond these adverse effects by offering protection against the deterioration of stomatal cells. Under drought stress in corn, melatonin treatment increases stomatal length and width, increasing the capacity of plants to maintain their physiological functions ([Li et al., 2021](#)). [Li et al. \(2015\)](#) presented contrasting results, showing that drought stress do not effect stomata amount in apples. However, exogenous application of melatonin helped maintain increased turgor pressure and allows stomata open. This discrepancy may be attributed to the variation in melatonin regulatory mechanisms across various plants ([Li et al., 2021](#)). It highlights that the amount, effects, and phenomena of melatonin deed vary among plant species, although there is limited documentation of the morpho physiological stimuli of various plants in drought stress.

8.4.1.2 Melatonin under Salt Stress

This is a significant threat to agriculture and causes substantial economic loss worldwide. Increasing evidence suggests that melatonin enhances salt resistance in different plant species, including maize, wheat, cucumber, tomato, and rice ([Ke et al., 2018](#)). It is due to action of melatonin as an effective antioxidant, which helps suppress peroxidative biochemical pathways in plants exposed to stress. Additionally, melatonin often interacts with other signaling molecules, such as nitric oxide (NO), to regulate redox balance. For instance, [Kaur and Bhatla \(2016\)](#) observed a significant interaction between melatonin and NO in modulating glutathione (GSH) levels and GSH in cotyledons of sunflower plants induced by salinity stress. Sustaining ion homeostasis, particularly the balance between K^+ and Na^+ , is critical under salt stress. It significantly upregulate NHX1 and AKT1, two important ion channels involved in ion sustainability, in plants under salinity salt stress, thereby improving salinity tolerance. Melatonin supplements also reduced the concentration of Na^+ and Cl^- in rice plants, while enhancing the expression of *OsSOS1* in roots and *OsCLC1* and *OsCLC2* ([Li et al., 2017b](#)).

Supplementation of melatonin is believed to regulate K^+/Na^+ homeostasis by triggering triacylglycerol degradation, fatty acid β -oxidation, and energy conversion to sustain plasma membrane H^+ -ATPase efficacy, which in turn helps maintain K^+/Na^+ balance in sweet potato ([Li et al., 2017b](#)). This is consistent with earlier proteomic studies, such as one in which melatonin enhanced effects of salt stress during physiological growth of seeds in cucumber by insisting energy synthesis ([Zhang et al., 2017](#)). Recent research has identified the signaling molecules that mediate melatonin's role in promoting cellular K^+ accumulation under salt stress, particularly in a salt-resistant rice ([Liu et al., 2020](#)). Melatonin enhances

salt tolerance in rice by stimulating an OsRBOHF-dependent ROS burst, which stimulate cascade of stress-induced genes and enhance the expression of K⁺ acceptance transporters. These findings suggest that melatonin plays a multifaceted and crucial importance in orchestrating salt resistant in plants.

Salt stress drastically impacts quality and quantity of plants, but the exogenous role of phyto-melatonin has been shown to alleviate these adverse effects, enhancing plant morphophysiological, biochemical, and molecular activities ([Liang et al., 2015](#)). Current investigations has laid emphasis on the importance of phyto-melatonin in extenuating salt stress, there is still limited information on its regulation of seed germination and related genes in salt stress ([Ke et al., 2018](#)). Seed germination is critical stage in plant development, as it regulates the successful launch of the new varieties. Earlier reports have found that seeds having phyto-melatonin exhibit enhanced germination rates and increased free radical scavenging activity compared to untreated controls ([Zhang et al., 2017](#)). For example, phyto-melatonin enhances the germination rate of *Zizyphus* under abiotic stress by promoting antioxidant activity and facilitating the involvement of osmolytes to scavenge reactive oxygen species (ROS) ([Li et al., 2021](#)).

In cotton, melatonin treatment improves seed germination by kerbing the translation of phyto-melatonin signaling genes, which leads to changes in metabolic processes and reduces seedling damage ([Cai et al., 2017](#)). Additionally, phyto-melatonin treatment is responsible for decrease in ABA and enhance GA amount in early stages of germination in contrast to NaCl supplementation ([Li et al., 2016](#)). These findings confirm that exogenous phyto-melatonin decrease detrimental efficacy of salt in physiological growth of seed, thus promoting better seedling establishment under salt stress. The role of phyto-melatonin across different growth pathways is yet

to be studied. Earlier reports have shown that melatonin can promote ethylene production via *ACS1* and *MYB108A* being strongly triggered by phyto-melatonin for grapevine maturation. Additionally, phyto-melatonin-related genes like *COMT* have been shown to mitigate the drastic outcomes of salt stress and enhance crop productivity (Li et al., 2017). The *COMT* genes, involved in the biosynthesis of phyto-melatonin, have been identified in various plants, including *Populus tremuloides*, *Hordeum vulgare*, *Chrysanthemum grandiflorum*, and *Lolium perenne* ([Ke et al., 2018](#)). However, the engagement of *COMT* in melatonin biosynthesis has primarily been studied in experimental plants, like monocots like rice, and dicots including *Solanum lycopersicum* (tomato) and *Arabidopsis thaliana* ([Li et al., 2016](#)). These studies indicate the potential for broader applications of phyto-melatonin in improving salt stress tolerance across various plant species, but more studies is at urge to fully understand its role and regulation during different growth stages. For instance, salt stress has been shown to decrease chlorophyll amount and photosynthetic efficacy while increasing ROS accumulation and disrupting photoperiod control ([Yin et al., 2013](#)). Melatonin application perished the growth of green bean seedlings by enhancing photosynthetic efficacy and mitigating oxidative disruption induced by ROS. This was achieved through the activation of antioxidant defense mechanism in plants.

Several research emphasize the importance of melatonin in adaptive stimuli to salt stress across different floras ([Chen et al., 2019](#); [Liang et al., 2018](#)). Still many of these findings remain observational, lacking comprehensive physiological and molecular support ([Liu et al., 2020](#)). In rice, melatonin has been found to alleviate salt stress by promoting potassium (K⁺) retention in plant roots, a critical component of tissue tolerance mechanisms. This process involves OsRBOHF-dependent ROS

signaling, which activates stress-stimulated genes and increases the metabolism of K⁺ absorbent transporters, mainly *OsHAK5*, in root tips ([Liu et al., 2020](#)). Potassium (K⁺) is a crucial nutrient for physiological growth, but its availability is often diminished under salt stress, compounding the drastic effects on plant physiological development ([Chen et al., 2019](#); [Liu et al., 2019](#)). Melatonin's function in maintaining K⁺ homeostasis underscores its potential as a pivotal regulator in enhancing plant salinity tolerance. These findings align are in agreement of Huang et al. ([2019](#)), which indicated that salt -stimulated respiratory burst oxidase homolog (RBOH)-stimulated synthesis of H₂O₂ is critical for stress signaling and plant acclimation to salt stress. Despite this, research exploring the specific importance of OsRBOHF-dependent ROS signaling in activating stress-induced genes and boosting the expression of K⁺ absorbent channels in root tips remain limited, particularly across a broader range of crops under abiotic stress conditions. This highlights a gap in research that could be addressed by identifying genes responsive to OsRBOHF-dependent ROS signaling to enhance K⁺ ion absorb in the root tips of various plants under diverse stresses ([Yu et al., 2014](#)).

Additionally, melatonin has been shown to promote ethylene biosynthesis, with its application strongly inducing the expression of *MYB108A* and *ACSI* genes through grape berry ripening, suggesting its broader regulatory influence under stress conditions ([Xu et al., 2019](#)). Further investigations into these pathways could offer new strategies to improve crop resilience and productivity in challenging environments. The *MYB108A* and *ACSI* genes, which play critical roles as transcription factors and regulators in ethylene production, have been shown to be induced by melatonin application ([Dong et al., 2011](#)). Among these, *ACS* genes are particularly noteworthy under abiotic stress conditions as they regulate

ethylene biosynthesis to mediate stress responses in plants. For example, the salt-stimulated gene *VviACSI* has been recognized as a key regulator of ethylene production under saline conditions ([Xu et al., 2019](#)). Similarly, *ACSa* and *ACSI* have been highlighted as primary targets for enhancing salt resistant in crops like corn and tobacco ([Cao et al., 2006](#)).

Interestingly, the combination of melatonin and 1-aminocyclopropane-1-carboxylic acid (ACC, an ethylene precursor) reported to improve salt tolerance in grapevines. This suggests that ethylene biosynthesis plays a role in the melatonin-induced enhancement of salt tolerance ([Xu et al., 2019](#)). Despite these findings, the precise mechanisms and functional roles of *MYB108A*, *ACSI*, *ACSa*, and *VviACSI* in ethylene production, particularly in response to melatonin under various abiotic stresses, remain largely unexplored. Further research is necessary to elucidate how these genes contribute to ethylene-mediated stress responses across different plant species and environmental conditions. Such insights could provide valuable strategies for improving stress resilience in crops through targeted manipulation of melatonin and ethylene signaling pathways.

8.4.1.3 Melatonin under Low Temperature Stress

Ambient temperature significantly influences plant development, with both cold and heat stress disrupting cellular homeostasis by altering redox status, leading to ROS accumulation and peroxidative membrane damage ([Sung et al., 2003](#); [Penfield, 2008](#); [Liu et al., 2018](#)). It has been reported that exogenous melatonin application can enhance these adverse effects in different crops, including rice, tomato, Bermudagrass, and *Lupinus albus*. Melatonin effectively counteracts temperature stress by promoting germination and physiological development of plant ([Wang et al., 2018](#)). For instance, genetically modified rice expressing human *SNAT* showed significantly higher cold resistant than wild-type plants ([Kang et al., 2010](#)).

Cold stress induces drastic oxidative disruption in plants such as wheat seedlings; however, melatonin application has been shown to mitigate these effects by increasing the efficacies of antioxidant enzymes viz SOD, CAT, ascorbate APX and glutathione GR.

This enzymatic boost reduces ROS levels and lipid peroxidation, thereby protecting cellular structures ([Turk et al., 2014](#)). Additionally, melatonin treatment increases the accumulation of natural products, including amino acids, organic acids, and sugars, which further improve plant resilience ([Li et al., 2017a](#)). When molecular analysis has been done it was observed that, melatonin activates the expression of cold-related genes that contribute to enhanced stress tolerance. These include *C-repeat-binding factors (CBFs)* and *Drought Response Element Binding factors (DREBs)*, which are critical for cold signaling pathways, as well as cold-responsive genes like *COR15a*. Other activated genes include *CAMTA1*, engaged in chilling and drought-stress tolerance, and *ZAT10* and *ZAT12*, which regulate ROS-scavenging antioxidant systems ([Bajwa et al., 2014](#); [Guo et al., 2018](#); [Wang et al., 2018](#)). These findings underscore melatonin's multifaceted importance in enhancing plant resistant to temperature-induced stress.

Cold stress, which occurs between 0°C and 12°C, significantly impairs the morpho-physiological, biochemical, molecular, and metabolic activities of plants, ultimately reducing crop growth and yield ([Bhat et al., 2022](#); [Gusain et al., 2023](#)). One of the key effects of cold stress is a decrease in membrane fluidity, disruption of antioxidant enzyme activity, and a loss of metabolic homeostasis ([Wu et al., 2022](#)). These changes compromise the structural and functional integrity of plant cells, with photosynthesis being one of the most affected processes. Cold stress disrupts the carbon–oxygen cycle, limits CO₂ presence, and decreases the synthesis of photosynthetic pigments, leading to a reduction in photosynthetic efficiency ([Li et al.,](#)

[2021](#); [Xu et al., 2022](#)). The decline in chlorophyll content during cold stress results in chlorosis in leaves, which is a visible symptom of impaired physiological processes ([Manasa et al., 2022](#)). Because chlorophyll levels reflect the efficiency of photosynthesis, they are considered a critical indicator of plant health ([El-Hendawy, et al., 2022](#)). Treatment with phyto-melatonin is reported to moderate the effects of cold stress on plants. Studies have reported that melatonin-treated plants maintain an enhanced chlorophyll level than non-treated plants in similar stress conditions. This suggests that melatonin helps to stabilize chloroplast function and prevents pigment degradation during low-temperature stress.

Furthermore, cold stress exacerbates oxidative stress through the overproduction of ROS, which has superoxide anion, OH⁻ radicals, and H₂O₂ ([Tasgin et al., 2006](#)). The accretion of ROS exacerbates cellular damage, including LPO, protein denaturation, and nucleic acid fragmentation. Melatonin, with its potent antioxidant properties, scavenges ROS and enhances the efficacy of antioxidant enzymes, thereby shielding plants from oxidative disruption and promoting resilience under cold stress conditions. Cold stress significantly affects plant cellular structures and processes due to the higher accretion of ROS. This oxidative stress damages DNA, causes lipid peroxidation, and oxidizes proteins, severely impairing physiological development ([Cao et al., 2015](#)). To mitigate these effects, plants activate a robust defense system that includes non-enzymatic antioxidants like proline and glutathione, as well as enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) ([Ghaderian, et al., 2015](#)).

Phyto-melatonin gained lot of attention as a potent regulator in enhancing plant flexibility to cold stress. It reduces the unnecessary production of ROS, thereby alleviating oxidative damage and maintaining cellular

homeostasis. Several studies have highlighted its importance in promoting physiological growth. For example, phyto-melatonin enhances the morpho-physiological activities of crops like corn and cucumber, promoting seed germination and early development under cold stress. In rice, it regulates antioxidant enzyme activities and cold-responsive gene expression to mitigate cold stress. Moreover, phyto-melatonin-treated seeds in early-planted spring maize exhibited improved seedling growth and yield under cold stress. In pepper plants, phyto-melatonin alleviated cold stress by enhancing root traits, plant growth, gas exchange, pigment molecule levels, and the expression of associated stress-response genes ([Altaf et al., 2023](#)).

It suggest that phyto-melatonin recovers crop productivity by modulating morpho-physiological and molecular processes. Further, the accurate pathways through that phyto-melatonin operates across different crops under cold stress remain inadequately explored. Further research is required to unravel these pathways and to optimize the application of phyto-melatonin in agriculture for cold-stress mitigation. Cold stress significantly challenges crop productivity, particularly in temperate zones and high-altitude regions ([Bhat et al., 2022](#)). Plants unprotected to low temperatures practice disruptions in key physiological, and biological mechanism, such as reductions in membrane turgidity, metabolic imbalances, and diminished enzyme activities ([Wu et al., 2022](#)). Among these, photosynthesis is especially vulnerable to cold stress, decrease in temperatures impair crucial gears of this process, leading to reduced photosynthetic efficiency and plant growth.

One of the noticeable impacts of cold stress is a decline in chlorophyll content, which often results in leaf chlorosis. Chlorophyll content serves as a critical indicator of the functionality of plant physiological processes, particularly photosynthesis ([Kaura et al., 2022](#)). However, melatonin

treatment has been shown to counteract these effects, maintaining or increasing chlorophyll concentrations in plants under cold stress conditions, thereby supporting photosynthetic activity ([Yang et al., 2021](#)). Cold stress also leads to excessive accumulation or inefficient detoxification of reactive oxygen species (ROS), such as H₂O₂, O₂⁻, and OH. These ROS induce oxidative damage, including protein oxidation and lipid peroxidation, which compromise cellular integrity and reduce plant growth ([Ghaderian et al., 2015](#); [Nahar et al., 2015](#)). To mitigate ROS-induced damage, plants generated urbane antioxidant pathways, encompassing antioxidants like SOD, POD, and CAT, including non-enzymatic antioxidants like proline and glutathione ([Ghaderian et al., 2015](#)).

Melatonin's role in alleviating cold stress involves enhancing these antioxidant defense mechanisms, thereby reducing oxidative stress and preserving cellular function. Further investigations could provide deeper knowledge of molecular pathways through which melatonin confers temperature stress tolerance, paving the way for its broader application in agriculture to protect crops in challenging climates. Cold stress significantly impairs seed germination and seedling germinations, but exogenous melatonin application has been shown to mitigate these adverse effects across different crops. For instance, melatonin promotes germination in cucumber seeds and enhances development of corn seedlings in low temperature ([Posmyk et al., 2009](#)). In Arabidopsis, melatonin application delays leaf senescence caused by low temperature, ensuring sustained photosynthetic efficiency and plant vitality ([Shi et al., 2015b](#)). Bermuda grass treated with melatonin demonstrated enhanced cold resistance through improved cell membrane stability, regulated photosynthesis, and better biochemical pathways ([Khalid et al., 2022](#)). Additionally, melatonin acts as a dual-purpose agent: a prior line protective source against oxidative

damage and an endogenous device of oxidative stress, as evidenced in studies across various plant species. For example, melatonin enhances photosynthetic carbon accumulation in barley by bolstering antioxidant defenses in cellular organelles during cold stress ([Li et al., 2016](#)). The beneficial effects of melatonin on seed priming are attributed to its capacity to fortify antioxidant defense systems, leading to improved seedling growth and germination under cold stress. Despite these findings, knowledge regarding the response of specific crops, such as waxy corn, to melatonin priming under cold stress remains limited. Future research should focus on exploring these crop-specific responses to fully harness melatonin's potential for agricultural productivity in cold climates.

8.4.1.4 Melatonin under High-Temperature Stress

Heat stress, a critical abiotic stress, can elevate endogenous melatonin levels in plants, enhancing thermos-tolerance through melatonin's potent antioxidant properties. This increase in melatonin helps plants mitigate oxidative damage caused by heat, contributing to improved survival and growth under stressful conditions ([Liang et al., 2018](#)). In tomato seedlings, exogenous melatonin enhance heat-stimulated disruption by balancing redox homeostasis and regulating the biosynthesis of polyamines and nitric oxide, which are critical for stress adaptation. Melatonin's involvement in heat stress responses is mediated by heat shock factors (HSFs), such as HSFA2 and HSA32, which regulate the expression of heat shock proteins (HSPs). HSPs like HSP90 and HSP101 are integral to melatonin-mediated thermotolerance, as they assist in protein folding, stabilization, and repair under heat stress conditions ([Shi et al., 2015b](#)).

Further, melatonin induces autophagy and HSP expression to protect cellular proteins from heat-induced denaturation in tomato. Autophagy mechanisms triggered by melatonin ensure the degradation or refolding of

damaged proteins, maintaining cellular integrity and function under high temperatures. These findings highlight melatonin's pivotal role in protecting plants from heat stress, suggesting its potential application in improving crop resilience in regions prone to high temperatures. Further research should explore the molecular pathways linking melatonin with HSFs and HSPs to broaden our understanding of its thermotolerance mechanisms across different plant species. High levels of heat stress are known to elevate endogenous melatonin concentrations in plants, which enhances thermotolerance due to melatonin's potent antioxidant properties. This thermos-protective role has been demonstrated in various studies. For instance, research on *Arabidopsis* showed that melatonin application enhance physiological development rate under heat stress near about 31% to 40%, highlighting its potential role in stress mitigation during early plant development ([Liang et al., 2018](#)). Phyto-melatonin synthesis is also reported in seed germination. In cucumber seeds, melatonin production peaks 14 hours after germination, suggesting a crucial role during this early growth phase ([Zhang et al., 2014](#)). Furthermore, melatonin recovers germination ability by enhancing soluble sugar consumption, promoting the production of novel proteins, and increasing the activity of enzymes like amylase and α -amylase, as seen in melon and *Limonium bicolor* seeds ([Li et al., 2019](#)). These findings underscore the multifaceted importance of melatonin in boosting seed development and early growth under heat stress. However, other investigations across a broader variety of crop varieties are necessary to complete comprehend its molecular pathways and practical applications in agricultural systems facing temperature extremes.

Recent research has illuminated how melatonin tremendously alleviates the impact of high temperature on plant through various mechanisms. One key aspect is its high potency in maintaining seed viability and promoting

germination capacity even under high heat stress conditions ([Hernández-Ruiz et al., 2005](#)). When plants are treated with elevated temperatures, enzymes SOD, POD and CAT are enhanced to combat oxidative stress. Melatonin plays a crucial role in modulating this response by inhibiting the accumulation of harmful reactive oxygen species (ROS), particularly hydrogen peroxide (H_2O_2), which is detrimental to plant cells during stress ([Wang et al., 2022](#)). Additionally, melatonin has been shown to regulate the biosynthesis of gibberellins (GAs), particularly by upregulating genes like GA20ox and GA3ox, having role in GA production. This increase in gibberellin content, particularly GA4, is beneficial for physiological development under heat stress. On the other hand, melatonin treatment also under express NECD2, a gene required for abscisic acid (ABA) biosynthesis. This down-regulation may be a mechanism to reduce ABA levels, which are typically associated with stress responses that can inhibit growth ([Zhang et al., 2014](#); [Li et al., 2019](#)).

However, specific molecular pathways behind the up- and down-regulation of these genes in response to melatonin under heat stress are still not fully understood. Further studies are required to explore how melatonin precisely cooperates with these pathways at the cellular level and its broader implications for plant stress tolerance. Heat stress can disrupt the balance between antioxidants in plants, causing accumulation of ROS and results in significant disruption to cell membranes ([Sun et al., 2021](#)). The exogenous submission of melatonin has been shown to alleviate the hostile effects of heat stress on plant development, particularly in tomatoes and rice, where it reversed the damage to shoot and root growth induced by high temperatures ([Wang et al., 2018](#)). It also have importance in regulating redox homeostasis and modulating the biosynthesis of nitric oxide (NO) and polyamines in tomato seedlings under heat stress ([Jahan et al., 2021](#)).

In Arabidopsis, heat shock proteins (HSPs), including HSP90 and heat shock factors (HSFA2 and HSFA32), have been identified as key contributors to melatonin-mediated heat stress responses. These HSPs help prevent the aggregation and degradation of cellular proteins, allowing them to refold properly and maintain cellular activity in high temperature ([Shi et al., 2015b](#); [Xu et al., 2016](#)). HSPs act as molecular chaperones, protecting cells against protein degradation and stress-induced protein aggregation, thereby enhancing cell survival and maintaining cellular function during periods of elevated heat stress. Further precise mechanisms by which HSPs and heat shock factors (HSFs) interact with melatonin to mediate heat stress tolerance is still limited, and further research is needed to elucidate these pathways and their broader implications for plant resilience under heat stress.

8.4.1.5 Melatonin under Heavy Metal Stress

Increasing levels of heavy metal pollutants are a significant environmental concern, as they cause stress and toxicity in plants, ultimately affecting the ecosystem. Melatonin has emerged as an effective tool for shielding plants from metal toxicity. For example, pre-sowing treatment provide defense to red cabbage (*Brassica oleracea rubrum*) seedlings from Cu⁺² toxicity ([Posmyk et al., 2009](#)), while foliar scattering of melatonin deliberated resistance to cadmium stress in *Nicotiana tabacum* ([Wang et al., 2018](#)). The improved plant recital observed after including upregulation of melatonin biosynthetic genes, proves that variation in melatonin levels in plants will be promising strategy for developing crops with enhanced tolerance to heavy metal stress. Melatonin primarily boosts resistance against heavy metal toxicity by increasing antioxidant activity. For instance, exogenous melatonin treatment triggered the efficacy of antioxidant enzymes, thus decreased ROS production in wheat seedlings under aluminum stress.

These findings highlight melatonin's potential as a defensive agent against heavy metal toxicity, suggesting its broader application as a source for refining plant resilience to environmental stresses caused by pollution. Melatonin plays a crucial importance in refining redox homeostasis in plants facing metal toxicity, particularly through its involvement in nitric oxide (NO) signaling. NO is known to improve plant tolerance to toxic metals like cadmium and lead through reducing oxidative stress. It has been reported that melatonin-mediated NO signaling helps plants cope with these stresses by mitigating the disruption due to ROS ([Kaya et al., 2019](#)). Additionally, melatonin production itself is influenced by NO, particularly under metal toxicity, suggesting a close relationship between melatonin and NO signaling ([Lee et al., 2017](#)). Beyond enhancing antioxidant defenses, melatonin also helps plants mitigate heavy metal toxicity by assisting the elimination of heavy metals inside cells. Several studies have highlighted that melatonin reduces cadmium absorption in plants like *Arabidopsis* by moderating the expression of heavy metal transporter genes. These include genes related to ABC transporters and PCR2, which are involved in the movement and compartmentalization of toxic metals ([He et al., 2004](#)). Further research proves that melatonin contributes to heavy metal stress resistance in plants by controlling multiple mechanisms. These include enhancing antioxidant defense systems, promoting NO signaling, and modulating the transport and sequestration of heavy metals, making melatonin a promising tool for improving plant resilience to heavy metal contamination. The potential use of melatonin in phytoremediation efforts, particularly in pollutant-tolerant plants like *Water hyacinth* (*Eichhornia crassipes*), holds significant promise. As an aquatic plant capable of surviving in highly polluted environments, *Water hyacinth* is often used for removing pollutants such as phosphorus, pesticides, and heavy metals from

industrial and agricultural wastewater ([Rezania et al., 2015](#)). Its ability to thrive in contaminated conditions is partly due to the high melatonin levels naturally present in the plant, which help it cope with oxidative stress and metal toxicity ([Tan et al., 2007](#)).

Further it has been proved that it can enhance plant resistance to contaminants, making it a valuable tool for improving phytoremediation processes. For example, [Tan et al. \(2007\)](#) demonstrated that melatonin application in copper-enriched soil tremendously improved the viability of *Pisum sativum* (pea) plants. Similarly, [Tal et al. \(2011\)](#) found that exposure to heavy metals like cadmium, lead, and zinc enhanced melatonin contents in algae, and increased metal-stimulated disruption to seedlings. These findings suggest that melatonin's ability to promote plant growth and alleviate toxicity could assist to extended well-organized phytoremediation, as plant development rates and contaminant tolerance are key factors in the effectiveness of this process. Incorporating melatonin into phytoremediation strategies could therefore enhance the ability of plants to detoxify polluted environments, especially in aquatic or metal-contaminated soils, providing a sustainable method for addressing environmental pollution.

Melatonin's capacity to enhance pollutant mobilization, preoccupation, and exclusion in plants offers significant potential for its application in phytoremediation. Studies, such as those by Cao et al. ([2018](#)), have shown that melatonin treatment can increase the amount of key compounds like glutathione and phytochelatins in plants. These compounds play crucial roles in chelating heavy metals like Cu^{2+} , promoting their sequestration in vacuoles and trapping them in cell walls. This process limits the toxic accumulation of heavy metals in the cytoplasm, thereby protecting the plant and reducing metal toxicity. Melatonin's effects have been observed not only in copper but also in increasing the production of other toxic metals

such as cadmium, arsenic, mercury, and lead in various plant species. This suggests that melatonin could be especially beneficial when applied to hyper-accumulating plants-those that naturally accumulate high levels of contaminants offering an innovative approach to phytoremediation. In these plants, melatonin could potentially boost the plant's ability to uptake and detoxify heavy metals, thus improving the recovery of polluted soils.

The concept of using melatonin as a phytoremediation bioagent is promising, especially in soils enriched with heavy metals or other pollutants. A normalized concept illustrating the importance of melatonin in shielding plants in contrast to abiotic stress would show melatonin's protective effects through enhanced antioxidant activity, modulation of metal transporter genes, and facilitation of pollutant sequestration in plants. Heavy metals, while essential for plant metabolism in trace amounts (e.g., iron, copper, molybdenum, and nickel), can be toxic at higher concentrations, hindering plant growth and yield. In contrast, metals like arsenic, cadmium, and lead are not required by plants and are highly detrimental to their development. These toxic heavy metals disrupt several physiological processes, including photosynthesis and free radical scavenging efficacy, and can impair carbohydrate metabolism and the Calvin cycle. Furthermore, the accumulation of reactive oxygen species (ROS) during heavy metal stress inhibits root growth and accelerates leaf senescence, ultimately damaging the plant.

Exogenous role of phyto-melatonin is reported to alleviate the drastic impacts of heavy metal toxicity by enhancing the plant's physiological and biochemical responses. Studies have demonstrated that phyto-melatonin improves plant productivity in heavy metal stress by restoring nutrient amount, redox potential, and osmotic balance. For instance, melatonin treatment has been found to improve seed germination in crops like

cucumber and red cabbage, and enhance free radical scavenging efficacy in soybeans under stress conditions. Melatonin's protective role also involves reducing the translocation of heavy metals within plants. It overexpress genes related with melatonin production, such as the HSfA1a gene, which is known to enhance resistance to cadmium toxicity in tomato plants. This suggests that melatonin can mitigate heavy metal-induced toxicity by promoting the plant's internal defense mechanisms. Other than these promising research, the precise pathways through which melatonin influences the expression of genes responsible for its biosynthetic pathways, particularly under heavy metal stress, still require further investigation. Expanding our understanding of how these genes interact in various crops could lead to more efficient use of melatonin in agricultural practices to enhance plant tolerance to heavy metals.

Heavy metals, like Zn, Cd, Fe and Cu, are necessary for plant growth and metabolism in trace amounts. However, when their concentrations exceed a certain threshold, they become toxic, hindering plant development and reducing yield. Heavy metal stress negatively impacts photosynthesis, enzyme activities involved in the Calvin cycle, and carbohydrate metabolism, all of which are critical for plant physiological development ([Hasan et al., 2019](#)). Additionally, excessive accumulation of ROS during heavy metal stress damages plant cells, particularly affecting root growth and promoting leaf senescence, which can ultimately reduce overall plant vitality.

Recent research proves that the exogenous role of melatonin can help plants cope with heavy metal stress by enhancing their morphophysiological activities, improving growth and yield. Melatonin not only triggers the synthesis of endogenous melatonin in plants but also helps build tolerance to heavy metal toxicity. One of the ways melatonin exerts its

protective effects is by boosting antioxidant enzyme efficacy and stimulating the ascorbate-glutathione pathway, which has crucial importance in mitigating oxidative stress caused by ROS accumulation. In wheat plants exposed to cadmium (Cd) toxicity, the role of exogenous melatonin was found to increase the levels of its endogenous level, which in turn enhanced root and shoot growth despite the toxic outcomes of Cd. It proves that melatonin is crucial for improving plant tolerance to heavy metal stress by enhancing metabolic processes and strengthening antioxidant defenses. The increase of endogenous melatonin has tremendous importance in mitigating cadmium (Cd) toxicity in plants by balancing hydrogen peroxide (H_2O_2) homeostasis and stimulating free radical scavenging defense mechanism. For instance, in wheat, melatonin helps to restore redox balance and protect cells from oxidative damage induced by Cd stress. Additionally, melatonin enhances H^+ -ATPase activity, increases the content of phytochelatin and glutathione, and facilitates the sequestration of Cd in plant cells, all of which contribute to mitigating Cd toxicity in tomatoes ([Hasan et al., 2015](#)).

Melatonin also influences sulfur biosynthesis, which is vital for plant resistance to Cd stress. In tomato plants, melatonin deficiency leads to reduced sulfur amount and enhanced Cd synthesis, underlining the importance of melatonin in maintaining sulfur homeostasis for heavy metal detoxification ([Hasan et al., 2019](#)). Moreover, the overexpression of its genes increase resistance of Arabidopsis plants to heavy metal stress, suggesting that modulating melatonin levels through genetic engineering could improve stress resistance in other crops as well. In rice, various gene families, including NRAMP, HMA, MTP, YSL, and ZIP, are involved in heavy metal stress responses. These genes play roles in reducing the uptake and accumulation of heavy metals, such as Cd, in plant tissues, ultimately

contributing to better plant health and reduced metal toxicity. However, the detailed molecular pathways through which melatonin regulates these genes, as well as its overall role in improving morphophysiological and biochemical activities under heavy metal stress in crops like cotton, rice, and others, remain poorly understood and warrant further investigation.

8.4.1.6 Ultraviolet Stress

Ultraviolet (UV) radiation, particularly due to the depletion of the ozone layer, is increasingly harmful to plant growth and productivity. Elevated UV levels can severely hinder plant morpho-physiological activities, ultimately reducing crop yields. For example, increased UV radiation reduces the production of key photosynthesis proteins, including chlorophyll a/b binding proteins, having importance in photosynthetic efficiency and overall plant health ([Ahmad et al., 2023](#) and [Bera et al., 2022](#)).

Phyto-melatonin, a potent antioxidant, has been shown to mitigate the drastic impacts of UV radiation on plants. In *Arabidopsis thaliana*, phyto-melatonin reduced UV-induced stress by regulating various signaling pathways. It enhances the expression of transcription factors, including HY5, HYH, and RUP/2, which are responsible for plant's stimuli to UV stress. Additionally, phyto-melatonin influences the ubiquitin-degrading enzyme COP1, which plays a role in modulating UV stimuli in plants ([Wei et al., 2019](#) and [Yao et al., 2021](#)).

One of the key ways in which phyto-melatonin protects plants from UV radiation is by regulating antioxidant defense systems. It assist to neutralize ROS generated by UV exposure, preventing oxidative damage. For instance, in *Malus hupehensis* (a type of apple), phyto-melatonin control drastic outcomes of UV stress on photosynthetic apparatus including chlorophyll content, leaf membrane integrity, stomatal conductance, and gas exchange, thus improving overall plant physiological development ([Wei et](#)

[al., 2019](#)). The phyto-melatonin enhances plant resilience to UV stress, the underlying mechanisms-specifically how it regulates various signaling pathways and the extent of its impact across different crops are still not fully understood. Further research is needed to elucidate these processes and to apply phyto-melatonin more effectively in crop protection against UV radiation.

8.4.1.7 CO₂ Stress

Phyto-melatonin has crucial significance in regulating redox balance and increasing plant tolerance under nitrogen dioxide (NO₂) stress. NO₂ is absorbed by plants from the atmosphere, where low concentrations can be beneficial by providing nitrogen for growth or acting as a signal molecule to promote crop productivity. However, elevated levels of NO₂ can be toxic to plants, causing to the synthesis of ROS and reactive nitrogen species (RNS), which can cause significant oxidative disruption to plant cells ([Takahashi and Morikawa, 2019](#)).

The oxidative stress caused by NO₂ disrupts various biochemical pathways, like photosynthesis and carbon fixation, which in turn affects the plant's metabolism. However, low levels of ROS have action to act as signaling moieties, helping to regulate plant responses to stress if kept in balance with the plant's antioxidant systems ([Abbas et al., 2018](#); [Wang et al., 2020](#)).

Phyto-melatonin helps mitigate NO₂-stimulated oxidative stress by regulating key biochemical pathways. It progresses the content of proteins engaged in thioredoxin-peroxiredoxin and ascorbic acid-glutathione pathway, which are involved in maintaining redox balance in plant cell ([Wang et al., 2022](#)). Additionally, phyto-melatonin enhances the polyamine biosynthetic cycle by increasing the expression of important enzymes like SAMS1, SAMS2, and SAMS3, has main importance in polyamine

synthesis in plant leaves. Polyamines are important for stress tolerance, as they are involved in cellular stabilization, protection against oxidative damage, and regulation of physiological development in adverse environment ([Wang et al., 2022](#)). Phyto-melatonin also influences abscisic acid (ABA) signaling and calcium (Ca²⁺) signaling pathways, which are vital for stress response mechanisms. It regulates calmodulin binding transcription factors like CAMTA12 and NtCaM in calcium signal transduction, which are essential for the plant's adaptive responses to abiotic stress ([Wang et al., 2022](#)). This proves that phyto-melatonin could be a valuable source for improving crop tolerance to NO₂ stress, thus supporting agricultural yield. However, more lot of investigations in needed to understand the defensive role of phyto-melatonin, particularly its role with various abiotic stress signaling pathways and influences overall crop resilience under these conditions.

8.4.2 Importance of Melatonin in Response to Biotic Stresses

Earlier reports proved that melatonin fortifies plants against biotic stress ([Arnao and Hernández-Ruiz, 2014, 2015](#)), which causes serious yield and economic losses in crops worldwide. Despite the well-known role of melatonin as a biocide that inhibits the proliferation of microbes, the role of melatonin in the pathogenicity of agricultural microbes has been reported [Yin et al., \(2013\)](#) suggested that melatonin-treated apple plants have appearance of disrupted leaves and delayed pathogen extension in contrast to the control. Research on pathogenesis-related proteins, it has been reported that phenylalanine ammonia-lyase (PAL), chitinase, and β -1,3-glucanase assist in disease resistance. Further reports proved that melatonin assist plants against pathogen invasion by controlling plant innate immunity

to stimulate defense mechanism ([Shi et al., 2015b](#)). A *SNAT* knockout *Arabidopsis* mutant proved decreased endogenous melatonin amount and shown strappingly stifled defense gene expression with enhanced susceptibility to *P. syringae* than the wild type ([Lee et al., 2015](#)). Further, melatonin treatment recovered the tolerance of cotton to *Verticillium dahlia* by assisting metabolic flux into the phenylpropanoid, mevalonate, and gossypol pathways, results in lignin and gossypol synthesis ([Li et al., 2019](#)). It also enhance capacity and reduce the treatment of synthetic pesticides needed for crop resistance (Casado-Zapico et al., [2010](#)). Therefore, the combined application of melatonin including other fungicides proves a new bio-control pathway for agricultural prospectives.

Melatonin is also known in plants to fight against biotic stress, particularly in stimulating innate immunity against microbes attack. The defense mechanisms activated by melatonin are tightly connected to the well-established plant immune signaling cycles involving salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). These phytohormones are central to both pathogen-associated molecular pattern (PAMP) - and effector-stimulated immunity ([Glazebrook, 2005](#)). Research has shown that melatonin-triggered plant immunity is impaired in mutants that are imperfect in SA and ethylene signaling, suggesting that these pathways are crucial for the full expression of melatonin's defense functions ([Lee and Back, 2019](#)). Specifically, melatonin has been found to upregulate genes associated with SA, JA, and ET signaling cycles, important for plant's immune response. A study of *Arabidopsis* treated with melatonin revealed significant upregulation of genes involved in these pathways, highlighting the close interaction between melatonin and these critical phytohormones ([Lee et al., 2014](#)).

Further evidence of melatonin's role in immune signaling comes from research using the *Arabidopsis*–*Pseudomonas syringae* plant–bacterial pathogen interaction system. In this system, melatonin enhanced the expression of pathogen-related genes that are dependent on SA and ethylene, but these effects were not observed in mutants lacking functional SA or ethylene pathways ([Lee et al., 2014](#)). Additionally, SNAT (serotonin N-acetyltransferase) knockout *Arabidopsis* mutants, which are deficient in melatonin biosynthesis, showed reduced SA amount and enhanced vulnerability to *P. syringae*. This was because of failure in inducing defense genes like PR1, ICS1, and PDF1.2, which are crucial for pathogen resistance ([Lee et al., 2015](#)). An early response to pathogen attack involves an oxidative burst, which appears to trigger an increase in melatonin levels within plant cells. This suggests that melatonin might not only act as an antioxidant but also as a signaling moiety that enhances the plant's defense mechanisms in response to biotic stress ([Arnao and Hernández-Ruiz, 2015](#)). Overall, melatonin contributes significantly to plant immunity, particularly in its coordination with traditional signaling pathways like SA, JA, and ethylene, providing plants with a more robust defense against microbial pathogens.

Melatonin's role in plant immunity extends beyond its interactions with phytohormones like salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). It is increasingly recognized that other regulators, such as NO, ROS, and mitogen-activated protein kinases (MAPKs), are also crucial in melatonin-mediated immune responses. Studies have shown that NO, ROS, and MAPKs are key components in the early and late stages of plant defense, helping coordinate a plant's response to pathogen attack. The interaction between melatonin and ROS/NO signaling cycles is a key aspect of melatonin's function in biotic defense. ROS and NO are synthesized

during pathogen infection and are involved in various defense mechanisms, like triggering of defense-related genes and the triggering of an oxidative burst. Melatonin enhances this response by interacting with NO and ROS signaling networks. For example, melatonin has been shown to improve resistance to band virus infection in rice through a NO-dependent cycles ([Lu et al., 2019](#)). In Arabidopsis, it works in concert with NO to overexpress several SA- co-related genes, which helps confer battle to pathogens ([Shi et al., 2015b](#)).

This cooperation between melatonin, NO, and ROS highlights the complex signaling network that orchestrates plant immunity. Melatonin has both a signaling molecule and an antioxidant, increasing the plant's ability to combat biotic stress by regulating ROS and NO levels, as well as interacting with key immune pathways such as those involving SA. This underscores the potential of melatonin as a natural agent in enhancing plant resistance to microbial pathogens and improving overall plant health ([Figure 8.3](#)).

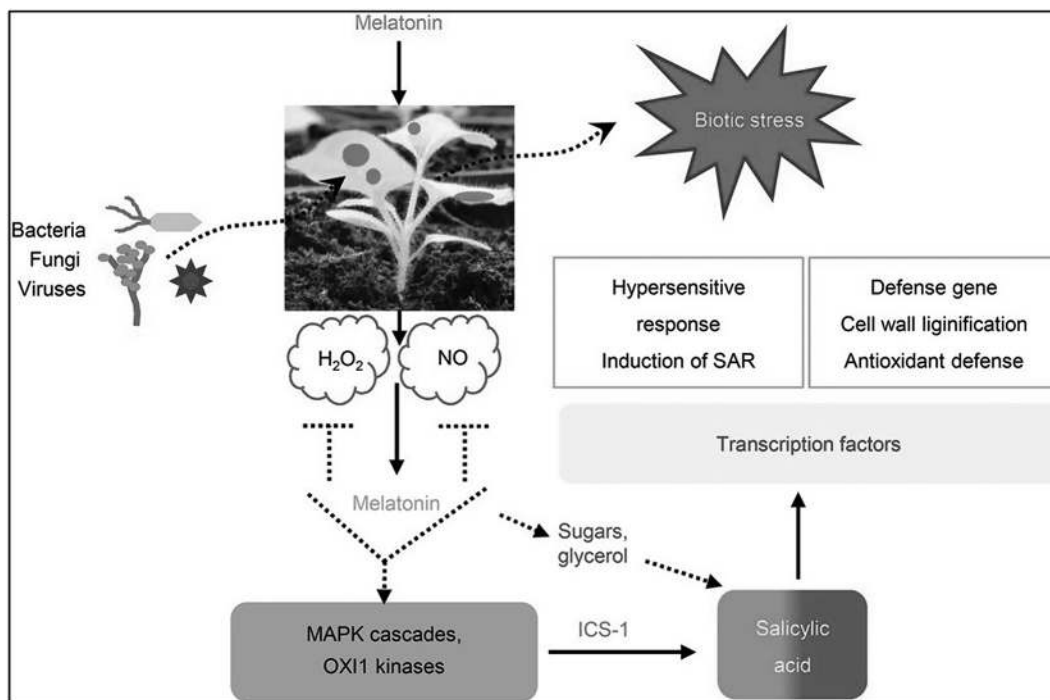


FIGURE 8.3 The role of melatonin in protecting plants from biotic stress: signal transduction and mechanisms.

The involvement of MAPKs (Mitogen-Activated Protein Kinases) in melatonin-mediated immunity in plants highlights the complex signaling network that regulates plant defense responses. MAPKs serve as critical molecular devices of stress signals and are integral to melatonin's role as a signaling compounds that stimulates various defense pathways. Current studies by Lee and Back ([2016](#)) revealed that melatonin activates MAPK cascades by signaling through MKK4/5/7/9 kinases, which phosphorylate and stimulate MPK3 and MPK6. These stimulated MAPKs, in turn, activate key transcription factors, leading to the expression of defense-correlated genes and the triggering of plant defense responses.

The interaction of melatonin with these signaling molecules creates a finely tuned system that helps plants adapt to biotic stress. In the *Arabidopsis-Pseudomonas syringae*, melatonin has been shown to function provides resistance in defense through signaling pathways, with its activity stimulated by an amalgamation of hydrogen peroxide (H₂O₂) and nitric oxide (NO). This process involves MAPK cascades and oxidative signal-inducible 1 (OXI1) kinases, which are accountable for initiating the innate immunity response triggered by melatonin ([Arnao and Hernández-Ruiz, 2018](#)).

The present sympathetic of melatonin's role in plant immunity suggests that it operates through a combination of phytohormonal pathways, having those associated to salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and NO, including MAPK signaling. This cooperation enhances the plant's defense pathways against pathogens. However, much remains to be explored, especially concerning the connections between melatonin and other components of plant immune systems. Further research, particularly

through genomic and proteomic tactics, will be essential to undo the detailed mechanisms by which melatonin interacts with SA, JA, ethylene, NO, and their respective moieties. Understanding these interactions could lead to more effective strategies for improving plant resistance to pathogens.

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9

Interplay between Phytomelatonin with Gasotransmitters under Environmental Extremes

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DOI: [10.1201/9781003527633-9](https://doi.org/10.1201/9781003527633-9)

9.1 INTRODUCTION

Due to their sessile nature, plants encounter a number of strenuous and stressful environmental factors during their life cycles, including abiotic stressors like drought, salinity, floods, temperature extremes, and heavy metals, among others. As a result, agricultural crop productivity is greatly reduced and limited globally ([Khan et al. 2021](#)). Nonetheless, these stressors cause a variety of plant reactions, such as modifications to cellular metabolism, gene expression, growth rates, and crop yields. Various mechanisms have been established by plants to deal with these stresses, and they adapt to these conditions by causing a number of distinct physiological, cellular, and molecular changes ([Yadav et al. 2020](#), [Zhou et al. 2021](#); [Kumari et al. 2024](#)).

Phytohormones are one of the defensive molecules generated by plants to mitigate the detrimental consequences of abiotic stresses ([Nazir et al. 2023](#)).

In recent years, melatonin (Mel) has long been recognized as an animal hormone and identified in all organisms including bacteria, algae, fungi, insects and invertebrates ([Samanta and Roychoudhury 2023](#); [Yusuf et al. 2024](#)). Subsequently, it was found to be a crucial component of plant stress resilience and defense-related studies, found in every part of plants such as leaves, roots, shoots, fruits and seeds ([Samal et al. 2024](#)). The word ‘phytomelatonin’ was first established in 2004 ([Blask et al. 2004](#); [Zhao et al. 2021](#); [Wang et al. 2022](#)). The discovery of the first phytomelatonin receptor, candidate G-protein-coupled receptor 2/phytomelatonin receptor (CAND2/PMTR1), in *Arabidopsis thaliana* (Arabidopsis), demonstrated that phytomelatonin is a novel phytohormone ([Wei et al. 2018](#)). However, it is now generally accepted that phytomelatonin protects plants from a variety of stresses ([Arnao et al. 2022](#)). Auxin, gibberellin, cytokinin, abscisic acid, ethylene, brassinosteroids, jasmonic acid, salicylic acid, and plant growth regulators like nitric oxide (NO), polyamines, γ -aminobutyric acid (GABA), and serotonin have all been shown to interact with phytomelatonin in a way that regulates stress alleviation and maintains basal growth in harsh environmental conditions ([Samanta and Roychoudhury 2023](#)).

Additionally, phytomelatonin is involved in various physiological activities including the regulation of photo-responses, circadian rhythm, root growth, seed germination and leaf senescence (Arnao and Hernández-Ruiz [2020](#), [2021](#); [Tyagi et al. 2023](#)). It is very ascertained that phytomelatonin act as a strong ROS-scavenging molecule, emerging as a ‘phyto-protectant’ that safeguards critical of several environmental stresses like salt stress, heat, cold, drought, heavy metals, low nitrogen and UV-B radiation ([Kaya et al. 2020](#); [Turk and Genisel 2020](#); [Bidabadi et al. 2020](#); [David et al. 2021](#); [Yan et al. 2021](#); [Wang et al. 2021](#); [Haskirli et al. 2021](#);

[Fan et al. 2022](#); [Samanta and Roychoudhury 2023](#)). Additionally, phytemelatonin can improve antioxidant enzyme activity, preserve mitochondrial homeostasis, and make oxygen physiologically acceptable. It also acts as the first line of defense against reactive oxygen species (ROS) and reactive nitrogen species (RNS) ([Mannino et al. 2021](#)). The ability of phytemelatonin to directly scavenge hydrogen peroxide (H_2O_2), hydroxyl radical ($HO\cdot$), peroxyxynitrite anion ($ONOO^-$), hypochlorous acid ($HOCl$), singlet oxygen ($^1O_2$), and superoxide anion (O^{2-}) has been reported, thereby minimizing stress-associated damages ([Khanna et al. 2023](#)). Additionally, it was observed that several crop cultivars high in phytemelatonin show higher stress tolerance due to fewer ROS levels and therefore, reduced oxidative stress ([Khan et al. 2023](#)).

Unquestionably, signaling molecules are no longer limited to molecules with a solid or liquid base. In 2003, a new idea known as the “gasotransmitter” was put forth, which referred to endogenously produced gaseous molecules like nitrous oxide (NO), carbon monoxide (CO), and hydrogen sulphide ([Wang 2003](#)). These gaseous signaling molecules are now widely acknowledged to play essential and vital roles in a variety of physiological processes ([Feng et al. 2021](#)). Despite the fact that the idea of gasotransmitters in plants is relatively new, our knowledge of their functions is growing quickly. Gasotransmitters have been shown to control plant growth and improve resistance to abiotic stressors in plants. A substantial body of evidence indicates that phytemelatonin is predominantly synthesized within the mitochondria and chloroplasts. The synthesis of phytemelatonin is a complicated process that is coherent with the metabolism of other phytohormones. Several studies have observed a correlation between phytemelatonin and endogenous auxin. For example, the exogenous application of phytemelatonin has been shown to increase

endogenous levels of phytohormones in comparison to untreated plants in the species *Brassica juncea* (mustard) ([Park et al. 2021](#)) and *Plantago ovata* (blond plantain) ([Chakraborty and Raychaudhuri 2025](#)). On the other hand, endogenous phytomelatonin has been shown to lower auxin content in plants based on genetic data. For example, endogenous phytomelatonin levels were higher in *Arabidopsis* seedlings overexpressing apple acetylserotonin O-methyltransferase (ASMT), whereas endogenous auxin content was lower ([Zuo et al. 2014](#)). These findings suggest that the synthesis of phytomelatonin and auxin may be competitive, implying that changes in auxin content could influence phytomelatonin metabolism in plants. Given that auxin biosynthesis may be regulated by certain gasotransmitters, such as hydrogen molecule (H_2) ([Wu et al. 2020](#)), H_2S ([Zhang et al. 2020](#)), and NO ([Sanz et al. 2015](#)), a potential interrelationship between phytomelatonin and gasotransmitters may exist. Gasotransmitters are molecules that perform key parts in regulating plant development and stress responses. The principal gasotransmitters include NO, CO, and H_2S ([Khatoon et al. 2023](#); [Martínez-Lorente et al. 2022](#)). However, NO and H_2S are considered air pollutants and hazardous to human health. Despite all, these gases are toxic in high concentrations but beneficial at low concentrations. NO, in particular, a potent signaling molecule found in mitochondria and chloroplasts, similar to phytohormones ([Khan et al. 2021](#)). These sites point to a potential interaction between NO and phytomelatonin. It has been established that treating plants with phytomelatonin can change their endogenous NO levels and vice versa. Phytomelatonin can either induce NO production or scavenge NO and can increase NO levels by upregulating NO synthase (NOS) gene expression. Additionally, phytomelatonin has been found to scavenge various RNS, including $ONOO^-$, and peroxynitrous acid ([Mansoor et al. 2024](#)).

Phytomelatonin can also reduce excessive RNS generated in both chloroplasts and mitochondria, and provides a potential on-site form of protection ([Wang et al. 2022](#)).

Sulfur (S) is an essential component of numerous biological compounds including amino acids (cysteine and methionine), as well as certain cofactors, polysaccharides, lipids, and iron–sulfur clusters, making it vital for all living organisms. H₂S, the third novel gaseous signaling molecule, found after NO and CO, plays a crucial part in enhancing plant resilience to abiotic stress ([Kumari et al. 2023](#)). The enzyme sulfite reductase (SiR) is a key player in the production of H₂S in plants. The chloroplast is widely considered to be the exact location of SiR at the subcellular level ([Wang et al. 2022](#)). SiR is pivotal for plants to assimilate exogenously absorbed sulfates, as its substrate is sulfite ([Oshanova et al. 2021](#)). Another key enzyme, L-/D-cysteine desulphydrase (L/DCD) is primarily responsible for the degradation of cysteine to produce H₂S ([Thakur & Anand 2021](#)). In *Arabidopsis*, sir mutants exhibit significantly retarded growth, indicating that SiR is a critical enzyme in the reductive sulfate assimilation pathway ([Forieri et al. 2021](#)). The synthesis of H₂S in plants may be affected by phytomelatonin levels. For example, phytomelatonin application has been shown to increase the H₂S content in *Capsium annuum* (pepper) plants and *Triticum aestivum* (wheat) ([Kaya et al. 2020](#); [Iqbal et al. 2021](#)). Similar findings were observed in *Cucumis sativus* (cucumber) and *Solanum lycopersicum* (tomato) where phytomelatonin enhanced the activities of LCD and DCD ([Mukherjee and Bhatla 2021](#); [Sun et al. 2021](#)). Furthermore, exogenous H₂S treatments have been found to significantly elevate endogenous phytomelatonin content ([Wang et al. 2021](#)). This chapter will explore the relationship between phytomelatonin and gasotransmitters

particularly NO and H₂S in the context of different abiotic stresses ([Figure 9.1](#)).

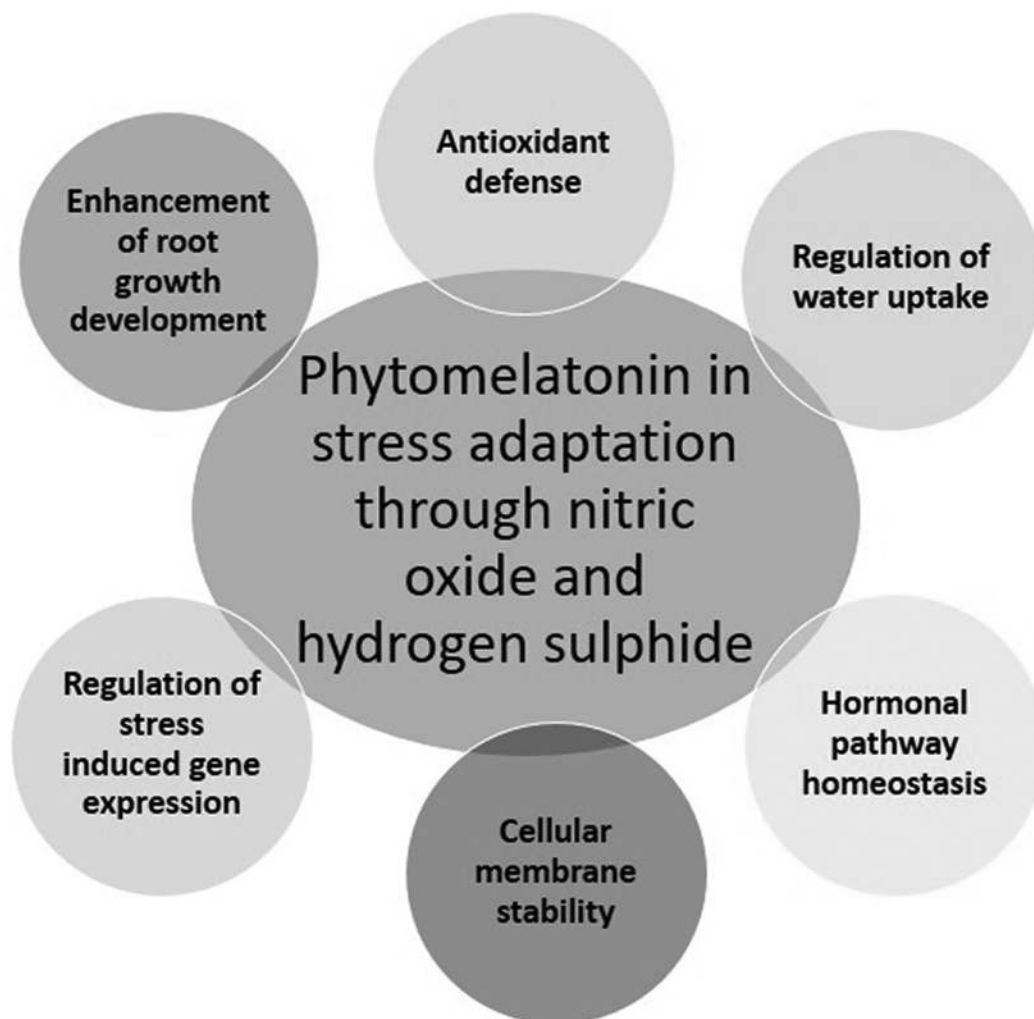


FIGURE 9.1 A general overview of effect of phytomelatonin with nitric oxide (NO) and hydrogen sulphide (H₂S) during abiotic stresses. [↗](#)

9.2 PHYTOMELATONIN AND GASOTRANSMITTERS UNDER ENVIRONMENTAL CONSTRAINTS

9.2.1 Phytomelatonin and Gasotransmitters under Salt Stress

9.2.1.1 Nitric Oxide (NO)

Irrational irrigation and over-fertilization aggravate salt stress, a common environmental problem that restricts crop growth by interfering with photosynthesis and antioxidant defense systems, which lowers agricultural output. Its effects include increasing senescence, slowing development, preventing seed germination, and even causing cell death ([Sun et al. 2021](#); [Wang et al. 2022](#)). It is therefore evident that enhancing plant resilience to salt stress represents a crucial avenue of investigation.

Phytomelatonin is one of the main biochemical and molecular mechanisms that plants have developed to react to salt stress at the cellular and whole-plant levels. By controlling the production and activity of antioxidant enzymes, phytomelatonin has been shown in numerous studies to improve plant resistance to salt stress ([Samanta and Roychoudhury 2023](#)). Moreover, phytomelatonin plays a role in maintaining ion homeostasis under conditions of high salt salinity, reducing the uptake of excess sodium (Na^+) and chloride (Cl^-) by the plants. Phytomelatonin has been demonstrated to safeguard total chlorophyll content and offset the decline in net photosynthetic rate induced by salt stress in *Avena sativa* (oat) ([Gao et al. 2019](#)) and tomato seedlings ([Altaf et al. 2021](#); [Samanta and Roychoudhury 2023](#)). Similarly, gasotransmitters have been demonstrated to possess analogous functions to those of phytomelatonin. For instance, the interaction between phytomelatonin and NO signaling pathways can control seedling growth via decreasing tyrosine nitration of proteins, and reducing peroxynitrite content and maintaining the oxidative equilibrium during salt stress in sunflower seedlings ([Arora and Bhatla 2017](#)). Increased phytomelatonin content in rapeseed (*Brassica napus*) causes a signaling

cascade in response to salt stress, which raises nitrate reductase (NR)- and NO-associated 1 (NOA1)-dependent NO concentrations. This improves salt stress tolerance by restoring ion and redox homeostasis ([Zhao et al. 2018](#)). Studies also revealed that exogenous application of phyto melatonin (150 μM) improved nitrogen (N) metabolism and endogenous NO content in tomato seedlings under NaCl stress by upregulating the expression of enzymes participating in N metabolism ([Ghorbani et al. 2023](#)). Also, in sunflower (*Helianthus annuus*) seedling exposed to 120 mM NaCl stress revealed phyto melatonin regulated CAT activity by interacting with NO, thereby, scavenging H_2O_2 ([Kaur & Bhatla 2023](#)). Studies by [Yin et al. \(2022\)](#) demonstrated that exogenous phyto melatonin (100 μM) supplementation induced isoflavone accumulation and improved growth in soybean plants exposed to salt stress (60 mM). Also, phyto melatonin increased the activity of NR which subsequently led to an increase in NO content ([Figure 9.2](#) and [Table 9.1](#)).

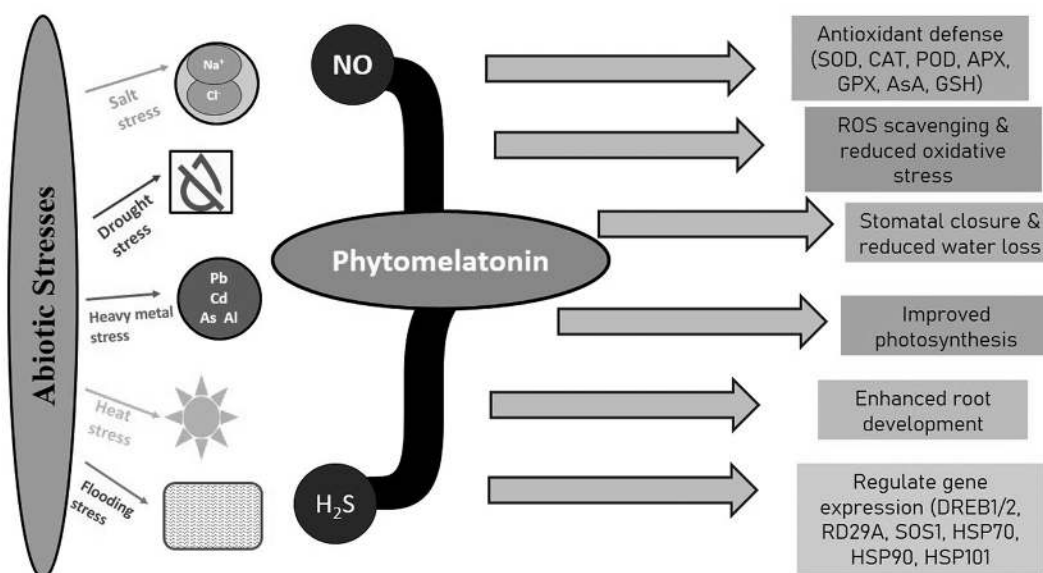


FIGURE 9.2 The relationship between phyto melatonin and nitric oxide (NO) and hydrogen sulphide (H_2S) in mitigating abiotic stresses, such as salinity, heat, drought, heavy metals and flooding. Phyto melatonin and nitric oxide (NO) and hydrogen

sulphide (H_2S) work synergistically to enhance plant stress tolerance. **Abbreviations:** APX: Ascorbate Peroxidase; AsA: Ascorbic Acid; CAT: Catalase; DREB: Dehydration-Responsive Element-Binding Protein; GPX: Glutathione Peroxidase; GSH: Glutathione; H_2S : Hydrogen Sulphide; HSP: Heat Shock Proteins; NO: Nitric Oxide; NOS: Nitric Oxide Synthase; NR: Nitrate Reductase; NPR1: Nonexpressor of Pathogenesis-Related Genes 1; POD: Peroxidase; RD29A: Responsive to Desiccation 29A; ROS: Reactive Oxygen Species; SOD: Superoxide Dismutase; SOS1: Salt Overly Sensitive 1. [!\[\]\(b39c89771cd6fb2128a8c57aa7d97f9a_img.jpg\)](#)

TABLE 9.1 Potential of Phytomelatonin with Nitric Oxide (NO) in Elu
Responses to Develop Abiotic Stress Resilience in Crop Pla

Botanical Name	Type of Stress (Conc.)	Phytomelatonin (Conc.)	Nitric Oxide (Conc.)	Effect
<i>Helianthus annuus</i> (Sunflower)	Salt stress; 120 mM	15 μ M	SNP; 250 μ M	Regulate seedling growth, decreased peroxynitri content, tyrosine nitration of proteins an maintain oxidative homeostasi
<i>Brassica napus</i> (Rapeseed)	Salt stress; 200 mM	1 μ M	10 μ M	Elevated N levels and maintain io homeostasi
<i>Arabidopsis thaliana</i> (Arabidopsis)	Drought stress	Endogenous	Endogenous NO generation	Stomatal closure, mutants dic not show N and ROS formation v PMTR1 mediated signaling

Botanical Name	Type of Stress (Conc.)	Phytomelatonin (Conc.)	Nitric Oxide (Conc.)	Effect
<i>Capsicum annuum</i> (Pepper)	Drought stress	0.1 mM	Endogenous NO generation	Enhanced nitrogen metabolism enzymes such as NR, NiR, Gs, GOGAT; increased antioxidant defense system
<i>Glycine max</i> (Soybean)	Drought stress	100 μ M	SNP; 100 μ M	Enhanced plant biomass, photosynthesis efficiency, water content while reducing ROS accumulation through decreased lipid peroxidation; improved antioxidant enzyme activities, and decreased abscisic acid (ABA) concentration

Botanical Name	Type of Stress (Conc.)	Phytomelatonin (Conc.)	Nitric Oxide (Conc.)	Effect
<i>Medicago sativa</i> (Alfalfa)	Drought stress induced by withholding water for 7 days	10 μ M	Endogenous NO generation	Higher amounts of secondary metabolites, photosynthesis, amino acid production, carbon fixation, and differentially expressed proteins (DEPs)
<i>Zea mays</i> (Maize)	Lead (Pb); 0.1 mM	0.10 mM	Endogenous NO generation	Increased H_2O_2 and MDA levels suppressed plant growth; increased F levels were seen when treated with cPTIO; NO scavenger

Botanical Name	Type of Stress (Conc.)	Phytomelatonin (Conc.)	Nitric Oxide (Conc.)	Effect
<i>Triticum aestivum</i> (Wheat)	Cadmium (Cd); 100 μ M	100 μ M	Endogenous NO generation	Improved p growth, enhanced l Ca^{2+} and K content, an reduced M H_2O_2 , and Cd levels
<i>Catharanthus roseus</i> (Periwinkle)	Cadmium (Cd); 200 mg/kg	100 μ M	SNP; 200 μ M	Increased POD, CAT activities, increased s biomass, C and b conte reduced electrolyte leakage
<i>Solanum lycoperscium</i> (Tomato)	Aluminium (Al); 148 μ M	150 μ M	Endogenous NO generation	Enhanced enzymatic antioxidati activity, reduced RC increased photosynth capability & increased phytochela

Botanical Name	Type of Stress (Conc.)	Phytomelatonin (Conc.)	Nitric Oxide (Conc.)	Effect
<i>Solanum lycopersicum</i> (Tomato)	Cadmium (Cd); 200 μ M	100 μ M	SNP; 100 μ M	Upregulate gene expression AAO, AAOH, APX1, APX2, DHAR1, DHAR2, MDHAR and GR genes, reduced H ₂ MDA, DH and GGSG
<i>Solanum lycopersicum</i> (Tomato)	Heat stress; (42°C)	100 μ M	Endogenous NO generation	Decreased ROS production, increased antioxidant enzymatic activities, elevates PA, NO, NR and NOS
<i>Spinacea oleracea</i> (Spinach)	Flooding stress	50 and 100 μ M	50 and 100 μ M NO	Enhanced ROS scavenging antioxidant defense sys

Abbreviations: AAO: Aldehyde Oxidase; AAOH: Aldehyde Alcohol Dehydrogenase; APX: Ascorbate Peroxidase; Ca²⁺: Calcium Ion; CAT: Catalase; Chl a: Chlorophyll a; Chl b: Chlorophyll b; Cptio: 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; DHA:

Dehydroascorbate; **DHAR**: Dehydroascorbate Reductase; **GOGAT**: Glutamate Oxaloacetate Transaminase; **GR**: Glutathione Reductase; **Gs**: Stomatal Conductance; **MDA**: Malondialdehyde; **MDHAR**: Monodehydroascorbate Reductase; **NiR**: Nitrite Reductase; **NOS**: Nitric Oxide Synthase; **NR**: Nitrate Reductase; **PAs**: Polyamines; **POD**: Peroxidase; **ROS**: Reactive Oxygen Species; **SNP**: Sodium Nitroprusside

9.2.1.2 Hydrogen sulphide (H₂S)

In addition to NO, phyto melatonin-regulated salt tolerance is also influenced by H₂S. By dramatically raising LCD/DCD activity, phyto melatonin causes the production of H₂S. This, in turn, enhances photosynthetic efficiency, antioxidant enzyme activity, and upregulated antioxidant enzyme gene expression in pepper ([Kaya et al. 2020](#)) and cucumber ([Sun et al. 2021](#)), all of which lead to improved salt tolerance. Hypotaurine, an H₂S scavenger, can partially counteract these positive effects of phyto melatonin, suggesting that H₂S plays a role in downstream signaling of phyto melatonin under salt stress ([Kaya et al. 2020](#)). Research has shown that H₂S helps plants tolerate stress by interacting with NO, H₂O₂, and mitogen-activated protein kinases (MAPKs). For instance, H₂S functions as a downstream signal of H₂O₂ in faba beans (*Vicia faba*) and contributes to the closure of stomata brought on by salt stress ([Ma et al. 2021](#)). Additionally, it was found that phyto melatonin and H₂S worked together to mitigate the growth loss in tomatoes caused by salt stress. In particular, it was discovered that exogenous phyto melatonin therapy helped to regulate early H₂S signaling. By boosting LCD-dependent H₂S generation, phyto melatonin can also lessen electrolytic leakage in tomatoes ([Mukherjee and Bhatla 2021](#)). Additionally, [Siddiqui et al. \(2021\)](#) demonstrated that an H₂S-dependent mechanism is involved in phyto melatonin-mediated salt stress tolerance in tomato seedlings. By lowering oxidative stress through manipulation of ROS formation and

regulation of superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT) activity, this combination action improves the plant's capacity to mitigate the negative effects of salt stress. These results imply that H₂S has a positive regulatory function under abiotic stress and interacts with other signaling molecules. Nevertheless, it is still unclear exactly how H₂S contributes to phyto melatonin-induced salt tolerance in plants ([Figure 9.2](#) and [Table 9.2](#)).

TABLE 9.2 Potential of Phytomelatonin with Hydrogen Sulphide (H₂S)
Multiple Responses to Develop Abiotic Stress Resilience in Crop

Botanical Name	Type of Stress (Conc.)	Phytomelatonin (Conc.)	Hydrogen Sulphide (H₂S) (Conc.)	Effect
<i>Cucumis sativus</i> (Cucumber)	Salt stress; 150 mM	100 µM	Endogenous H ₂ S generation	Elevated L-/cysteine desulphydrase activity, which controls photosynthetic efficiency, antioxidant enzyme activity and gene expression of antioxidant enzymes
<i>Capsicum annuum</i> (Pepper)	Salt stress; 100 mM	100 µM	Endogenous H ₂ S generation	Tolerance induced by L involved with downstream signal cross-talk between NC and H ₂ S
<i>Solanum lycopersicum</i> (Tomato)	Salt stress; 120 mM	30 µM	H ₂ S donor NaHS (100 µM)	Reduced EL Upregulated activity of L cysteine desulphydrase

Botanical Name	Type of Stress (Conc.)	Phytomelatonin (Conc.)	Hydrogen Sulphide (H₂S) (Conc.)	Effect
<i>Solanum lycopersicum</i> (Tomato)	Salt stress; 200 mM	30 µM	Endogenous H ₂ S	Increased L-DES activity reduced oxidative str through modulation ROS genera and induced enzymatic antioxidative defences
<i>Solanum lycoperscium</i> (Tomato)	40% water level	30µM	Endogenous H ₂ S generation	Improved K ⁺ /Na ⁺ , decreased N including lip peroxidation electrolyte leakage, and oxidative damage

Botanical Name	Type of Stress (Conc.)	Phytomelatonin (Conc.)	Hydrogen Sulphide (H₂S) (Conc.)	Effect
<i>Solanum lycoperscium</i> (Tomato)	Six days of water deprivation caused drought stress	30 µM	Endogenous H ₂ S generation	Enhanced H ₂ S accumulation, increased activity of mitochondrial enzymes, antioxidant defense, and expression of HSP17.6 and HSP70
<i>Solanum lycoperscium</i> (Tomato)	Cadmium (Cd); 200 µM	100 µM	SNP; 100 µM	Upregulated gene expression of AAO, AAOH, APX, APX6, DHAR1, DHAR2, MDHAR and GR genes, reduced H ₂ O ₂ , MDA, DHA and GGSG.
<i>Matthiola incana</i> (Stock)	Cadmium (Cd); (50 mg/l)	100 µM	H ₂ S; 1.5 mM	Recovery in growth, photosynthesis, reduced lipid peroxidation, and MDA content

Botanical Name	Type of Stress (Conc.)	Phytomelatonin (Conc.)	Hydrogen Sulphide (H₂S) (Conc.)	Effect
<i>Carthamus tinctorius</i> (Safflower)	Lead (Pb); 50 µM	100 µM	H ₂ S; 100 µM	Reduced MDA, H ₂ O ₂ , LOX activity and increased AsA-GSH cycle and increased phytochelatin
<i>Capiscum annuum</i> (Pepper)	Arsenic (As); 0.1 mM	0.10 mM	Endogenous H ₂ S generation	Increased biomass, chlorophyll content, chlorophyll a/b ratio, antioxidant enzymes' activities, ABA levels, vacuolar accumulation of As, reduced MDA and H ₂ O ₂ levels

Botanical Name	Type of Stress (Conc.)	Phytomelatonin (Conc.)	Hydrogen Sulphide (H₂S) (Conc.)	Effect
<i>Solanum lycoperscium</i> (Tomato)	Chromium (Cr); 25 μ M	30 μ M	Endogenous H ₂ S generation	Improved phytochelatin H ⁺ -ATPase activity, reduced RO levels, electrolyte leakage and lipid peroxidation and improve nitrogen metabolism AsA-GSH c

Botanical Name	Type of Stress (Conc.)	Phytomelatonin (Conc.)	Hydrogen Sulphide (H ₂ S) (Conc.)	Effect
<i>Zea mays</i> (Maize)	Chromium (Cr); 100 µM	100 µM	Endogenous H ₂ S generation	Elevated cell wall polysaccharide content, enhanced peroxidase activity, better antioxidant enzyme activity, higher endogenous H ₂ S levels, increased Cd tolerance, and upregulated L-/D-cysteine desulfhydrase (LCD/DCD) gene expression
<i>Triticum aestivum</i> (Wheat)	Heat stress; (25°C)	100 µM	Endogenous H ₂ S generation	Reduced oxidative stress via decreasing TBARS and H ₂ O ₂ , increased photosynthesis and carbohydrate metabolism

Abbreviations: **AAO**: Aldehyde Oxidase; **AAOH**: Aldehyde Alcohol Dehydrogenase; **APX**: Ascorbate Peroxidase; **Ca²⁺**: Calcium Ion; **CAT**: Catalase; **Chl a**: Chlorophyll a; **Chl b**: Chlorophyll b

b; **Cptio**: 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; **DHA**: Dehydroascorbate; **DHAR**: Dehydroascorbate Reductase; **GOGAT**: Glutamate Oxaloacetate Transaminase; **GR**: Glutathione Reductase; **Gs**: Stomatal Conductance; **MDA**: Malondialdehyde; **MDHAR**: Monodehydroascorbate Reductase; **NiR**: Nitrite Reductase; **NOS**: Nitric Oxide Synthase; **NR**: Nitrate Reductase; **PAs**: Polyamines; **POD**: Peroxidase; **ROS**: Reactive Oxygen Species; **SNP**: Sodium Nitroprusside.

9.2.2 Phytomelatonin and Gasotransmitters under Drought Stress

9.2.2.1 Nitric Oxide (NO)

One of the crucial abiotic stresses is drought stress which negatively influences plant productivity and development. Presence of insufficient moisture for proper plant growth and development caused by a prolonged absence of precipitation mixed with increased evapotranspiration leads to agricultural drought conditions. Plant growth and development are greatly impacted by drought, which results in considerable reductions in crop growth rate and biomass build-up. Drought-related impacts on crop plants include decreased stomatal behavior, diminished nutrient and water interactions, smaller leaves, slowed stem elongation and root growth, and decreased cell division and expansion. These factors all contribute to decreased crop yield and decreased water usage efficiency. Phytomelatonin, an important regulator of plant physiological responses, aids in mitigating drought-induced oxidative damage by modulating the levels of ROS and maintaining cellular redox homeostasis. Studies have shown that phytomelatonin treatment can lower the levels of harmful ROS and NO, thereby reducing nitro-oxidative stress. This is achieved through the upregulation of antioxidant enzymes such as SOD, glutathione reductase (GR), CAT, and ascorbate peroxidase (APX), which work together to neutralize ROS and stabilize cellular structures. Furthermore,

phytomelatonin influences the expression of genes associated with proline biosynthesis, enhancing osmo-protection and stress tolerance. The combined action of phytomelatonin and NO helps to maintain nitro-oxidative homeostasis, improve water retention, and support stomatal function, thereby sustaining plant growth and productivity under drought conditions. This synergistic relationship underscores the potential of phytomelatonin as a priming agent in agriculture to enhance drought tolerance through its interaction with NO and the regulation of redox and osmo-protective pathways. Research evidences revealed that phytomelatonin-treated *Zea mays* (maize) displayed increased levels of carbon fixation, photosynthesis, amino acid biosynthesis, secondary metabolites, and differentially expressed proteins (DEPs) in comparison to untreated controls, according to iTRAQ proteomic investigations. It has been demonstrated that pre-treating alfalfa (*Medicago sativa*) with phytomelatonin produces priming effects that increase the resistance to drought stress. When compared to untreated, drought-stressed plants, the rhizosphere's phytomelatonin treatment greatly enhanced the plant tolerance to drought, as seen by improved plant phenotypes, increased stomatal conductance, and higher chlorophyll fluorescence. Significantly, phytomelatonin pre-treatment reduced stressed levels of NO, H₂O₂, and lipid peroxidation, suggesting systemic mitigation of drought-induced nitro-oxidative stress ([Antoniou et al. 2017](#)). ROS and RNS metabolism are regulated by phytomelatonin at both the enzymatic and transcriptional levels, which helps maintain nitro-oxidative balance ([Wang et al. 2013](#)). Studies have indicated that phytomelatonin, via the phytomelatonin receptor 1 (PMTR1), causes stomatal closure by inducing the generation of ROS. Because NO scavengers like carboxy-PTIO (cPTIO) eliminate exogenous phytomelatonin-induced stomatal closure and NO production, this process

is closely related to NO. Furthermore, nitrate reductase (*nia1*, *nia2*) and NO-associated 1 (*noa1*) mutant lines do not show phytoalexin-induced stomatal closure, highlighting the NO-dependent nature of this activity. Results show that phytoalexin-induced NO generation is reduced in *rhohC* and *rbohD/F* mutants of NADPH oxidase homologs, suggesting that NO functions in this pathway downstream of ROS. Remarkably, the ROS levels of NR mutant plants do not significantly differ from those of wild-type plants, suggesting that NO contributes to the production of ROS that is triggered by phytoalexin. Furthermore, since phytoalexin does not induce the creation of ROS and NO in the guard cells of *PMTR1* mutant lines, it is crucial to remember that NO generation in the PMTR1-mediated signaling pathway happens after ROS. All of these findings point to a crucial role that phytoalexin and NO play in controlling stomatal closure, which is necessary to augment drought tolerance in plants by influencing the production of ROS and NO via PMTR1-mediated signaling pathways ([Wang et al. 2023](#)). Additionally, the study conducted on pepper (*Capsicum annuum*) plants delves into the role of phytoalexin (0.1 mM) in enhancing drought tolerance in revealing a pivotal connection with nitric oxide (NO). When subjected to drought stress, plants exhibited significant decline in various growth parameters, including dry weight, relative water content, leaf water potential, PSII efficiency, chlorophyll, soluble protein, and nitrogen content in both leaves and roots. Concurrently, drought stress elevated levels of oxidative stress markers such as H₂O₂ and malondialdehyde (MDA), alongside increased nitrate, ammonium, free amino acids, soluble sugars, proline, and glycine betaine. The activities of antioxidant enzymes like peroxidase (POD), glutathione S-transferase (GST), and catalase (CAT) also spiked, reflecting the plant response to increased oxidative damage by enhancing nitrogen metabolism by

activating enzymes crucial for nitrogen assimilation, such as NR, nitrite reductase (NiR), glutamine synthetase (GS), glutamate synthetase (GOGAT), and glutamine dehydrogenase (GDH). This enzymatic activation not only improved nitrogen metabolism but also reduced the content of NO_3^- , NH_4^+ , and free amino acids. According to these findings, NO is a known signaling molecule implicated in a number of plant stress responses, such as the control of antioxidant defenses and nitrogen metabolism, and its interaction with phytomelatonin is essential to improving drought tolerance ([Kaya & Shabala 2023](#)). However, research on the functions of phytomelatonin-NO signaling during drought stress is limited, and comprehensive studies are needed to understand the underlying mechanisms ([Figure 9.2](#) and [Table 9.1](#)).

9.2.2.2 Hydrogen sulphide (H_2S)

Almost all living organisms, including plants, create H_2S endogenously. It is an essential gasotransmitter that actively supports plant growth and development, senescence, and tolerance to a variety of abiotic stimuli, including drought stress in many plant species. H_2S can boost the activity and gene expression of antioxidant defense enzymes, which lowers lipid peroxidation, prevents unchecked ROS production, and ultimately increases plant output under stress. Additionally, H_2S modulates a variety of physio-biochemical processes and stress tolerance/adaptation by interacting with other molecules such as phytohormones, calcium ions (Ca^{2+}), H_2O_2 , NO, ROS, and MG. In the study by [Imran et al. \(2021\)](#), exogenous phytomelatonin (100 μM) and SNP (100 μM) improved drought tolerance in soybean by enhancing plant biomass, photosynthetic efficiency, and water content while reducing ROS build-up through decreased lipid peroxidation, improved antioxidant enzyme activities, and decreased abscisic acid (ABA) content. Similarly, H_2S has been known to mitigate

drought stress through antioxidant defense, modulation of ABA levels, and enhancement of photosynthetic performance. This study indicated that phytemelatonin induced NO regulation, characterized by increased S-nitrosoglutathione and altered expression of NO synthesis-related genes, also parallels the known actions of H₂S in drought stress response, suggesting a potential cross-talk where both phytemelatonin and H₂S could synergize to enhance drought resilience. In study conducted by [Khan et al. \(2024\)](#) on tomato plants having 40% water levels, by activating the Na⁺-H⁺ antiporter and H⁺-ATPase, phytemelatonin treatment at 30 μM was found to considerably affect important physiological processes. This improved the K⁺/Na⁺ ratio by facilitating an increase in potassium (K⁺) influx and a decrease in sodium (Na⁺) outflow. This control performed a crucial function in decreasing the negative consequences of drought stress, including lipid peroxidation, electrolyte leakage (EL), and oxidative damage. Meanwhile, phytemelatonin improved nitrogen metabolism and strengthened both enzymatic and non-enzymatic antioxidant defense mechanisms strengthening the plant resistance to drought-induced stress. The work emphasizes the role that H₂S plays in this adaptive response as the application of hypotaurine, an H₂S scavenger, reversed the positive effects of phytemelatonin and restored the unfavorable conditions brought on by drought stress. This indicates that the cooperative action of phytemelatonin and H₂S is pivotal for activating the drought stress tolerance mechanisms in plants. Furthermore, research on tomato plants under drought stress revealed that ATP synthase and ATPase activities were downregulated, whereas mitochondrial enzymes like pyruvate dehydrogenase, malate dehydrogenase, and citrate synthase showed increased activity. Applying 30 μM phytemelatonin to drought-stressed plants increased H₂S buildup, which in turn increased HSP17.6 and HSP70 expression, antioxidant

defense, and mitochondrial enzyme activity. Reduced ROS and less damage were the results of this phytemelatonin-induced improvement. However, even in the presence of phytemelatonin, the negative effects of drought stress returned when DL-propargylglycine decreased H₂S production, reversing the positive effects of phytemelatonin. These findings suggest that the protective effects of phytemelatonin under drought stress are mediated through the action of endogenous H₂S, highlighting a crucial interaction between phytemelatonin and H₂S in plant defense mechanisms against drought stress. This implies a broader regulatory network where phytemelatonin and H₂S collaboratively modulate antioxidant defenses and stress-responsive signaling pathways to confer enhanced drought tolerance ([Figure 9.2](#) and [Table 9.2](#)).

9.2.3 Phytemelatonin and Gasotransmitters under Heavy Metal Stress

9.2.3.1 Nitric Oxide (NO)

Anthropogenic activities through industrial wastes, sewage disposal, usage of fertilizers lead to accumulation of heavy metals such as iron (Fe), lead (Pb), copper (Cu), nickel (Ni) etc. in soil leads to heavy metal stress in plants affecting plant productivity. Remediating heavy metal-contaminated soils is usually a challenging to remediate. Phytemelatonin being one of the many defense compounds found naturally in plants, seems to have a great deal of bio stimulatory potential. Numerous studies have found that NO serves as a signaling molecule in plants' reactions to environmental stressors. Phytemelatonin and NO may have a link where phytemelatonin may help increase tolerance to heavy metal stress as reported in available studies. Experiment on maize plants under Pb stress demonstrated that treatment with phytemelatonin (0.1 mM) increases tolerance under Pb

stress via regulating the endogenous generation of NO. Pb toxicity usually results in increased levels of stress indicators such as proline, H₂O₂, MDA, EL, leaf Pb, and endogenous NO, while decreasing plant biomass, PSII maximum efficiency (Fv/Fm), total chlorophyll, leaf K⁺, Ca, and leaf water potential. Experiments employing NO scavengers and the NO-specific inhibitor cPTIO helped to clarify the relationship between phyto melatonin and NO in reducing Pb toxicity. Since cPTIO reduced phyto melatonin-induced tolerance to Pb toxicity, the data demonstrated that NO is necessary for phyto melatonin-mediated defensive mechanisms. The interaction between phyto melatonin and NO is demonstrated by raised H₂O₂ and MDA concentrations, decreased activity of certain antioxidant enzymes, suppression of plant growth, and increased lead content in leaves when phyto melatonin treatment is coupled with cPTIO. It is evident from this that phyto melatonin improves maize's ability to withstand Pb poisoning by regulating endogenous NO levels, which in turn triggers antioxidant defenses and lessens Pb-induced oxidative damage ([Okant & Kaya 2019](#)). Moreover, under cadmium (Cd) stress, wheat plants exhibit decreased growth, chlorophyll content, PSII maximum efficiency (Fv/Fm), leaf water potential, and essential nutrients such as K and Ca. Concurrently, Cd toxicity leads to increased levels of leaf MDA, H₂O₂, EL, Cd accumulation, and endogenous NO. Phyto melatonin treatment, at concentrations of 100 µM, alleviates these toxic effects by improving plant growth, enhancing leaf Ca²⁺ and K⁺ content, and reducing MDA, H₂O₂, and leaf Cd levels. Further investigations using the NO scavenger cPTIO combined with phyto melatonin treatments demonstrated that the phyto melatonin induced tolerance to Cd stress is mediated through NO. The presence of cPTIO reversed the beneficial effects of phyto melatonin, leading to reduced endogenous NO levels and negating the improvements in growth and stress

markers. Additionally, the combination of phytomelatonin and cPTIO resulted in growth inhibition and higher leaf Cd content. Thus, it is clear that phytomelatonin improves wheat's resistance to Cd by inducing endogenous NO, which is essential for initiating antioxidant defense and reducing oxidative stress brought on by Cd ([Kaya et al. 2019](#)). Also, co-application of phytomelatonin (100 μ M) and NO, applied via foliar spray as sodium nitroprusside (SNP; 200 μ M), significantly enhance the tolerance of *Catharanthus roseus* (L.) G. Don (periwinkle) plants to Cd (200 mg/kg) toxicity. The antioxidant enzymes POD and CAT were activated, shoot biomass was increased, chlorophyll a and b content were raised, EL was decreased, and critical cation homeostasis was preserved.

Although Cd accumulated mostly in the roots, Cd accumulation in both the roots and the shoots was enhanced by the combined phytomelatonin and SNP treatment. This increased antioxidant enzyme activity and better mineral homeostasis together enhanced Cd tolerance ([Nabaei & Amooaghaie 2020](#)). Experimental evidences by the study of tomato plants against aluminum (Al; 148 μ M) toxicity revealed the crucial interplay between phytomelatonin (150 μ M) and NO significantly enhances the defense mechanisms. Al stress usually results in decreased growth, biomass, and photosynthetic pigments in the roots and leaves as well as elevated concentrations of MDA, EL, superoxide anion, H₂O₂, and Al. Nevertheless, by lowering internal ROS levels, phytomelatonin treatment of Al-stressed tomato plants increases the activity of antioxidant enzymes, safeguarding cell membranes and photosynthetic pigments. Al is immobilized in the roots and its translocation to the leaves is decreased by phytomelatonin treatment, which also stimulates the formation of phytochelatins, glutathione, and NO in the leaves and roots. When NO is scavenged using cPTIO, the positive effects of phytomelatonin are reduced,

suggesting that NO is essential for defense responses triggered by phytomelatonin. Therefore, by controlling internal NO levels, phytomelatonin helps tomato seedlings adapt to Al stress, demonstrating the complementary function of both phytomelatonin and NO in boosting plant resistance to Al toxicity ([Ghorbani et al. 2023](#)). Tomato seedlings less development when exposed to Cd stress (300 μ M), but when exogenous phytomelatonin or NO is applied at an ideal dosage of 100 μ M, seedling growth is much enhanced. The NO scavenger cPTIO inhibits the beneficial effects of phytomelatonin on seedling growth under Cd stress, suggesting that NO plays a crucial role in the growth response triggered by phytomelatonin. The growth response induced by phytomelatonin appears to be dependent on NO, as the NO scavenger cPTIO inhibits the positive effects of phytomelatonin on seedling growth under Cd stress. By lowering H₂O₂, MDA, DHA, and oxidized glutathione (GSSG), phytomelatonin and NO treatments also lessen oxidative damage. They also increased the levels of ascorbic acid (AsA) and glutathione (GSH), enhanced the ratios of AsA/DHA and GSH/GSSG, and increased the activity of antioxidant enzymes such as monodehydroascorbic acid reductase (MDHAR), glutathione reductase (GR), ascorbic acid oxidase (AAO), and dehydroascorbic acid reductase (DHAR). Additionally, both increased the expression of genes such AAO, AAOH, APX1, APX6, DHAR1, DHAR2, MDHAR, and GR that are involved in the metabolism of ROS and the AsA-GSH cycle. To mitigate oxidative damage and promote seedling growth under Cd stress, these findings imply that phytomelatonin-mediated NO improves Cd tolerance via controlling the AsA-GSH cycle and ROS metabolism ([Xu et al. 2023](#)) ([Figure 9.2](#) and [Table 9.1](#)).

9.2.3.2 Hydrogen sulphide (H₂S)

H₂S being one of the crucial gasotransmitters participates a vital function in mitigating heavy metal stress in plants by enhancing detoxification processes, activating antioxidant defense systems, and modulating gene expression to improve overall plant health and resilience. Few research evidences have revealed that phytemelatonin and H₂S synergistically contribute for enhancing plant resilience against heavy metal stresses by regulating growth, development, and stress response mechanisms through intricate signaling pathways and antioxidant activities. Together, phytemelatonin (100 µM) and H₂S (1.5 mM) reduced Cd stress (50 mg/l) in *Matthiola incana* (stock) plants by restoring a number of morpho-physiological and biochemical traits, including growth, photosynthesis, Cd-induced oxidative stress, ROS accumulation, EL, and MDA content. These effects may be ascribed to phytemelatonin and H₂S-induced antioxidant plant defense activities, which successfully lessen the intensity of oxidative stress indicators ([Zulfiqar et al. 2024](#)). Research by [Haghi et al. \(2022\)](#) revealed that co-application of phytemelatonin (100 µM) and H₂S (100 µM) alleviated Pb toxicity (50 µM) in *Carthamus tinctorius* (safflower) seedlings by significantly reducing MDA and H₂O₂ content and lipooxygenase (LOX) activity and increasing the AsA-GSH cycle and phytochelation triggered by Pb by stimulating metal-binding ligands metabolism which subsequently mitigated Pb toxicity by decreasing its absorption and translocation. Further, research revealed that As toxicity (0.1 mM) in *Capiscum annuum* (pepper) plants was alleviated by the treatment of phytemelatonin (0.10 mM) through production of increased intrinsic H₂S proved through hypotaurine (0.1 mM), increased biomass, chlorophyll a, chlorophyll b, antioxidant enzymes' activities, As accumulation in vacuoles and decreased concentrations of MDA and H₂O₂ ([Kaya et al. 2022](#)). Also, similar results were shown in tomato seedlings under Cr stress (25 µM)

when application of phyto melatonin (30 μM) improved phytochelation, H^+ -ATPase activity, reduced ROS levels, EL and lipid peroxidation and improved nitrogen metabolism and AsA-GSH cycle ([Khan et al. 2023](#)). Phyto melatonin (100 μM) treatment of Cr-stressed (100 μM) maize plants increased endogenous H_2S levels and increased Cr tolerance, as demonstrated by increased expression of the *LCD/DCD* gene, increased content of cell wall polysaccharides, increased pectin methylesterase activity, and improved antioxidant enzyme activity ([Yang et al. 2024](#)) ([Figure 9.2](#) and [Table 9.2](#)).

9.2.4 Phyto melatonin and Gasotransmitters under Temperature Stress

9.2.4.1 Nitric Oxide (NO)

Globally, one of the main environmental factors limiting plant growth, metabolism, and productivity is high temperature (HT) stress. Because of protein misfolding and intracellular protein denaturation, high temperatures are linked to a marked decrease in transcription and translation. Moreover, increased ROS formation, which results in oxidative stress, is one of the main effects of heat stress. In the end, this reduces photosynthesis by increasing the generation of thiobarbituric acid reactive substances (TBARS) and H_2O_2 , decreasing the accumulation of total soluble sugars, starch, and enzymes involved in carbohydrate metabolism ([Medina et al. 2021](#); [Iqbal et al. 2021](#); [Wang et al. 2022](#)). By lowering TBARS and H_2O_2 concentration, phyto melatonin has been shown to mitigate oxidative stress (Arnao and Hernández-Ruiz [2014](#), [2015](#)). According to research, exogenous application of phyto melatonin (100 μM) with 75 μM SNP decreased the accumulation of ROS, MDA, and EL in cucumber seedlings exposed to cold stress (5°C). This resulted in photoprotection for both photosystems

and increased the expression of genes involved in the chilling response, including C-repeat-binding factor (CBF1), inducer of CBF expression (*ICE1*), and cold-responsive (COR47) ([Feng et al. 2021](#)). By preserving redox homeostasis and controlling the synthesis of endogenous polyamines and NO, phytomelatonin (100 μ M) therapy has been demonstrated to improve tomato seedlings' tolerance to heat stress (42°C). By assisting the seedlings in overcoming the negative consequences of heat stress, these systems improve development and survival in trying circumstances ([Jahan et al. 2019](#)). ([Table 9.1](#) and [Figure 9.2](#)).

9.2.4.2 Hydrogen sulphide (H₂S)

According to studies, the application of hypotaurine, an H₂S scavenger, dramatically eliminates the phytomelatonin's ameliorative effect, suggesting that H₂S signaling is involved. Similarly, the interaction between H₂S and phytomelatonin promotes the metabolism of carbohydrates ([Iqbal et al. 2021](#)). In wheat, exogenous phytomelatonin (100 μ M) mitigated heat stress (40°C)-induced oxidative damage and increased H₂S production, suggesting that phytomelatonin mediated H₂S plays a role in alleviating oxidative stress. However, when H₂S was inhibited by its inhibitor, the beneficial effects of phytomelatonin were attenuated, indicating that the interaction between H₂S and phytomelatonin, and perhaps phytomelatonin, regulates heat stress signaling by acting upstream of H₂S in wheat ([Iqbal et al. 2021](#); [Khan et al. 2022](#)). ([Figure 9.2](#); [Table 9.2](#)).

9.2.5 Phytomelatonin and Gasotransmitters under Flooding Stress

9.2.5.1 Nitric Oxide (NO)

Flooding stress in plants is followed by a chain of physiological alterations, mainly attributed to the lack of oxygen supply in the root zone ([Jia et al. 2021](#)). Hypoxia evokes a highly complex signaling cascade, and two important molecules in the regulation of the stress response are NO and phytoalexin. Stress triggers the synthesis of NO, a vital and adaptable signaling molecule that is crucial for regulating a wide range of physiological functions, including stomatal closure, root elongation, and antioxidant defense systems. During flooding conditions, it is produced because of low oxygen availability in plants as a secondary messenger that participates a significant part in the regulation of stress-related genes and proteins ([Zhou et al. 2021](#)). The interaction of NO with phytoalexin could occur in at least two ways: it may serve as a precursor in the biosynthesis of phytoalexin. NO can induce the production of key enzymes responsible for phytoalexin biosynthesis, thus increasing its concentration within the plant under stress ([Martínez-Lorente et al. 2022](#)). It is thought that such an interaction optimizes plant ability to control its internal environment when oxygen supply is low. In addition, NO and phytoalexin function redundantly in controlling ROS formation in flooding. Flooding stress results in generating the production of ROS that may subsequently lead to the deterioration of the cellular functions through breaking metabolic procedures. Phytoalexin decreases the cell injury by protecting with antioxidant functions by stimulating antioxidants including SOD, CAT activities that removes hazardous ROS activity in cells and by modulating NO, could it be responsible for enhancing some antioxidants activities against such cell-injury. This coordinated action maintains cellular homeostasis and leads to better growth of plants under hypoxic conditions. This synergy between NO and phytoalexin not only supports better growth but also enhances the plant's capacity to survive prolonged flooding

events. By interfering with aerobic root respiration and causing oxygen deprivation, flooding causes metabolic and physiological alterations in plants ([Seymen et al. 2024](#)). Furthermore, flooding alters the soil's gas exchange, producing CO₂ pressure that has an adverse effect on root growth and specific metabolic functions ([Patel et al. 2014](#)).

According to a number of research ([Wang et al. 2021](#); [Seymen et al. 2023](#)), applying phyto melatonin and NO decreases ROS activity and accumulation in cells. By reducing ROS-induced cellular damage, strengthening the antioxidant defense system, and halting cell death in roots under flooding stress, these applications aid in the growth of plants ([Wang et al. 2021](#); [Can 2023](#)). In *Spinacea oleracea* (Spinach) plants, research indicates that applying phyto melatonin (50 µM) and NO (50 µM) improved total chlorophyll content, actual photosynthetic efficiency, and chlorophyll fluorescence by mitigating the negative effects of flooding stress ([Seymen et al. 2024](#)) ([Figure 9.2](#); [Table 9.1](#)).

9.2.5.2 Hydrogen Sulphide (H₂S)

In response to flooding stress, plants utilize various signaling molecules to mitigate the negative effects. Phyto melatonin and H₂S play very important roles in their interactive and synergistic effects towards enhancing plant tolerance against flooding stress ([Seth et al. 2023](#)). The nature of a plant-derived indoleamine like phyto melatonin has associated antioxidant properties that help combat ROS, either by scavenging free radicals or by activating antioxidant enzymes, thereby better managing oxidative damage and maintaining cellular integrity during stress. Additionally, it alters how genes linked to stress are expressed, improving the plant's overall ability to withstand stress. Plants treated with both phyto melatonin and H₂S showed improved growth, better photosynthetic efficiency, and enhanced root function, which led to greater resilience and survival under flooding

conditions ([Samal et al. 2024](#)). Therefore, the interaction between phytomelatonin and H₂S is a critical approach for the mitigation of flooding stress and opens up possibilities for enhancing the flood tolerance of plants in flooded areas. According to [Cheng et al. \(2013\)](#), exogenous H₂S pretreatment greatly reduces hypoxia-induced root tip death in pea (*Pisum sativum*) seedlings by boosting the antioxidant system's capacity. By safeguarding the root tips, which are essential for absorbing water and nutrients, this treatment increases the plant's resistance to hypoxic stress and improves overall plant survival and growth in low oxygen environments. Exogenous NO therapy has been shown to dramatically increase endogenous hydrogen H₂S buildup, which in turn increases the hypoxia tolerance of maize seedling roots exposed to submersion. Furthermore, exogenous H₂S application raises endogenous H₂S levels, enhancing the plant's ability to defend itself against free radicals. Because of its increased antioxidant capacity, which helps to eliminate excess ROS, the plant can withstand stress better ([Peng et al. 2016](#)). According to a recent study, applying phytomelatonin to hot peppers under flooding stress circumstances enhanced their shoot and root lengths as well as their chlorophyll, photosynthesis, proline, and protein contents ([Khosravi et al. 2023](#)). Applications of NO and phytomelatonin considerably reduced the adverse effects of flooding stress in spinach, according to [Seymen et al. \(2024\)](#). Interestingly, these treatments also controlled pigment content, which improved the antioxidant defense mechanism that scavenges ROS ([Figure 9.2](#) and [Table 9.2](#)).

9.3 CONCLUSION AND FUTURE PROSPECTS

Phytomelatonin greatly increases plant resistance to several environmental stressors, including salt, drought, heavy metals, severe temperatures, and flooding, when combined with NO and H₂S. These three signaling molecules coordinate an intricate web of physiological and biochemical processes that protect plants from environmental stressors. By directly scavenging ROS and controlling the actions of antioxidant enzymes, phytomelatonin functions as a strong antioxidant. It intensifies the plant's stress response systems when paired with NO and H₂S. By controlling stomatal closure, NO helps to improve nutrient uptake and decrease water loss during droughts. In addition, H₂S reduces oxidative stress and lessens the absorption and translocation of harmful metals, which reduces the toxicity of heavy metals. When combined, these molecules promote cellular homeostasis, activate detoxification pathways, and affect the expression of genes linked to stress tolerance. Under harsh environmental circumstances, phytomelatonin, NO, and H₂S work in concert to provide an integrated defensive mechanism that improves plant growth, survival, and production. This all-encompassing strategy presents a viable path toward cultivating crops with enhanced resistance to environmental stresses and climate change.

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10

Omic(s), Transgenic, and Bioinformatics Approaches/Tools to Up-regulate Phytomelatonin-Mediated Environmental Stress-Tolerance in Crop Lines

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DOI: [10.1201/9781003527633-10](https://doi.org/10.1201/9781003527633-10)

10.1 INTRODUCTION

The global climate will continue to rise over the next decade, affecting crop yields. The development of stress-tolerant plants by genetic modification is crucial. Plants face a variety of environmental challenges, including increased reactive oxygen species (ROS) production, damage to genetic material and cell structures ([Kumari et al., 2024](#)). In reaction to environmental stressors, the plant develops defensive compounds ([Thiruvengadam et al., 2024](#)). Melatonin is secreted in response to environmental stimuli ([Seth et al., 2023](#)). It was long assumed to be found

only in humans and animals until 1995 when it was discovered in plants ([Janas and Posmyk, 2013](#)). It is a plant growth regulator with the molecular formula N-acetyl-5-methoxytryptamine. Melatonin is synthesized in animals' pineal glands, while in plants, it is produced in mitochondria and chloroplasts a ([Tyagi et al., 2023](#)). According to research, flowers contain the most melatonin, while roots and fruits contain the least ([Mannino et al., 2021](#)). Tryptophan is a precursor to melatonin production. Melatonin production from tryptophan occurs in plants via two major routes, which differ at the intermediary N-acetylserotonin and 5-methylhydroxytryptamine. Melatonin production involves enzymes: tryptophan decarboxylase; TCC, tryptophan dihydroxylase; TPH, tryptamine 5-hydroxylase; T5H, serotonin N-acetyltransferase; SNAT, acetylserotonin O-methyltransferase; ASMT and Caffeic acid 3-O-methyltransferase; COMT ([Back et al., 2016](#)).

Melatonin enhances photosynthetic activity and antioxidant enzyme activity under the adverse environmental conditions. Exogenous melatonin use during drought stress increased crop yield by 18%. Melatonin-treated plants enhanced their photosynthetic ability by 48% during cold stress, 44% during drought stress, and 42% during salt stress conditions ([Yang et al., 2023](#)). Recent studies showed the role of melatonin in heat tolerance, salt tolerance, growth regulation, root formation etc. ([Tian et al., 2025](#); [Zhang et al., 2025](#); [Cai et al., 2025](#); [Maity et al., 2025](#)). The breeding approaches to enhance endogenous melatonin content involve the marker assisted breeding of varieties with high melatonin content. In a recent study, a marker gene SiWRKY67 was identified in sesame by using genome-wide association study (GWAS). The overexpression of SiWRKY67 resulted in melatonin accumulation (up to 0.452 ng/g FW) in hairy root lines ([Wang et al., 2022](#)). However, the endogenous amount of melatonin has been

increased through genetic alteration of melatonin production genes to explore their effect on various biotic and abiotic conditions. The melatonin biosynthesis genes have been overexpressed or silenced to investigate their impact under various stressors. Overexpression of melatonin biosynthesis genes resulted in increased endogenous melatonin content, providing tolerance to multiple stresses such as salinity, heat, cold, drought, and heavy metal stress ([Colombage et al., 2024](#)). Melatonin's significance in adverse environmental conditions including biotic and abiotic stresses, has been shown in several model plants, including rice, tomato, tobacco, and *Arabidopsis*. However, it requires more investigation in other plant species. The candidate gene for melatonin biosynthesis namely *TDC*, *TPH*, *T5H*, *SNAT* and *COMT/ASMT*), could be used to develop crops resistant for various conditions such as drought, high temperature, cold, heavy metal and other biotic factors. This chapter provides detailed information on the role of melatonin in various abiotic and biotic stress conditions as a result of increased endogenous melatonin content achieved through genetic modification in many crops including *Arabidopsis*, rice, tobacco, tomato, and watermelon.

10.2 DIFFERENT APPROACHES TO TARGET PHYTOMELATONIN BIOSYNTHESIS PATHWAY TO MODULATE ENDOGENOUS LEVEL OF PHYTOMELATONIN IN PLANTS

Changing climatic conditions impose single or multiple stressors on approximately 95% of the world's agricultural land ([Khan et al., 2021](#); [Cramer et al., 2011](#)). Plants produce endogenous phytomelatonin in

response to abiotic and biotic stressors which is synthesized in mitochondria and chloroplast. Tryptophan is a precursor to melatonin which is produced by the shikimic acid pathway for the synthesis of aromatic amino acids in plants. The enzymes involved include tryptophan decarboxylase; TDC, tryptophan hydroxylase; TPH, tryptamine 5-hydroxylase; T5H, serotonin N-acetyltransferase; SNAT, N-acetylserotonin methyltransferase; ASMT, and caffeic acid O-methyltransferase; COMT. In *Hypericum perforatum*, an additional pathway of melatonin production occurs, involving tryptophan hydroxylase (TPH) that convert tryptophan to 5-hydroxytryptophan followed the action of TDC enzyme that convert 5-hydroxytryptophan to tryptamine (Murch et al., 2000). Figure 10.1 depicts different routes for melatonin biosynthesis in plants. The chemical structure of key intermediates of the melatonin biosynthesis pathway is given in Table 10.1.

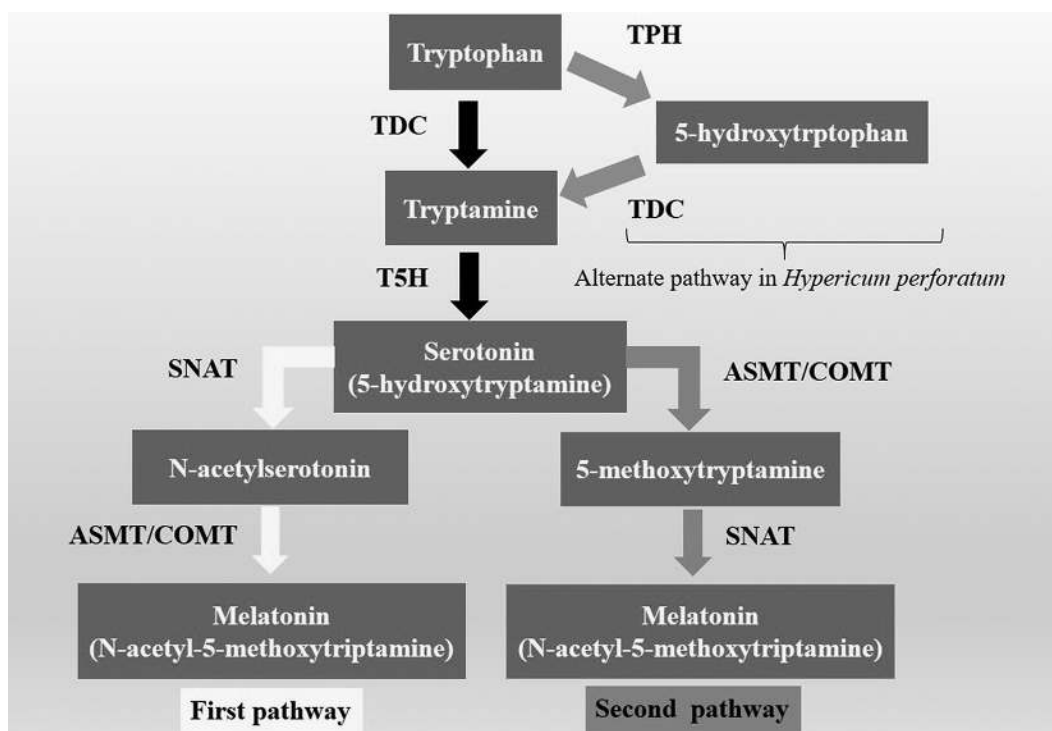
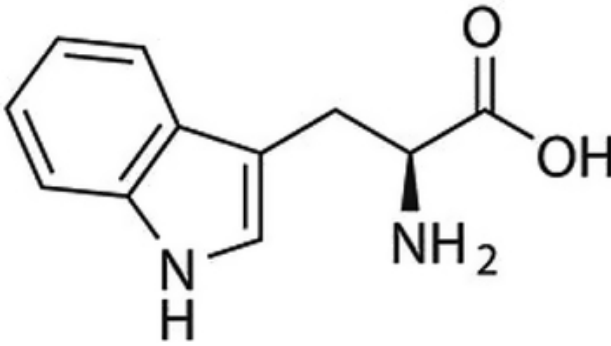
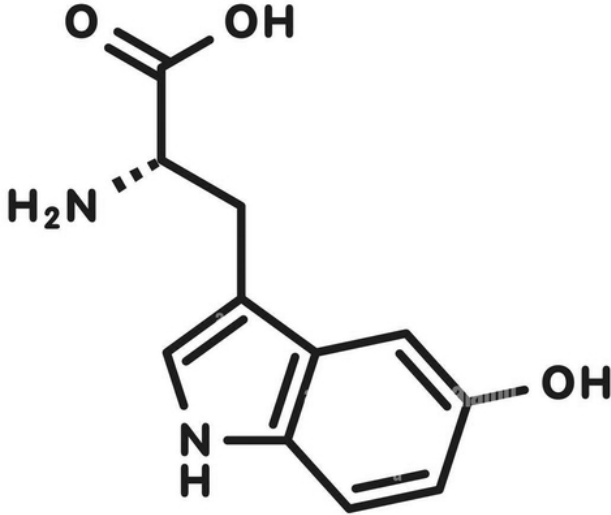
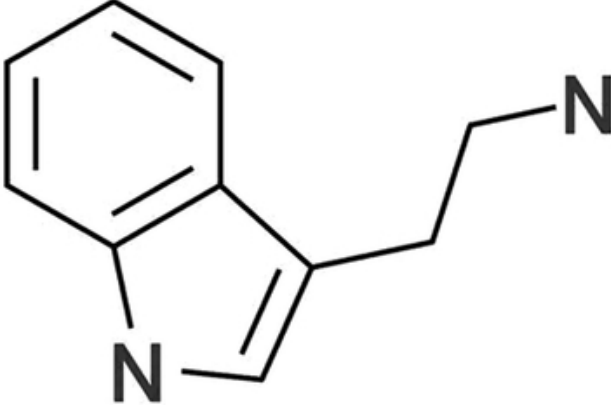
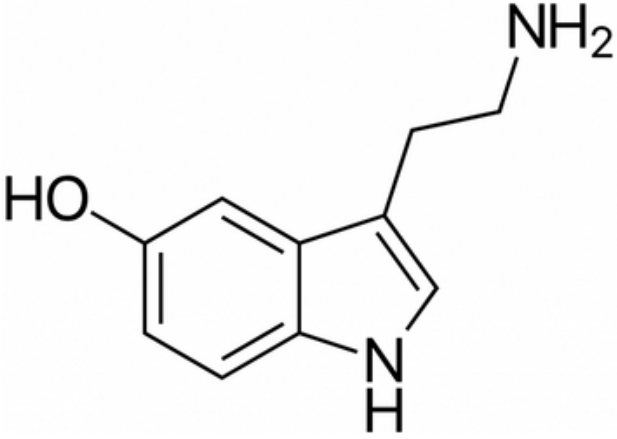
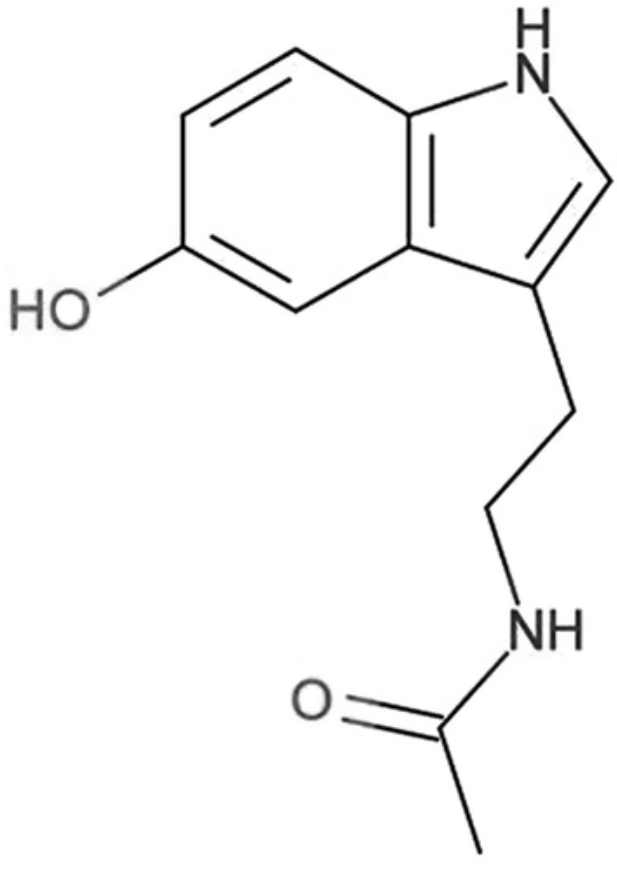
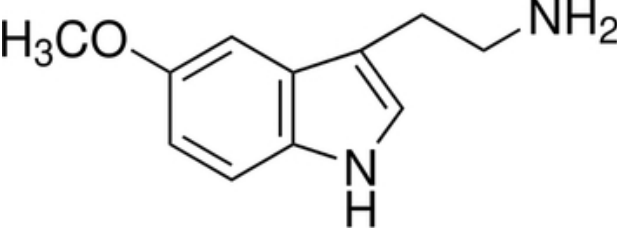


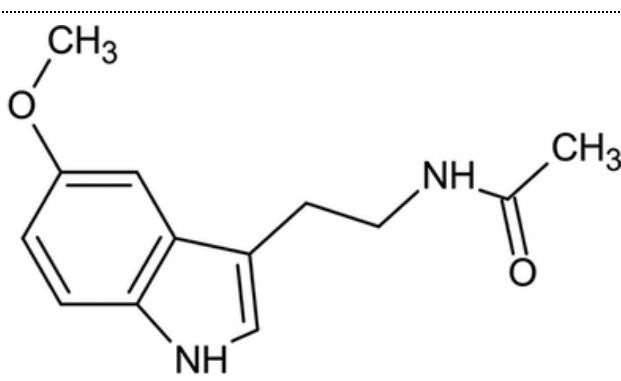
FIGURE 10.1 Melatonin biosynthesis pathway in plants. Two routes, as depicted in the picture, connect serotonin to methionine

via N-acetylserotonin and 5-methyltryptamine. *Hypericum perforatum* has an alternative pathway in which an intermediary known as 5-hydroxytryptophan is involved. The enzymes involved include tryptophan decarboxylase; TDC, tryptophan hydroxylase; TPH, tryptamine 5-hydroxylase; T5H, serotonin N-acetyltransferase; SNAT, N-acetylserotonin methyltransferase; ASMT, and caffeic acid O-methyltransferase; COMT. [!\[\]\(125d701e9425b54c764340b5671b38cd_img.jpg\)](#)

TABLE 10.1 The Chemical Structure of Key Intermediates of Melatonin Biosynthesis Pathway [📄](#)

Compound Name	Chemical Structure
Tryptophan	 <chem>NC(Cc1c[nH]c2ccccc12)C(=O)O</chem>
5-Hydroxytryptophan	 <chem>NC(Cc1c[nH]c2cc(O)ccc12)C(=O)O</chem>
Tryptamine	 <chem>NCc1c[nH]c2ccccc12</chem>

Compound Name	Chemical Structure
Serotonin (5-hydroxytryptamine)	 The chemical structure of Serotonin (5-hydroxytryptamine) consists of an indole ring system. A hydroxyl group (HO-) is attached to the 5-position of the benzene ring. A 2-aminoethyl chain (-CH₂-CH₂-NH₂) is attached to the 3-position of the indole ring. <chem>NCCc1c[nH]c2ccc(O)cc12</chem>
N-Acetylserotonin	 The chemical structure of N-Acetylserotonin is a tryptophan derivative. It features an indole ring system with a hydroxyl group (HO-) at the 5-position of the benzene ring. The 3-position of the indole ring is substituted with a 2-(N-acetylamino)ethyl chain (-CH₂-CH₂-NH-C(=O)-CH₃). <chem>CC(=O)NCCc1c[nH]c2ccc(O)cc12</chem>
5-Methoxytryptamine	 The chemical structure of 5-Methoxytryptamine consists of an indole ring system. A methoxy group (H₃CO-) is attached to the 5-position of the benzene ring. A 2-aminoethyl chain (-CH₂-CH₂-NH₂) is attached to the 3-position of the indole ring. <chem>NCCc1c[nH]c2ccc(OC)cc12</chem>

Compound Name	Chemical Structure
Melatonin (N-acetyl-5-methoxytryptamine)	

10.2.1 Genetic Modification of Melatonin Biosynthesis Genes to Upregulate Phytomelatonin-Mediated Environmental Stress-Tolerance in Plants

Endogenous phytomelatonin levels fluctuate in response to environmental stressors. Melatonin modulates a range of developmental activities in plants, including rooting, blooming, fruiting, and senescence, under stressful conditions. Melatonin's significance in stress tolerance has been shown in plant species with high endogenous melatonin levels ([Martinez et al., 2018](#)). As a result, manipulating the endogenous melatonin level will help achieve the desired outcomes. The *AANAT* gene, which encodes arylalkylamine N-acetyltransferase, is a limiting enzyme in melatonin production. It transforms N-acetylserotonin into melatonin. *AANAT* gene from *Chlamydomonas reinhardtii* was overexpressed in Micro-Tom tomato, resulting in elevated melatonin levels ([Okazaki et al., 2009](#)). Melatonin levels in transgenic plant leaves were greater (10.1 to 40.3 ng/g FW) than in wild-type leaves (4.6 ng/g FW). Melatonin levels were found to correlate with *AANAT* mRNA expression levels. Melatonin acts as a regulatory hub, altering phytohormone levels in adverse conditions to aid plant survival

([Arnao and Hernández, 2021](#)). The influence of phytohormones on phytomelatonin synthesis was examined in rice, with ABA and methyl jasmonate treatment resulting in significant expression of rice *ASMT* mRNA. The treatment with N-acetylserotonin (1 mM) increased the melatonin concentration in the leaves of transgenic plants ([Park et al., 2013](#)). Melatonin production occurs in the pineal gland in animals, and the precursor for melatonin synthesis is tryptophan obtained from the diet, whereas in plants, the precursor is tryptophan obtained through the shikimate pathway. The genetic modification of genes involved in melatonin biosynthesis and their role in plant growth, development, senescence, biotic and abiotic stress tolerance is discussed in this section.

10.2.2 Plant Growth, Development and Senescence

Melatonin is a ROS scavenger that plays important role in plant growth and developmental processes, such as root and shoot growth, flowering, fruiting, and stomatal closure ([Arnao and Hernández-Ruiz, 2006](#); [Wei et al., 2018](#)). In vertebrates, an enzyme called hydroxyindole-O-methyltransferase transforms N-acetyl serotonin into melatonin. N-acetyl serotonin is produced by a rate limiting enzyme, serotonin N-acetyltransferase. The ectopic expression of human *SNAT* in rice increased melatonin levels. Higher levels of *SNAT*-specific activity (0.11 to 71 pkat/mg protein), *SNAT* m-RNA, N-acetylserotonin (0.5–2.9 ng/g FW), and melatonin content (0.4 to 2.7 ng/g FW) was present in transgenic plants compared to wild type plants. Exogenous serotonin supplementation raised melatonin levels in transgenic plants, showing that serotonin availability is a critical barrier to melatonin synthesis.

Sheep *SNAT* was overexpressed in rice resulted in higher melatonin accumulation (up to 5.3 ng/g FW) than the wild type. Under continuous

light, these transgenic plants showed increased seminal root elongation and root biomass, which could be attributed to phytochrome or auxin signaling ([Park and Back, 2012](#)). In other studies, ectopic overexpression of sheep *SNAT* resulted in higher melatonin levels (0.34–0.87 ng/g) in transgenic rice compared to wild type. The transgenic plants produced more biomass, altered plant height, increased seedling growth, and reduced grain yield, all of which are linked to the pleiotropic action of endogenous melatonin on rice overall development and growth ([Byeon and Back, 2014](#)). The ovine *AANAT* gene was expressed ectopically in switch grass. The RNA-seq analysis identified 946 differentially expressed genes, with a total of 737 upregulated and 209 downregulated genes. The gene ontology research found that these genes have two broad functions: stress tolerance and growth regulation. However, KEGG maps found signaling pathways (estrogen signaling pathway, MAPK signaling pathway) and secondary metabolite biosynthesis processes (flavonoids, steroids, phenylpropanoids, and stilbenoids) associated with melatonin metabolism. Overexpression of *TDC* delayed senescence, whereas suppression accelerated the process ([Kang et al., 2009](#)). *TDC* encodes tryptophan decarboxylase, which produces serotonin, a precursor to melatonin. Microarray investigation of sheep *SNAT*-expressing transgenic rice revealed a total of 464 differentially expressed genes. Signaling-related genes and transcription factors were increased, while genes linked with senescence were downregulated ([Byeon et al., 2013](#)).

10.2.3 Herbicide Induced Oxidative Stress Tolerance

Melatonin's significance in butafenacil herbicide-induced oxidative stress tolerance was investigated in transgenic rice expressing *SNAT* ([Park et al.,](#)

[2013](#)) Butafenacil is a herbicide belonging to pyrimidinedione class of compounds that targets the chlorophyll biosynthetic pathway, specifically the protoporphyrinogen IX oxidase enzyme. It creates singlet oxygen, which causes membrane lipid peroxidation and cell death. *SNAT*-expressing transgenic rice had increased levels of melatonin 96 hours after butafenacil treatment. Butafenacil administration enhanced the expression of *TDC*, *T5H*, and *ASMT* genes which are involved in the melatonin biosynthesis. Melatonin is a powerful antioxidant. Butafenacil-challenged *SNAT*-expressing transgenic plants had lower H₂O₂ levels, malondialdehyde levels (MDA), chlorophyll loss, and stronger antioxidative capacity than wild-type plants ([Park et al., 2013](#)).

10.2.4 Heat Stress Tolerance

Plant growth is temperature-dependent, with temperatures above the optimum causing heat stress and temperatures below the optimum causing cold stress ([Lohani et al., 2022](#); [Khan et al., 2023](#)). [Wang et al., \(2020\)](#) studied the role of melatonin in thermotolerance by overexpressing *SlSNAT* (*Solanum Lycopersicum*) in tomato lines. *SlSNAT* (f *Solanum Lycopersicum*) overexpressing tomato lines produced three to four times more melatonin. These transgenic tomatoes improved thermotolerance by increasing ribulose biphosphate carboxylase oxygenase (RuBisCO) and RuBisCO activase levels while decreasing ROS (O₂^{·-} and H₂O₂) levels. During heat treatment, overexpression lines showed increased production of *Hsfs* and heat shock proteins (HSPs), specifically HSP21, HSP70, and HsfA2. *SlSNAT* association with *HSP40* during heat stress was validated using the bimolecular fluorescence complementation assay and yeast two-hybrid experiment. Melatonin's significance in thermotolerance has previously been documented in transgenic tomato plants overexpressing the

ASMT gene. Melatonin levels increased as temperatures rose (up to 40 °C). Transgenic plants exhibited reduced heat-induced photoinhibition, electrolyte leakage, and insoluble, ubiquitinated, and aggregated proteins, but increased heat shock protein production and autophagosome formation. These activities aid in the protein folding and prevention of aggregation of denatured proteins under heat stress conditions ([Xu et al., 2016](#)).

Tomato plants overexpressing *COMT1* demonstrated increased CO₂-induced melatonin production. Elevated CO₂ (800 µmol mol⁻¹) resulted in enhanced melatonin levels (6.8-fold). Under increasing CO₂ and heat stress conditions, heat shock protein expression increased in *COMT1* overexpressing tomato lines ([Hasan et al., 2023](#)). Melatonin's contribution in high temperature tolerance was linked to polyamine and nitric oxide production. The pretreatment of tomato seedlings with 100 µM melatonin reduced lipid peroxidation, membrane damage, and H₂O₂ generation. Melatonin-treated seedlings exhibited higher heat shock protein expression, polyamine levels, and nitric oxide content under heat stress treatment ([Jahan et al., 2019](#)). The *COMT1* silenced tomato lines were compromised during oxidative stress; however, when these plants were given exogenous melatonin, endogenous melatonin levels increased, and the redox balance and antioxidant enzyme levels were preserved during heat stress ([Ahammed et al., 2019](#)).

10.2.5 Cold Stress Tolerance

Cold stress occurs when the temperature falls below the optimum in plants. Melatonin protects from the negative effects of cold stress in the plants. The transgenic plant increased chlorophyll content (1.9–2.5 times) and expanded roots and leaves, indicating the function of melatonin in cold stress ([Kang et al., 2010](#)). Melatonin's significance in cold stress and its

synergistic effect with jasmonic acid were demonstrated in tomato plants. Methyl jasmonate therapy increased the expression of *SNAT* and *AMST*, while exogenous melatonin administration increased jasmonic acid buildup under cold stress conditions. Melatonin and methyl jasmonate foliar treatment resulted in reduced electrolyte loss and H₂O₂ generation under cold-treatment conditions. These findings show that melatonin and jasmonate act synergistically under cold stress conditions ([Ding et al., 2022](#)).

10.2.6 Drought Stress Tolerance

The amount of water supplied determines plant growth and development. Drought stress occurs when there is insufficient water to support plant growth. Micro-Tom tomato overexpressing ovine *HIOMT* and *AANAT* genes resulted in greater melatonin concentration (15.63–31.38 ng/g FW) compared to wild type (13.80 ng/g FW). These transgenic plants were subjected to drought conditions for 20 days; wild-type non-transgenic plants did not recover, whereas transgenic plants recovered completely in 48 hours after watering. This demonstrated the involvement of melatonin in drought stress tolerance ([Wang et al., 2014](#)). The activation of these two genes in tobacco increased melatonin synthesis (up to 50 µg/g dry weight) and showed protection to the DNA damage induced by UV-B radiation in the plants ([Zhang et al., 2012](#)). Melatonin's significance in drought stress tolerance was extensively investigated in transgenic tobacco overexpressing *CrCOMT* from *Carex rigescens*. Tobacco with *CrCOMT* overexpression demonstrated enhanced seed germination, survival rate, membrane stability, and relative water content under drought stress. Transgenic lines with higher proline accumulation and antioxidant enzyme activity showed less damage from ROS-induced oxidative stress during drought stress ([Li et al.,](#)

[2022](#)). The transgenic tomato overexpressing the *SlCOMT1* gene produced greater melatonin levels (30–35 pg/ml) than the wild type (27.365 pg/ml). Transgenic plants showed high proline content and lower levels of superoxide and H₂O₂ compared to wild-type plants ([Liu et al., 2019](#)). Under drought conditions, this transgenic tobacco produced more melatonin. The wheat *COMT* gene was overexpressed in *Arabidopsis*, resulting in higher melatonin content and drought resistance. Transgenic plants expressed drought-responsive genes such as *AtDREB2A*, *AtKIN1*, *AtKIN2*, *AtRAB18*, and *AtRD29A* at much higher levels than wild-type plants ([Yang et al., 2019](#)).

10.2.7 Salt Stress Tolerance

Salt stress inhibits plant growth and developmental processes that affect the yield of the plants ([Kumari et al., 2023](#)). Salt stress give rise to ion toxicity and osmotic stress in plants ([Zhu, 2016](#)). Cucumber lines overexpressing *SNAT* had 2–3 times the amount of melatonin compared to wild type. During salt stress conditions, these lines produced more malondialdehyde, proline, antioxidant enzymes, and reduced reactive oxygen species accumulation. Higher expression of *HSP90*, *SOD*, *CAT* and Pyrroline-5-carboxylate synthase (*P5CS*) was detected in *SNAT* overexpressing lines, which collectively play all of which play role in tolerance to salt stress ([Qi et al., 2020](#)). The grape *SNAT* gene was overexpressed in *Arabidopsis*, resulting in better performance during salt stress. *SNAT* gene overexpression lines resulted in elevated melatonin levels and better growth performance in *Arabidopsis*. Transgenic plants had higher fresh weight, higher germination rate, and longer root length, less leaf wilting, lower malondialdehyde content, and lower H₂O₂ content in salt stress conditions ([Wu et al., 2021](#)). [Zheng et al. \(2017\)](#) showed that the chloroplast is

involved in melatonin production. Under salt stress, the *MzSMT9* gene was upregulated in transgenic *Arabidopsis* that was expressing it ectopically. Melatonin role in salt stress tolerance is mediated by reducing lipid peroxidation, producing less reactive oxygen species, and increasing photosynthetic efficiency in transgenic plants. Transgenic tobacco overexpressing *MzASMT1* (from *Malus zumi* Mats) boosted melatonin concentration. These transgenic lines exhibited increased relative water content, chlorophyll content, proline content, an enhanced antioxidant system, and decreased ROS accumulation during salt stress conditions ([Zhuang et al., 2020](#)).

10.2.8 Heavy Metal Stress Tolerance

Cadmium (Cd), chromium (Cr), arsenic (As), and lead (Pb) etc. are considered as heavy metals that cause adverse effects to the plants even in small quantities ([Hoque et al., 2021](#)). The contribution of melatonin in cadmium (Cd) stress tolerance was investigated in alfalfa. Exogenous melatonin treatment was associated with reduced Cd toxicity through improved seedling development and root elongation. Transgenic *Arabidopsis* overexpressing alfalfa *SNAT* produced more melatonin under Cd stress. Melatonin regulates the transporters involved in Cd accumulation. Exogenous melatonin elevated the expression of *ABC* transporters and *PCR2* transcripts in alfalfa seedlings, while transgenic *Arabidopsis* upregulated *PDR8* and *HMA4*. Melatonin-induced Cd exclusion in root tissues is facilitated by these transporters. The Cd-induced redox balance was enhanced through increased Cu/Zn superoxide dismutase (SOD), which was regulated by miR398a and miR398b. As a result, melatonin helped to improve Cd tolerance ([Gu et al., 2017](#)). The mechanism of Cd stress tolerance was linked to the long non-coding RNA.

The major lncRNAs control the pectin and cellulose metabolism gene expression, ROS scavenging, brassinosteroid biosynthesis, salicylic acid-mediated defense response, and cell wall modification genes in response to Cd absorption ([Qiu et al., 2024](#)). Rice transgenic lines overexpressing *SNAT3* genes exhibited higher melatonin concentration and better performance under Cd stress. These transgenic plants had greater amounts of endoplasmic reticulum (ER) chaperones BiP3 relative to wild type, that contribute to cadmium stress tolerance ([Lu et al., 2024](#)). Rice plants overexpressing *OsSNAT1* resulted in delayed leaf senescence, higher grain output due to increased panicle number, and Cd tolerance ([Lee and Back, 2017](#)). Exogenous melatonin supplementation raised endogenous melatonin concentration in rice, which influenced arsenic stress tolerance ([Samanta et al., 2021](#)). The mechanism involves increased arsenic bioaccumulation through the expression of arsenic transporters (*OsNramp1*, *OsLsi1*, *OsPT2*, *OsPT8*) and improved utilization of thiol machinery such as cysteine, glutathione, and phytochelatins.

10.2.9 Biotic Stress Tolerance

Plant diseases cause a 20–40% loss in agricultural production ([Sahu et al., 2023](#)). Applying melatonin to combat pathogen attacks is a more environmentally friendly and cost-effective technique than chemical control methods. Melatonin's involvement in powdery mildew resistance was examined using an *Arabidopsis* transgenic line overexpressing the grape *SNAT2* gene. *SNAT2* overexpression in *Arabidopsis* resulted in increased melatonin accumulation and resistance to powdery mildew than the wild type. Higher levels of salicylic acid pathway (*PR1* and *NPRI*) and jasmonic acid pathway (*PDF1.2* and *COII*) marker genes were observed in transgenic plants compared to wild-type plants. This suggests that the SA

and JA pathways participate in *SNAT2*-mediated plant defense in *Arabidopsis* transgenic line ([Yu et al., 2019](#)).

10.2.10 Multiple Stresses Tolerance

In field conditions, plants may be subjected to a different stress combinations namely heat and drought, drought and salinity, salinity and iron deficiency, and so on. Few studies have been undertaken on the contribution of melatonin in the combined stress conditions in plants. The *COMT1* gene in watermelon is involved in melatonin production. Overexpression of the *COMT1* gene in watermelon increased the melatonin concentration by 7-fold compared to wild type. *COMT1* expression was elevated under drought, salt and low temperature stress conditions ([Chang et al., 2021](#)). Overexpression of and *MzSMT9* (*Malus zumi Mats*) in *Arabidopsis* has been linked to drought and salinity stress. *MzSMT1* overexpression resulted in 2–4 times greater melatonin levels than the wild-type. This reduced ROS generation and increased tolerance to drought stress ([Zuo et al., 2014](#)). [Figure 10.2](#) demonstrates the mechanism underlying the melatonin-induced changes in plants under various abiotic stressors.

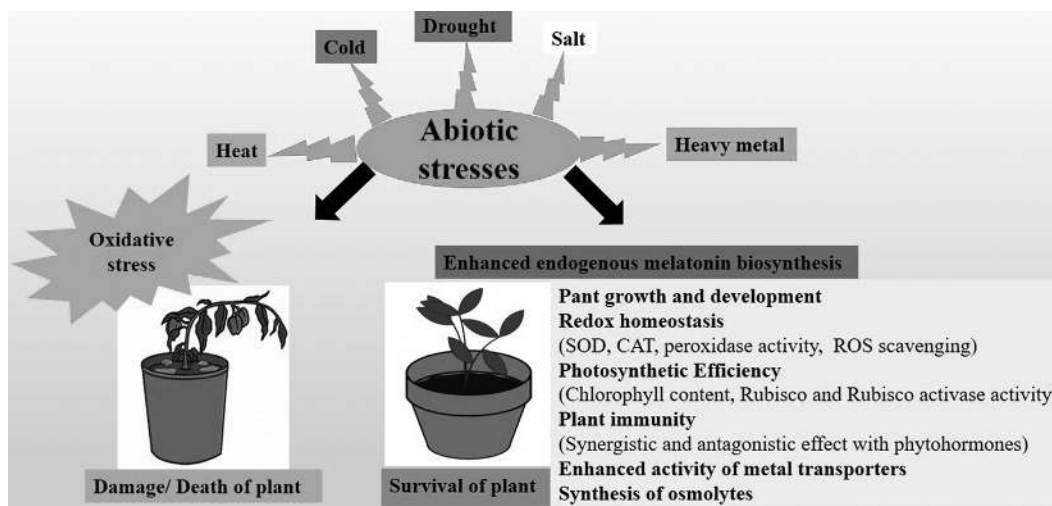


FIGURE 10.2 The mechanism of melatonin induced changes in plants under abiotic stresses. [!\[\]\(a3ea015cc5581cad732d1eb81613fe7b_img.jpg\)](#)

10.3 AVAILABLE DATA OF TRANSGENIC PLANTS SHOWING PHYTOMELATONIN-MEDIATED ENVIRONMENTAL STRESS-TOLERANCE

Melatonin's role in abiotic stress tolerance has been investigated in several model plants, including *Arabidopsis*, tobacco, and rice. [Table 10.2](#) summarizes the existing data on genetic alteration of endogenous melatonin production. To create climate-resilient crop variants, the function of the melatonin biosynthesis gene must be validated through genetic engineering of other vegetable and forestry plants.

TABLE 10.2 Melatonin Transgenic Plants Showing Phytomelatonin-Environmental Stress-Tolerance [!\[\]\(d637afc69e59bab3905899caa44fd7b5_img.jpg\)](#)

Transgenic Plants	Gene Modification	Source	Changes in Melatonin Content	Effect/Role
Rice	<i>SNAT</i>	Sheep	15–2-fold increase	Tolerance to herbicide induced oxidative damage
	<i>SNAT1</i> overexpression	Endogenous	58–90 ng/g FW compared to wild-type, 48 ng/g FW	Cd tolerance
	<i>OsCOMT</i> -Cas9 mutants	Endogenous	Melatonin deficient	Enhanced leaf senescence
Tomato	<i>COMT1</i> overexpression	Endogenous	200–250 pmol g ⁻¹ FW compared to wild type, 150–200 pmol g ⁻¹ FW	Lower Carbendazim phytotoxicity

Transgenic Plants	Gene Modification	Source	Changes in Melatonin Content	Effect/Role
	<i>SlCOMT1</i> overexpression	Endogenous	30–35 pg/mL compared to wild-type, 27.365 pg/ml	Salt stress tolerance
	<i>COMT1</i> overexpression	Endogenous	6.8 fold increase	Improved thermotolerance
	<i>SIM3H</i> overexpression	Endogenous	Decreased melatonin content	Accelerated leaf senescence
	<i>SISNAT</i> overexpression	endogenous	3–4 fold higher than wild-type	Improved thermotolerance
Tobacco	<i>NtCOMT1</i> expression	Endogenous	Higher than wild-type	Drought tolerance
	<i>T5H2</i> overexpression	<i>Morus notabilis</i>	0.015–0.020 µg/g than the wild type, 0.005–0.010 µg/g	Salt tolerance
	<i>CrCOMT</i> overexpression	<i>Carex rigescens</i>	11.23–12.16 ng/g	Drought stress tolerance

Transgenic Plants	Gene Modification	Source	Changes in Melatonin Content	Effect/Role
			Compared to wild-type, 8.4 ng/g	
	<i>MzASMT1</i>	<i>Malus zumi</i> Mats	Higher (10–12 ng/g FW) than wild type (8–10 ng/g FW)	Salt stress tolerance
<i>Arabidopsis</i>	<i>SNAT</i> overexpression	<i>Vitis vinifera</i>	7.89–8.11 ng/g compared to wild-type, 5.13 ng/g	Salt tolerance
	<i>ASMT</i> gene	1.2 fold higher than wild-type	2.5 fold increase	Cd stress tolerance
	<i>COMT</i> overexpression	Wheat	86–84–95.01 ng/mL compared to wild-type, 60.54 ng/mL	Drought resistance

Transgenic Plants	Gene Modification	Source	Changes in Melatonin Content	Effect/Role
	<i>SNAT2</i> overexpression	<i>Vitis vinifera</i>	Higher (4–5 ng/g FW) than wild type (2ng/g FW)	Powdery mildew resistance
	<i>MzSMT9</i> overexpression	<i>Malus zumi</i> Mats	Four times greater than wild type	Salt and drought stress tolerance
Watermelon	<i>COMT1</i> overexpression	endogenous	2.2–7 fold increase compared to wild type	Salt, Drought, and cold stress resistance

Note: *NAT*, serotonin N-acetyltransferase; *OsCOMT*-Cas9, *Oriza sativa*, caffeic acid O-methyltransferase, CRISPR-associated protein 9; *SLM3H*, *Solanum lycopersicum*, melatonin 3-hydroxylase; *T5H2*, tryptophan 5-hydroxylase; *ASMT*, N-acetylserotonin methyltransferase, FW, fresh weight.

10.3.1 Rice

The significance of phytomelatonin in plant growth regulation and developmental processes, as well as environmental stressors, has been extensively studied in Rice. The overexpression of sheep *NAT* gene in rice plants, resulted in enhanced melatonin levels and herbicide tolerance. The transgenic rice plants have greater antioxidant activities (catalase, CAT and superoxide dismutase, SOD activity), which contributed to reduced ROS

levels under herbicide treatment ([Park et al., 2013](#)). Transgenic rice overexpressing melatonin biosynthesis genes, specifically tryptophan decarboxylase (*TDC*), led to a 31-fold increase in melatonin content as well as increased concentration of melatonin intermediates such as tryptamine, N-acetylserotonin and 5-hydroxytryptophan ([Byeon et al., 2014](#)). Melatonin's contribution in growth and developmental processes under adverse environmental conditions was investigated by generating mutants of melatonin production genes in rice. *OsCOMT*-Cas9 mutants were produced, resulting in enhanced leaf senescence. The DNA methylome study revealed that hypo-CG DNA methylation areas changed gene expression in melatonin-deficient transgenic rice. These genes comprised phosphofructokinase, a smaller subunit of Rubisco, and sucrose synthase. RNA-seq research indicated that S-adenosyl methionine (SAM) biosynthesis genes are downregulated in methionine-deficient mutants. Changes in the SAM pathway were associated with delayed leaf senescence ([Lu et al., 2024](#)).

10.3.2 Tomato

Tomato is a popular horticultural fruit that is utilized as a model plant for growth and developmental studies ([Zhan et al., 2018](#)). The function of melatonin in leaf senescence was examined in tomato plants overexpressing the *TDC* gene. The reduced leaf senescence of these transgenic plants was associated with the downregulation of genes involved in chlorophyll breakdown, including pheophytinase (*PPH*) and pheophorbide oxygenase (*PAO*). It maintained excellent photosynthetic efficiency by increasing Rubisco and Rubisco activase activity while decreasing photosystem II complex photoinhibition ([Wang et al., 2024](#)). Melatonin's role in pesticide metabolism was investigated in tomato lines that overexpressed *COMT1*.

COMT1 overexpression raised melatonin levels. The lower Carbendazim phytotoxicity was linked to the increased antioxidant capacity of *COMT1* overexpressing tomato lines. These transgenic plants stimulated the ascorbate-glutathione cycle, which was involved in detoxification of carbendazim insecticide mediated by glutathione-S-transferase enzyme ([Yan et al., 2019](#)). Overexpression of *COMT1* in tomato raised melatonin levels and imparted salt tolerance. Melatonin's role in salt stress tolerance was linked to maintaining ionic balance, activating the SOS (salt overly sensitive) pathway, increasing antioxidant capacity (catalase, peroxidase and superoxide dismutase activity), higher glutamate and ascorbate levels, and upregulation of stress-responsive genes (*AREB1*, *MAPK1*, *WRKY33*, and *CDPK1*) in the high salt environment ([Sun et al., 2020](#)).

Melatonin's role in leaf senescence was investigated using the melatonin degradation gene, *M3H* (melatonin 3-hydroxylase) overexpressing tomato plants. These transgenic lines exhibited lower melatonin levels and hastened senescence. Melatonin reduces the expression of senescence-related genes and thus regulates the leaf senescence ([Yu et al., 2022](#)). Melatonin application improves nutrition and agronomic qualities. It enhances color, flavor, antioxidant content, shelf life, and total nutritional value. The melatonin biosynthesis gene was discovered and characterized in tomatoes. Overexpression of *ASNT5*, *ASMT7*, *COMT2*, and *SNAT* genes in tomato led to increased melatonin synthesis. The red light treatment increased expression of the *ASMT5* and *COMT2* genes, which increased melatonin production. The molecular mechanism was linked to the interaction of *SlphyB2* and *SLPIF4*, which facilitates *SPIF4* breakdown by the 26S proteasome complex and removes the inhibition of *SlCOMT2*, resulting in melatonin accumulation during red light treatment ([Zhang et al., 2023](#)).

10.3.3 Tobacco

Tobacco is a model plant for studying novel gene activities involved in abiotic and biochemical stress tolerance ([Schaeffer et al., 2012](#)).

Indoleamine 2, 3-dioxygenase (*IDO*), a gene implicated in tryptophan degradation, was identified from rice and expressed in the tobacco plant. The transgenic plants revealed a lower levels of melatonin ([Okazaki et al., 2010](#)). The transgenic tobacco with the melatonin biosynthesis gene *NtCOMI* produced higher melatonin. These plants showed less wilting, an active antioxidant system, and higher expression of drought-responsive genes ([Yao et al., 2022](#)). Two *SNAT* genes in tobacco were discovered and characterized: *NbSNAT1* and *NbSNAT2*. Overexpression of these two genes in the tobacco plant did not result in an increase in melatonin content due to catabolic enzyme activity ([Back et al., 2021](#)). The ectopic expression of another mulberry melatonin biosynthesis gene, *T5H2*, tryptamine-5-hydrolase, in tobacco resulted in increased melatonin concentration. The function of this gene in salt stress resistance was explored during salt stress. *T5H2* overexpressing tobacco transgenic lines displayed higher expression of catalase and superoxide dismutase antioxidant enzymes, increased proline content, and reduced malondialdehyde content than the wild type. Transgenic tobacco overexpressing the *T5H2* gene grew quicker under salt stress. This gene may be a candidate for producing salt-resistant plants ([Zhu et al., 2022](#)).

10.3.4 *Arabidopsis*

Arabidopsis is a model plant for molecular biology research. Melatonin's significance in plant growth, development, and senescence has been discovered. However, the perception and signaling of melatonin remain an area of investigation. Cassava melatonin receptor was recently identified,

and its overexpression in *Arabidopsis* was related to the plant development and senescence delay. Transgenic *Arabidopsis* produced more biomass, more photosynthetic activity, more chlorophyll content, and fewer reactive oxygen species (hydrogen peroxide and superoxide concentration) than the wild type ([Cheng et al., 2024](#)). NADPH oxidase produces ROS and initiates ROS signaling, which is critical for salt stress resistance in *Arabidopsis*. The respiratory burst oxidase homolog (Rboh) gene encodes NADH oxidase, which is implicated in ROS-mediated signaling and salt tolerance. In *Arabidopsis*, this gene was altered, causing salt sensitivity even in the presence of melatonin. Melatonin treatment activated an antioxidant defense system and SOS-driven Na⁺ efflux in wild-type *Arabidopsis*, which was mediated by RbohF-dependent ROS signaling. Thus, melatonin-induced salinity tolerance was linked to RbohF-dependent ROS signaling in *Arabidopsis* ([Chen et al., 2017](#)). Melatonin's contribution to development and growth was previously explained. [Lee et al. \(2019\)](#) recently explored the contribution of melatonin in blooming by developing an *Arabidopsis* *SNAT2* knockout mutant. *SNAT2* mRNA was expressed in flowers. The *SNAT2* knockout mutants have lower melatonin levels than the wild type. The mutant has lower expression of FLOWERING LOCUS T (*FT*) and entkaurene synthase (*KS*), gibberellin biosynthesis genes ([Lee et al., 2019](#)). The *ASMT* gene was discovered and characterized in *Arabidopsis*. Melatonin concentration increased in *Arabidopsis* after cadmium treatment ([Byeon et al., 2016](#)). Melatonin's effect on circadian rhythm and its downstream signaling pathway were investigated in *Arabidopsis* ([Shi et al., 2016](#)). The microarray data analysis revealed that *SNAT* and *COMT* transcript levels varied throughout the day. Melatonin levels and diurnal fluctuations influence downstream signals such as *C-repeat binding factors* (*CBFs*)/ *Drought response element Binding 1 factors* (*DREB1s*). The CBF

knockout lines showed compromised resistance against *Pseudomonas syringae* pv. Tomato (Pst) strain DC3000 and lost gene expression of *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*). These results showed that melatonin and *CBF/DREB1s* are crucial for diurnal changes including *CCA1* expression and disease resistance.

10.3.5 Watermelon

Watermelon (*Citrullus lanatus*) is a major horticultural crop. Melatonin's significance in abiotic stress tolerance has been previously investigated ([Li et al., 2017](#), [Nawaz et al., 2020](#)). The complexity of genetic transformation resulted in the identification and cloning of only a few melatonin synthesis genes in watermelon. The effect of melatonin on leaf senescence was investigated in watermelon overexpressing the *ASMT* gene. The transgenic line had decreased MDA and ROS levels despite expressing more antioxidant enzymes. *ASMT* overexpression transgenic lines showed lower expression of the senescence-associated gene *SAG20* and the cell death-related gene. Melatonin was found to reduce leaf senescence by inhibiting ABA (abscisic acid) synthesis and promoting IAA (auxin) synthesis ([Jing et al., 2022](#)).

10.4 FUTURE PROSPECTS

Phytomelatonin is a novel growth regulator that is being explored in plants for greater tolerance to environmental challenges. The different isoforms of melatonin biosynthetic enzymes play a function in various environmental stressors. The researchers must investigate the many roles that these isoforms play, as well as the factors that influence melatonin production. These regulatory mechanisms can be modified via genetic engineering to enhance endogenous melatonin content in plants. The most recent studies

conducted in the melatonin-induced changes of phytochrome levels ([Di et al., 2024](#); [Kakkar, 2023](#); [Zhang et al., 2023](#)).

More research is needed to better understand the connection between melatonin and phytohormones requires further investigation to gain a full picture of the hormones' synergistic and antagonistic roles, which ultimately affect plant growth and development. Melatonin receptor, *PMTR1*, was found in 2018 ([Wei et al., 2018](#)). The signaling pathway includes the G-protein and MAPK cascades, and ROS and calcium may play a role in downstream signaling. However, the detailed signaling process must be investigated. The transcription factors and response regulators involved in melatonin signaling are yet unclear. The melatonin synthesis genes in the endoplasmic reticulum and cytoplasm has motivated an investigation into the melatonin presence in vascular tissues ([Kao et al., 2024](#); [Zhu et al., 2024](#)). It will be valuable for studying melatonin's role in nutrition uptake and transport. Melatonin's concentration-dependent effect had been shown under various environmental conditions, hence there is a need for a simple detection method in plant samples. Melatonin transgenic plants studies are known for a few species, including rice, tomato, tobacco, *Arabidopsis*, and watermelon, and they need to be expanded to other crops.

10.5 CONCLUSION

Melatonin has recently been studied for its function in plant growth and development, redox homeostasis, photosynthesis, osmoregulation, plant immunology, and phytoremediation under adverse conditions. Melatonin biosynthesis genes can be overexpressed or silenced to help overcome environmental stress. Potential target areas include the modification of melatonin biosynthesis genes. However, the discovery of the melatonin receptor opens the door to investigating additional downstream candidates

such as regulators, transcription factors, chaperones, and so on. Melatonin biosynthesis genes that have been validated for their significance in many crops, including rice, tomato, tobacco, *Arabidopsis*, watermelon, and soybean, under abiotic and biotic stress tolerance, could be employed as breeding targets.

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